Transcription Regulation in Eukaryotes

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WORKSHOP VII

Transcription Regulation in Eukaryotes

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"Transcription Regulation in Eukaryotes"
Eds P. Chambon, T. Fukasawa, R. D. Kornberg and C. Coath. HFSP, Strasbourg, 1999

HFSP WORKSHOPS

The HFSP workshops were initiated by **Michel Cuénod** (*Secretary-General, HFSPO*) with the support of the Board of Trustees and the Council of Scientists. The workshops, held in English, are organized at the HFSPO office in Strasbourg on specific scientific topics of particular timeliness in the two fields of research covered by HFSP: brain functions and biological functions at the molecular level. The guiding principles are general significance and novelty of the topic, treatment by leading experts, emphasis on discussion, and broad international, intercontinental and interdisciplinary representation. There is no general audience but the reports, edited by the organizers, present the issues discussed in a format attractive to specialists, post-doctoral trainees and students.
The present report is based on a meeting held in Strasbourg from December 3–5, 1998.

Acknowledgements

The proposal for the workshop came from **Pierre Chambon** (*Strasbourg*), **Toshio Fukasawa** (*Tokyo*) and **Roger Kornberg** (*Stanford*) and the meeting was organized by them with the help of the HFSP staff.

Our thanks go to the five rapporteurs who wrote the initial reports of the talks given at the meeting:
Geneviève Almouzni (*Paris*): Kingston, Hörz, Struhl, Egly
Stefan Björklund (*Umeå*): Kornberg, Sakurai, Green, Svejstrup
Irwin Davidson (*Strasbourg*): Roeder, Meisterernst, Tora, Sentenac
Karl Nightingale (*Cambridge*): Workman, Wu, Becker, Tjian
Kerstin Weiss (*Zürich*): Richmond, Travers, Allis, Cairns

These reports were edited and the book produced by **Chris Coath** (*Norwich*) and **Jennifer Altman** (*London*).

We thank **Valérie Dupé** (*London*) and **Olivia Wendling** (*Strasbourg*) for technical support at the meeting, **Stuart Molloy** (Resolution Design Ltd, *London*) for the artwork, **Alissa Surges** (*Ann Arbor*) for compiling the bibliography and **Solange Metzger** (*HFSPO, Strasbourg*) for her unfailing support in administration and production.

We acknowledge the following publishers for their permission to reproduce copyrighted figures: Academic Press (*Fig. 9* from *The Journal of Molecular Biology*); The American Society for Microbiology (*Fig. 62* from *Molecular and Cellular Biology*); Cell Press (*Figs 18, 19, 20, 58* and *Table 7* from *Cell*; *Figs 21, 36, 37, 38, 41, 42, 60, 68, 69, 70* from *Molecular Cell*); Cold Spring Harbor Laboratory Press (figure in *Box 1* from *Cold Spring Harbor Symposium on Quantitative Biology*; *Fig. 61* from *Genes and Development*); The National Academy of Sciences USA (*Figs 43, 44* from *The Proceedings of the National Academy of Sciences USA*).

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GLOSSARY AND ABBREVIATIONS

Terms shown in italics are explained elsewhere in the Glossary.

DNA structure and chromosome organization

B-form DNA	the classic right-handed helical DNA structure, with an axial repeat of about 34 Å and approximately 10 base pairs per turn
chromatid	one half of a replicated chromosome
chromatin	the packaged eukaryotic chromosome in which the DNA is highly organized into *chromatosomes*; see *higher-order structure*
chromatosome	*nucleosome* with linker *histone* H1 (H5 in avian cells) bound to *linker DNA*
duplex DNA	double-stranded DNA
dyad	an axis of two-fold rotational symmetry; in the case of the core *nucleosome* particle, the dyad provides the reference point for the major core *histone* contacts; cf. *midpoint*
euchromatin	diffuse region of *interphase chromatin* in which genes are actively transcribed; note that it also contains inactive regions
heterochromatin	a highly compact, transcriptionally inactive portion of the *chromatin* in *interphase*; see also *euchromatin* and *higher-order structure*
higher-order structure	supranucleosomal organization of the *chromatin*; DNA, organized into *nucleosomes* joined by *linker DNA* and associated *histone* H1, i.e., *chromatosome*, is further condensed into a fibre of diameter 30 nm, which itself is folded in some manner
histones	small group of highly conserved basic proteins that bind DNA and form core of a *nucleosome*
homeobox	conserved DNA sequence of 180 nucleotides found in coding region of many genes that encodes the homeodomain, a helix–turn–helix DNA-binding protein structure

linker DNA	DNA connecting *nucleosomes*
major groove	exterior surface of DNA, important in DNA–protein interactions; major groove is the side with the C4 of the pyrimidine and N7 of purine; *minor groove* with O2 of pyrimidine and N3 of purine
midpoint	centre of the DNA region protected by the core *histones* and the central globular domain in the *chromatosome*; cf. *dyad*
minor groove	see *major groove*
nucleosome	basic repeating subunit in eukaryotic *chromatin* comprising a *histone* octamer core of two copies each of H2A, H2B, H3 and H4, and an average of 180–200 *bp* of DNA; see *higher-order structure*
promoter	transcription-controlling domain in a gene; the promoter generally contains the binding site for RNA *polymerase*, the *TATA box*, and sites for the binding of regulatory proteins, i.e., *transcription factors*, *activators* and *repressors*
TATA box	DNA motif with the 7 *bp* consensus sequence TATA(A/T)A(A/T) found in many eukaryotic *promoters*; *TBP* binds the TATA box as the first step in the formation of the *preinitiation complex*
UAS	upstream activation sequence; in yeast can be used as a synonym for *enhancer* in other eukaryotes

Transcription

activator	protein product of a regulatory gene that induces expression of a target gene(s) usually by binding to the activation sequence of that gene or by interaction with transcription factors; cf. *repressor*
adaptor	intermediary factor that transduces a signal from DNA-bound regulatory proteins, e.g., *activators*, to the *basal transcription* machinery
basal transcription	transcription in in vitro systems consisting of RNA polymerase, the basal transcription factors and naked DNA template; also used to describe in vivo transcription observed in the absence of known *activators*
cis-acting element (sequence)	a DNA sequence that affects its own activity or the activity of a gene (sequence) in the same chromosome

coactivator	generally proteins that enhance in vitro or in vivo transcription in some way in addition to the effect of *activators*
CTD	C-terminal repeat domain of *RNA polymerase* II; a heptapeptide repeated 26–52 times at the C terminus of the largest subunit of *RNA polymerase* II; the activity of the polymerase is regulated by the phosphorylation of this repeated heptapeptide sequence, which is necessary for transcription
enhancer	binding site for a regulatory protein(s) or *activator* that determines the efficiency of transcription
elongation	process by which the RNA transcript chain is sequentially lengthened by *RNA polymerase* using DNA as the template
helicase	an ATP-dependent enzyme that can unwind a nucleic acid *duplex*
holoenzyme	see *holo-RNA polymerase* II
holopolymerase	see *holo-RNA polymerase* II
holo-RNA polymerase II	core *RNA polymerase* II associated with the *Mediator* complex; note that there is some controversy associated with this term, as it is sometimes intended to designate the *polymerase* associated with basal factors in a high-molecular mass complex whose existence in vivo remains to be established
kinase	an enzyme that catalyses phosphorylation, e.g., the kinase in *transcription factor* TFIIH phosphorylates the *CTD* of *RNA polymerase* II
promoter	transcription-controlling domain in a gene; the promoter generally contains the binding site for *RNA polymerase*, the *TATA box*, and sites such as *enhancer* or *UAS* for the binding of regulatory proteins, i.e., *transcription factors*, *activators* and *repressors*; synonym for 'core promoter', the upstream region containing the *TATA box*, the initiator or binding sites for any protein required for the accurate initiation of transcription

promoter opening	process by which the region of the gene containing the *promoter* is made accessible to regulatory proteins; often refers to the promoter region becoming single-stranded so that *RNA polymerase* can proceed from initiation to elongation
repressor	protein product of a regulatory gene that prevents expression of a target gene(s) usually by binding to the operator sequence of that gene or by interaction with activators, histones or transcription factors; cf., *activator*
RNA polymerases	enzymes that catalyse the formation of polymers; in eukaryotes there are three types of RNA polymerase responsible for gene transcription: I synthesizes most rRNA; II synthesizes most mRNA and some *snRNA*; III synthesizes precursors of 5S rRNA, tRNA and the remaining *snRNA*s and cytosolic RNAs
topoisomerase	enzyme that catalyses the conversion between topological isomers of DNA and generally reduces the level of positive or negative supercoiling; topoisomerase I cleaves one DNA strand whereas topoisomerase II cleaves both DNA strands
transcriptional silencing	negative regulation of transcription; local repression of gene expression by a change in *chromatin* structure, e.g., in the telomere or another region in *heterochromatin*

Transcriptional complexes

ACF	ATP-dependent *chromatin* assembly and remodelling factor
CHRAC	*chromatin* accessibility complex
CRSP	cofactor required for Sp1 activation
Mediator	general transcription regulatory complex, found first in yeast, with homologues in many eukaryotes, including *CRSP* and *SMCC*; forms *holoenzyme* with *RNA polymerase* II
NURF	nucleosome remodelling factor
PIC	see *preinitiation complex*

preinitiation complex	complex of general transcription factors, i.e., TFIID, B, F and E, and *RNA polymerase* II assembled at the *promoter* sufficient for *basal transcription*; the complex can support a low level of transcription without *activators*
RSC	remodels the structure of *chromatin*
SMCC	Srb- and Med-containing coactivator complex
SWI/SNF	switching mating types/sucrose non-fermenting
TFTC	TBP-free TAF-containing complex

Histone-modifying complexes/proteins

CBP	*CREB*-binding protein; also known as p300
CREB	cyclic AMP response element binding factor
HAT	histone acetyltransferase
NuA4	*nucleosome*-associated–*histone* H4
PCAF	p300/*CBP*-associated factor
SAGA	Spt1-Ada-Gcn5-acetyltransferase

Other transcription factors

AAD	acidic activation domains; transcriptional activation domain rich in acidic amino acids found in some *transcription factors*
ARC	*activator*-recruited cofactor
BAF	Brg1- or Brahma-associated factor
CAF	*chromatin* assembly factor
CAK	Cdk-activating kinase
GAGA factor	specific eukaryotic transcription factor that binds to GA/CT-rich sites
hTAF, yTAF, dTAF	human, yeast, *Drosophila TAF*s. The prefix is used only where confusion is likely, otherwise it is assumed the TAF is from the organism discussed in that article
ISWI	imitation switch

PAF	*PCAF*-associated factor
TAF	*TBP*-associated factor; TAF_{II}, TAFs for class II genes, i.e., those transcribed by *RNA polymerase* II
TBP	*TATA* box binding protein
TF	see *transcription factor*
transcription factor	a protein that participates in gene transcription, often by binding to a specific DNA sequence, e.g., TFIID
TTF-1	transcription termination factor 1
USA	upstream stimulatory activity
VP16	viral protein 16 from herpes simplex 1, also known as etoposide; an *activator*

Genetics and cell cycle

Conventions

gene names	in italics throughout the text
dominant	all capitals (yeast, human); first letter capital (*Drosophila*, mouse); all lower case (nematodes)
recessive	all capitals (human); all lower case (*Drosophila*, yeast, mouse, nematodes)
proteins	unless stated otherwise, the protein coded by a gene uses the same name/abbreviation but in regular type with first letter always capital and following letters lower case, except for nematodes (all capitals) and in humans when the protein name is an acronym
alleles	variants of a gene found in the normal population; as individuals carry two copies of each gene, one on each pair of chromosomes, they may have identical (*homozygous*) or different (*heterozygous*) alleles
conditional alleles	*allele* that affects the *phenotype* only under specific conditions, e.g., a *temperature-sensitive* mutation

Δ	used to indicate a deletion strain; see *gene knockout*. Usage: Δ before the deleted gene in bacteria and after in yeast; mammals do not use this terminology
G1/S phase	phase in cell cycle between G1 phase, during which cells prepare for DNA replication, and S phase, when DNA is replicated
gene knockout	complete loss of function of a gene in vivo, usually achieved by making a transgenic organism in which the gene has been made nonfunctional by targeted disruption or replacement with a nonfunctional copy; also known as a deletion
genetic rescue	confirmation of the possible function of a gene through restoring function by introducing a cDNA for the normal gene into an organism in which the gene is defective
heterozygous	organism that has different *alleles* of the gene under study, or one allele that carries a mutation or has been deleted
homozygous	organism carrying two identical *alleles* or mutations of a gene, or with both alleles deleted (cf. *heterozygous*)
interphase	part of cell cycle made up of G1, S and G2 phases; see *G1/S phase*
kinetochore	protein complex that attaches to the microtubules and helps to segregate DNA during mitosis
lacZ reporter	a gene is fused to bacterial *lacZ*, which encodes β-galactosidase, so that the expression of that gene is detected by a coloured reaction product
null	an *allele* of the gene that has been deleted, disrupted or mutated, so that it does not produce any trace of the encoded protein
open reading frame	a DNA or RNA sequence between a start signal and termination codon that can be translated into a polypeptide
phenotype	physical manifestation of *wild type*, mutant or deleted gene

temperature sensitive	*conditional allele* that expresses its mutant *phenotype* only at a higher (restrictive or nonpermissive) temperature but shows a *wildtype* phenotype at a lower temperature
transcript	RNA or product of transcription of a particular gene
ts	see *temperature sensitive*
wildtype	organism with no known mutation in the gene under study

General terms and abbreviations

acetyl CoA	acetyl coenzyme A, an acetyl group carrier
BAH	bromo-adjacent homology region/domain
bp	base pairs; measure of size of piece of DNA
Cre/Lox	a method to direct single-copy site-specific integration of exogenous DNA into the genome (Sauer and Henderson, 1990); Cre recombinase is a bacteriophage P1 protein that catalyses a site-specific recombination between a pair of specific LoxP sequences without the need of cofactors
downregulation	reduction, not cessation, in the effect being studied, e.g., gene expression
downstream	1. location of a motif or domain in a gene nearer the 3′ end of the sequence than a reference site; gene sequences are read from the amino (NH_2) terminal, also called the 5′ end, to the carboxy (C) terminal or 3′ end; 2. later reactions in a biochemical cascade or pathway
epitope tagging	method that allows a protein to be identified in the cell and to investigate the effects of altering specific amino acids in that protein; a fusion protein is engineered containing a short peptide, the epitope, that can be recognized by an antibody or purified by immunological procedures from the cell
FLAG	a specific type of epitope tag; see *epitope tagging*
footprinting	technique to identify position of DNA sequences bound by particular proteins

Hsp70	heat-shock protein with M_r ~70K; a family of stress-induced proteins, some of which are induced in response to heat
kb	kilobase = 1000 base pairs; measure of size of piece of DNA
MAPK	*mitogen*- (or messenger-) activated protein *kinase*
-mer	denotes number of units in a molecule; e.g., 12-mer oligonucleotide indicates a molecule with 12 nucleotides
mitogen	an agent that can induce mitosis
M_r	relative molecular mass, no units; an M_r of 1000 is represented by 1K; M_r is numerically equivalent to daltons (1K = 1kDa)
P_i	inorganic phosphate
Sarkosyl	an anionic detergent (sodium lauroylsarcosine or sodium *N*-lauroylsarcosinate) commonly used to disrupt multiprotein complexes; it can prevent protein–protein interactions without disrupting protein complexes already formed
snRNA	small nuclear RNA
TRAP	thyroid hormone receptor-associated proteins
TRRAP	transformation/transcription domain-associated protein
upregulation	the opposite of *downregulation*
upstream	opposite direction to *downstream*
VDR	vitamin D3 receptor
WD40 repeat proteins	a 40 amino-acid repeat usually ending in Trp-Asp, first described in the guanine nucleotide binding protein β-subunit, thought to be involved in protein–protein interactions

INTRODUCTION

Roger D. Kornberg

Gene regulation in eukaryotes begins with the binding of sequence-specific activator and repressor proteins to DNA elements termed enhancers, operators and silencers. It was once thought that such DNA-binding proteins regulated transcription by direct interaction with the basal transcription machinery at promoters. This view was altered by the discovery of global regulators, so-called because of their effects on the transcription of many promoters, which play an intermediary role and transduce information from regulatory DNA elements to promoters. The current intense interest in these molecules, reflected by the involvement of many laboratories and a burgeoning literature, prompted the organization of the Human Frontier Workshop reported in this volume.

Global regulators came to light in genetic screens in yeast and from biochemical studies in yeast, *Drosophila* and mammals. The SWI, SNF, SPT, SRB* and GAL11 families, comprising over 50 members, were identified in genetic screens and, with the biochemical isolation of NURF, CHRAC, ACF, SAGA, RSC,* Mediator and other multiprotein complexes, over 100 global regulatory molecules are now known. More than half of these molecules interact with chromatin, perturbing its structure in ways that activate or repress transcription, e.g., by disrupting nucleosome structure. One of the areas generating most excitement in the field is the complexes that have enzymatic activities directed towards chromatin. SAGA and other global regulatory proteins possess histone acetyltransferase activities, believed to play a role in activation, and repression is brought about, at least in part, by deacetylation, e.g., by the Sin3–Rpd3 histone deacetylase complex.

* see *Glossary* for full names and definitions.

Another target of global regulators is the chief transcription enzyme, RNA polymerase II. The Srb proteins, members of the GAL11 family and newly discovered Med proteins combine to form a 20-subunit complex, termed Mediator, which interacts with the C-terminal repeat domain of polymerase II. The C-terminal domain is a feature unique to this enzyme and essential for transcriptional activation in both yeast and mammalian cells in vivo. Mediator conveys regulatory information to the polymerase that modulates the frequency of initiation of transcription. During elongation of the RNA chain, Mediator is replaced on the C-terminal domain by (an)other regulatory complex(es). The binding of the TATA-binding protein (TBP) to the TATA element of polymerase II promoters, an early step in the initiation of transcription, is regulated by other proteins termed TBP-associated factors (TAFs). The relative contributions of Mediator and TAFs to transcriptional regulation is an important topic currently being investigated.

The proceedings of the workshop have been ably reviewed (Björklund *et al.*, 1999). Here I introduce the main themes covered in this book — the mechanisms of chromatin remodelling and transcriptional activation and their universality — and results published since the meeting.

Chromatin-remodelling and chromatin-modifying complexes

The X-ray structure of the nucleosome core particle (Richmond), i.e., the basic unit of chromatin, comprising DNA wrapped around a histone octamer, and the structural basis of the interaction between linker histone H5 and nucleosome (Travers) place studies on chromatin in context. Particularly interesting is the lack of a defined conformation of the histone 'tails', amino- and carboxy-terminal domains extending from the body of the core particle. They make no contribution to its structure but rather serve as sites for interaction with regulatory enzymes and proteins.

One important chromatin-remodelling complex, *Drosophila*

NURF, requires the histone tails for its activity (Wu). NURF and another chromatin-remodelling complex, CHRAC (Becker), alter chromatin structure by catalysis of nucleosome 'sliding' and consequent exposure of DNA sequences previously covered by nucleosomes (Hamiche *et al.*, 1999; Längst *et al.*, 1999). The mode of action of RSC, and by inference other members of the SWI/SNF family of remodelling complexes, differs from that of NURF and CHRAC in several regards (Kingston, Cairns, Kornberg): the histone tails are not required but rather nucleosomal DNA may be may attacked from the ends to form an 'activated' state of the nucleosome, characterized by a grossly perturbed histone–DNA interaction. These complexes can also catalyse histone octamer transfer to naked DNA, apparently by means of the same activated intermediate (Lorch *et al.*, 1999).

The histone tails are also targets of chromatin-modifying complexes. Acetylation and deacetylation of histone H3 and H4 tails are a primary basis for transcriptional control by coactivators and co-repressors. These important regulatory proteins may themselves possess catalytic activity, such as p300/CBP (see *Glossary*) which is a histone acetyltransferase, or they may attract catalytic factors, e.g., the yeast and mammalian co-repressors that bridge between DNA-binding proteins and histone deacetylase complexes (Struhl). The major histone acetyltransferase complex, SAGA, is brought to promoters in vitro by direct interaction with VP16 and Gcn4 activation domains, which potentiates both acetylation and transcription of nucleosomal templates (Workman). Histone acetyltransferases may also influence RNA chain elongation (Svejstrup). Phosphorylation of histone tails may play an important transcriptional regulatory role but has been little studied. Discovery of a histone-tail kinase that is a downstream target of the mitogen-activated protein kinase cascade indicates that histone phosphorylation may be an endpoint in the nucleus of a cellular signalling pathway (Allis).

The most pertinent questions are the consequences of all

histone modifications for chromatin structure and how they influence transcription. A role for the histone tails in higher-order coiling, i.e., structure at the highest level (see *Glossary*), of chromatin fibres is indicated by the lack of involvement in core particle structure, together with other observations. There is evidence for both direct interaction of the tails with adjacent nucleosomes and interaction with other proteins which, in turn, create higher-order structures. In the yeast *PHO5* promoter, an interplay between the configuration of a nucleosomal array and histone acetylation in vivo has been revealed (Hörz).

Mediator

Disruption of chromatin structure by complexes that remodel and modify chromatin allows promoters to interact with RNA polymerases and general transcription factors. Although a large and probably complete set of these transcription proteins has been identified in a wide range of organisms, most aspects of the transcription mechanism remain obscure. However, similarities have now been demonstrated between transcription by the different RNA polymerases of eukaryotic cells (Sentenac) and connections established between transcription, DNA repair and human disease (Egly).

As well as influencing the chromatin structure of promoters, transcriptional activator and repressor proteins are believed to affect the assembly and function of transcription-initiation complexes. This dual role of DNA-binding regulatory proteins is reflected in a duality of global regulators. In addition to the complexes that remodel and modify chromatin, important for transcription of many promoters, the necessity for a transcriptional mediator may be even more widespread. Mediator activity was originally identified in yeast extracts that were required for a partially reconstituted RNA polymerase II transcription system to respond to activator proteins and is now known to reside in a 20-subunit complex containing Srb, Gal11 and other previously

described proteins implicated by genetic evidence in transcriptional control (Kornberg). Mediator interacts with both RNA polymerase II and general transcription factors, such as TFIIE and TFIIH (Sakurai); analysis of a yeast mutant shows that Mediator is required for transcription of almost all promoters in vivo.

One of the revelations of the workshop was the universality of Mediator in diverse systems. Multiprotein complexes isolated from mouse and human cells by affinity methods or on the basis of a requirement for transcriptional activation all contain a set of related proteins (Kornberg, Roeder, Tjian). Another multiprotein complex, termed TRAP or DRIP, which plays an intermediary role in transcriptional regulation by nuclear receptors, was reported after the meeting to be essentially the same as human Mediator, also known as SMCC (M. Ito *et al.*, 1999; Roeder, this volume).

Other coactivators that interact with the basal transcription machinery and that may play global regulatory roles as well have been isolated. Principal among these are the TAF_{II} complex and the upstream stimulatory activity fraction. The regulatory roles of several TAF_{II}-containing complexes that have been isolated from both yeast and human cells (Workman, Tora) are under investigation by genetics and other means (Green, Struhl, Tora). The TAF_{II} complex, as well as a negative-acting component of the upstream stimulatory activity fraction, NC2 (Meisterernst), interact directly with TBP, modulating its function in an undetermined manner.

The current intense interest in global regulators may be only a prelude to even greater activity in the future, as studies of diverse transcriptional regulatory systems converge on the importance of these molecules. The emphasis to date has been on discovery and an astonishing number and variety of molecules have been identified. Now the molecules are being classified and their functional significance determined. This consolidation should soon reveal a large part of the overall picture of eukaryotic gene regulation.

PART I

CHROMATIN STRUCTURE IN GENE REGULATION

Introduction

Karl Nightingale

The packaging of the DNA template in eukaryotic cells raises an intriguing and complex biological question: how does the cell fit the two-metre-long DNA molecule into the nucleus while ensuring that the genome is optimally organized, particularly as the pattern of gene activity in a typical cell is constantly changing? This remarkable feat of biological engineering is performed by organizing the DNA template into a highly complex and dynamic protein scaffold termed chromatin. Yet despite its necessary complexity, the basic structure of chromatin is extremely simple, consisting of a regular array of nucleosomes spaced along the DNA at an average, between species, of about 180–200 base-pair intervals. Each nucleosome, the fundamental building block of chromatin, consists of a compact ball of eight highly folded core histone proteins, two each of histones H2A, H2B, H3 and H4, around which usually 146 base pairs of DNA are wrapped in almost two complete turns. The high-resolution structure of the nucleosome is described by Timothy Richmond. The linker histone H1 stabilizes the DNA on the nucleosome core by binding at the point where the DNA enters and exits the core. H1 bound to the nucleosome forms a unit known as the chromatosome and usually binds an additional 20 base pairs of DNA. The length of the linker DNA between chromatosomes varies between species and cells but the average is 55 base pairs.

The assembly of DNA into a nucleosome and then a chromatosome gives a basic level of compaction of the DNA molecule but further condensation of the chromatin fibre is promoted by subsequent interactions between nucleosomes (*Fig. 1*).

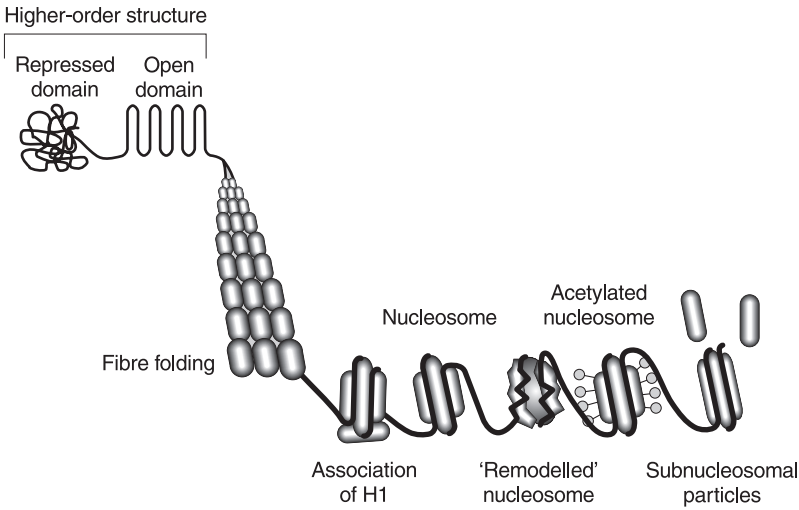

Figure 1. *Rendering chromatin more permissive for transcription. Several proteins or multiprotein complexes modulate transcriptional activity by their effects on chromatin structure. These include linker histone H1, which acts as a transcriptional repressor. In contrast, both acetylation of nucleosomal histones and chromatin remodelling activities can preclude transcriptional activation. See text for details.*

These initially produce a solenoid of about 30 nm in diameter, termed the 30 nm fibre; after further refolding this yields the ultimate level of condensation seen in the metaphase chromosome, known as the higher-order structure. Thus the fundamental structure of chromatin provides an elegant means of packaging the genome.

However, the functions of chromatin are not merely restricted to packaging DNA; the chromatin framework is also used in transcriptional regulation. This is immediately apparent from the high level of protein complexity superimposed upon the underlying array of nucleosomes in vivo, reflecting the various structural and functional requirements of the different regions of the DNA template. The heterogeneity arises not only from the

histone proteins, with specific variants and modifications, but also from the non-histone proteins that are associated with chromatin in specific functional states.

In this section, several apparently discrete but potentially inter-related chromatin-mediated mechanisms contributing to transcriptional regulation are considered (reviewed in Workman and Kingston, 1998). The proteins and multi-subunit complexes discussed seem to have direct effects on chromatin structure, which may simplistically be imagined to be through two non-exclusive ways: globally over entire genes or large regions of chromatin (chromatin domains); and/or locally, at specific nucleosomes associated with precise DNA sequences, such as transcription-factor binding sites.

• *Linker histones* bind nucleosomes together. Histone H1, or the related protein H5 in chicken, is the general family name for several abundant and highly related lysine-rich proteins that bind at or near the nucleosome dyad and stabilize the DNA entering and leaving the nucleosome. Andrew Travers discusses recent experiments pinpointing the location of histone H5 when bound to the nucleosome and discusses its role in transcriptional repression.

• *Histone modification.* Histones are subject to several post-translational modifications at highly conserved residues in the flexible amino- and carboxy-terminal 'tails' (Van Holde, 1989; *Table 1*). These tails extend from the central histone core and interact with many of the components of chromatin, including the linker DNA, adjacent nucleosomes and non-histone proteins.

Table 1. *Post-translational modifications of histones.*

Histone	Sites	Modification	Functional role(s)
H4, H3, H2A, H2B	Lysine	Acetylation	Transcriptional regulation Dosage compensation Chromatin assembly
H1, H4, H3, H2A, H2B	Serine	Phosphorylation	Unknown
H2A, H2B	Lysine	Ubiquitination	Unknown
H3, H4	Lysine	Methylation	Unknown
H1, H3, H2B	–	ADP-ribosylation	Unknown

They contribute functionally to both transcriptional activation and repression and are required for the higher-order folding of the chromatin fibre. Modification of the histone tails is therefore likely to have important consequences for both the structure of chromatin and the process of transcription in chromatin.

Histone acetylation is a highly dynamic modification and increases in acetylation can affect both chromatin structure and function. Histone hyperacetylation reduces the ability of nucleosome arrays to fold into higher-order structures in vitro, indicating that this chromatin forms an 'open' or extended structure; functionally, histone acetylation has been biochemically linked to transcription. David Allis reviews the occurrence of histone acetyltransferases (HAT) and Jerry Workman summarizes recent advances on the composition of several HAT complexes and their role in transcriptional regulation.

Both active and potentially active genes are assembled into chromatin with increased acetylation of histones, whereas transcriptionally inactive heterochromatin contains little acetylation. However, this correlation does not hold in all cases, indicating that the acetylation of specific residues on specific histones, rather than global or 'bulk' acetylation, may be functionally important. Interest in histone acetylation has increased with the recent discovery that a number of transcriptional coactivators and a component of the RNA polymerase II machinery have HAT activity in vitro, indicating that histone acetylation could be targeted to promoter regions (Allis, Workman). Conversely, complexes containing histone deacetylases are recruited to promoter regions by transcriptional repressors.

Histone phosphorylation also seems to have several roles in the cell. One example, described by Allis, is the specific phosphorylation of histone H3, which has roles both in the pathway leading to gene activation in response to mitogens and in the cell cycle.

• *Chromatin remodelling complexes.* As the DNA template is assembled into chromatin, nucleosomes prevent transcriptional

activators and basal transcription factors from binding to their cognate sites. Thus chromatin is associated with transcriptional repression, which can be overcome by mechanisms that 'remodel' nucleosomes to allow transcription factors access to DNA. Recently, multi-subunit chromatin remodelling complexes have been identified that use the energy of ATP hydrolysis to accomplish this remodelling (*Table 2*). These complexes have been identified either in yeast genetic screens designed to find transcriptional coactivators, such as SWI/SNF (Bradley Cairns and Roger Kornberg), or were isolated using biochemical chromatin disruption assays from *Drosophila* (NURF, described by Carl Wu; CHRAC by Peter Becker) or RSC in yeast (Cairns and Kornberg).

At present the concept of chromatin remodelling is used to describe several diverse and potentially different observations in various assays and the detail of the molecular process(es) is unclear. However, the functional picture is beginning to emerge as the component subunits of the chromatin remodelling complexes are identified and characterized (Becker, Cairns, Kingston, Wu). Recent biochemical studies on the mechanism of nucleosome disruption or chromatin remodelling by the CHRAC

Table 2. *Chromatin remodelling complexes.*

Factor	Organism	ATPase	Size (M_r)	Number of subunits	In vivo function
SWI/SNF	*Saccharomyces cerevisiae*	Swi2/Snf2	2,000K	11	Transcription of specific genes
Brahma complex	*Drosophila*	Brm (Brahma)	2,000K	Unknown	Essential for cell viability
Mammalian SWI/SNF	Human	Brg1	2,000K	9–12*	Unknown
		hBrm	2,000K	9–12*	
RSC	*S. cerevisiae*	Sth1	1,000K	15	Essential for mitotic growth
NURF	*Drosophila*	ISWI	500K	4	Unknown
CHRAC	*Drosophila*	ISWI	670K	5	Unknown
ACF	*Drosophila*	ISWI	220K	2	Unknown

M_r, relative molecular mass; * subunit heterogeneity.

complex (Becker) or by the related yeast RSC (Cairns/Kornberg) and human SWI/SNF complexes (Kingston), give some insight into the process of nucleosome remodelling and subsequent transcription factor binding. Lastly, Wolfram Hörz describes in vivo studies that examine the process of chromatin disruption associated with transcriptional activation at a specific promoter, the *PHO5* gene in yeast.

The nucleosome core structure at 2.0 Å resolution

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in collaboration with

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The nucleosome is the elemental repeating entity of chromatin and is responsible for the most fundamental level of DNA organization in the nuclei of eukaryotic cells. First visualized by electron microscopy as 'beads-on-a-string', the overall structure of the nucleosome was determined 25 years ago (Kornberg, 1974). The structure of the nucleosome core particle was solved using X-ray crystallography, first to a molecular resolution of 7 Å (Richmond *et al.*, 1984) and more recently at 2.8 Å (Luger *et al.*, 1997). The nucleosome core has a relative molecular mass of 206,000 (M_r 206K) and consists of a protein octamer comprising a tetramer of two copies of histones H3 and H4 bound by two dimers of histones H2A and H2B and a roughly equal mass of DNA. In chromatin the nucleosome cores are linked by short stretches of DNA bound by the linker histones H1/H5. Using X-ray data obtained at 2.8 Å and 2.0 Å spacings, we describe the structure of the nucleosome core particle and indicate the importance of water molecules and divalent ions in shaping this structure.

X-ray structure

The X-ray structure of the nucleosome core particle at 2.8 Å resolution (*Fig. 2*) shows that 147 bp of DNA are wrapped in 1.65 left-handed superhelical turns around the histone octamer. To accommodate this path, the DNA double helix has to bend and twist substantially, making it highly distorted compared to canonical B-form DNA. The histone protein chains are divided into three types of structures: rigid, folded α-helical domains, named the histone fold; histone-fold extensions, which interact with each other and the histone folds; and flexible histone tails,

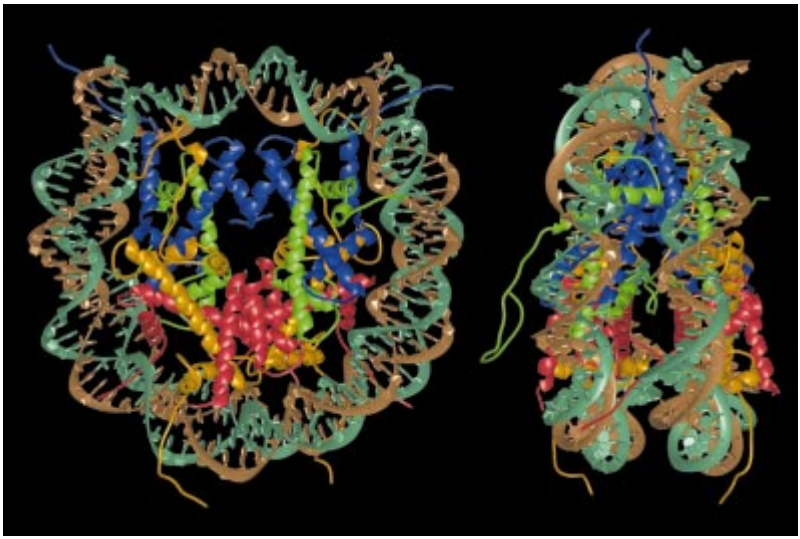

Figure 2. *The structure of the nucleosome, the universal repeating unit of DNA packaging in eukaryotic cells. The crystal structure of the core particle at 2.8 Å resolution shows that the DNA double helix, effectively 147 bp in two chains (dark green and brown), is wound around a histone octamer (two copies each of H2A, orange; H2B, red; H3, blue; and H4, light green) in 1.65 left-handed superhelical turns. The left view is down the DNA superhelix axis. The right view is orthogonal to the DNA superhelix and to the overall molecular pseudo-fold axis. Although the histone-fold domains, their extensions and the DNA can be seen fully in the electron density map, only about one-third of the histone tail regions are seen. Modified from Luger* et al.*, 1997.*

which are the N-terminal regions of the histone proteins. The structure refined to 2.0 Å provides almost three times more X-ray data than at 2.8 Å, allowing us to include approximately 1,000 solvent water molecules and ions, which make important contributions to the protein–DNA and protein–protein interactions throughout the complex (*Fig. 3*).

The histone-fold domains form crescent-shaped heterodimers, H3 with H4 and H2A with H2B that provide extensive interaction interfaces. The contacts within the dimers are made between the first two of the three α-helices that comprise the histone fold. The C termini of the second and third α-helices of the histone folds combine with each other to join the halves of the H3/H4 tetramer, through H3–H3 interactions, and add

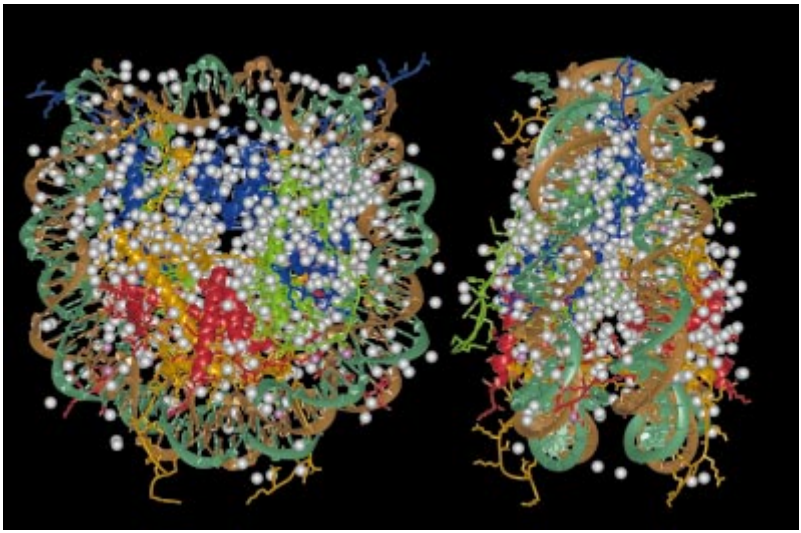

Figure 3. *The 2.0 Å X-ray structure of the nucleosome core particle allows the addition of nearly 1,000 well-ordered water molecules and salt ions (white and magenta spheres) to the over 12,000 atoms in protein and DNA seen in the structure at 2.8 Å* (Fig. 2). *The water molecules are important in stabilizing histone protein and DNA structure as well as adding many indirect protein–DNA interactions. Red and orange, H2A–H2B dimer; blue, H3 histone-fold domain; light green, H4 tail. X-ray data were obtained at the European Synchrotron Radiation Facility in Grenoble, France.*

the two H2A/H2B dimers to complete the octamer, through H4–H2B interactions. Networks of ordered water molecules are found between the histone subunit interfaces of H3 and H2A (*Fig. 4*) and of H4 and H2B and these further stabilize the octamer structure. The histone-fold extensions contribute to the nucleosome core particle structure in several ways, from octamer formation to DNA binding. One or more of the extensions are likely to be involved in internucleosomal contacts in the higher-order structure or in interactions with non-histone factors.

The flexible N-terminal tails of histones reach out between and around the gyres of the DNA superhelix to contact neighbouring particles in the crystal. About one-third of these flexible tails is apparent in the electron density maps calculated from the X-ray diffraction data. The disordered state of the tails, particularly at their sites of post-translational modifications — acetylation, methylation and phosphorylation (see Aliss, Workman, this volume) — indicates that the tails are primarily involved in higher-order chromatin organization. The transition between the more compact higher-order structure and the relatively open nucleofilament, i.e., beads-on-a-string, may be induced through modifications introduced by transcriptional coactivators as part of the overall control of gene expression.

The X-ray structure indicates that these regions of the protein chains are most likely to make internucleosomal interactions. The contact between the H4 N-terminal tail and an acidic patch created by H2A/H2B at the octamer surface of a neighbouring nucleosome core particle is essential for crystal formation. At 2.0 Å resolution, a manganese ion is seen to be bound in perfect octahedral coordination, forming a bridge from the H2A–H2B dimer of one nucleosome core particle to the H4 tail and H3 histone-fold domain of another (*Fig. 5*). These interactions may be relevant to the higher-order structure, a hypothesis that can be tested in vitro by using site-directed mutagenesis of defined segments of higher-order structure.

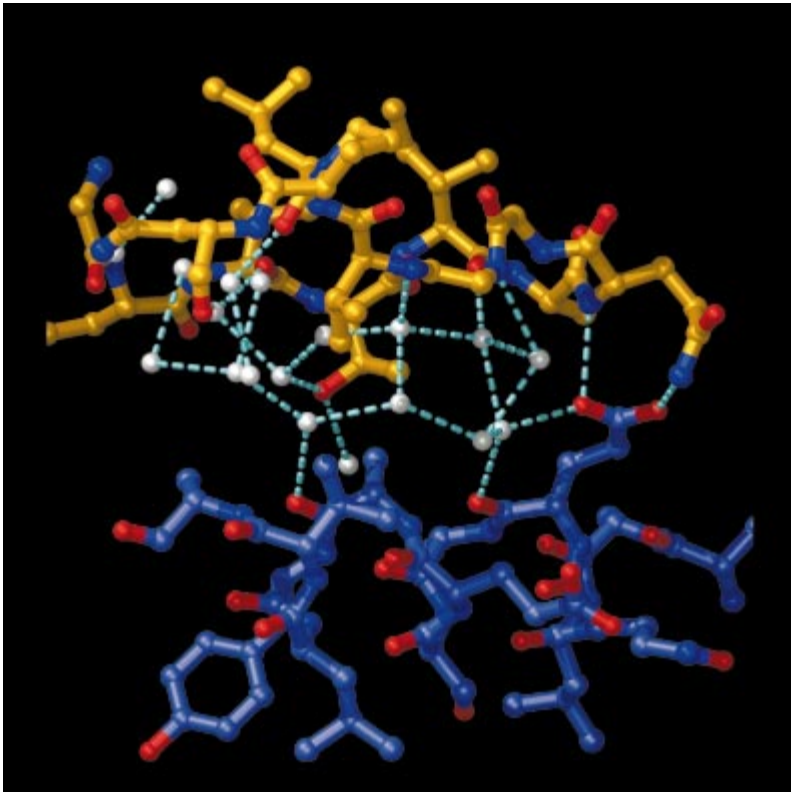

***Figure 4**. A complicated network of water molecules bridges between each pair of histone H2A (orange) and H3 (blue) molecules. Networks of water molecules (white) stabilize the interactions of the histones with each other within the nucleosome core. In the region of the histone octamer shown, the hydrogen bonds are in the interface between dimer and tetramer sub-assemblies.*

The four pairs of histone-fold domains in the histone octamer organize only 121 bp of DNA in the nucleosome core superhelix, not the entire 147 bp. One α-N histone-fold extension, i.e., on the most N-terminal α-helix in the H3 sequence, occurs just before each H3 histone fold and binds the first and last remaining 13 bp of the DNA superhelix. The odd number of base pairs in the DNA

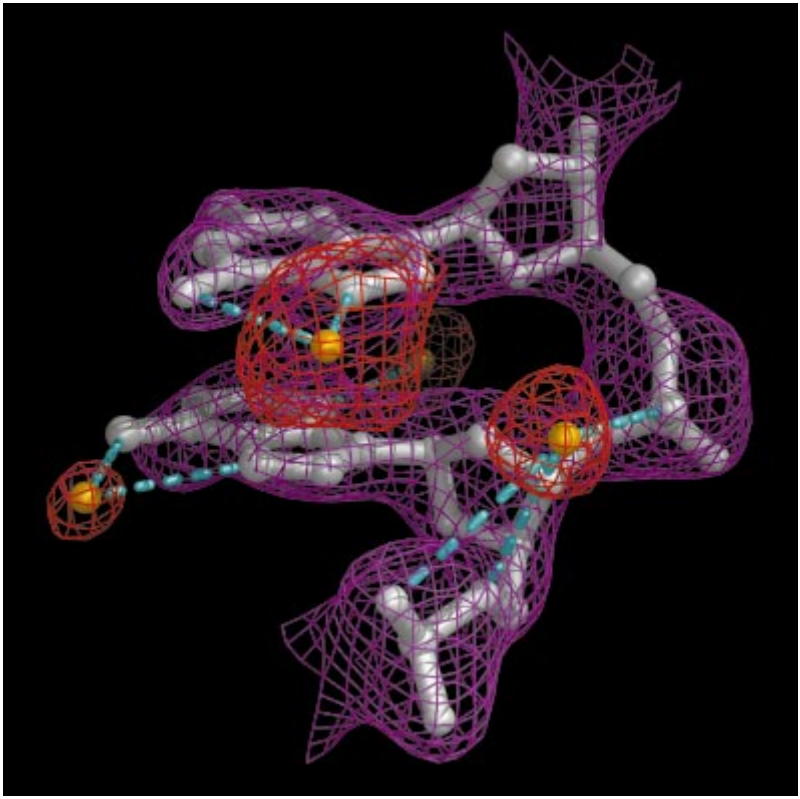

Figure 5. *The divalent manganese ions (35–50 mM; yellow) used in the crystallization of the nucleosome core particle bind DNA most frequently at the guanine bases (N7 atoms), although in this example a manganese ion is tightly bound between adjacent core particles. Manganese ions are identified by electron density greater than that for water molecules and their characteristic octahedral coordination of ligands.*

superhelix, with one base pair lying on the molecular dyad axis of the whole particle, agrees with the results from high-resolution mapping of the histone octamer on DNA (Flaus and Richmond, 1998). A variety of interactions occur between protein and DNA at their 14 contact sites: three are made by each of the H3/H4 and H2A/H2B histone-fold pairs and two by the H3 α-N extensions.

Many basic side-chain interactions occur with the DNA phosphate groups and hydrophobic side-chain interactions with deoxyribose groups. The 2.0 Å crystal structure reveals the location of several solvent molecules that aid the interaction of the DNA with protein. However, the direct hydrogen bonds from the amide groups on the histone main chain to the phosphate oxygen atoms seem to specify the DNA conformation most rigorously. The character and number of these interactions indicate that the path of the DNA superhelix and the structure of the DNA double helix are largely independent of DNA sequence.

The energetics of nucleosome stability depend on the difference in energy for DNA in its free state compared to its state bound at the different sites along the nucleosome superhelix path. Overall, for example, the DNA must change its twist from 10.5 bp per turn in solution to 10.3 bp per turn in the nucleosome and bend through 594°. Certain sequences can accommodate this better than others. Despite a probable invariance of DNA structure at each binding site within the nucleosome core, certain DNA sequences crystallize better than others. The human centromeric α-satellite DNA used to determine the crystal structure of the nucleosome core particle may possess the advantage that it has an intrinsic curvature that maintains a histone-bound state at the ends of the superhelix, the weakest binding site. The sequence-dependent propensity for a terminus of the DNA superhelix to release from the surface of the histone octamer may be important for the access of transcription factor proteins to specific DNA sequences bound in the nucleosome.

Histone octamer sliding models

Examination of the X-ray structure of the nucleosome core particle has enabled us to discriminate between possible mechanisms of histone octamer 'sliding' along DNA. A simple model is that the octamer simply turns around the DNA superhelix axis inside the gyres of DNA. However, this would be impossible

without the supercoils expanding in diameter as well as in separation, because arginine side chains penetrating the minor groove and histone tails passing between the gyres in aligned minor grooves would prevent this rotation. A second mechanism, which almost certainly occurs, is the DNA 'peeling' off from one side of the particle while the other side remains bound, then the particle rebinding further along the DNA, causing a bubble to form on the surface of the octamer. The bulged DNA could work its way around the octamer by repeated release of one end or the other until the octamer is centred on a different sequence. This mechanism has been invoked to explain how RNA polymerase can transcribe the DNA bound in a nucleosome without releasing the histone octamer (Studitsky *et al.*, 1997). The polymerase resides in the bubble on the nucleosome.

A third possibility comes from the difference in DNA structure on opposite sides of the core particle. One half of the DNA superhelix has a distortion that does not appear in the other half, despite the general twofold symmetry that relates the halves of the particle. This distortion comprises overwinding of 12 bp of DNA, stretching the double helix to fit a length spanned by 13 bp in the symmetrically related region on the opposite side of the DNA superhelix. Although the observed distortion is near the centre of the DNA superhelix, five double-helical turns from the nearest DNA terminus, its origin probably lies in the stretching of the DNA that permits close packing of DNA termini between particles in the crystal. The DNA overwinding then propagates to the observed site, which better accommodates it. This difference in structure on the two sides of the core particle indicates that twist diffusion can occur in the DNA along the surface of the nucleosome (first suggested by Van Holde and Yager, 1985). If this is the case, then the DNA double helix resembles a flexible screw with the arginine side chains and histone tail segments tracking in the minor grooves. This mechanism could also account for the nucleosome sliding activity of chromatin remodelling factors (see Becker, this volume).

Nucleosomal location of the linker histone and its role in transcriptional repression

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in collaboration with

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Condensed chromatin consists of chromatosomes, each of which contains a histone octamer (nucleosome) and a lysine-rich linker histone H1/H5 bound to 168 bp of DNA. The binding of the linker histone H1/H5 facilitates the proper folding of a nucleosome array into this higher-order structure. One facet of the regulation of transcriptional activity could be competition between the linker histone and the activating transcription factors. Here we describe experiments that locate the position of the central globular domain of the linker histone in the chromatosome particle. We show that defined positions adopted by core nucleosome particles on *Xenopus* somatic oocyte 5S rRNA genes are important determinants of the relative affinities for the linker histone and the activating transcription factor, TFIIIA. Thus core nucleosome positions can directly influence the activation and repression of particular genes, probably through a combination of effects at single nucleosomes, in which the DNA transcriptional position is stabilized by histone H1 binding, and in nucleosomal arrays, where linker-histone binding promotes the folding of chromatin into higher-order structures.

Position of the linker histone

The linker histones contain basic C- and N-terminal tails flanking a central globular domain that is sufficient for chromatosome formation. Their exact location in the chromatosome is contentious (for review, see Vignali and Workman, 1998). The globular domain consists of a three-helix bundle, helices I–III, homologous to helix–turn–helix DNA-binding proteins. Helix III is thought to be a recognition site and to bind in the major

groove of the DNA, and a cluster of basic amino-acid residues opposite the recognition helix may be a secondary binding site (Ramakrishnan *et al.*, 1993; Cerf *et al.*, 1994). This structure indicates a model in which the globular domain binds two duplexes, i.e., both strands of DNA in each DNA double-stranded molecule, thus bridging two adjacent DNA gyres (*Fig. 6*).

In agreement with these predictions, mutations in the secondary binding site prevent the globular domain of histone H5 binding to two duplex DNA molecules and forming chromatosomes (Goytisolo *et al.*, 1994). In addition, the binding site of H5 on mixed-sequence chicken DNA chromatosomes has been mapped using a protein–DNA photo-crosslinking method to crosslink to the DNA at specific cysteine residues substituted for serine in the wildtype protein domain (Zhou *et al.*, 1998). In particular, serines in helix I (amino-acid 29), helix II (amino-acid 41) and

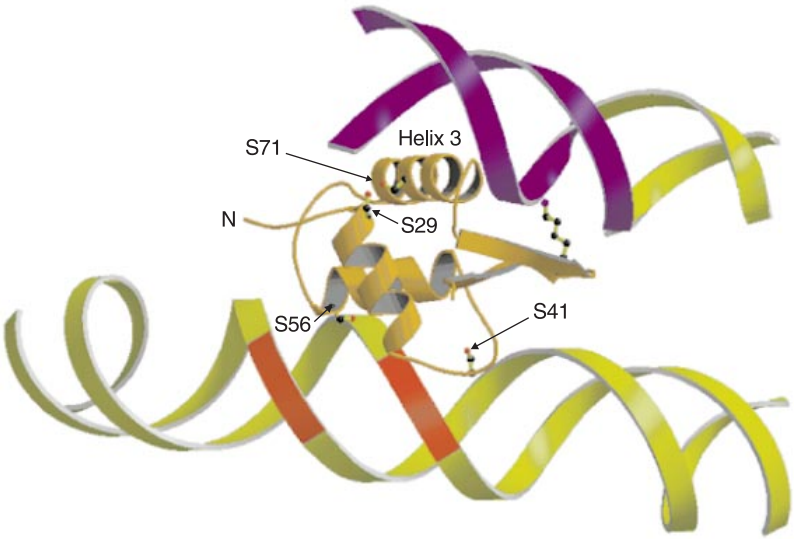

Figure 6. *Model for the binding of the globular domain of histone H5 to the chromatosome. Helix III binds in a major groove close to one terminus (purple) of chromatosomal DNA whereas the loop between helix I and helix II contacts the DNA close to the midpoint of the DNA (near the dyad; red).*

the recognition helix III (amino-acid 71) were mutated and found to be near the nucleosomal DNA. Thus helix III binds in the major groove of the first helical turn of chromatosomal DNA at the exit or entry point of the nucleosomal DNA. Meanwhile the secondary DNA binding site on the opposite face of the globular domain of H5 contacts the nucleosomal DNA close to its midpoint, or dyad (*Fig. 6*).

By exploiting the ability of some serine-to-cysteine mutants to self-dimerize, it was inferred that helix I of the globular domain of H5 faces the solvent and helix II faces the nucleosome. In bulk chromatin, the globular domain of the linker histone thus forms a bridge between one terminus of chromatosomal DNA and the midpoint, bringing the C terminus of the globular domain to lie on the outside of the particle between one end of chromatosomal DNA and the central gyre (*Fig. 7b*; Lambert *et*

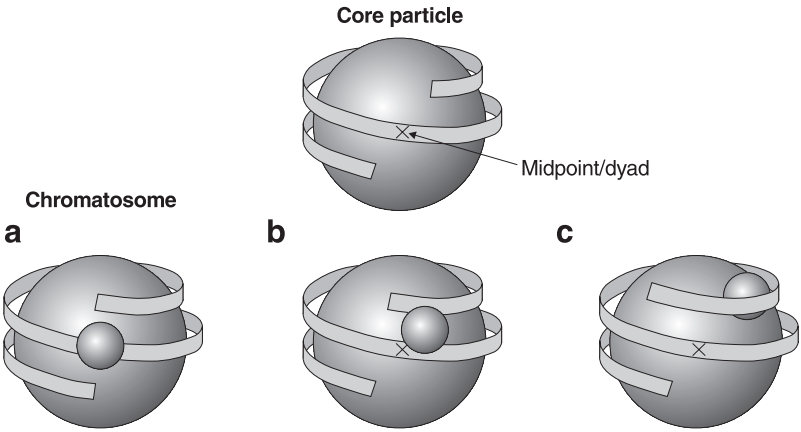

***Figure 7**. Models for the placement of the globular domain of linker histone on the core particle. **a**, the globular domain (small sphere) binds centrally to the nucleosome dyad; **b**, the globular domain is asymmetrically placed, forming a bridge between one terminus of the chromatosomal DNA and the DNA close to the dyad; **c**, the globular domain binds only to one duplex, about 2.5 double-helical turns from one DNA terminus.*

al., 1991; cf. the simple model in *Fig. 7a* proposed by Allan *et al.*, 1980). The two contact points of the linker histone with the nucleosomal DNA are separated by one superhelical turn. This bridging model does not distinguish between two possible positions of the globular domain, one where the linker histone binds to the DNA entering the nucleosome, the other where it binds to the exiting DNA in addition to the nucleosome dyad sequences. However, it excludes the possibility of two globular domains binding as a dimer, for which there is no experimental evidence.

Our results differ significantly from the model for the chromatosome on the *Xenopus borealis* 5S RNA gene (Hayes, 1996; Pruss *et al.*, 1996). In this model, the globular domain binds on the inside of one DNA gyre at a single internal site 65 bp from the nucleosomal midpoint or dyad and about two helical turns from the proximal terminus of chromatosomal DNA, so that no contact is made with the dyad region itself (*Fig. 7c*; Pruss *et al.*, 1996). This model depends critically on the assumption that the 5S RNA chromatosome adopts a single dominant translational position, which determines the exact DNA sequences that make contact with the octamer. However, accurate mapping of chromatosome dyads on this sequence and that of the closely related *Xenopus laevis* somatic 5S RNA genes reveals multiple equivalent translational positions both with and without histone H1 (Panetta *et al.*, 1998).

Role of histone H1 binding in transcriptional regulation

In *Xenopus* somatic cells, histone H1 effects the transcriptional repression of oocyte-type 5S RNA genes without altering the transcription of the somatic-type 5S RNA genes. Using a direct method for mapping nucleosome dyads, site-directed hydroxyl radical cleavage of the DNA sequence in contact with an EDTA moiety conjugated to residue 47 of histone H4 (Flaus *et al.*, 1996), we have mapped multiple nucleosome positions after in-vitro reconstitution of both the oocyte and somatic 5S genes of

X. borealis (*Fig. 8*). These nucleosome positions determine binding of both transcription factor and H1, allowing the transcription factor TFIIIA to bind more efficiently to nucleosomes on the somatic 5S RNA gene than to those on the oocyte 5S RNA gene.

A significant observation is that, in a binding competition

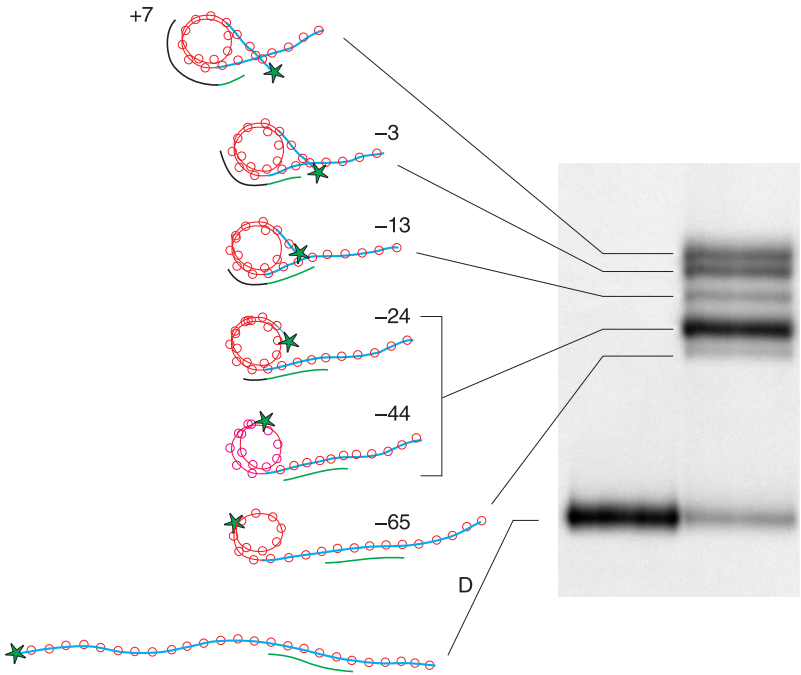

Figure 8. *Multiple nucleosome positions on the somatic 5S rDNA. Right, the preparative fractionation of free DNA (D, left lane) and nucleosomal complexes (right lane) on a 5% polyacrylamide gel. Left, representation of the nucleosome dyad positions present in each gel band. The dyads are numbered relative to the start point of 5S RNA transcription and deduced from site-directed hydroxyl-radical cleavage. Red circles every 10 bp of the DNA helix axis indicate positions on the 5S rDNA fragment. The region bound by the histone octamer is shown in red and the free DNA in blue. The TFIIIA binding site is indicated by a green line where exposed and a black one where covered by a positioned nucleosome. Green star, position of the radiolabel. Modified from Panetta* et al.*, 1998.*

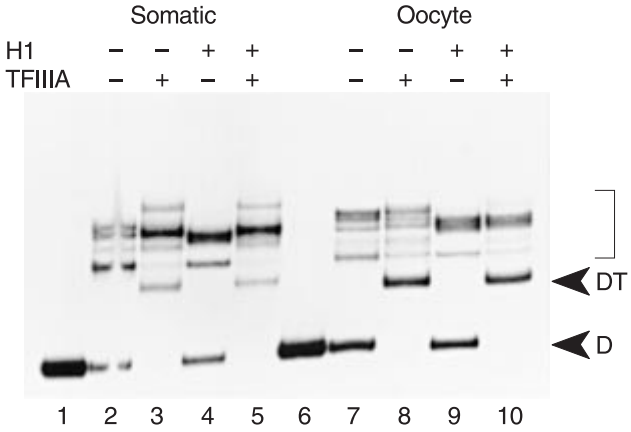

Figure 9. *H1 and TFIIIA bind differentially to the somatic and oocyte nucleosomes. Somatic (lanes 1–5) and oocyte (lanes 6–10) were incubated with TFIIIA (lanes 3 and 8), H1 (lanes 4 and 9) and with both H1 and TFIIIA (lanes 5 and 10). The positions of free 5S rDNA (D) and the TFIIIA–DNA complex (DT) are indicated. Bracket, position of the complexes between nucleosomes and TFIIIA or H1. The complexes were fractionated on a 5% polyacrylamide gel. Reproduced with permission from Panetta* et al., *1998.*

experiment between TFIIIA and H1, TFIIIA preferentially binds to the somatic nucleosome whereas H1 preferentially binds to the oocyte nucleosome and prevents TFIIIA binding (*Fig. 9*). This is a strong indication that nucleosome positioning is important in the regulation of transcription of 5S RNA genes, as has also been shown for the corresponding genes in *X. laevis* (Howe and Ausio, 1998; Sera and Wolffe, 1998). It provides a molecular mechanism for the selective repression of the oocyte 5S RNA genes by H1. Studies are required to test whether H1 and TFIIIA binding is similarly regulated when the 5S RNA genes are present in nucleosomal arrays and under conditions where the histones are differentially acetylated.

Linking histone modifications to gene activation

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in collaboration with

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The chromatin fibre has to undergo two fundamentally different transitions: it has to increase condensation to yield the chromosome structure in mitosis and meiosis, and it has to unfold to allow the transcription or replication machinery access to the nucleosomal DNA. A correlation between gene regulation and chromatin structure has been established using biochemical and genetic studies. In eukaryotes, two highly conserved and possibly linked mechanisms relieve nucleosomal repression: chromatin remodelling (see Becker, Hörz, Kingston, Wu, this volume) and post-translational histone modification, in particular histone acetylation (see Workman, this volume) and phosphorylation (*Fig. 10*). Covalent modifications of histones seem to be important in gene regulation and thus have significant implications for eukaryotic biology. As well as allowing regulatory factors to interact specifically with nucleosomes, differential 'marking' of the various histone tails with covalent modifications may influence nucleosome–nucleosome interactions, thus modulating the higher-order organization of chromatin.

Acetylation of lysines in the N-terminal histone tails causes structural changes in individual nucleosomes and chromatin, and influences the interaction with the modified nucleosomes of non-histone proteins, such as regulatory factors (Mizzen and Allis, 1998). Combinations of post-translational histone modifications, including methylation, ADP-ribosylation, ubiquitination or glycosylation in addition to acetylation and phosphorylation (*Table 1*), provide a challenge for determining the participants

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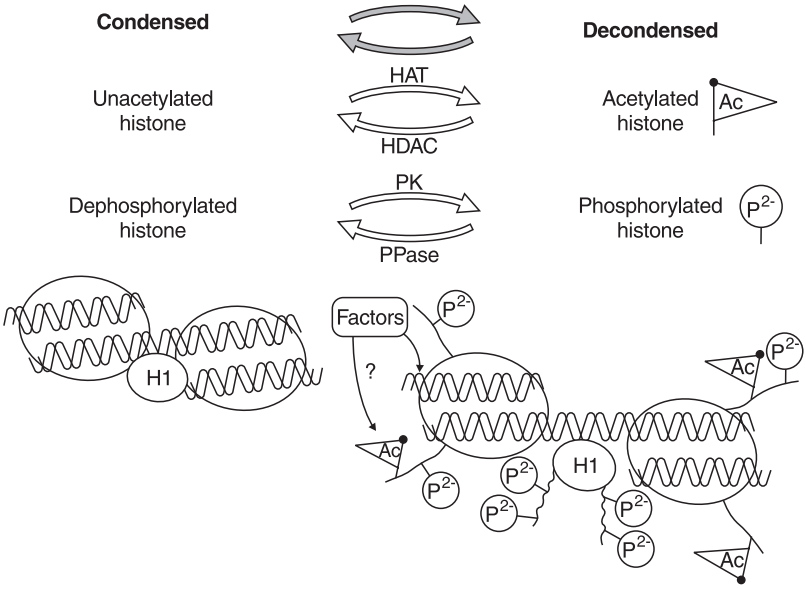


Figure 10. *Proposed roles of histone acetylation and phosphorylation in modulating chromatin structure. Left, condensed chromatin, in which DNA transcription and replication are inactive, is characterized by low levels of post-translationally modified histones. Right, acetylation of the N-terminal tails of core histones (Ac) by histone acetyltransferases (HAT) and phosphorylation of core and linker histone tails (P^{2-}) by protein kinases (PK) attenuate their interactions with DNA or other chromatin components. This leads to decondensation of chromatin fibres and increases accessibility to DNA that is recognized by factors regulating chromatin activity. Modified histone tails themselves may also be recognized by regulatory factors. Histone deacetylases (HDAC) remove acetyl groups (Ac) and protein phosphatases (PPase) remove phosphoryl groups from the histone tails, enhancing their binding to nucleosomal DNA or other chromatin components and promoting a return to the condensed, quiescent, basal state. The positions shown for the globular domain of H1 and modified core histone tails are arbitrary and are not intended to reflect experimental data.*

and targets in chromatin-mediated eukaryotic gene regulation. Here we review the occurrence of histone acetyl transferases (HAT), enzymes involved in acetylation, and report the first evidence that a core histone is physiologically phosphorylated by a

mitogen-activated protein kinase. We also provide new insights into a poorly understood mechanism that links histone phosphorylation to activation by mitogens.

Histone acetylation

Several protein complexes responsible for acetylation have been identified and isolated in a range of eukaryotes, from yeast to humans. Their catalytic subunits, the acetyltransferases, are highly conserved. The first-known transcription-associated HAT was cloned from the ciliated protozoan *Tetrahymena thermophila* (Brownell *et al.*, 1996) and is significantly homologous to the yeast *S. cerevisiae* protein Gcn5. Characterization of Gcn5 function in vitro and in vivo indicated that transcriptional activation is linked to HAT activity (Kuo *et al.*, 1998; L. Wang *et al.*, 1998; see Hörz, this volume). Gcn5 homologues in various mammals and in *Drosophila* were identified by the domain containing a previously described putative acetyl-CoA-binding motif (Neuwald and Landsman, 1997; *Fig. 11*).

Several other transcriptional coactivators, such as the human p300/cyclic AMP response element binding factor (CREB)-binding protein (CBP), p300/CBP-associated factor (PCAF) and $TAF_{II}250$, also have intrinsic HAT activity. Yeast Esa1, a protein homologous to the SAS2 and SAS3 proteins that are involved in yeast mating-type transcriptional silencing, and the related human Tip60, a protein that interacts with the Tat transactivator of the human immunodeficiency virus (HIV), are members of the MYST family of proteins, named after the 'founding members': MOZ, YBF2/SAS3, SAS2 and Tip60 (*Fig. 11*); these all can acetylate histones in vitro (Kuo and Allis, 1998; Smith *et al.*, 1998*a*,*b*).

HATs are the catalytic core of nucleosome-acetylating multiprotein complexes, such as the SAGA or the NuA4 complex (Grant *et al.*, 1997; see Workman, this volume). Recent evidence that SAGA and PCAF histone-modifying complexes share com-

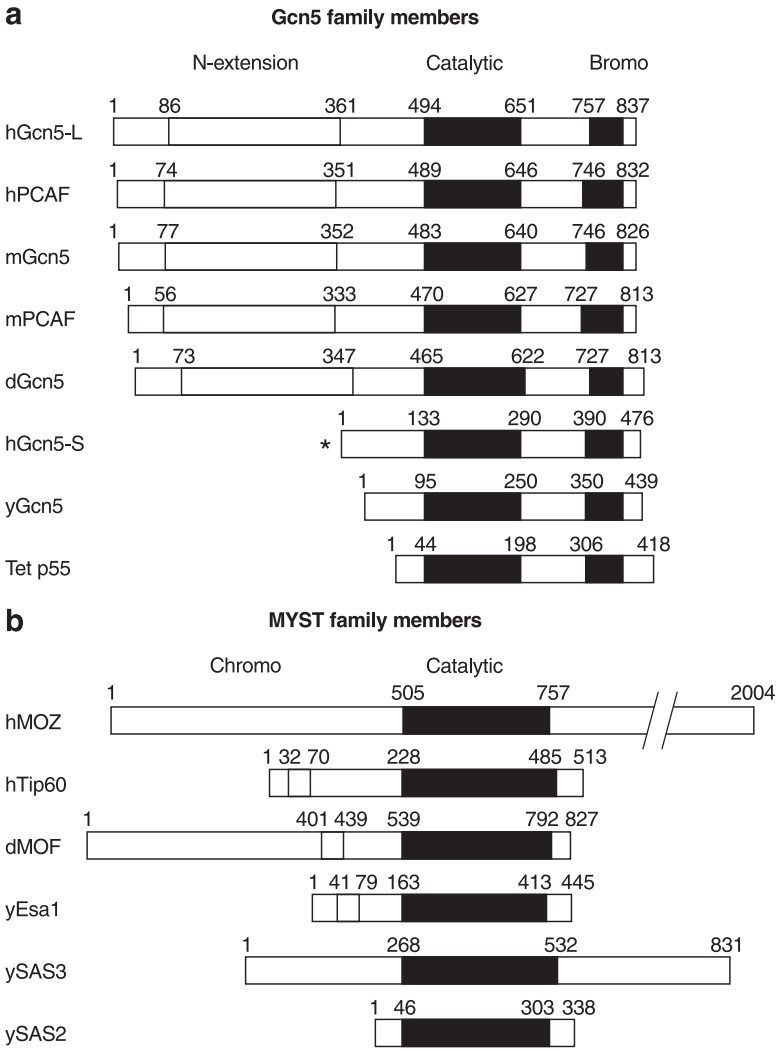


Figure 11. *Features of recently described members of* ***a****, the Gcn5 and* ***b****, the MYST families of histone acetyltransferases. The proteins are aligned according to homology of a previously described putative acetyl-CoA-binding motif in the catalytic domain (Neuwald and Landsman, 1997). Recent evidence indicates multiple forms of hGcn5-S (asterisk). d,* Drosophila*; h, human; m, mouse; y, yeast.*

mon factor requirements with the polymerase II transcription apparatus reinforces our view that chromatin structure and transcriptional regulation are interdependent (Grant *et al.*, 1998*a*; Ogryzko *et al.*, 1998).

Histone phosphorylation

Widespread phosphorylation of histones, particularly H1 and H3, correlates with mitosis in mammalian cells and may function in mitotic and meiotic chromosome condensation (Hendzel *et al.*, 1997; Wei *et al.*, 1998; 1999). In mammalian cells, a subset of H3 molecules is rapidly and transiently phosphorylated in response to mitogenic and other stimuli that activate expression of immediate-early genes, such as c-*fos* and c-*jun* (Mahadevan *et al.*, 1991). Thus H3 phosphorylation may have a role in decondensing chromatin to facilitate transcriptional activation, similar to that proposed for histone acetylation and interphase phosphorylation of linker histones (reviewed in Mizzen *et al.*, 1999). To investigate this possibility, we made a synthetic peptide comprising part of the amino-acid sequence of the histone H3 N-terminal tail domain found in yeast, human and mouse, and phosphorylated the residue corresponding to serine 10 of the complete histone H3 sequence. An antibody raised against the phosphorylated peptide is highly selective and reactive and is a remarkable marker of mitosis in a range of eukaryotic cells (Wei and Allis, 1998). In *Tetrahymena*, mutation of this residue from serine to alanine causes the chromosomes in the micronucleus to loosen, compromising mitotic segregation (Wei *et al.*, 1999), a significant indication that phosphorylation at this site is highly conserved and functionally important.

During the well-known immediate-early response to mitogens, the phosphorylation at Ser10 is stimulated by treatment with mitogens. The antibody raised against the phosphorylated peptide (anti-PS10) reacts with histone H3 in mouse fibroblasts stimulated by epidermal growth factor (EGF) or the phorbolester

12-*O*-tetradecanoylphorbol-13-acetate (TPA) in a fast, transient response corresponding to the immediate-early response to mitogens (Mahadevan *et al.*, 1991). The antibody staining seems to be specific to euchromatic nuclear domains, i.e., decondensed, actively transcribed chromatin domains, so phosphorylation at Ser 10 may, like histone acetylation, have a role in decondensing the chromatin fibre to facilitate gene expression.

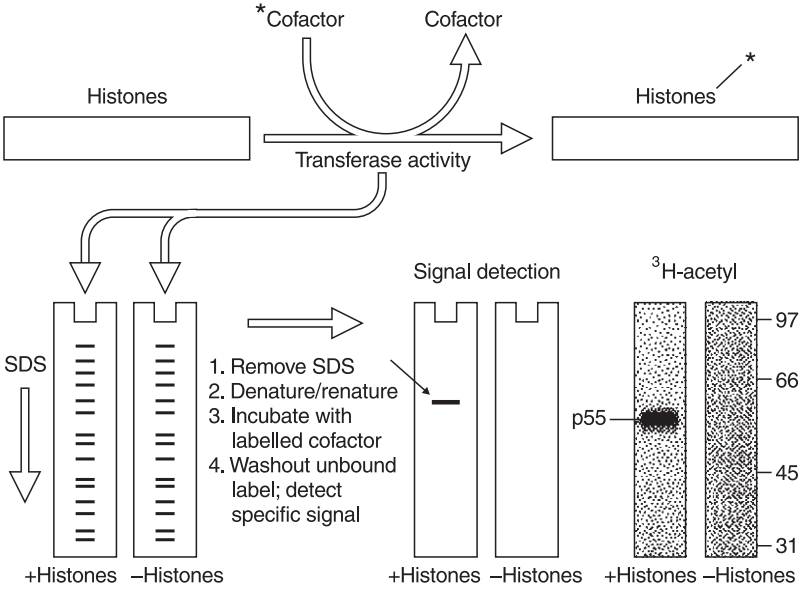


Figure 12. *Principles of the activity gel assay for detecting histone-modifying enzymes. Top, the method is based on the detection of modification using a radioisotopic cofactor, e.g., γ^{32}P-ATP or ^{3}H-acetyl-CoA; asterisks, radiolabel. Below, the* M_r *of polypeptides with intrinsic modifying activity is determined by electrophoresis on SDS gels containing histones. After denaturing and renaturing the resolved proteins, gels are incubated with radioisotopic cofactor and processed for autoradiography to detect modified histones surrounding active species in the gels. A schematic example showing the detection of the* Tetrahymena *p55 HAT in crude macronuclear extract is shown on the right. We used this method to demonstrate that Rsk-2 possesses intrinsic H3 kinase activity (see text) and in theory it could be used, with the appropriate radiolabelled cofactor, for other histone-modifying enzymes, such as histone methyltransferases.*

The kinases responsible for histone-specific phosphorylation have remained elusive. An 'activity gel assay', developed initially to identify the histone-modifying activity of p55 HAT, has been modified to detect other polypeptides with intrinsic histone phosphorylation activity (*Fig. 12*). In a histone H3 in-gel kinase assay using radiolabelled ATP, nuclear extracts from EGF-stimulated fibroblasts selectively yielded an intense band corresponding to a polypeptide with M_r ~90K. Antibodies raised against $p90^{rsk}$, a human protein targeted by the mitogen-activated protein kinase (MAPK) signalling pathway, reacted with the 90K polypeptide band. The H3 kinase was thus tentatively identified as Rsk-2. An activity test on free and nucleosomal histones revealed that Rsk-2 specifically phosphorylates Ser10, in contrast to other kinases, which are able to phosphorylate all histones.

Thus, Rsk-2, a member of the $p90^{rsk}$ family of mitogen-activated kinases implicated in cell proliferation (see *Fig. 13*), is the kinase responsible for mitogen-stimulated H3 phosphorylation in human cells. In agreement with this, in starved embryonic stem cells of *rsk-2* knockout mice, the Rsk-2+ phenotype can be rescued using human Rsk-2 cDNA. In stimulated cells, selective chromatin immunoprecipitation with anti-PS10 antiserum promises to be a powerful approach to the identification of DNA sequences for immediate-early genes that require Rsk-2 for induction.

Mutations in the *RSK-2* gene are causally linked to Coffin-Lowry syndrome, an X-linked disorder characterized by mental retardation and developmental skeletal deformations in humans. Cells derived from individuals with Coffin-Lowry syndrome, i.e., Rsk-2-deficient, fail to phosphorylate H3 after mitogen stimulation (Sassone-Corsi *et al.*, 1999), although during mitosis H3 phosphorylation is normal. Two independent kinase pathways therefore seem to target histone H3 for phosphorylation during mitosis and mitogenic stimulation. A chromatin remodelling step involving histone H3 phosphorylation, possibly in concert with histone acetylation of mitogen-regulated genes (*Fig. 13*), may

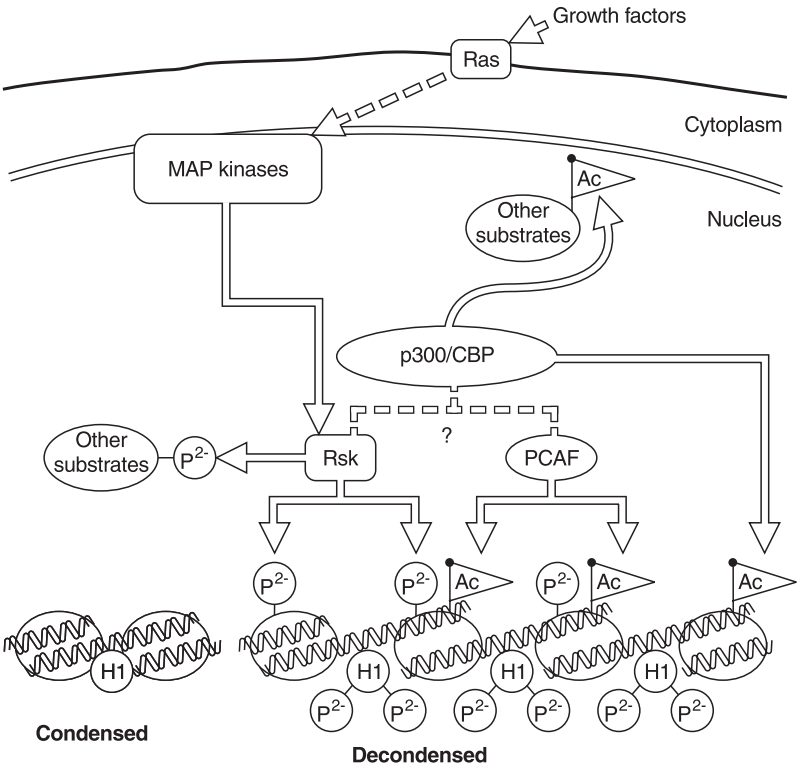


Figure 13. *Possible interactions involved in coordinated recruitment of histone kinase and acetyltransferase activities to chromatin. When activated by MAP kinases, p90^{rsk} proteins are translocated to the nucleus, where Rsk-2, in particular, phosphorylates H3 in the nucleosome in response to mitogenic stimuli. This phosphorylation may be targeted to nucleosomes acetylated by p300/CBP and/or PCAF because p90^{rsk} proteins can bind to a site on p300/CBP that is near an interaction site for PCAF. Both these events are thought to promote chromatin decondensation and facilitate transcriptional activation (see text and* Fig. 10*). Each of these modifying activities may target other substrates as well as histones. If some of these interactions are correct, DNA sequences, i.e., mitogen-stimulated target genes, that are closely associated with modified histones, should be recoverable with chromatin immunoprecipitation using modification-specific antibodies. Ac, acetyl group; P^{2-}, phosphoryl group.*

thus be a critical part of a signalling pathway that, when deregulated, is intimately linked to human disease.

Function and composition of transcription adaptor histone acetyltransferase complexes

Jerry L. Workman

Reversible acetylation of the N-terminal tails of the histones has long been linked biochemically with actively transcribed chromatin. Several multi-subunit HAT complexes have been identified in yeast, including the Spt-Ada-Gcn5-acetyltransferase (SAGA), Ada, nucleosome-acetylated–histone H3 (NuA3) and H4 (NuA4) complexes. These large complexes have different subunit compositions and substrate specificities, indicating that they have distinct functional roles. Interest in this field increased rapidly when the yeast transcriptional adaptor protein Gcn5 was discovered to have HAT activity (Brownell *et al.*, 1996), which is essential for its functional effect. However, despite its potent HAT activity with purified histones (H3 and H4), Gcn5 cannot modify histones when they are assembled into a nucleosome.

The observation that yeast extracts contain HAT activities that could modify nucleosomal histones prompted studies to identify these activities. Here I review the progress in identifying subunits and biochemical experiments that give insight into how these acetyltransferases contribute to transcriptional regulation. These studies support the idea that the HAT complexes act as transcriptional adaptors and are recruited by transcriptional activators to the promoter through direct interactions with the cofactor subunits. The subsequent activation of transcription is likely to be through several separate mechanisms but the acetylation of adjacent, promoter-bound nucleosomes clearly contributes to this process.

Identification and subunit composition of HAT complexes

Biochemical fractionation of yeast extracts identified four HAT complexes that can acetylate histones when assembled in nucleosomes (*Fig. 14*; Grant *et al.*, 1997). These had M_rs of 400–1,800K and displayed different histone specificities, indicating that they are distinct complexes. This was confirmed by subsequent purification and subunit identification (*Table 3*).

SAGA complex. The SAGA complex is a large HAT with an apparent M_r of 1,800K, that specifically acetylates nucleosomal histones H3 and H2B. Evidence from immunological and mass spectroscopy studies has identified a large number of the components of this complex, which can be classified into four groups (*Fig. 15*; Grant *et al.*, 1997; 1998*a,b*):

• GCN5-ADA *gene products.* Early immunological studies confirmed that SAGA contained the HAT Gcn5, as well as the Ada proteins, Ada1, -2, -3 and Ada5/Spt20. These had previously been shown to be components of a transcriptional adaptor termed the Ada complex, which functionally interacts with the transcriptional activator Gcn4 and the VP16 activation domain of the herpes virus (*Fig. 16*). The SAGA complex cannot form in *gcn5*Δ, *ada2*Δ or *ada3*Δ yeast strains, i.e., mutant strains lacking *gcn5*, *ada2* or *ada3* genes, confirming that the SAGA complex contains Ada.

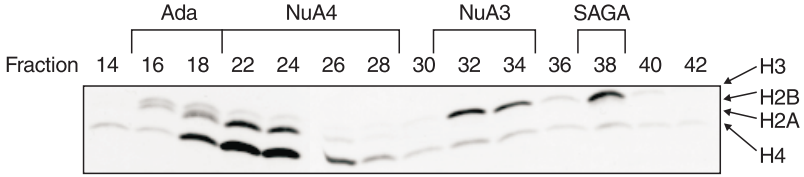


Figure 14. *Identification of multiple HAT activities in yeast. The fluorogram shows a gel on which histones have been separated from nucleosomes, all labelled with ^{3}H-acetyl-CoA during incubation and eluted at the indicated fractions from a MonoQ fast-protein liquid chromatography (FPLC) column. Four distinct HAT complexes with different histone specificities were eluted from this column with increasing salt solution, corresponding to the Ada, NuA4, NuA3 and SAGA complexes.*

Table 3. *Properties and known components of the SAGA, Ada, NuA3 and NuA4 HAT complexes.*

Complex	SAGA	Ada	NuA3	NuA4
Size (M_r)	1,800K	800K	400K	1,300K
Substrate	H3/H2B	H3/H2B	H3	H4/H2A
Interactions	Activators TBP	?	?	Activators
Catalytic subunit	Gcn5	Gcn5	?	Esa1
Additional subunits	Ada1,-2,-3 Spt3,-7,-8 Spt20/Ada5 TAF_{II}20/17, -25/23,-60, -61/68,-90 Tra1	Ada2,-3		

• SPT *gene products linked to TATA box binding protein* (*TBP*). SAGA contains the SPT gene products Spt3, Spt7 and Spt8, proteins in a class that is thought to affect TBP function. This activity is clearest for Spt3 and Spt8, which contribute to SAGA interactions with TBP (Roberts and Winston, 1996; Sterner *et al.*, 1999). As *ADA5* has previously been shown to be identical to *SPT20*, the Ada and Spt proteins may function through a common physiological pathway.

• *TBP-associated factors* (*TAF_{II}s*). SAGA also contains a number of TAF_{II}s, including TAF_{II}90, -68/61, -60, -25/23 and 20/17. Inactivation of TAF_{II}-68 in a yeast temperature-sensitive mutant led to an apparent loss in mass to 1,000K–1,200K and a reduction in the amount of TAF_{II}-90 and Spt3 in the complex, establishing that these proteins are an integral component of SAGA. Furthermore, inactivation of TAF_{II}-68 leads to a severe disruption in SAGA nucleosomal HAT activity and in the ability of the complex to activate transcription from a chromatin template in vitro (Grant *et al.*, 1998*a*).

• *Tra1*. The largest subunit of the SAGA complex is the 3,744 amino-acid protein Tra1 (Grant *et al.*, 1998*b*; Saleh *et al.*, 1998). Tra1 is the yeast homologue of the human transformation/transcription domain-associated protein TRRAP, a novel

member of the family of phosphatidylinositol 3-kinases that are mutated in ataxia telangiectasia (McMahon *et al.*, 1998). Tra1 is a potential target within SAGA for interactions with transcription activators.

This association of multiple classes of transcriptional cofactor proteins, Adas, Spts, TAF_{II}s and Tra1, is a strong indication that SAGA acts as a transcriptional adaptor complex. The large number of adaptor protein subunits similarly indicates that SAGA can respond to a range of stimuli and interact with several activators, with the potential to regulate a broad range of

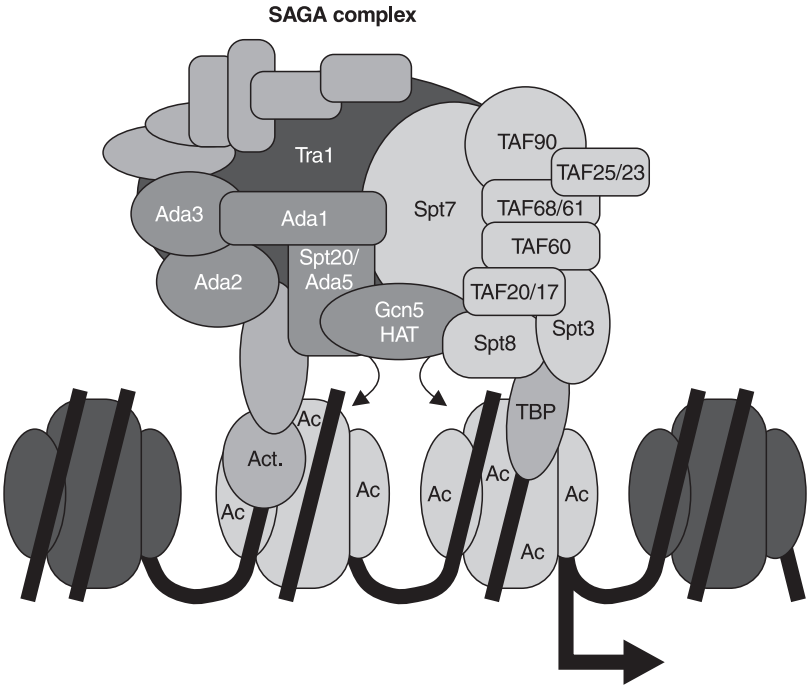


Figure 15. *The confirmed components of the SAGA transcriptional adaptor HAT complex, which contains multiple transcriptional regulatory proteins, including components implicated in interactions with TBP (Spts and TAFs), transcription activators (Adas and Tra1) and histone acetylation (Gcn5). Ac, acetylated histones; Act., activator protein.*

promoters. The role of the Gcn5 HAT activity in this transcriptional adaptor complex has been examined biochemically (see below).

Ada complex. A second HAT, the 800K Ada complex, has a similar substrate specificity to SAGA, nucleosomal histones H3 and H2B, and also contains the HAT Gcn5 and the Ada proteins Ada2 and Ada3. The relationship between the Ada and SAGA complexes is unclear but the Ada complex, which does not contain the Spt proteins, may be a sub-complex of the larger SAGA complex. An alternative possibility is that the complexes, although having Gcn5 and the Ada proteins as common subunits, may have distinct nucleosomal HAT activities with different functions. The exact relationship between these HATs should be resolved when the unknown subunits of each complex and the degree to which they are common or unique have been identified.

NuA3 and NuA4 complexes. In contrast to the SAGA and Ada complexes, little is known about the subunit composition of the 400K NuA3 and 1,300K NuA4 complexes. These HATs have different substrate specificities, NuA3 modifying nucleosomal histone H3 and NuA4 modifying histones H4/H2A. The functional HAT in the NuA4 complex has been identified as the essential yeast protein Esa1 (J. Côté, unpublished observations), a homologue of the HAT MOF1 in *Drosophila*, which is involved in X-chromosome dosage compensation. The HAT protein in NuA3 and the remaining subunits in both complexes remain to be identified.

Functional properties of HAT complexes

The identification of multiple classes of transcriptional coactivator proteins in Ada and SAGA indicated that these HAT complexes were likely to interact with activator proteins. This has been examined by glutathione-*S*-transferase (GST) pull-down assays, in which the four native yeast HAT complexes were incubated with GST fused to the VP16 activation domain of herpes virus or the yeast transcriptional activator Gcn4 (Utley *et al.*,

1998). Both SAGA (see *Fig. 16*) and NuA4 can bind both activators but NuA3 and the Ada complex, which contains at least three of the same subunits as SAGA, cannot bind either activator protein.

Thus these transcriptional activators seem to recruit the SAGA or NuA4 complexes through contacts with their transcriptional adaptor subunits, thereby targeting HAT activity to adjacent nucleosomes. This was tested by reconstituting a mononucleosome template with a single Gal4-binding site 20 bp from the end of the DNA fragment. When this template was incubated with increasing amounts of Gal4–VP16 in the presence of HAT complexes and ^{3}H-acetyl-CoA, the binding of the activator to the nucleosome increased the level of histone acetylation by SAGA and NuA4 but not by the NuA3 or Ada complexes, consistent with the activator-binding studies.

The recruitment of HAT complexes also has transcriptional effects. This was initially analysed using VP16-driven transcription from a model template containing a minimal E4 promoter containing five Gal4 sites. When this template was assembled into a spaced nucleosome array, transcription was highly

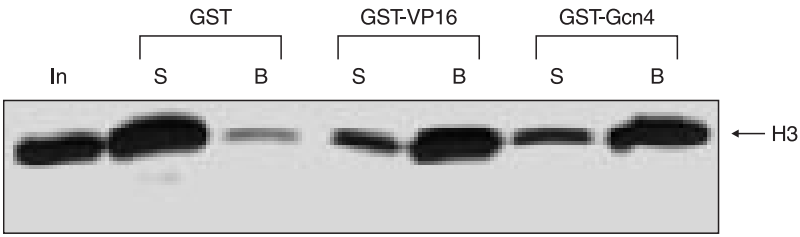


Figure 16. *Interaction of the SAGA complex with the acidic activation domain of herpes virus activator VP16 and the yeast activator Gcn4. SAGA failed to interact with glutathione-*S*-transferase (GST) alone but interacted with the GST–VP16 and GST–Gcn4 fusion proteins. GST pull-down experiments using purified SAGA were used to assay for SAGA interaction indicated by acetylation of histone H3 in nucleosomes. The gel shows 10% of the input activity (In), 25% of the pull-down supernatant (S) and 50% of the activity recovered on the glutathione beads (B).*

repressed. However, in the presence of Gal4–VP16, both SAGA and NuA4 stimulated transcription from this template in an acetyl-CoA-dependent manner (Ikeda *et al.*, 1999; Utley *et al.*, 1998). Complementary in vitro transcription studies on a defined chromatin template containing the HIV-1 promoter, which has a clear correlation between acetylation and transcriptional activation in vivo (Steger *et al.*, 1998), showed that all four HAT complexes, SAGA, Ada, NuA3 and NuA4, can stimulate transcription from this chromatin template in an acetyl-CoA-dependent manner (*Fig. 17*) and that the transcriptional stimulation by NuA4 and Ada results at least in part from the acetylation of histones.

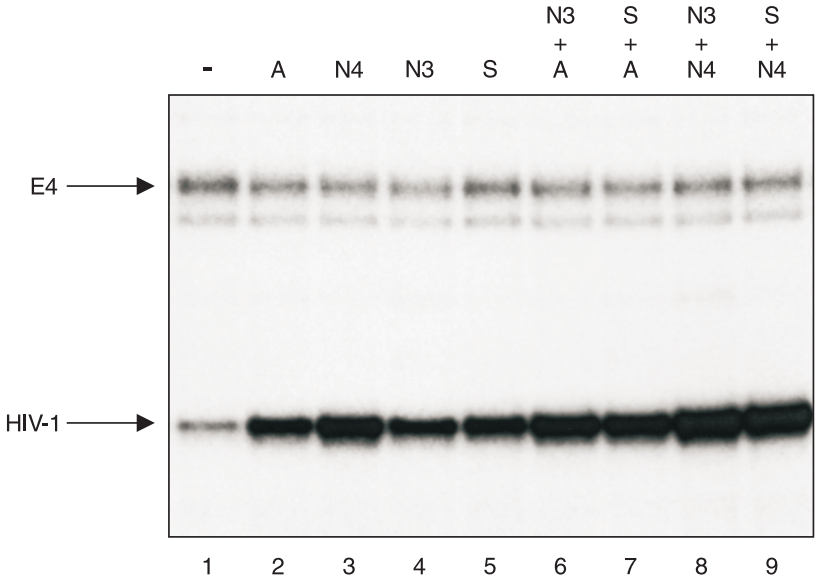


Figure 17. *Demonstration of transcriptional activation by native yeast HAT complexes using the primer extension analysis of transcripts from the HIV-1 promoter embedded in a spaced nucleosome array. The Ada (A), NuA4 (N4), NuA3 (N3) and SAGA (S) HAT complexes all enhanced transcription from the HIV-1 template in an acetyl-CoA-dependent reaction (compare lanes 2–5 to lane 1). Addition of more than one HAT complex resulted in an additive stimulatory effect (lanes 6–9). E4, promoter (TATA box and initiation site) with five Gal4 sites.*

ATP-dependent chromatin remodelling by NURF and transcriptional activation

Carl Wu

in collaboration with

Toshio Tsukiyama and Gaku Mizuguchi

The assembly of the DNA template into chromatin is associated with transcriptional repression. Much of this is repression of transcriptional initiation because nucleosomes prevent the binding of transcriptional activators and the basal transcription factors to their cognate sites. Eukaryotic cells have developed several ways of overcoming this chromatin-mediated binding inhibition to enable gene transcription to take place, including mechanisms that disrupt or 'remodel' nucleosomes to permit transcription factors to bind to the underlying DNA sequence. For example, in *Drosophila* a four-subunit chromatin remodelling complex, nucleosome remodelling factor (NURF), facilitates the binding of sequence-specific transcription factors to their cognate sites in chromatin and so stimulates transcription from chromatin templates. We describe here progress in the identification of the component subunits of NURF and our biochemical studies on its role in transcriptional activation from chromatin templates.

Isolation and properties of NURF

NURF was initially identified using a biochemical assay to examine the mechanism of nucleosome disruption mediated by the binding of the GAGA factor to sites in the promoter of the *Drosophila* heat-shock protein gene, *hsp70,* when this is assembled into chromatin (Tsukiyama *et al*., 1994). We did this using a chromatin-assembly extract derived from *Drosophila* embryos to assemble a plasmid containing the intact *hsp70* gene (Becker and Wu, 1992). The extract contains many of the structural and enzymatic activities necessary for chromatin assembly and

assembles regular, physiologically spaced chromatin on plasmid templates.

To isolate NURF from the extract, *hsp70* templates were assembled into chromatin and purified by gel filtration, which removes both the extract and ATP (*Fig. 18*; Tsukiyama and Wu, 1995). The purified chromatin was incubated with recombinant GAGA factor and the chromatin digested with micrococcal nuclease (MNase), which cleaves chromatin in the linker DNA and generates a nucleosomal ladder in regularly spaced chromatin (*Fig. 18*, lane 1). This assay showed that GAGA factor can bind only in the presence of ATP (*Fig. 18*, compare lanes 2 and 4) and that binding is sensitive to treatment with the detergent Sarkosyl (sodium lauroylsarcosine, 0.05%; *Fig. 18*, compare lanes 4 and 8), indicating that an ATP-dependent cofactor was inactivated or removed from the chromatin by Sarkosyl treatment. Adding back the *Drosophila* embryo extract restores nucleosome disruption mediated by GAGA-factor binding (*Fig. 18*, lane 12), so this was used as an assay to fractionate the extract.

NURF was isolated in a fraction with ATP-dependent activity that restored the nucleosome disruption mediated by GAGA-factor binding to the *hsp70* promoter in Sarkosyl-treated chromatin templates. Highly purified NURF can facilitate GAGA-factor binding and only about one NURF per 40 nucleosomes is required to catalyse activity. At this low concentration, chromatin is disrupted only in the presence of GAGA factor and only at the promoter region to which GAGA factor binds (*Fig. 19a*, top), not at regions within the gene coding region (*Fig. 19a*, bottom). When NURF concentration is close to stoichiometric, i.e., 1 per 5 nucleosomes, disruption of the *hsp70* promoter increases (*Fig. 19a*). At this concentration there is also a global effect on chromatin even in the absence of GAGA factor, seen as a smearing of the nucleosomal ladder, together with an apparent decrease in nucleosome spacing indicated by compression of the ladder (*Fig. 19b*).

Using very pure mononucleosome templates reconstituted

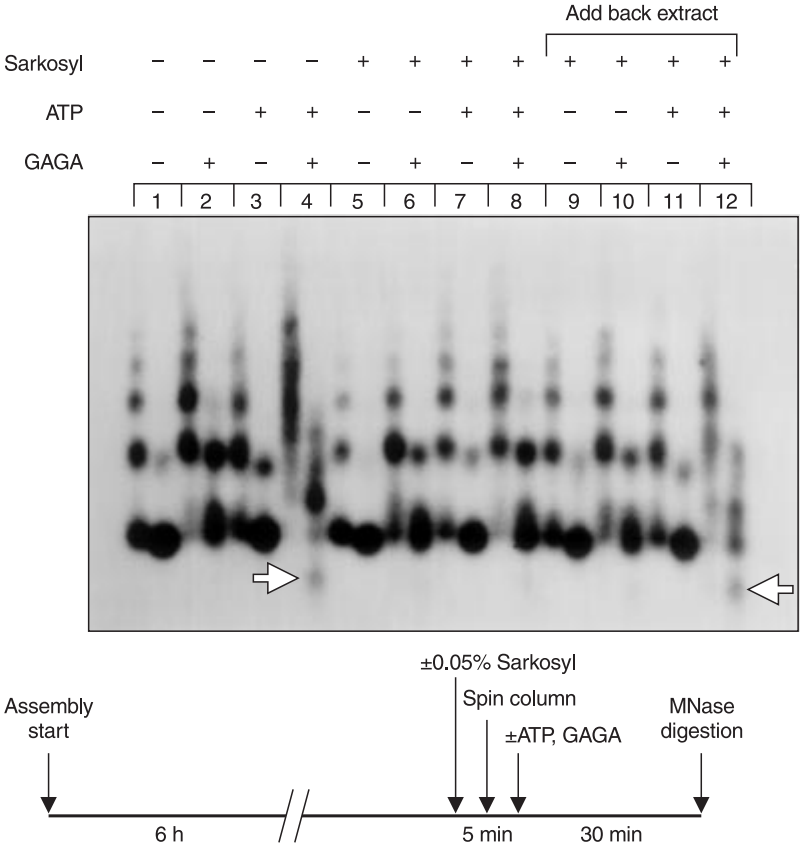


Figure 18. *Nucleosome disruption at the* hsp70 *promoter requires GAGA factor, a Sarkosyl-sensitive factor (NURF) and ATP. Chromatin remodelling is indicated by a smearing of the nucleosomal DNA ladder. In the absence of GAGA factor, the* hsp70 *promoter is assembled into a regular nucleosome array (lane 1); GAGA-factor binding disrupts this nucleosome ladder and produces a smaller fragment corresponding to the protection produced by GAGA factor alone (lane 4, arrow). Bottom, experimental protocol: MNase digestion of Sarkosyl-treated* hsp70 *chromatin and DNA blot hybridization with an* hsp70 *promoter oligonucleotide. Nucleosome perturbation mediated by GAGA-factor binding was visualized by Southern blotting and hybridization of this digest using an oligonucleotide corresponding to the GAGA-factor binding site. For further details, see text. Reproduced with permission from Tsukiyama and Wu, 1995.*

from purified histones, we confirmed that NURF does not require other components of chromatin for activity. It disrupted the 10 bp repeat pattern of DNase I cleavage characteristic of nucleosomal DNA in an ATP-dependent manner, and facilitated the binding of GAGA factor to mononucleosome templates, as assessed by DNase I footprinting (Tsukiyama and Wu, 1995).

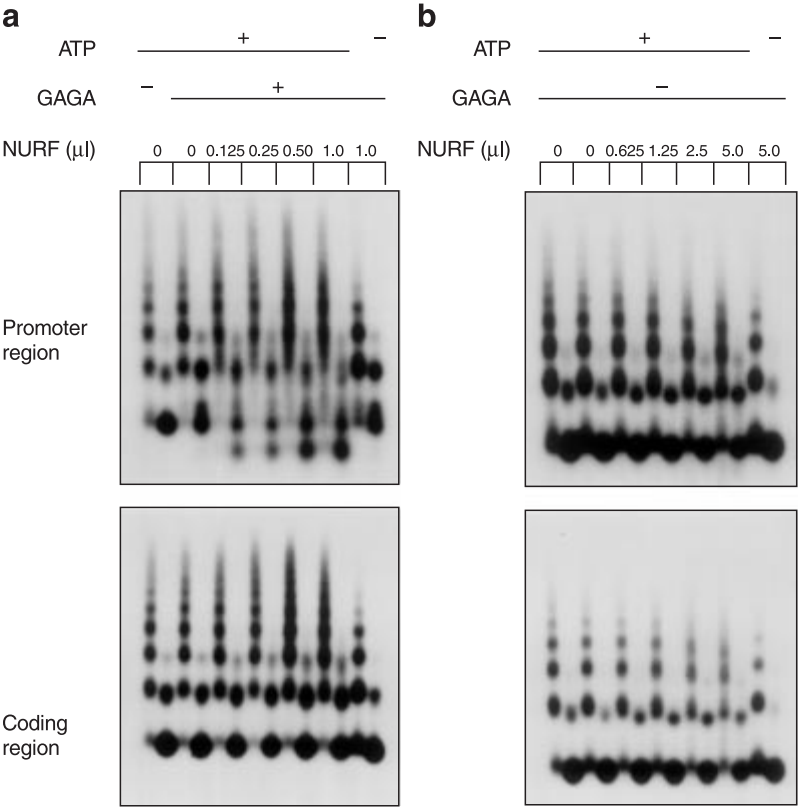


Figure 19. *Actions of NURF that are dependent and independent of GAGA factor.* ***a****, GAGA factor, low NURF levels and ATP mediate promoter-specific disruption of nucleosome organization.* ***b****, higher NURF levels and ATP in the absence of GAGA factor cause global effects on promoter and coding regions. Chromatin remodelling is indicated by a smearing of the nucleosomal DNA ladder. See text for details. Reproduced with permission from Tsukiyama and Wu, 1995.*

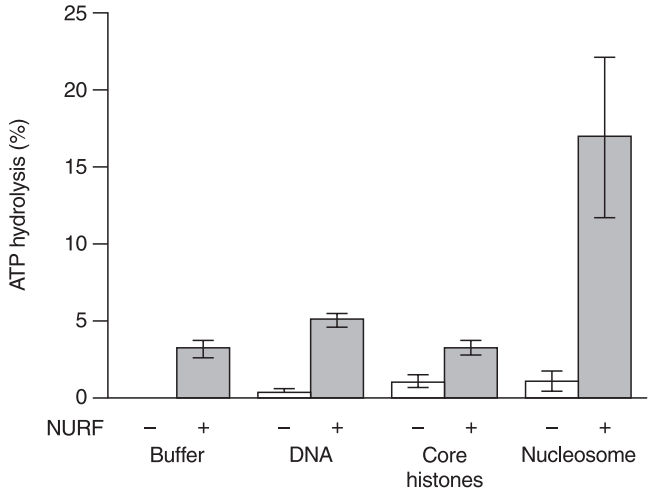


Figure 20. *ATPase activity of NURF is stimulated by nucleosomes but not by free DNA or free histones. Buffer, control. Reproduced with permission from Tsukiyama and Wu, 1995.*

The NURF complex contains a low, constitutive ATPase activity that is not stimulated either by free DNA or by histones; however, the ATPase activity of the complex was stimulated fivefold by incubation with reconstituted mononucleosomes (*Fig. 20*). As this was not observed with an unreconstituted mixture of DNA and histones, stimulation of the ATPase activity seems to require some aspect of the structure of assembled nucleosomes.

Subunit composition of NURF

NURF contains four main polypeptides, with apparent M_rs of 215K, 140K, 55K and 38K, that cosediment in a complex of about 500K (Tsukiyama and Wu, 1995). Three of these subunits have been identified, giving insights into the mechanism of NURF activity. The 140K subunit (NURF-140), which is responsible for ATP hydrolysis, is imitation switch (ISWI; Tsukiyama *et al.*, 1995), which is also found in two other *Drosophila* chromatin remodelling complexes, ATP-dependent chromatin assembly and remodelling complex (ACF; T. Ito *et al.*, 1997) and

chromatin accessibility complex (CHRAC; Varga-Weisz *et al.*, 1997; see Becker, this volume). Purified ISWI alone at high concentrations is active in several chromatin remodelling assays and in NURF this protein is likely to have a function similar to the key role it has in CHRAC (Corona *et al.*, 1999; see Becker, this volume).

The 55K subunit is related to RbAp48, a WD, or Trp-Asp, repeat protein that has highly conserved repeating units usually ending in Trp-Asp. RbAp48, which was originally identified by its in-vitro association with the retinoblastoma protein, is a common component of several chromatin-associated complexes, including chromatin assembly factor (CAF-1) and several mammalian histone deacetylase complexes (Martínez-Balbás *et al.*, 1998). The related yeast protein Hat2 is a subunit of the cytoplasmic HAT and yeast MSI1 is a subunit of yeast CAF-1. Thus NURF-55 and its homologues probably have a structural role, perhaps acting as a platform for the assembly of complexes involved in chromatin metabolism.

The 38K subunit is homologous to inorganic pyrophosphatase (Gdula *et al.*, 1998). Both recombinant NURF-38 alone and the purified NURF complex have inorganic pyrophosphatase activity but inhibition of this activity with sodium fluoride has no noticeable effect on the ability of NURF to remodel chromatin. As the ATPase activity of NURF, which is essential for chromatin remodelling, releases inorganic phosphate rather than pyrophosphate, the activities of NURF-140 and -38 seem to be biochemically uncoupled. Hence NURF-38 may have a structural or regulatory role in the NURF complex or NURF may have been adapted to deliver pyrophosphatase activity to transcriptionally or replicatively active regions of chromatin. These polymerization processes may be inhibited by the accumulation of unhydrolysed pyrophosphate during nucleotide incorporation, so NURF pyrophosphatase activity could promote the forward reaction by the efficient removal of this metabolite.

We have recently cloned the largest subunit, NURF-215,

which is encoded by a very large, new open reading frame giving a polypeptide of predicted M_r of 301K. Experiments aimed at the expression of this gene, with the goal of reconstituting the NURF complex from recombinant proteins, are currently underway.

Role of NURF in transcriptional activation

We have found that NURF-mediated chromatin remodelling activity on chromatin templates can facilitate access of transcription factors to their sites in the chromatin and so activate transcription. A simple model promoter consisting of five tandemly repeated Gal4-binding sites upstream of a minimal adenovirus E4 core promoter (Mizuguchi *et al.*, 1997) was assembled into chromatin using a *Drosophila* embryo extract and separated from the extract and ATP by gel filtration (see *Fig. 21a*, top). To examine chromatin remodelling, saturating amounts of Gal4 derivatives were incubated with the chromatin template in the presence of ATP before examining the transcriptional activity of the templates in a *Drosophila* nuclear extract. The chromatin template was highly repressive to transcription in the absence of Gal4 and became only weakly active in the presence of Gal4(1–147), which contains only the activator DNA-binding domain. In contrast, a derivative containing the Gal4 DNA-binding domain fused to the activation domain of heat-shock factor (Gal4–HSF) led to transcriptional activation (*Fig. 21a*, bottom, lane 3).

Gal4–HSF-mediated transcriptional activation was sensitive to Sarkosyl treatment of the chromatin template (*Fig. 21a*, lane 6) but could be rescued by the addition of purified NURF and ATP (*Fig. 21a*, lane 9). Even at sub-stoichiometric concentration, i.e., 1 NURF to 16 nucleosomes, NURF activated transcription (*Fig. 21a*, lane 10). The nucleosomes were remodelled in the promoter region, as smeared nucleosomal ladders were observed in the presence of NURF and Gal4(1–147) and Gal4–HSF activators in the promoter region (*Fig. 21b*, top), compared with the lack of remodelling in a distal region of chro-

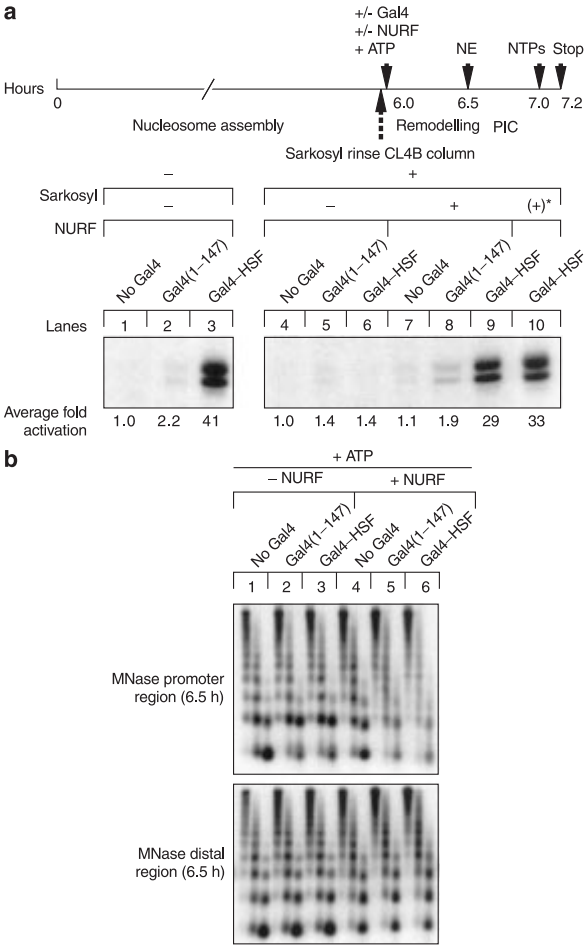


Figure 21. *Chromosome remodelling allows transcriptional activation. In a reconstituted plasmid chromatin containing five tandem Gal4 sites and the adenovirus E4 core promoter, transcriptional activation follows ATP-dependent nucleosome disruption by Gal4–HSF and NURF.* ***a****, experimental scheme and primer extension analysis of transcription from chromatin templates.* ***b****, MNase digestion analysis of the chromatin structure shows that chromatin remodelling, indicated by a smearing of the nucleosomal DNA ladder, occurs only over the promoter region (top) and not over the distal region (bottom). NE, nuclear extract; NTP, nucleoside triphosphate; PIC, preinitiation complex. See text for details. Reproduced with permission from Mizuguchi* et al.*, 1997.*

matin (*Fig. 21b*, bottom). Purified NURF can restore a large proportion of the transcriptional activation by Gal4–HSF on untreated chromatin templates, indicating that the Sarkosyl-sensitive activity required for transcriptional activation can be largely satisfied by NURF. It is of interest that activation was poor with Gal4(1–147) despite strong remodelling at the promoter region, indicating that the activation domain of HSF has an important role in relieving chromatin-dependent repression.

CHRAC, a chromatin remodelling complex driven by the ATPase ISWI

Peter B. Becker

in collaboration with

Cedric Clapier, Davide Corona, Gernot Längst and Patrick Varga-Weisz

The chromatin remodelling complex of *Drosophila*, NURF (see Wu, this volume), was isolated in an assay designed to identify activities that enable the GAGA factor, a specific eukaryotic transcription factor that binds to GA/CT-rich sites, to access its binding site in a specific promoter sequence. Using this approach, however, we could not distinguish between two possibilities: that the chromatin remodelling activity is actively recruited to promoter regions by the transcription factor or that chromatin remodelling is an untargeted process providing a 'window of opportunity' for proteins to gain access to the DNA. To identify chromatin remodelling activities, an alternative assay, which uses restriction-enzyme cleavage, was developed for examining protein access to multiple sites in chromatin. This has the advantage that restriction enzymes, which are prokaryotic proteins, cannot have evolved mechanisms to overcome chromatin-mediated repression. Using this assay, a five-subunit complex, the chro-

matin accessibility complex (CHRAC), has been isolated from *Drosophila* nuclear extract (Varga-Weisz *et al.*, 1997).

CHRAC is an ATP-dependent chromatin remodelling complex that facilitates the access of DNA-binding proteins to sites in chromatin. It can also realign nucleosomes to generate regular, evenly spaced nucleosome arrays. We review the progress made in identifying the component subunits of this complex and discuss experiments indicating that the ATPase subunit ISWI is the functional core of CHRAC's chromatin remodelling activity.

Isolation of CHRAC

The restriction-enzyme assay is based on the assembly of regular, physiologically spaced and highly complex chromatin by extracts from *Drosophila* embryos (Becker and Wu, 1992). Assembled chromatin templates are passed over a gel-filtration column to remove the extract and ATP, and incubated with the restriction enzyme *Dra*I. Under these conditions, the chromatin largely resists *Dra*I cleavage, consistent with previous observations that nucleosome assembly can inhibit cleavage by restriction enzymes.

Cleavage was stimulated when ATP was added back during incubation with *Dra*I, indicating that an ATP-dependent activity in chromatin facilitated access by the restriction enzyme to sites in the chromatin template. This activity was inactivated by treating the chromatin with Sarkosyl to remove ATP-dependent chromatin remodelling activities but reappeared when *Drosophila* nuclear extract and ATP were added back. Using this assay to fractionate *Drosophila* nuclear extract resulted in the isolation of CHRAC (Varga-Weisz *et al.*, 1997).

Chromatin remodelling activities of CHRAC

Biochemical studies show that CHRAC has two apparently different actions on chromatin: it increases protein access to chromatin templates and increases the regularity of an irregular nucleosomal array.

Increased protein access to chromatin templates. When designing the assay used to isolate CHRAC, we assumed that increased cleavage of chromatin templates by *Dra*I was a satisfactory model for general access by proteins to sites in chromatin. This proved to be so for two dissimilar sequence-specific DNA-binding prot-eins: the SV40 replication protein T-antigen and transcription termination factor 1 (TTF-1), a factor required for polymerase I transcription and termination on ribosomal genes.

• *SV40 T-antigen*. SV40 replication has an absolute requirement for the binding of an initiation factor, T-antigen, at the origin of replication but replication is inhibited by chromatin assembly. Experiments using chromatin templates assembled using *Drosophila* embryo extract showed that T-antigen could gain access to its binding site in chromatin in the presence of a Sarkosyl-sensitive, ATP-dependent cofactor (Becker and co-workers in collaboration with Claudia Gruss and co-workers, University of Konstanz). This activity could be replaced by purified CHRAC, which can also facilitate T-antigen binding in chromatin and leads to increased replication on these templates (Alexiadis *et al*., 1998).

• *TTF-1.* Polymerase I transcription of the mouse ribosomal genes requires the binding of TTF-1 to a site (T_o) in the promoter region. This protein is chromatin-specific, co-operating with ATP-dependent chromatin remodelling activities to position nucleosomes within the promoter. This nucleosome remodelling correlates with transcription, indicating that a specific chromatin architecture is required for initiation (Längst *et al*., 1998). Biochemical studies showed that purified CHRAC can facilitate TTF-dependent nucleosome remodelling in the ribosomal DNA promoter (Corona *et al*., 1999). This assay was performed using chromatin templates assembled with polyglutamic acid, which contain only histones and DNA, confirming that additional chromatin-bound proteins are unnecessary for CHRAC remodelling activity.

Increased nucleosome regularity. CHRAC was originally identified as a complex that facilitated protein access to sites in chromatin, a process that is probably associated with nucleosome removal or disassembly. However, it can also increase the regularity or order in chromatin. This activity was identified using Sarkosyl-stripped chromatin: the detergent disrupts the regularity of nucleosome spacing, generating a DNA smear when analysed by micrococcal nuclease (MNase) digestion. Addition of ATP and purified CHRAC to this chromatin realigns the nucleosomes, generating a regular nucleosome ladder (*Fig. 22*; Varga-Weisz *et al.*, 1997).

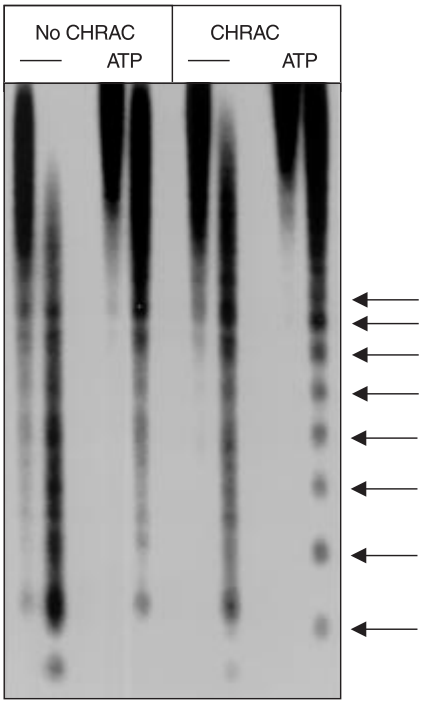


***Figure 22**. CHRAC is a nucleosome spacing factor. Regularly spaced nucleosomes are a hallmark of physiological chromatin and are detected by cleavage of chromatin with MNase, which generates a regular ladder of protected fragments (arrows). CHRAC can generate regularly spaced nucleosome arrays from irregular nucleosomes in the presence of ATP*

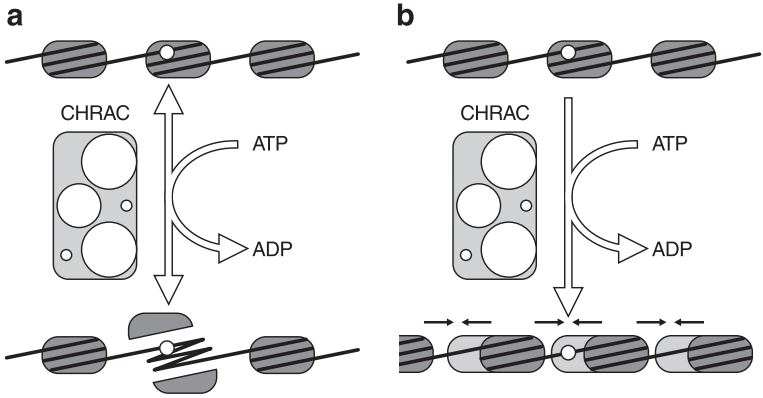


Figure 23. *Two proposals for how CHRAC increases the accessibility of nucleosomal DNA to transcription factors while improving the regularity of chromatin.* ***a****, model assumes that CHRAC is a nucleosome assembly/disassembly factor that influences the equilibrium between nucleosomes and partially disassembled sub-nucleosomal particles.* ***b****, model assumes that CHRAC increases nucleosome mobility and so introduces a dynamic element into chromatin.*

Two broad models for the mechanism of CHRAC action are consistent with these two forms of chromatin remodelling activity: the complex can act either by dissociating nucleosomes into sub-nucleosomal components, thereby enabling access for proteins, and reassembling them elsewhere; or by facilitating nucleosome movement or sliding along the DNA (*Fig. 23*).

Identification and role of CHRAC subunits

The CHRAC complex has five subunits, with apparent M_rs of 175K, 160K, 130K, 18K and 14K (*Fig. 24*; Varga-Weisz *et al.*, 1997). Mass spectroscopy of subunit-derived tryptic peptides and immunological analysis demonstrated that p160 and p130 are ATPases: topoisomerase II and ISWI, respectively. Co-immunoprecipitation studies using FLAG-tagged (see *Glossary*) ISWI confirm that these reside in a single complex. Subunits p175, p18 and p14 are being identified. ISWI is likely to be the functional core of CHRAC and the other subunits probably either

perform structural or regulatory roles, or target the complex to specific chromatin regions or nuclear processes.

• *ISWI*. This subunit is also found in NURF (see Wu, this volume) and the ATP-dependent chromatin assembly and remodelling complex (ACF; T. Ito *et al.*, 1997), as well as in CHRAC, indicating that it has a central role in chromatin remodelling activity. ISWI alone can recognize some aspects of nucleosome structure and trigger ATP-dependent chromatin remodelling. Comparing the biochemical properties of recombinant ISWI with purified CHRAC revealed that, surprisingly, ISWI is active in all the CHRAC-associated assays: it has nucleosome-stimulated ATPase activity and can remodel chromatin. The latter was demonstrated by its ability to facilitate GAGA-factor binding and TTF-1-dependent nucleosome rearrangement and to generate nucleosome regularity in irregularly spaced chromatin (Corona *et al.*, 1999).

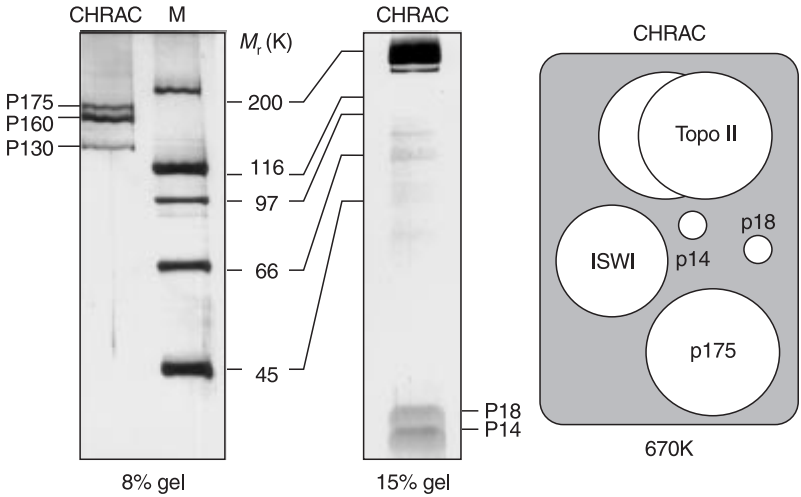


Figure 24. *Purification of CHRAC yields a 670K complex of five subunits (right); topoisomerase II (topo II) is shown as a dimer. Left, purified CHRAC was separated on different SDS gels to reveal the larger (8% gel) and smaller subunits (15% gel). The p160 and p130 subunits have been identified as the ATPases topoisomerase II and ISWI. M, molecular mass markers.*

• *Topoisomerase II* has roles in a number of cellular processes, including the removal of catenates after replication, chromosome condensation and decondensation, kinetochore assembly and the separation of sister chromatids at mitosis. It is not clear, however, why topoisomerase II, an enzyme involved in DNA topology, is in a chromatin remodelling complex, as its inactivation with the specific inhibitors, VP-16 (also called etoposide) and VM-26, had no effect on CHRAC chromatin remodelling

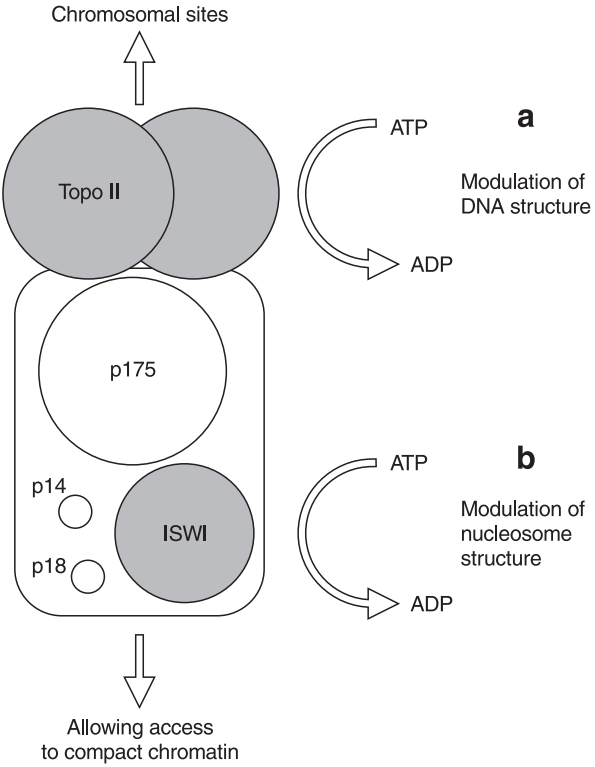


Figure 25. *Two explanations for the significance of the association in CHRAC of enzymes that modulate both (**a**) DNA topology and (**b**) chromatin structure, given that topoisomerase II has been shown not to be mechanistically involved in CHRAC remodelling activity (see text). The CHRAC complex is shown on the left.*

activity. The protein forms a dimer, so CHRAC probably contains two molecules of topoisomerase II, consistent with the increased strength of the p160 band in purified CHRAC (*Fig. 24*) and the estimated size of the complex, 670K, obtained from gel-filtration chromatography. The topoisomerase II present in CHRAC is active, because the DNA strand breakage and re-ligation activity of topoisomerase II, assessed by ATP-dependent relaxation of supercoiled plasmid DNA, elutes precisely with CHRAC at the final purification step.

Two models have been proposed: either chromatin remodelling by ISWI facilitates the binding of topoisomerase II to chromatin, thereby enhancing its function, or topoisomerase II helps to target CHRAC to specific chromosomal sites (*Fig. 25*). Identification of the remaining unknown subunits in CHRAC and further biochemical studies on its chromatin remodelling activity will give a clearer picture of how topoisomerase II contributes to CHRAC function.

New components of the chromatin remodelling complexes RSC and SWI/SNF

Bradley R. Cairns

in collaboration with

Roger Kornberg, Fred Winston, Paul Tempst and Alisha Schlichter

Two related large protein complexes that utilize ATP for remodelling the structure of chromatin have been isolated from the yeast *S. cerevisiae*, the 11-subunit SWI/SNF complex and the 15-subunit RSC complex, both with M_r ~1,000K (*Fig. 26*). SWI/SNF was characterized after copurification of proteins identified in genetic screens for mutants defective in mating-type

switching (*swi*) and mutants defective in sucrose fermentation (sucrose non-fermenting, *snf*; Cairns *et al.*, 1994; Côté *et al.*, 1994). RSC, so-named because of its ability to remodel the structure of chromatin, was isolated because its subunits have sequence homology with those of the SWI/SNF complex (Cairns *et al.*, 1996). Both complexes contain a DNA-dependent ATPase that can be stimulated by both free and nucleosomal DNA with comparable affinities. RSC is 10 times more abundant than SWI/SNF and most of the genes encoding RSC subunits are essential for mitotic growth.

We report here combined genetic and biochemical approaches to the functions of RSC and SWI/SNF and the identification and characterization of some subunits in each complex: Rsc1 and 2, which are unique to RSC, and the actin-related proteins Arp7 and Arp9, which are common to RSC and SWI/SNF.

RSC, SWI/SNF and chromatin remodelling

Detailed information about the functions of the two complexes is only now emerging. In contrast to SWI/SNF, many components of RSC are essential for viability, indicating that RSC may have a more general role than SWI/SNF in chromatin remodelling, although its essential targets are unknown. Suppressors of *swi/snf* mutant phenotypes are found in genes coding for histones or other chromatin components, indicating a direct interaction between chromatin and the remodelling complex. Several homologues of SWI/SNF and RSC components have been identified in humans and in *Drosophila*, where they are implicated in cell-cycle control, mitotic growth or nuclear matrix integrity, but their evolutionary relationship to the yeast complexes is still poorly understood (Winston and Carlson, 1992; Workman and Kingston, 1998).

Study of RSC function was initially hampered by difficulties in separating the different forms of RSC–DNA and RSC–nucleosome complexes. This has been resolved by the demonstration

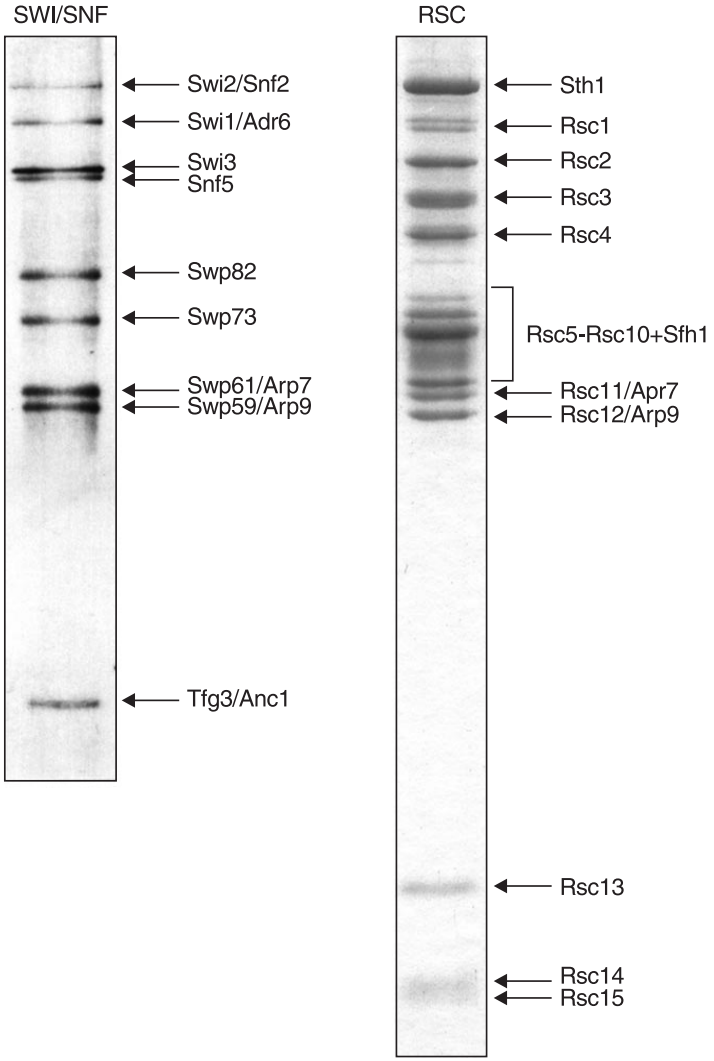


Figure 26. *The budding yeast* S. cerevisiae *contains two related ATP-dependent chromatin remodelling machines, SWI/SNF and RSC. SWI/SNF is composed of 11 proteins, 9 of which are visible on this gel, and RSC is composed of about 15. They both contain the actin-related proteins Arp7 and Arp9. A solidus indicates alternative names for the same protein rather than two proteins.*

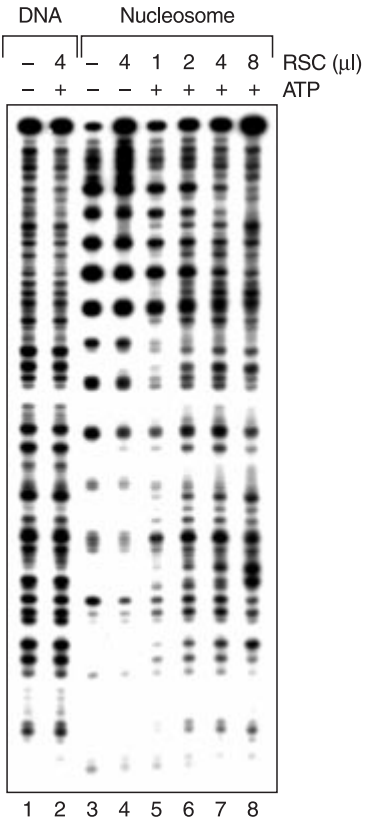


Figure 27. *RSC utilizes ATP to remodel chromatin in vitro. In the presence of ATP, RSC alters the rotational phase of DNA on the nucleosomal surface. This is the standard assay for in vitro chromatin remodelling and is a clear demonstration of the activity of RSC. See text for details.*

that, in the absence of ATP, the affinity constants for RSC–DNA and RSC–nucleosome interactions are similar (Lorch *et al.*, 1998). Whereas the RSC–DNA interaction is ATP-independent, ATP not only stimulates RSC–nucleosome interactions but also converts the complex to a slower-migrating form. As this form is more readily accessible to restriction endonucleases and DNase I, ATP seems to perturb the structure of the RSC–nucleosome

complex, producing what is referred to as the activated form. The persistence of this form is independent of the presence of ATP. Removal of RSC results in an altered nucleosome conformation, which can be converted to the starting material by adding back both ATP and RSC. The activated RSC–nucleosome complex, rather than the altered form lacking RSC, may be an intermediate in the pathway leading to transcriptional activation.

Chromatin remodelling involves the perturbation of nucleosomes at transcription initiation sites to facilitate the assembly of functional preinitiation complexes. The increase in accessibility is revealed by increased sensitivity to DNase I cleavage (*Fig. 27*). The remodelling requires ATP hydrolysis, which has been attributed to the Swi2/Snf2 component of SWI/SNF and its homologue Sth1 in RSC (*Fig. 26*). Four models of the role of 'chromatin remodelling machines' in activation of transcription in chromatin have been deduced from genetic and biochemical evidence (*Fig. 28*; for review, see Cairns, 1998). Yeast SWI/SNF may utilize all four models: yeast SWI/SNF interacts with transcriptional activators (*Fig. 28a,b*), facilitates nucleosome sliding (*Fig. 28c*), and interacts with transcriptional repressors Hir1 and Sin1 (*Fig. 28d*). Additional experiments should determine the promoters at which these modes are utilized, and how each of these modes is regulated.

Newly identified RSC and SWI/SNF components

Two components unique to RSC have been identified and characterized by combined genetic and biochemical approaches: Rsc1 and Rsc2 (Cairns *et al.*, 1999). The two proteins are 50% identical and each contains two bromodomains. The bromodomain is a 110 amino-acid motif that interacts with acetylated histone tails and is present in many proteins associated with transcriptional control and histone modification, e.g., in p300/CBP, $TAF_{II}250$, Gcn5, Snf2 and Sth1 (Winston and Allis, 1999). Rsc1 and Rsc2 also contain a bromo-adjacent homology

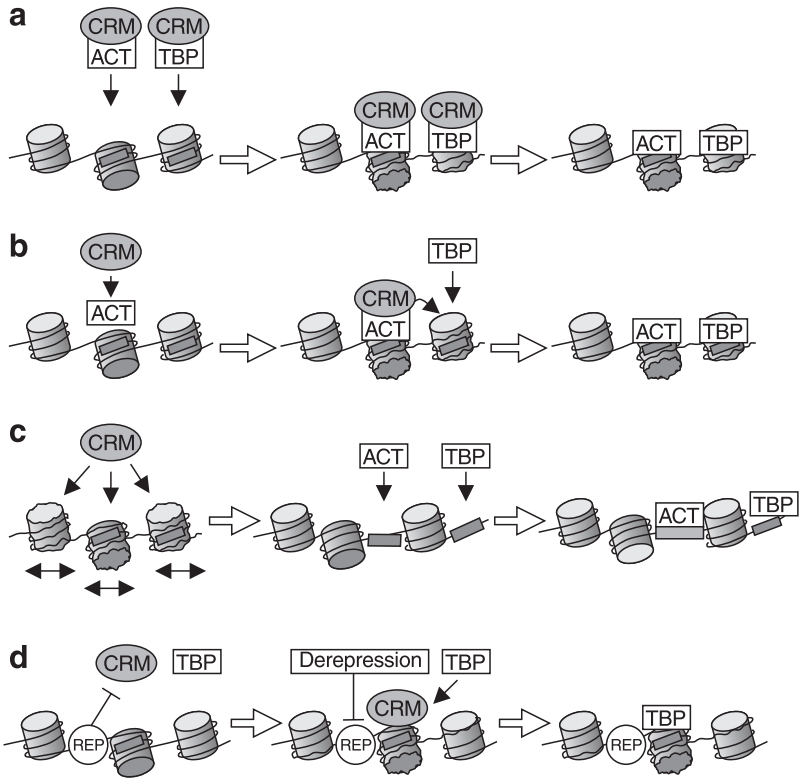


Figure 28. *Models for how chromatin remodelling machines (CRM) can facilitate binding of factors to nucleosomal DNA. A hypothetical promoter consisting of a binding site for a transcriptional activator (ACT) and the TATA-binding protein (TBP) are assembled into a nucleosome array. Nucleosomes perturbed by a CRM remain associated with DNA but dissociation could also occur.* ***a****, CRM facilitates activator or TBP binding.* ***b****, activators bind without CRM involvement, but subsequently recruit CRM to remodel neighbouring nucleosomes.* ***c****, CRM action facilitates nucleosome mobility, uncovering binding sites.* ***d****, a DNA-bound transcriptional repressor (REP) stabilizes the nucleosome and represses CRM/ACT action/binding until derepression signals block REP function. Once derepression has occurred, facilitated binding of ACT, TBP or other factors by the CRM might occur by any of the mechanisms in models* ***a–c****.*

motif that is present in the *Drosophila* transcriptional activator Ash1, as well as in proteins of unknown function in the nematode *Caenorhabditis elegans* and the yeast *Schizosaccharomyces pombe* (*Fig. 29*). Assaying growth on caffeine (caffeine sensitivity is a phenotype in many yeast strains with defects in osmoregulation or cAMP signalling) showed that the Ash1-related region and one of the two bromodomains, BD2, are required for growth on media containing caffeine. Deletion analysis indicates that

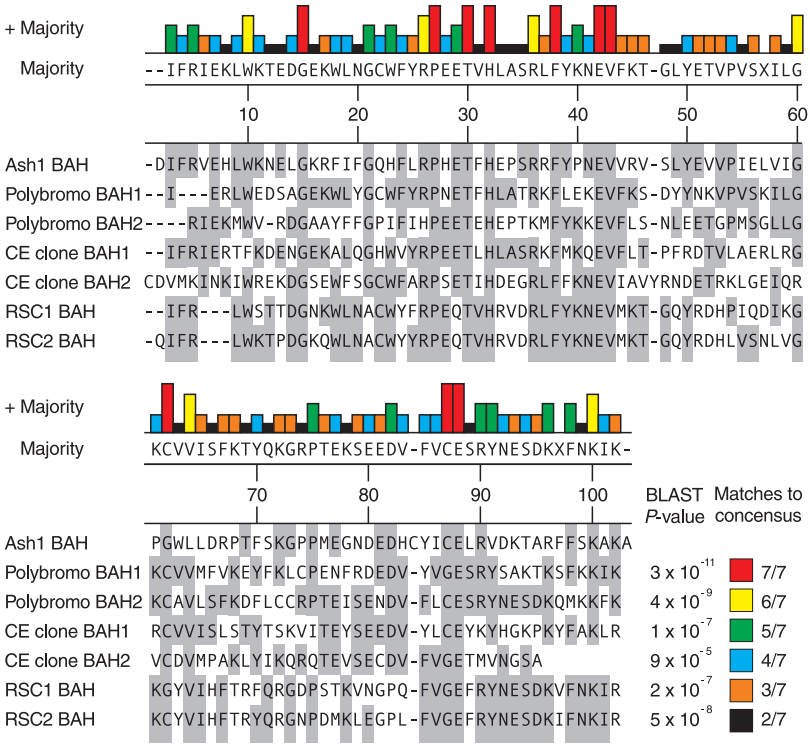


Figure 29. *The bromo-adjacent homology (BAH) domain, a new motif found in factors involved in chromatin remodelling. Five proteins bearing BAH domains are aligned: Ash1,* Drosophila *homeotic transcriptional activator; Rsc1,* S. cerevisiae *member of RSC; polybromo, chicken, possible member of human SWI/SNF; CE,* C. elegans, *unknown function. BLAST (basic local alignment search tool), comparison of amino-acid sequences against a protein sequence database.*

the C terminus of either Rsc1 or Rsc2 is sufficient for assembly into the RSC complex, whereas neither the bromodomain nor the bromo-adjacent homology domain is required for assembly.

Cells with a mutation in either *rsc1* or *rsc2* (single mutant) exhibit slow mitotic growth and are sensitive to elevated caffeine concentrations. Although single mutants are viable, the mutations in both *rsc1* and *rsc2* are lethal, indicating that the two proteins share a common essential function. Immunoprecipitation studies indicate

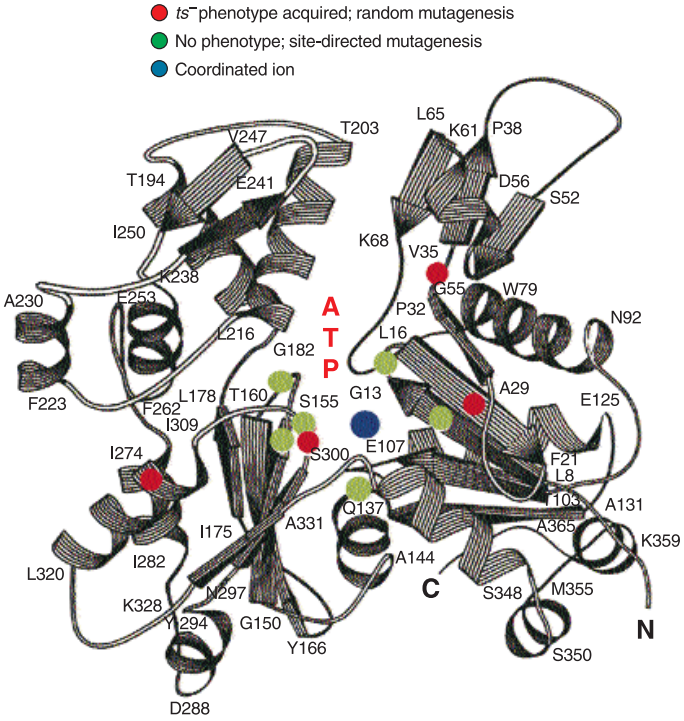


Figure 30. *Locations of, and phenotypes conferred by, mutations in* ARP7 *superimposed on the crystal structure of actin. Locations of substitutions are based on sequence alignments. Red dots, locations of the* ARP7 ts⁻ *substitutions, which align with residues important for the structure of actin. Green dots, locations of site-directed replacements performed with the* ARP7 *mutants designed to impair ATP hydrolysis and which conferred no phenotype. Blue dot, location of the divalent ion. Modified from Kabsch* et al.*, 1990.*

that there are distinct RSC complexes, one containing Rsc1, the other Rsc2, which explains this synergistic lethality.

SWI/SNF and RSC share two components that cofractionate during purification. These are the actin-related proteins Arp7, which is known as Rsc11 in RSC and Swp61 in SWI/SNF, and Arp9, known as Rsc12 and Swp59 (*Fig. 26*; Cairns *et al.*, 1998). Both *ARP7* and *ARP9* are genes essential for growth in the common laboratory yeast strain S288C, whereas cells of W303, another common laboratory strain lacking *ARP7* or *ARP9,* exhibit extremely poor growth. Temperature-sensitive (ts^-) mutations in each gene (*Fig. 30*) were isolated to study their functions.

Although the structural relationship to actin is supported by the predicted amino-acid changes, Arp7 and Arp9 seem to differ from actin in their lack of ATP hydrolytic activity. Arp7 and Arp9 are homologous to domains 1 and 3 of actin, which bind and hydrolyse ATP (actin domains 2 and 4 are involved in protein–protein binding). By analogy to mutants abolishing ATP hydrolysis of actin, several site-directed mutations in Arp 7 and 9 were predicted to impair ATP binding or hydrolysis. However, they did not seem to affect the essential growth function of Arp7 or Arp9. Further evidence of a role for these proteins in transcriptional regulation is provided by the observation that isolated ts^- mutants alter the site where transcription is initiated in vivo.

Regulation of human chromatin remodelling

Robert E. Kingston

in collaboration with

Gavin Schnitzler, Michael Phelan and Steven Brown

The five related complexes, SWI/SNF, NURF, RSC, CHRAC and ACF, that facilitate transcription by altering nucleosome structure (for review, see Cairns, 1998; see Becker, Cairns, Wu, this

volume) increase either the binding of transcription factors or the access of restriction enzymes to DNA (for review, see Workman and Kingston, 1998). They are all ATP-dependent and are assumed to catalyse the formation of a remodelled nucleosome, the precise histone configuration of which is unknown. In humans, two separate nucleosome remodelling complexes, referred to collectively as the human SWI/SNF complexes (hSWI/SNF), have been isolated (Kwon *et al.*, 1994; W. Wang *et al.*, 1996*a,b*).

By incubating the purified hSWI/SNF complex with reconstituted nucleosomes, we have found a nucleosome with previously unknown structure. This form and the standard form are in a dynamic equilibrium that seems to be created by the hSWI/SNF complex. Preferential binding of transcriptional activators to the new form and the displacement of the equilibrium by repressors or activators both indicate a possible mechanism for the activation and repression of transcription. We also report here that the SWI/SNF complex has an active catalytic core of four conserved subunits, which catalyse the remodelling of the nucleosome core. The remaining subunits probably have a regulatory or targeting function.

A new form of remodelled nucleosome

To purify homogeneous hSWI/SNF complex with high specific activity, we used a helper-free retrovirus system to establish a cell line that produces an epitope-tagged copy of the smallest subunit of the complex, Ini1, the human homologue of yeast Snf5 protein (Sif *et al.*, 1998). In human cells, Ini1 is the only Snf5 homologue found in association with hSWI/SNF complexes containing either of the closely related ATPases, Brg1 and hBrm. Using an anti-FLAG affinity gel cell lines with the highest level of the FLAG-tagged Ini1 protein were selected and rapidly purified. The isolated complex had ATP-dependent remodelling activity and disrupted both nucleosome and plasmid chromatin. These activities

were indistinguishable from the previously characterized hSWI/SNF fractions prepared by conventional chromatography.

Using gel-shift analysis, stable products were detected after incubation of reconstituted nucleosomes with hSWI/SNF (Schnitzler *et al.*, 1998). Addition of high salt or cold competitor DNA after the reaction and before electrophoresis helped to resolve two species, one with the same mobility as the core particle and the other a slower-migrating form (*Fig. 31a*). The presence of the new form was dependent on both the SWI/SNF complex and ATP but did not seem to be bound to SWI/SNF. It also displayed a distinct DNase I footprinting pattern when compared to a standard nucleosome (*Fig. 31b*). Protein analysis revealed that the new form contained the four core histones. It migrated faster than a standard mononucleosome on a glycerol gradient and biochemical characterization indicated that it may correspond to a dimer. Similar results have been obtained with the RSC remodelling complex from yeast (Lorch *et al.*, 1998; see Cairns, this volume).

Because a substantial proportion of the input nucleosome remained in the reaction, the standard nucleosome could be in equilibrium with the new form. To test this, the new form was converted back to the standard migrating nucleosome using a low level of hSWI/SNF complex in the presence of ATP (*Fig. 31a*). That the SWI/SNF complex can catalyse a back reaction was confirmed by comparing the DNase I digestion patterns of the gradient-isolated new form to that of standard nucleosome in the presence and absence of hSWI/SNF. In the absence of either the complex or ATP, the altered pattern with the new band (*Fig. 31b*, lanes 1 and 2) was clearly distinct from that of the standard nucleosome, which showed the pattern repeating at every 10 bases typical of a nucleosome in a DNase I analysis (*Fig. 31b*, lanes 4 and 5). With the hSWI/SNF complex and ATP present, both the new form and the standard nucleosome generated an intermediate pattern (*Fig. 31b*, cf. lanes 3 and 6), indicating

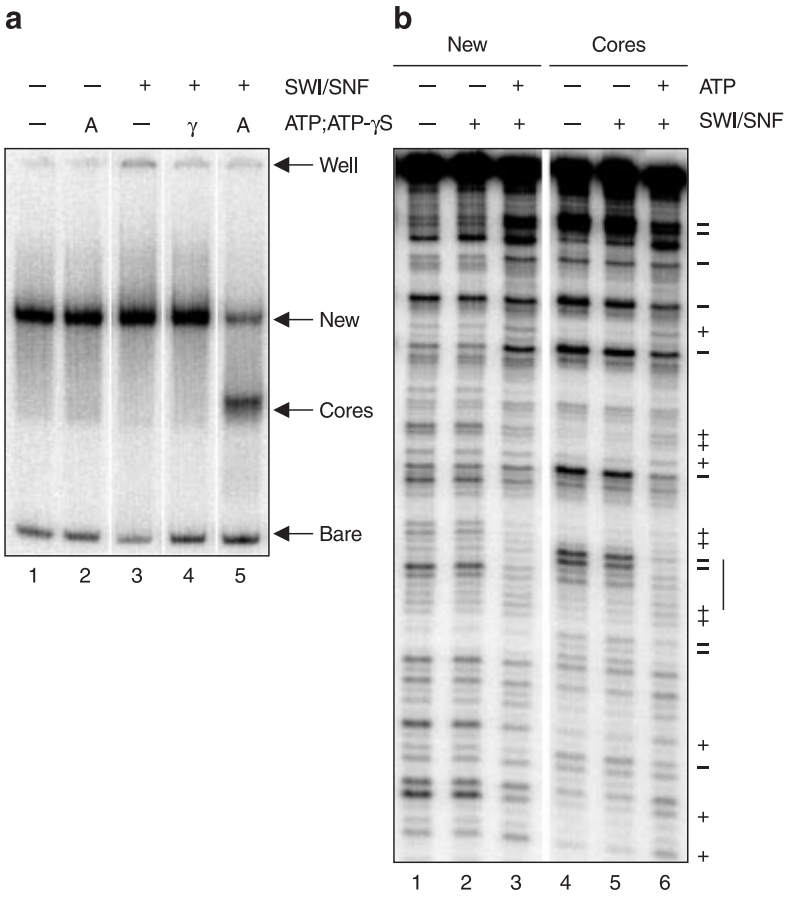


Figure 31. *SWI/SNF can catalyse the reformation of a standard nucleosome core from a remodelled nucleosome core.* ***a****, the remodelled nucleosome form was isolated on a glycerol gradient and characterized by native gel electrophoresis (lane 1). This species (New) was converted to a standard nucleosome core in the presence of ATP and SWI/SNF (Cores; lane 5) but not when ATP-γS was used (γ; lane 4).* ***b****, the new form has a different DNase digestion pattern from the standard nucleosome core when analysed by denaturing gel electrophoresis (compare lanes 1 and 2 to lanes 4 and 5). The DNase digestion pattern in the presence of active SWI/SNF and ATP is similar to the DNase digestion pattern of the standard form (lane 6) and the new form (lane 3). Increases (+) and decreases (–) in DNase cleavage are indicated to the right of the gel. Bar indicates positions protected when SWI/SNF is present. Modified from Schnitzler* et al.*, 1998.*

that, in the presence of ATP, the hSWI/SNF complex can create a dynamic equilibrium between the standard nucleosome structure and the new form.

To determine whether the new form can facilitate the binding of a transcriptional activator in vivo, we reconstituted mononucleosomes containing a single Gal4-binding site with high or low affinity, isolated the new form as described above and assessed Gal4 binding by DNase I footprinting. There was preferential binding on the new form, irrespective of whether the binding site was low or high affinity (Schnitzler *et al.*, 1998). A strong implication of these results is that the standard nucleosome can exist in equilibrium with alternative structures and that a complex involved in gene regulation can affect the transition between these structures (*Fig. 32*). In addition, the equilibrium can potentially be driven in either one of two directions to establish states that are either repressed, in the presence of repressors, or active, in the presence of activators. In this context the remodelling machine may be a way simply to make the chromatin more fluid, which can be used in the mechanisms of both activation and repression.

Defining the catalytic subunits in the hSWI/SNF complex

The hSWI/SNF complex is a multipolypeptide complex which contains from eight up to fifteen subunits. We have identified the four core catalytic subunits of the complex and reconstructed the core using recombinant proteins; this recombinant core has similar activity to that of the intact 'normal' holo complex (*Fig. 33*). Three of the subunits, Brg1- or Brahma-associated factor (BAF) 155, BAF170 and Ini1, are highly conserved between yeast and human and are always present in both the hSWI/SNF complexes. The fourth is the ATPase subunit, which can be either Brg1 or the related hBrm protein. Complexes contain either Brg1 or hBrm, not both, and the two types of complex are likely to have different activities and biological roles.

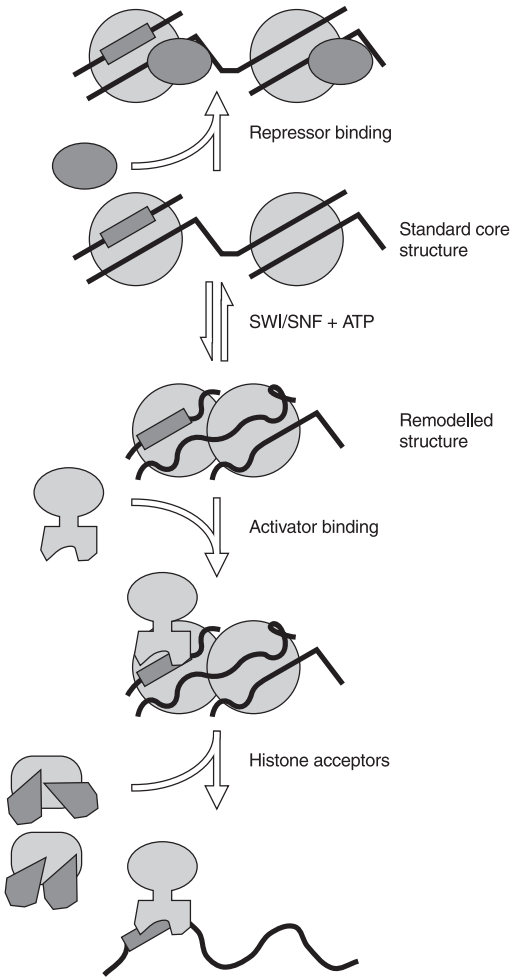


Figure 32. *A possible model for SWI/SNF action in vivo. SWI/SNF may use the energy of ATP hydrolysis for continuous interconversion between the standard and remodelled core structure of the nucleosome. Any protein that binds with higher affinity to the remodelled structure, e.g. an activator, will drive the equilibrium toward the remodelled form, whereas any protein that binds with higher affinity to the standard state, e.g. a repressor, will drive the reaction towards the standard form. Thus SWI/SNF may create a fluid chromatin environment that can be fixed in either a remodelled configuration or a standard configuration depending upon the local concentrations and activities of regulatory proteins.*

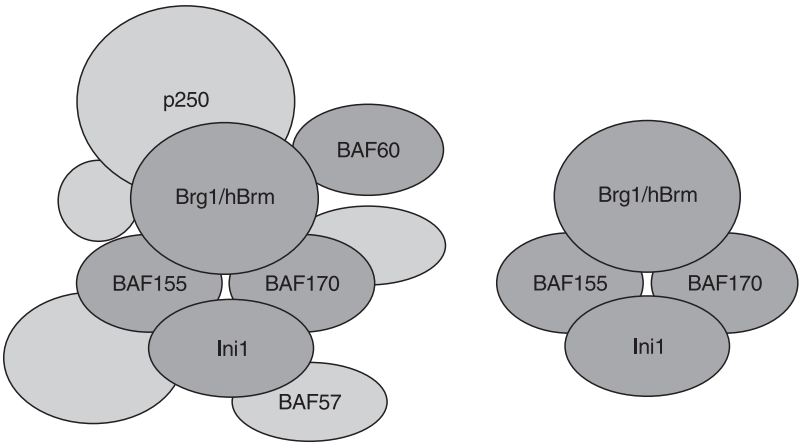


Figure 33*. The intact human SWI/SNF complex (holo complex, left) contains a core of subunits (core, right) that are sufficient for ATP-dependent chromatin remodelling.*

The ATP-dependent remodelling activity was reconstituted from only the conserved subunits, using recombinant proteins produced by a baculovirus expression system. Tagged Ini1 and untagged BAF155 and BAF170 were coexpressed after a mixed infection and isolation of the complex was facilitated by the tagged Ini1 protein. Brg1 was produced at high levels by itself as an epitope-tagged (FLAG) version. The four subunits were co-purified from a mixture of extracts from cells infected with the three subunits and cells infected with Brg1 (Phelan *et al.*, 1999). The remodelling activity of this recombinant core complex was compared with that of the holo complex using the plasmid supercoiling assay (*Fig. 34;* Germon *et al.,* 1979). A circular DNA was reconstituted into chromatin using histones in the presence of topoisomerase to relax constraint arising from the deposition of each nucleosome. After deproteinization, the reconstituted minichromosomes gave rise to highly supercoiled molecules, which were identified by their migration properties on an

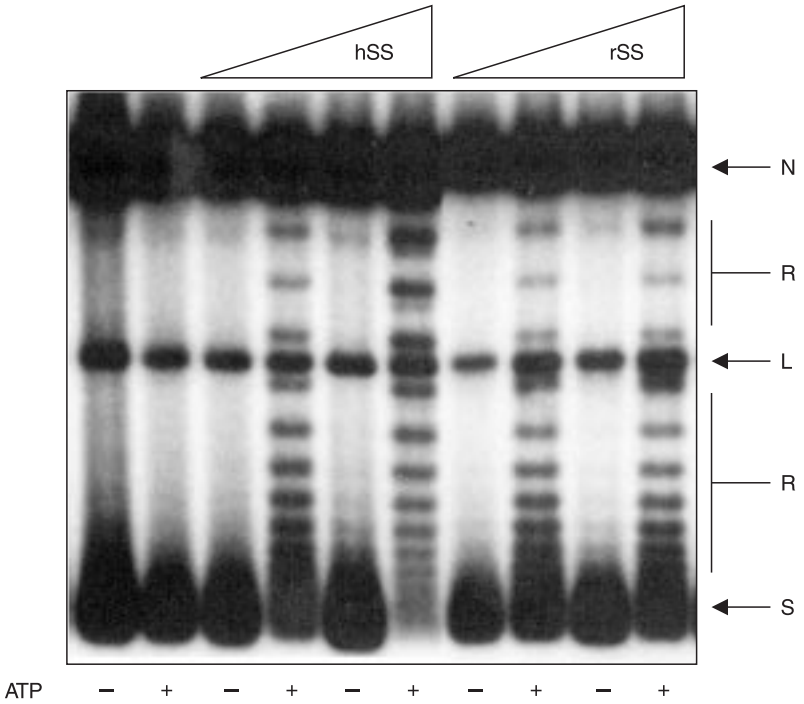


Figure 34. *Reconstituted hSWI/SNF (rSS) has similar activity to native, intact hSWI/SNF (hSS). Brg1, BAF170, BAF155 and Ini1 were overproduced and purified from baculovirus and reconstituted into a complex, the activity of which was compared to intact SWI/SNF isolated from HeLa cells by measuring changes in supercoiling of nucleosomal templates. The reconstituted complex had two- to fourfold less activity than the intact complex. S, supercoiled molecules; N, nicked DNA; R, relaxed topoisomers; L, linear DNA.*

agarose gel (*Fig. 34*, band S). When the reconstituted minichromosome was incubated with either the natural SWI/SNF complex or the recombinant core in the presence of topoisomerase and a source of ATP, the changed migration properties of the final deproteinized product was easily detected as relaxed topoisomers (*Fig. 34*, bands R).

It is not yet clear exactly which changes are promoted in terms of topological constraints at the level of individual remodelled particles. However, the change in topology detected with both the

holo and core complexes provides a useful assay for defining the catalytic component(s) of the core subunits. The isolated Brg1 subunit was remarkable in that it alone could support remodelling in this assay, although at a tenfold lower efficiency than an equimolar concentration of the holo complex. The three subunits, BAF155, BAF170 and Ini1, stimulated this activity. We assume that the additional subunits in the holo complex (see *Fig. 33*), are likely to have regulatory roles. Because Brg1-related polypeptides are part of other complexes, this family of polypeptides may represent a basic module to create remodelling.

Chromatin remodelling in vivo

Wolfram Hörz

in collaboration with

Philip D. Gregory, Martin Münsterkötter and Gudrun Ertinger

The control of chromatin organization with respect to transcriptional regulation is being investigated in detail (for review, see Gregory and Hörz, 1998). Genetic studies in yeast have indicated that nucleosomes are involved in the repression of transcription, e.g., depletion of histone H4 prevented the formation of intact nucleosomes and hence the activation of promoters (Han *et al.*, 1988). Understanding how nucleosomes undergo transition from repressed to active transcriptional states is thus a matter of considerable interest.

The acid phosphatase *PHO5* promoter of yeast provides a tractable in vivo model of the mechanism of chromatin remodelling during gene activation at a regulated promoter (Svaren and Hörz, 1997). The repressed gene is packaged in a positioned array of nucleosomes. When the gene is activated by phosphate starvation, a transition in chromatin state precisely affects the structure of four nucleosomes, so that critical parameters of the transition from closed to open chromatin can be identified. The

transcription factor Pho4 binds to the chromatin at an early step in the chromatin remodelling process. Auxiliary factors, such as ATP-dependent remodelling by the SWI/SNF complex (see Cairns, Kingston, this volume) or the enzymatic activity of a complex with Gcn5 HAT (Brownell *et al.*, 1996; see Allis, Workman, this volume) are not essential for the activation of the gene under normal conditions. As we show here, however, Gcn5 HAT activity becomes necessary under conditions that are submaximal for inducing gene activation and in its absence a new chromatin pattern appears that may correspond to randomized nucleosomes. Thus, Gcn5, the catalytic subunit of a HAT, directly affects the remodelling of chromatin in vivo.

Remodelling factors at the PHO5 *promoter*

Starving the yeast *S. cerevisiae* of phosphate triggers a 50-fold increase in the production of acid phosphatase, 90% of which is the product of the *PHO5* gene. The gene's promoter contains two upstream activator sequence elements (UASp1 and UASp2) that are critical for its activation (*Fig. 35*). They are bound by Pho4, a basic helix–loop–helix (bHLH) transactivator (Svaren *et al.*, 1994), an interaction facilitated by Pho2, a homeobox DNA-binding protein that cooperatively binds with Pho4 at Pho4-binding sites (Barbaric *et al.*, 1998).

Under repressive, i.e., high phosphate, conditions, two negative regulators, Pho80 and Pho85, form a kinase complex that maintains Pho4 in a phosphorylated state, preventing its location in the nucleus (Hirst *et al.*, 1994; O'Neill *et al.*, 1996). Therefore, phosphate starvation or the deletion of the genes encoding these negative regulators can activate the *PHO5* promoter. Without the negative regulators, the promoter is submaximally activated, at ~30–50% of the level found in starvation conditions. Activation of the promoter leads to a chromatin transition in which four nucleosomes are disrupted and the entire promoter becomes accessible (*Fig. 35*). This is a dynamic

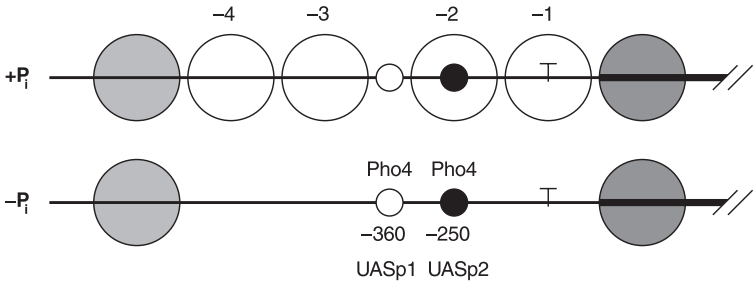


Figure 35. *Chromatin transition at the* PHO5 *promoter when the gene is activated by phosphate starvation ($-P_i$) or disruption of the genes* PHO80 *or* PHO85*, which encode negative regulators. In the repressed state ($+P_i$), the promoter is organized in an array of positioned nucleosomes. Four nucleosomes, –1 to –4, are disrupted on activation and the entire promoter becomes accessible. Upstream activator sequences UASp1 and UASp2 are Pho4 binding sites. T, site of TATA box; –360 and –250, distance in bp from the coding sequence; shaded circles, nucleosomes that persist when* PHO5 *is activated. Modified from Gaudreau* et al.*, 1997.*

process that does not require replication (Schmid *et al.*, 1992) or transcription (Fascher *et al.*, 1993).

Two lines of evidence indicated that histone acetylation states could influence the activation of the *PHO5* promoter: the promoter is sensitive to the mutation of the potentially acetylated residues of histone H4; and strains cannot reach maximal activation or repression after deletion of the gene encoding Rpd3, the human homologue of which has a histone deacetylase activity (Taunton *et al.*, 1996). A direct test of the role of histone acetylation in the control of *PHO5* promoter states ideally requires the manipulation of a specific enzyme involved in histone acetylation.

The discovery and characterization of a nuclear HAT activity in *Tetrahymena thermophila* that has striking similarity to the yeast Gcn5 transcriptional coactivator (Brownell *et al.*, 1996) provided the jumping-off point. The effect of deleting *GCN5* on the activation of the *PHO5* promoter was examined by creating yeast strains with the *GCN5* gene disrupted and assaying the activation of the promoter by measuring acid phosphatase activity. *PHO5* was induced either by phosphate starvation or the use

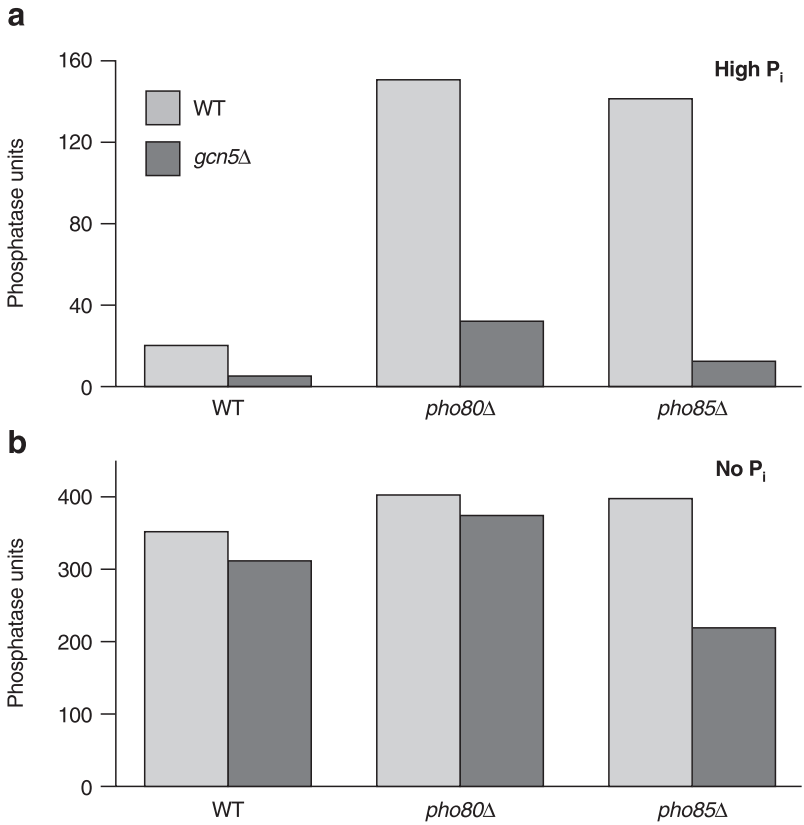


Figure 36. *Disrupting* GCN5 *diminishes the activity of the* PHO5 *promoter.* ***a****, in an otherwise wildtype (WT) strain, activity under repressing conditions (high* P_i*) drops even further in the absence of Gcn5 (strain* gcn5Δ*; dark bars).* ***b****, under fully inducing conditions, when no phosphate is present (no* P_i*),* GCN5 *disruption has only a small effect. In the absence of the negative regulators Pho80 (strain* pho80Δ*) and Pho85 (strain* pho85Δ*),* PHO5 *activity is about 30% of the fully induced level. Reproduced with permission from Gregory* et al.*, 1998.*

of strains with either of the negative regulators, *PHO80* or *PHO85*, disrupted. Under repressive conditions, i.e., high phosphate (*Fig. 36a*), Gcn5 was essential for the constitutive phosphatase activity in strains without either *PHO80* or *PHO85*. This is only 30% of the maximal level obtained in the fully inducing

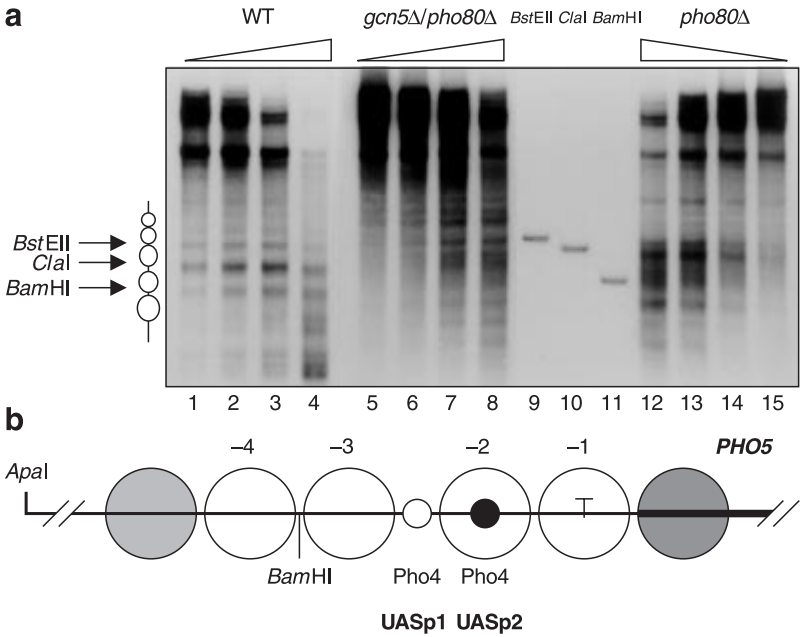


Figure 37. *Chromatin analysis of the* PHO5 *promoter.* ***a****, lanes 1–4, standard wildtype repressed pattern (see* Fig. 35 *top). Lanes 12–15, pattern of the active promoter with the four disrupted nucleosomes (see* Fig. 35 *bottom), here as a consequence of a* PHO80 *disruption. Lanes 5–8, a new pattern appears when Gcn5 is absent with* PHO80 *disrupted, i.e., in a* gcn5Δ/pho80Δ *strain, and the phosphate level is high, the conditions under which activity drops significantly because of lack of Gcn5 (see* Fig. 36a*). The new pattern differs from both the closed and the open pattern and consists of randomly positioned nucleosomes across the promoter. Lanes 9–11, digests with restriction enzymes as markers.* ***b****, the chromatin organization of the repressed* PHO5 *promoter. The open circles are removed under wildtype conditions. T, TATA box. Reproduced with permission from Gregory* et al.*, 1998.*

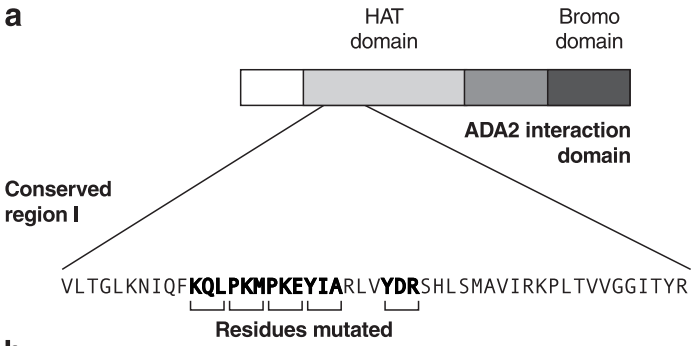
conditions of phosphate starvation (*Fig. 36b*), when the dependence of the *PHO5* promoter on Gcn5 could not be detected. The critical role of Gcn5 for the activation of the *PHO5* promoter under fully inducing conditions was demonstrated using another approach: by weakening the promoter through mutation of either of its UAS elements in *lacZ*-reporter plasmids driven by *PHO5* promoter variants (Barbaric *et al.*, 1998).

The nucleosomal organization of the *PHO5* promoter was determined by DNase I analysis in the presence and absence of Gcn5 (*Fig. 37*). The wildtype strain produced a standard repressed pattern with discrete bands, revealing a positioned array of nucleosomes (*Fig. 37a*, lanes 1–4). Disruption of *PHO80* resulted in a classic pattern of the active promoter with four disrupted nucleosomes (*Fig. 37a*, lanes 12–15). In the absence of Gcn5 together with *PHO80* disruption, a new pattern appeared that differed from both the closed and open pattern (*Fig. 37a*, lanes 5–8); it can be interpreted as a result of randomly positioned nucleosomes across the promoter (*Fig. 37b*).

Under repressive high-phosphate conditions in a strain lacking both *GCN5* and *PHO80*, this new chromatin structure had exceptionally low accessibility to all restriction enzymes tested, an increased protection that is consistent with the loss of nucleosomal positioning. The discovery of this type of nucleosomal organization indicates that Gcn5 may be involved in the modulation of chromatin in vivo. A similar pattern at the *PHO5* promoter for transitions between transcriptional states independent of DNA replication has previously been reported (Schmid *et al.*, 1992), so the new pattern may result from a rapid equilibration between the open and the closed state.

Nucleosomal organization requires Gcn5 HAT activity

The effects on chromatin and transcriptional activity observed in the *GCN5*-deleted strains are attributable solely to the loss of the HAT activity. Point mutations in Gcn5 that abolish HAT activity had the same effect on *PHO5* promoter activity as loss of the entire protein and were sufficient to generate the new chromatin structure at the *PHO5* promoter (*Fig. 38*). Thus, the new pattern at the *PHO5* promoter must result from histones with a decreased acetylation level (L. Wang *et al.*, 1998). This observation supports the connection between HAT activity and the activation of the *PHO5* promoter.



b

Mutant	HAT activity (%)	Acid phosphatase activity	
		High P_i	No P_i
Gcn5	100	154	590
gcn5Δ	22	39	451
KQL	23	35	448
PKM	19	50	571
PKE	114	150	489
YIA	62	81	557
YDR	100	141	497

***Figure 38**. Point mutations in Gcn5 that abolish HAT activity produce the same pattern of chromatin at the* PHO5 *promoter as loss of the entire Gcn5 protein, implying that this pattern is due to histones with a decreased level of acetylation. **a**, diagram of yeast Gcn5, indicating the domains of the protein and conserved region I within the HAT domain which contains residues critical for HAT activity. The substitution mutants, named according to groups of amino acids mutated to alanine, are shown in brackets. **b**, the HAT activity of each mutant tested is reported as a percentage of wild-type (Gcn5) and the* PHO5 *promoter activity in these mutants is shown in units of acid phosphatase activity. Reproduced with permission from Gregory* et al.*, 1998.*

Comparison of other genes in the phosphatase gene family in terms of their dependence on Gcn5 will be interesting. *PHO10* and *PHO11*, located at the telomere, also code for secreted acid

phosphatases and are regulated by phosphate depletion in much the same way as *PHO5*. However, the fully activated levels of expression of these two genes are considerably lower than that of *PHO5*. An alkaline phosphatase gene, *PHO8*, is also regulated by Pho4 and undergoes a chromatin transition at the promoter (Barbaric *et al*., 1992). However, chromatin opening is only partial and some nucleosome-mediated protection persists even when the gene is fully activated.

The requirement for Gcn5 HAT activity in the remodelling of chromatin and transcriptional activation in vivo is well supported by these studies using the *PHO5* promoter. Further work is needed to analyse whether the effect is mediated through a specific targeting of acetylation within the promoter region. Because Gcn5 is part of several complexes (see Allis, this volume), it will be critical to establish whether mutations affecting its partners in these complexes also result in a similar chromatin phenotype at the *PHO5* promoter. This model yeast promoter promises more useful information on the importance of various chromatin remodelling machines in vivo, particularly as genetic manipulation is possible.

PART II

FACTORS REQUIRED FOR ACTIVATED TRANSCRIPTION

Introduction

Stefan Björklund

Gene transcription first requires that the DNA template, packaged into chromatin, becomes accessible for the binding of transcriptional activators and repressors at their cognate sites in the promoter and for the formation of a preinitiation complex at the transcription start site (see Part I). The following articles focus on subsequent steps in the process leading to synthesis of a mature mRNA: the communication between regulatory proteins and the general transcription factors, mainly for the transcription of protein-encoding genes by RNA polymerase II.

Eukaryotic RNA polymerase II by itself is unable to recognize a promoter. However, correct initiation and transcription can be reconstituted in vitro from homogenous RNA polymerase II and five highly purified factors: TBP, transcription factors IIB (TFIIB), TFIIE, TFIIF and TFIIH. These are highly conserved from yeast to mammalian cells. It is generally believed that they assemble on the promoter to form the preinitiation complex in a stepwise mode initiated by the binding of TBP to the conserved TATA sequence that lies 20–30 nucleotides upstream from the transcription start site of most genes in eukaryotes (see *Box 1*).

Since the identification of the general transcription factors, a prime interest has been to understand how they interact and what their functions in the transcription process are. The first insight came from the determination of the atomic structure of TBP alone and complexed with TFIIB bound to the TATA element (reviewed in Burley, 1996). Recently published data complement and extend this structure (Fu *et al.*, 1999; Poglitsch *et*

al., 1999). Combining the known structure of DNA–TBP–TFIIB with this new structure of RNA polymerase II in a complex with TFIIB shows that the DNA is located in the active centre channel of the polymerase by the interactions between the general transcription factors, enabling initiation about 30 bp downstream from the TATA box.

With the development of in vitro transcription systems reconstituted from recombinant or highly purified general transcription factors, it became clear that intermediary factors were needed to transduce signals from DNA-bound transcriptional regulatory proteins, i.e., activators and repressors, to the basal transcription machinery. These intermediary factors have been given different names, such as coactivators, adaptors or mediators. Here they are termed global transcription regulators to emphasize their general importance for regulated transcription of most genes as opposed to transcriptional activators or repressors that exert their effect locally at certain promoters through sequence-specific interaction with DNA.

Several global transcription regulators such as TAF_{II}s, Mediator and upstream stimulatory activity (USA) were originally identified as important components required for this process. Here, Roger Kornberg describes the yeast Mediator and identifies a specific Mediator domain that is essential for activation by Gal4 and Gcn4. The generality of the Mediator complex has been questioned because it was first identified only in yeast cells. Mediator-like complexes have now been identified in mammalian cells as essential for transcriptional regulation by such a broad spectrum of transcriptional regulators as the thyroid hormone receptor (TRAP; see Roeder, this volume), Sp1 (cofactor required for Sp1 activation (CRSP); see Tjian, this volume), the vitamin D receptor (DRIP; Rachez *et al.*, 1999), the adenovirus E1A protein (mammalian Mediator, Boyer *et al.*, 1999; mouse Mediator, see Kornberg, this volume) and SREBP (ARC, see Tjian, this volume). The occurrence and gen-

eral importance of Mediator in mammalian cells is reported by Kornberg, Robert Tjian and Robert Roeder. Hiroshi Sakurai describes the domains in TFIIE and Gal11, a subunit of the yeast Mediator, that are essential for their mutual interaction and also shows that Gal11 and TFIIE act cooperatively to stimulate phosphorylation of the RNA polymerase II CTD by the kinase activity of TFIIH.

The USA was originally identified as a partially purified fraction from human cells that acts synergistically with TAF_{II}s in activated transcription in vitro. Several positive and negative cofactors have subsequently been derived from the USA fraction and Michael Meisterernst summarizes recent results on the positive cofactors PC2, PC4, PC6 and the negative cofactor NC2.

Recent results obtained in vivo have indicated that the TAF_{II} complex does not play as general a role in transcriptional activation as was originally proposed (Moqtaderi *et al.*, 1996*a*; Walker *et al.*, 1996). However, some TAF_{II}s seem to have a rather general effect whereas others act more specifically on a small set of promoters. This might be because the former TAF_{II}s, in addition to being components of TFIID, are also found as subunits of other transcription factors with global effects, i.e., PCAF, TBP-free TAF-containing complex (TFTC) and SAGA. Kevin Struhl and Michael Green report on studies on the function of TAF_{II}s at specific promoters in vivo, using the microarray technique for whole-genome transcription analyses. Their studies, in combination with results obtained by Rick Young and co-workers (Holstege *et al.*, 1998), are starting to reveal this fuller picture of TAF_{II} function in transcription. Làszlò Tora reports on the first studies of the in vivo function of a TAF_{II} subunit in mammalian cells, which reveal that human $TAF_{II}30$ is required for cell-cycle progression and for parietal endodermal differentiation.

The general transcription factor TFIIH comprises nine subunits and contains the kinase activity responsible for phosphorylation of the C-terminal repeat domain (CTD; see *Glossary*) of

the largest subunit of RNA polymerase II (Feaver *et al.*, 1994*b*; Roy *et al.*, 1994). Mutations in human TFIIH subunits are associated with the genetic disorders xeroderma pigmentosum, Cockayne's syndrome and trichothiodystrophy. Jean-Marc Egly discusses how the study of these mutations is helping to define a role for TFIIH in transcription, DNA repair and cell-cycle regulation.

Once transcription is initiated, mRNA synthesis proceeds through several steps: synthesis of the first phosphodiester bond, promoter clearance, elongation and termination. Elongation is the only step considered here: Jesper Svejstrup discusses the composition of an elongating form of RNA polymerase II and implicates a chromatin remodelling activity as important for its function. To close, André Sentenac compares RNA polymerase III with polymerase II. Recruitment of polymerase III to a promoter involves a cascade of protein–protein interactions in which TFIIIC acts both as enhancer and promoter-binding factor to overcome nucleosomal repression, perhaps through an intrinsic HAT activity. The similarity of the polymerase II and III systems is emphasized by the involvement of PC4 and topoisomerase I, two known polymerase II coactivators, in mammalian TFIIIC function.

The Mediator complex and in vitro transcriptional activation in yeast

Roger D. Kornberg

in collaboration with

Lawrence C. Myers and Claes M. Gustafsson

The view of how transcriptional activator proteins transmit their signals to the basal transcription machinery has recently under-

gone a dramatic change. Most activators have commonly been thought to contact specific TAFs in the TFIID complex but several experiments have cast doubt on this. First, the Mediator complex has been shown to be necessary and sufficient for regulated transcription in the absence of TAFs (Oelgeschläger *et al.*, 1998). Second, in vivo studies indicate that yeast TAFs are important for transcription of a very limited set of genes (Moqtaderi *et al.*, 1996*a*; Walker *et al.*, 1996). Third, large protein complexes comprising subunits with high homology to the yeast Mediator subunits have recently been found in higher eukaryotes (see Roeder, Tjian, this volume). Lastly, determination of the structures of the yeast and mouse complexes separately or in complex with RNA polymerase II reveals striking similarities (Asturias *et al.*, 1999), so they are likely to be functional counterparts. Thus Mediator-like complexes seem to be a ubiquitous part of the transcriptional activating mechanism and may take the role, previously ascribed to TAFs, of global coactivators.

We describe the identity of the subunits of the purified yeast Mediator transcription complex and their various roles, including interaction with the CTD of RNA polymerase II, and show that some subunits are essential for normal transcriptional function. We have also identified a mammalian homologue of the yeast complex. We conclude that the Mediator complex is obligatory for regulated transcription in vivo and that the individual subunits determine the responses to different transcriptional regulatory proteins.

The yeast Mediator complex

Mediator was originally found in a crude protein fraction isolated from *S. cerevisiae* that could relieve activator inhibition of transcription, known as squelching, in an in vitro transcription system based on a nuclear extract (Kelleher *et al.*, 1990). A requirement for the Mediator fraction in the activation of

transcription was demonstrated using an in vitro transcription system composed of semipurified general transcription factors (Flanagan *et al.,* 1991). About the same time, the *SRB* genes were identified as suppressors of the cold-sensitive phenotype in yeast mutants that had the CTD of the largest RNA polymerase II subunit shortened from 26 to 11 heptapeptide repeats; several of the Srb proteins were subsequently found to associate with RNA polymerase II in a large multiprotein complex (Thompson *et al.,* 1993). Purification of Mediator to homogeneity revealed a complex of about 20 polypeptides, including Srbs 2 and 4–7 (Kim *et al.,* 1994; see *Table 4*).

Purified Mediator has three biochemical activities in a fully reconstituted in vitro transcription system: it stimulates basal transcription, i.e., transcription in the absence of activators, about tenfold; it enables stimulation by transcriptional activation; and it stimulates phosphorylation of the CTD. That it forms a stable complex with the CTD of RNA polymerase was established by three independent criteria. First, the Mediator can be purified to homogeneity either as a separate entity by displacement or by using monoclonal antibodies specific for the CTD or as a complex with the polymerase. Second, several polypeptides in the Mediator were originally identified as CTD-interacting proteins (Srbs) by genetic means. Third, the phosphorylation of

Table 4*. Mediator subunits divided into three groups according to their proposed functions determined from genetic and biochemical experiments.*

CTD binding	Repression	Activation
Srb2	Gal11	Med1
Srb4	**Rgr1**	Med2
Srb5	Sin4	Med4
Srb6	Pgd1	**Med6**
Srb7	Rox3	**Med7**
	Nut1	Med8
	Nut2	Med11
	Cse2	

Bold type, yeast proteins for which mammalian homologues have been identified.

the CTD of the holopolymerase by the TFIIH kinase is ~50-fold higher than that for the core polymerase (see Sakurai, this volume).

All 20 subunits of the Mediator complex have now been identified by a combination of immunoprecipitation, amino-acid sequencing and western blotting (Myers *et al.*, 1998). They have been divided into three subgroups based on structural/functional studies and physical properties (*Table 4*). The first is composed of the Srb proteins that are thought to interact with the CTD. The second consists of basic proteins encoded by genes that repress transcription. The third group consists of acidic proteins, now termed Med, encoded by previously unidentified genes. About half the subunits of the yeast Mediator complex are encoded by genes essential for yeast viability. Mammalian homologues of five subunits have been identified (*Table 4*).

The role of Med proteins in transcriptional activation has been analysed using Mediator complex isolated from yeast mutant strains lacking individual subunits (Y. Li *et al.*, 1995; Myers *et al.*, 1998). The pattern of activation by these different Mediator mutants correlates to their subunit composition.

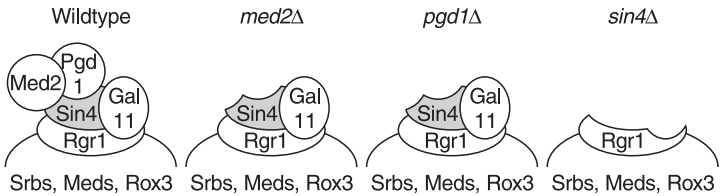


Figure 39. *Structure–function relationship of wildtype and mutant Mediator–RNA polymerase II complexes. The subunit organization of the Sin4/Rgr1 module of Mediator is based on the results shown in* Table 5 *and in the text. This model, however, does not preclude the existence of weak interactions among Med2, Pgd1, Sin4 and other subunits that do not withstand the rigours of purification.* Δ*, strains with particular genes deleted.*

Table 5*. Functional analysis of wildtype and mutant Mediator complexes in a purified yeast in vitro transcription system. Subunit organization is shown in* Fig. 39*. Fold activation by transcriptional activators VP16 and Gcn4 is shown as the ratio of full-length transcripts in the presence of activator from a template bearing the appropriate activator-binding sequence, to transcripts in the absence of the activator. This ratio was normalized by division by the ratio obtained from a second template lacking the appropriate activator-binding sequence.*

	Activation ratio			
	Wildtype	***med2*Δ**	***pgd1*Δ**	***sin4*Δ**
VP16 activation	30	1.7	1.8	1.1
Gcn4 activation	8.2	6.4	6.9	1.1

Mediator from either the *med2* or *pgd1* deletion strain lacks both the Med2 and Pgd1 proteins, whereas Mediator isolated from the *sin4* deletion strain lacks Sin4, Med2, Pgd1 and also Gal11 (*Fig. 39*; see Sakurai, this volume). Purified Mediator complexes lacking the Med2, Pgd1 or Sin4 subunits were unable to support transcriptional activation by the acidic activator protein Gal4–VP16 in vitro (*Table 5*). In contrast, only Mediator lacking Sin4 was defective for activation by another acidic transcriptional activator, Gcn4.

Because the mutant Mediator complexes from *med2* or *pgd1* deletion strains have no defects in stimulating either the basal activity or CTD phosphorylation, the separate subdomains that compose Mediator must have different functions. The electron-microscopical structure of the Mediator complex purified from the *sin4* deletion strain supports a modular structure because one of the three Mediator subdomains is lacking.

The involvement of the Mediator in transcriptional regulation in vivo has been demonstrated using the *med2* deletion strain. This strain is unable to activate a reporter gene driven by a promoter containing binding sites for Gal4 under conditions where the same reporter construct is fully activated in the wildtype strain (*Table 6*). Furthermore, microarray analysis of global gene expression shows that deletion of *med2* affects the expression of

more than this one gene: supporting the results obtained in vitro, cells lacking Med2 are impaired in expression of several of the *GAL* genes required for galactose metabolism. However, *SRB4* deletions had a much more general effect on gene expression, in line with the observation that *SRB4* is an essential gene whereas *MED2* is not. This provides further support for the idea that, although the Mediator complex is obligatory for regulated transcription in vivo, the individual subunits determine the responses to different transcriptional regulatory proteins.

Table 6. *Med2 is required for activation by Gal4–VP16 both in vitro and in vivo. Levels of β-galactosidase activity in vivo were assayed in strains with a plasmid containing Gal4–VP16 under control of a promoter and the* lacZ*-reporter plasmid. The units of activity are normalized to cell absorbance at 600 nm and are the means from at least three replicate assays. For detailed results obtained in vitro, see* Table 5.

	Fold activation	
	Wildtype	***med2*Δ**
In vitro	30	1.7
In vivo	1,156	138

The mammalian Mediator complex

Our identification of a mammalian Mediator complex (Jiang *et al.,* 1998) supports the contention that the Mediator is generally part of the transcriptional activation machinery. The mouse complex was reveated by chromatographic coelution of the mammalian counterparts of the Med7 and Srb7 proteins. The purified complex also contained the mammalian Rgr1 and Med6 proteins and eight other subunits. During purification, the Med7 and Srb7 proteins eluted in different side fractions. Although these fractions have not been purified further because of technical difficulties, their existence indicates there may be several Mediator-like complexes in higher eukaryotes (see also Roeder, Tjian, this volume).

Amino-acid sequencing of the additional subunits did not reveal any other homologues to yeast Mediator subunits, casting

doubt on whether the purified complex is a true functional counterpart to the yeast Mediator. The most compelling evidence for the homology comes from the similar shape and overall size of the two complexes at ~40 Å resolution, determined by electron microscopy. When bound to the corresponding RNA polymerase II, both complexes seem to be divided into three subdomains. Moreover, addition of the core RNA polymerase induces a similar conformational change in both complexes, the Mediator adopting a crescent-like structure that covers much of the polymerase.

Regulation of transcription through general and gene-specific coactivators

Robert G. Roeder

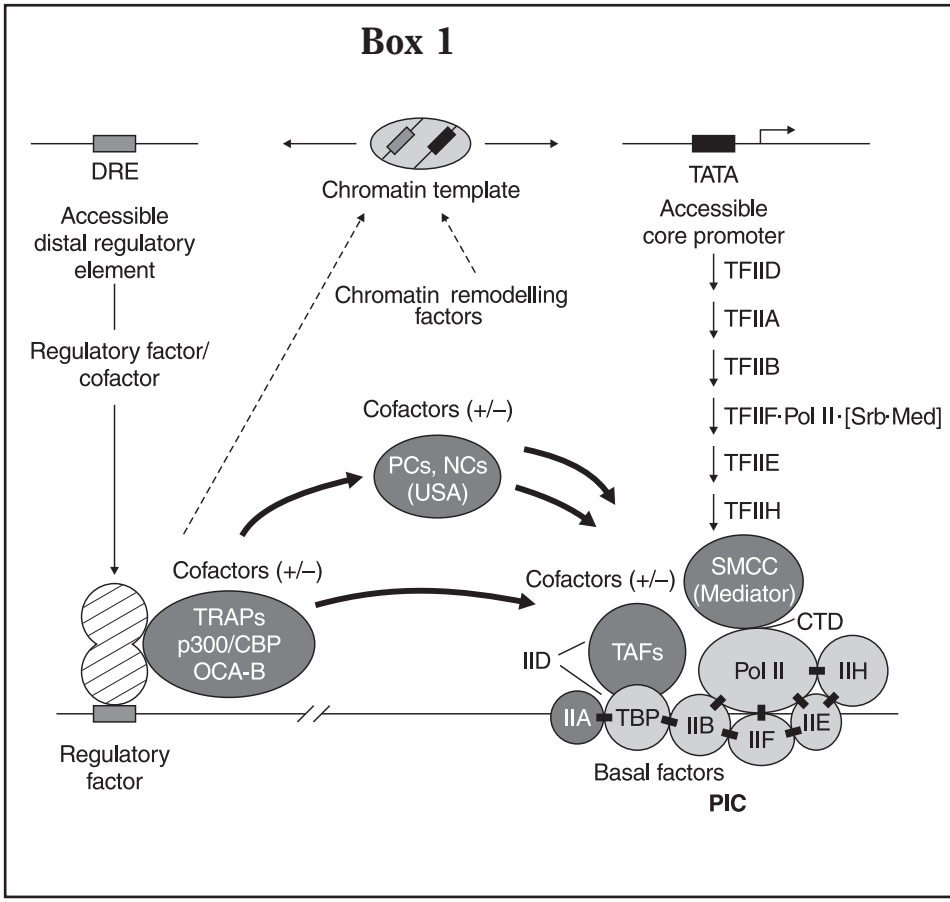
Biochemical analyses in cell-free systems from mammalian cells have shown that the regulation of transcription by RNA polymerase II involves concerted interactions of gene-specific regulatory factors that bind to distal control elements, general initiation factors that act through common core promoter elements to form a preinitiation complex, and a large variety of cofactors that mediate functional interactions between these components (summarized in *Box 1*; see Roeder, 1998). The TAF_{II} class of coactivators, which are TBP-associated subunits of TFIID, and the USA-derived positive cofactors seem indispensable for significant activation in transcription systems reconstituted with purified factors. However, TBP alone can fully support both basal and activator-mediated transcription when added to a TFIID-depleted HeLa cell nuclear extract (Oelgeschläger *et al.*, 1998), indicating that other coactivators present in the crude extract, but not in the reconstituted systems, can substitute for TAF_{II} function in activation. I describe here a candidate for all or part of this alternative coactivator activity, a Srb- and Med-

containing cofactor complex (SMCC) that contains human homologues of a small subset of proteins found in the yeast Mediator (Gu *et al.*, 1999). SMCC functions either to repress or to enhance activation in purified reconstituted systems, depending on the TFIIH concentration, and acts synergistically with the USA-derived positive cofactors.

Studies using the nuclear receptor for thyroid hormone show that multi-subunit coactivator complexes can also be stably associated with the activators themselves (Fondell *et al.*, 1996). A high M_r complex of thyroid hormone receptor-associated proteins (TRAPs) specifically associates (through the TRAP220 subunit) with the liganded thyroid hormone receptor in HeLa cells. The thyroid hormone receptor–TRAP complex mediates transcriptional activation from DNA templates in a purified reconstituted system with a dependency upon general positive cofactors but not upon TAF_{II}s (Fondell *et al.*, 1999). On the basis of these observations, and other reports of nuclear receptor coactivators containing histone acetyltransferase activities (Torchia *et al.*, 1998), I propose a multistep model for transcriptional activation by nuclear receptors. Moreover, as TRAP and mammalian SMCC complexes have proved to be virtually identical in structure and function (Ito *et al.*, 1999), a pleasing unification of various mammalian and yeast coactivator studies is emerging.

TAF_{II}-dependent and -independent activation in vitro

Many experiments in metazoan transcription systems reconstituted with highly purified natural and/or recombinant general transcription factors and a diverse series of activators have shown that TAF_{II}s are absolutely required for efficient activator function, as evidenced by activation with TFIID but not with TBP (Burley and Roeder, 1996). In such systems TAF_{II}s function synergistically with other coactivators present in the partially purified USA fraction (*Fig. 40*; Meisterernst *et al.*, 1991; Chiang



et al., 1993). USA-derived positive cofactors that act synergistically with TAFs include the M_r 15K positive cofactor 4 (PC4; Malik *et al.*, 1998) and the M_r 500K PC2 complex (Kretzschmar *et al.*, 1994; see Meisterernst, this volume). In the case of PC4, the coactivator has been shown to interact both with activators and with components of the general transcriptional machinery, e.g., TFIIA and RNA polymerase II, thus serving an adaptor function (Ge and Roeder, 1994; Malik *et al.*, 1998). The ability of TAF_{II}s to act as coactivators has been ascribed to their

A comprehensive summary of the stepwise assembly pathway for a preinitiation complex (PIC). The scheme shows general transcriptional factors (light grey) on a simple TATA-containing class II core promoter and three classes of cofactors (dark grey) that variously modulate the functions of DNA-binding regulatory factors (hatched) on corresponding target genes. Interactions between specific basal factors are indicated by short solid bars. The pathway, established using purified factors, begins with recognition of the TATA-element by the TBP component of TFIID. The assembly and/or function of the PIC may be regulated either by direct interactions between PIC components and DNA-bound regulatory factors; or by direct interactions between PIC components and certain cofactors, e.g., NC2, acting alone; or by direct interactions between PIC components and cofactors that are recruited to the promoter by interactions with DNA-bound regulatory factors, in which case the cofactors act as adaptors transmitting signals from DNA-bound regulatory factors to general factors. In mammals, the cofactors include a group associated with the basal transcriptional machinery: TFIIA, the TAF_{II} components of TFIID and the Srb- and Med-containing cofactor complex (SMCC or Mediator) that may associate with RNA polymerase II (Pol II); a group associated with gene-specific regulatory factors, including OCA-B, p300/CBP and TRAPs and others not indicated; and a miscellaneous group of potentially more general positive and negative cofactors (PCs and NCs). The three groups are not necessarily mutually exclusive and include both positively and negatively acting cofactors (see this article). These cofactors also include targeted histone acetyltransferases, e.g., p300/CBP, and deacetylases that, along with distinct ATP-dependent factors such as NURF and SWI/SNF complexes, are involved in chromatin remodelling (dashed arrows) before or concomitant with regulatory factor binding and PIC assembly (for review, see Roeder, 1998). Reprinted with permission from Roeder, 1998.

R.G. Roeder

specific interactions with the activation domains of many transcriptional activators (Verrijzer and Tjian, 1996). However, genetic experiments unexpectedly indicated that several TAF_{II}s were not generally required for transcriptional activation in yeast (Moqtaderi *et al.*, 1996; Walker *et al.*, 1996), leading to the proposal that other factors, such as the Mediator complex (see Kornberg, this volume), could provide necessary and sufficient coactivator functions in cells, as well as alternative activation pathways.

The above observations prompted a re-evaluation of mam-

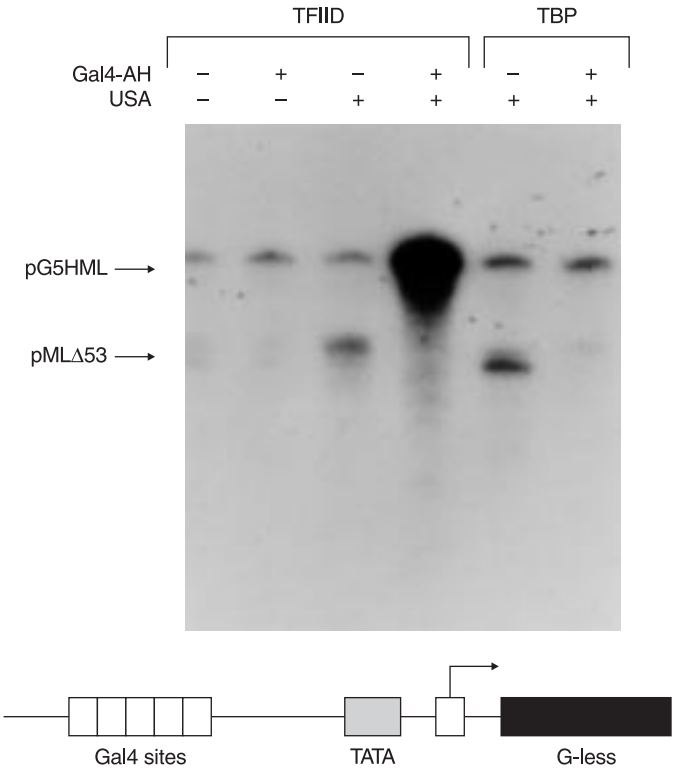


Figure 40. *Optimal activator function in a cell-free system reconstituted with purified general initiation factors requires both TAF_{II} components of TFIID and a positive cofactor(s) in USA. In vitro transcription from an HIV-1 TATA-containing core promoter with five Gal4 sites (pG5HML) and the adenovirus major late core promoter (pMLΔ53) was reconstituted with recombinant or partially purified general initiation factors, including either affinity purified TFIID or recombinant TBP in the presence (+) or absence (–) of Gal4-AH and/or a four-column USA fraction (Ge and Roeder, 1994). Modified from Meisterernst* et al.*, 1991 and Chiang* et al.*, 1993.*

malian TAF_{II} function in cell-free systems containing a more physiological complement of nuclear proteins. HeLa cell nuclear extracts were depleted of TFIID using antibodies directed against TBP and $TAF_{II}100$ (Oelgeschläger *et al.*, 1998). The resulting extracts were almost completely free of TBP and the

TFIID-specific TAF_{II}s, such as $TAF_{II}250$, $TAF_{II}135$ and $TAF_{II}55$, whereas a large fraction of $TAF_{II}31$ and TAF_{II} 20/15, which are also present in the PCAF/Gcn5 and TFTC complexes (see Tora, this volume), remained in the extract (*Fig. 41a*). The extracts lacked detectable transcriptional activity both in the presence and absence of activators. However, basal transcription and activation by both the acidic activation domain (AAD) of the viral activator VP16 and the proline-rich activation domain of the mammalian activator CTF-1, assayed as Gal4 fusion proteins, could be fully restored not only by addition of immunopurified TFIID but also by an equimolar amount of recombinant TBP alone (*Fig. 41b*; Oelgeschlager *et al.*, 1998). Thus, in a crude extract with a more natural complement of nuclear factors, TAF_{II}-independent transcription can be observed with at least some activators. Interestingly, however, the addition of TBP alone does not restore activation by the glutamine-rich Sp1 activator, indicating that Sp1 has a more rigorous requirement for TAF_{II}s (M. Guermah and R.G. Roeder, unpublished observations).

Mammalian SMCC modulates transcriptional activation in vitro

If the TAF_{II} components of TFIID are not always required for activation in nuclear extracts, what are the necessary cofactors? In yeast, the Mediator complex is essential for activation (reviewed in Myer and Young, 1998; see Kornberg, this volume). Mammalian homologues of several yeast Mediator subunits, including Srb7, Srb10 and Srb11, have been identified (see *Table 4*). In contrast to immunodepletion of TFIID-specific TAF_{II}s, partial immunodepletion of Srb7 from HeLa cell extracts results in a reduction of activation by the VP16 AAD (Oelgeschläger *et al.*, 1998). The high degree of conservation of several Srb proteins from yeast to mammals, as well as indications of Srb proteins in mammalian holoenzyme preparations (reviewed in Parvin and Young, 1998), indicated the existence of a Mediator-like complex in mammalian cells. To identify such a

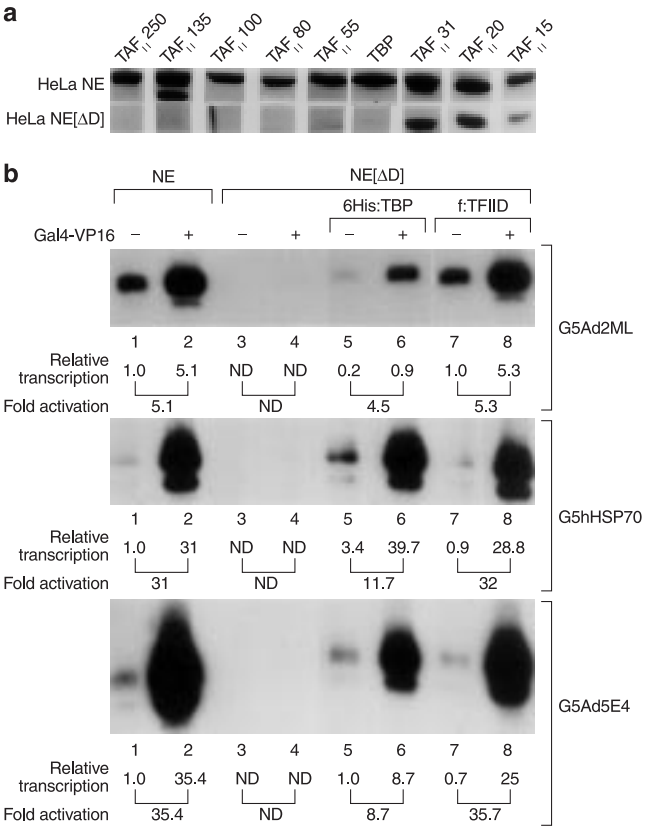


Figure 41. *TAF_{II}-independent transcriptional activation by Gal4-VP16 in HeLa nuclear extract.* ***a****, immunoblot analysis of untreated HeLa nuclear extract (HeLa NE) and HeLa nuclear extract depleted of TFIID (NE[ΔD]) to show that the extracts were almost completely free of the TFIID-specific TAF_{II}s but contained a large fraction of TAF_{II}31 and TAF_{II}20/15, which are also present in the PCAF/Gcn5 and TFTC complexes (see Tora, this volume).* ***b****, activation of transcription from the Ad2ML, human HSP70 and Ad5E4 core promoters, which contain upstream Gal4 sites, by Gal4–VP16. Basal transcription and activation, as observed in untreated HeLa nuclear extract (NE; lanes 1 and 2), can be restored to TFIID-deficient and transcriptionally inactive HeLa nuclear extract (NE[ΔD]; lanes 3–8) supplemented with either 6His:TBP (lanes 5 and 6) or epitope-tagged TFIID (f:TFIID; lanes 7 and 8). ND, not detectable. Reprinted with permission from Oelgeschläger* et al.*, 1998.*

complex, stably transformed HeLa cell lines expressing epitope-tagged versions of either Srb7, Srb10 or Srb11 were established. All three Srb proteins copurified from these cell lines irrespective of which were tagged, indicating that these proteins exist in a common complex. Gel filtration of cellular extracts showed the presence of the Srb proteins in either a low M_r complex or a second much higher M_r complex of around 1,500K (Gu *et al.*, 1999).

Immunoblot analysis showed that the high M_r Srb-containing complex, designated SMCC, did not contain basal factors, CBP, BRCA-1 or SWI/SNF proteins, all of which had previously been suggested to be components of a mammalian RNA polymerase II holoenzyme. However, SMCC did contain, in addition to the three SRB proteins, homologues of the yeast regulatory proteins Med6, Med7, Rgr1, Nut2 and Soh1, as well as a protein of M_r 220K (TRAP220) that is also present in the TRAP (see below) and CRSP (see Tjian, this volume) complexes (Gu *et al.*, 1999). Several of the SMCC components, Med6, Med7 and Rgr1, were also found in a human complex (NAT) that represses activated transcription (Sun *et al.*, 1998) and in a murine Mediator complex (Jiang *et al.*, 1998).

Homologues of several of the SMCC components were previously identified as components of the yeast Mediator complex. These include components that act either as negative or as positive regulatory factors and in either a gene-specific or a global fashion, suggesting that similar properties might pertain to the human counterparts. SOH1 is not known to be a component of the yeast mediator complex but was identified genetically as a suppressor of mutations in HPR1, a component of some preparations of the yeast polymerase II holoenzyme (reviewed in Gu *et al.*, 1999). In yeast, SOH1 also interacts genetically with two RNA polymerase II subunits and with TFIIB, raising the possibility that human SOH1 might show similar functions, and thus facilitate SMCC interactions with RNA polymerase II and TFIIB in the preinitiation complex. Despite these numerous parallels

between SMCC and yeast Mediator, mammalian RNA polymerase II was found associated with SMCC only when the immunopurification was performed at low ionic strength and no direct SMCC–RNA polymerase II interactions were observed with the respective purified proteins.

The function of SMCC was tested in an in vitro transcription system with highly purified/recombinant basal factors, PC4 and a synthetic AAD (Gu *et al.*, 1999). Addition of SMCC at high levels of TFIIH diminished activated transcription in this system, in a similar way to that observed with the NAT complex (Sun *et al.*, 1998). Activated transcription was also repressed by SMCC with an RNA polymerase II lacking the CTD of the largest subunit. Repression therefore does not involve phosphorylation of the CTD by the cyclin/kinase components, Srb10 and Srb11, of SMCC. However, SMCC does phosphorylate PC4, which inactivates its coactivator function and stimulates its single-stranded (ss)DNA-binding activity (see Meisterernst, this volume), indicating a potential mechanism by which SMCC represses activated transcription mediated by PC4.

Intrinsic SMCC coactivator function for a synthetic AAD (AH) was observed when transcription assays were performed in the reconstituted system containing basal factors and either intact (form IIA) or CTD-less (form IIB) RNA polymerase II and TFIIH either at limiting concentration or in its absence (*Fig. 42a*). Significantly, there was also a strong synergy between SMCC and PC4 under these conditions (*Fig. 42b*). SMCC-dependent activation was seen not only with the synthetic AAD, but also with the VP16 AAD and the mammalian p53 activation domain, indicating broader coactivator functions for SMCC (*Fig. 42c*). Moreover, it was possible to show a direct SMCC–p53 interaction that was abolished by a double amino-acid substitution that inactivates the p53 activation domain (Gu *et al.*, 1999). This interaction provides a mechanism by which SMCC may be recruited to function at specific promoters. It is not yet clear

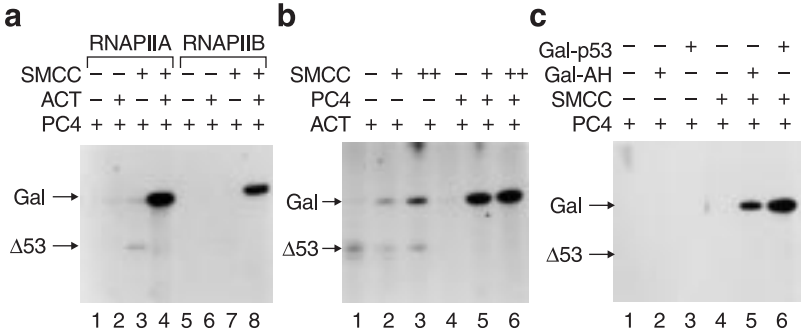


Figure 42. *Coactivator function of SMCC.* ***a****, in vitro transcription reactions were reconstituted with recombinant TFIIA, TFIIB, TFIIE and TFIIF, affinity-purified TFIID, highly purified RNA polymerase IIA (intact) or IIB (CTD-less), the pG5HML (Gal) test template and the pMLΔ53 (Δ53) reference template. PC4, Gal4–AH (ACT) and SMCC in reactions as indicated.* ***b****, coactivator assay for SMCC, indicating the synergy between SMCC and PC4. In vitro transcription reactions as in (****a****) except RNA polymerase IIA was included in all reactions and PC4 only in lanes 4–6. SMCC concentration as indicated: +, 1 µl; ++, 2.5 µl.* ***c****, in vitro transcription reactions as in (****a****) to show that SMCC mediates activation by the natural activation domain in p53, assayed as a Gal4-p53 fusion protein, as well as by Gal4-AH. All reactions contained PC4 and in lanes 4–6 contained 2.5 µl SMCC. Activators as indicated. Reprinted with permission from Gu* et al.*, 1999.*

whether SMCC can function with TBP in the absence of TAF_{II}s and therefore whether TAF_{II} and SMCC functions are fully redundant.

The TRAP complex mediates activation by nuclear receptors

The nuclear receptors for steroid and non-steroid hormones are a class of transcriptional activators, the activity of which is modulated by ligand binding. Nuclear receptors are composed of distinct functional domains involved in DNA and ligand binding. Several models for transcriptional regulation have emerged from studies of the receptors. In the absence of their cognate ligands, the receptors for all-*trans* retinoic acid and thyroid hormone can repress transcription. The unliganded retinoic acid and thyroid

hormone receptors interact with co-repressor proteins which themselves are part of larger complexes containing histone deacetylases, leading to the idea that gene repression may involve nuclear receptor-targeted local histone deacetylation and chromatin condensation (Torchia *et al.*, 1998).

On ligand binding, the conformation of the nuclear receptor ligand-binding domain changes to create a new surface formed by the juxtaposition of three α-helices. This creates a hydrophobic cleft, which allows the nuclear receptor ligand-binding domain to interact with a series of proteins that contain a short amino-acid motif, Leu-X-X-Leu-Leu (LXXLL), which docks in this cleft. Among the proteins that interact with the nuclear receptors in a ligand-dependent manner are the coactivators CBP, PCAF, TIF-2, SRC-1 and ACTR, all of which have HAT activity. These observations have led to the idea that gene activation may involve nuclear receptor-targeted local histone acetylation and chromatin decondensation (Torchia *et al.*, 1998).

Most of these nuclear receptor-interacting proteins have been identified by two-hybrid screen or direct in vitro binding of proteins to immobilized receptors. However, a new class of proteins involved in thyroid hormone receptor function has been identified using a different approach. Because the purified recombinant receptor does not activate transcription from DNA templates in vitro under conditions in which many other activators are active, a search was made for proteins that may interact with the receptor and allow it to function in vitro.

Stably transformed HeLa cell lines were established that express epitope-tagged thyroid hormone receptor. When the tagged receptor is purified from these cells after treatment with thyroid hormone, a set of at least 10 major TRAP proteins ranging in M_r from 240 to 80K is found stably associated with the receptor (Fondell *et al.*, 1996). Subsequent gel filtration showed that even in the absence of the receptor, the TRAP components form a complex of high native M_r (Yuan *et al.*,

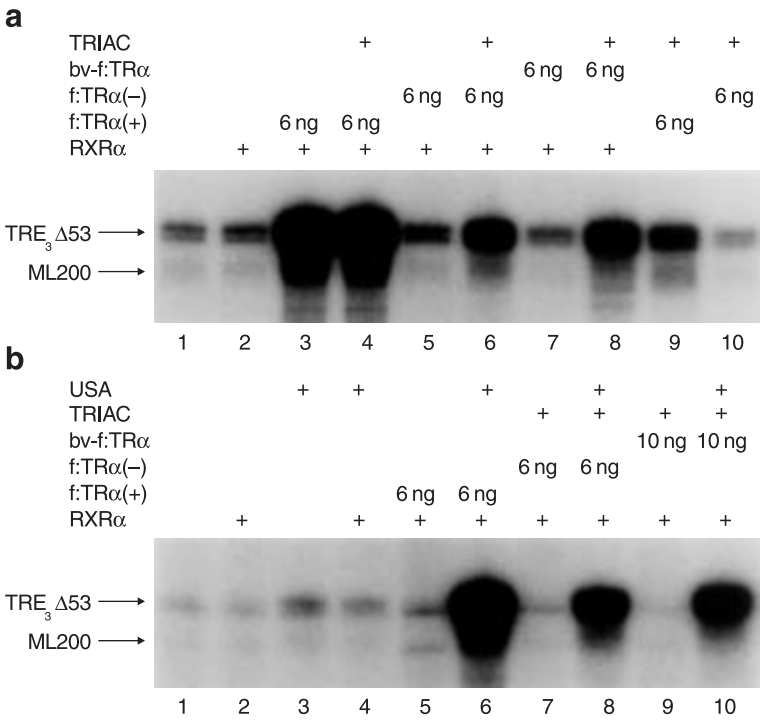


Figure 43. *Analyses of the thyroid hormone receptor (TR)–TRAP complex in a purified transcription system.* ***a****, liganded TR plus TRAPs enhance activation in vitro. Immunopurified epitope-tagged TRα (f:TRα) from cells grown in the absence (f:TRα(–)) or presence (f:TRα(+)) of thyroid hormone was added as indicated to transcription reactions containing purified basal transcription factors and RNA polymerase II. Recombinant f:TRα (bv-f:TRα), RXRα (20 ng) and the thyroid hormone TRIAC (2 μM final concentration) were added as indicated. Each reaction also contained the USA fraction (250 ng), TRE*$_3$*Δ53 promoter containing three thyroid-hormone response elements upstream of the adenovirus major late core promoter (50 ng) and ML200 reference promoter (20 ng).* ***b****, activation by liganded TR requires USA activity. In vitro transcription as described in (****a****) in the presence or absence of USA. Reprinted with permission from Fondell* et al.*, 1996.*

1998). In contrast to recombinant receptor and epitope-tagged receptor purified from cells in the absence of thyroid hormone, the receptor–TRAP complex markedly activates transcription in vitro in a retinoid X receptor (RXR)-dependent manner (*Fig. 43a*). A seemingly identical complex has also been identified through its ability to bind selectively to the liganded vitamin D3 receptor (VDR) in vitro. The complex containing VDR and VDR-interacting proteins (DRIPs) is moderately active in transcription in vitro (Rachez *et al.*, 1998).

Most importantly, the TRAP complex was found to be devoid of other known nuclear receptor coactivators, such as p300/CBP, TIF-2 and SRC-1. A further characterization of TRAP components, including cognate cDNA cloning, revealed many with LXXLL motifs. However, only the TRAP220 component, containing two LXXLL motifs, was found to interact significantly with thyroid hormone receptor in a ligand-dependent manner (Yuan *et al.*, 1998). These results indicate that TRAP220 may mediate the principal contact between the thyroid hormone receptor and the TRAP complex. In a further analysis, TRAP220 showed strong ligand-dependent or ligand-enhanced interactions with the retinoic acid receptor (RAR), RXR, VDR and peroxisome proliferator-activated receptors α and γ, whereas it interacted only weakly, albeit specifically, with the oestrogen receptor (Yuan *et al.*, 1998). As with thyroid hormone receptor, these receptors showed only very weak interactions with TRAP100, which contains many LXXLL motifs, consistent with other studies showing context effects of LXXLL motif interactions with nuclear receptors. Thus, although the TRAP220 shows some preference in receptor interactions, the results indicate a broader role for TRAP220 and the TRAP complex in nuclear receptor functions.

Another significant finding is that transcriptional activation by the thyroid hormone receptor (TR)–TRAP complex in a purified reconstituted system absolutely requires other coactivators, showing that the requirement for a specialized coactivator does

not obviate the requirement for the general coactivators. Thus, on naked DNA templates, activation by the TR–TRAP complex absolutely requires the USA fraction (*Fig. 43b*) or, for optimal activity, a combination of the derived PC2 and PC4 (Fondell *et al.*, 1996; 1999). However, the activation is TAF_{II}-independent, indicating a functional redundancy between TAF_{II}s and the TRAP complex (Fondell *et al.*, 1999).

A multistep model for activation by nuclear receptors

Our observations, along with those of other laboratories (reviewed by Torchia *et al.*, 1998 and Fondell *et al.*, 1999), indicate that activation by nuclear receptors may be a multistep process (*Fig. 44*). In a first step, ligands interact with promoter-bound nuclear receptors to facilitate dissociation of co-repressors and associated histone deacetylases. In a second step, liganded receptors interact with a series of coactivators that, through associated HAT activators, allow a local modification of chromatin structure. The receptors may then interact with TRAP proteins that in turn act in concert with other general coactivators to enhance preinitiation complex formation, with RNA polymerase II and general initiation factors, and transcription initiation. The view that the TRAPs and the other nuclear receptor interacting factors perform different functions is strengthened by the fact that CBP enhances activation by nuclear receptors on chromatin templates but not on naked DNA templates, whereas the TRAP complex can promote activation using naked DNA templates.

An interesting but still unanswered question concerns the mechanisms by which the HAT-containing coactivators on the promoter might be displaced by the TRAPs. One possibility is that HATs and TRAPs both interact reversibly with liganded receptors following co-repressor disassociation but that only HAT cofactor interactions allow chromatin modifications that are prerequisites for stable interactions of general initiation factors at core promoter elements. Concerted synergistic inter-

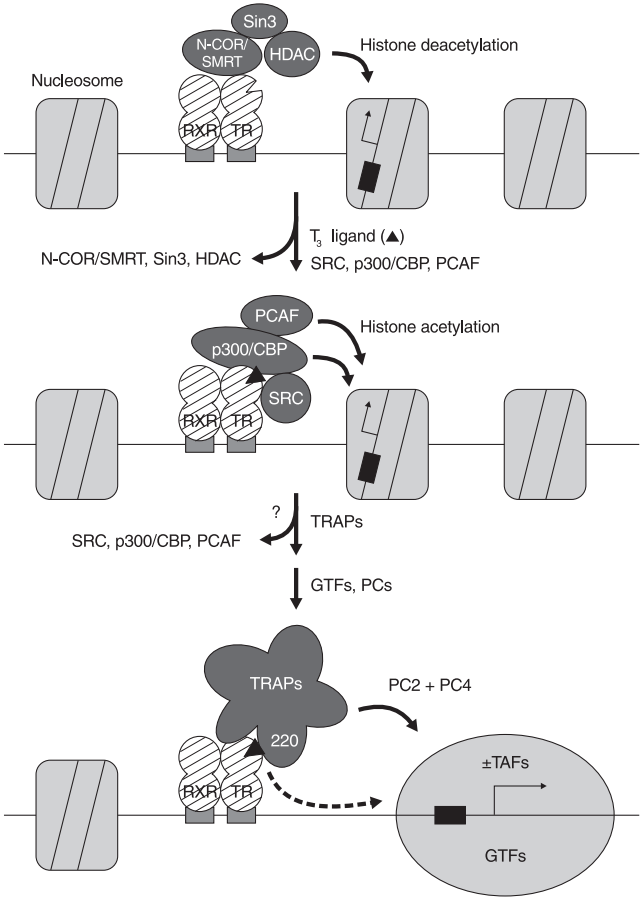


Figure 44. *Multistep model for gene activation by thyroid hormone receptor (TR), based on studies showing ligand-independent TR-mediated repression through TR-interacting co-repressors (N-COR/SMRT, Sin3) and the associated histone deacetylase (HDAC), followed by ligand-dependent dissociation of co-repressors and corresponding association of coactivators with HAT activity (p300/CBP, SRC-1-related factors, PCAF). Following ligand-dependent association of TRAPs, and with probable displacement of HAT coactivators, the complex acts in conjunction with general coactivators PC2 and PC4 to mediate transcription from DNA templates by RNA polymerase II and general initiation factors. GTF, general transcription factor; T_3, thyroid hormone. See text for details. Reprinted with permission from Fondell* et al.*, 1999.*

actions between general initiation factors (including RNA polymerase II), TRAPs and other coactivators may then shift the equilibrium to favour TRAP displacement of HATs and formation of a stable preinitiation complex.

Convergence of coactivator studies

Intriguingly, TRAP220 was also identified as a component both of SMCC (Gu *et al.*, 1999) and of CRSP, a complex that is required, with TAF_{II}s, for Spl activity on DNA templates (see Tijan, this volume) and that seems to be related to the PC2 component of USA (S. Malik and R.G. Roeder, unpublished observations). These results raised the interesting possibility that various components may be shared among various cofactor complexes, just as some TAF_{II}s are common to TFIID and PCAF/Gcn5 complexes (see Tora, Workman, this volume), or that they may be mixed and matched to create complexes of varying specificity and function. It seemed important in this regard to determine the full extent of components shared between TRAP/DRIP, SMCC, CRSP and PC2 complexes, and whether SMCC and CRSP can also interact with nuclear receptors and thereby facilitate crosstalk between different activators through TRAP220. Indeed, recent results have established that the TRAP and SMCC complexes are virtually identical in structure and function (M. Ito *et al.*, 1999) and that PC2 is made up of a large subset of the TRAP/SMCC components (S. Malik and R.G. Roeder, unpublished observations). Although somewhat surprising, these findings represent a pleasing convergence and unification of various mammalian and yeast coactivator studies. However, although the TRAP complex, and the related complexes subsequently described by us and others, contain apparent homologues of several yeast Mediator components and ostensibly function by similar mechanisms, the vast majority of the component subunits have no yeast counterparts and probably reflect metazoan-specific functions and mechanisms.

Transcriptional activation by Sp1: a biochemical journey

Robert Tjian

In collaboration with

Anders Näär and Soojin Ryu

Transcriptional activation by upstream activators is a multistep process requiring several classes of cofactor that interface with the basal transcription machinery. Here we review the evolution of biochemical studies on the regulation of gene expression by the human promoter selectivity factor, Sp1, that have revealed the complexity of this process. Sp1 is a glutamine-rich transcriptional activator required for the efficient transcriptional activation of many cellular and viral genes by RNA polymerase II.

Over the past decade a variety of biochemical approaches have revealed that several factors contribute to Sp1-mediated transcriptional activation (Näär *et al.*, 1999), which include the TAF_{II}s and a new multi-subunit complex termed cofactor required for Sp1 activation (CRSP; Pugh and Tjian, 1990). Other requirements, demonstrated by reconstitution of synergistic activation of the native low-density lipoprotein receptor promoter induced by Sp1 and the cholesterol-regulated transcription factor SREBP-1a, include chromatin assembly and a SREBP-binding multiprotein coactivator containing CBP, called activator-recruited cofactor (ARC), as well as TFIID.

CRSP has several components in common with other transcriptional coactivator complexes, e.g. Mediator, NAT and TRAP (see Kornberg, Roeder, this volume). This sharing of components seems to be emerging as a common theme in transcriptional regulation: e.g., a subset of TAF_{II}s are found in both the SAGA and TFIID complexes (see Workman, this volume) and several distinct chromatin remodelling complexes contain the common ATPase ISWI subunit (see Becker, Wu, this volume). Thus transcriptional cofactor complexes such as CRSP may act

through a combinatorial mechanism, interacting with a variety of proteins at multiple promoters.

TFIID and the TAF_{II}s

Initial biochemical studies used partially purified basal transcriptional factors, RNA polymerase II and a simple model template to identify essential factors needed to reconstitute Sp1-activated transcription in vitro. The template contained three tandem Sp1-binding sites upstream of a minimal adenovirus major late promoter (Pugh and Tjian, 1990; *Fig. 45*). The general transcription factor TFIID was thought to be the first factor recruited to the core promoter region and thus likely to be a target for activators. Biochemical fractionation showed that purified TFIID was indeed needed for activated transcription and could not be replaced by recombinant TBP alone.

TFIID is a complex consisting of TBP and several TAF_{II}s (see Green, Struhl, Tora, this volume). The function of individual TAF_{II}s was examined by purifying separate subunits of TFIID

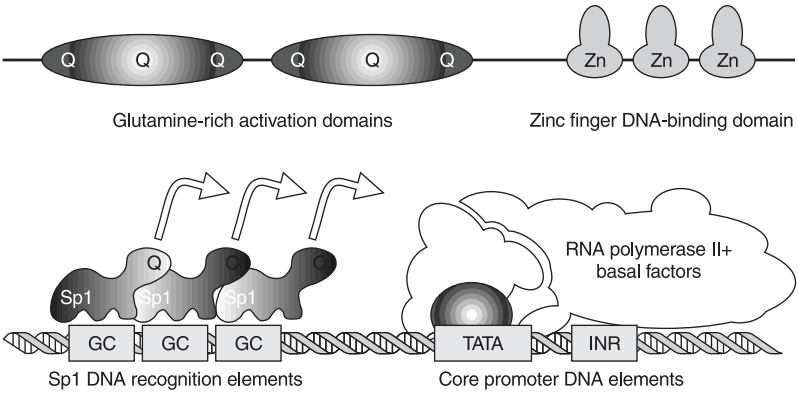


Figure 45. *Top, structure of transcriptional activator Sp1, indicating the glutamine-rich transcriptional activation domains and the zinc finger DNA-binding domain. Bottom, the process of transcriptional activation on a simple model template containing three Sp1-binding sites (GC boxes).*

(Dynlacht *et al.*, 1991), cloning them and producing recombinant proteins. This revealed that distinct classes of activator can bind different TAF_{II}s; Sp1 directly interacts with *Drosophila* $TAF_{II}110$ ($dTAF_{II}110$; human homologue $TAF_{II}130$) through its glutamine-rich activation domains (Hoey *et al.*, 1993), whereas the *Drosophila* transcription factor NTF-1 binds $dTAF_{II}150$ and $dTAF_{II}60$ (Chen *et al.*, 1994*a*).

The contribution of individual TAFs to the function of TFIID in transcriptional activation was reconstituted biochemically using subsets of recombinant TAF_{II}s to assemble partial TFIID complexes (Chen *et al.*, 1994*a*). A minimal TFIID complex containing TBP and $TAF_{II}250$ supported basal transcription in the purified transcription system but additional TAF_{II}s were required for activated transcription. These varied for different classes of activators, e.g., Sp1 activation was supported by a partial TFIID complex containing TBP, $TAF_{II}250$, $TAF_{II}150$ and $TAF_{II}110$ and assembly of different partial complexes showed that $TAF_{II}110$, the subunit that contacts Sp1, is necessary. In contrast, response to NTF-1 required $TAF_{II}150$ and $TAF_{II}60$ in partial complexes. Thus direct contact between activators and the TAF_{II}s in TFIID seemed to correlate with transcriptional activation. However, partial TFIID complexes could not fully reconstitute the high level of transcription observed with reconstituted holo-TFIID, so the TAF_{II}s that are not directly involved in contacting a specific activator may also contribute to TFIID coactivator function or stability.

The functional roles of some of the other TAF_{II}s are also being clarified: $TAF_{II}150$ recognizes and binds to certain core promoter sequences and may act in promoter specificity by stabilizing TFIID binding at certain promoters (Verrijzer *et al.*, 1994; 1995). $TAF_{II}250$ may be structural, acting as a core subunit contacted by TBP and the other TAF_{II}s but it could also be engaged in activated transcription by transmitting an activation signal, possibly through a conformational change. In addition

$TAF_{II}250$ interacts with core promoter elements; contains multiple kinase domains; can phosphorylate several proteins; and has HAT activity; however, the physiological substrates of these activities remain unclear.

The TAF_{II} components of TFIID clearly play multiple roles in activated transcription (Goodrich *et al.*, 1996), including promoter selectivity through direct contact with DNA and functioning as a coactivator by making direct contact with diverse classes of activator that transmit the activating signals to the basal machinery. The mechanism of this signal transduction is unclear but is likely to involve conformational change within the TFIID complex and/or the modulation of enzymatic activities resulting in the formation of a stable and active preinitiation complex at the promoter.

Cofactor for Sp1 activation (CRSP)

The development of purer and more active transcription systems permitted a more refined analysis of the factor requirements for Sp1-mediated transcriptional activation. Using a simple model template and a combination of recombinant and purified factors, we found that an activity present in a crude phosphocellulose fraction of HeLa extracts is required (*Fig. 46*; Ryu *et al.*, 1999). This was identified as a new, multiprotein complex, which we term CRSP with M_r ~700K and consisting of at least 7 subunits: p200, p150, p130, p77, p70, p33a and p33b. The presence of these subunits in a cofactor complex was confirmed by affinity purification using anti-p150 antibodies, which immunodepletes a high-salt-resistant complex containing the CRSP subunits from a crude HeLa fraction. The antibody also inhibits CRSP activity, providing evidence that these subunits are responsible for this activity.

Several of the CRSP components, subunits 130, 77, 70 and 33b, have not previously been identified; others are already known as components of other complexes involved in transcrip-

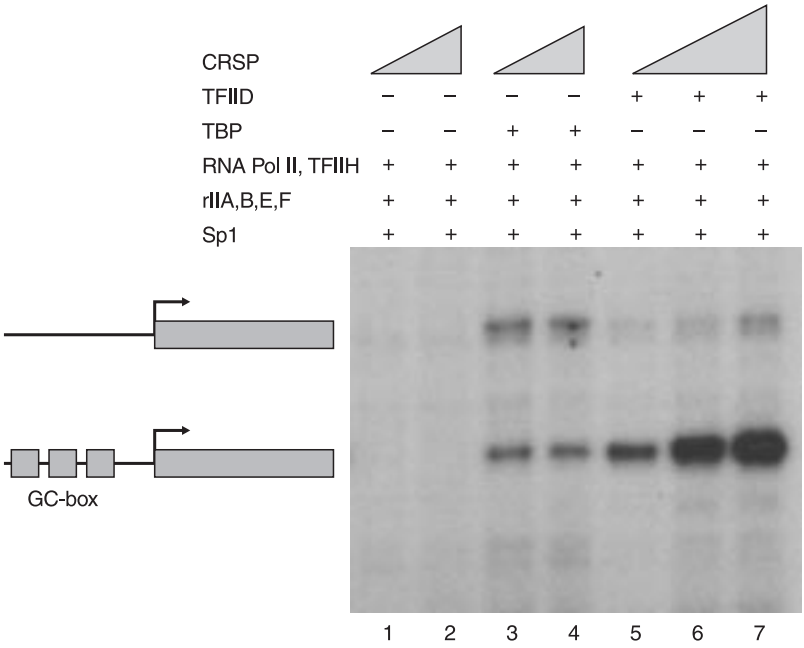


***Figure 46**. The reconstitution of Sp1 activation requires the basal transcriptional factors, RNA polymerase II and TFIID, and is stimulated by the CRSP complex. Transcription from a simple model promoter containing three Sp1-binding sites (GC box) and a minimal adenovirus E1b promoter (lower band) or from a control template lacking Sp1-binding sites (upper band) is displayed. Triangles, increasing concentrations; r, recombinant factors TFIIA, B, E and F. Modified from Ryu* et al.*, 1999.*

tional regulation (*Fig. 47*). For example, CRSP 150 contains a small stretch of homology to yeast Rgr1 and CRSP 33a seems to be the human homologue of Med7. Both are components of the yeast Mediator complex and the negative transcriptional regulator NAT complex (Sun *et al.*, 1998). CRSP 130, 77 and 33b have no homology to known proteins. CRSP also associates with a 200K protein homologous to TRIP2/PBP, a component of the TRAP/DRIP coactivator complex (see Roeder, this volume).

We are now using biochemical studies to examine how CRSP stimulates Sp1 activation. The complex does not appear to contact the activator directly but does interact with both individual

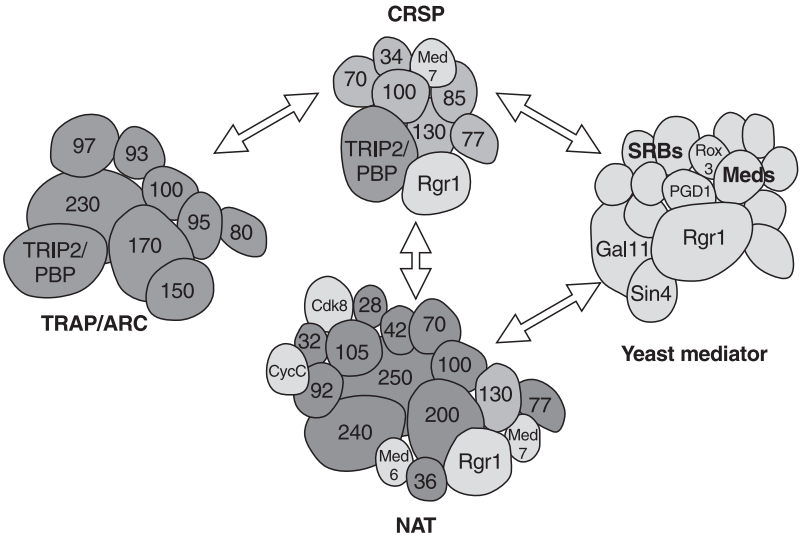


Figure 47. *Subunit composition of CRSP and its relationship to other transcriptional regulatory complexes. Two CRSP subunits, 150 and 33a, share homology with Rgr1 and Med7, which are also components of the yeast Mediator and NAT complexes, whereas CRSP 200 (TRIP2/PBP) is also present in the TRAP/DRIP/ARC complex.*

TAF_{II}s and holo-TFIID (see *Fig. 49*). CRSP 70, which is encoded by a newly found gene, exhibits some sequence homology to the transcriptional elongation protein TFIIS, so we are curious to see whether CRSP may also act at a step after transcriptional initiation.

Synergistic activation by Sp1 and SREBP-1a

Although our studies of Sp1 (see above) have used a single activator on a simple model promoter, in vivo Sp1 often cooperates with other transcriptional activators on complex promoters. We are investigating cofactor requirements for Sp1 activation in the context of more complex promoters by recapitulating the synergistic activation by Sp1 and the sterol-regulated factor SREBP-1a at the promoter of the low-density lipoprotein receptor gene (Näär *et al.*, 1998*a*, *b*). In a purified human transcription

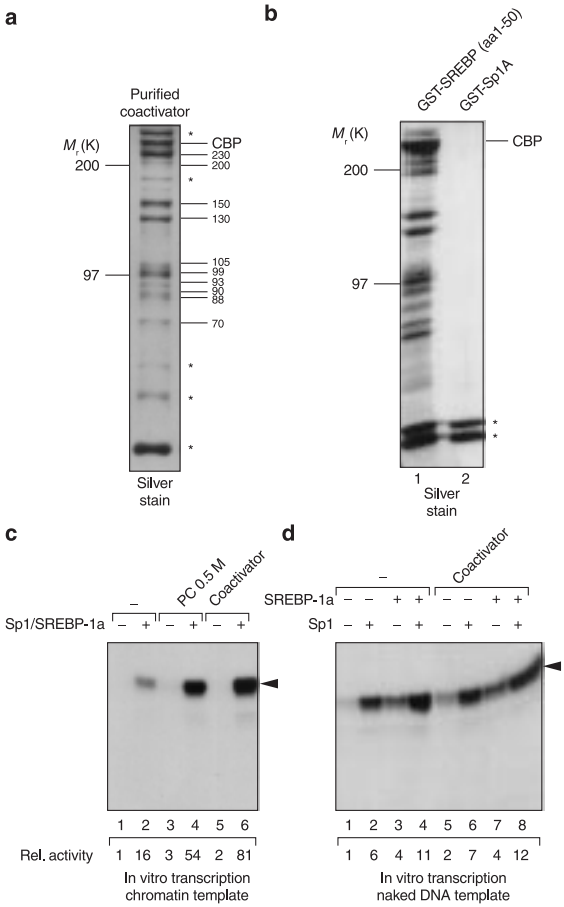


Figure 48. *A coactivator, ARC, is required for synergistic Sp1 and SREBP-1a activation on the promoter of the low-density lipoprotein receptor gene.* ***a****, components of the purified coactivator. *, nonspecific proteins that do not consistently copurify with this activity.* ***b****, a large multi-subunit complex (M_r 2,000K, not shown) containing CBP associates selectively with the SREBP-1a activation domain. HeLa nuclear extract was incubated with affinity resins containing the activation domains of SREBP-1a (GST–SREBP amino acids 1–50) or Sp1 (GST–Sp1A) and the bound fractions analysed by SDS–PAGE. *, two nonspecific proteins that bind the GST portion of the fusion proteins.* ***c****, purified coactivator stimulates transcription from the promoter assembled into chromatin. Transcriptional activity was assessed by primer extension in the absence (–) or presence (+) of Sp1 and SREBP-1a and either in the absence of coactivator (lanes 1, 2), with a partially purified coactivator fraction (lanes 3, 4) or purified coactivator (lanes 5, 6).* ***d****, coactivator does not affect transcription from naked DNA templates.*

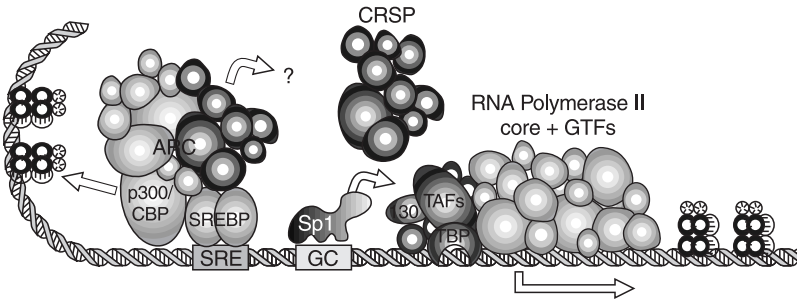


Figure 49. *Model of cofactor requirements for Sp1-activated transcription and for synergistic activation by Sp1 and SREBP-1a in the low-density lipoprotein receptor promoter. In HeLa extracts the glutamine-rich domain of Sp1 makes direct contacts with $hTAF_{II}130$ within TFIID, transmitting information to the basal transcriptional machinery and leading to increased transcription initiation. CRSP contributes to this process through an unknown mechanism. Chromatin-mediated repression of transcription could be relieved by the recruitment of ARC/CBP coactivator by SREBP, which may facilitate the access of the transcriptional machinery to the template. The HAT activity of CBP may participate in chromatin remodelling.*

system, chromatin, TAF_{II}s and a new SREBP-binding coactivator activity are all required to mediate efficient synergistic activation by Sp1 and SREBP-1a. Purification of the SREBP-binding cofactor to near homogeneity (*Fig. 48a*) identified a large multiprotein coactivator we have called activator-recruited cofactor (ARC) that selectively interacts with the SREBP activation domain (*Fig. 48b*). This activity contains the transcriptional coactivator and the acetyltransferase CBP but the complex cannot be replaced by either recombinant CBP or the CBP-associated transcriptional coactivator PCAF.

As the coactivator can mediate high levels of synergistic activation by SREBP-1a and Sp1 on chromatin templates but not on naked DNA (cf. *Fig. 48c* and *d*), we think this large complex may be involved in overcoming chromatin-mediated repression. However, its mechanism of action remains unclear but identification of the remaining components of the ARC complex, as indicated in the proposed model (*Fig. 49*), should help to resolve this in the future.

Structure and function of proteins that modulate RNA polymerase II transcription

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in collaboration with

Keiko Ikeda, Jun Xie and Martine Couart

Several proteins beside the general transcription factors and coactivators such as the TAF_{II}s and the Med and Srb proteins can act as transcriptional coactivators in vitro (reviewed in Kaiser and Meisterernst, 1996). We have characterized the positive cofactors PC2 and PC4 (see Roeder, this volume) in vitro and the negative regulator NC2 in vitro and in vivo in yeast. We show here that PC2 is involved in the formation of the preinitiation complex and that PC4 contains two partially distinct functional domains: an N-terminal domain involved in enhancing activated transcription through interaction with transcriptional activation domains and general transcription factors, and a C-terminal domain that binds single-stranded DNA and represses transcription. Another coactivator, PC6, seems to be essential for activation by the viral acidic activator VP16 in vitro. It may be a specificity factor for one of the VP16 activation regions in a similar way that CBP is specific for another region, although its precise role is uncertain. We also show that NC2 repression is essential in yeast and NC2 may compete with TFIIA for binding to TBP in vivo as well as in vitro.

The RNA polymerase II coactivator PC2

Transcriptional coactivators required for activator function in vitro were originally identified in a crude USA fraction from ion exchange chromatography of HeLa cell nuclear extracts. Further purification showed that the USA fraction contained several activities: positively acting coactivators PCs 1–4 and PC7 and negatively acting factors NC1 and NC2 (Meisterernst *et al.*, 1991). After purification to homogeneity, PC1 was identified as

poly-ADP-ribose polymerase and PC3 as topoisomerase I. Others, such as PC2 and PC7, have not been purified to homogeneity but are likely to correspond to large multiprotein complexes (Kaiser and Meisterernst, 1996). Recent work indicates that PC2 has subunits homologous to those in Mediator, as well as to human TRAP and DRIP/ARC complexes, indicating PC2 may be related to yeast Mediator (M. Meisterernst, unpublished observations).

Although PC2 has not been completely purified, biochemical experiments have revealed that it is a general coactivator that will potentiate activation by transcriptional activators, such as the glutamine-rich activator Sp1 (see Tjian, this volume), in in vitro systems reconstituted with highly purified endogenous and/or recombinant general transcription factors (Kretzschmar *et al.*, 1994*b*). PC2 acts during preinitiation complex formation because activation does not occur if it is added to the transcription reaction after the general transcription factors and assembly of the preinitiation complex. Because activity is diminished but not fully lost if it is added after TFIID but before the other general transcription factors, PC2 seems to act, in part, early in the activation process, possibly on the binding of TFIID.

The requirement for PC2 cannot be eliminated by using premelted promoter templates in which the nucleotides around the RNA initiation site are not paired. The use of premelted templates alleviates the requirement for TFIIH and TFIIE and allows transcription to be performed in a more defined system. Under these conditions, the synthetic AAD AH-peptide fused to Gal4 activator can activate transcription in a PC2-dependent manner when only TBP, TFIIB, polymerase II and TFIIF are included in the transcription reaction.

Interaction of the VP16 AAD with CBP

PC6, to which we have recently added the term MOVE (for mediator of VP16 enhancer), is a coactivator fraction that can be

separated from the other positive cofactors at an early stage of chromatography. Its activity is required for specific activation of the VP16 activator and it must be a distinct cofactor for VP16 because it cannot be substituted by PC2, PC4 or PC5.

As PC6-MOVE could not be purified by ion-exchange chromatography, we used affinity chromatography of extracts in a column containing the immobilized VP16 AAD. This resulted in the extract losing the specific ability to support activation by VP16, indicating that PC6-MOVE or a PC6-MOVE-like activity had been removed and confirming that this activity is essential for transcriptional activation of region H1 (see below) of VP16 in vitro. Immunoblot analysis using antibodies against the known transcription factors present in the starting extract showed that the VP16 column did not efficiently deplete any of the general transcription factors or TAF_{II}s but did remove the closely related p300/CBP proteins. Although CBP binds to another region (H2) of the VP16 AAD, it did not coelute with the PC6-MOVE fraction and is therefore not the PC6-MOVE activity.

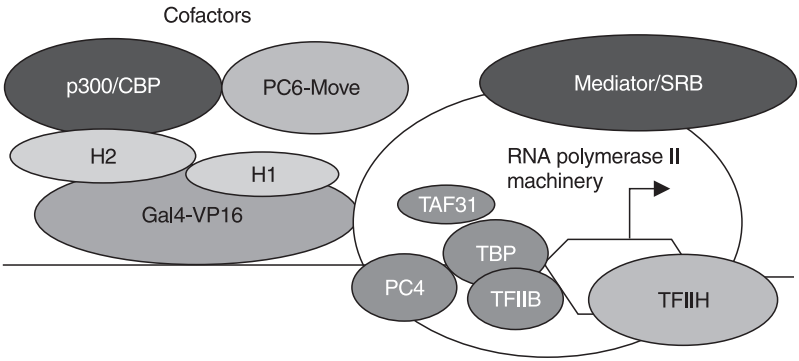

Figure 50. *Summary of the transcription factors that are established as interacting directly with VP16 or that are required for VP16 activity in vitro. Direct interaction between the VP16 acidic activator domain (made up of regions H1 and H2) and p300/CBP, TAF31, PC4, TBP and TFIIB are indicated. PC6-MOVE is required for the transcriptional activation of the H1 activation subdomain of VP16.*

The activation domain of VP16 can be divided into two subdomains, H1 in the N-terminal region and H2 in the C-terminal region (*Fig. 50*). Mutation of a phenylalanine residue in the H1 region, which abolishes activation in vitro, did not affect VP16–CBP interactions. In contrast, a triple amino-acid substitution in the H2 domain abolished VP16–CBP interaction and the H2 domain alone was sufficient for binding to CBP, although deletion of this domain did not affect transcriptional activation in vitro in the absence of chromatin.

CBP has HAT activity and can potentiate transcriptional activation in vitro on chromatin templates by activators such as the oestrogen receptor. This indicates that the VP16 AAD has a bipartite structure involved in modulating distinct stages of the activation process. First, the H2 domain may recruit the CBP HAT activity to modify the chromatin structure. The general transcription factors and coactivators such as PC6-MOVE are then recruited to the modified template by interactions with the H1 domain. VP16 also stimulates RNA elongation through interactions with TFIIH. The ability of the VP16 AAD to enhance multiple steps of the activation process may account for the very high activity observed in vivo with this activator.

The positive cofactor PC4

PC4 has been purified to homogeneity and its corresponding cDNA has been cloned. It is a 15K protein comprising 127 amino acids that can both stimulate activated transcription and, at higher concentrations, repress basal transcription in vitro (Kretzschmar *et al.*, 1994*a*; Ge and Roeder, 1994; see Roeder, this volume). PC4 can be divided into a structured C-terminal domain and a probably unstructured N-terminal domain containing many lysine and serine residues. Full homologues of PC4 are found in many organisms including *C. elegans*, although in yeast only the structured C-terminal domain is conserved.

The structure of the PC4 C-terminal domain at the atomic

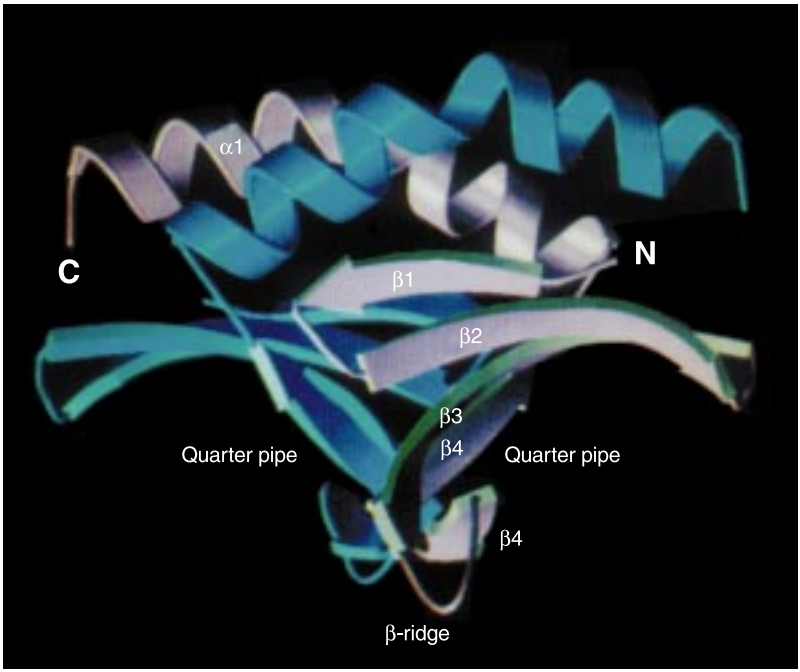

Figure 51. *Ribbon representation of the crystal structure of the PC4 C-terminal domain deduced from X-ray crystallography data. The quarter-pipe ssDNA-binding channels and the principal α-helical and β-sheet regions are indicated.*

level (*Fig. 51*; Brandsen *et al.*, 1997) shows that the domain dimerizes to form two 'quarter-pipe' structures, each acting as a single-stranded (ss) DNA-binding channel. PC4 binds ssDNA with 100-fold higher affinity than for double-stranded DNA and its C-terminal domain resembles replication protein A, which binds strongly to ssDNA (Werten *et al.*, 1998*a*). One of the functions of PC4 may therefore be to bind to melted DNA duplexes in open promoters or during DNA replication, recombination or repair.

We used the structural data to design amino-acid substitutions in exposed residues of the predicted DNA-binding surface in order to disrupt ssDNA binding (Werten *et al.*, 1998*b*). These

mutations do not, however, affect coactivator function, indicating that ssDNA binding is not required for this. Instead, these mutants affect the ability of PC4 to repress transcription through binding to ssDNA. This ability, interestingly, is antagonized by TFIIH, perhaps through an intrinsic helicase, indicating that TFIIH may be in part an anti-repression factor.

The N-terminal domain of PC4 is required to promote activation because the C-terminal domain alone is inactive. PC4 binds to several activation domains and to TFIIA and enhances activation by facilitating the binding of TFIID, perhaps through its interaction with TFIIA and also by acting at an undefined later stage of the formation of the preinitiation complex, possibly through direct interaction with polymerase II itself (Kaiser *et al.*, 1995). Coactivator activity is, however, abolished upon hyperphosphorylation of serines in the N-terminal domain by casein kinase II and perhaps by other more physiologically relevant kinases, such as TFIIH or Srb10/11. The coactivator function of PC4 is also abolished by mutation of four lysine residues in the N-terminal domain, some of which are targets for acetylation, indicating that PC4 function may be regulated in other ways.

In vivo analysis of human NC2α and NC2β in yeast

Negatively acting factors repress basal transcription in an activator-reversible manner, so enhancing the apparent stimulation by the activator. The negatively acting NC2 factor has two subunits, NC2α and β, and the heterodimer binds to the TBP–DNA complex. This prevents the subsequent association with TFIIA and TFIIB and alters the conformation of the DNA, which blocks formation of the preinitiation complex and transcription (*Fig. 52*). As the α subunit contains a histone-fold motif homologous to that of histone H2A and the β subunit one to that of H2B, NC2 is composed of a histone-like pair (Goppelt *et al.*, 1996).

Homologues of the NC2α and β subunits are found in yeast and the β-subunit homology extends beyond the histone-fold

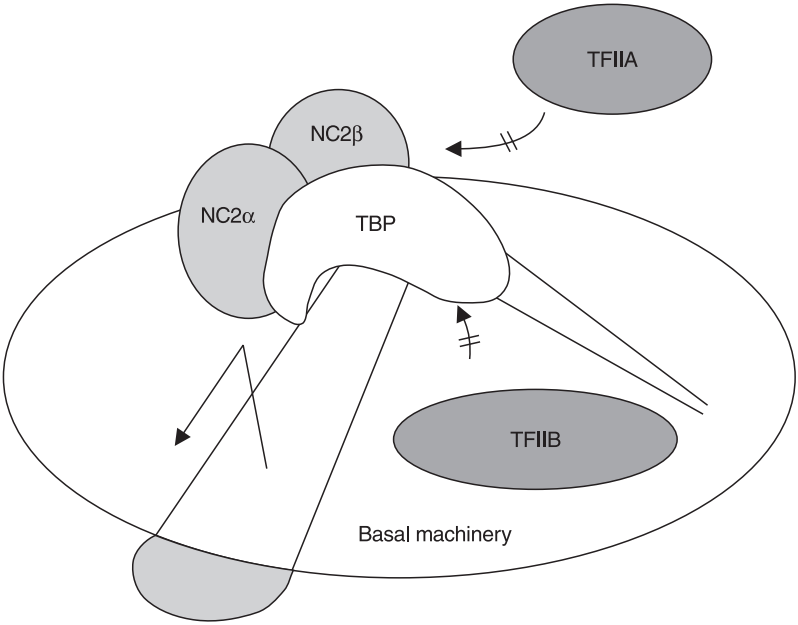

Figure 52. *Model of the mechanism of repression of polymerase II transcription by NC2. NC2α and β bind to TBP, blocking the subsequent association with TFIIA and TFIIB (crossed arrows) as well as altering the shape of the DNA in the preinitiation complex (large arrow).*

regions. Null mutations in the yeast NC2 proteins are lethal but mutations in both the α and β subunits can be complemented by their human counterparts. Deletion analysis shows that the histone-fold region of the α subunit is sufficient for complementation, whereas the β subunit requires additional conserved C-terminal sequences. Amino-acid substitutions in the histone-fold dimerization interface of either subunit result in mutants that fail to complement, so dimerization through this surface is required for function in vivo. Amino-acid substitutions in the β subunit on the presumed exposed face of the long α2-helix of the histone fold are interesting because they also abolish complementation but do not affect dimerization. This mutated NC2

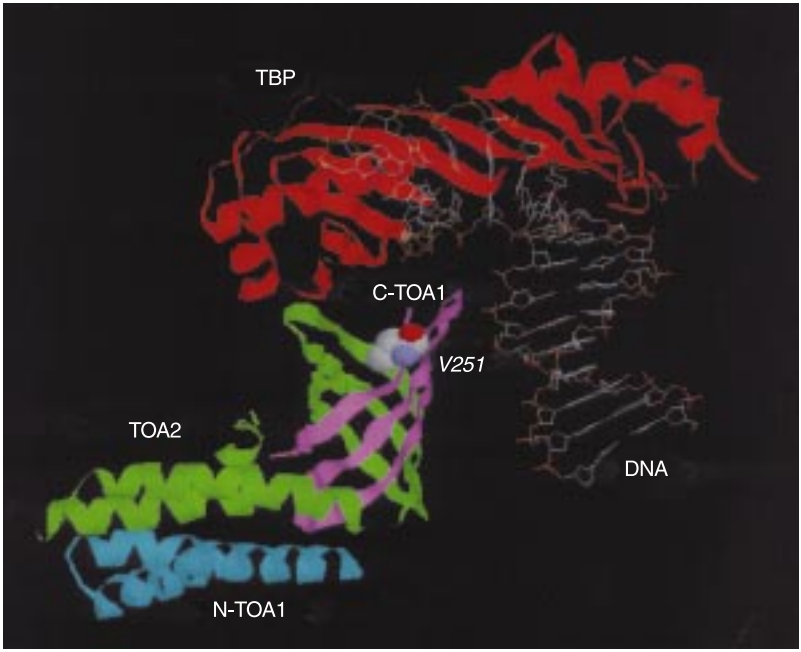

Figure 53. *Ribbon representation of the crystal structure of the TBP–TFIIA–DNA complex showing the location of a single amino-acid substitution, Val-to-Phe at position 251 (V251), in the TOA1 subunit of TFIIA. This form is a suppressor of human NC2β expression in yeast. Blue, N terminus of TOA1; pink, C terminus of TOA1; green, TOA2 subunit; red, TBP.*

complex does not repress basal transcription in vitro, indicating that yeast viability requires the ability of NC2 to act as a transcriptional repressor.

Yeast cells grow normally when the human NC2β subunit is expressed from a high-copy-number plasmid but poorly when it is expressed from a low-copy-number plasmid. This enabled us to use a suppressor-screen technique to identify proteins that may be involved in NC2 function. One of these suppressors, which has a cold-sensitive phenotype, contained a Val-to-Phe mutation in the yeast TFIIA subunit TOA1 at position 251, which is in the TOA1–TOA2 dimerization interface (*Fig. 53*). Recombinant TFIIA containing this amino-acid substitution

failed to antagonize the binding of NC2 to TBP and did not alleviate transcriptional repression by NC2. So, when limiting amounts of NC2 are present in yeast, transcription is not efficiently repressed and growth is compromised. This defect can be rescued by mutations in TFIIA which weaken its interaction with TBP and make it a less efficient competitor for NC2.

Functional correlation between Gal11, TFIIE and TFIIH in *S. cerevisiae*

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in collaboration with
Toshio Fukasawa

The *GAL11* gene was first identified as a positive regulator for the expression of genes encoding proteins involved in galactose metabolism (Nogi and Fukasawa, 1980; Suzuki *et al.*, 1988). Subsequent studies, however, have revealed that *GAL11* may have more general effects, both positive and negative, on the regulated transcription of several functionally unrelated genes, as well as on the basal transcription machinery (Sakurai and Fukasawa, 1997). In line with these results, Gal11 has been identified as a component of the yeast RNA polymerase II holoenzyme (Kim *et al.*, 1994) and more specifically as a subunit of the Mediator complex (see Kornberg, this issue).

Yeast strains lacking *GAL11* (*gal11*Δ strains) are viable but their growth on a rich medium is temperature sensitive and they have a variety of phenotypes, including slow utilization of galactose, sucrose and non-fermentable carbon sources. These phenotypes correlate well with a reduced expression of the genes involved in the respective metabolic pathways, e.g., the expression of *GAL1*, *GAL7* and *GAL10* is reduced to 5–20% of wildtype,

expression of *SUC2*, encoding invertase, is reduced to 30%, and expression of *CYC1*, encoding cytochrome *c*, is reduced to 30%. Because transcription of genes involved in histidine synthesis and of genes encoding mating hormones is also affected, *gal11*Δ strains are deficient in amino-acid biosynthesis and have sporulation and mating defects (Sakurai and Fukasawa, 1997; Suzuki *et al.*, 1988).

A loss-of-function mutation in *GAL11* had the same effect as a mutation in the *SPT13* gene, one of a family of genes involved in transcriptional repression. Cloning the *GAL11* and *SPT13* genes showed that they are identical, so Gal11 could be a negative factor (Fassler and Winston, 1989; see Workman, this issue). From the in vivo observations (above) and in vitro studies described below, the apparent negative effect of *GAL11* may be an indirect consequence of the loss of Gal11 function. Gal11 stimulates basal and activator-induced transcription in yeast nuclear extracts and basal transcription in a fully defined yeast transcription system reconstituted from highly purified or recombinant general transcription factors. Gal11 may thus interact directly with one or several of the general transcription factors. We discuss Gal11 function in terms of its interactions with the subunits of the transcription factors TFIIE and TFIIH.

Gal11 and TFIIE interactions and CTD phosphorylation

Affinity chromatography and immunoprecipitation experiments demonstrated a specific interaction between Gal11 and TFIIE (Sakurai *et al.*, 1996*b*). We have mapped the mutual binding regions in Gal11 and TFIIE (Sakurai *et al.*, 1996*b*; Sakurai and Fukasawa, 1997). The Gal11 protein has two separate domains, A and B, both essential for its function (*Fig. 54*), which have been identified using different *GAL11* deletion mutants in vivo. Domain A has also been implicated in the binding of Gal11 to the RNA polymerase II holoenzyme (Barberis *et al.*, 1995). The large subunit of yeast TFIIE, encoded by *TFA1*, interacts specifically with domain B of Gal11, whereas the small TFIIE subunit,

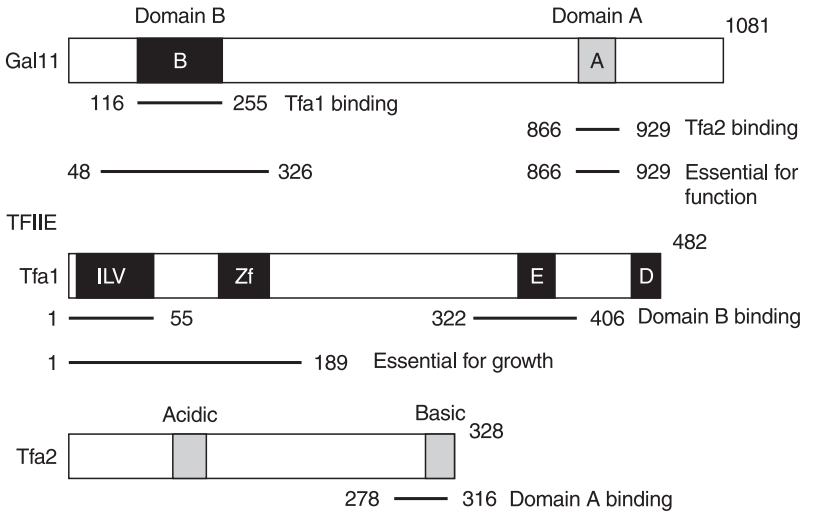

Figure 54. *Maps of the domains of Gal11 and TFIIE lined up to show their respective sites of interaction. Yeast TFIIE is a heterodimer of Tfa1 and Tfa2 subunits. Tfa1 has hydrophobic (ILV, rich in isoleucine, leucine and valine), zinc finger (Zf), glutamic acid-rich (E) and aspartic acid-rich (D) regions. Tfa2 has acidic and basic regions. Domain A of Gal11 binds to the basic region in Tfa2 and domain B binds to the ILV and E regions in Tfa1.*

encoded by *TFA2*, interacts with domain A (*Fig. 54*). The Tfa1 subunit of TFIIE contains four characteristic regions (see *Fig. 54* for details), two of which, ILV and E, are involved in binding to domain B of Gal11. The Tfa2 subunit contains a basic region in its C terminus that forms part of the region binding to domain A of Gal11.

To study the function of the interaction between TFIIE and Gal11 in vivo we constructed yeast with a mutation, *TFIIE-ΔC*, in which TFIIE lacks two of its three Gal11-binding regions, ILV, E and the basic region (Sakurai and Fukasawa, 1997). These cells produced versions of both TFIIE subunits truncated at their C termini, which cannot interact with Gal11, and showed phenotypes that resembled those of a *GAL11* deletion. A yeast strain containing the *TFIIE-ΔC* mutation and a *GAL11* deletion showed

no additional phenotypes, indicating that Gal11 acts exclusively through interactions with TFIIE. In confirmation, in a reconstituted in vitro transcription system Gal11 stimulated transcription in the presence of TFIIE but not TFIIE-ΔC.

TFIIE and Gal11 act cooperatively to stimulate CTD phosphorylation (Sakurai and Fukasawa, 1998). The CTD of the largest subunit of RNA polymerase II is phosphorylated by a kinase within TFIIH during the transcription process from initiation to elongation (Dahmus, 1996). The RNA polymerase II holoenzyme is a 50-fold better substrate than the core RNA polymerase II for CTD phosphorylation by TFIIH (Kim *et al.*, 1994) because it contains Mediator (see Kornberg, this volume). We found that TFIIE stimulated CTD phosphorylation of the holoenzyme a further 10-fold but did not stimulate CTD phosphorylation of the core polymerase or the holoenzyme lacking Gal11; however, addition of recombinant Gal11 to the deficient holoenzyme restored the high level of phosphorylation mediated by TFIIE.

To identify regions in TFIIE that participate in the stimulation of the kinase activity of TFIIH, we repeated these experiments using separate TFIIE subunits and found that only the Tfa1 subunit was required to stimulate CTD phosphorylation. In addition, domain B of the Gal11 protein was essential, so stimulation of phosphorylation depends on the interaction between Tfa1 and domain B. A Gal11 protein lacking domain A produced only a threefold increase in CTD phosphorylation, a reduction that cannot be explained by an inability of the Tfa1 subunit to interact with Gal11; it might, however, result from a less stable interaction between Gal11 and the RNA holopolymerase.

Core promoter specificity in activated transcription

As expected for a general transcription factor, both subunits of TFIIE are required for yeast growth (Feaver *et al.*, 1994*a*). To study the function of TFIIE in vivo, a temperature-sensitive

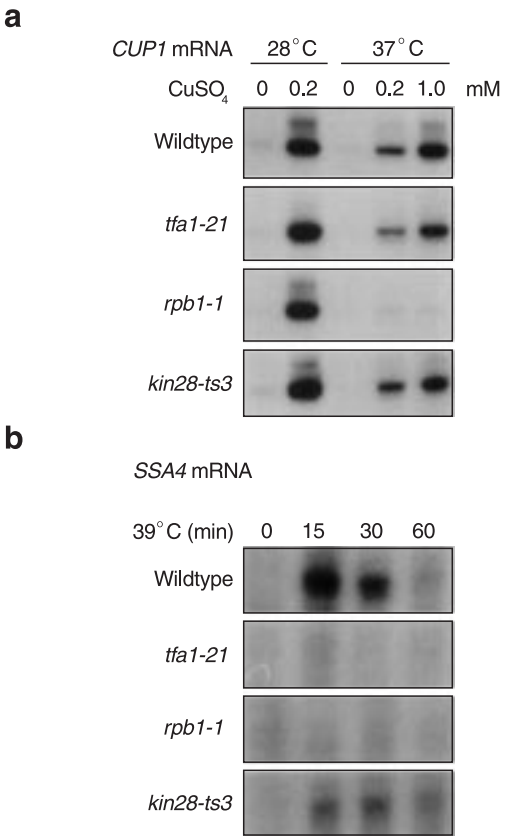

Figure 55. *Transcriptional activation of* CUP1 *is independent of TFIIE and Kin28 but* SSA4 *shows some dependence on TFIIE.* ***a****, transcription of* CUP1*, encoding copper metallothionein, is induced by copper sulphate in* tfa1-21 *and* kin28-ts3 *cells but not in* rpb1-1 *cells at 37 °C.* ***b****, transcriptional activation of* SSA4*, encoding Hsp70, by heat shock is inhibited by the* tfa1-21 *mutation. By contrast, transcription of* SSA4 *is activated in* kin28-ts3 *cells but only at about 30% of that in wildtype cells. Modified from Sakurai and Fukasawa, 1999.*

mutant of the Tfa1 subunit, *tfa1-21*, was isolated (Sakurai *et al.*, 1997). When *tfa1-21* cells are shifted to the restrictive temperature, synthesis of bulk poly(A)$^+$ RNA and various specific mRNAs ceases, indicating that TFIIE is necessary for mRNA

synthesis from most genes. However, transcription of both *GAL80*, a negative regulator of galactose-inducible genes, and *HIS3*, a histidine-synthesizing enzyme, was maintained after the temperature shift. Constitutive transcription of *GAL80* is mediated by the initiator element and of *HIS3* by the non-consensus TATA sequence, indicating that TFIIE is dispensable for transcription from several TATA-lacking promoters. Although Gal11 is necessary for full expression of several genes (see above), it is not needed for transcription of *GAL80* and *HIS3* (Sakurai *et al.*, 1996*a*). Thus TFIIE and Gal11 show similar gene specificity relating to the core promoter structure.

These results led us to investigate whether the *tfa1-21* mutation affected the induction of *CUP1* and *SSA4* (Sakurai and Fukasawa, 1999). *CUP1* encodes copper methallothionein and its expression can be induced by addition of copper sulphate to the growth media. *SSA4* encodes Hsp70 and is induced by heat shock. Transcriptional activation of both genes is independent of the CTD, the CTD kinase Kin28, which is a subunit of TFIIH, and CTD-associated Mediator components such as Gal11, Srb4 and Srb6 (Lee and Lis, 1998; McNeil *et al.*, 1998; Sakurai and Fukasawa, unpublished observations).

In cells with a mutation in the largest subunit of RNA polymerase II (*rpb1-1*), copper-inducible transcription of *CUP1* was inhibited at the restrictive temperature (37 °C). However, *CUP1* was induced in *tfa1-21* cells and also in cells with a recessive temperature-sensitive mutation in *KIN28* (*kin28-ts3* cells), after shifting to the restrictive temperature. Thus TFIIE and Kin28 seem to be dispensable for *CUP1* activation (*Fig.* 55). We constructed hybrid promoters to determine if the requirements for TFIIE and Kin28 were localized to the core promoter or to an upstream activation sequence (UAS). These promoter constructs contained the UAS and core promoter of the TFIIE/Kin28-dependent *GAL7* promoter and the TFIIE/Kin28-independent *CUP1* promoter in different combinations. We found that the

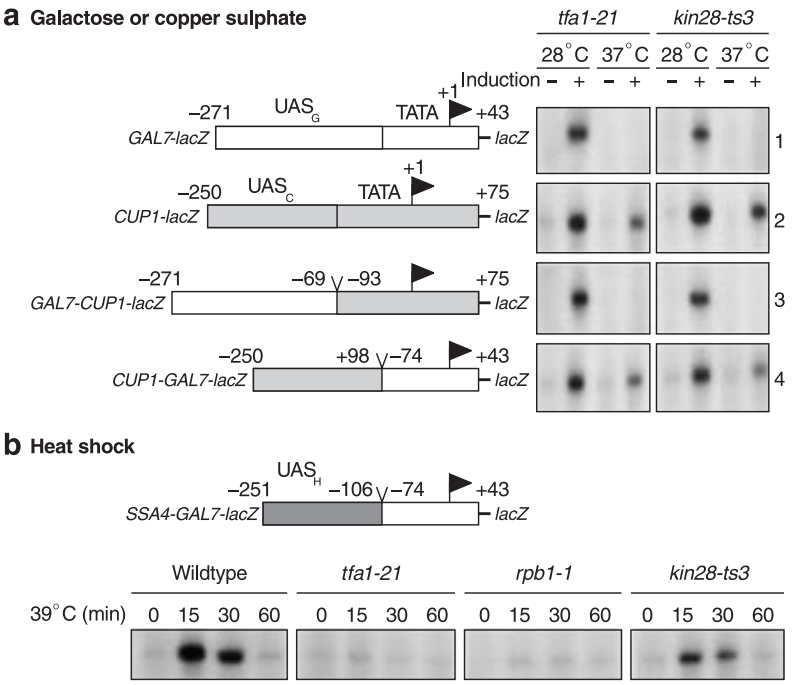

Figure 56. *Upstream activation sequences (UAS) are determinants of the requirement for TFIIE or Kin28.* ***a****, transcription of* GAL7–lacZ *and/or* CUP1–lacZ *fusion genes was induced by the addition of galactose (UAS$_G$) or copper sulphate (UAS$_C$) to the culture.. The* GAL7 *gene is one of the galactose-inducible genes and its activation is dependent on both TFIIE and Kin28 (line 1). Transcription of the reporter genes containing the UAS (line 4) but not the core promoter (line 3) of* CUP1 *is induced after inactivating TFIIE or Kin28.* ***b****,* SSA4–GAL7–lacZ *fusion gene induced by heat shock (UAS$_H$). Kin28 is not essential for activation mediated by the* SSA4 *UAS. Modified from Sakurai and Fukasawa, 1999.*

determinant for the TFIIE/Kin28 independence of *CUP1* was located within its UAS (*Fig. 56a*). In contrast, the requirement for induction of *SSA4* was slightly different. Like *CUP1, SSA4* induction was independent of Kin28 (*Fig. 55*), which was mediated by the UAS (*Fig. 56b*). In the *tfa1-21* cells, however, the induction of *SSA4* or *SSA4-GAL7-lacZ* was almost undetectable (*Figs 55, 56b*).

Thus TFIIE is necessary for activation mediated by the *SSA4* UAS whereas Kin28 is dispensable, unlike activation mediated by the *CUP1* UAS, for which both TFIIE and Kin28 are dispensable. Transcriptional activators on the *CUP1* UAS may have an unknown function that allows the UAS to circumvent the requirements for these factors, as well as for the CTD and Mediator (see above). CTD and Mediator also seem to be dispensable for activation of *SSA4* and we propose that the transcriptional machinery forms on the promoter through mediation by the *SSA4* UAS without the involvement of the CTD, Kin28 or Mediator; however, the transcription initiation step requires TFIIE as an integral component of the basal initiation machinery.

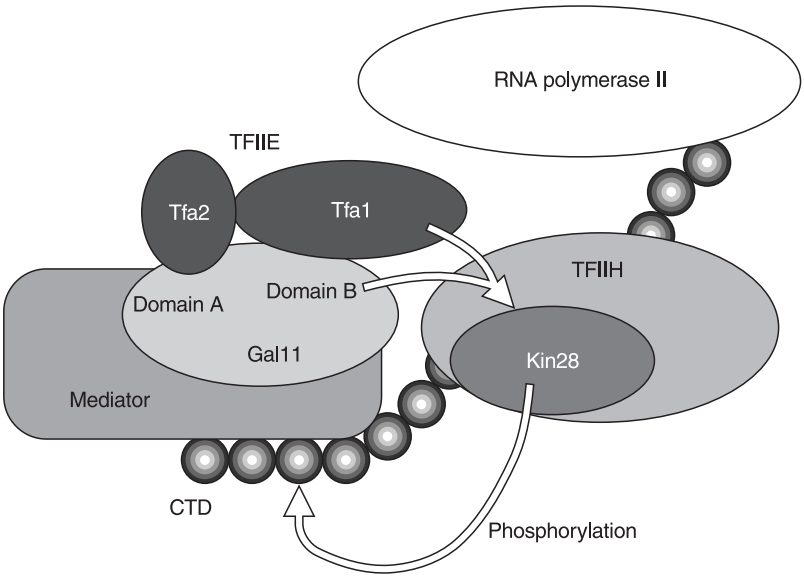

Figure 57. *Model of Gal11–TFIIE–TFIIH interactions and CTD phosphorylation. Interaction between domain B of Gal11 and the Tfa1 subunit of TFIIE stimulates RNA polymerase II CTD phosphorylation by Kin28, a CTD kinase subunit of TFIIH, which is part of the complex between RNA polymerase II and Mediator (see Kornberg, this volume). This interaction is necessary for transcription of most but not all genes in yeast; see text for details.*

Based on these results, we have constructed a model of the interactions between Gal11, TFIIE, TFIIH and the CTD (*Fig.* 57). In the RNA polymerase II holoenzyme, Gal11 helps the interaction with TFIIE, which enhances CTD phosphorylation catalysed by Kin28, the CTD kinase resident in TFIIH. This interaction is necessary for transcription of most but not all yeast genes in vivo. The requirements for TFIIE, the CTD, Kin28 and mediator are determined by the activator/UAS interaction and presumably by the structure of the core promoter. Elucidation of the molecular mechanism underlying such gene-specific requirements for components of the transcription machinery should provide further insight into transcriptional regulation.

Functional analysis of the components of yeast TFIID in vivo

Michael R. Green

in collaboration with
Wu-Cheng Shen, Xiao-Yong Li and Lynne M. Apone

TBP helps to initiate transcription in eukaryotes by interacting with the TATA sequence located about 20–30 nucleotides away from the 5′ side of the transcription start site of most genes. When biochemically fractionated from extracts of human cells, TBP coeluted with a set of associated TAFs with which it forms the TFIID complex, whereas most TBP in yeast was found as a monomeric protein (Poon and Weil, 1993). As the TAF_{II}s in higher eukaryotes are thought to be coactivators essential for transmitting signals from transcriptional activator proteins to the basal transcription machinery, it seemed likely that yeast and mammalian cells use different mechanisms to regulate transcrip-

tion. However, a fraction of the TBP present in yeast was later shown by affinity purification to be bound to TAF_{II}s (Poon and Weil, 1993).

With the identification of the genes encoding the human and *Drosophila* TAF_{II}s, the sequencing of the entire yeast genome and the characterization of immunoaffinity-purified yeast TFIID, yeast homologues of most of the TAF_{II}s in higher eukaryotes have been identified (Reese *et al.*, 1994; Poon *et al.*, 1995; *Box 2*). Most of the genes encoding TAF_{II}s are essential for yeast viability indicating that the corresponding protein is involved in an essential and non-redundant function. Some yeast TAF_{II}s, e.g., $TAF_{II}90$, $TAF_{II}150$ (also known as TSM-1) and $TAF_{II}145$, affect the transcription of limited sets of genes, whereas others, such as $TAF_{II}17$, have a more general action. Here we compare the functions of $TAF_{II}145$ and $TAF_{II}17$, which differ not only in their specificity but also in the location of their determinants. $TAF_{II}145$ affects particularly genes involved in progression through the cell cycle from G1 to S (see *Glossary*). We also discuss some recent experiments to resolve the effect of transcriptional activators on occupancy of TBP at the TATA box.

Functional studies of yeast $TAF_{II}145$ in vivo

We studied the function of TAF_{II}s in vivo by constructing yeast mutants either with a temperature-sensitive mutation in one of the genes encoding a TAF_{II} or with the gene under the control of a conditional allele that can be inactivated by depletion or temperature. A specific TAF_{II} subunit can then be analysed at different time points after inactivation (Walker *et al.*, 1997). Such inactivation of individual y TAF_{II}s surprisingly did not lead to defects in transcription of most genes transcribed by RNA polymerase II, whereas transcription of most genes rapidly ceased after genes encoding general transcription factors or subunits of the Mediator complex were inactivated. This result contrasts with several experiments in vitro that implied TAF_{II}s were essen-

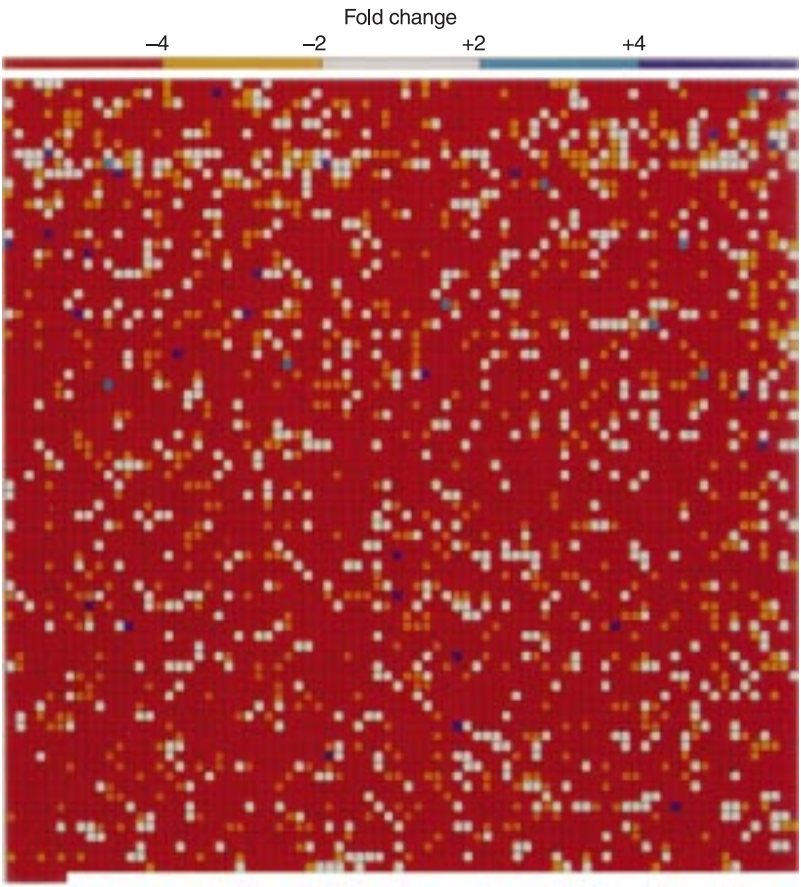

***Figure 58**. Microarray technique showing changes in expression of the yeast open reading frames after inactivation of RNA polymerase II using a mutant strain carrying a temperature-sensitive mutation in the largest RNA polymerase II subunit. Reproduced with permission from Holstege* et al.*, 1998.*

tial for transcriptional regulation of most, if not all, class II genes in yeast (Walker *et al.*, 1996).

The inactivation of one of the subunits, $TAF_{II}145$, was particularly interesting as it led to a specific arrest of cells in the G1/S phase of the cell cycle. Thus $yTAF_{II}145$, like its human homologue $TAF_{II}250$, is required for G1/S progression, indicat-

ing a functional conservation through evolution. The expression of *CLN1*, *CLN2*, *PCL1* and *PCL2*, which encode cyclin proteins needed for progression from G1 to S, was downregulated rapidly after inactivation of yTAF$_{II}$145 (Walker *et al.,* 1997). *RPS* genes, encoding proteins of the small ribosomal subunit, are also dependent on yTAF$_{II}$145 function (Shen and Green, 1997).

We are also using the yeast microarray technique for whole-genome transcription analysis to detect the effect of yTAF$_{II}$145 inactivation on gene expression. This technique analyses the expression from the entire set of about 6,200 yeast open reading frames under different conditions, i.e., growth in different media or in a mutant versus the corresponding wildtype strain. Inactivation of RNA polymerase II abolished the transcription of nearly all these genes, indicating the general importance of RNA polymerase II in transcription (*Fig. 58*). In contrast, when TAF$_{II}$145 was inactivated, the expression of 1,400 genes increased or decreased (*Fig. 59*) and 540 of these showed decay kinetics similar to those observed after inactivation of RNA polymerase II. Thus TAF$_{II}$145 is directly required for proper expression of about 10% of the genes in yeast but not required for the other 90%. The genes that were most clearly dependent on TAF$_{II}$145 for expression encoded proteins involved in cell-cycle control or chromosome metabolism (*Table 7*).

To identify the determinants for TAF$_{II}$145 dependence, chimerical promoters were constructed where the UAS for a typical TAF$_{II}$145-dependent promoter was fused to the core promoter of a TAF$_{II}$145-independent gene and vice versa. These experiments showed that the determinant for TAF$_{II}$145-dependence is localized to the core promoter rather than to the UAS, a somewhat surprising result given that several reports suggest a role for the TFIID complex, i.e., TBP and TAF$_{II}$s, in contacting upstream-bound transcriptional activator proteins (Burley and Roeder, 1996; Pugh, 1996; Ranish and Hahn, 1996). However, it agrees well with results that indicate the TFIID complex makes

Table 7. *Genes that require $TAF_{II}145$ function.*

Gene	Description	Fold reduction†
Cell cycle		
*DDC1**	DNA damage checkpoint protein	10
YER066W	Similar to CDC4, which degrades G1 cyclins	9
SPO1	Possible role in spindle pole body duplication	8
*LTE1**	GDP/GTP exchange factor	8
*MKK2**	Kinase involved in cell wall integrity	8
*BIM1**	Possible role in early spindle pole body assembly	8
*MDM1**	Involved in mitochondrial segregation	7
*CTR9**	Required for normal expression of G1 cyclins	7
*PAC1**	Possible role in spindle pole body orientation	6
*SCP160**	Involved in control of chromosome transmission	6
CDC13	Telomere-binding protein	6
*TOP3**	DNA topoisomerase III	5
*TRX1**	Thioredoxin I	5
ARD1	*N*-Acetyltransferase	5
*SCC2**	Required for sister chromatid cohesion	5
*CLB2**	G2/M cyclin	5
*KIP2**	Kinesin-related protein	5
*MEC1**	Cell-cycle checkpoint protein	4
RAD9	DNA repair checkpoint protein	4
*SPC98**	Spindle pole body component	4
*BCK1**	Kinase involved in cell wall integrity	4
DNA repair		
*RAD3**	Involved in nucleotide excision repair	8
*YHR031C**	Possible role in chromosome repair	7
*RAD5**	Involved in DNA repair	6
*HSM3**	Involved in mismatch repair	6
*RAD50**	Involved in recombinational repair	5
*EXO1**	Involved in mismatch repair	5
*MSH3**	Involved in mismatch repair	5
YER041W	Similar to DNA repair protein Rad2	5
REV1	Involved in translesion DNA synthesis	4
HDF2	Involved in DNA end-joining repair pathway	4
MSH6	Involved in mismatch repair	4
DNA synthesis		
*MCM3**	Involved in replication initiation, MCM/P1 family	13
RLF2	Chromatin assembly complex, subunit 2	9
*MCM6**	Involved in replication initiation, MCM/P1 family	9
REV7	DNA polymerase subunit zeta	7
*MIP1**	Mitochondrial DNA-directed DNA polymerase	6
*CDC47**	Involved in replication initiation, MCM/P1 family	6
*CDC5**	Kinase	5
*CDC46**	Involved in replication initiation, MCM/P1 family	5
*RFC1**	DNA replication protein RFC large subunit	5
*CAC2**	Chromatin assembly complex, subunit 1	5

*Gene exhibits equivalent dependence on $TAF_{II}145$ and Rpb1 for normal expression.
†Reduction in expression after $TAF_{II}145$ inactivation. Reproduced with permission from Holstege *et al.*, 1998.

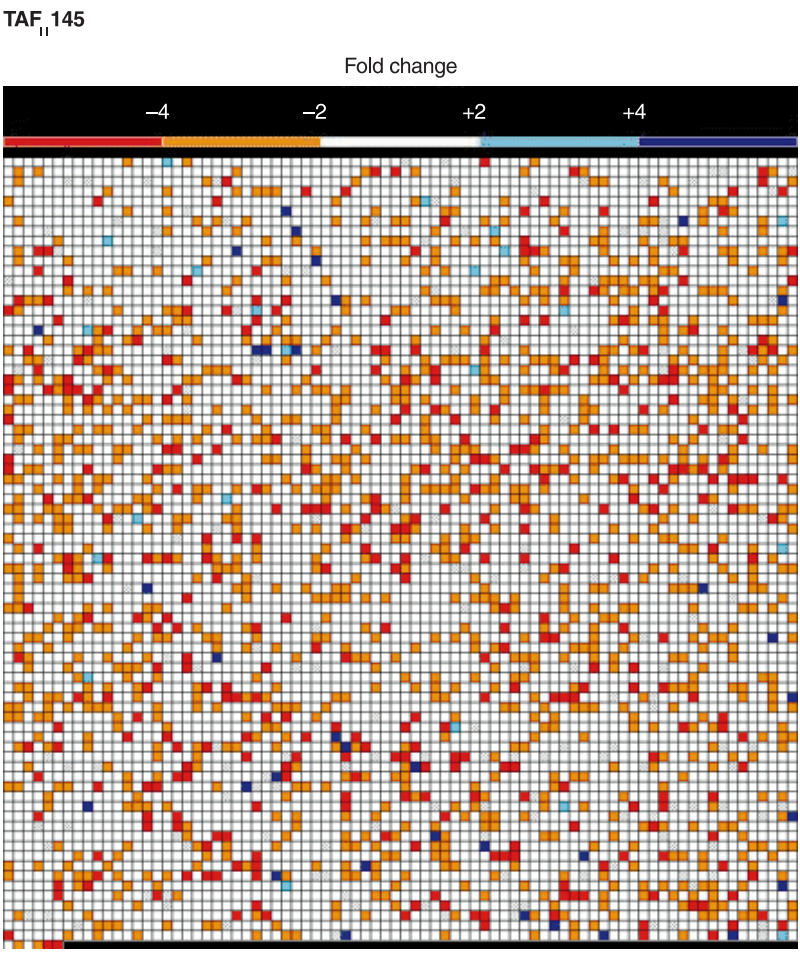

Figure 59. *Microarray technique showing changes in expression of the yeast open reading frames after inactivation of yeast* $TAF_{II}145$ *using a mutant strain carrying a temperature-sensitive mutation in yeast* $TAF_{II}145$.

contact with the core-promoter DNA surrounding the TATA box (Oelgeschlager *et al.,* 1996).

The microarray assays also showed that the expression of a limited number of genes increased after inactivation of $TAF_{II}145$ (*Fig.* 59). This set of genes had some features in common with genes that are repressed under normal growth conditions. *HXT5*

encodes an alternative hexose transport protein that is induced when glucose is removed from the medium, *ARG3* is normally not expressed unless cells are grown in the absence of arginine, *PRB1* is induced when cells are deprived of nutrients or induced to enter the stationary phase and the *DAN1* gene is expressed under anaerobic conditions and completely repressed during aerobic growth. These genes could have been induced as a result of secondary effects caused by downregulation of one of several genes after $TAF_{II}145$ inactivation. However, induction is more likely to be direct because it is as rapid as the effects on the expression of genes that are downregulated after $TAF_{II}145$ inactivation; and it is specific for $TAF_{II}145$ and is not seen after inactivation of other TAF_{II}s or in *cdc* mutants that also arrest in the G1 phase of the cell cycle. Lastly, the same results are obtained when using constructs containing only the core promoter of the respective genes.

Functional studies of $yTAF_{II}17$ in vivo

Yeast $TAF_{II}17$ is the homologue of human $TAF_{II}32$ and *Drosophila* $TAF_{II}40$ and is one of the TAF_{II}s containing a histone-fold motif similar to that found in histone H3. The human and *Drosophila* homologues can make protein–protein interactions with TFIIB and acidic activators (Klemm *et al.,* 1995) but also make DNA contacts with sequences downstream from the TATA box to the downstream promoter element (Burke and Kadonaga, 1996). $TAF_{II}17$ has also been identified more recently as a subunit of the yeast SAGA complex (Grant *et al.,* 1998; see Workman, this volume).

We studied the in vivo function of $yTAF_{II}17$ by generating a temperature-sensitive mutation in yeast *$TAF_{II}17$* combined with the microarray technique. Inactivating $TAF_{II}17$ produced a twofold or more downregulation of 72% of all genes, so its effects are more general than those previously obtained by inactivating $yTAF_{II}90$ and $yTAF_{II}145$. Most of these transcripts

showed decay kinetics similar to those observed after inactivation of RNA polymerase II. However, no general pattern of dependency of specific genes on particular TAF_{II}s has emerged. For example, expression of *ADH1*, encoding alcohol dehydrogenase, is completely dependent on $TAF_{II}17$ but not affected by $TAF_{II}145$ inactivation; *RPS5* is dependent on both $TAF_{II}17$ and $TAF_{II}145$; *PCL1*, encoding G1 cyclin, is dependent on $TAF_{II}145$ but not $TAF_{II}17$; and expression of *DED1*, encoding a putative RNA helicase of the DEAD-box family, is independent of both $TAF_{II}17$ and $TAF_{II}145$.

The microarray technique as we used it is limited in that it monitors the effect of inactivation only on genes that are expressed under normal growth conditions; it does not show changes in genes whose expression is induced in response to other stimulation. Studies of various inducible genes showed that they also differ in their requirement for particular TAF_{II}s. For example, the heat-shock-inducible *SSA4* and the copper-inducible *CUP1* genes (see Sakurai, this volume) are independent of $TAF_{II}17$, whereas induction of *HIS4* by 3-aminotriazole requires $TAF_{II}17$. The determinants for $TAF_{II}17$-dependence, analysed using chimerical promoters, seem to be located within the UAS, unlike those for $TAF_{II}145$ (Apone *et al.*, 1998).

Effects of activators on TFIID binding to the TATA box

These results question the established view that transcriptional activators operate by interacting with TAF_{II}s in the TFIID complex to affect the interaction of TFIID with the TATA box. In this model (see *Box 1*), the binding of TBP in the presence of TFIID is the key step in assembling the preinitiation complex, which subsequently leads to activation of transcription. We therefore decided to examine the effect of transcriptional activators on occupancy of TBP at the TATA box. In prokaryotes, activators can work in two ways, either bringing the RNA polymerase to the promoter or acting on RNA polymerases already present on the promoter. In vitro exper-

iments in eukaryotic systems have indicated that when activators are added, they can act on promoters to which the TBP is already bound. Footprinting analysis shows that in some cases TBP is bound to the promoters before induction, in other cases it is not (Chen *et al.*, 1994*b*). Our results show a clear correlation between transcription levels and TBP occupancy at the TATA box for a large set of promoters in combination with mutations in genes encoding:

- general transcription factors, i.e., TFIIB;
- inhibitors of TBP–TATA interactions, i.e., *MOT1*;
- TAF_{II}s;
- subunits of the mediator complex, i.e., *SRB4* (X. Li *et al.*, 1999; see Struhl, this volume).

Discussion: The significance of these results is disputed because the experiments were performed under conditions that allowed transcription to take place, which makes it impossible to determine whether the effect of the transcriptional activators on TBP binding to the TATA box was direct. It could be an indirect consequence of activated levels of transcription induced by pathways other than those operating directly on TBP. Furthermore, the method used does not detect protein associated with the gene during the activation process but rather proteins present on the transcribed gene at steady state.

Mechanism for in vivo transcriptional activation and repression in yeast

Kevin Struhl

in collaboration with
Zarmik Moqtaderi, David Kadosh, Laurent Kuras and Marie Keaveney

Transcriptional activation involves the binding of a transcriptional activator to a *cis*-acting sequence, termed an enhancer. Most activators have a modular organization, with a DNA-binding domain that targets the factor to a specific enhancer sequence and an activation domain that permits activation of transcription of the promoter under control of the enhancer sequence. Activation domains may enhance transcription by recruiting the polymerase II machinery (Ptashne and Gann, 1997), by altering the conformation of components of the polymerase II machinery (Roberts and Green, 1994) and by modifying chromatin structure (reviewed in Workman and Kingston, 1998). The relative importance of these three mechanisms has yet to be estimated.

We discuss here three aspects of the in vivo stimulation of transcription by transcriptional activators in yeast: recruitment of the polymerase II transcription apparatus; recruitment of TBP, an important step in the activation process; and the importance of various TAFs that are required for the response to activators. We also provide evidence for the intimate relationship of transcription with some specific chromatin modifications to support a mechanism of transcription repression through localized histone deacetylation.

Recruitment of the RNA polymerase II machinery

To estimate the importance of the polymerase recruitment mechanism, we transferred activation domains from their normal location on the enhancer-bound protein (*Fig. 60a*) to different

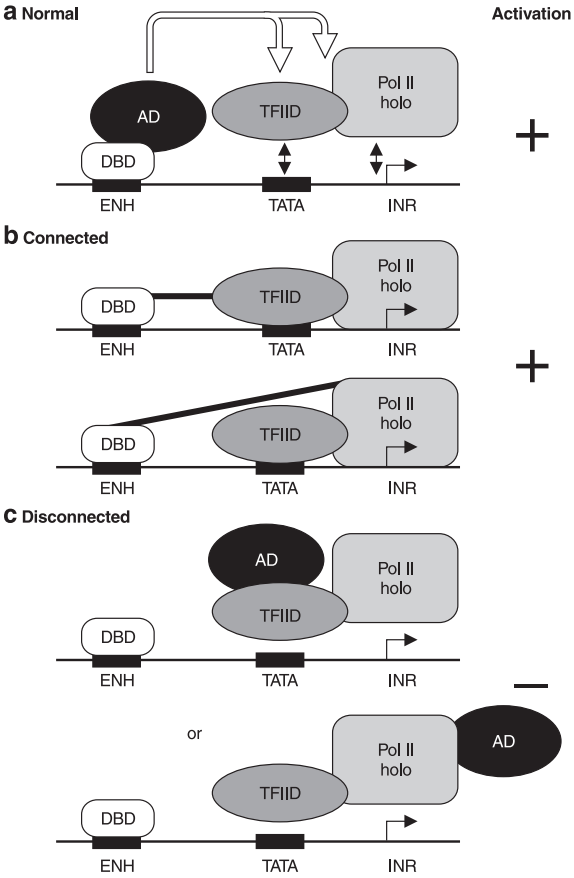

Figure 60. *Normal organization of transcriptional activators and the polymerase II machinery at promoters and connected and disconnected constructs used to examine the polymerase recruitment mechanism.* ***a****, the physiologically relevant organization, in which activator proteins bind enhancer elements (ENH) through specific DNA-binding domains (DBD) and stimulate transcription through functionally autonomous activation domains (AD). Open arrows, interactions between activation domains and the TFIID and/or the polymerase II holoenzyme (Pol II holo) complexes; the direct targets within these complexes are not specified. Filled arrows between TFIID and the TATA element and between the polymerase II holoenzyme and the mRNA initiation site (INR) indicate activators increasing recruitment of the polymerase II machinery to promoters.* ***b****, in* →

components of the RNA polymerase II machinery, either in the TFIID complex or in the polymerase II holoenzyme complex (*Fig. 60c*). Alternatively, the need for the activator was bypassed by connecting the enhancer-bound protein to a component of either TFIID or the polymerase II holoenzyme (*Fig. 60b*; Keaveney and Struhl, 1998). The TFIID components include TBP and approximately ten associated factors, among which are two TAFs required for cell viability in yeast: TAF17, homologue of *Drosophila* TAF17; and TAF23, homologue of human TAF30. Two components of the holoenzyme, Srb2 and Gal11 (see Sakurai, this volume) that are not essential for growth were also used to anchor activation domains. The function of these constructs was assayed using either the *GAL1* promoter and measuring β-galactosidase activity, or the *HIS3* promoter and measuring growth on selective media.

The polymerase II machinery containing a functionally autonomous activation domain could neither activate transcription from core promoter nor synergize with other activators to support high levels of transcription in the cases we tested. Thus the presence of the activation domain within the polymerase II machinery does not bypass the need for a DNA-binding protein to interact with enhancer sequences, indicating that the DNA-binding domain can serve as an anchor to recruit the polymerase II machinery (Keveaney and Struhl, 1998).

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the connected construct, transcriptional activation is achieved (+) in the complete absence of an activation domain by physically connecting (thick bold line) a component of either TFIID or the polymerase II holoenzyme to an enhancer-bound protein, which artificially recruits the polymerase II machinery to promoters and bypasses the need for an activation domain. ***c****, in the disconnected construct, the activation domain is transferred from its normal location on the enhancer-bound protein to a component of either TFIID or the polymerase II holoenzyme; transcriptional activation does not occur (–). Reproduced with permission from Keaveney and Struhl, 1998.*

By measuring TBP occupancy at several promoters under various conditions in vivo with or without activation we established that TBP is generally required for polymerase II transcription. To do this, we used yeast strains producing a protein tagged with TBP and immunoprecipitated chromatin with antibodies directed against the tag. Under activation conditions, the *GAL1* and heat-shock genes displayed high occupancy and quantitative measurement of various genes showed a tight correlation between gene activity and TBP occupancy, indicating that TBP occupancy may be a limiting step in the mechanism of transcriptional activation. The *CYC1* and *COX5a* promoters were exceptions to this rule as they displayed a constitutive level of TBP occupancy with or without induction and so may have a distinct mode of regulation. Conversely, genes that can be repressed also showed a correlation between TBP occupancy and gene activity. Other issues include the exact target of an activator in vivo; whether one or more targets are involved; and, if more than one, whether they all contribute to the activation. Artificial recruitment experiments indicate several targets within the promoter (Gaudreau *et al.*, 1999).

The importance of TAFS specifically found in TFIID, i.e., TAFs 19, 40, 67 and 130 (see *Box 2*), was evaluated by removing the TAFs using a 'copper-induced double shut-off', which causes the repression of TAF transcription, so that pre-existing TAF protein becomes a target for ubiquitin-mediated degradation (Moqtaderi *et al.*, 1996*b*). Although we did not investigate the selective effects on transcription of depleting each TAF, they all affected the *HIS3* promoter, which has one weak and one strong TATA box, by reducing transcription only from the weak one (Moqtaderi *et al.*, 1998). Hence specific features of the core promoter region can be critical. Because TFIID is the only complex known to contain these TAFs, it is probably critical for the function of the *HIS3* core promoter. Yeast cells with TBP replaced by a human TBP are interesting because they displayed

properties very similar to those of cells with mutations in the TAFs specific to TFIID, further supporting the argument that TFIID has an important role in the function of core promoter.

In contrast, depletion of TAF17, which is common to the TFIID and SAGA HAT complexes, had a more dramatic effect, causing a decrease in transcription of most, although not all, genes. This may be the result of a combination of defects in both the TFIID and SAGA complexes and indicates that TAF17 is broadly required for transcription (Apone *et al.*, 1998; see Green, this volume).

Transcriptional repression through targeted deacetylation

Specific sequences with particular properties in terms of their nucleosomal organization may help regulate transcription, providing a link between chromatin organization and the regulation of transcription. Repetitive sequences, such as the poly-d(A–T) tracks that are the most common element in yeast promoter, are intriguing as they are important in transcription regulation in vivo. Such sequences could prevent the formation of stable nucleosomes, so making the sequences more accessible for a potential transcription factor. However, no specific factor binding to these sequences has been identified; the AT-binding factor 1 does not bind to the perfect poly-d(A–T) track.

Recent work has focused on the repression of transcription involving deacetylation of histones (reviewed in Struhl, 1998). Proteins with the properties of histone deacetylase were identified as homologues of the product encoded by the *RPD3* gene in yeast (Taunton *et al.*, 1996). Yeast cells lacking Rpd3 are viable but show selective defects of gene expression, so a key question is how the selectivity/specificity is obtained. The mechanism of acetylation could be untargeted, generally targeted to a promoter over large sequence regions or tightly targeted to very specific sites within a promoter (*Fig. 61*).

In yeast, the transcription of one set of genes, which includes

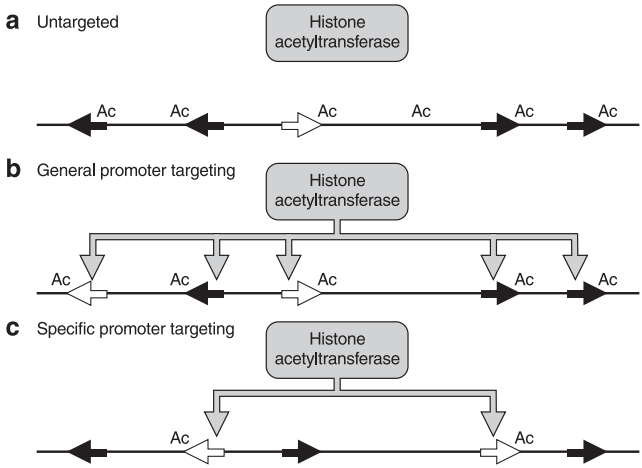

Figure 61. *Three ways in which histone acetyltransferases could selectively affect transcriptional activity. For each model, sites of acetylation (Ac) are indicated with respect to five promoters that either are (unfilled arrows) or are not (filled arrows) affected by acetylation.* ***a****, histone acetyltransferases are not targeted and histone acetylation occurs at promoter and non-promoter regions.* ***b****, histone acetyltransferases are generally targeted to promoters (grey arrows) because they associate with a general component of the polymerase II transcription machinery.* ***c****, histone acetyltransferases are targeted to specific promoters (grey arrows) by gene-specific activator proteins, leading to selective effects on transcription. In* ***a*** *and* ***b****, selective effects on transcription can be attributed to inherent differences in the state of histone acetylation of the promoters. Reproduced with permission from Struhl, 1998.*

those involved in meiosis, depends on Sin3 and Rpd3. Repression of the transcription of these genes provides an interesting way to approach these questions. A zinc-finger DNA-binding protein, Ume6, depends on Sin3 and Rpd3 to direct transcriptional repression. Eighty amino acids in the repression domain of Ume6 are necessary and sufficient to achieve complete repression (Kadosh and Struhl, 1997). Coimmunoprecipitation demonstrated that this region is critical for binding to Sin3, which in turn binds to the histone deacetylase Rpd3. The repression depends on the catalytic domain of the histone deacetylase, which is defined as the 30 amino acids conserved in

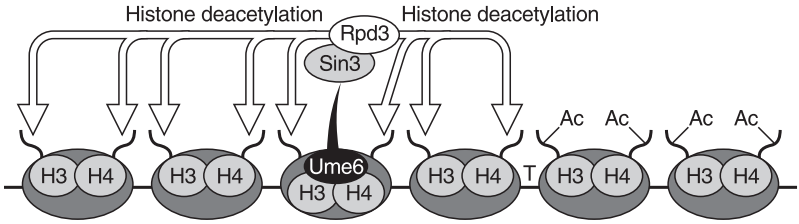

Figure 62. *Creation of a repressive chromatin domain by targeted recruitment of the Sin3–Rpd3 histone deacetylase complex. The Ume6 repressor binds URS1, shown in the context of a nucleosomal template, and recruits the Sin3–Rpd3 co-repressor complex to the promoter. As a consequence, histones H3 and H4 are deacetylated at lysines 5 and 12 and to a lesser extent lysine 16 over a range of one to two nucleosomes from the site of recruitment (indicated by absence of Ac). Arrows, sites of frequent histone deacetylation. Nucleosomes further downstream and upstream are not specifically deacetylated (indicated by Ac). The region of local histone deacetylation is defined with respect to a hypothetical promoter (see text) that includes the UAS element but probably ends upstream of the TATA elements (T). Analogous regions of other Sin3–Rpd3 repressed promoters could vary in length and position. The TATA elements are indicated in the spacer region for clarity; there is no information on the nucleosomal position of these TATA elements in vivo. Reproduced with permission from Kadosh and Struhl, 1998.*

both Rpd3 homologues and polyamine deacetylases, in which four histidines are highly conserved (Kadosh and Struhl, 1998). The deacetylase complex containing a mutation in this domain of the catalytic subunit was inactive in vitro on a synthetic peptide substrate.

Using chromatin immunoprecipitation, we have examined the nature and extent of the perturbation of chromatin in the vicinity of the repressed genes. Transcriptional repression was associated with promoter-specific deacetylation of histones H4 and H3. Precisely mapping the preferentially deacetylated domain delineated a region of 288 bp with its centre at the URS1 element, the Ume6-binding site. These data support a model in which limited deacetylation is promoted by specific targeting of the deacetylase activity through an adaptor, here Sin3, to a specific DNA-binding protein (*Fig. 62*).

The role of $TAF_{II}30$-containing complexes in vertebrate gene regulation

Làszlò Tora

in collaboration with

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The observation that transcriptional activation in reconstituted animal cell-free transcription systems is not supported by TBP but requires TFIID (Goodrich *et al.*, 1996) has triggered an intensive study of the TAF_{II}s as essential transcriptional coactivators. In human HeLa cells, several TAF_{II}-containing TFIID complexes with distinct functional properties have been described (Brou *et al.*, 1993*a*; 1993*b*; Jacq *et al.*, 1994). Using antibodies against $TAF_{II}30$, we found two TFIID populations that differ in their subunit composition: both TFIIDα and TFIIDβ contain TAF_{II}s 250, 135, 80, 55 and 28, whereas TAF_{II}s 30, 20 and 18 are preferentially associated with TFIIDβ (Jacq *et al.*, 1994; Mengus *et al.*, 1995).

We show here that $TAF_{II}30$ is also present in another complex containing a subset of TAF_{II}s, as well as other components that include the Gcn5-L HAT enzyme, although it lacks TBP. This TBP-free, TAF-containing (TFTC) complex is interesting because it accurately engages RNA polymerase II to promoters to allow transcription in vitro. We also demonstrate that $TAF_{II}30$ is required for cell-cycle progression and for parietal endodermal differentiation through conditional disruption of the two alleles of the $TAF_{II}30$ gene in F9 embryonal carcinoma cells. Thus $TAF_{II}30$ is critical in the regulation of a subset of cell-cycle and differentiation genes in vivo.

Complexes containing $TAF_{II}30$

$TAF_{II}30$ is present in several distinct complexes (Wieczorek *et al.*, 1998). HeLa cell extracts were immunopurified with a monoclonal antibody directed against specific $TAF_{II}30$, the precipitated

material eluted with a peptide corresponding to the epitope of this antibody and the eluted fraction reprecipitated with an antibody against TBP. The supernatant fraction surprisingly still contained $TAF_{II}30$, so not all $TAF_{II}30$ is associated with TBP. This fraction, named TFTC, also contained TAF_{II}s 135, 100, 80, 55, 31 and 20 and several unidentified components but TBP, $TAF_{II}250$ and $TAF_{II}28$ were absent (*Fig. 63*; Wieczorek *et al.*, 1998).

TFTC binds specifically to promoter sequences, substituting for TBP and TFIID in both activated and basal transcription from TATA-containing and TATA-less promoters in vitro (Wieczorek *et al.*, 1998). This indicates that TBP is not absolutely required for transcription in vitro. TFTC is also devoid of a second TBP-like factor that is highly homologous to the core domain of TBP. We therefore question the long-standing assumption that TBP is a universal and indispensable transcription factor (Dantonel *et al.*, 1999).

The non-TAF_{II} components of TFTC include Gcn5-L, an isoform of the human homologue of the yeast Gcn5 HAT enzyme (Brand *et al.*, 1999). TFTC is therefore similar to the yeast SAGA and human PCAF complexes (Grant *et al.*, 1998; Ogryzko *et al.*, 1998; *Box 2*; see Allis, Workman, this volume). There are several striking parallels in the composition of the TFIID, TFTC, PCAF and SAGA complexes. All contain a HAT enzyme, a protein with WD40 repeats (see *Glossary*) and at least five histone-fold motifs (*Box 2*). The PCAF and SAGA complexes contain $TAF_{II}31$, $TAF_{II}30$ and $TAF_{II}20$ or their yeast homologues, as well as the PCAF subunit or the highly related yeast Gcn5. In addition, SAGA contains the yeast homologues of $TAF_{II}100$ and $TAF_{II}80$, both of which are found in TFTC. The PCAF complex on the other hand contains PCAF-associated factor (PAF) 65β, which like $TAF_{II}100$ contains WD40 repeats, and PAF65α, which like $TAF_{II}80$ contains a histone-fold motif complementary to that of $TAF_{II}31$. On the other hand, TFTC could be a mixture of PCAF and a second uncharacterized complex containing

Box 2

Comparison of subunit composition of TAF_{II}-containing complexes

	Containing TBP			Lacking TBP			
Protein characteristics	**hTFIID**	**dTFIID**	**yTFIID**	**ySAGA**	**hTFTC**	**hPCAF/ Gcn5**	**hSTAGA**
***a*, TAF_{II}s**							
HAT and bromo domain	250 (CCG1)	250 (230)	145 (130)	–	–	–	–
Initiator/DNA binding	150	150	TSM1	–	150	ND	–
–	135	110	–	–	135	–	–
WD40 repeats	100	80	90	90	100	100*	–
Histone H4-like	80 (70)	60 (62)	60	60	80	– (PAF65α)	–
–	55	ND	67	–	55*	ND	ND
Histone H3-like	31 (32)	40 (42)	17 (20)	17 (20)	31 (32)	31 (32)	31 (32)
–	30	ND	25 (23)	25	30	30	30
–			30 (ANC1)				
Histone-like	28	30β	40	–	–	ND	–
Histone H2B-like	20 (15)	30α (28/22)	68 (61)	68 (61)	20 (15)	20 (15)	20 (15)
Histone H4-like	18	ND	19 (FUN81)	–	–	ND	–
TATA box binding	TBP	TBP	TBP	–	–	–	–
***b*, Spts, Adas, Gcn5 and other components**							
HAT and bromo domain				yGcn5	hGcn5	hPCAF/ Gcn5-S	Gcn5
				yAda1	ND	ND	ND
				yAda2	–	hAda2	ND
				yAda3	hAda3	hAda3	hAda3
				ySpt3	hSpt3	hSpt3	hSpt3
				ySpt7	ND	ND	ND
				ySpt8	ND	ND	ND
				ySpt20 (yAda5)	ND	ND	ND
WD 40 repeats				ND	hPAF65β	hPAF65β	hPAF65β
				yTRA1	hTRRAP	hTRRAP	hTRRAP
				Other uncharacterized proteins			

The numbers in parenthesis indicate alternative nomenclatures used in the literature.
ND, not determined; Ada, alteration deficiency in activation, and Spt, suppressor of Ty insertion, are proteins found in various yeast genetic screens
*, factor is substoichiometric in the respective complex.

Subunit composition of TAF_{II}-containing complexes: ***a***, left, the composition of TFIID complexes from human (h), *Drosophila* (d) and yeast (y); right, a comparison of the TAF_{II}s present in the SAGA, TFTC, PCAF and hSTAGA (human Spt–TAF–Gcn5–acetyltransferase) complexes. The TAF_{II}s in human TFIID are arranged vertically by their apparent M_r from 250K to 18K; TAF_{II}s homologous to each are shown on the same line. ***b***, other proteins found in the complexes.

Some of the characteristic features of each protein are indicated on the left. The TFIID, TFTC, PCAF and SAGA complexes all contain a histone acetyltransferase (HAT) enzyme, either $TAF_{II}250$, hGcn5-L, PCAF or yGcn5; a WD40 repeat protein (see *Glossary*), such as $hTAF_{II}100$, PAF65β or $yTAF_{II}90$; and five or more histone-fold motifs: in TFIID these are found in $hTAF_{II}80$, 31, 28, 20 and 18; in TFTC, PCAF and STAGA they are in $hTAF_{II}80$ (or PAF65α), $TAF_{II}20$ and hSpt3, which contains two complementary histone-fold motifs analogous to those of $hTAF_{II}28$ and $hTAF_{II}18$ of the TFIID complex (Birck *et al.*, 1998); in SAGA, they are in $yTAF_{II}68$, 60, 17 and 3. hSTAGA is found in HeLa cells and acetylates histone H3 (Martinez *et al.*, 1998); its role is not yet clear. Some data taken from Struhl and Moqtaderi, 1998.

L. Tora, K. Struhl

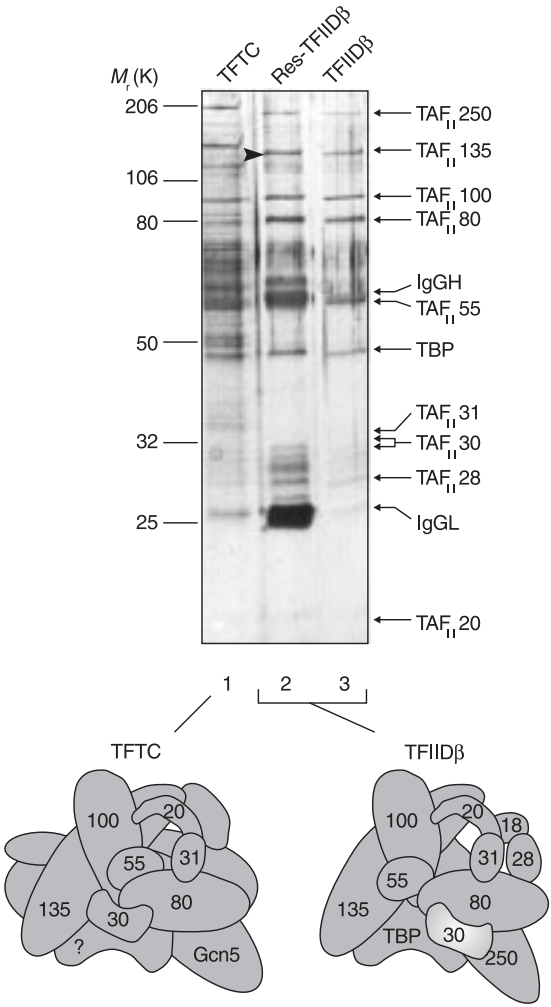

Figure 63. *TAF_{II}s are present in the TBP-free, TAF_{II}-containing (TFTC) complex. Above, TFTC remained in the supernatant after immunoprecipitation (lane 1), whereas the TFIIDβ complex bound to the anti-TBP antibody resin (Res; lane 2), from which it was eluted (lane 3).* M_r *standards are shown on the left and the positions of the TAF_{II}s and TBP on the right. IgGH and IgGL, immunoglobulin heavy and light chains. Below: the subunit compositions of the two complexes are shown schematically. Subunit composition of the complexes were revealed by silver nitrate staining of an SDS polyacrylamide gel.*

$TAF_{II}30$ along with $TAF_{II}135$, $TAF_{II}100$ and $TAF_{II}80$, which have not been reported as PCAF components.

The physiological significance of the conservation of these structural motifs is unclear. The histone-fold proteins were originally proposed to form an octamer-like structure (Xie *et al.*, 1996) but at least five histone-fold motifs in each complex are now known, which is too many to be accommodated in a simple octamer-like model. The histone fold does nevertheless provide a very tight interaction interface, perhaps facilitating the high-affinity interactions required for the formation of these stable macromolecular complexes. The similarity among these complexes does, however, indicate that they have similar and perhaps even overlapping roles in gene regulation (*Fig. 64*).

Requirement for $TAF_{II}30$ in cell-cycle progression

Because genetic experiments in yeast have shown that TAF_{II}s are essential for viability (Moqtaderi *et al.*, 1996*b*), the classic knock-out strategy seemed to be inappropriate for testing the role of TAF_{II}s in mammalian cells in vivo. Instead, we have used a conditional Cre/LoxP-mediated strategy (Sauer and Henderson, 1990; see *Glossary*) to inactivate $TAF_{II}30$ in mouse F9 embryonal carcinoma cells. The production of only a very short N-terminal $TAF_{II}30$ peptide was achieved with a targeting vector designed to insert a LoxP site in the intron between exons 1 and 2 in the mouse *$TAF_{II}30$* gene, and a neomycin-resistance gene (*Neo*^{r}) flanked by LoxP sites in the intron between exons 2 and 3. Treatment with the Cre recombinase then causes deletion of exon 2. One *$TAF_{II}30$* allele was modified with this vector by homologous recombination and when the gene encoding the Cre recombinase was introduced into cells harbouring the modified allele, the *Neo*^{r} gene was deleted, sometimes with exon 2 as well. These *$TAF_{II}30^{+/-}$* heterozygous cells were fully viable, so the second allele also had to be inactivated. The second allele was targeted using a similar vector in a cell clone with the *Neo*^{r} gene deleted, leaving exon 2 surrounded by

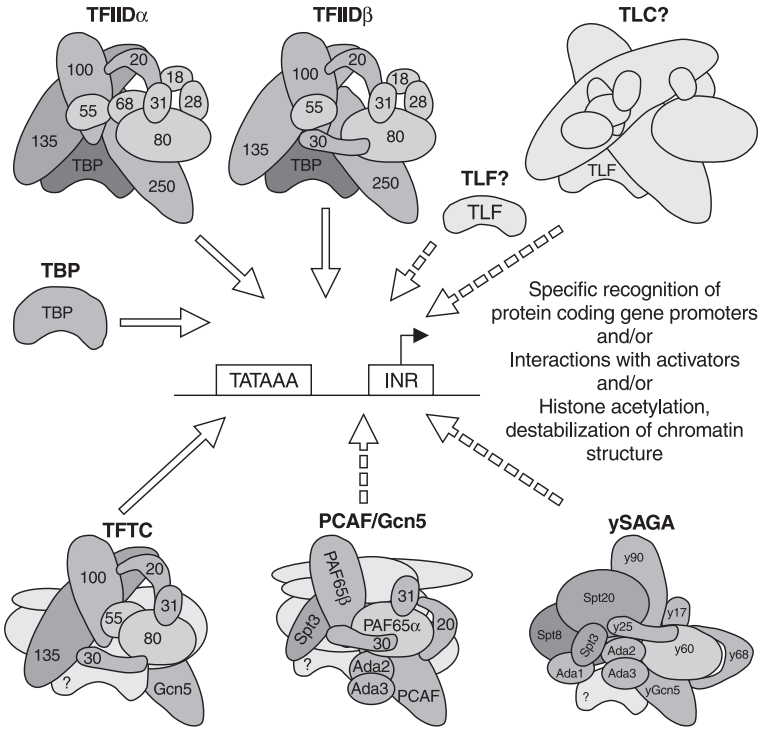

Figure 64. *Possible roles of TAF_{II}-containing complexes in regulating transcription. The subunit composition of the different complexes is shown schematically. Dashed arrows indicate that the human PCAF and the yeast (y) SAGA complexes have not yet been shown to function in the initiation of transcription by RNA polymerase II. TLF, TBP-like factor; TLC, hypothetical TBP-like factor complex; INR, initiator sequence. Ada, alteration deficiency in activation, and Spt, suppressor of Ty insertion, are proteins found in various yeast genetic screens.*

LoxP sites. After treatment with the Cre recombinase, no clones of $TAF_{II}30^{-/-}$ homozygous cells were obtained, indicating that the cells are not viable and that $TAF_{II}30$ is essential for cell survival (Metzger *et al.*, 1999).

That $TAF_{II}30$ is required for cell-cycle progression in mammalian cells was established by creating cells in which the expression of h$TAF_{II}30$ could be controlled. The h$TAF_{II}30$ cDNA

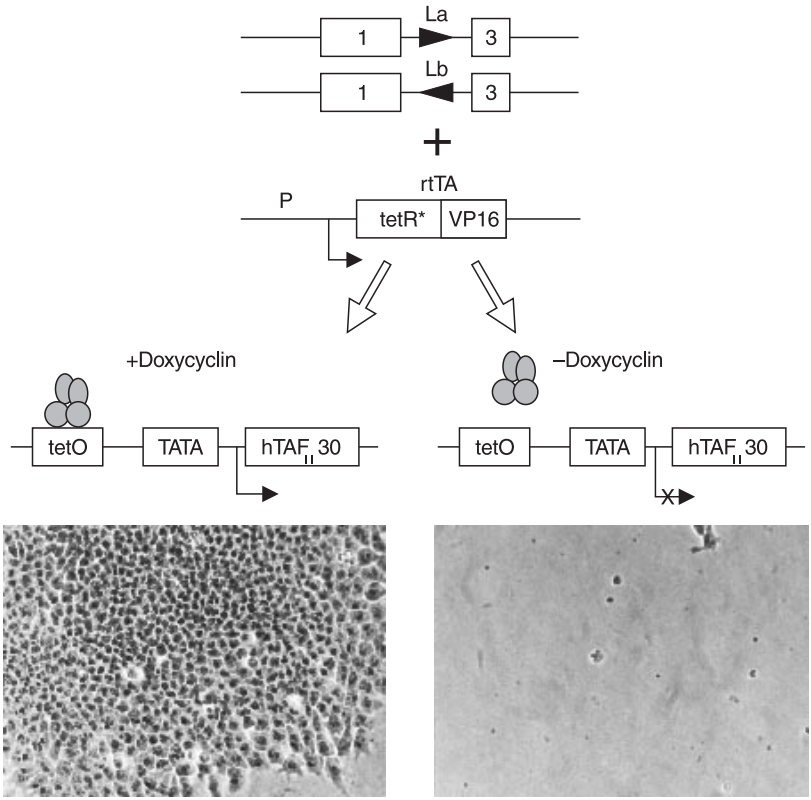

Figure 65. *F9 embryonal carcinoma cells lacking* $TAF_{II}30$ *are not viable. Top,* $TAF_{II}30^{-/-}$ *F9 cells (alleles La and Lb deleted for exon 2) carrying a* $hTAF_{II}30$ *cDNA inducible with the tetracycline analogue, doxycyclin, were constructed. The reverse tetracycline repressor molecule bound to the promoter of the* $hTAF_{II}30$ *transgene in the presence of doxycyclin or unbound in the absence of doxycyclin is shown schematically. Transcription of this cDNA rescued the* $TAF_{II}30^{-/-}$ *cells. Bottom, cells are viable when doxycyclin is present (left) but most cells died after 5 days in the absence of doxycyclin (right). P, promoter; rtTA, reverse tetracycline (tet) repressor; tetR*, tet repressor; VP16, VP16 acidic activator domain; tetO, tet operator.*

was stably introduced under the control of a promoter inducible with doxycyclin, an analogue of the antibiotic tetracycline, into cells containing two *$TAF_{II}30$* alleles surrounded by LoxP sites and producing the reverse tetracycline-repressor activator protein. Thus the production of the $hTAF_{II}30$ protein could be induced by the addition of doxycyclin (*Fig. 65*). After treating these cells with Cre recombinase, we isolated *$TAF_{II}30^{-/-}$* cell clones that were viable because they produce the $hTAF_{II}30$ protein. However, production of $hTAF_{II}30$ protein was shut off in these cells when doxycyclin was removed from the culture medium and within 2 days $hTAF_{II}30$ could not be detected. After 5 days the *$TAF_{II}30^{-/-}$* cells were blocked in the G_1/G_0 phase of the cell cycle and underwent apoptosis (*Fig. 65*).

We have also shown that $TAF_{II}30$ is necessary for the activation of a subset of genes required in the differentiation of F9 cells into parietal endoderm. Treatment of F9 cells with all-*trans* retinoic acid promotes their differentiation into primitive endodermal-like cells, whereas retinoic acid and dibutyryl cyclic AMP together promote differentiation into parietal endodermal cells. The differentiation is associated with the activation of many target genes and changes in both cell morphology and cell cycle. After withdrawal of doxycyclin, the *$TAF_{II}30^{-/-}$* cells can still, surprisingly, differentiate into primitive endodermal-like cells but they cannot differentiate into parietal endoderm (Metzger *et al.*, 1999).

The requirement for $TAF_{II}30$ both in progression through the cell cycle and in differentiation indicates that it is involved in the correct regulation of a subset of cellular promoters. It remains to be seen whether the defects in the *$TAF_{II}30^{-/-}$* F9 cells result from perturbing the function of the TFIID complex or the TFTC/PCAF complex, or a combination of both.

TFIIH and the transcription-repair syndromes

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in collaboration with

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TFIIH is a large protein complex involved in transcription, DNA repair and cell-cycle regulation (Hoeijmakers *et al.*, 1996; Svejstrup *et al.*, 1996). Mutations in TFIIH subunits, such as the two DNA helicases/ATPases xeroderma pigmentosum complementation groups B and D (XPB and XPD), have been associated with the genetically heterogeneous human diseases commonly referred to as repair syndrome diseases: xeroderma pigmentosum, Cockayne's syndrome and trichothiodystrophy (Bootsma and Hoeijmakers, 1993; reviewed in Semenza, 1998).

A diverse range of clinical defects are observed in these syndromes, some of which can be explained by defects in nucleotide-excision repair, a highly conserved pathway that is largely involved in the repair of lesions in DNA caused by ultraviolet radiation. For example, characteristics of xeroderma pigmentosum include hyperpigmentation of skin exposed to the sun, cutaneous abnormalities and a predisposition to skin cancer. However, the developmental retardation and neurological abnormalities that are also features of this syndrome are less clearly related to the repair function and may arise from the transcription function of the TFIIH complex.

To identify the relationship between genotype and clinical phenotype, we have investigated the enzymatic functions of the TFIIH complex by purifying normal and mutated forms of its subunits and assaying the properties of the complex. The complex was first isolated from cells derived from patients but more recently we have used a baculovirus expression system to produce either recombinant wildtype or mutant TFIIH to analyse the functions of various subunits. We outline here that the p44 subunit is a regulatory factor for the XPD helicase; Cdk7 is

involved in the phosphorylation of the CTD of RNA polymerase II; and opening of the promoter to allow reading of the coding strand depends on XPB.

TFIIH subunits, enzymatic activities and phenotypes

Nine subunits of TFIIH have been cloned and sequenced (Gérard *et al.*, 1991; *Fig. 66*) and are highly conserved from yeast to human (Marinoni *et al.*, 1997). The core TFIIH is a complex that resists high-salt extraction and contains five subunits: p89/XPB, p62, p52, p44 and p34. The remaining subunits, which dissociate from the complex at high-salt concentrations, are p80/XPD and the ternary kinase complex, consisting of Cdk7 (MO15), cyclin H and MAT1 (for review, see Nigg, 1996), which together are referred to as Cdk7-activating kinase (CAK)/XPD (Drapkin *et al.*, 1996; Rossignol *et al.*, 1997).

We have shown that the enzymatic activities of TFIIH come from the Cdk7 kinase, which phosphorylates components of the basal transcription machinery, and the helicases XPD and XPB; XPD is a 5′-to-3′ helicase, whereas XPB is a weak 3′-to-5′ helicase (Schaeffer *et al.*, 1993; Coin *et al.*, 1999). Recombinant XPB and XPD were produced in insect cells using baculovirus infection and immunopurified with antibodies against XPB or XPD. The helicase activity of recombinant XPD increased when integrated into the TFIIH complex (Coin *et al.*, 1998*a*,*b*) and we have identified p44 as the subunit that interacts with XPD and regulates its helicase activity. This was achieved by co-infecting insect cells with two baculoviruses, one producing XPD, the other one of the core subunits of TFIIH. The only recombinant protein that co-immunoprecipitated with XPD from lysates of these cells was p44. Although this had no helicase activity itself, the helicase activity of an immobilized XPD was directly dependent on the amount of p44 present.

In patients, mutations in the XPD protein have been mapped to the C-terminal domain; trichothiodystrophy patients also have

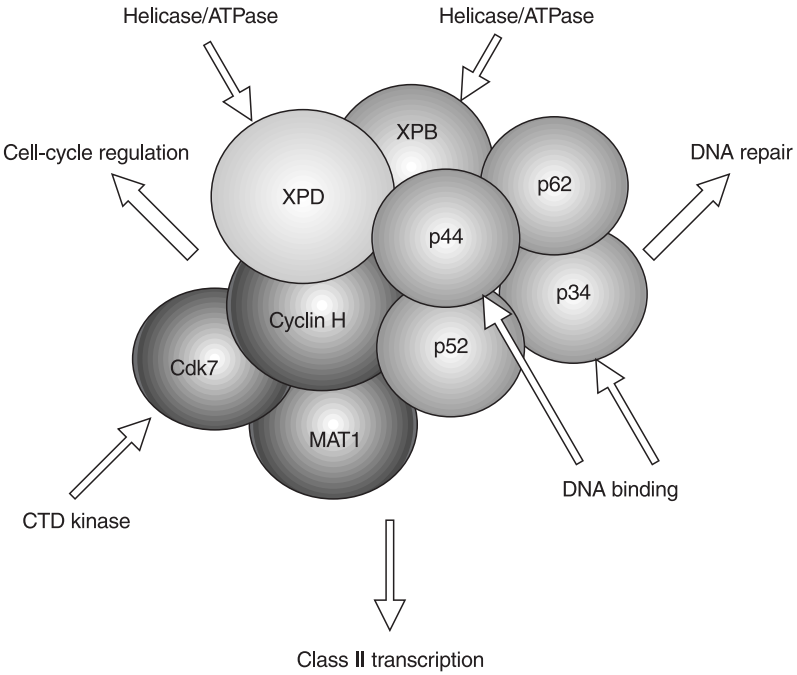

Figure 66. *TFIIH contains nine subunits ranging in size from XPB with* M_r *89K to MAT1 with* M_r *32K. The subunits resolve into two subcomplexes, the core (medium shading) and the Cdk7-activating kinase (CAK) complex (dark shading), plus the single XPD subunit (light shading).*

mutations in this domain (Taylor *et al.*, 1997). Similar mutations in the baculovirus expression system all had altered interactions with p44, leading to a weaker helicase activity, confirming that they affect the XPD–p44 interaction. Western-blot analysis of TFIIH purified from a cell line derived from patients carrying a mutated XPD demonstrated modified stoichiometry of the complex: this TFIIH lacks part of the CAK complex (Coin *et al.*, 1998*a*,*b*).

A similar study of the critical region in XPB was based on the mapping of mutations in the XPB gene found in patients with very severe disease, involving major problems in the nucleotide excision repair pathway as well as developmental abnormalities (Hwang *et al.*, 1996). We tested the consequences of these

mutations in enzymatic assays in vitro using TFIIH from cell lines derived from patients. The stoichiometry and composition of the isolated complex was not perturbed and its kinase and DNA-dependent ATPase activities were essentially similar to normal TFIIH. However, transcription was severely impaired, revealing the crucial role of XPB in this assay; footprinting indicated a defect in the opening of the promoter.

A complete reconstitution of the TFIIH complex

We have constructed a complete recombinant TFIIH complex using the baculovirus expression system (Tirode *et al.*, 1999). The five subunits corresponding to the core TFIIH are absolutely required to obtain transcription in vitro and the ATPase and helicase activities in XPB are necessary. The absence of XPB, but not of XPD, completely impairs the initiation of transcription. Analysis of the promoter opening at the initiation site, –10 to +15 from the transcription start point, revealed that XPB is critical at this step in the transcription process, confirming the footprinting analysis. The phosphorylation of the CTD of the largest subunit in RNA polymerase II is independent of this opening, which indicates that this phosphorylation is not related to the initiation of transcription.

We also tested the role of the CAK kinase in transcription. A holo-TFIIH stimulated transcription equally well from the adenovirus major late promoter and from the dehydrofolate reductase promoter. Transcription from the latter depends on phosphorylation of the CTD of the largest subunit in RNA polymerase II and the CAK complex was required for transcription from this promoter indicating that CAK is involved in phosphorylating the RNA polymerase II subunit. It is also required for transcription from the adenovirus promoter indicating that the physical presence of the CAK complex is required for optimum transcription; in this case CAK may stabilize the formation of the preinitiation complex.

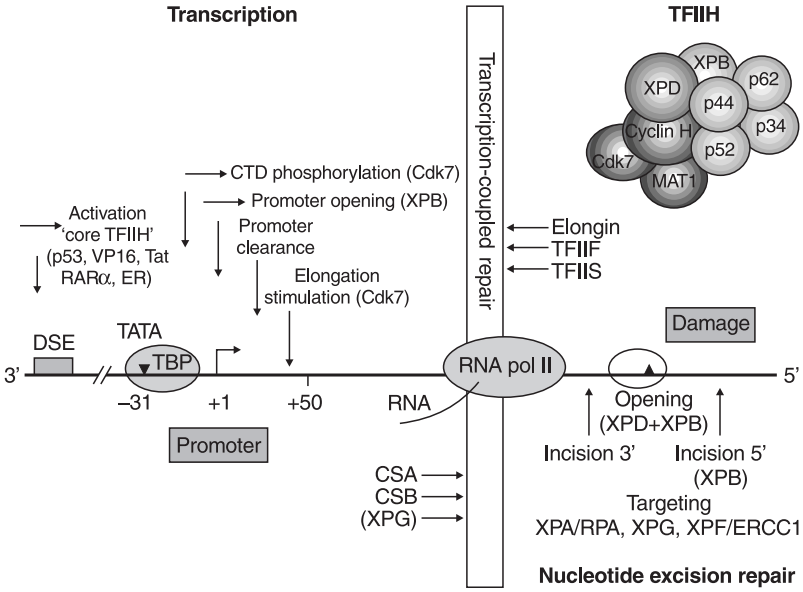

Figure 67. *TFIIH is probably involved at several levels in transcription, from the formation of the transcription preinitiation complex to the incision of damaged strands. Enzymes such as XPB and XPD unwind DNA for promoter opening and excision of a damaged strand. Cdk7 phosphorylates the C-terminal domain (CTD) of RNA polymerase II to activate transcription and probably to regulate some processing. TFIIH may also be targeted by some regulators of the transcription process, e.g., p53, VP16 and nuclear receptors. The action of TFIIH in the mechanism of transcription-coupled repair is unknown. RARα, retinoic acid receptor α; DSE, downstream element; ER, oestrogen receptor; CSA, CSB, XPA, XPG, XPF, RPA and ERCC1 are nucleotide excision repair factors.*

On the basis of these and other experiments, we have been able to assign properties to the various TFIIH components and determine their importance in both transcription and nucleotide excision repair (*Fig. 67*). The core TFIIH is involved in transcriptional activation because interactions with various activators have been detected, including the acidic activator VP16, the p53 protein, HIV Tat protein, retinoic acid receptor α and oestrogen receptor (Rochette-Egly *et al.*, 1997); CTD phosphorylation is dependent on Cdk7 (Coin *et al.*, 1999); promoter opening

depends on XPB; and promoter clearance is dependent on Cdk7 (Moreland *et al.*, 1999). In nucleotide excision repair XPD and XPB are both required for the opening at the lesion site and XPB is involved in the 5′ incision (Constantinou *et al.*, 1999).

Elongator, a component of the elongating RNA polymerase II holoenzyme

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in collaboration with

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Once a preinitiation complex forms (see *Box 1*), the synthesis of mRNA requires four steps: synthesis of the first phosphodiester bond, promoter clearance, elongation and termination. Most work has focused on the termination step, including 3′-end processing of the mRNA transcript (Neugebauer and Roth, 1997), but surprisingly little is known about the factor requirements for the elongation of transcripts and the mechanisms controlling transcription through chromatin DNA.

We have identified and characterized a distinct form of RNA polymerase II involved in elongation and established that it forms a complex with elongator proteins. The Elongator complex interacts specifically with the phosphorylated form of the CTD of RNA polymerase II and may act by increasing the ability of the polymerase to progress along the DNA, although it does not seem directly to affect the rate of transcription.

Identification of the Elongator complex

The CTD is a unique feature of the largest subunit of RNA polymerase II and is essential for yeast viability. It is thought to be important in the transition from initiation to elongation because only the non-phosphorylated form of the polymerase can enter

the preinitiation complex, whereas the hyperphosphorylated form has been found in ternary complexes composed of RNA polymerase II, DNA and RNA (Cadena and Dahmus, 1987; O'Brien *et al.,* 1994; Svejstrup *et al.,* 1997). As most, if not all, general transcription factors remain at the promoter after the transition from initiation to elongation, phosphorylation of the CTD could induce the partition of the polymerase and the general transcription factors by introducing a net negative charge at the CTD. It has not been clear whether the Mediator complex (see Kornberg, this volume) remains bound to the polymerase during elongation but RNA polymerase II isolated from the DNA fraction of a whole-cell yeast extract was both hyperphosphorylated and lacked bound Mediator (Svejstrup *et al.,* 1997). Release of the polymerase from the ternary complex by incubation with the elongation factor TFIIS further indicated that this is the elongating form of the polymerase (Svejstrup *et al.,* 1997).

The elongating form of the polymerase was characterized by a purification that included adjusting the salt concentration of the whole-cell extract to disrupt the DNA binding of most proteins; RNA polymerases engaged in ternary elongation complexes remain stable at higher ionic strengths (Gnatt *et al.,* 1997; Svejstrup *et al.,* 1997). The elongating form of the polymerase had an M_r of 500–600K compared with ~400K for the core RNA polymerase and coeluted with additional polypeptides, at least three of which seemed to be stoichiometric to the polymerase subunits. The polypeptides have apparent M_rs of 150K, 90K and 60K (*Fig. 68*) and we have named them elongator proteins (Elp) 1–3. Peptide sequencing showed that Elp1 (150K) is encoded by a previously uncharacterized open reading frame on yeast chromosome XII (YLR384C).

A free form of the Elongator complex, without the polymerase, was purified from the soluble fraction using an antibody raised against Elp1. The native size of the free Elongator complex is M_r ~600K, indicating that each polypeptide is present in the

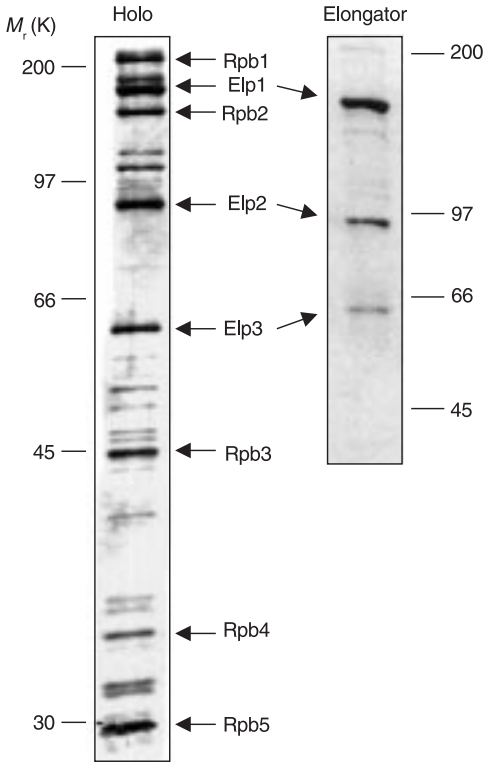

***Figure 68**. Purification and separation of the RNA polymerase II elongating holoenzyme and the elongator proteins associated with it in the Elongator complex. After separation of the elongating form of the polymerase from the soluble material by high-speed centrifugation, the RNA polymerase was released from the DNA/RNA by treatment with nucleases and extraction with ammonium sulphate. The RNA polymerase holoenzyme was purified over five chromatographic steps ending with a gel filtration (left). The polypeptides designated Elp1, Elp2 and Elp3 (M_rs 150K, 90K and 60K) are part of the Elongator complex, which is found both in free form (right) and in the holoenzyme. Rpb1–5, subunits of polymerase. Modified from Otero* et al.*, 1999.*

complex as a dimer. Free Elongator was also detected continuously throughout the purification from the DNA-bound fraction. Despite using phosphatase inhibitors in all buffers, the CTD seemed to become dephosphorylated at a rate that correlated

with the dissociation of free Elongator complex. Purified Elongator interacts directly with core RNA polymerase II to form a complex: when applied separately to a gel-filtration column, Elongator eluted at M_r 650K and core polymerase at 400K but preincubation of the two complexes resulted in coelution of the proteins at a position equivalent to ~500K (Otero *et al.*, 1999).

Functional studies of the Elongator complex

Many other factors involved in elongation of transcription, i.e., TFIIS, Spt4 and elongin, are not essential for yeast viability (Archambault *et al.,* 1992; Malone *et al.,* 1993; Aso and Conrad, 1997). Likewise, *elp1* is not an essential gene in yeast but *elp1*Δ cells, i.e., cells in which *elp1* is deleted, did display a pronounced delay in adapting to changed growth conditions. For example, the *elp1*Δ strain was much slower to adapt than the wildtype strain when glucose in the medium was substituted by galactose, sucrose or raffinose, or when the inositol concentration was reduced. However, once they had adapted to the new conditions *elp1*Δ cells had the same doubling time as the wildtype cells (Otero *et al.,* 1999).

The involvement of the Elps in transcriptional elongation was demonstrated in vivo, using the sensitivity of yeast strains with mutations in genes encoding proteins involved in transcription elongation to 6-azauracil or mycophenolic acid (Exinger and Lacroute, 1992). These drugs deplete the cellular pools of ribonucleotide triphosphate substrates for RNA polymerase II, mainly UTP and GTP, but have no marked effects on wildtype cells (*Fig. 69a*). However, they clearly affect yeast growth when an additional constraint on elongation, e.g., a mutation in a gene encoding an elongation factor such as *TFIIS*, *SPT4* or *SPT5*, is imposed. Growth of a strain (*sii*Δ) that is hypersensitive to high doses of the drug was only slightly affected by low doses of 6-azauracil (*Fig. 69b*) and an Elongator-deleted strain (*elp1*Δ) shows little sensitivity to the drug (*Fig. 69c*). By contrast, *sii*Δ/*elp1*Δ

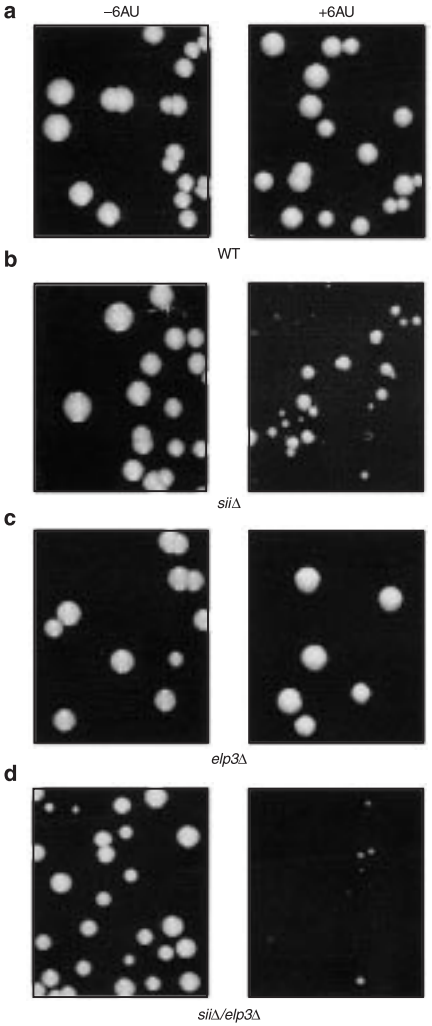

Figure 69. *Genetic evidence of a role for the Elongator complex in elongation in vivo is provided by the in vivo elongation phenotype of yeast cells lacking an Elongator component (Elp3). Yeast cells grown on 6-azauracil (6-AU, 50 μg ml*$^{-1}$*) are sensitive to mutations in elongation factors because they have a smaller pool of free nucleotides for transcription.* ***a****, wildtype control.* ***b****, cells of the* sii*Δ strain are sensitive to 6-AU.* ***c****, Elongator-deleted strain (*elp3*Δ) shows little sensitivity to 6-AU.* ***d****,* sii*Δ/*elp3*Δ double deletions are hypersensitive to 6-AU. Similar results are seen with* elp1*Δ and* elp2*Δ. Modified from Otero* et al.*, 1999.*

double-deletion mutants were hypersensitive to the drug, indicating a drug-induced synthetic lethal phenotype (*Fig. 69d; elp3*Δ, which gives similar results to *elp1*Δ, is shown). Similar results were obtained with mycophenolic acid, and the hypersensitive phenotype of the double-deletion strain was rescued by supplying guanine to the media.

That Elongator is important for normal gene activation was established by determining whether the slow-adaptation phenotype of *elp1*Δ correlated with delayed induction of genes encoding key enzymes in the pathways that are activated by changes in growth conditions. In wildtype cells, expression of the *PHO5* gene, which is induced when phosphate is limiting in the medium (see Hörz, this volume), became apparent 90 minutes after shifting from high- to low-phosphate medium. In the *elp1*Δ strain, although the *PHO5* gene seemed to be induced to the same level as in the wildtype, the time to reach full induction was significantly delayed. Similar results were obtained for the *GAL1/10* gene after shifting from glucose to galactose and for the *INO1* gene after shifting from high to low concentrations of inositol.

The effect of the Elongator complex in vitro was studied using naked terminal transferase-tailed transcripts, where transcription by RNA polymerase II can initiate independently of general transcription factors. The only difference when Elongator was present was the appearance of fewer pause sites compared to core polymerase alone. However, in similar assays using nucleosome templates the core polymerase paused at many distinct positions on the template but these pause sites were not detected when elongating holoenzyme was used.

The identification of the Elongator complex and its specific interaction with the phosphorylated form of the CTD of RNA polymerase II indicates that the protein interactions of RNA polymerase II form a cycle from initiation through elongation to termination and back (*Fig. 70*). Most, if not all, polymerases at the promoter have the Mediator complex bound to their unphos-

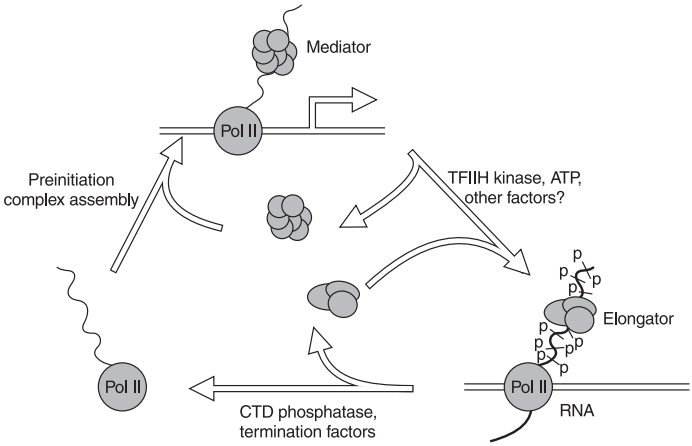

***Figure 70**. Model for a transcription cycle in which the association of polymerase with the Mediator and Elongator complexes is governed by CTD phosphorylation. Mediator is associated with non-phosphorylated RNA polymerase II during initiation but is displaced at the transition to elongation where Elongator associates with hyperphosphorylated RNA polymerase II. The reassociation of Mediator with the polymerase is made possible by CTD dephosphorylation mediated by CTD phosphatase. See text for details. Modified from Otero* et al.*, 1999.*

phorylated CTD, which allows them to receive signals from proteins that regulate transcription (see Kornberg, this volume). After formation of the preinitiation complex with the general transcription factors, interactions between polymerase and the other factors have to be disrupted. Phosphorylation of the CTD by TFIIH probably leads to dissociation of the Mediator and the general transcription factors from the polymerase. The Elongator complex then binds preferentially to the phosphorylated CTD, possibly replacing Mediator, and may protect the CTD from dephosphorylation as long as elongation proceeds. Inhibition of elongation, either at internal pause sites or at the termination sites downstream from the coding sequence, could lead to dephosphorylation of the CTD, subsequently releasing free RNA polymerase II to enter a new round of transcription.

Molecular interactions leading to the recruitment of RNA polymerase III

André Sentenac

in collaboration with

Christophe Carles, Christine Conesa and Michel Werner

RNA polymerases I, II and III have similarities in their subunit composition, with some subunits in common and some that are related, although there are others that are specific to one polymerase (see *Fig. 71*). In addition, accurate and regulated transcription by each polymerase requires a cofactor complex containing TBP (Margottin *et al.*, 1991; Hernandez, 1993). Consequently, common principles may govern the molecular mechanism by which each polymerase is recruited to promoters and engaged in an active transcription complex.

RNA polymerase III transcribes transfer (t) RNAs, 5S RNA and a number of small stable RNAs such as the U6 small nuclear (sn) RNA in yeast. It has been purified from both yeast and mammalian cells and yeast polymerase III comprises at least 17 subunits (Sentenac, 1985), making it the most complex of the three nuclear enzymes (*Fig. 71*).

Combining yeast genetics and biochemistry with mutant polymerase III enzymes in an in vitro system, we have determined the functions of three RNA polymerase III-specific subunits. We report here that interactions between the C34 subunit and the 70K component of TFIIIB (TFIIIB70) are required for recruitment of polymerase III as well as for open complex formation. This contact is not, however, unique because a second specific subunit, C17, also interacts with TFIIIB70. In contrast, the C11 subunit is essential for the intrinsic RNA cleavage activity of polymerase III and has a role in elongation and termination.

The RNA polymerase III transcription complex

As with polymerases I and II, accurate and regulated polymerase

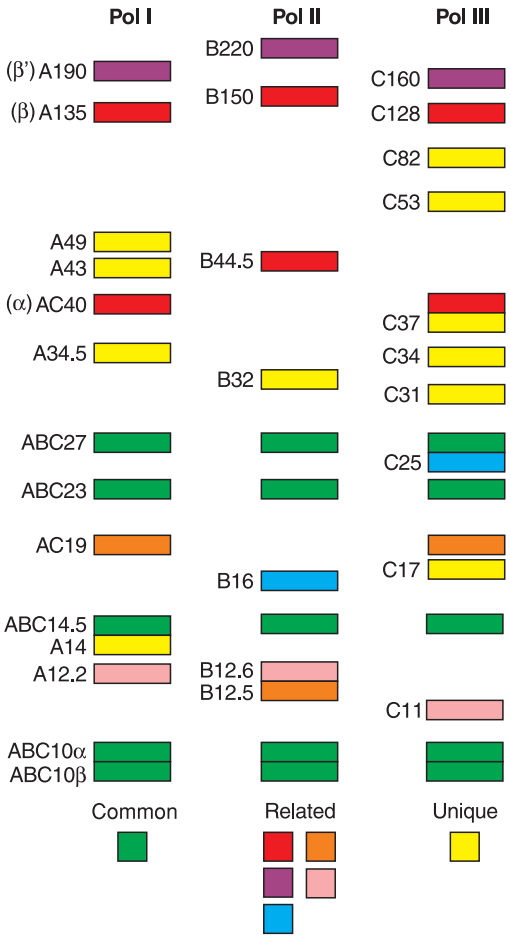

Figure 71. *Schematic comparison of the polypeptide components of yeast RNA polymerases I, II and III. Common, related and unique subunits are indicated by differences in shading.*

III initiation requires several other factors (Brun *et al.*, 1997). Polymerase III transcription in yeast is remarkable because all 27 of the proteins involved are absolutely essential and null mutations in the corresponding genes are lethal (*Table 8*). The prototype zinc-finger protein TFIIIA specifically recognizes an

internal element in the 5S RNA gene, whereas TFIIIC is responsible for recognition of the intragenic promoter elements in tRNA genes, named the A and B blocks. The positioning of the B block can be variable and is functional even when placed downstream of the transcription terminator. Once TFIIIC is bound to the B block, it also binds to the A block and the intervening DNA will be 'looped out' if the A and B blocks are far apart.

Table 8. *The polypeptides required for polymerase III transcription in yeast, determined by genetic analysis. The protein components of polymerase III and its auxiliary factors and the corresponding genes are summarized.*

Polypeptides	M_r (K)	Gene
RNA polymerase III		
C160	162	*RPO31* (*RPC160*)
C128	129	*RET1*
C82	74	*RPC82*
C53	47	*RPC53*
AC40	38	*RPC40*
C37	32	*RPC37*
C34	36	*RPC34*
C31	28	*RPC31*
ABC27	25	*RPB5*
C25	24	*RPC25*
ABC23	18	*RPB6* (*RPO26*)
AC19	16	*RPC19*
C17	18	*RPC17*
ABC14.5	17	*RPB8*
C11	13	*RPC11*
ABC10α	8	*RPC10*
ABC10β	8	*RPB10*
Transcription factors		
TFIIIA	50	*TFC2*
TFIIIB		
TFIIIB90/B″	68	*TFC5*
TFIIIB70/BRF1	67	*PCF4* (*BRF1, TDS4*)
TBP	27	*SPT15*
TFIIIC		
τ138	132	*TFC3*
τ131	120	*TFC4* (*PCF1*)
τ95	74	*TFC1*
τ91	75	*TFC6*
τ60	68	*TFC8*
τ55	49	*TFC7*

Yeast TFIIIC is a multiprotein complex consisting of six subunits (*Table 8*; *Fig. 72*). TFIIIC binding relieves the repression of polymerase III transcription by chromatin (Marsolier *et al.*, 1995) and in mammalian cells, three subunits of TFIIIC exhibit HAT activity with different histone specificities, consistent with a possible role of TFIIIC HAT activity in TFIIIC-dependent chromatin anti-repression (Kundu *et al.*, 1999).

Binding of TFIIIC allows recruitment of TFIIIB, the equivalent of the general transcription factors for polymerase II. In yeast, TFIIIB is not stable. It comprises TBP, TFIIIB90 and TFIIIB70, the homologue of the polymerase II transcription factor TFIIB (see *Fig. 72*), which are assembled when recruited by interactions with the τ131 subunit of TFIIIC (Chaussivert *et al.*, 1995; Ruth *et al.*, 1996). The TFIIIB–DNA complex is very stable and can engage polymerase III in multiple rounds of transcription; this high efficiency in vitro is due to the rapid recycling of polymerase III on the same template after termination (Dieci and Sentenac, 1996). One proposal is that the polymerase is directly transferred from the termination site to the promoter–TFIIIB complex and some TFIIIB–polymerase III interaction may be specifically involved in the efficient recycling of the polymerase.

Recruitment of polymerase III involves a cascade of protein–protein interactions where, by analogy to the polymerase II system, TFIIIC acts as an enhancer and a promoter-binding factor. This analogy can be carried further because PC4 (see Meisterernst, this volume) and topoisomerase I, two known polymerase II coactivators, are also involved in mammalian TFIIIC function (Z. Wang and Roeder, 1998). As in the polymerase II system (see, for example, Kornberg, Roeder, Tjian, this volume), transcription can be observed when TFIIIC or TFIIIB subunits fused to a DNA-binding domain, e.g., that of Gal4, are artificially recruited to the promoter (Marsolier *et al.*, 1994).

RNA polymerase III subunit C34 in polymerase recruitment

Two-hybrid assays and genetic analysis in yeast and direct physical interaction studies have been used to map the protein–protein interactions between the subunits of the polymerase III transcription machinery (*Fig. 72;* Flores *et al.*, 1999). Most interactions between polymerase III and the TFIIIC and TFIIIB subunits involve the polymerase III-specific subunits C34, C17 and C53, which is interesting because it indicates how polymerase-specific recruitment to different genes may be achieved. We have used yeast genetics to provide direct evidence for a role of C34 in polymerase III recruitment.

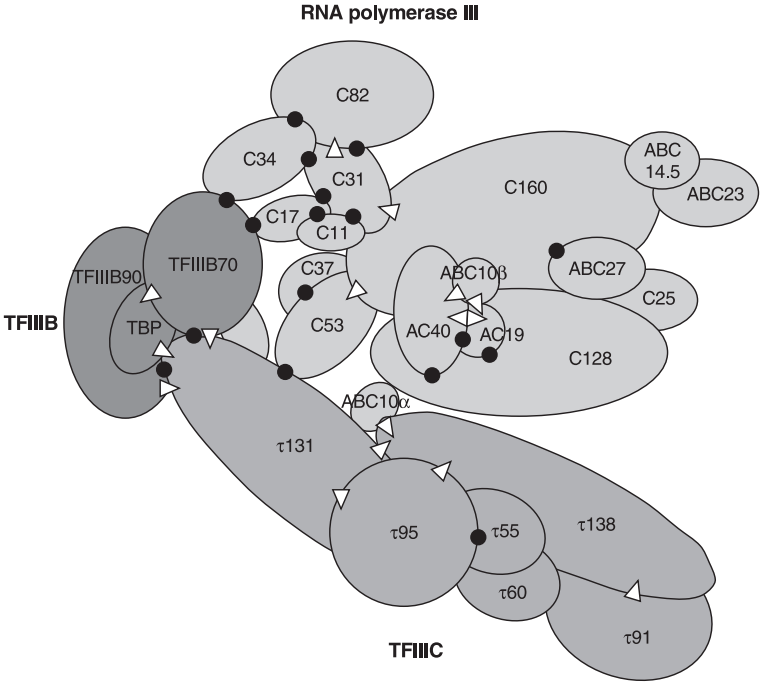

Figure 72. *The subunit composition and protein–protein interactions in the yeast polymerase III transcription machinery. Light grey, RNA polymerase III; mid grey, TFIIIC; dark grey, TFIIIB. Contacts have been defined either by genetic analysis (triangles) or by yeast two–hybrid analysis (filled spots).*

Although null mutations in the yeast *RPC34* gene, which encodes the C34 subunit, are lethal, we have generated several conditional alleles by site-directed mutagenesis, with phenotypes of slow or cryosensitive growth. Most of these alleles result in the loss of the ability to interact with TFIIIB70 and/or C82 in two-hybrid assays (Brun *et al.*, 1997). One, *rpc34-1124*, in which two negatively charged residues were changed to alanine, exhibited a cryosensitive phenotype at 16 °C and a selective loss of the C34–TFIIIB70 interaction in two-hybrid assays. Studies of this strain have provided evidence that the C34–TFIIIB70 interaction is required for recruitment of polymerase III (Brun *et al.*, 1997). The purified polymerase III had a normal subunit composition and equivalent enzymatic activities to wildtype polymerase III at saturating concentrations of a poly-d(A–T) template with ATP and UTP substrates. In contrast, when a tRNA gene was used as a template, a much higher concentration of polymerase III was required to give normal levels of correctly initiated in vitro transcription. The loss of the C34–TFIIIB70 interaction may, therefore, result in defective recruitment, which can be compensated for by increased enzyme concentration.

Another allele, *rpc34-1109*, in which two lysine residues in C34 were replaced by alanine, revealed that the C34–TFIIIB70 interaction is also required to influence post-recruitment events, such as open complex formation (Brun *et al.*, 1997). This strain exhibits slow and cryosensitive growth, loss of the TFIIIB70 interaction and reduced interaction with the C82 subunit. The reduced transcription of the tRNA gene in vitro with this polymerase III could not be corrected by increased enzyme concentration, unlike *rpc34-1124*. Preinitiation complexes made with the mutant enzyme had a fivefold reduction in open complex formation, where the DNA is melted in the initiation region, compared with that of the wildtype.

Similar conclusions about interactions between TFIIIB and polymerase III in recruitment and open complex formation have

been reached by studying mutations in TFIIIB (Kassavetis *et al.*, 1998). In mammals, three polymerase III-specific subunits, homologous to the yeast subunits C82, C34 and C31, interact with each other to form a subcomplex designated the core enzyme. The polymerase III without this subcomplex can transcribe DNA nonspecifically but, because the missing subunits normally dictate interactions with components of TFIIIB, it cannot specifically be recruited to polymerase III promoters (Z. Wang and Roeder, 1997).

C17 is another essential subunit in polymerase III that interacts with TFIIIB70. Two-hybrid and co-immunoprecipitation experiments have shown that the N-terminal region of TFIIIB70, which is homologous to TFIIB, is required for interaction with C17 (M.-L. Ferri, G. Peyroche, M. Siaut, O. Lefebvre, C. Carles, C. Conesa and A. Sentenac, unpublished). Polymerase III recruitment may, therefore, involve multiple interactions between TFIIIB70 and distinct polymerase III subunits. Subunits C17 and C34 have no counterpart in polymerase II, although they interact with the TFIIB-like part of TFIIIB70.

RNA polymerase III subunit C11 and RNA cleavage

The role of subunit C11 in polymerase III activity differs from that of C34. Replacing the gene for C11 in *S. cerevisiae* by that of *S. pombe* results in a temperature-sensitive phenotype. The polymerase III enzyme isolated from this strain, designated polymerase IIIΔ, lacks the C11 subunit. Although the *S. pombe* gene partially complements the deletion of the *S. cerevisiae RPC11* gene, the *S. pombe* C11 protein does not associate with the *S. cerevisiae* polymerase III as stably as the native protein and so is lost during purification (Chédin *et al.*, 1998).

The amino-acid sequence of C11 contains a region with strong homology to the evolutionarily conserved zinc-ribbon structure in the polymerase II elongation factor, TFIIS, which stimulates the backtracking required to promote release of

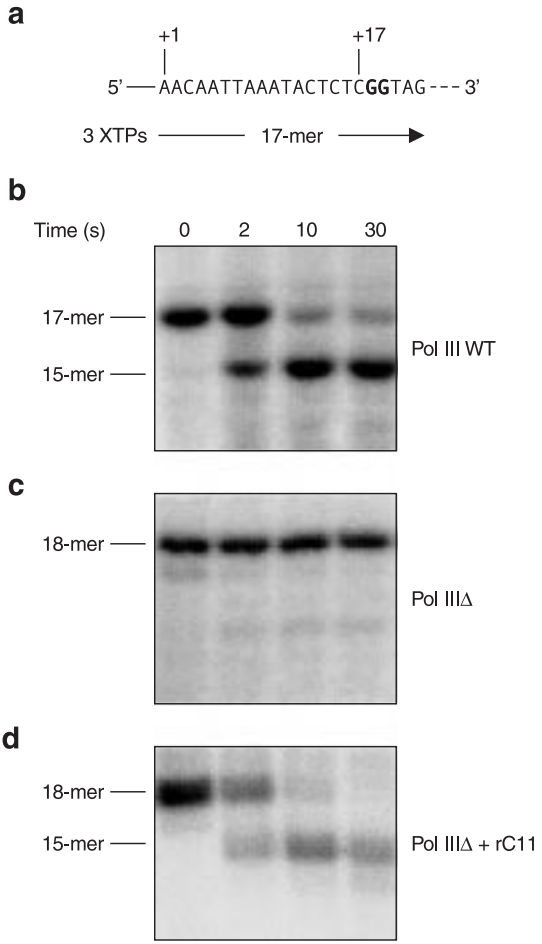

Figure 73. *Comparison of in vitro transcription with wildtype polymerase III (Pol III) and polymerase III lacking C11 (Pol IIIΔ) reveals that the mutant enzyme fails to excise mispaired nucleotides in newly generated transcripts.* ***a****, the sequence, up to nucleotide +22, of the RNA-like strand template used for in vitro transcription reactions. XTPs, ATP + UTP + CTP; the occurrence of G residues in the template is shown in bold; +1, start site; +17, arrest site in the absence of GTP.* ***b****, transcript cleavage by the halted wildtype polymerase III after the nucleotides were removed.* ***c****, cleavage fails when polymerase IIIΔ was used.* ***d****, purified recombinant (r)C11 rescued cleavage by polymerase IIIΔ.*

paused polymerase II. We have shown that C11 is essential for the intrinsic polymerase III RNA cleavage activity, similar to the activity of TFIIS with polymerase II, by analysing the properties of the polymerase IIIΔ enzyme in vitro using a template where transcription can proceed in the absence of GTP to position +17 (*Fig. 73a*). Transcription with the wildtype enzyme in the absence of GTP generated a 17-mer product that could partially be degraded to a 15-mer product by the stalled enzyme when incubated in the absence of nucleotides (*Fig. 73b*). In contrast, the polymerase IIIΔ enzyme generated a stable 18-mer product (*Fig. 73c*), so the mutant enzyme seems to incorporate an additional mispaired nucleotide that it cannot excise. Addition of recombinant C11 corrected the defect: the 18-mer product was rapidly degraded to a 15-mer product (*Fig. 73d*).

The failure of the polymerase IIIΔ enzyme to excise the mispaired nucleotide that is added at the pause results in an inability to leave the paused state, because the mutant enzyme cannot generate full-length transcripts. Transcription in the absence of GTP allowed initiation and pausing of the polymerase IIIΔ at position 18; adding GTP then allowed the synthesis of the full-length transcript. Although the wildtype enzyme resumed transcription and generated the full-length transcript, polymerase IIIΔ could not generate the full-length transcript unless recombinant C11 was present.

Termination of polymerase III transcription occurs at defined termination sites downstream of the transcribed genes. The polymerase IIIΔ enzyme cannot recognize and terminate at these sites (*Fig. 74*). Polymerase III termination involves several rounds of cleavage and synthesis that effectively slow the polymerase down. The absence of C11 probably results in defective cleavage and failure of the enzyme to slow down. Support for this idea comes from the observation that slowing down also occurs at limiting nucleotide concentration, allowing correct termination to take place, and reducing the nucleotide concentra-

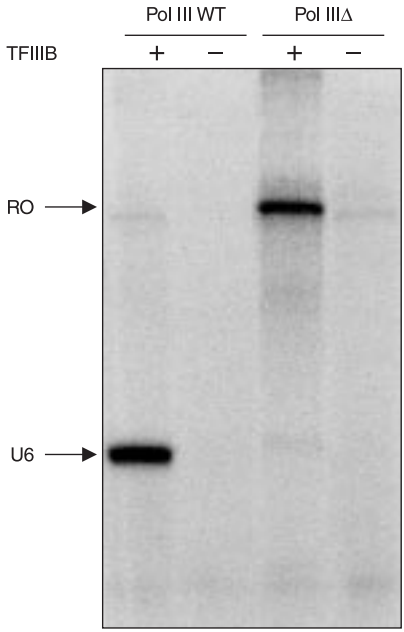

Figure 74. *Defective termination of transcription by polymerase IIIΔ. Accumulation of run-off (RO) transcript indicates the inability of the mutant enzyme to recognize and terminate at the termination site. However, the wildtype enzyme generated a correctly terminated transcript (U6) with little run-off product. In vitro transcriptions using a linearized template containing the U6 snRNA gene with wildtype polymerase III or polymerase IIIΔ. The requirement for TFIIIB demonstrates the transcriptional specificity of polymerase IIIΔ.*

tion corrected the termination defect of the polymerase IIIΔ. The RNA cleavage activity of polymerase III seems to be essential for releasing the stalled enzyme and removing the kinetic barriers to the termination process. We propose that C11 allows polymerase III to switch between RNA elongation and RNA cleavage modes.

CONCLUSION

Toshio Fukasawa

We have aimed to answer a question that is central to the field of eukaryotic transcription: how a gene-specific activator exerts its effect on selected promoter(s) in the highly condensed chromosome. The process of regulated transcription in eukaryotes can *a priori* be divided into at least two stages. First, a gene in a repressed state, because of the packaging of the chromatin, becomes accessible to specific DNA-binding regulatory proteins and/or the basal transcription machinery; and second, the transcription machinery starts to operate under the control of gene-specific regulatory proteins. When this workshop was planned in late 1997, about half the known global transcription regulators were reported to interact with chromatin, indicating they are involved in the first stage, whereas the remaining factors were reported to interact with RNA polymerase II indicating involvement in the second stage. In practice, the activity of the first group of regulators can only be determined in vivo or in vitro with nucleosomal templates that mimic chromatin structure, whereas the members of the second group can be studied readily in vitro because they are active with naked DNA templates.

The structure of the nucleosome revealed by X-ray diffraction gives an idea of the spatial complexity we are dealing with, as well as information about the ionic interactions. The crystal structure of the nucleosome core particle analysed at 2.8 Å, published only two years ago (Luger *et al.*, 1997), provided a basis for the positioning of the nucleosome in the chromosome. The current 2.0 Å resolution map reveals fine details of nucleosome structure and indicates that manganese ions may play a role in the formation of higher-order chromatin structure (Richmond). Furthermore, approximately 1,000 water molecules per nucleosome contribute significantly to DNA–protein interactions. These findings may furnish a basis on which to explain how RNA polymerase can transcribe the DNA bound in a nucleosome with-

out releasing the histone octamer. Combining this with information about the role of linker histone HI/H5 in the structure of the chromatosome (Travers) provides a hint of the higher-order structure and gives insights into how this may regulate the access of transcription factors to the DNA.

Many of the global regulators are striking for their size, forming large multi-subunit complexes, with M_rs sometimes reported to be in the order of millions. Rigorous biochemical verification is required before the existence of a new complex can be established: purification until the constituent components become reproducibly constant and each component is present in a stoichiometric amount. Several outstanding examples of such well characterized complexes are described here, e.g., Mediator, CHRAC, SAGA and NURF, although not all the known complexes could be included.

One remarkable feature of the global regulators is their multiplicity. Distinct complexes with subunits in common with similar activities have been isolated from the same organism, e.g., SAGA, Ada, NuA3 and NuA4 are all HAT complexes isolated from yeast (Allis, Workman); BAF (Kingston), NURD, NUD and RSF (not discussed in this volume) are ATP-dependent chromatin-remodelling complexes isolated from HeLa cells; and NURF, CHRAC and ACF (Becker, Wu) are chromatin-remodelling complexes in *Drosophila*. The significance of the multiplicity remains unknown: it may represent functional differentiation, overlap or redundancy but some of the complexes could merely be artefacts of isolation.

Another important aspect is the universality of these complexes. As functionally and structurally similar complexes have been isolated from a range of organisms, they must generally be essential for transcription. The Mediator complex provides a good example as the two complexes isolated from human cells on the basis of a mediator function, SMCC/TRAP (Roeder) and CRSP (Tjian), contain several subunits homologous with yeast

Mediator and with each other. Many complexes have structural similarities, containing common or homologous subunits, e.g., SWI/SNF and RSC have Arp7 and Arp9 in common, although the other subunits are exclusive to one or the other (Cairns). Some also have similar functions, e.g., yeast RSC (Kornberg) and human SWI/SNF (Kingston) both alter nucleosome structure when incubated with reconstituted mononucleosomes and ATP.

Chromatin remodelling

The regulators that interact with chromatin fall into two classes, one with HAT/deacetylase activity and the other with ATP-dependent chromatin-remodelling activity. Functional studies of the HAT complexes show that they enhance transcription activated by Gal4–VP16 in an acetyl CoA-dependent fashion on a nucleosomal template that is highly repressed when no HAT activity is available. A prerequisite for this activation is the interaction between a subunit of the HAT complex and the activator (Workman). One possible model for the function of chromatin-altering complexes proposes that the complexes target promoter regions, whereas another model considers that chromatin modification is not targeted but sets up an environment for proteins to gain access to the DNA. The first model has more support. For example, in yeast the depletion of Gcn5, the component responsible for HAT activity in the SAGA or Ada complexes, alters the chromatin structure of the promoter region of *PHO5* in close association with its expression (Hörz); however, it affects expression of only 5% of the genes in the whole genome (Holstege *et al.*, 1998).

The ATP-dependent chromatin-remodelling complexes catalyse the formation of a remodelled nucleosome. For example, *Drosophila* NURF can render an otherwise completely repressed nucleosomal template susceptible to an activator as efficiently as a naked DNA template (Wu). Interestingly, *Drosophila* CHRAC has the same ATPase subunit as NURF, ISWI, although its

functional core seems to be another ATPase, topoisomerase II (Becker). As in the case of the HAT complexes, a similar question arises of how the chromatin remodelling complex can be targeted to selected genes. As no chromatin-modifying proteins have been found in SMCC (Roeder) or in Mediator (Kornberg), the remodelling complex does not seem to be recruited to the target genes as a combined complex at the same time as Mediator. Roeder proposes a multistep model in which histone-modifying complexes are first recruited to the target gene by ligand-activated thyroid hormone receptor, facilitating access of SMCC to the gene. A paper published after the workshop (Cosma *et al.*, 1999) shows that a similar scheme fits the recruitment of the ATP-dependent chromatin-remodelling SWI/SNF complex to the upstream region of the *HO* endonuclease promoter. Activation occurs at defined stages of the cell cycle, after binding of the Swi5 activator, which in turn recruits SAGA. Both complexes facilitate the binding of the second activator, SBF, which triggers the formation of preinitiation complexes at the *HO* promoter. A question that remains unanswered is how an activator binds to a gene in the first place. Does a gene-specific factor with a strong affinity for its cognate element bind to the cognate sequence without altering chromatin structure or is the sequence in a naked DNA region at the outset?

Other steps in transcription, other polymerases

Although most of the studies cited in this volume are devoted to regulatory mechanisms at the initiation step, the elongation step is also an important target for transcriptional regulation in eukaryotes. An increasing number of factors involved in elongation have been identified, which also often form multi-subunit complexes (see Yamaguchi *et al.*, 1999 and references therein). The purification of a complex comprising three subunits from an elongating form of RNA polymerase II fits this picture (Svejstrup). The complex replaces Mediator as the polymerase

passes from initiation to elongation but its functions remain to be clarified.

We have not described recent progress on the regulation of transcription by RNA polymerases I and III in this volume, except for the regulatory mechanism of polymerase III (Sentenac). Because the species of RNA polymerase are evolutionary related, sharing several subunits with each other, there must be many analogies among their regulatory mechanisms. The best known is that TBP is an important component of the initiation complex formed by all of them. Another interesting analogy between the polymerase II and polymerase III systems, as emphasized by Sentenac, is that PC4 (see Meisterernst, Roeder) and topoisomerase I, two known polymerase II coactivators, are involved in the function of the polymerase III initiation factor TFIIIC, a complex required for the recruitment and enhancement of polymerase III (Z. Wang and Roeder, 1998). While pursuing analogies among the three RNA polymerases, the question of how the functional differentiation among these polymerases is achieved must be answered, i.e., how each RNA polymerase specifically transcribes its target genes. Upstream activating factor, a multi-subunit complex apparently corresponding to TFIIIC in the polymerase I system (Nomura, 1998), has recently been shown to play an important role in the mechanism by which the genes encoding ribosomal RNA are exclusively transcribed by polymerase I in yeast (Vu *et al.*, 1999) .

Complexity and future perspectives

Most of the component proteins of the transcription complexes described in this volume have been identified and their respective genes sequenced but their exact roles are still poorly understood. It is uncertain whether all the subunits are required for function or if some merely provide the complex with structural integrity. One way to answer this is by reconstituting a complex in its active form in vitro from recombinant components, as Egly

reports for the basal transcription factor TFIIH. Studying this construct has not only solved several fundamental questions about the role of the subunits in transcription and DNA excision repair but also clarified the association between TFIIH and inherited human disorders, such as xeroderma pigmentosum, Cockayne's syndrome and trichothiodystrophy.

Because most in vitro experiments with the global regulators use artificial activators and promoters, the results need to be confirmed by experiments in vivo using native activators and/or promoters. For this purpose, cells must be specifically depleted of the protein in question and the effect on as many genes as possible determined. Such experiments have now been achieved in yeast for Mediator components (Kornberg, Sakurai) and for TAFs (Green, Struhl) indicating that most, if not all, of the subunits are vital for normal transcription. However, such experiments are not easy in mammalian cells because not only is selective depletion of gene function technically difficult but, more seriously, many of the global regulators seem to be essential for cell division. Nevertheless, this problem is being resolved by using techniques, such as the Cre/Lox method, that conditionally disrupt gene function at a defined stage, enabling Tora to demonstrate that $TAF_{II}30$ is required both for cell-cycle progression and for parietal endoderm differentiation of mouse F9 embryonal carcinoma cells.

Most of the eukaryotic transcription complexes have probably now been identified and any that remain should be discovered soon. The factors that are necessary and sufficient for basal transcription in vitro are defined and the structure of the nucleosome refined. The big task ahead is to understand how these factors act and when and where they regulate target genes during the life of an organism. One of the main themes that emerged from the Workshop is the universality and similarity between complexes in the same and different organisms. It is even possible that the differences between eukaryote and prokaryote

transcriptional mechanisms are not as great as currently assumed. This convergence will facilitate learning more about the regulation of transcription, as insights gained from one organism can be tested in and extended to others. With so many powerful techniques at our disposal, the prospects for the next few years are exciting indeed.

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ISBN : 92-9164-006-9
ISSN : 1026-9819
Achever d'imprimer en décembre 1999
sur les presses de l'imprimerie Valblor à Strasbourg

HUMAN FRONTIER SCIENCE PROGRAM

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Human Frontier Workshop on Global Transcription Regulators of Eukaryotes, Strasbourg, December 3-5, 1998
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Middle row: R. Kornberg, M. Green, S. Björklund, R. Roeder, H. Sakurai, K. Weiss, G. Almouzni, D. Allis, A. Travers, J.-M. Egly, L. Tora
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