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Global Transcription Regulators of Eukaryotes

Meeting Review

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The fields of transcriptional regulation and chromatin structure and function have for a long time been separate with too little interdisciplinary communication. The two fields have now merged with advances in the complete description of all factors that are required for basal, nonregulated transcription, the availability of highly purified in vitro transcription systems, and the purification of several large complexes with chromatin remodeling activities. Thus, it is now possible to start studying the biologically important question: How is transcription regulated in a chromatin context? To discuss this issue, scientists from the two fields met in Strasbourg, France (December 3–5, 1998) at a workshop titled "Global Transcription Regulators of Eukaryotes," arranged by the International Human Frontier Science Program. We have chosen to focus this report on the most recent results presented at the workshop and would like to refer the reader to the given citations for further discussion of already published work.

Chromatin Structure

The organization of DNA in an intricate, dynamic nucleoprotein assembly termed chromatin is accomplished by a remarkable feat of biological engineering. Despite its complexity, the fundamental organization of chromatin is surprisingly simple. It is composed of repeating units termed nucleosomes, whose existence and basic structure were proposed 25 years ago (Kornberg, 1974; Oudet et al., 1975). The nucleosome core is composed of an octamer of four highly folded proteins, H2A, H2B, H3, and H4 (two of each protein), and of 147 base pairs of DNA wrapped around the octamer. A nucleofilament is then formed by linking nucleosome cores by short stretches of DNA bound in part by the "linker" histones H1 and H5.

Timothy Richmond presented data on the nucleosome core structure at 2.0 and 2.8 Å resolution (Luger et al., 1997). The structure reveals that DNA is wrapped in 1.65 left-handed superhelical turns around the histone octamer, leading to a significant deformation of the DNA double helix. The refined structure at 2.0 Å resolution includes approximately 1000 solvent water molecules and ions, which make important contributions to the DNA structure and DNA–protein interactions. The octamer structure and the DNA at the contact sites are particularly well ordered and defined, whereas the noncontacting DNA shows more flexibility. This suggests that all nucleosomes might adopt the same structure, independent of the DNA sequence. Networks of ordered water molecules are found at the histone–subunit interfaces and at histone–DNA contact sites. They might thus modulate the dissociation of the octamer into dimers and tetramers, depending on salt concentration and entropic effects, and possibly permit the DNA to detach over as much as one half of the superhelix to allow transcription of nucleosomal DNA without being completely dissociated from the octamer.

Formation of higher order chromatin is dependent on divalent ions, and interestingly, the 2.0 Å structure locates a manganese ion bound in perfect octahedral coordination between two core particles. Only about one third of the flexible N-terminal histone tails is visible in the structure and reaches out between and around the gyres of the DNA superhelix. The disordered structure of the tails suggests that they are primarily involved in higher order interactions.

The location of linker histone binding relative to the core particle DNA has been the subject of several studies yielding conflicting results. Andrew Travers reported results on the binding site of linker histone H5 on mixed sequence chicken DNA chromatosomes, structural units of chromatin in chicken erythrocytes comprised of a nucleosome core, its DNA extension, and one linker histone molecule. The globular domain of the linker histone appears to form a bridge between one terminus of chromatosomal DNA and the dyad of the nucleosome. Proper folding of a nucleosome array into a higher order structure is facilitated by linker histone H1/H5 binding and might regulate transcription factor access to DNA by inducing repositioning of octamers. Precise mapping of nucleosome positions after in vitro reconstitution of both the oocyte and somatic *Xenopus borealis* 5S genes into chromatin indicated substantially different nucleosome positioning on these genes (Panetta et al., 1998). In addition, binding competition experiments showed that the transcription factor TFIIIA preferentially binds to the somatic nucleosome whereas H1 preferentially binds to the oocyte nucleosome and thus prevents TFIIIA binding. These results suggest that nucleosome positioning

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plays a key role in the regulation of 5S RNA transcription and provide a molecular mechanism for the selective repression of the oocyte 5S RNA genes by H1.

Histone Modifications, Chromatin Remodeling, and Their Involvement in Transcription Regulation

Several proteins and multisubunit complexes act directly on chromatin structure to regulate transcription. The effects on transcription could be envisioned in two nonexclusive ways: globally over large chromatin domains or entire genes and/or locally at single nucleosomes specified by DNA-bound transcription factors.

Histone Tail Modifications

The N- and C-terminal tails of histones are flexible, extend out from the nucleosome core, and contain several conserved residues that can be posttranslationally modified. The histone tails are known to interact with other chromatin components and contribute to both transcriptional activation and repression. The most intensively studied histone tail modification has so far been the acetylation of lysine residues.

Only three years have past since the cloning of the first transcription-associated histone acetyltransferase (HAT) (Brownell et al., 1996). Despite displaying a potent HAT activity with purified histones, it was shown that purified recombinant Gcn5 could not efficiently modify histones in a nucleosome context. Since then, a plethora of transcriptional coactivator complexes from both human and yeast have been biochemically purified and shown to have intrinsic HAT activity (see Struhl and Moqtaderi, 1998). In yeast *Saccharomyces cerevisiae*, for example, several complexes including SAGA, Ada, NuA3, and NuA4 have been described. The SAGA complex is a 1.8 MDa complex that shows specificity for nucleosomal histone H2B and H3 acetylation. The constituent subunits of SAGA can be classified into four groups: *GCN5-Ada* gene products, *SPT* gene products, TAF_{II} proteins, and *Tra1*, a homolog of the human TRRAP protein. The SAGA and Ada complexes share several subunits whereas others are still unidentified (Grant et al., 1998). Characterization of these will tell us if Ada is a unique entity or a subcomplex of SAGA.

Much less is known about the subunits of the NuA3 (0.4 MDa) and NuA4 (1.3 MDa) complexes. Functional studies of the HAT complexes described by Jerry Workman showed that the SAGA and NuA4 complexes bind directly to both the VP16 and GCN4 activation domains while the Ada and NuA3 complexes bound to neither of the activators. Incubation of a mononucleosome template containing a single Gal4-binding site with increasing amounts of Gal4-VP16 in the presence of HAT complexes showed that binding of activator to the nucleosome increased the level of histone acetylation. This was only observed with the SAGA and NuA4 complexes, consistent with the activator binding studies. A direct effect of HAT complexes on transcription was demonstrated using a chromatin template composed of a minimal E4 promoter with five Gal4 sites. This highly repressed template is activated by Gal4-VP16 together with either SAGA or NuA4, in an acetyl-CoA-dependent manner. While all four yeast HAT complexes can stimulate transcription of a chromatin template due to general acetylation effects, targeting of SAGA and NuA4 to the promoter by Gal4-VP16 increases the degree of stimulation.

Data presented by David Allis, done in collaboration with Paolo Sassone-Corsi, strongly argued that phosphorylation of histones might prove to play an equally important role for transcriptional regulation. An antibody against the highly conserved N-terminal tail, specifically phosphorylated at serine 10, can be used as a powerful in situ marker for mitotic nuclei in diverse eukaryotes (Wei et al., 1998). However, this antibody is also highly specific for euchromatic nuclear domains after mitogen stimulation. These are defined as decondensed and actively transcribed chromatin domains and argue for the involvement of histone tail phosphorylation in transcriptional regulation. The “activity in-gel assay” developed to identify histone acetyltransferases (HATs) was modified to identify Rsk-2 as the histone H3-Ser10 kinase following mitogenic stimulation. Rsk-2 is a 90 kDa protein that is specifically targeted by the MAPK signaling pathway. In addition, mutations in the human *RSK-2* gene are causally linked to the Coffin-Lowry syndrome (CLS), an X-linked disorder characterized by mental retardation and skeletal deformations in humans (Trivier et al., 1996). Interestingly, cells derived from CLS patients are Rsk-2 deficient and fail to exhibit H3 phosphorylation after mitogen stimulation while the mitotic H3 phosphorylation is normal.

ATP-Dependent Chromatin Remodeling Complexes

Genetic and biochemical studies in yeast, *Drosophila melanogaster*, and human cells have identified a new class of multisubunit complexes that utilize ATP to remodel nucleosomal structure and facilitate transcription (Table 1). It is assumed that these complexes catalyze the formation of a remodeled nucleosome, but the nature of this remodeled structure in terms of histone configuration has remained elusive.

Swi/Snf and RSC. The yeast SWI/SNF complex was identified by copurification of proteins identified in genetic screens for mating-type switching (swi) and sucrose nonfermenting (snf) mutants. The SWI/SNF complex is comprised of 11 subunits, has a molecular weight of about 2 MDa, and contains Swi2/Snf2 as its catalytic subunit. The RSC (remodels structure of chromatin) complex is about 1 MDa in molecular weight and is composed of 15 subunits, six of which are homologous or identical to SWI/SNF subunits. The RSC complex includes a DNA-dependent ATPase homologous to Swi2/Snf2, encoded by *STH1*. Despite these similarities, RSC is about ten times more abundant than SWI/SNF, and most genes encoding RSC subunits are essential for mitotic growth.

Bradley Cairns described the identification and characterization of several new RSC subunits. Rsc1 and Rsc2 each contain two bromodomains, a 110–amino acid motif of unknown function that is found in several proteins involved in histone modification and transcriptional regulation. Furthermore, Rsc1 and Rsc2 are both highly homologous to the chicken protein polybromo and contain a bromodomain-associated homology (BAH) motif also found in the *Drosophila* transcriptional activator protein Ash1. It was shown that the BAH and at least one of the bromodomains were essential for Rsc1 and

Table 1. ATP-Dependent Nucleosome Remodeling Machines

Organism	Complex	Name	Molecular Mass	No. of subunits	ATPase
Saccharomyces cerevisiae	SWI/SNF	Switching mating type sucrose-fermenting	2 MDa	11	SWI2/SNF2
	RSC	Remodels the structure of chromatin	1 MDa	15	STH1
Homo sapiens	BAF (mammalian SWI/SNF)	BRG1- or hbrm-associated factors	2 MDa	9–12	BRG1
			2 MDa	9–12	hbrm
	NuRD	Nucleosome remodeling histone deacetylase complex	1 MDa	7	Mi-2
	NRD	Nucleosome remodeling and deacetylating	ND	6	CHD3/4
	NURD	Nucleosome remodeling and histone deacetylation	1.5 MDa	18	CHD4
	RSF	Remodeling and spacing factor	500 kDa	2	hSNF2h (homolog of *Drosophila* ISWI)
Xenopus laevis	Mi-2 complex		1–1.5 MDa	6	Mi-2 (Snf2 superfamily member)
Drosophila melanogaster	Brahma complex		2 MDa	ND	BRM
	NURF	Nucleosome remodeling factor	500 kDa	4	ISWI
	CHRAC	Chromatin accessibility complex	670 kDa	5	ISWI
	ACF	ATP-dependent chromatin assembly and remodeling factor	220 kDa	4	ISWI

Mi-2, NuRD, NRD, and NURD all have reported ATP-dependent remodeling and histone deacetylase activity. Several complexes have common subunits.

Rsc2 function. Immunoprecipitation experiments indicated that two RSC complexes exist, one containing Rsc1 and the other Rsc2. This result may underlie the observation that mutants lacking either *RSC1* or *RSC2* are viable, whereas the lack of both genes causes lethality. Arp7 and Arp9 are two essential, actin-related proteins that are subunits of both the ySwi/Snf and RSC complexes. Analysis of temperature-sensitive mutations in *ARP7* and *ARP9* supports a functional role for both proteins in RSC, SWI/SNF, and transcriptional regulation. However, in contrast to actin, extensive mutation of the predicted ATP-binding sites in Arp7 and Arp9 showed no phenotype (Cairns et al., 1998).

Roger Kornberg and Robert Kingston both reported recent results on the effects of yeast RSC and human Swi/Snf on nucleosomal DNA. Incubation of either of the complexes with reconstituted mononucleosomes and ATP resulted in the appearance of an altered nucleosome species. In the case of RSC, formation of an "activated" complex was shown to precede the appearance of the altered nucleosome. In both systems, incubation of the altered nucleosome form with ATP and yRSC or with hSwi/Snf resulted in conversion back to the starting material. These results suggest that yRSC and hSwi/Snf make chromatin more dynamic, catalyzing equilibrium between multiple structural states. For a further discussion of these results, see Travers (1999) and references therein.

The Nucleosome Remodeling Factor. Carl Wu described the nucleosome remodeling factor (NURF), a large (500 kDa) chromatin remodeling complex, originally identified as an activity that facilitated transcription factor binding to its sites in chromatin in an ATP-dependent reaction (Tsukiyama et al., 1994). Purified NURF was found to be a nucleosome-stimulated ATPase containing four major polypeptides (215, 140, 55, and 38 kDa). The 140 kDa ATPase imitation switch (ISWI) is a protein also present in the *Drosophila* chromatin remodeling complexes ACF and CHRAC and is likely to be the chromatin remodeling subunit of the complex (see below). NURF-55 is similar to RbAp48, a WD repeat protein found in a number of chromatin-associated complexes, and is assumed to act in a structural role. Surprisingly, the third subunit (NURF-38) has been identified as a protein homologous to inorganic pyrophosphatase. NURF-38 does show inorganic pyrophosphatase activity, but this does not appear to contribute to chromatin remodeling, at least in vitro. However, it may be that NURF is adapted to deliver pyrophosphatase activity to regions of chromatin undergoing active transcription or replication to allow removal of accumulated unhydrolyzed pyrophosphate, which is inhibitory to these processes.

Recent biochemical studies have demonstrated that NURF can activate transcription from chromatin templates in vitro as a direct result of its facilitating activator binding, strongly suggesting that this complex has a role in transcriptional activation from chromatin templates also in vivo. However, fundamental questions remain regarding the mechanism of the chromatin remodeling reaction, and whether this complex is actively recruited to promoters. The recent cloning of the largest NURF subunit (NURF-215) is likely to give insight into these questions.

The Chromatin Accessibility Complex. Using a similar biochemical approach to examine protein access to chromatin, Peter Becker and colleagues have isolated the chromatin accessibility complex (CHRAC), a chromatin remodeling complex (~670 kDa), which was able

to both facilitate protein access to sites in chromatin and which also, perhaps surprisingly, increases the regularity of nucleosome spacing via ATP-dependent mechanisms. CHRAC contains five subunits, two of which are ATPases: topoisomerase II (Topo II) and ISWI. Recombinant ISWI alone is active in all CHRAC-associated assays, suggesting that ISWI is the functional core of CHRAC that can recognize nucleosomes and trigger chromatin remodeling (Corona et al., 1999).

The function of Topo II, an enzyme involved in DNA topology, in CHRAC remains open. Topo II is present in CHRAC as an active dimer, but its activity does not contribute to chromatin remodeling in vitro. Two hypotheses for proposed function of Topo II in CHRAC are that chromatin remodeling facilitates Topo II binding to chromatin, thereby enhancing its function, or Topo II targets CHRAC to specific chromosomal sites. The remaining subunits in CHRAC are in the process of identification and should give some insight into the mechanism of chromatin remodeling activity.

Chromatin Remodeling and Transcriptional Regulation In Vivo

Phosphate starvation of *S. cerevisiae* triggers a 50-fold increase in acid phosphatase activity due to increased transcription of the *PHO5* gene, which becomes activated by binding of the activator proteins Pho4 and Pho2 to the *PHO5* promoter. The binding is inhibited by phosphorylation of Pho4 through the cyclin-CDK complex Pho80/Pho85 when phosphate is present in the growth media. Either phosphate starvation or deletion of the genes encoding the negative regulators results in a localized disruption of four nucleosomes in the *PHO5* promoter whereupon the promoter becomes activated. Wolfram Hörz presented in vivo data showing that activation of the *PHO5* promoter is dependent on the Gcn5 protein under specific conditions. Activation of PHO5 by PHO80 disruption rather than phosphate starvation leads to a loss of nucleosomal positioning over the *PHO5* promoter and failure to activate PHO5 in strains lacking Gcn5 HAT activity.

The connection between histone deacetylation and transcriptional repression was also studied in vivo. Yeast strains lacking the histone deacetylase Rpd3 are viable but show selective effects on gene expression. However, it was unclear whether deacetylation was untargeted or localized to specific promoter sequences. Kevin Struhl presented a study on transcription repression mediated by Ume6, which is dependent on both Sin3 and Rpd3. An 80 aa region in the Ume6 repression domain was shown to be sufficient for complete repression of a reporter gene and was also critical for interaction with Sin3, which in turn bound Rpd3. Repression was dependent on the Rpd3 histone deacetylase activity and associated with specific deacetylation of histones H3 and H4 at a limited region centered on the repressor-binding site (Kadosh and Struhl, 1998).

Structural and Functional Studies of General Transcription Factors

Roger Kornberg presented electron density maps obtained from paused RNA polymerase II/DNA/RNA ternary complexes showing that the DNA template is located within a cleft of about 25 Å in diameter in the elongating RNA polymerase, indicating that it represents the active site. The interaction surfaces on the polymerase for TFIIB and TFIIE were also described based on results from cocrystallography. TFIIB is located on the surface of the RNA polymerase II at about 110 Å (~30 bp) from the active center cleft. This led to the hypothesis that TFIIB, by interaction with the TATA box via TBP and with the polymerase, aligns the polymerase properly on the DNA template. This was tested by solving the structure of an RNA polymerase II–TFIIB complex to 6.5 Å resolution using X-ray crystallography. By combining these results with the previously reported high-resolution structure of a TATA DNA–TBP–TFIIB complex (Nikolov et al., 1995), it was shown that the TATA DNA is oriented so that linear extension leads across the surface of the polymerase to the active center cleft about 30 bp downstream.

The transcription factor TFIIH comprises nine subunits and is involved in both transcription, DNA repair, and cell cycle regulation. Mutations in human TFIIH subunits are associated with the genetic disorders xeroderma pigmentosum (XP), Cockayne's syndrome (CS), and trichothiodystrophy (TTD). Jean-Marc Egly described the importance of the interaction between the XPD helicase and the p44 subunits of TFIIH in these syndromes (Coin et al., 1998). Mutations in the C-terminal part of the XPD protein have been mapped in both XP and TTD patients. Interestingly, recombinant XPD proteins with the corresponding mutations showed a weaker interaction with p44, leading to a reduced XPD helicase activity (Tirode et al., 1999). TFIIH was also purified from cell lines derived from patients with mutations in the XPB helicase. In contrast to XPD, the TFIIH from these cell lines was not perturbed in subunit stoichiometry or composition and had essentially similar CTD kinase and DNA-dependent ATPase activities as wild-type TFIIH. However, transcription was severely impaired, thus indicating that some of the symptoms might be explained by deficiencies in the transcription apparatus rather than by defects in DNA repair.

André Sentenac highlighted some interesting analogies between the Pol II and Pol III systems. Recruitment of Pol III to a promoter involves a cascade of protein–protein interactions in which TFIIIC plays the role of both an enhancer and a promoter-binding factor that overcomes nucleosomal repression, perhaps via an intrinsic HAT activity. This analogy was emphasized by the finding that PC4 and topoisomerase I, two known Pol II coactivators, are also involved in mammalian TFIIIC function. He also reported on more recent studies that identify the C11 subunit of Pol III as necessary for the intrinsic RNA cleavage activity of Pol III, which is required for backtracking of artificially stalled polymerases and for recognition of defined termination sites downstream of a transcribed gene (Chédin et al., 1998).

Factors Involved in Transcriptional Regulation

By the time the general polymerase II transcription factors were obtained in a highly pure form from several organisms, it was obvious that they were insufficient to respond to the addition of transcriptional activators and repressors in vitro. Several cofactors/adaptors such as

TAF_{II}s, Mediator, and USA were identified as important components required for this process. However, questions have been raised concerning the original proposals about these complexes, as to how general a role the TAF_{II} complex plays in transcriptional activation, and the generality of the Mediator complex (since it was originally only identified in yeast cells). Several speakers at the meeting contributed to clarification of these issues. First, the biochemical purification of Mediator-like complexes from mammalian cells by three independent groups using different model systems validated its general importance for transcriptional regulation. Second, studies on the function of TAF_{II}s at specific promoters, along with recent findings of TAF_{II}s as subunits of several chromatin-remodeling complexes, provide a more mature view of TAF_{II} function.

The Mediator Complex

Mediator was originally described as a crude protein fraction isolated from *S. cerevisiae* that could relieve squelching (activator inhibition of transcription) in a nuclear extract–based in vitro transcription system. Later studies showed a requirement for the Mediator fraction in transcriptional activation. Other studies identified the *SRB* genes as suppressors of a truncation of the C-terminal domain (CTD) of RNA polymerase II and showed that several of the SRB proteins reside in a large protein complex containing RNA polymerase II (Thompson et al., 1993). These results converged with the purification of Mediator to homogeneity as a complex of 20 polypeptides, including five Srb proteins (Kim et al., 1994). The purified Mediator exhibited three biochemical activities in a reconstituted system: it stimulated basal transcription, it enabled stimulation of transcription by activators, and it stimulated CTD phosphorylation. Several independent results also indicated that Mediator formed a stable complex with the CTD.

All of the 20 yeast Mediator subunits have now been identified (Table 3). Roger Kornberg reported functional studies on Mediator isolated from strains lacking Med2, Pgd1, or Sin4. Structure–function correlations indicated that they, together with Gal11, constitute a specific subcomplex in Mediator that is involved in activation by the Gal4 and Gcn4 proteins. This specific subcomplex of Mediator is also involved in interaction with TFIIE. Results presented by Hiroshi Sakurai described the domains in TFIIE and Gal11 that are essential for this interaction and also showed that Gal11 and TFIIE act cooperatively to stimulate CTD phosphorylation (Sakurai and Fukasawa, 1998). This finding explains why expression of most genes is inhibited in a Gal11 strain, which exhibits temperature-sensitive growth on rich medium.

The generality of Mediator was strongly emphasized at the meeting by the identification of related Mediator complexes in mammalian cells by three different groups (Table 2). Robert Roeder reported that HeLa cell nuclear extracts that are depleted of TFIID (TBP + TAF_{II}s) only require addition of TBP to support both basal and activated transcription. In contrast, partial depletion of the human homolog of the yeast Mediator subunit Srb7 resulted in a corresponding reduction of activation by Gal4-VP16. The high conservation of certain Srb proteins suggested the existence of a Mediator-like complex in mammalian cells. This was confirmed both by

Table 2. Comparison of the Subunits of Yeast and Mouse Mediator with the Human Med1-Containing Coactivator Complexes SMCC and CRSP

YEAST MEDIATOR	MOUSE MEDIATOR	SMCC	CRISP	COMMENTS
–	–	TRAP220	TRAP220	Interacts with TR, PPAR, RXR, NAT-subunit
–	p160	–	–	N.S
Nut 1(p130)	–	–	–	Involved in negative transcriptional regulation
Gal11 (p130)	–	–	–	Req. for metab. of galactose and nonfermentable carbon sources
Rgr1 (p115)	Rgr1 (p110)	Rgr1 (p170) (=TRAP170)	Rgr1 (p150)	Required for repression of glucose-regulated genes. (see Sin4)
–	–	–	p130	N.S
Sin4 (p110)	–	–	–	In subcomplex with Gal11, Rgr1, Pgd1, Med 2
–	–	TRAP100	–	TRAP-subunit (see TRAP220)
–	p96	–	p100	CRSP100 N.S
Srb4 (p98)	–	–	–	Binds Med6 and Srb6. Target for Gal4-A.D
–	–	–	p85	N.S
–	p78	–	p77	N.A
Med1 (p70)	–	–	–	Interacts with Med2, neg. regulated by Srb10/11.
–	–	–	p70	N-term. similar to hTFIIS
[Srb10 (p63)]	p55	Srb10 (p53)	–	Cyclin/kinase pair with Srb11 Repr. of glucose reg. genes
Med2 (p62)	–	–	–	See Sin4, Med1. Required for Gal4-activation
Pgd1/Hrs1 (p48)	–	–	–	See Sin4
Med4 (p38)	–	–	–	N.A
[Srb11 (p38)]	–	Srb11 (p32)	–	See Srb10
Med6 (p36)	Med6 (p32)	Med6 (p33)	–	See Srb4. Subunit of hNAT
Srb5 (p35)	–	–	–	Req. for efficient transcr. init.
–	p34	–	p34	CRSP34 N.S
Med7 (p31)	Med7 (p36)	–	Med7 (p33)	N.A
Med8 (p30)	–	–	N.D.	N.A
Rox3/Ssn7 (p30)	–	–	N.D.	Inv. in glucose-regulated transcr.
–	p28a,b	–	N.D.	Homolog to dTRF prox. prot
Srb2 (p27)	–	–	N.D.	See Srb5
–	–	Soh1 (p25)	N.D.	Positive regulator, supressor of Hpr1
Med9/Cse2 (p20)	–	–	N.D.	Involved in chromosome segregation Required for basal *HIS4* expression
Med10/Nut2 (p19)	N.D	Nut2 (p18)	N.D.	Required for Gcn4-dependent basal and activated *HIS4* expression
Srb7 (p16)	Srb7 (p21)	Srb7 (p20)	N.D.	N.A
Med11 (p15)	N.D.	N.D.	N.D.	Required for MFα1 transcription
Srb6 (p15)				See Srb4

Proteins highlighted in red represent homologous subunits in the different complexes. Proteins in blue indicate subunits present in at least two of the complexes that are similar in size but have not yet been sequenced. Underlined yeast subunits are encoded by genes that are essential for mitotic growth, and numbers in parentheses indicate apparent or predicted molecular weights in kilodaltons. Note that Srb10 and Srb 11 (marked by brackets) are not bona fide subunits of the yeast mediator complex. ND, not determined; NS, not sequenced; NA, not functionally analyzed.

coimmunoprecipitation of the human homologs to Srb7, Srb10, and Srb11 and gel filtration of cellular extracts showing that all three proteins comigrated in a complex of about 1.5 MDa. The complex was designated Srb and Med–containing cofactor complex (SMCC), and peptide sequencing showed that it contained additional mammalian homologs of the yeast Mediator, and also a 220 kDa protein found both in TRAP and CRSP (Gu et al., 1999; Table 2). However, it lacked general transcription factors, CBP, BRCA1, or Swi/Snf proteins, all of which have been reported as components of mammalian polymerase holoenzymes. In contrast with the yeast Mediator complex, SMCC does not require the RNA polymerase II CTD to function.

Analysis of SMCC function in vitro also indicated that

it had a negative effect on transcription also when a polymerase lacking CTD was used. This indicated that the negative effect was not due to phosphorylation of the CTD by the Srb10/11 cyclin-kinase pair, as is the case in yeast, in which the Srb10 and Srb11 proteins are suggested to negatively regulate transcription by phosphorylation of the yeast RNA polymerase II in solution, thus preventing its incorporation into a functional preinitiation complex. However, SMCC was shown to phosphorylate PC4, which has been demonstrated to inhibit its coactivator function.

SMCC supported transcriptional activation in the reconstituted system when limiting TFIIH concentrations were used. Under these conditions, SMCC synergized with PC4 and was essential for activation by both Gal4-VP16 and the mammalian p53 protein. A direct SMCC–p53 interaction could be observed and was abolished by mutations in p53 that abrogate its activator function.

Roger Kornberg also described the purification of a mouse Mediator containing Srb7, Rgr1, Med6, Med7, and eight other proteins that did not show homology to any of the yeast Mediator subunits. Significant amounts of mouse Srb7 and Med7 eluted in side fractions throughout the purification procedure, indicating that mammalian cells might contain several different Mediator complexes. The most compelling evidence that the mouse complex is homologous to the yeast Mediator comes from electron microscopy structure determinations (Asturias et al., 1999). These results show that the mouse and yeast mediator complexes are similar in both shape and overall size. Furthermore, addition of the corresponding core RNA polymerase II induces a conformational change in both Mediators, resulting in a crescent-like structure that covers a large portion of the polymerase.

Previous results have shown that an in vitro transcription system composed of all RNA polymerase II general transcription factors and the TFIID complex (but not TBP alone) was sufficient to reconstitute Sp1-activated transcription. However, a reanalysis of this system reported by Robert Tjian indicated the requirement for an additional activity present in HeLa extracts. Fractionation of this activity identified a novel 700 kDa multiprotein complex termed CRSP (cofactor required for Sp1 activation) composed of both novel subunits and proteins previously identified as components of other complexes involved in transcriptional regulation (Ryu et al., 1999). Two subunits were homologous to the yeast Mediator subunits Rgr1 and Med7, and a third subunit was identified as TRAP220/TRIP2, which is also a subunit of both TRAP and SMCC (see below and above). In contrast, a 70 kDa subunit contained regions with homology to the transcriptional elongator protein TFIIS, indicating that CRSP might act at a step subsequent to transcriptional activation. Identification of the remaining CRSP subunits might reveal additional Mediator or SMCC proteins.

Tjian also reported on the factor requirements for synergy between Sp1 and the sterol-regulated factor SREBP-1A on chromatin templates. TAF_{II}s, a CRSP-containing fraction, and a novel SREBP-binding activity were all required to mediate efficient activation of the promoter. Purification of the SREBP coactivator to near homogeneity showed that it contained CBP. However, neither recombinant CBP nor PCAF could functionally replace the purified complex.

An interesting finding that originates from the work on the yeast Mediator was presented by Jesper Svejstrup. Purification of an elongating form of RNA polymerase II from yeast chromatin showed that its CTD was both hyperphosphorylated and lacked bound Mediator. The elongating polymerase was a holoenzyme, of which the elongator complex was the main component. Elongator consists of three polypeptides of 150, 90, and 60 kDa named elongator proteins (Elp) 1–3. In vivo studies of the Elp1 subunit showed that it was not essential for viability, but *elp1* cells were temperature sensitive and showed a slow adaptation to changed growth conditions (Otero et al., 1999). Consistent with a role in transcript elongation through chromatin, the purified holoenzyme could transcribe nucleosomal templates in vivo without the pause sites typically seen with core RNA polymerase II. The interaction of elongator with the phosphorylated form of the CTD may be incorporated in a cyclic model for RNA polymerase II as it goes from initiation through elongation and then back for a new round of transcription. In agreement with this, CTD phosphatase was shown to be a component of native, ternary complexes, indicating that it is bound to the holoenzyme during transcript elongation.

TAF_{II}-Containing Complexes

The transcription factor TFIID is comprised of the TATA-binding protein TBP and a set of evolutionarily conserved TBP-associated factors (TAF_{II}s). Although original in vitro observations in reconstituted mammalian systems suggested that TAF_{II}s were essential transcriptional coactivators, genetic results in yeast have challenged this simple view. Moreover, the identification of multiple complexes containing subsets of TAF_{II}s have complicated the interpretation of the genetic experiments.

Michael Green presented results on the effects of in vivo inactivation of yeast $TAF_{II}17$ and $TAF_{II}145$ using the microarray technique for whole-genome transcription analyses allowing determination of the expression of the entire set of 6200 yeast open reading frames in mutant and wild-type strains. Inactivation of $yTAF_{II}145$ affected the expression (up or down) of about 1400 genes, many encoding proteins involved in cell cycle control or chromosome metabolism. In contrast, both Michael Green and Kevin Struhl reported that inactivation of $yTAF_{II}17$, which is present also in the SAGA complex, affected the transcription of the majority of yeast genes. For a more detailed description of these results, please see Hahn (1998) and references therein.

Several complexes containing TAF_{II} subunits have been identified in HeLa cell extracts (Table 3). Laszlo Tora presented studies of the human $TAF_{II}30$ subunit and showed that two consecutive immunoprecipitations of a crude HeLa nuclear extract using monoclonal antibodies specific for TBP and $hTAF_{II}30$ resulted in the purification of a TBP-free TAF_{II}-containing complex (TFTC). TFTC contains both TAF_{II}s and proteins known as components of the PCAF/SAGA complex. Functional analysis of TFTC showed that it can substitute for TBP or TFIID in basal and activated transcription from both

Table 3. Comparison of TAF-Containing Complexes from Different Species

	TAF_{II}-Containing Complexes					
	Containing TBP			Lacking TBP		
Functions	yTFIID	dTFIID	hTFIID	ySAGA	hTFTC	hPCAF/GCN5
Protein kinase. HAT activity. Required for cell cycle progression.	145 (130)	230	250	—	—	—
Interacts with promoter DNA. Required for initiator function.	TSM1	150	150	—	ND	ND
Interacts with Sp1, E1A, and CREB. Coactivator for RAR, TR, and VDR.	—	110	135	—	135	—
Contains WD40 repeats. Interacts with TFIIFβ.	90	80	100	90	100	— (PAF65β)
Coactivator for p53, Histon H4 motif, and forms histone-like pair with $hTAF_{II}32$.	60	62	80 (70)	60	80	— (PAF65α)
Interacts with several activators, Sp1, VDR, and TR.	67 (68)	55	55	—	55	ND
Histone H3 motif and forms histone-like pair with $hTAF_{II}80$. Interacts with TFIIB and the VP16 AAD.	17 (20)	42	31 (32)	17 (20)	31 (32)	31 (32)
Interacts with the AF-2a domain of the ER. Required for ER activity in vitro.	25	ND	30	25	30	30
Coactivator for ER, VDR, RXR, and Tax. Forms histone pair with $hTAF_{II}18$.	40	30β	28	—	—	ND
Contains histone H2B–like motif.	68 (61)	30α	20 (15)	68 (61)	20 (15)	20 (15)
Contains novel type of histone fold. Forms histone-like pair with $hTAF_{II}28$.	19 (FUN81)	ND	18	—	—	ND
Binds to promoter TATA element.	yTBP	dTBP	hTBP	—	—	—
HAT	—	—		yGcn5	hGcn5-L	PCAF/Gcn5-s

ND, not determined.

TATA-containing and TATA-less promoters, challenging the dogma that TBP is a universal and indispensable transcription factor.

Cofactors Involved in Transcriptional Regulation by Nuclear Receptors

The nuclear receptors (NRs) are a well-studied class of transcriptional activators whose activity is modulated by ligand binding. They are composed of distinct functional domains involved in DNA binding, dimerization, ligand binding, and transcriptional activation or repression. In the absence of their cognate ligands, the NRs for all-trans retinoic acid (RAR) and thyroid hormone (TR) can repress transcription by interaction with corepressor proteins present in large complexes containing histone deacetylases. Conversely, several protein factors such as CBP, PCAF, TIF2, SRC, and ACTR, all of which exhibit HAT activity, have been shown to interact with NRs in a ligand-dependent manner. These observations have led to the idea that gene activation may involve NR-targeted local histone acetylation and chromatin decondensation (Torchia et al., 1998).

Robert Roeder described experiments designed to search for proteins that interact with the TR and allow it to function in vitro. Stably transformed HeLa cell lines expressing epitope-tagged TR were established. When TR is purified from these cells, a set of at least nine proteins (TR-associated proteins, TRAPs) is found stably associated with TR, but only from cells treated with thyroid hormone. A seemingly identical complex (DRIP) has also been identified by its ability to bind selectively to the liganded vitamin D3 receptor (VDR) in vitro (Rachez et al., 1998). Most significantly, both complexes are devoid of the other known NR coactivators (CBP, TIF-2, SRC, etc.). Identification of the components of TRAP (and DRIP) has shown that they contain a protein designated TRAP220 (Yuan et al., 1998), which interacts with the TR in a ligand-dependent fashion in vitro. An intriguing finding is that TR-TRAP transcriptional activation in vitro absolutely requires another coactivator such as PC4, but apparently not TAF_{II}s, showing that the requirement for a specialized coactivator does not obviate the requirement for general coactivators. The above observations suggest that activation by NRs may be viewed as a multistep process. In a first step, the NRs interact with a series of proteins possessing HAT activity, which allow a local modification of chromatin structure. The receptors may then interact with the TRAP proteins that then act in concert with the other general coactivators (Mediator/SMCC/CRSP) to enhance transcriptional initiation.

Cofactors Isolated from the USA Fraction

Ion exchange chromatography of HeLa cell nuclear extracts generates multiple fractions containing transcriptional coactivators required for activator function in vitro. These factors were originally identified in the crude USA (upstream-factor stimulatory activity) fraction. Further purification of the USA fraction showed that it contained both positive-acting coactivators PCs 1–6, and negative-acting factors NC1 and NC2 (Meisterernst et al., 1991). Michael Meisterernst reported results obtained from studies of PC4 and NC2.

PC4 has been purified to homogeneity and is a 15 kDa protein that can both stimulate activated transcription and, at higher concentrations, repress basal transcription. PC4 is divided into a structured C-terminal domain and a probably unstructured N-terminal domain.

The recent X-ray crystal structure of the C-terminal domain showed that it dimerizes and forms two quarter pipe structures that each resemble a single-stranded (ss) DNA-binding channel (Brandsen et al., 1998). PC4 does indeed bind ssDNA with a 100-fold higher affinity than double-stranded DNA and might therefore bind melted DNA duplexes in opened promoters or during DNA replication, recombination, or repair. However, mutations in PC4 that were generated to disrupt ssDNA binding had no effect on PC4 coactivator function, at least in vitro, but rather affected its ability to repress basal transcription.

Purification of NC2 to homogeneity showed that it is composed of two subunits, NC2α and NC2β. The NC2 heterodimer binds to the TBP–DNA complex and prevents the subsequent association with TFIIA and TFIIB. Homologs of NC2α and β are found in yeast, and both are essential for viability but can be complemented by their human counterparts. Expression of the human NC2β at a low level in yeast strains lacking NC2β results in a slow-growing phenotype and was used in a screen for suppressor mutations. One such suppressor was mapped to a mutation in the gene encoding the Toa1 subunit of the yeast TFIIA, located in the dimerization interface between Toa1 and Toa2. Recombinant TFIIA containing this mutation failed to antagonize the binding of NC2 to TBP and to alleviate transcriptional repression by NC2. These results support the idea that transcriptional repression by NC2 via competitive binding with TFIIA to TBP is essential for yeast viability.

Future Perspectives

The fractionation of cellular extracts has defined a minimal set of factors required for transcription of a naked DNA template in vitro, including some 30 polypeptides for unregulated transcription, and at least twice as many for regulated transcription. However, the situation in vivo is much more complex. It involves both accessibility of the general RNA polymerase II transcription factors and regulatory proteins to a highly organized template in the form of chromatin, and recruitment of specific factors that can remodel or modify chromatin both locally and over larger regions. The meeting on global transcriptional regulators of eukaryotes highlighted the necessity of combining multiple approaches using biochemical, genetic, and biophysical techniques. The structural determination of the nucleosome core particle has provided fundamental insights into the hurdles that have to be overcome to provide access to the DNA for general and regulatory transcription factors. This is just the beginning, since the core nucleosome structure represents the most basic level of DNA packaging. It does not include the highly modifiable histone tails whose roles in the formation of higher ordered chromatin structures and connections with factors involved in chromatin remodeling are only beginning to be unraveled. In parallel, the large basal transcriptional machinery structure is gradually being revealed at higher resolution and will certainly make a major contribution to a better understanding of the mechanistic details of transcription and how it is regulated.

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