From the Department of Medical Nutrition
Karolinska Institutet, Stockholm, Sweden

THE TRANSCRIPTION MACHINERY IN *SCHIZOSACCHAROMYCES POMBE* AND ITS REGULATION

Henrik Spåhr

Stockholm 2004

Published and printed by Karolinska University Press
Box 200, SE-171 77 Stockholm, Sweden

ISBN 91-7349-863-7

ABSTRACT

The Mediator complex acts as a bridge, conveying regulatory information from enhancers and other control elements to the general transcription machinery. The Mediator was originally identified in *Saccharomyces cerevisiae* and is required for the basal and regulated expression of nearly all RNA polymerase II dependent genes. Mediator-like complexes have also been identified in higher eukaryotes and shown to play an essential role in transcription regulation. However, most of the subunits identified in these mammalian complexes displayed low or no significant sequence similarity with Mediator subunits previously identified in yeast. Our specific aim was to purify Mediator from *Schizosaccharomyces pombe* and to compare its subunit composition and function to *S. cerevisiae* and mammalian Mediators to shed light on the mechanism and evolution of Mediator dependent transcription regulation.

In paper I and II, we purified the *S. pombe* Mediator in complex with RNA polymerase II. We showed that the *S. pombe* Mediator complex was considerably smaller than its *S. cerevisiae* counterpart containing only 13 subunits instead of 20. Three of the *S. pombe* subunits were species specific named PMC for Pombe Mediator Complex. Additionally, the *S. pombe* Mediator contained 10 subunits conserved in *S. cerevisiae* and 8 in metazoans. Genetics showed that the conserved subunits were essential for cell growth, whereas the species-specific subunits were non-essential. Our findings led us to propose that the Mediator consists of a set of core subunits conserved through evolution that is responsible for contacts with the general transcription machinery and a set of species-specific subunits that function as a dynamic interface for direct interactions with gene-specific activators.

In paper III we analyzed the function of a specific Mediator subcomplex. Mediator from mammalian cells has been isolated in two different forms, the larger TRAP/Mediator complex and the smaller PC2/CRSP complex. The TRAP/Mediator complex contains 4 additional proteins, TRAP230, TRAP240, Srb10 and Srb11, which are absent in PC2/CRSP. We developed a purification scheme for the larger form of the *S. pombe* Mediator using the so-called tandem affinity purification tag (TAP). Our new purification procedure allowed to identify a novel form of Mediator, which also contained homologues to TRAP230, TRAP240, Srb10 and Srb11, which we denoted the TRAP240/Mediator.

In paper IV we reconstituted a pure *in vitro* system for RNA polymerase II dependent transcription. We purified *S. pombe* general initiation factors TFIIB, TFIIF, TFIIE, and TFIIH to near homogeneity. These factors enabled highly purified RNA polymerase II to initiate transcription from the *S. pombe* alcohol dehydrogenase promoter (*adh1p*) when combined with *S. cerevisiae* TBP. We used the *in vitro* system to compare the activities of Mediator and the larger TRAP240/Mediator on basal transcription. We found that the smaller form of Mediator was able to stimulate transcription whereas the larger TRAP240/Mediator repressed transcription. Our studies lead us to propose a model for how the two forms of Mediator interact to regulate RNA polymerase II dependent transcription.

LIST OF PUBLICATIONS

I. Spåhr H, Bève J, Larsson T, Bergström J, Karlsson KA, Gustafsson CM. (2000)
Purification and characterization of the RNA polymerase II holoenzyme from *Schizosaccharomyces pombe*.
J Biol Chem. 275(2): 1351-1356

II. Spåhr H, Samuelsen CO, Baraznenok V, Ernest I, Huylebroeck D, Remacle JE, Samuelsson T, Kieselbach T, Holmberg S, Gustafsson CM. (2001)
Analysis of *Schizosaccharomyces pombe* Mediator reveals a set of essential subunits conserved between yeast and metazoan cells.
Proc Natl Acad Sci U S A. 98(21): 11985-90

III. Samuelsen CO, Baraznenok V, Khorosjutina O, Spåhr H, Kieselbach T, Holmberg S, Gustafsson CM. (2003)
TRAP230/ARC240 and TRAP240/ARC250 Mediator subunits are functionally conserved through evolution**.**
Proc Natl Acad Sci U S A. 27 (11): 6422-7

IV. Spåhr H, Khorosjutina O, Baraznenok V, Linder T, Samuelsen CO, Hermand D, Mäkelä TP, Holmberg S, Gustafsson CM. (2003)
Mediator influences *Schizosaccharomyces pombe* RNA polymerase II dependent transcription *in vitro*.
J Biol Chem. 278(51): 51301-6

TABLE OF CONTENTS

INTRODUCTION **1**

THE FLOW OF GENETIC INFORMATION 1
INITIATION OF TRANSCRIPTION IN PROKARYOTES 2
INITIATION OF TRANSCRIPTION IN EUKARYOTES 2
RNA polymerase II *4*
Transcription factor IID *7*
Transcription factor IIB *7*
Transcription factor IIF *8*
Transcription factor IIE *9*
Transcription factor IIH *9*
THE STAGES OF TRANSCRIPTION INITIATION 11
TRANSCRIPTION REGULATION 11
Revealing intermediary factors in transcription regulation *12*
Chromatin modifying Coactivators *13*
Transcription activation in vivo *14*
THE MEDIATOR COMPLEX 15
The discovery of Mediator *15*
Subunit composition of the Mediator complex *16*
Structural studies of the Mediator complex *17*
Subcomplexes in Mediator *18*
Mediator-like complexes in higher eukaryotes *19*
Proposed differences between yeast and human Mediators *21*
Activation *21*
Repression *22*

MAIN OBJECTIVES **23**

RESULTS AND DISCUSSION **24**

PAPER I AND II 25
PAPER III 26
PAPER IV 29
SUMMARY 29
A PUTATIVE MODEL FOR MEDIATOR DEPENDENT TRANSCRIPTION REGULATION 30

FUTURE INVESTIGATIONS **33**

ACKNOWLEDGEMENTS **35**

REFERENCES **37**

LIST OF ABBREVIATIONS

bp	base pair
BRE	TFIIB recognition element
CAK	cdk activating kinase
cdk	cyclin dependent kinase
CTD	Carboxy terminal domain
DPE	Downstream promoter element
GTF	General transcription factor
HAT	Histone acetyltransferase
HDAC	Histone deacetylase complex
Inr	Initiator
kD	kilodalton
mRNA	messenger RNA
NC	Negative cofactor
NER	Nucleotide excision repair
PC	Positive cofactor
PIC	Pre-initiation complex
RNAP	RNA polymerase
S. cerevisiae	*Saccharomyces cerevisiae*, budding yeast
S. pombe	*Schizosaccharomyces pombe*, fission yeast
Srb	Suppressor of RNAP B
TAF	TBP associated factor
TAP	Tandem affinity purification
TBP	TATA binding protein
TF	Transcription factor
UAS	Upstream activating sequence
USA	Upstream stimulatory activity

INTRODUCTION

THE FLOW OF GENETIC INFORMATION

Many genes contain information that specifies the expression of a specific protein. Thus, the genetic code of DNA ultimately directs the activity of proteins, which in turn are the essential machinery of life. DNA is, however, not the direct template for protein synthesis. Rather messenger RNA (mRNA) functions as an intermediate between DNA and protein expression. All forms of cellular RNA are synthesized by RNA polymerases from a DNA template in a step called transcription. Prokaryotes contain only one RNA polymerase whereas eukaryotes contain three types, which transcribe different sets of nuclear genes. RNA polymerase I (RNAP I) transcribes genes encoding ribosomal RNAs, RNA polymerase II (RNAP II) transcribes protein-encoding genes and RNA polymerase III (RNAP III) transcribes transfer RNA encoding genes. The synthesis of proteins according to the instructions given by the mRNA template has been named translation. Thus, the central dogma in molecular biology concerning the flow of genetic information in a cell is:

There has been remarkable conservation of the transcription process throughout evolution. These findings imply that what we learn about transcription in prokaryotes applies to our basic understanding of transcription in eukaryotic cells. Furthermore, the transcription machinery in simple eukaryotes, e.g. yeast, is virtually identical to the machineries found in mammalian organisms.

All cells in a multi-cellular organism contain the same set of genes, but the genes that are expressed vary from cell to cell. Therefore gene expression must be regulated in order to produce the right set of proteins in correct amounts. The content of proteins in a cell determines the phenotype and the type of tissue the cell gives rise to. Regulation of gene expression is far more complex in eukaryotes than in prokaryotes,

and takes place at many different levels, e.g. transcription, RNA processing, mRNA export, mRNA stability, and translation. The key step in the regulation of most genes is however at initiation of transcription.

INITIATION OF TRANSCRIPTION IN PROKARYOTES

RNA polymerases initiate transcription from specific DNA sequences, so called promoters. The simple monomeric RNA polymerase of bacteriophage T7 interacts sequence-specifically with promoter DNA and initiates transcription in the absence of additional protein factors (Kochetkov et al. 1998). Transcription initiation in prokaryotes is slightly more complex. The *E. coli* RNAP holoenzyme contains a 400 kD core complex comprising 5 subunits (α_1, α_2, β, β', ω), which associates with the dissociable initiation specific subunit, σ (Young et al. 2002). The core complex is responsible for template-directed RNA synthesis and also interacts with regulatory proteins, which modulate transcription levels. The σ factor has dual roles in transcription; it is responsible for promoter recognition as well as melting double stranded DNA. The core share both sequence and structural homology with its eukaryotic counterparts suggesting similar transcription mechanisms. However, the regulation of transcription seems fundamentally different between prokaryotes and eukaryotes. In contrast to eukaryotes, the DNA in prokaryotes is not packaged into the inaccessible chromatin structure (Struhl 1999). Regulatory factors therefore have free access to specific sequences immediately upstream of the initiation site. In prokaryotes the regulatory factors can contact and recruit RNAP directly whereas in eukaryotes additional factors are needed. In *E. coli* there are no less than 6 alternative σ factors, which are used to fine-tune the transcriptional output (Gruber and Gross 2003).

INITIATION OF TRANSCRIPTION IN EUKARYOTES

Promoters typically contain a core sequence element, to which DNA binding factors associates and forms a nucleation site for transcription complex formation. In eukaryotes, the most common and well-characterized RNAP II promoter core element is the TATA box. However, other promoters exist generally referred to as TATA-less promoters. In an evolutionary perspective yeast seems to be more dependent on a

TATA-box than higher eukaryotes where TATA-less promoters are more common (Smale and Kadonaga 2003). TATA-less promoters often contain an initiator element (Inr) that is a discrete core promoter element functionally similar to the TATA box that encompasses the transcription start site. Inr can function both independently or in combination with a TATA box. Another element found associated with many TATA-less promoters is the downstream promoter element (DPE). DPE is situated downstream of the transcription start site and always acts in conjunction with Inr.

In eukaryotes, RNAP II is dependent on other factors to recognize the core promoter elements and initiate transcription. A breakthrough in revealing these factors came when transcription could be reconstituted in a crude cell extract, supplemented with RNAP II and a DNA template (Weil et al. 1979). Through work in many different laboratories the extract could later be divided into a number of essential fractions, which were purified to homogeneity. This work led to the identification of five general transcription factors (GTFs) including transcription factors IIB, IID, IIE, IIF, and IIH that could support transcription *in vitro*. A simplified model has emerged for the initiation of transcription in eukaryotes (Fig1) (Buratowski 1994). The process begins when the TATA binding protein (TBP), a component of TFIID, recognizes and binds the TATA-box of the promoter and cause a bend of the DNA. The initial complex is subsequently bound by TFIIB. The bent DNA/TFIID complex makes it possible for TFIIB to contact DNA sequences both upstream and downstream of the TATA-box. The TFIID-TFIIB complex is then bound by RNAP II and TFIIF who are tightly associated in solution. After the formation of the TFIID-B-F-RNAP II complex, TFIIE is recruited to the complex followed by TFIIH, leading to formation of the pre-initiation complex (PIC). However, the sequential mode of assembly of the transcription machinery has been questioned and it has been proposed that the entire transcription machinery may be recruited as one single multi-protein complex to eukaryotic promoters (Koleske and Young 1995).

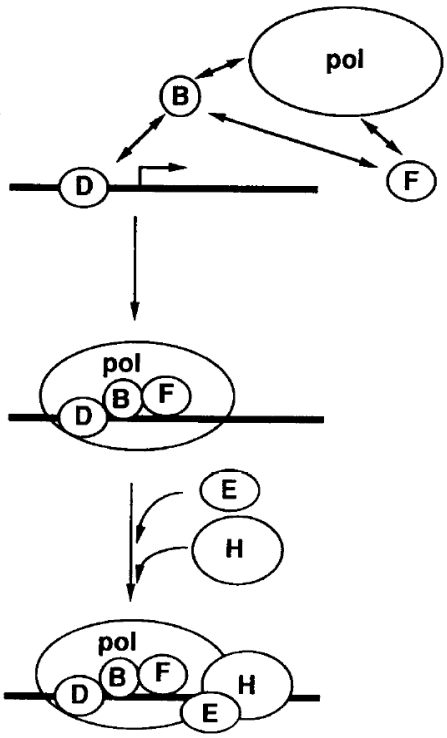

Figure 1. Assembly of the RNAP II transcription machinery.

RNA polymerase II

RNAP II is the molecular engine for all mRNA synthesis in eukaryotic cells. Transcription directed by RNAP II is not only the first step in protein expression, but is also an important target point for signal transduction pathways in cell regulation. RNAP II comprises 12 subunits denoted Rpb1 to Rpb12 making up a large protein complex around 500 kD in size. There is extensive structural conservation of RNAP II subunits among eukaryotes. That statement has been emphasized as six human RNAP II subunits can functionally replace their counterparts in yeast (McKune et al. 1995). Not only RNAP II shares a high degree of similarity in eukaryotes. All known cellular RNA polymerases including RNAP I-III are strikingly similar when it comes to subunit composition, amino acid sequence, and function. Only three subunits out of twelve appear to be specific for RNAP II (Hampsey 1998). The two largest subunits, Rpb1 and Rpb2, are the ones with most pronounced conservation. Moreover, they have homologues in the smaller and simpler bacterial RNA polymerase.

Structure of RNA polymerase II

The structure of RNAP II was recently solved and has generated a detailed understanding of the function of this giant enzyme complex. The size, complexity, and low abundance of RNAP II made determining its three-dimensional structure very difficult. After more than 15 years of experimental work on the subject, Kornberg and colleagues could in 2001 describe yeast RNAP II at 2.8 Å resolution in one case and at 3.3 Å resolution in the case of an RNAP II elongation complex (Cramer et al. 2001; Gnatt et al. 2001). Since human and yeast RNAP II share a high level of sequence identity that is evenly dispersed among subunits, at both surface and core positions, the yeast structure is a structural paradigm for all forms of RNAP II.

The two large subunits, Rpb1 and Rpb2, form the central mass of the enzyme and opposite sides of a positively charged cleft (Fig. 2). The positive charge makes contacts possible to the negatively charged DNA. The active site of RNAP II is located at the floor of the cleft, near the center of the enzyme. Just behind the active site, the DNA path is blocked by a protein "wall" forcing the DNA to bend in a nearly right angle. One side of the cleft (the Rpb1 side) is formed by a mobile 'clamp'. The clamp can be in an open position that allows DNA to enter the cleft. When closed, the clamp can sense the conformation of the DNA-RNA hybrid and separate them upstream of the transcription bubble. The separation allows the RNA to leave RNAP II through an exit groove (groove 1) situated between the base of the clamp and the wall. The active site of RNAP II is made up by subunits shared by bacterial RNAP that suggests analogous catalytic mechanisms. In contrast, there is no pronounced conservation of surface residues that may reflect the need for the eukaryotíc enzyme to interact with GTFs and regulatory factors not present in bacteria.

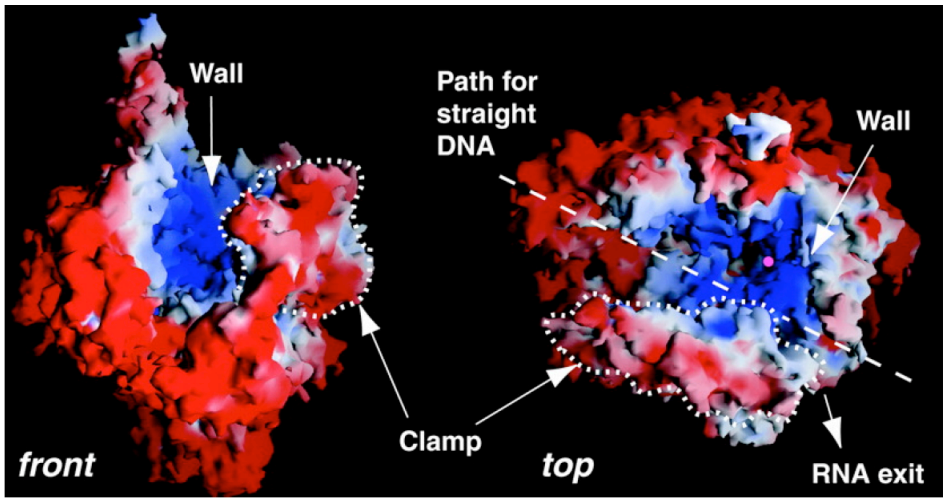

Figure 2. The structure of RNA Polymerase II. The surface of RNAP II is colored according to the electrostatic surface potential, with negative, neutral, and positive charges shown in red, white, and blue, respectively. The active site is marked by a pink sphere.

The Carboxy Terminal Domain of RNA polymerase II

The Carboxy Terminal Domain (CTD) of RNAP II consists of a conserved heptapeptide, with the sequence Tyr-Ser-Pro-Thr-Ser-Pro-Ser (Hampsey 1998). Although this sequence is essential for cell viability the number of repeats varies among organisms. Yeast CTD has 26 repeats, *Caenorhabditis elegans* 34 repeats, *Drosophila melanogaster* 43 repeats, and human 52 repeats. It thereby appears that the number of repeats reflects genome complexity. If the consensus CTD is truncated in budding yeast, it results in a temperature sensitive phenotype and impaired transcriptional activation (Scafe et al. 1990). The CTD exists in two different states, one hypophosphorylated and one hyperphosphorylated. Only the hypophosphorylated form can associate with the GTFs at the promoter, whereas the hyperphosphorylated form is involved in elongation (Cadena and Dahmus 1987). Although a number of kinases capable of phosphorylating CTD have been identified, the principle kinase involved appears to be the cyclin dependent kinase (cdk) of TFIIH (Feaver et al. 1994). It is believed that this step is important for the shift from initiation to elongation of transcription (Svejstrup et al. 1997). A phosphatase with the CTD as a target has been identified (Chambers and Dahmus 1994). It is thought to dephosphorylate RNAP II after elongation to allow re-entry for another round of transcription (Fig 3).

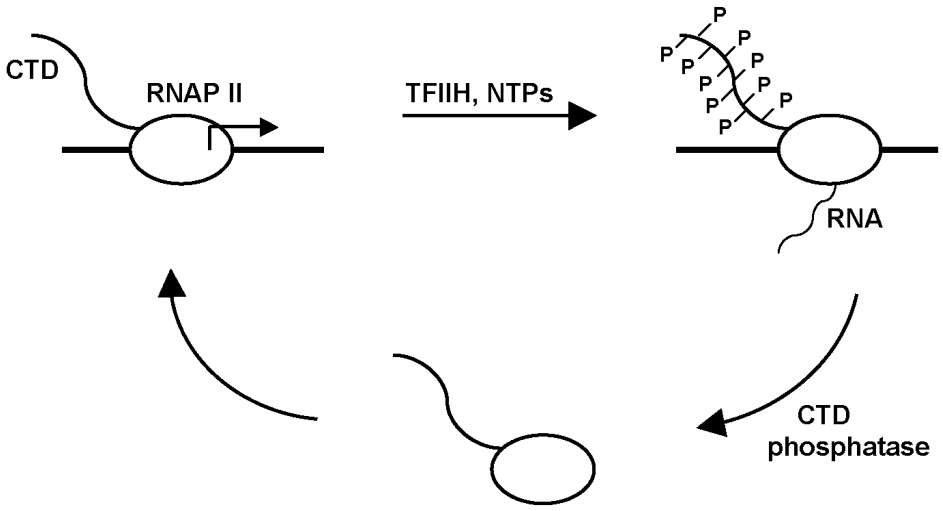

Fig 3. A round of RNAP II dependent transcription

Transcription factor IID

TFIID is a large (~750kD) multisubunit complex containing the TATA Binding Protein (TBP) and about ten TBP associated factors (TAFs). TBP is a single polypeptide, which upon binding to the TATA-box at the promoter causes a sharp bend of the DNA necessary for subsequent binding of other GTFs (Nikolov and Burley 1997). TATA-box recognition by TBP is a universal process in transcription initiation for all three eukaryotic RNA polymerases (Hernandez 1993). TBP is an essential transcription factor required for all RNAP II dependent transcription *in vivo* (Eisenmann et al. 1989). It is highly conserved among eukaryotic organisms and functionally interchangable between *in vitro* transcription systems from yeast to man (Buratowski et al. 1988; Cavallini et al. 1988).

The primary role for the TAFs appears to be to initiate transcription from promoters lacking a TATA motif. This model is supported by the finding that TFIID, but not TBP by itself, is able to target distinct set of promoters (Verrijzer et al. 1995; Burke and Kadonaga 1996).

Transcription factor IIB

TFIIB, which is a single polypeptide, enters the PIC subsequent to formation of the TBP-DNA complex and bridges RNAP II binding to the promoter (Buratowski et al. 1989). A subset of eukaryotic promoters contains a TFIIB recognition element

(BRE), which is situated upstream of the TATA-box (Lagrange et al. 1998). This element aids stabilization of the interaction between TBP and TFIIB onto DNA (Wolner and Gralla 2001) and has been demonstrated to alter the conformation of TFIIB upon binding (Fairley et al. 2002). TFIIB contains two conserved domains separated by a flexible linker region. A C-terminal domain interacts with TBP and contains a helix-turn-helix motif that binds to sequences flanking the 5' and 3' sides of the TATA element (Nikolov et al. 1995; Tsai and Sigler 2000). The N-terminal contains a zinc ribbon domain that is important for binding to RNAP II (Pardee et al. 1998) and a B-finger domain that affects transcription start site selection (See below).

TFIIB´s role in transcription start site selection

One important aspect of transcription is start site selection, i.e. the selection of the specific base pair (bp) in promoter DNA at which transcription should be initiated. RNAP II in pair with TFIIB from *Schizosaccharomyces pombe* (fission yeast) could be substituted for their counterparts in an *in vitro* transcription system from *Saccharomyces cerevisiae* (budding yeast), without loss of transcription activity. This substitution shifted the transcription start site from a location characteristic of budding yeast, 40-120 bp downstream of the TATA box, to the location found in fission yeast and most other eukaryotes including human at 25 bp downstream of the TATA-box (Li et al. 1994). In addition, genetic analysis has showed that mutations in the B-finger domain of TFIIB cause start shifts (Pinto et al. 1994; Hawkes and Roberts 1999). Recently a structure of RNAP II in complex with TFIIB has been solved (Bushnell et al. 2004). The B-finger protrudes all the way into the active site of RNAP II contacting template DNA where the fingertip have been suggested to perform fine setting of the start site.

Transcription factor IIF

TFIIF contains three subunits in yeast, but only homologues to the two largest subunits are present in the mammalian complex. The smallest subunit – Tfg3 - is the only GTF subunit in *S. cerevisiae*, which is encoded by a non-essential gene. Tfg3 is present both in TFIID and the Swi/Snf chromatin remodeling complex, establishing a connection between the basal and regulatory components of the transcription machinery. TFIIF is tightly associated with RNAP II and assists in stabilizing the PIC

(Greenblatt 1991; Conaway and Conaway 1993). In addition to its role at initiation, TFIIF interacts with the non-template strand following unwinding of the promoter keeping the DNA in an open conformation (Bushnell et al. 2004). Later TFIIF stimulates both the rate of elongation and the efficiency by which RNAP II passes through pausing sites (Bengal et al. 1991).

Transcription factor IIE

The dimeric TFIIE is the least understood GTF. Swapping experiments between fission and budding yeast *in vitro* transcription systems have demonstrated a functional link between TFIIE and TFIIH (Li et al. 1994). TFIIE cannot be individually swapped between these systems, but a fission yeast TFIIE-TFIIH pair can replace its counterpart in the budding yeast transcription system without loss of transcription activity. TFIIE interacts both with RNAP II (Van Mullem et al. 2002) and TFIIH, which indicates that it functions as a bridge between these factors (Maxon et al. 1994). It has been suggested that TFIIE functions as a checkpoint factor for formation of the PIC via its recruitment of TFIIH and regulation of its enzymatic activities (Ohkuma et al. 1995).

Transcription factor IIH

TFIIH contains nine subunits and is by far the largest and most complex of all the GTFs. TFIIH is also the only GTF containing defined enzymatic activities (Tirode et al. 1999). Two subunits, in human denoted ERCC2/XPD and ERCC3/XPB, contain DNA helicase activity with opposite polarity. Both promoter opening and promoter escape require the ERCC3/XPB helicase activity, which can be circumvented by negative supercoiling of the template DNA (Hawley and Roeder 1987). TFIIH also contains a cdk/cyclin complex, denoted Kin28/Ccl1 in *S. cerevisiae*, Cdk7/cyclin H in human, and Mcs6/Mcs2 in *S. pombe*. The cdk/cyclin pair phosphorylates the CTD of RNAP II and is important in the transition from initiation to elongation of transcription (Dahmus 1994; O'Brien et al. 1994). TFIIH can be divided into two subcomplexes: core TFIIH and the cdk/cyclin containing TFIIK. In addition to its role in transcription, TFIIH subunits take part in nucleotide excision repair (NER) and in cell cycle regulation (see below).

TFIIH in nucleotide excision repair

Cells are continuously threatened by the occurrence of DNA damage arising from exposure to irradiation and genotoxic chemicals. The DNA damage induces processing by DNA repair mechanisms, such as the NER pathway. The lesion in the DNA is removed via dual incision followed by new DNA synthesis using the intact strand as a template (Svejstrup et al. 1996). The connection between transcription and DNA repair became apparent when human TFIIH was shown to contain the helicase ERCC3/XPB previously implicated in DNA repair (Schaeffer et al. 1993). In addition, repair deficiency can be rescued in Rad3 and Ssl2 mutant extracts, which are the budding yeast counterparts of ERCC2/XPD and ERCC3/XPB respectively, with the addition of TFIIH (Wang et al. 1994). It has been reported that the core of TFIIH could form a complex with other gene products indispensable for NER creating a multi-protein repairosome (Svejstrup et al. 1995), but these findings have later been questioned (Araujo et al. 2001). The idea of transcription-coupled repair was strengthened with the discovery that DNA damage in actively transcribed genes is repaired at a much faster rate than the genome in general (Svejstrup 2002).

TFIIH in cell cycle regulation

Cell division proceeds through an ordered series of events constituting the cell cycle. The transition from one step to the next in the cell cycle is regulated by sequential activation of cdk:s (Kaldis 1999). A network of regulatory mechanisms controls the proper timing of the activity of the cdk:s including binding of subunits (cyclins and repressors), proteolysis and phosphorylation events. To become activated the cdk:s need to be phosphorylated, which is performed by a cdk activating kinase (CAK). Surprisingly, the CAK activity in human was identical to the TFIIK subcomplex of TFIIH adding yet another role to this intriguing complex. In *S. pombe*, Mcs6 reportedly has CAK activity, whereas the *S. cerevisiae* CAK is distinct from Kin28.

THE STAGES OF TRANSCRIPTION INITIATION

At initiation of transcription, the DNA double helix has to be unwound around the transcription start site. The unwinding creates a single-stranded transcription bubble, which allows RNAP II to access the DNA template. To form the open complex, TFIIH binds DNA and cause promoter opening through thermal breathing after introducing negative supercoiling or by a direct helicase action (Bushnell et al. 2004). TFIIF binds the non-template single stranded DNA to trap the open region, which allows RNAP II and TFIIB to bind the template strand and to select the proper transcription start site. RNAP II is then able to synthesize the first phosphodiester bonds of nascent RNA transcripts. When the transcripts are about 10 nucleotides long, RNAP II appears to get stalled and are left with two options; abortive transcription by releasing the DNA or promoter escape initiating elongation of transcription (Dvir et al. 2001). A possible explanation for abortive transcription is the competition between RNA of length greater than 10 residues and TFIIB for occupancy on the saddle of RNAP II between the active site and the RNA exit point (Bushnell et al. 2004). This competition likely also contributes to promoter escape by the release of TFIIB. In addition, promoter escape also requires the ATP-dependent helicase activity of TFIIH (Moreland et al. 1999). However, the mechanism that decides which route RNAP II takes is still an open question.

TRANSCRIPTION REGULATION

RNAP II and the GTFs alone can initiate basal transcription on a DNA template containing a promoter *in vitro*. Cells, however, need to respond to physiological changes in their environment to be able to adjust to the new conditions. An important way is to regulate the transcription levels and thereby produce more or less of a specific protein required to alter a process in the cell. In both the vicinity of the promoter region and at more distal positions, genes contain regulatory sequences, which control the rate of transcription. These regulatory sequences contain binding sites for factors that can either stimulate or repress transcription. How can the regulatory factors transfer their signals to the transcription machinery and which factor do they contact? These questions have been a matter of extensive research and debate for the last 15 years.

Revealing intermediary factors in transcription regulation

One of the first models presented for activated transcription in eukaryotes was the recruitment model. The recruitment model contends that activators function by recruiting components of the RNAP II machinery to promoter DNA by contacting them directly (Ptashne and Gann 1997), much similar to mechanisms of transcription activation in prokaryotes. Although there have been numerous reports on interactions between activators and GTFs (Hall and Struhl 2002), activated transcription *in vitro* is not supported by RNAP II and GTFs alone (Myers and Kornberg 2000). Instead, activators need to contact intermediary factors that can convey regulatory information to the transcription machinery. The intermediary factors are referred to as cofactors, which may function as a coactivator, corepressor or both. Cofactors are distinct from the GTFs in that they are dispensable for basal transcription *in vitro* and are distinct from activators and repressors in that they in most cases do not have the capacity to bind DNA with sequence specificity. Several targets for activators have been proposed over the years as a prerequisite for activated transcription *in vitro*:

1. The TAFs, which are the non-TBP components in TFIID (Dynlacht et al. 1991).
2. Upstream Stimulatory Activity (USA), which was found in mammalian cells (Meisterernst et al. 1991).
3. A multi-protein complex named Mediator, isolated from yeast, which associates with RNAP II forming a holoenzyme (Kelleher et al. 1990).

The model for TAFs in transcription has been revised in recent years. In the budding yeast *in vitro* transcription system, the TAFs are not required for transcription activation. Genetics suggested another role for the TAFs. Destruction of one of the larger TAFs important for the integrity of TFIID had no effect on transcription levels of most yeast promoters (Moqtaderi et al. 1996; Walker et al. 1996). At the minority of promoters where transcription was impaired, the TAFs were mapped to sequences surrounding the TATA-box at the promoter rather than regulatory sequences (Shen and Green 1997). The TAFs might affect transcription regulation at certain conditions, but the main role for the TAFs seems to be in promoter targeting rather

than transcription activation. The TAFs are not only members of the TFIID complex. In addition, certain TAFs are also components of histone acetyltransferase containing complexes like the yeast SAGA complex and the human STAGA, PCAF and TFTC complexes (Woychik and Hampsey 2002).

The USA fraction was upon further purification shown to contain several positive cofactors (PCs) as well as negative cofactors (NCs). These cofactors repress transcription in the absence of activators and stimulate transcription in the presence of activators (Meisterernst et al. 1991). One of them, PC2, could support transcription activation *in vitro* (Kretzschmar et al. 1994) and was later shown to have homology to the yeast Mediator complex (Malik et al. 2000).

The Mediator complex was isolated as an activity required for activated transcription in a reconstituted *in vitro* transcription system in budding yeast (Kelleher et al. 1990). Both positive and negative regulatory information are transferred from gene-specific activators and repressors to the transcription machinery through the Mediator (Myers and Kornberg 2000). TAFs have recently been shown to have a limited role in transcription activation, and so the Mediator complex is referred to as the general coactivator involved in RNAP II transcription regulation. In fact, Mediator is needed for almost all RNAP II dependent transcription *in vivo* (Holstege et al. 1998).

Chromatin modifying Coactivators

DNA in eukaryotes is packaged into chromatin (Kornberg 1974). The basic unit of chromatin is the nucleosome, which consists of around 150 bp genomic DNA wrapped around an octamer of the four histone proteins (H2A, H2B, H3, and H4). The chromatin structure of nuclear DNA prevents the GTFs to have access to the promoter. A large number of factors have been discovered that can relieve the restrictive environment of the chromatin structure and make it accessible for transcription. These factors can be divided into two groups: (1) ATP-dependent chromatin remodeling factors including the Swi/Snf complex and (2) histone acetyltransferases (HATs) including the SAGA complex. Swi/Snf contains an ATPase subunit that catalyzes the reorganization of chromatin to promote access of promoter sequences (Cairns et al. 1994). The SAGA complex comprises a subunit

with HAT activity and function by acetylating lysine residues of the histone tails and thereby weakening the interaction between the histones and DNA (Brownell and Allis 1996). Both chromatin remodeling and acetylation of histone tails are reversible processes. The Swi/Snf complex has been shown to both up-regulate and down-regulate protein expression in whole genome DNA array experiments, suggesting a role also in repression of transcription (Holstege et al. 1998). Histone deacetylase complexes (HDACs) have been identified to deacetylate histone tails and thereby again tighten the interaction between histones and DNA causing repression of transcription (Rundlett et al. 1996). In recent years, many more modifications have been shown to take place on histones and to regulate transcription including phosphorylation, methylation and ubiquitination (Fischle et al. 2003). The histone modifications are highly specific for particular amino acids and their position on the histone tail (Jenuwein and Allis 2001). This site specificity has lead to a hypothesis that histone tails contain a code for gene regulation. In agreement with the idea, multiple histone modifications appear to cross-talk with each other to provide binding sites for specific effector proteins. In turn, the effector proteins can regulate transcription levels directly or by the recruitment of other factors.

Transcription activation *in vivo*

For transcriptional activation to occur *in vivo* a complex machinery of GTFs, coactivators and activators have to interact and work together (fig 4). Activators are generally made up of two functionally separable domains, one for DNA binding and one for activation. Activators bind to upstream activating sequences (UAS) close to the promoter or enhancers at more distal positions. Several activators can bind to an enhancer at the same time leading to formation of nucleoprotein complexes called enhanceosomes. It is generally believed that transcription activation occurs in discrete steps. First, activators contact and recruit chromatin modifying coactivators to open up the chromatin template and make it accessible for the GTFs. The transcription levels can then be further stimulated, when activators contact the Mediator complex, which conveys the activating signal to the basal factors of the transcription machinery.

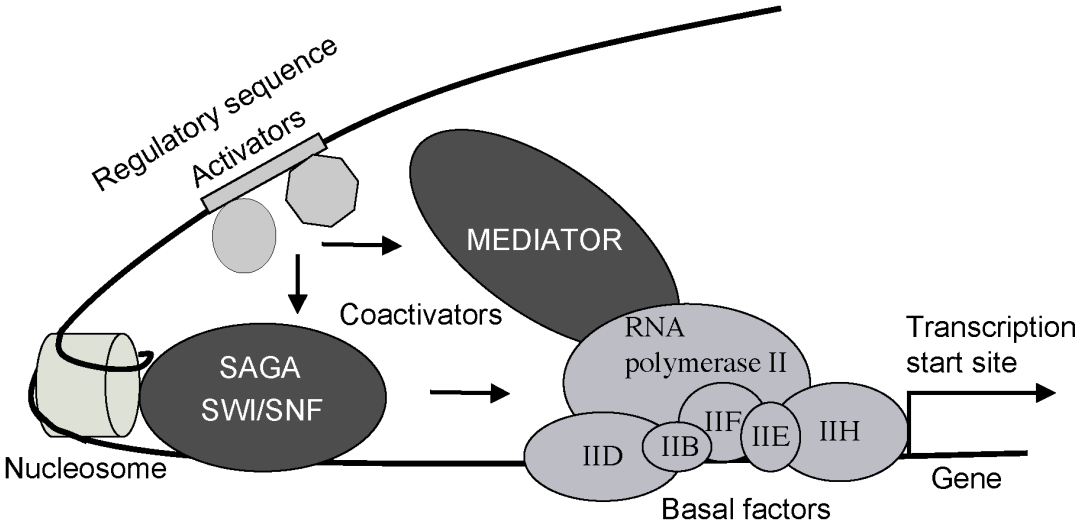

Fig 4. Transcription activation *in vivo*

THE MEDIATOR COMPLEX

The discovery of Mediator

The first sign of an intermediary complex came from *in vitro* transcription experiments where one activator could squelch the effect of another (Gill and Ptashne 1988). Even though large excess of GTFs were added, the squelching effect remained indicating that the binding target of activators were separated from the basal transcription machinery. This phenomena lead to a search for a factor that could enable a response to transcriptional activators in a pure *in vitro* transcription system. R. D. Kornberg and colleagues isolated a crude activity in the yeast *S. cerevisiae*, which could relieve the squelching effect (Kelleher et al. 1990; Flanagan et al. 1991). This factor was named Mediator on its ability to mediate the activating signal from activators to the transcription machinery. Further purification demonstrated that Mediator was in a holoenzyme form in complex with RNAP II (Kim et al. 1994). Later, the Mediator complex could also be purified as a discrete entity (Myers et al. 1998).

In an independent genetic study by R. A. Young and colleagues, genes were isolated on their ability to suppress the cold sensitive phenotypes caused by partial truncations of the CTD of the largest domain of RNAP II. These genes were named Srb for

suppressor of RNAP B (Nonet and Young 1989). Further analysis revealed that Srb proteins formed a large complex with RNAP II and some GTFs together termed RNAP II holoenzyme (Thompson et al. 1993; Koleske and Young 1994). The Young holoenzyme could also support activated transcription in an *in vitro* transcription system when supplemented with the missing GTFs. A link between the two different holoenzymes could be made when Mediator purified by the Kornberg laboratory was proved to contain Srb subunits. Upon further purification the Young holoenzyme was shown to contain Srb8-11 (Liao et al. 1995) and the chromatin remodeling complex Swi/Snf (Wilson et al. 1996). The hypothesis of the Young laboratory was that a preassembled holoenzyme complex containing the GTFs, Srbs, and Swi/Snf recognizes the promoter and initiate transcription. In the Kornberg model, the Mediator is a discrete complex making up a holoenzyme with RNAP II needed for activators to convey their signals to the GTFs at the promoter.

Several important functions have been described to Mediator, namely support of transcriptional activation, stimulation of basal transcription, and stimulation of TFIIH-dependent phosphorylation of the RNAP II CTD (Kim et al. 1994; Myers et al. 1998). Later, Mediator has also been shown to possess a HAT activity (Lorch et al. 2000).

Subunit composition of the Mediator complex

The Mediator complex in *S. cerevisiae* contains 20 polypeptides (table 1). Three groups of Mediator polypeptides can be distinguished:

1. The products of the SRB genes, which have been found in genetic screens for suppressors of partial truncations in CTD (Nonet and Young 1989).
2. The products of the GAL11, RGR1, SIN4, PGD1/HRS1, NUT1, NUT2, ROX3, and CSE2 genes, which were all discovered in genetic screens for mutations affecting transcription in positive as well as in negative ways.
3. The MED genes, which were all previously uncharacterized and whose products were identified through peptide sequencing (Kim et

al. 1994; Gustafsson et al. 1998; Myers et al. 1998).

The requirement of the individual subunits of Mediator differs significantly. Some are necessary for the expression of nearly all genes whereas others only are required for a subset of genes (Holstege et al. 1998). For instance, the essential protein Srb4 are needed for the expression of 93 % of all *S. cerevisiae* genes whereas Srb5, a non-essential protein, are necessary for the expression of 16 % of the genes.

TABLE 1

Mediator subunits in *Saccharomyces cerevisiae*

Subunit	Gene deletion phenotype	Protein mass (kD)	Activity
Nut1	Conditional	129	Histone acetyltransferase
Rgr1	Inviable	123	
Gal11	Conditional	120	
Sin4	Conditional	111	
Srb4	Inviable	78	
Med1	Conditional	64	
Med2	Conditional	48	
Pgd1/Hrs1	Conditional	47	
Srb5	Conditional	34	
Med7	Inviable	32	
Med4	Inviable	32	
Med6	Inviable	32	
Med8	Inviable	25	
Rox3	Inviable	25	
Srb2	Conditional	23	
Nut2	Inviable	18	
Cse2	Conditional	17	
Srb7	Inviable	16	
Med11	Inviable	15	
Srb6	Inviable	14	
(Srb8)	Conditional	167	
(Srb9)	Conditional	160	
(Srb10)	Conditional	63	Cyclin dependent kinase
(Srb11)	Conditional	38	Cyclin

Proteins within brackets corresponds to a loosely associated subcomplex of Mediator identified in the Young holoenzyme

Structural studies of the Mediator complex

The structure of the budding yeast Mediator has been solved using electron microscopy both as a discrete entity and in complex with RNAP II in a holoenzyme form (Fig5) (Asturias et al. 1999). Mediator alone appeared globular whereas in a holoenzyme, Mediator takes on an extended conformation that embraces RNAP II. Three Mediator domains were distinguishable in the RNAP II holoenzyme and named

head, middle, and tail. One contact point between Mediator and RNAP II appeared to be in the middle domain in close proximity to the CTD of RNAP II reinforcing earlier obsevations that the CTD is important for holoenzyme formation (Myers et al. 1998). The Mediator head domain creates the most pronounced interaction point to RNAP II. Despite limited sequence homology between subunit components, electron microscopy structures of several eukaryotic Mediator complexes, including human, suggest similarities in structural organization (Dotson et al. 2000).

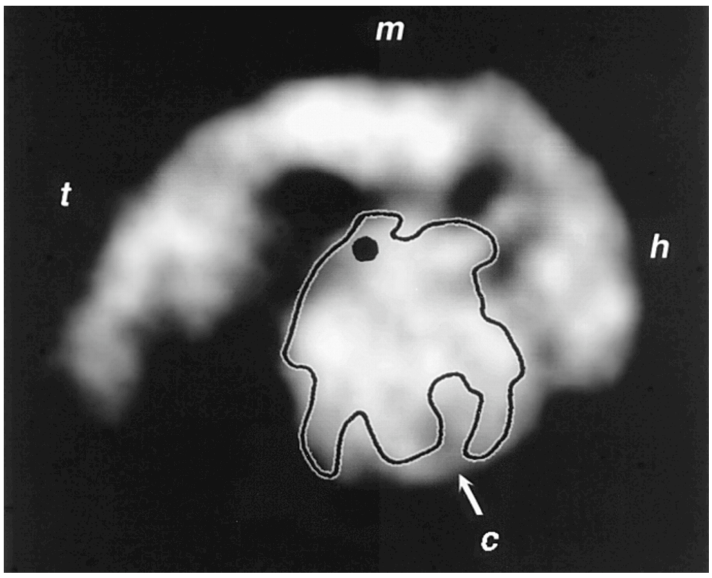

Figure 5. Holoenzyme formed by *S. cerevisiae* Mediator and RNA polymerase II. Mediator domains are head (h), middle (m), and tail (t). The globular structure corresponds to RNAP II, with the point of attachment of the CTD (dark circle) and the location of the DNA-binding channel (c) indicated.

Subcomplexes in Mediator

In biochemical experiments with urea treatment, the budding yeast Mediator could be divided into two distinct modules, the Srb4 and Rgr1 modules (Lee and Kim 1998). The Rgr1 module could be further divided into the Nut2 module and the Gal11 module as Sin4, Gal11, Med2, and Pgd1 subunits were lost in a Sin4 knockout strain (Myers et al. 1999). The Gal11 module was shown to correspond to the tail domain in the Mediator structure described above as the entire tail was diminished with a Sin4 knockout (Dotson et al. 2000). The Gal11 module interacts with the Nut2 module through Rgr1 establishing that the Nut2 module corresponds to the middle domain (Li et al. 1995). That leaves the Srb4 module that must comprise the head domain. Based on the structural studies and observed physical interactions a putative model for the

subunit organization of the Mediator complex is summarized in fig 6. In a RNAP II holoenzyme isolated by Young and colleagues, a fourth module containing Srb8-11 subunits have been implicated to interact with Mediator (Myer and Young 1998). This module is, however, absent in the Mediator preparation by Kornberg and colleagues (Myers et al. 1998). Srb8-11 has been suggested to be associated with the Mediator complex when cells are grown in exponential phase, but absent in stationary phase (Hengartner et al. 1998).

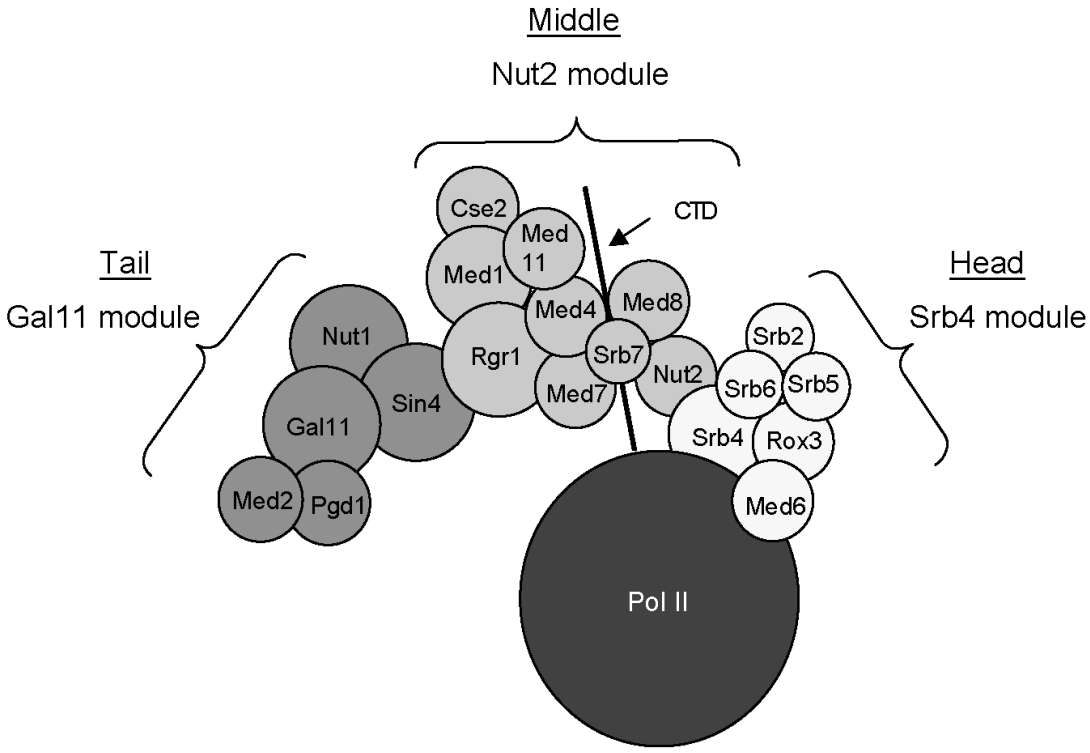

Fig 6. Subunit organisation of the Mediator complex in *S. cerevisiae*

Mediator-like complexes in higher eukaryotes

Given the general dependence for the yeast Mediator complex in transcription regulation, it was expected that a related complex would be present in cells of higher organisms as well. Indeed, based on the observations made in yeast, coactivator complexes containing homologues to Mediator subunits have been identified in mice, *Drosophila melanogaster*, *Caenorhabditis elegans*, and human. However, there are significant differences in subunit composition between Mediator complexes isolated from *S. cerevisiae* and metazoan cells. Human Mediator-like complexes have been

purified in several different laboratories, including TRAP (Fondell et al. 1996; Fondell et al. 1999), SMCC (Gu et al. 1999), ARC (Naar et al. 1999), DRIP (Rachez et al. 1999), NAT (Sun et al. 1998), PC2 (Malik et al. 2000), and CRSP (Ryu et al. 1999). These complexes were isolated with three different strategies: on their ability to bind to specific activators, capacity to stimulate transcription *in vitro*, or on their possession of homology to Mediator subunits. TRAP, ARC, and DRIP were all isolated based on their interaction with activators such as ligand bound thyroid hormone receptor (TRAP), SREBP and VP16 activators (ARC), and ligand bound vitamin D receptor (DRIP). The CRSP and PC2 complexes were purified on their ability to support transcription activation by the SP1 activator. Additionally, Mediator-like complexes were isolated based on homology to the yeast Mediator with either an affinity tag (SMCC) or an antibody (NAT). As more thorough biochemical studies have emerged the Mediator-like complexes seems to share many subunits. They can be divided into two groups according to their size. The larger 2 MDa complexes comprise NAT, DRIP, ARC, SMCC, and TRAP that are highly similar or even identical (Ito et al. 1999). The other group contains the smaller 500-700 kD CRSP and PC2 complexes that also appears to be identical.

Activities of Mediator-like complexes

Mediator-like complexes belonging to both groups have previously been shown to activate transcription. Additionally, SMCC and NAT complexes can repress both activated and basal levels of transcription (Sun et al. 1998; Gu et al. 1999). The capacity of the larger form of Mediator (TRAP/ARC) to activate transcription has, however, recently been questioned. Tjian and coworkers could show that the ARC preparation isolated with activator affinity columns containing VP16 or SREBP comprised two and not one complex, which was initially thought (Taatjes et al. 2002). These complexes corresponded to the larger ARC and the smaller CRSP identified earlier (Naar et al. 1999; Ryu et al. 1999). Interestingly, the ARC complex could not support activated transcription with the SREBP activator, whereas CRSP could.

Proposed differences between yeast and human Mediators

There are significant differences in subunit composition between Mediator complexes isolated from *S. cerevisiae* and human cells. In yeast, the Mediator complex can be purified both as a discrete entity and as a holoenzyme in complex with RNAP II. In contrast, the human Mediator-like complexes are always isolated in free form. The role of CTD also appears to differ between yeast and metazoan Mediator. In the presence of RNAP II lacking CTD, the *S. cerevisiae* Mediator cannot stimulate basal transcription or support transcriptional activation (Myers et al. 1998). In contrast, *in vitro* studies of human Mediator have demonstrated activated transcription using a CTD-less polymerase (Gu et al. 1999). The molecular basis for the observed differences remains unclear. Combined these observations have raised the possibility that Mediator-like complexes in metazoan cells may be significantly different both in structure and function from yeast Mediator (Gu et al. 1999). This matter will be further discussed in the results and discussion section.

Activation

The exact molecular mechanism of Mediator dependent transcriptional activation is still unclear. However, Mediator appears to function as a bridge in the aspect of transferring the activating signal from activators to the transcription machinery (Kim et al. 1994). Mediator was first showed to aid stimulation of transcription from the activators Gal4-VP16 and Gcn4 in a highly purified *S. cerevisiae in vitro* transcription system (Flanagan et al. 1991). Most activators appear to contact the Gal11 module in the tail domain of the Mediator complex. Interactions are formed between Gal11 and the activators VP16, Gcn4, and Gal4 as well as between Hrs1/Pgd1 and Gcn4 (Park et al. 2000). However, activators contact other modules of the Mediator as well as Gal4 additionally contacts Srb4 in the head domain (Koh et al. 1998).

There have been reports indicating that Mediator after releasing RNAP II during early elongation forms a scaffold together with TFIID, TFIIA, TFIIE, and TFIIH at the promoter that awaits re-initiation of transcription (Yudkovsky et al. 2000). Activators like Gal4-Vp16, stabilize such a scaffold, suggesting one model for activated transcription.

Recent electron microscopy structures of the human Mediator-like CRSP complex purified over different activator affinity columns, showed different conformations of CRSP caused by the activator interactions (Taatjes et al. 2002). The structural data indicate that activators influence coactivator conformation and thereby alter transcription levels.

Repression

Genetic screens have implicated Mediator in the negative regulation of transcription complementing its role in activation. Eight of the Mediator genes (*SRB8-11, GAL11, SIN4, RGR1*, and *ROX3*) were identified in searches for negative regulators of transcription (Carlson 1997). Mutations in these genes increased transcription levels from various promoters including *HO*, *CYC*, and *GAL* as well as weakening the repression of a subset of genes by the corepressor Cyc8-Tup1 (Lee et al. 2000). Biochemical studies have further suggested Srb10 and 11 to phosphorylate the CTD of RNAP II prior to PIC formation inhibiting its recruitment to the promoter (Hengartner et al. 1998).

MAIN OBJECTIVES

Considering Mediator's fundamental role in transcription and its regulation in yeast, our belief was that this complex and its mechanism of action would be evolutionary conserved among eukaryotes. In order to substantiate this hypothesis and to shed light on the molecular mechanism of transcription regulation, our specific aims were:

- Develop a purification scheme to isolate the Mediator complex from *S. pombe* and identify all subunits with mass-fingerprinting / MALDI-TOF

- Genetic characterization of genes encoding *S. pombe* Mediator subunits

- Compare the subunit composition between *S. pombe, S cerevisiae*, and human Mediators

- Purify alternative forms of the *S. pombe* Mediator

- Reconstitute a *S. pombe* derived *in vitro* transcription system and test the activities of the alternative forms of Mediator in this system

RESULTS AND DISCUSSION

As described in the introduction, Mediator was isolated as a factor essential for expressing and regulating nearly all RNAP II dependent genes. The Mediator complex was first discovered and purified from *S. cerevisiae*. However, genes encoding homologues to some of the *S. cerevisiae* Mediator subunits were also present in *Drosophila melanogaster*, *Caenorhabditis elegans*, mouse, and human suggesting that Mediator would be conserved also in higher eukaryotes. A number of Mediator-like complexes could later also be isolated from human cells. However, most of the subunits identified in these mammalian complexes displayed low or no significant sequence similarity with Mediator subunits previously identified in yeast. The low degree of conservation at the primary sequence level raised the possibility that Mediator-like complexes in metazoan cells would be significantly different both in structure and in function from yeast Mediator. To address this question and to better understand the molecular mechanisms of the Mediator complex, we decided to employ a comparative biology approach. Comparisons between the two yeasts, *S. cerevisiae* and *S. pombe* had previously been employed to study the eukaryotic cell cycle and differentiation. *S. pombe* is thought to be such an early offshoot of the standard ascomycete lineage that it has been dubbed an `archaeascomycete' (Forsburg 1999). Thus, *S. cerevisiae* and *S. pombe* are highly diverged from one another in evolution. In fact, the evolutionary distance between *S. pombe* and *S. cerevisiae*, is about the same as between *S. pombe* and human or between *S. cerevisiae* and human. Both yeasts are genetically tractable and an extensive collection of molecular tools is available for their study. Additionally, *S. pombe* has closer resemblance to human than *S. cerevisiae* in the aspect of transcription start site selection described previously. Combined, these different aspects made us choose *S. pombe* as our model organism. We decided to purify Mediator from *S. pombe* and to compare its subunit composition and function to *S. cerevisiae* and human Mediators to shed light on the mechanism behind Mediator dependent transcription regulation.

PAPER I AND II

We raised polyclonal antibodies against three putative Mediator components in *S. pombe* (Med7, Srb4, and Nut2) to use immunoblot analysis in the development of a purification scheme for the Mediator complex. Eventually, we could purify the *S. pombe* Mediator to near homogeneity from 96 l of yeast cell culture using classical chromatography over BioRex70, DEAE-Sepharose, Hydroxyapatite, MonoQ, Heparin-Sepharose, and Superose 12. Throughout the purification, the Mediator complex migrated together with RNAP II forming a holoenzyme. We next identified the unknown subunits of *S. pombe* Mediator with MALDI-TOF mass fingerprinting and subsequently confirmed these results with immunoblotting. The *S. pombe* Mediator was considerably smaller than its *S. cerevisiae* counterpart containing only 13 subunits instead of 20 (table 2). Apparently, the *S. pombe* Mediator lacked the entire tail domain comprising the Gal11 module in *S. cerevisiae* (Fig7A). As expected, the primary sequence similarities to Mediator subunits identified in other cells varied among the subunits. With the subunit identification of the *S. pombe* Mediator, we were able to analyze for homologues in *S. cerevisiae* and metazoans. Three of the *S. pombe* subunits were species specific named PMC for Pombe Mediator Complex. Additionally, the *S. pombe* Mediator contained 10 subunits conserved in *S. cerevisiae* and 8 in metazoans. Genetics showed that the conserved subunits were essential for cell growth, whereas the species-specific subunits were non-essential. By comparing the subunit composition between yeast Mediators it was evident that only the essential subunits in *S. cerevisiae* had a counterpart in *S. pombe*. Our findings led us to propose a model where Mediator consists of a core conserved through evolution that is responsible for contacts with the general transcription machinery and a set of species-specific subunits that make up a dynamic interface for gene-specific activators. Structures of Mediator complexes have been solved with electron microscopy in *S. cerevisiae*, mouse and human cells (Asturias et al. 1999; Dotson et al. 2000) and the similarities are striking despite the low degree of conservation at the primary sequence level. The Mediator head region makes out most contacts with RNAP II and is also the region where the subunit conservation of eukaryotic Mediators are most pronounced. The tail region is the largest part of Mediator in *S. cerevisiae* and consists of only non-essential subunits that are needed for a number of activators including Gal4, VP16 and Gcn4. Combined, these studies were in favor of the Mediator core model indicating that this fundamental complex

would be conserved from yeast to man. The *S. pombe* Mediator therefore proved to be a valuable tool to clarify a closer relationship between yeast and metazoan Mediators than was previously assumed.

TABLE 2

***S. pombe* Mediator subunit composition**

Subunit	Gene deletion viability	Protein mass (kD)	*S. cerevisiae* homologue	Metazoan homologue
Pmc1/spRgr1	ND	90	Rgr1	Trap170/Med150
spSrb4	Inviable	62	Srb4	Trap80/Med78
spMed7	Inviable	51	Med7	hMed7/Med34
Pmc2	Viable	49		
Pmc3	Viable	40		
Pmc4/spMed4	Inviable	38	Med4	Trap36/Med36
Pmc5/spMed6	ND	30	Med6	hMed6/Med33
Pmc6	Viable	29.5		
spMed8	Inviable[1]	29	Med8	Arc32
spRox3	ND	21	Rox3	
spSrb6	ND	17.5	Srb6	
spNut2	ND	16	Nut2	hNut2/Med10
spSrb7	ND	15.5	Srb7	hSrb7/Med17
(spTrap240)	Viable	139	Srb9	Trap240/Med240
(spSrb8)	Viable	131	Srb8	Trap230/Med230
(spSrb10)	Viable[2]	41	Srb10	hSrb10/Cdk8
(spSrb11)	ND	26	Srb11	hSrb11/CycC

Proteins within brackets corresponds to a submodule present in the larger Srb8-11/Mediator complex

ND, Not determined

[1]Zilahi et al, Curr genet. 2000

[2]Watson and Davey, Yeast 1998

PAPER III

We next analyzed the function of a specific Mediator subcomplex. Mediator from mammalian cells has been isolated in two different forms, the larger TRAP/Mediator complex and the smaller PC2/CRSP complex. The TRAP/Mediator complex contains 4 additional proteins, TRAP230, TRAP240, Srb10 and Srb11, which are absent in PC2/CRSP. Homologues to Srb10 and Srb11 are encoded in the *S. cerevisiae* genome and together with the Srb8 and Srb9 proteins they form a distinct unit (Borggrefe et al. 2002), with a close functional relationship to the Mediator complex. The physical association of Srb8, -9, -10, and –11 with other Mediator components was unclear, since these proteins were absent from all highly purified Mediator preparations. The proteins were, however, present in the Young holoenzyme, but so were also many GTFs and the Swi/Snf complex. It was also unclear if *S. cerevisiae* Srb8 and Srb9 were the bona fide homologues to mammalian TRAP230 and TRAP240, since the

primary sequence similarities between these proteins were extremely low. We developed a purification scheme for the larger form of the *S. pombe* Mediator using the so-called tandem affinity purification (TAP) tag. Our new purification procedure allowed to identify a novel form of Mediator that also contained homologues to Srb8, TRAP240, Srb10 and Srb11, which we denoted the spTRAP240/Mediator. Subunit characterization of the *S. pombe* spTRAP240/Mediator demonstrated that it was purified only in free form, devoid of RNAP II (Fig7B). This was in contrast to the smaller form of Mediator, which was always purified together with RNAP II forming a holoenzyme. We concluded that Srb8, TRAP240, Srb10 and Srb11 proteins make up a specific submodule, which negatively regulates the interaction between Mediator and RNAP II. The most predominant form of mammalian Mediators is isolated in complex with TRAP230, TRAP240, Srb10 and Srb11. Our findings therefore helped to explain why studies of mammalian Mediators in the past had failed to demonstrate direct interactions with RNAP II. In agreement with our findings, the large ARC complex was unable to interact with RNAP II whereas the smaller CRSP could (Naar et al. 2002). Gene knockouts of *S. pombe* Srb8 or Trap240 showed identical phenotypes and DNA microarray analysis reveals that spSrb8 and spTrap240 are involved in the control of the expression of the same distinct subset of genes. One of the most affected genes is the homologue of *S. cerevisiae FLO1*. This gene, which is also up-regulated in the *S. cerevisiae* Srb10 kinase dead mutant strain (Holstege et al. 1998), encodes a cell-wall protein, which adheres to cell-wall components and causes aggregation of cells. The up-regulation of *FLO1* could thus explain the flocculation phenotype observed for the *srb8–11* gene deletions in both *S. cerevisiae* and *S. pombe*. The close biochemical and functional relationship between spTrap240 and spSrb8, led us to conclude that the Srb8-11 complex is conserved in evolution and that TRAP230 and TRAP240 are the bona fide homologues to *S. cerevisiae* Srb8 and Srb9.

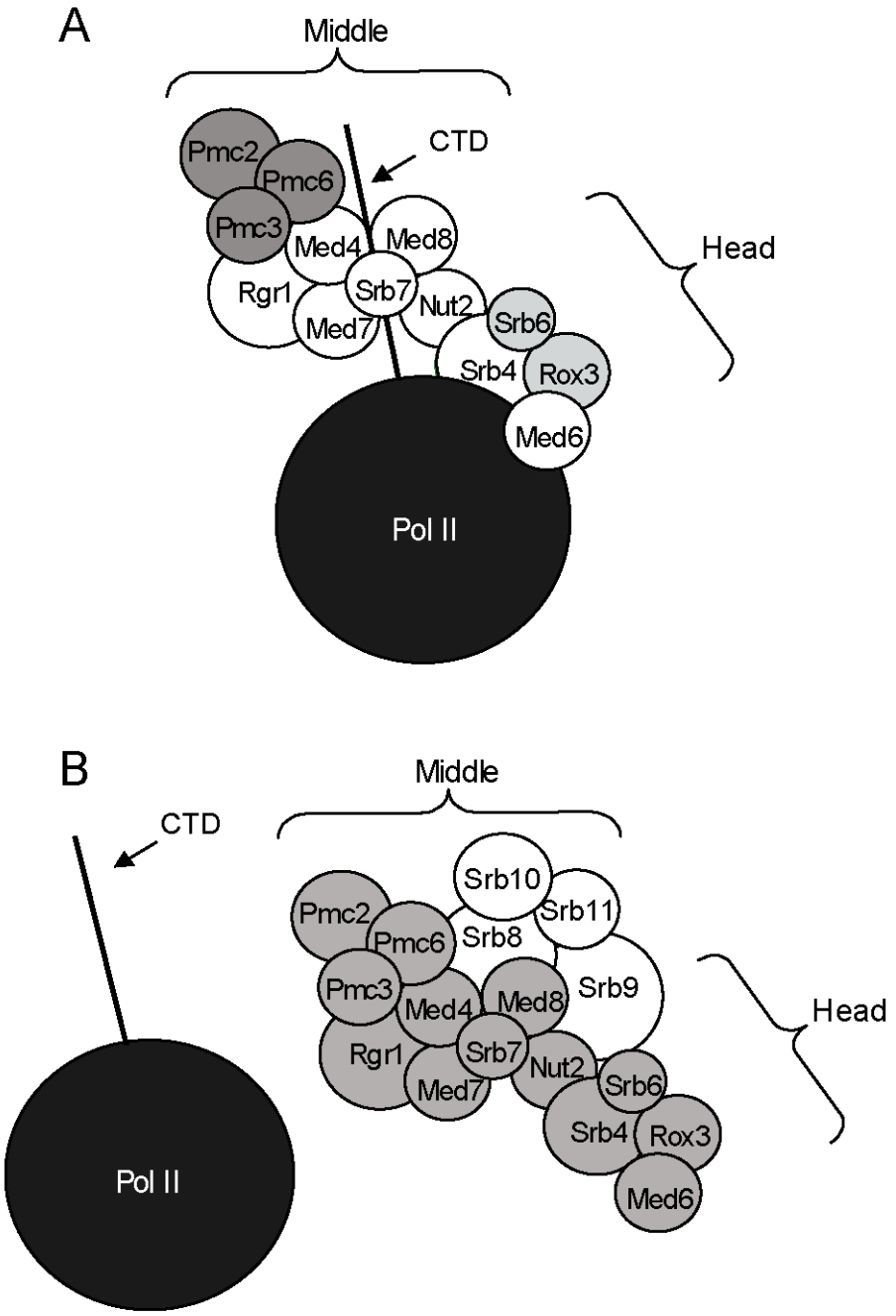

Fig 7. Tentative organization of the two forms of *S. pombe* Mediator. **A**. The small form of Mediator where subunits unique to *S. pombe* are shown in dark grey. The light colored subunits define essential Mediator components identified in both *S. cerevisiae* and *S. pombe*. The components in white also have homologues in metazoan Mediator and may represent a conserved Mediator core. **B**. The large form of Mediator where the Srb8-11 submodule specific to this form is shown in white. The large form of Mediator is unable to bind RNAP II.

PAPER IV

Finally, we wanted to test the activities of the two forms of Mediator in a pure *in vitro* transcription system. Since no such system was available for *S. pombe*, we had to reconstitute it by ourselves. We initiated different strategies for the isolation of the GTFs depending on the number of subunits they contained. A his-tagged version of the single subunit of TFIIB was expressed in recombinant form in *E. coli* cells and purified over Ni^{2+}- and MonoS columns. HisTFIIE containing two subunits and the three subunits of hisTFIIF were expressed in SF9 cells using the baculovirus system. Both transcription factors were purified over Ni^{2+} followed by a DEAE purification step for TFIIE and MonoQ for TFIIF. A recombinant strategy was not possible for RNAP II and TFIIH depending on their large size and complexity. RNAP II was purified to near homogeneity over an 8WG16 antibody column directed against its CTD. TFIIH contain two submodules that can easily be separable from each other, TFIIK containing the cyclin dependent kinase cyclin pair and the core of TFIIH. We therefore purified TFIIH in two parts introducing TAP tags in Pmh1 of TFIIK and Tfb2 of core TFIIH. Both complexes were purified over IgG and Calmodulin columns. The highly purified TFIIB, TFIIF, TFIIE, and TFIIH enabled RNAP II to initiate transcription from the *S. pombe* alcohol dehydrogenase promoter (*adh1p*) when combined with *S. cerevisiae* TBP. All transcription factors were essential for transcription *in vitro* except TFIIH where 33% transcriptional activity was observed without this factor. The same result has also been observed in other transcription systems on supercoiled templates and is probably due to the lack of need of TFIIH's capacity to melt DNA. We used our newly established *in vitro* transcription system to monitor the effects of Mediator on basal transcription. We found that the smaller form of Mediator was able to stimulate transcription whereas the larger Srb8-11/Mediator, repressed transcription.

SUMMARY

In summary, our studies have helped to define Mediator as a conserved entity involved in global regulation of transcription in all eukaryotes. We have proposed the core Mediator model, which suggests that Mediator is a dynamic interface between gene specific activators and repressors and the highly conserved basal transcription machinery. We have also demonstrated that Mediator exists in two forms, core

Mediator and Srb8-11/Mediator and that these two forms are evolutionary conserved. Furthermore, we have established the first pure *in vitro* system for *S. pombe* RNAP II dependent transcription and characterized the effects of our two forms of Mediator in this system.

A PUTATIVE MODEL FOR MEDIATOR DEPENDENT TRANSCRIPTION REGULATION

Based on results by others and us, it is possible to propose a model for Mediator dependent transcription regulation (fig 8). Mediator can exist in two different forms: one form in complex with RNAP II and one form in complex with the Srb8-11 module. Mediator containing the Srb8-11 module appears to be the predominant form in mammalian cells and represents a substantial fraction of Mediator in *S. pombe*. It is therefore likely that the Srb8-11 module has a general role in Mediator-dependent transcription. Perhaps, non-activated Mediator complexes all contain Srb8-11 and repress transcription before activation. To respond to physiological signals, activators contact the large form of Mediator containing the Srb8-11 module and recruit the complex to a specific promoter. Individual activators interact with specific Mediator subunits. Most often these interactions take place with the group of species-specific subunits, which has evolved to respond to specific environmental requirements during the course of evolution. That Mediator is recruited in free form, without RNAP II, is supported by findings at the yeast HO promoter and at Drosophila heat shock promoters (Bhoite et al. 2001; Cosma et al. 2001; Park et al. 2001). At the promoter, activator bound Srb8-11/Mediator together with TFIIB and TBP forms a DNA-protein structure, which is recognized by RNAP II. Recruitment of RNAP II leads to dissociation of the Srb8-11 module. The dissociation of Srb8-11 is catalyzed by CTD, which functions as an allosteric regulator of the Mediator structure. This idea is supported by the observation that GST-CTD bound to glutathione beads binds core Mediator, but dissociates Srb8-11 from the *S. pombe* Srb8-11/Mediator complex (Olga Khorosjutina and Vera Baraznenok, unpublished results). Perhaps, activators and repressors influence the ability for CTD to dissociate the Srb8-11 module, since Mediator adapts different conformations depending on the nature of the interacting activator (Taatjes et al. 2002). An activator would cause a conformation that weakens the interaction between Mediator and the Srb8-11 module and a repressor would have the opposite effect. Repressive effects on basal levels of transcription can also be

obtained by Srb10's capacity to phosphorylate the CTD of RNAP II to prevent its binding to the promoter. Most likely, the CTD is also dependent on other factors for the dissociation of Srb8-11 as the large form of Mediator is unable to support activated transcription *in vitro* in both the human (Taatjes et al. 2002) and in the *S. pombe* system (unpublished result). The release of the Srb8-11 sub-module causes a conformational change in Mediator from a compact to an open form, allowing interactions between Mediator and RNAP II. The Mediator subunits that establish binding to RNAP II as well as the rest of the transcription machinery likely belong to the conserved core in the middle and head domains of Mediator, as suggested by the general importance of these subunits for transcription *in vivo*.

Finally, Mediator stimulates the TFIIH dependent CTD kinase activity. This leads to hyper-phosphorylation of CTD and the interaction between CTD and Mediator is broken. RNAP II dependent transcription is now initiated, but Mediator remains bound to activators and GTFs at the promoter (Yudkovsky et al. 2000). Free, hypophosphorylated RNAP II may now be recruited to the "activated" promoter and another round of transcription is initiated.

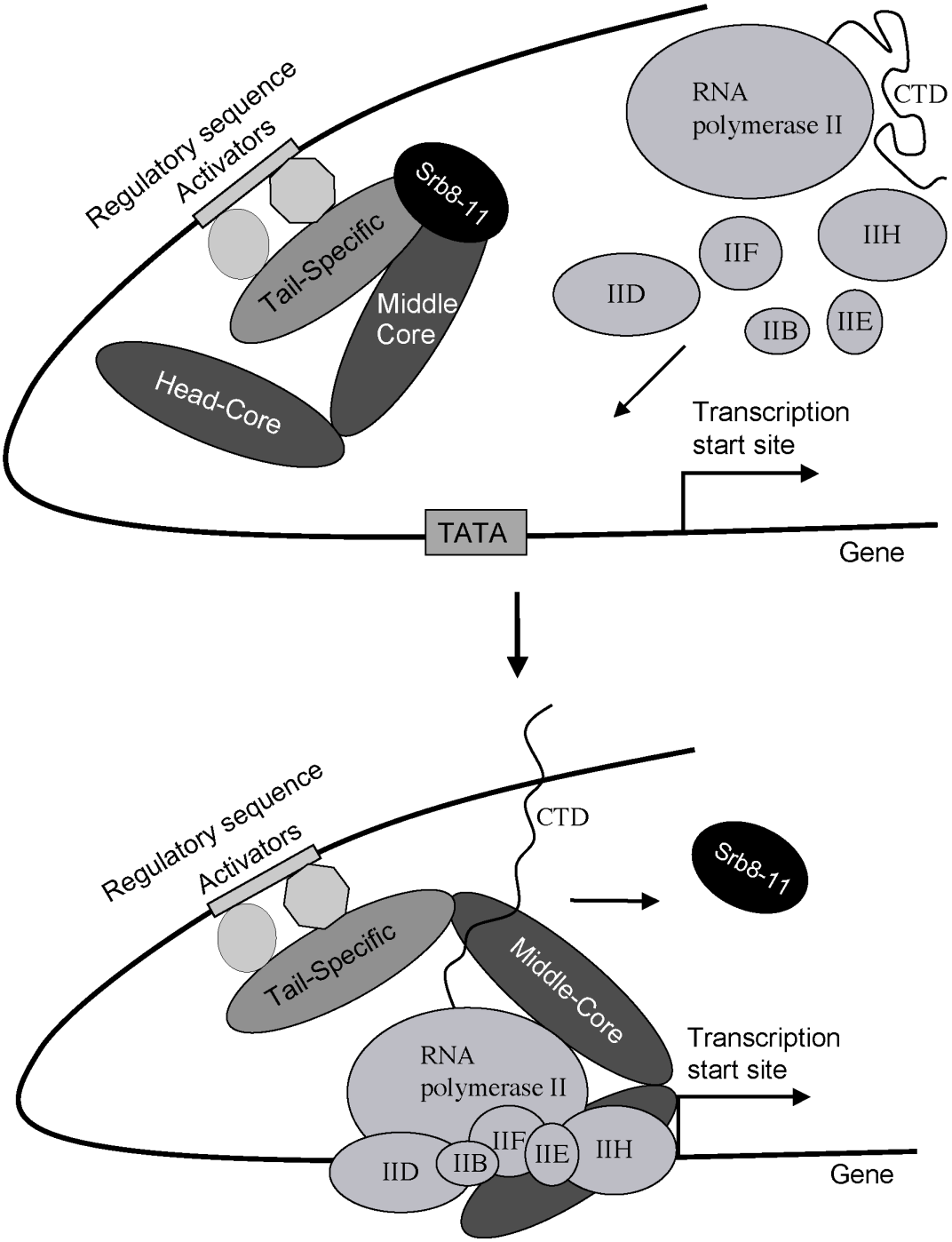

Fig 8. Speculative model for Mediator dependent transcription activation. The large form of Mediator is recruited by activators to a specific promoter. The CTD of RNAP II dissociates the Srb8-11 module causing a conformational change in Mediator. The open form of Mediator enable binding to RNAP II leading to activation of transcription. The Mediator middle and head domains are mainly made up of conserved subunits representing the core, here shown in dark grey. Colored in light grey is the Mediator tail domain where species specific subunits are most pronounced.

FUTURE INVESTIGATIONS

In the previous section, a speculative model for the mechanism of Mediator dependent transcription regulation has been presented. The most natural way to continue the investigations would be to further explore the role of Srb8-11 module in Mediator to test the proposed model.

In our previous studies we have seen that Srb8-11 inhibit the interaction between Mediator and RNAP II. The CTD of RNAP II appear to facilitate the dissociation of Srb8-11 from Mediator as we have seen that GST-CTD coupled to glutathione beads binds Mediator, but Srb8-11 get excluded. Does that process really take place in a transcription reaction? One way to test this would be to introduce free CTD in an *in vitro* transcription assay both in the presence of the large form of Mediator containing Srb8-11 and a transcriptional activator. The CTD's possibility to transform the inactive large form of Mediator to a transcriptionally active form by dissociating Srb8-11 could be monitored in such an experiment.

We have showed that the core Mediator is able to increase basal transcription levels whereas the Srb8-11 containing Mediator represses transcription *in vitro*. The repressive effect appear to originate from the kinase activity of Srb10 as it is dependent on preincubation allowing the CTD of RNAP II to become phosphorylated before the DNA template is added (Paper IV). In addition, a Srb10 kinase defective mutant is unable to repress transcription (Unpublished result). However, whether the repressive effect of Srb8-11/Mediator in activated transcription also comes from the kinase activity of Srb10 or is due to a structural role of the Srb8-11 blocking the interaction to RNAP II is still an open question. A simple experiment to answer this question would be to monitor the ability for the Srb10 kinase mutant form of Mediator to support activated transcription *in vitro*.

Is it the Srb8-11 module that keeps Mediator in a compact conformation? Would the Mediator complex adapt an open conformation upon the dissociation of Srb8-11 or is the presence of RNAP II required? A structural approach using electron microscopy might answer these questions. By performing comparative structural studies between Mediator and the larger Srb8-11/Mediator, the different conformations adapted in

these complexes could be revealed. In addition, by introducing a tag, like HA, in one of the subunits in Srb8-11, the exact location of this subcomplex in the Mediator structure could be shown in the presence of a gold plated antibody directed against the tag.

ACKNOWLEDGEMENTS

I have had an excellent time in "group CG" at the department of Medical Nutrition at NOVUM. I would like to thank all the people that are working at the department for creating such a warm and friendly atmosphere. There seems to be an endless flow of nice people here! I especially would like to thank:

My supervisor **Claes Gustafsson** for your remarkable enthusiasm and support. You have a broad knowledge and the ability to find a positive angle at any problem. Every time I walk out of your office after discussing various matters, I feel more positive and confident. You are simply the best supervisor anyone could wish for!

All members of "group CG" for making my time as a Ph.D student so pleasant. **Jenny Béve** with whom I began my ride as a Ph.D. student in Göteborg before the group moved to Stockholm. **Tomas Linder** for being a good friend and sharing my interest in music and visits to cultural events outside the lab like festivals, concerts and films. **Olivera Werngren** for keeping track of everything in the lab. My knowledge about things related to the 70's would be defective without you. **Olga Khorosjutina**, **Vera Baraznenok**, **Xuefeng Zhu** and **Tomas Linder** for joyful scientific discussions and project collaborations.

The "almost" group members **Maria Gustafsson**, **Jenny Korhonen** and **Martina Gaspari** for making the working atmosphere even nicer.

My former office-mate **Ingrid Dinnetz** for all the laughs.

Steen Holmberg and **Camilla Samuelsen** for fruitful collaborations.

Julian Walfridsson for help with scientific matters as well as all the good times at conferences, concerts and at the gym.

Jan-Åke Gustafsson for providing excellent research facilities at the department of Medical Nutrition.

The efficient administrative staff, **Marie Franzén**, **Monica Ahlberg**, **Lena Magnell**, **Stina Nordman**, **Kerstin Claesson**, **Marie Johansson**, and **Ylva Ekendahl** for having the things done almost before I asked for them.

Gunnel Almberger and **Lars Nybom** for making orders and keeping the department functioning in general.

Rikard Hofslagare for helping me with computer related issues.

Lady Sepulveda för dina "gröna fingrar" och utmärkta lab service.

All my friends outside the lab and their families. Especially, **Andreas**, **Magnus**, **Ann-Sofie**, **Katrin**, **Helena**, **Matthias**, **Susanne**, **Petter** and **Johan**. Thank you for being such wonderful friends!

Mina etablerade och nyvunna släktingar, **Farmor**, **Gunnie**, **Åke**, **Joakim**, **Ingrid**, **Sven-Olof** och **Therese**.

Mamma och **Pappa** för att ni har stöttat mig i allt jag har företagit mig.

Min **Syster**, **Jens** och **Jakob** för att ni alltid finns där för mig.

Véronique som ger mig så mycket glädje och lycka i mitt liv. Je t'aime.

REFERENCES

Araujo, S.J., E.A. Nigg, and R.D. Wood. 2001. Strong functional interactions of TFIIH with XPC and XPG in human DNA nucleotide excision repair, without a preassembled repairosome. *Mol Cell Biol* **21**: 2281-91.

Asturias, F.J., Y.W. Jiang, L.C. Myers, C.M. Gustafsson, and R.D. Kornberg. 1999. Conserved structures of mediator and RNA polymerase II holoenzyme. *Science* **283**: 985-7.

Bengal, E., O. Flores, A. Krauskopf, D. Reinberg, and Y. Aloni. 1991. Role of the mammalian transcription factors IIF, IIS, and IIX during elongation by RNA polymerase II. *Mol Cell Biol* **11**: 1195-206.

Bhoite, L.T., Y. Yu, and D.J. Stillman. 2001. The Swi5 activator recruits the Mediator complex to the HO promoter without RNA polymerase II. *Genes Dev* **15**: 2457-69.

Borggrefe, T., R. Davis, H. Erdjument-Bromage, P. Tempst, and R.D. Kornberg. 2002. A complex of the Srb8, -9, -10, and -11 transcriptional regulatory proteins from yeast. *J Biol Chem* **277**: 44202-7.

Brownell, J.E. and C.D. Allis. 1996. Special HATs for special occasions: linking histone acetylation to chromatin assembly and gene activation. *Curr Opin Genet Dev* **6**: 176-84.

Buratowski, S. 1994. The basics of basal transcription by RNA polymerase II. *Cell* **77**: 1-3.

Buratowski, S., S. Hahn, L. Guarente, and P.A. Sharp. 1989. Five intermediate complexes in transcription initiation by RNA polymerase II. *Cell* **56**: 549-61.

Buratowski, S., S. Hahn, P.A. Sharp, and L. Guarente. 1988. Function of a yeast TATA element-binding protein in a mammalian transcription system. *Nature* **334**: 37-42.

Burke, T.W. and J.T. Kadonaga. 1996. Drosophila TFIID binds to a conserved downstream basal promoter element that is present in many TATA-box-deficient promoters. *Genes Dev* **10**: 711-24.

Bushnell, D.A., K.D. Westover, R.E. Davis, and R.D. Kornberg. 2004. Structural basis of transcription: an RNA polymerase II-TFIIB cocrystal at 4.5 Angstroms. *Science* **303**: 983-8.

Cadena, D.L. and M.E. Dahmus. 1987. Messenger RNA synthesis in mammalian cells is catalyzed by the phosphorylated form of RNA polymerase II. *J Biol Chem* **262**: 12468-74.

Cairns, B.R., Y.J. Kim, M.H. Sayre, B.C. Laurent, and R.D. Kornberg. 1994. A multisubunit complex containing the SWI1/ADR6, SWI2/SNF2, SWI3, SNF5, and SNF6 gene products isolated from yeast. *Proc Natl Acad Sci U S A* **91**: 1950-4.

Carlson, M. 1997. Genetics of transcriptional regulation in yeast: connections to the RNA polymerase II CTD. *Annu Rev Cell Dev Biol* **13**: 1-23.

Cavallini, B., J. Huet, J.L. Plassat, A. Sentenac, J.M. Egly, and P. Chambon. 1988. A yeast activity can substitute for the HeLa cell TATA box factor. *Nature* **334**: 77-80.

Chambers, R.S. and M.E. Dahmus. 1994. Purification and characterization of a phosphatase from HeLa cells which dephosphorylates the C-terminal domain of RNA polymerase II. *J Biol Chem* **269**: 26243-8.

Conaway, R.C. and J.W. Conaway. 1993. General initiation factors for RNA polymerase II. *Annu Rev Biochem* **62**: 161-90.

Cosma, M.P., S. Panizza, and K. Nasmyth. 2001. Cdk1 triggers association of RNA polymerase to cell cycle promoters only after recruitment of the mediator by SBF. *Mol Cell* **7**: 1213-20.

Cramer, P., D.A. Bushnell, and R.D. Kornberg. 2001. Structural basis of transcription: RNA polymerase II at 2.8 angstrom resolution. *Science* **292**: 1863-76.
Dahmus, M.E. 1994. The role of multisite phosphorylation in the regulation of RNA polymerase II activity. *Prog Nucleic Acid Res Mol Biol* **48**: 143-79.
Dotson, M.R., C.X. Yuan, R.G. Roeder, L.C. Myers, C.M. Gustafsson, Y.W. Jiang, Y. Li, R.D. Kornberg, and F.J. Asturias. 2000. Structural organization of yeast and mammalian mediator complexes. *Proc Natl Acad Sci U S A* **97**: 14307-10.
Dvir, A., J.W. Conaway, and R.C. Conaway. 2001. Mechanism of transcription initiation and promoter escape by RNA polymerase II. *Curr Opin Genet Dev* **11**: 209-14.
Dynlacht, B.D., T. Hoey, and R. Tjian. 1991. Isolation of coactivators associated with the TATA-binding protein that mediate transcriptional activation. *Cell* **66**: 563-76.
Eisenmann, D.M., C. Dollard, and F. Winston. 1989. SPT15, the gene encoding the yeast TATA binding factor TFIID, is required for normal transcription initiation in vivo. *Cell* **58**: 1183-91.
Fairley, J.A., R. Evans, N.A. Hawkes, and S.G. Roberts. 2002. Core promoter-dependent TFIIB conformation and a role for TFIIB conformation in transcription start site selection. *Mol Cell Biol* **22**: 6697-705.
Feaver, W.J., J.Q. Svejstrup, N.L. Henry, and R.D. Kornberg. 1994. Relationship of CDK-activating kinase and RNA polymerase II CTD kinase TFIIH/TFIIK. *Cell* **79**: 1103-9.
Fischle, W., Y. Wang, and C.D. Allis. 2003. Histone and chromatin cross-talk. *Curr Opin Cell Biol* **15**: 172-83.
Flanagan, P.M., R.J. Kelleher, 3rd, M.H. Sayre, H. Tschochner, and R.D. Kornberg. 1991. A mediator required for activation of RNA polymerase II transcription in vitro. *Nature* **350**: 436-8.
Fondell, J.D., H. Ge, and R.G. Roeder. 1996. Ligand induction of a transcriptionally active thyroid hormone receptor coactivator complex. *Proc Natl Acad Sci U S A* **93**: 8329-33.
Fondell, J.D., M. Guermah, S. Malik, and R.G. Roeder. 1999. Thyroid hormone receptor-associated proteins and general positive cofactors mediate thyroid hormone receptor function in the absence of the TATA box-binding protein-associated factors of TFIID. *Proc Natl Acad Sci U S A* **96**: 1959-64.
Forsburg, S.L. 1999. The best yeast? *Trends Genet* **15**: 340-4.
Gill, G. and M. Ptashne. 1988. Negative effect of the transcriptional activator GAL4. *Nature* **334**: 721-4.
Gnatt, A.L., P. Cramer, J. Fu, D.A. Bushnell, and R.D. Kornberg. 2001. Structural basis of transcription: an RNA polymerase II elongation complex at 3.3 A resolution. *Science* **292**: 1876-82.
Greenblatt, J. 1991. RNA polymerase-associated transcription factors. *Trends Biochem Sci* **16**: 408-11.
Gruber, T.M. and C.A. Gross. 2003. Multiple sigma subunits and the partitioning of bacterial transcription space. *Annu Rev Microbiol* **57**: 441-66.
Gu, W., S. Malik, M. Ito, C.X. Yuan, J.D. Fondell, X. Zhang, E. Martinez, J. Qin, and R.G. Roeder. 1999. A novel human SRB/MED-containing cofactor complex, SMCC, involved in transcription regulation. *Mol Cell* **3**: 97-108.
Gustafsson, C.M., L.C. Myers, J. Beve, H. Spahr, M. Lui, H. Erdjument-Bromage, P. Tempst, and R.D. Kornberg. 1998. Identification of new mediator subunits in the RNA polymerase II holoenzyme from Saccharomyces cerevisiae. *J Biol Chem* **273**: 30851-4.
Hall, D.B. and K. Struhl. 2002. The VP16 activation domain interacts with multiple transcriptional components as determined by protein-protein cross-linking in vivo. *J Biol Chem* **277**: 46043-50.
Hampsey, M. 1998. Molecular genetics of the RNA polymerase II general transcriptional machinery. *Microbiol Mol Biol Rev* **62**: 465-503.
Hawkes, N.A. and S.G. Roberts. 1999. The role of human TFIIB in transcription start site selection in vitro and in vivo. *J Biol Chem* **274**: 14337-43.

Hawley, D.K. and R.G. Roeder. 1987. Functional steps in transcription initiation and reinitiation from the major late promoter in a HeLa nuclear extract. *J Biol Chem* **262**: 3452-61.
Hengartner, C.J., V.E. Myer, S.M. Liao, C.J. Wilson, S.S. Koh, and R.A. Young. 1998. Temporal regulation of RNA polymerase II by Srb10 and Kin28 cyclin-dependent kinases. *Mol Cell* **2**: 43-53.
Hernandez, N. 1993. TBP, a universal eukaryotic transcription factor? *Genes Dev* **7**: 1291-308.
Holstege, F.C., E.G. Jennings, J.J. Wyrick, T.I. Lee, C.J. Hengartner, M.R. Green, T.R. Golub, E.S. Lander, and R.A. Young. 1998. Dissecting the regulatory circuitry of a eukaryotic genome. *Cell* **95**: 717-28.
Ito, M., C.X. Yuan, S. Malik, W. Gu, J.D. Fondell, S. Yamamura, Z.Y. Fu, X. Zhang, J. Qin, and R.G. Roeder. 1999. Identity between TRAP and SMCC complexes indicates novel pathways for the function of nuclear receptors and diverse mammalian activators. *Mol Cell* **3**: 361-70.
Jenuwein, T. and C.D. Allis. 2001. Translating the histone code. *Science* **293**: 1074-80.
Kaldis, P. 1999. The cdk-activating kinase (CAK): from yeast to mammals. *Cell Mol Life Sci* **55**: 284-96.
Kelleher, R.J., 3rd, P.M. Flanagan, and R.D. Kornberg. 1990. A novel mediator between activator proteins and the RNA polymerase II transcription apparatus. *Cell* **61**: 1209-15.
Kim, Y.J., S. Bjorklund, Y. Li, M.H. Sayre, and R.D. Kornberg. 1994. A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. *Cell* **77**: 599-608.
Kochetkov, S.N., E.E. Rusakova, and V.L. Tunitskaya. 1998. Recent studies of T7 RNA polymerase mechanism. *FEBS Lett* **440**: 264-7.
Koh, S.S., A.Z. Ansari, M. Ptashne, and R.A. Young. 1998. An activator target in the RNA polymerase II holoenzyme. *Mol Cell* **1**: 895-904.
Koleske, A.J. and R.A. Young. 1994. An RNA polymerase II holoenzyme responsive to activators. *Nature* **368**: 466-9.
Koleske, A.J. and R.A. Young. 1995. The RNA polymerase II holoenzyme and its implications for gene regulation. *Trends Biochem Sci* **20**: 113-6.
Kornberg, R.D. 1974. Chromatin structure: a repeating unit of histones and DNA. *Science* **184**: 868-71.
Kretzschmar, M., G. Stelzer, R.G. Roeder, and M. Meisterernst. 1994. RNA polymerase II cofactor PC2 facilitates activation of transcription by GAL4-AH in vitro. *Mol Cell Biol* **14**: 3927-37.
Lagrange, T., A.N. Kapanidis, H. Tang, D. Reinberg, and R.H. Ebright. 1998. New core promoter element in RNA polymerase II-dependent transcription: sequence-specific DNA binding by transcription factor IIB. *Genes Dev* **12**: 34-44.
Lee, M., S. Chatterjee, and K. Struhl. 2000. Genetic analysis of the role of Pol II holoenzyme components in repression by the Cyc8-Tup1 corepressor in yeast. *Genetics* **155**: 1535-42.
Lee, Y.C. and Y.J. Kim. 1998. Requirement for a functional interaction between mediator components Med6 and Srb4 in RNA polymerase II transcription. *Mol Cell Biol* **18**: 5364-70.
Li, Y., S. Bjorklund, Y.W. Jiang, Y.J. Kim, W.S. Lane, D.J. Stillman, and R.D. Kornberg. 1995. Yeast global transcriptional regulators Sin4 and Rgr1 are components of mediator complex/RNA polymerase II holoenzyme. *Proc Natl Acad Sci U S A* **92**: 10864-8.
Li, Y., P.M. Flanagan, H. Tschochner, and R.D. Kornberg. 1994. RNA polymerase II initiation factor interactions and transcription start site selection. *Science* **263**: 805-7.
Liao, S.M., J. Zhang, D.A. Jeffery, A.J. Koleske, C.M. Thompson, D.M. Chao, M. Viljoen, H.J. van Vuuren, and R.A. Young. 1995. A kinase-cyclin pair in the RNA polymerase II holoenzyme. *Nature* **374**: 193-6.
Lorch, Y., J. Beve, C.M. Gustafsson, L.C. Myers, and R.D. Kornberg. 2000. Mediator-nucleosome interaction. *Mol Cell* **6**: 197-201.

Malik, S., W. Gu, W. Wu, J. Qin, and R.G. Roeder. 2000. The USA-derived transcriptional coactivator PC2 is a submodule of TRAP/SMCC and acts synergistically with other PCs. *Mol Cell* **5**: 753-60.
Maxon, M.E., J.A. Goodrich, and R. Tjian. 1994. Transcription factor IIE binds preferentially to RNA polymerase IIa and recruits TFIIH: a model for promoter clearance. *Genes Dev* **8**: 515-24.
McKune, K., P.A. Moore, M.W. Hull, and N.A. Woychik. 1995. Six human RNA polymerase subunits functionally substitute for their yeast counterparts. *Mol Cell Biol* **15**: 6895-900.
Meisterernst, M., A.L. Roy, H.M. Lieu, and R.G. Roeder. 1991. Activation of class II gene transcription by regulatory factors is potentiated by a novel activity. *Cell* **66**: 981-93.
Moqtaderi, Z., Y. Bai, D. Poon, P.A. Weil, and K. Struhl. 1996. TBP-associated factors are not generally required for transcriptional activation in yeast. *Nature* **383**: 188-91.
Moreland, R.J., F. Tirode, Q. Yan, J.W. Conaway, J.M. Egly, and R.C. Conaway. 1999. A role for the TFIIH XPB DNA helicase in promoter escape by RNA polymerase II. *J Biol Chem* **274**: 22127-30.
Myer, V.E. and R.A. Young. 1998. RNA polymerase II holoenzymes and subcomplexes. *J Biol Chem* **273**: 27757-60.
Myers, L.C., C.M. Gustafsson, D.A. Bushnell, M. Lui, H. Erdjument-Bromage, P. Tempst, and R.D. Kornberg. 1998. The Med proteins of yeast and their function through the RNA polymerase II carboxy-terminal domain. *Genes Dev* **12**: 45-54.
Myers, L.C., C.M. Gustafsson, K.C. Hayashibara, P.O. Brown, and R.D. Kornberg. 1999. Mediator protein mutations that selectively abolish activated transcription. *Proc Natl Acad Sci U S A* **96**: 67-72.
Myers, L.C. and R.D. Kornberg. 2000. Mediator of transcriptional regulation. *Annu Rev Biochem* **69**: 729-49.
Naar, A.M., P.A. Beaurang, S. Zhou, S. Abraham, W. Solomon, and R. Tjian. 1999. Composite co-activator ARC mediates chromatin-directed transcriptional activation. *Nature* **398**: 828-32.
Naar, A.M., D.J. Taatjes, W. Zhai, E. Nogales, and R. Tjian. 2002. Human CRSP interacts with RNA polymerase II CTD and adopts a specific CTD-bound conformation. *Genes Dev* **16**: 1339-44.
Nikolov, D.B. and S.K. Burley. 1997. RNA polymerase II transcription initiation: a structural view. *Proc Natl Acad Sci U S A* **94**: 15-22.
Nikolov, D.B., H. Chen, E.D. Halay, A.A. Usheva, K. Hisatake, D.K. Lee, R.G. Roeder, and S.K. Burley. 1995. Crystal structure of a TFIIB-TBP-TATA-element ternary complex. *Nature* **377**: 119-28.
Nonet, M.L. and R.A. Young. 1989. Intragenic and extragenic suppressors of mutations in the heptapeptide repeat domain of Saccharomyces cerevisiae RNA polymerase II. *Genetics* **123**: 715-24.
O'Brien, T., S. Hardin, A. Greenleaf, and J.T. Lis. 1994. Phosphorylation of RNA polymerase II C-terminal domain and transcriptional elongation. *Nature* **370**: 75-7.
Ohkuma, Y., S. Hashimoto, C.K. Wang, M. Horikoshi, and R.G. Roeder. 1995. Analysis of the role of TFIIE in basal transcription and TFIIH-mediated carboxy-terminal domain phosphorylation through structure-function studies of TFIIE-alpha. *Mol Cell Biol* **15**: 4856-66.
Pardee, T.S., C.S. Bangur, and A.S. Ponticelli. 1998. The N-terminal region of yeast TFIIB contains two adjacent functional domains involved in stable RNA polymerase II binding and transcription start site selection. *J Biol Chem* **273**: 17859-64.
Park, J.M., H.S. Kim, S.J. Han, M.S. Hwang, Y.C. Lee, and Y.J. Kim. 2000. In vivo requirement of activator-specific binding targets of mediator. *Mol Cell Biol* **20**: 8709-19.
Park, J.M., J. Werner, J.M. Kim, J.T. Lis, and Y.J. Kim. 2001. Mediator, not holoenzyme, is directly recruited to the heat shock promoter by HSF upon heat shock. *Mol Cell* **8**: 9-19.

Pinto, I., W.H. Wu, J.G. Na, and M. Hampsey. 1994. Characterization of sua7 mutations defines a domain of TFIIB involved in transcription start site selection in yeast. *J Biol Chem* **269**: 30569-73.
Ptashne, M. and A. Gann. 1997. Transcriptional activation by recruitment. *Nature* **386**: 569-77.
Rachez, C., B.D. Lemon, Z. Suldan, V. Bromleigh, M. Gamble, A.M. Naar, H. Erdjument-Bromage, P. Tempst, and L.P. Freedman. 1999. Ligand-dependent transcription activation by nuclear receptors requires the DRIP complex. *Nature* **398**: 824-8.
Rundlett, S.E., A.A. Carmen, R. Kobayashi, S. Bavykin, B.M. Turner, and M. Grunstein. 1996. HDA1 and RPD3 are members of distinct yeast histone deacetylase complexes that regulate silencing and transcription. *Proc Natl Acad Sci U S A* **93**: 14503-8.
Ryu, S., S. Zhou, A.G. Ladurner, and R. Tjian. 1999. The transcriptional cofactor complex CRSP is required for activity of the enhancer-binding protein Sp1. *Nature* **397**: 446-50.
Scafe, C., D. Chao, J. Lopes, J.P. Hirsch, S. Henry, and R.A. Young. 1990. RNA polymerase II C-terminal repeat influences response to transcriptional enhancer signals. *Nature* **347**: 491-4.
Schaeffer, L., R. Roy, S. Humbert, V. Moncollin, W. Vermeulen, J.H. Hoeijmakers, P. Chambon, and J.M. Egly. 1993. DNA repair helicase: a component of BTF2 (TFIIH) basic transcription factor. *Science* **260**: 58-63.
Shen, W.C. and M.R. Green. 1997. Yeast TAF(II)145 functions as a core promoter selectivity factor, not a general coactivator. *Cell* **90**: 615-24.
Smale, S.T. and J.T. Kadonaga. 2003. The RNA polymerase II core promoter. *Annu Rev Biochem* **72**: 449-79.
Struhl, K. 1999. Fundamentally different logic of gene regulation in eukaryotes and prokaryotes. *Cell* **98**: 1-4.
Sun, X., Y. Zhang, H. Cho, P. Rickert, E. Lees, W. Lane, and D. Reinberg. 1998. NAT, a human complex containing Srb polypeptides that functions as a negative regulator of activated transcription. *Mol Cell* **2**: 213-22.
Svejstrup, J.Q. 2002. Mechanisms of transcription-coupled DNA repair. *Nat Rev Mol Cell Biol* **3**: 21-9.
Svejstrup, J.Q., Y. Li, J. Fellows, A. Gnatt, S. Bjorklund, and R.D. Kornberg. 1997. Evidence for a mediator cycle at the initiation of transcription. *Proc Natl Acad Sci U S A* **94**: 6075-8.
Svejstrup, J.Q., P. Vichi, and J.M. Egly. 1996. The multiple roles of transcription/repair factor TFIIH. *Trends Biochem Sci* **21**: 346-50.
Svejstrup, J.Q., Z. Wang, W.J. Feaver, X. Wu, D.A. Bushnell, T.F. Donahue, E.C. Friedberg, and R.D. Kornberg. 1995. Different forms of TFIIH for transcription and DNA repair: holo-TFIIH and a nucleotide excision repairosome. *Cell* **80**: 21-8.
Taatjes, D.J., A.M. Naar, F. Andel, 3rd, E. Nogales, and R. Tjian. 2002. Structure, function, and activator-induced conformations of the CRSP coactivator. *Science* **295**: 1058-62.
Thompson, C.M., A.J. Koleske, D.M. Chao, and R.A. Young. 1993. A multisubunit complex associated with the RNA polymerase II CTD and TATA-binding protein in yeast. *Cell* **73**: 1361-75.
Tirode, F., D. Busso, F. Coin, and J.M. Egly. 1999. Reconstitution of the transcription factor TFIIH: assignment of functions for the three enzymatic subunits, XPB, XPD, and cdk7. *Mol Cell* **3**: 87-95.
Tsai, F.T. and P.B. Sigler. 2000. Structural basis of preinitiation complex assembly on human pol II promoters. *Embo J* **19**: 25-36.
Van Mullem, V., M. Wery, M. Werner, J. Vandenhaute, and P. Thuriaux. 2002. The Rpb9 subunit of RNA polymerase II binds transcription factor TFIIE and interferes with the SAGA and elongator histone acetyltransferases. *J Biol Chem* **277**: 10220-5.
Verrijzer, C.P., J.L. Chen, K. Yokomori, and R. Tjian. 1995. Binding of TAFs to core elements directs promoter selectivity by RNA polymerase II. *Cell* **81**: 1115-25.

Walker, S.S., J.C. Reese, L.M. Apone, and M.R. Green. 1996. Transcription activation in cells lacking TAFIIS. *Nature* **383**: 185-8.
Wang, Z., J.Q. Svejstrup, W.J. Feaver, X. Wu, R.D. Kornberg, and E.C. Friedberg. 1994. Transcription factor b (TFIIH) is required during nucleotide-excision repair in yeast. *Nature* **368**: 74-6.
Weil, P.A., D.S. Luse, J. Segall, and R.G. Roeder. 1979. Selective and accurate initiation of transcription at the Ad2 major late promotor in a soluble system dependent on purified RNA polymerase II and DNA. *Cell* **18**: 469-84.
Wilson, C.J., D.M. Chao, A.N. Imbalzano, G.R. Schnitzler, R.E. Kingston, and R.A. Young. 1996. RNA polymerase II holoenzyme contains SWI/SNF regulators involved in chromatin remodeling. *Cell* **84**: 235-44.
Wolner, B.S. and J.D. Gralla. 2001. TATA-flanking sequences influence the rate and stability of TATA-binding protein and TFIIB binding. *J Biol Chem* **276**: 6260-6.
Woychik, N.A. and M. Hampsey. 2002. The RNA polymerase II machinery: structure illuminates function. *Cell* **108**: 453-63.
Young, B.A., T.M. Gruber, and C.A. Gross. 2002. Views of transcription initiation. *Cell* **109**: 417-20.
Yudkovsky, N., J.A. Ranish, and S. Hahn. 2000. A transcription reinitiation intermediate that is stabilized by activator. *Nature* **408**: 225-9.