

MEDIATOR OF TRANSCRIPTIONAL REGULATION

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■ **Abstract** Three lines of evidence have converged on a multiprotein Mediator complex as a conserved interface between gene-specific regulatory proteins and the general transcription apparatus of eukaryotes. Mediator was discovered as an activity required for transcriptional activation in a reconstituted system from yeast. Upon resolution to homogeneity, the activity proved to reside in a 20-protein complex, which could exist in a free state or in a complex with RNA polymerase II, termed holoenzyme. A second line of evidence came from screens in yeast for mutations affecting transcription. Two-thirds of Mediator subunits are encoded by genes revealed by these screens. Five of the genetically defined subunits, termed *Srbs*, were characterized as interacting with the C-terminal domain of RNA polymerase II *in vivo*, and were shown to bind polymerase *in vitro*. A third line of evidence has come recently from studies in mammalian transcription systems. Mammalian counterparts of yeast Mediator were shown to interact with transcriptional activator proteins and to play an essential role in transcriptional regulation.

Mediator evidently integrates and transduces positive and negative regulatory information from enhancers and operators to promoters. It functions directly through RNA polymerase II, modulating its activity in promoter-dependent transcription. Details of the Mediator mechanism remain obscure. Additional outstanding questions include the patterns of promoter-specificity of the various Mediator subunits, the possible cell-type-specificity of Mediator subunit composition, and the full structures of both free Mediator and RNA polymerase II holoenzyme.

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INTRODUCTION

Early studies of gene regulation in eukaryotes identified activator proteins that bind to DNA sequences upstream or downstream of promoters and stimulate the initiation of transcription. It was at first thought that eukaryotic activators, like the corresponding proteins of prokaryotic cells, exert their effects directly, through contact with components of the transcription machinery. Then genetic and biochemical studies in yeast revealed the existence of global transcriptional regulators that go between activators (or repressors) and the transcription machinery (1). Recent work has identified counterparts of yeast global regulators in higher organisms.

Two broad classes of global regulators may be distinguished, those that affect the chromatin template and those that function through RNA polymerase and associated proteins. Members of the first class include SWI/SNF and ISWI chromatin-remodeling complexes, histone acetyltransferase complexes, and histone deacetylase complexes (2, 3). The second class is represented by the Mediator complex in yeast, and by one or several closely related complexes in mammalian cells. It is generally believed that the two classes of global regulators function in distinct stages, with alteration of the chromatin template preceding the stimulation of RNA polymerase activity. According to this view, Mediator plays the ultimate role in regulation, executing the final decision whether to initiate transcription and determining the frequency of the process. In keeping with such a role, Mediator contains both positive and negative control proteins, which may in some cases be one and the same.

Until recently, subunits of TFIID, specifically the TATA-binding protein (TBP) associated factors (TAFs), were regarded as the major, if not exclusive, targets of transcriptional activator proteins in the RNA polymerase II transcription machinery. This view was derived from studies with partially purified transcription

proteins from higher organisms. A better definition of the role of TAFs has come from genetic analysis in yeast. Conditional mutation or destruction of the larger TAFs, believed to form the scaffold for assembly of the entire TFIID complex, had no effect on transcription of most yeast promoters (4, 5). At the minority of promoters whose transcription was impaired, TAF dependence was shown to reside in sequences in the vicinity of the TATA box (6). While a contribution to regulation is not ruled out, TAFs clearly play an important role in promoter recognition, augmenting the minimal sequence specificity of TBP.

Genetic studies have been no less informative about the role of Mediator. A generation of genetic analysis in yeast identified mutations in more than a dozen proteins with pleiotropic effects on transcription. Although they were revealed by disparate genetic screens, the discovery of Mediator united these proteins in a common biochemical entity. There were a number of important implications. First, the multiprotein Mediator shown to be required for transcriptional regulation *in vitro* also plays a major role in regulation *in vivo*. Second, as many of the genetic screens were for negative as well as positive regulatory proteins, Mediator may support both types of regulation *in vivo*. Third, based on evidence for the interaction of Mediator with RNA polymerase II, the entire family of proteins must exert their effects through a polymerase holoenzyme.

The mechanisms by which Mediator influences transcription are still largely obscure. The simplest idea, drawn from studies of transcription control by catabolite gene activator protein (CAP) and bacteriophage lambda repressor in prokaryotes, is that activator-Mediator interaction recruits RNA polymerase and associated proteins to promoters (7). There are, however, also examples in prokaryotes of activation by an increase in the rate of isomerization of a polymerase-promoter complex, and other steps in the transcription initiation process could be affected as well. Still fewer ideas and virtually no experimental evidence have been put forward to explain the role of Mediator in the negative regulation of transcription.

The purposes of this review are to trace the evolution of the Mediator idea, to evaluate its current status, to summarize the information available from genetic, biochemical, and structural studies concerning Mediator subunit organization and activity, and to indicate some of the outstanding questions concerning Mediator function in transcriptional regulation. The emphasis is necessarily on yeast Mediator, for which the most information is available. The emerging picture of mammalian Mediator, while still lacking in detail, contributes important insights as well.

DISCOVERY OF MEDIATOR

The existence of Mediator was revealed by functional studies in a yeast RNA polymerase II transcription system (8, 9). The first evidence was indirect, based on the capacity of one activator to interfere with another in yeast and mammalian cells *in vivo* (10, 11). Such interference was attributed to the sequestration of a factor(s), present in a limiting amount, required for activated transcription.

Attention focused initially on general transcription factors and RNA polymerase II as direct targets of activators and the likely basis of activator interference. TBP, TFIIB, and RNA polymerase were all shown to bind activator proteins (12–14). When activator interference was reproduced in a yeast nuclear extract (8), the general transcription factors and RNA polymerase were all added, individually, in excess, to the transcription reaction, but interference persisted, showing that none of these components represented the limiting activator target. Rather, a distinct, crude yeast fraction was identified whose addition to the reaction would relieve interference. The activity in this fraction, termed Mediator, was thought to serve as an adaptor between activator proteins and the basal transcription machinery. This inference was borne out by the demonstration that the same crude yeast fraction was required for transcriptional activation in a system reconstituted from partially purified transcription proteins (15).

The next major advance came from a parallel line of work, whose origins lay in yeast genetic analysis (16). Extragenic suppressors of RNA polymerase II C-terminal domain (CTD) truncation mutations were identified by virtue of the cold-sensitive phenotype of the mutations. The products of four dominant suppressors, termed *Srb2*, *Srb4*, *Srb5*, and *Srb6*, were shown to interact in a large complex and bind to the polymerase CTD. The relationship of this complex to Mediator was not immediately apparent, for two reasons. First, the *Srb* complex was reported to contain TBP, and *Srbs* were thought on this basis to be candidates for TAFs in yeast. Mediator, however, was shown to be distinct from the TBP-TAF complex. Second, the *Srb* complex was isolated in association with about 2% of the cellular RNA polymerase II, raising the possibility that it only functioned at a minority of polymerase II promoters.

The pursuit of Mediator was brought to a conclusion by the fractionation of the system to homogeneity. The isolation of Mediator depended on the complete resolution of all the general transcription factors. The greatest challenge was purification in a fully active state of TFIIF, the largest and most complex of the general transcription factors; even at the penultimate stage of purification, TFIIF fractions were contaminated with Mediator. Soon after the yeast transcription system was reconstituted with a complete set of homogeneous proteins (17, 18), Mediator was purified to homogeneity as well, in the form of a 20-subunit complex (19). The connection with the *Srb* complex was made by the finding that *Srb2*, *Srb4*, *Srb5*, and *Srb6* were subunits of the pure Mediator.

Nine of the additional sixteen Mediator subunits proved to be products of previously identified genes, all of which had been recovered from screens for mutations affecting yeast RNA polymerase II transcription (Table 1 and discussion below). In some cases the screens were for mutations affecting negative regulation, suggesting a role of Mediator in repression. It remains to be determined whether this role is direct, or whether Mediator is important for the expression of other genes involved in repression.

Seven Mediator subunits are encoded by novel genes, named *MED* for Mediator. Mutational analysis of *MED2* and *MED6* has established their involvement

in transcriptional regulation in vivo (20, 21). Genetic studies were also important for addressing the question of whether Mediator dependence resides in core promoter sequences or upstream regulatory elements. Early work showed that *SRB2* functions through an upstream activating sequence (UAS) at the *INO1* promoter (22, 23), and more recent studies have demonstrated *MED2* function through a *GAL* UAS (21).

A further question addressed through combined genetic and biochemical analysis pertains to the mechanism of Mediator action. Deletion of the nonessential *MED2* gene caused a similar impairment of transcriptional activation in vitro and in vivo (21). This similarity provided strong evidence for relevance of the mechanism in vitro to that in vivo. There was previously a concern about the physiologic relevance of activated transcription in all in vitro systems, since the conditions of the transcription reaction (template DNA level, ionic strength, and so forth) were commonly manipulated to observe activation.

Biochemical studies of purified Mediator revealed three functional activities (19). In addition to enabling activated transcription, Mediator stimulates basal transcription about 10-fold and stimulates phosphorylation of the polymerase CTD by TFIIF kinase 30- to 50-fold. The effect on basal transcription may relate to an apparent requirement of *Srb2* and *Srb5* for transcription in a nuclear extract (16). Other Mediator proteins, including *Med2*, *Pgd1*, *Gal11*, and *Sin4*, are dispensable for the stimulation of basal transcription, and for the stimulation of CTD phosphorylation as well (21).

Purified Mediator binds to the polymerase CTD (24), although additional Mediator-polymerase interactions likely occur as well (25). Purification from yeast yields both free Mediator and Mediator-RNA polymerase II complex, referred to as holoenzyme (19). The relative amounts of these two forms vary from one preparation to another, and often most Mediator, as well as most polymerase, is isolated as holoenzyme. This finding appeared contradictory to the report mentioned above that Mediator is present in yeast at a level only about 2% that of RNA polymerase II (16). The contradiction would be resolved if the majority of polymerases in the cell were engaged in transcription and lacked associated Mediator. The transcribing polymerase, bound to DNA and therefore insoluble, would be lost during purification. Consistent with this idea, polymerase recovered from the insoluble fraction of a yeast cell lysate was devoid of Mediator (26). The enzyme was hyperphosphorylated on the CTD, a hallmark of the transcribing polymerase, and was associated with a novel set of proteins termed *Elongator* (27). These findings led to the proposal of a Mediator cycle (26), in which RNA polymerase II holoenzyme enters a preinitiation complex, undergoes phosphorylation by TFIIF, and releases Mediator, which binds free polymerase to form holoenzyme and repeat the cycle.

Mediator is general in two regards: it performs an essential role in transcription at almost all promoters in yeast, and corresponding complexes have been identified in a range of organisms from yeast to humans (discussed below). The requirement for transcription in yeast was shown with the use of a temperature-sensitive mutant in the *SRB4* gene (28). Raising the mutant strain to the restrictive temperature

TABLE 1 Yeast genes for Mediator subunits and related proteins

Gene (metazoan homolog)	Essential for viability	Protein mass (kD)	Complex	Functional notes
<i>NUT1</i>	No	129	yMed	Participates in negative regulation of <i>HO</i>
<i>RGR1</i> (82)	Yes	123	yMed	Required for glucose repression, C terminus anchors Mediator subcomplex containing Sin4, Gal11, Pgd1, Med2
<i>GAL11</i>	No	120	yMed, yPAF	Dual role in repression and activation
<i>SIN4</i>	No	111	yMed	Dual role in repression and activation
<i>SRB4</i>	Yes	78	yMed	Required for all polymerase II transcription
<i>MED1</i>	No	64	yMed	Role in activation negatively regulated by Srb10/11
<i>MED2</i>	No	48	yMed	Required for specific activators
<i>PGD1</i>	No	47	yMed	Suppressor of hyperrecombination, interacts with Med2
<i>SRB5</i>	No	34	yMed	Required for efficient transcription initiation
<i>MED6</i> (20)	Yes	32	yMed	Interacts with Srb4 and shows specific defects in activation
<i>MED7</i> (24)	Yes	32	yMed	
<i>MED4</i>	Yes	32	yMed	
<i>ROX3</i>	Yes	25	yMed	Involved in glucose repression and regulation of stress response
<i>MED8</i>	Yes	25	yMed	
<i>SRB2</i>	No	23	yMed	Interacts with Srb4 and Srb5
<i>NUT2</i> (50)	Yes	18	yMed	Involved in repression and activation
<i>CSE2</i>	No	17	yMed	Involved in stimulation of basal transcription

(continued)

TABLE 1 (Continued)

Gene (metazoan homolog)	Essential for viability	Protein mass (kD)	Complex	Functional notes
<i>SRB7</i> (47)	Yes	16	yMed	Recessive suppressor of CTD truncation
<i>MED11</i>	Yes	15	yMed	
<i>SRB6</i>	Yes	14	yMed	Binds <i>Srb4</i> and required for polymerase II transcription
<i>SRB8</i>	No	167	yHolo	Important for negative regulation
<i>SRB9</i>	No	160	yHolo	Important for negative regulation
<i>SRB10</i> (76)	No	63	yHolo	Important for negative regulation
<i>SRB11</i> (76)	No	38	yHolo	Important for negative regulation
<i>CCR4</i>	No	95	yPAF	Involved in <i>ADH</i> expression and member of <i>NOT</i> repression complex
<i>HPR1</i>	No	88	yPAF	Affects expression and hyperrecombination through elongation
<i>PAF1</i>	No	52	yPAF	<i>PAF1</i> deletion synthetic lethal with <i>ccr4Δ1</i> and <i>hpr1Δ1</i>
<i>CDC73</i>	No	44	yPAF	
<i>BDF1</i>	No	77	mMed	
<i>SOH1</i>	No	14	hTRAP	Suppressor of <i>hpr1Δ1</i> phenotype

resulted in an immediate cessation of transcription at all promoters tested (see below for details).

Exceptions to the rule of *SRB4* dependence have been identified, such as *CUP1* and *SSA1* (29), but even these genes require some components of Mediator, such as Rgr1, for transcriptional activation (30). A functional distinction among Mediator components was further revealed by fusion of a DNA-binding domain to Mediator subunits for “artificial recruitment” to promoters. Fusions to *Srb7*, *Gal11*, *Nut2*, and *Med6* all served to activate transcription in the *srb4^{ts}* background, whereas fusions to *Srb5* and *Srb6* did not (30). These results are consistent with biochemical evidence for a division of Mediator into a module containing Rgr1 and *Gal11* and a module containing *Srb2*, *Srb4*, *Srb5*, and *Srb6* (31, 32). The possibility has been

raised that these modules may occur not only in Mediator but also as separate, functional subcomplexes (30).

Beyond the 20-subunit Mediator defined by biochemical studies, additional interacting proteins have been identified. The products of the four recessive suppressors of CTD truncation phenotypes, *Srb8*, *Srb9*, *Srb10*, and *Srb11*, play roles in negative transcriptional regulation (33). Evidence for a connection with Mediator comes, in part, from the finding that artificial recruitment of Med1 activates transcription in *srb11* cells (34).

MAMMALIAN MEDIATORS

Evidence for the evolutionary conservation of Mediator has come from three directions: functional studies in human systems, the isolation of activator-coactivator complexes from human cells, and the pursuit of mammalian homologs of yeast Mediator proteins. Functional studies began with the USA fraction from human cells (35), whose active component was further resolved as PC2 (36). The first purification of mammalian Mediator to homogeneity was accomplished for TRAP, the coactivator complex for a nuclear receptor, on the basis of its association with liganded thyroid hormone receptor (37). Finally, the isolation of homologs of yeast Med7, *Srb7*, and *Srb10* from mouse and human cells yielded a number of related complexes (Table 2), including SMCC, which proved to be identical with TRAP (38).

To date, seven Mediator complexes have been isolated from mouse and human cells by independent procedures in six laboratories (Table 2). Most recently, a

TABLE 2 Mammalian and *S. pombe* Mediator complexes^a

Yeast (<i>S. cerevisiae</i>) homolog	TRAP/SMCC (37, 38, 83)	mMed (82)	NAT (84)	DRIP/ARC (85, 86)	CRSP (87)	SUR2 (88)	<i>S. pombe</i> (89)
Rgr1	+	+	+	+	+		+
Med4							+
Med6	+	+	+	+			
Med7	+	+	+	+	+	+	+
Nut2	+		+				+
Srb4							+
Srb7	+	+	+				
Srb10	+		+			+	
Srb11	+		+			+	
Soh1	+						

^a + denotes the demonstrated occurrence of a homolog of the yeast protein in the mammalian or *S. pombe* complex.

Mediator complex has been isolated from *Schizosaccharomyces pombe* as well (Table 2). Six of the mammalian complexes include homologs of yeast Mediator subunits Rgr1 and Med7, and several contain homologs of yeast Srb7, Srb10, Srb11, Nut2, and Med6. One human complex also includes a homolog of yeast Soh1. Five of the human complexes were shown to support transcriptional activation in reconstituted systems. This remarkable convergence has established Mediator as a major conduit of regulatory information from enhancers to promoters in higher cells.

None of the Mediator complexes isolated from higher cells so far contains homologs of Srb2, Srb4, Srb5, or Srb6, the products of dominant suppressors of CTD truncations. An entire module of the yeast complex is apparently lacking, or replaced by different or widely divergent proteins. Some of the human complexes, such as CRSP, appear to contain subsets of subunits found in others, suggesting either the loss of additional modules or the independent existence and function of subcomplexes. The possibility of cell-type-specific Mediator subunits, modules, or entire complexes has been considered but not so far demonstrated.

The question arises of whether a mammalian complex, fewer than half of whose subunits are clearly defined homologs of yeast Mediator subunits, is a physical and functional counterpart of the yeast complex or only distantly related. Electron microscopy and image averaging of single particles in negative stain in projection at about 40-Å resolution were informative in this regard (25). Yeast Mediator appeared compact, ellipsoidal in shape, with dimensions of about 400 by 200 Å. In the presence of RNA polymerase II, Mediator unfolded to a crescent of density, which partially surrounded the polymerase in the holoenzyme. Mouse Mediator was virtually identical in all respects. So despite considerable variation in primary structure between yeast and mammalian Mediators, apparent conservation of quaternary structure and a similar mode of interaction with RNA polymerase II indicate a true correspondence between the two complexes.

YEAST MEDIATOR PROTEINS

Srb2, Srb4, Srb5, Srb6

As already mentioned, a screen for suppressors of a CTD truncation mutation that restore growth in the cold revealed a set of genes for Mediator polypeptides. These SRB (suppressor of RNA polymerase B) genes also suppressed the other phenotypes and transcription defects associated with CTD truncation in an allele-specific manner. All dominant gain-of-function suppressors isolated were alleles of four genes, *SRB2*, *SRB4*, *SRB5*, and *SRB6* (16).

The initial cloning and characterization of *SRBs* 2, 4, 5, and 6 identified the products of these genes among a group of proteins associated with RNA polymerase II (16). All four of these Srb proteins proved to be components of a minimal, functional Mediator complex (19, 24). Yeast cells lacking *SRB4* or *SRB6* are inviable,

while deletion of *SRB2* or *SRB5* causes a slow-growth phenotype (doubling time about 2.5 times longer than wild type), as well as cold sensitivity, heat sensitivity, and inositol auxotrophy (16, 39). The same phenotypes are associated with CTD truncation, suggesting a role of the CTD in bringing Srb2 and 5 to promoters. A whole genome expression profile of the *srb5* deletion strain showed that under normal growth conditions, 16% of the approximately 5400 genes analyzed displayed a greater than twofold defect in expression compared to the wild-type strain (40). Several other Mediator subunits analyzed by whole genome profiles (see below) also appear to be involved in the regulation of subsets of yeast genes.

By contrast, Srb4 and Srb6 appear to play general roles in transcription. Cells containing the recessive temperature-sensitive (*ts*) mutations *srb4-138* or *srb6-107* rapidly cease all mRNA synthesis upon shifting to a restrictive temperature, as shown by Northern analysis of specific genes (28) and by expression profiles of the whole genome (40) (not yet available for the *srb6-107* strain). Comparison of mRNA decay rates upon shifting to the restrictive temperature of the *srb4^{ts}* strain with an *rpb1^{ts}* strain, whose defect results in a general loss of RNA polymerase II transcription, revealed significant differences for only two genes out of 5361 analyzed (40).

Biochemical and genetic studies suggest that the general requirement for Srb4 and Srb6, and hence for Mediator, at polymerase II promoters is due to antagonism by Mediator of general negative regulators of transcription. Biochemical studies have shown a requirement for Srb2 and Srb5, and so presumably for Mediator, for basal transcription in a crude extract (16) but no such dependence in a system reconstituted from highly purified transcription proteins (17, 19). Biochemical studies have further shown that the RNA polymerase II CTD is required for basal transcription in a crude extract but not in a reconstituted system (41). Thus the CTD appears, through its association with Mediator, to overcome a general repressor of transcription. Genetic studies identified recessive suppressors of the *srb4^{ts}* mutation in *BUR6* (*NCB1*), *NCB2*, *NOT1*, *NOT3*, *NOT5*, and *CAF1*, which encode subunits of *NC2* and the *NOT* complex (42). *NC2* (43) and *Not* proteins (44) are general negative regulators that interact with TBP.

The genetic similarities of *SRBs* 2, 4, 5, and 6 are supported by biochemical evidence that the recombinant gene products can form a complex (45). Srb4 directly binds both Srb2 and Srb6, while Srb5 is retained in the complex through an interaction with Srb2. This structural relationship may account for especially similar phenotypes of *srb2 Δ 1* and *srb5 Δ 1* strains. Binding studies have further shown an affinity of the Srb2,4,5,6 complex for the activation domain of Gal4, and photo-cross-linking experiments point to a specific interaction with Srb4 (45). These results are supported by genetic experiments identifying *SRB4* gain-of-function mutations that compensate for a *gal4* mutation with a partial activation defect. Functional assays of Gal4 activation in vitro will be important to substantiate this claim.

Srb7

SRB7, an essential yeast gene, was identified by a recessive mutation restoring viability of CTD truncation mutants (46). Occurrence of the gene product as a stoichiometric member of the yeast Mediator complex differentiates *SRB7* from other recessive suppressors of CTD truncation, *SRBs* 8, 9, 10, 11 (see below). Despite the genetic interaction between the CTD of RNA polymerase II and *SRB7*, as well as other *SRBs*, none of the Srb proteins nor any of the Srb protein subcomplexes so far studied binds to the CTD in vitro. There has also been no report of a direct interaction between Srb7 and the Srb2,4,5,6 subcomplex.

SRB7 is noteworthy for its human homolog, 35% identical in amino acid sequence (47). Antibodies against human *SRB7* have been used to purify mammalian RNA polymerase II holoenzymes. Evidence of an important role for Mediator in mammalian cells has come from studies of *SRB7* in mice. *SRB7* is expressed in all mouse tissue types, and disruption of the gene in embryonic stem cells reveals that it is essential for cell viability and for murine embryonic development (48).

Med6, Med7

Like *SRB7*, *MED6* and *MED7* are essential yeast genes (20) with homologs in higher eukaryotes. Studies of transcriptional activation in both nuclear extracts and with RNA polymerase II holoenzyme derived from a *med6^{ts}* strain suggest that Med6 plays a role in the activated transcription of a subset of yeast genes. Defects in VP16-activated transcription in vitro at the restrictive temperature could be corrected by addition of the recombinant protein (20). An expression profile of the whole genome of the *med6^{ts}* strain grown in rich medium at a restrictive temperature revealed that about 10% of all genes are dependent on *MED6* for wild-type levels of expression. Expression of genes for mating pheromones was especially defective under restrictive conditions. A similar defect was observed in expression profiles for the whole genome of *srb5 Δ 1* (40) and *med2 Δ 1* strains (see below) (21). More than 90% of the genes with expression defects in either the *med6^{ts}* or *srb5 Δ 1* strains were, however, independent of one another. The connection between *srb5 Δ 1* and *med6-ts* may be explained by a defect in *SRB2* expression in the *med6-ts* strain. *MED6* also exhibits a close connection with other *SRBs*, as mutations in *MED6* and *SRB6* were identified as dominant suppressors of the *srb4^{ts}* mutation, and the products of both genes interact with Srb4 (42). Conversely, mutations in *SRB4* were identified as dominant suppressors of the *med6^{ts}* mutation (32).

Med7 is the only yeast Mediator subunit whose homologs have been identified in all Mediator complexes purified from higher cells so far (Table 2). The human Med7 homolog is 32% identical and 60% similar to the yeast protein (24). Depletion of Med7 in yeast cells, by the use of a tetracycline-repressible promoter, resulted in complete arrest of cell division, and the arrested cells were larger than wild type and showed elongated buds (49). This morphology is distinctive among Mediator

mutants, contrasting, for example, with a clumpy phenotype of mutants with a Sin4/Gal11 module deletion.

Nut1, Nut2

NUT2, an essential yeast gene with higher organism homologs [25% identity and 47% similarity with *C. elegans NUT2* (50)], was identified together with the nonessential *NUT1* gene in a screen for mutations affecting the negative regulation of a Swi4-dependent reporter gene (51). The transcription factors Swi4 and Swi6 bind to the URS2 region of the *HO* promoter and are required for activation of *HO* transcription. Expression of a URS2-*lacZ* reporter gene was constitutive in a *nut2^{ts}* strain at a restrictive temperature. Other *nut2* alleles exhibited this phenotype only in the presence of a *nut1* mutation, implying a degree of functional redundancy. Furthermore, the phenotype was observed for mutants in the Mediator genes *SIN4*, *ROX3*, and *RGR1* (51), consistent with the identification of Nut2 as a stoichiometric Mediator component (50). As with many screens for mutations affecting promoter-specific, negative regulation of transcription by a URS, the *NUT* screen also identified alleles of *SRB8*, *SRB9*, *SRB10*, and *SRB11*.

In addition to its role in repression, *NUT2* may also play a role in transcriptional activation. Studies both in vivo and in vitro suggest that *NUT2* is necessary for induction of the histidine biosynthesis genes by the transcriptional activators *GCN4* and *BAS2* (52). A putative dual role in activation and repression has been observed for several other Mediator genes including *GAL11*, *SIN4*, *ROX3*, and *RGR1*.

Rgr1

RGR1 is essential for viability in yeast and has homologs in higher organisms [22% identity and 44% similarity between yeast and *C. elegans* genes (53)]. *RGR1* was originally identified in a mutant screen for resistance to glucose repression; thus the RGR designation (54). *RGR1* has since been found necessary for negative regulation of meiosis genes, *HO* expression, and genes lacking a UAS, as well as for $\alpha 2$ repression (for review see 33). *RGR1* mutations have also been shown to impair the activation of certain genes. Biochemical studies established the association of *RGR1* with Mediator and also demonstrated that the C terminus of Rgr1 anchors a subset, or module, of Mediator components (31) comprising Sin4, Gal11, Pgd1, and Med2 (21). These studies provided an explanation for the similar phenotypes of mutations in the genes for these Mediator subunits in diverse genetic screens (33). The function of the N terminus of Rgr1, which is essential for viability, is unknown. This uncharacterized function is likely to be important in higher cells as well since the three regions of greatest homology between yeast and *C. elegans RGR1* all lie in the N terminus. The C terminus (residues 745–1081) is not essential for viability, though it accounts for all the phenotypes described above. Mutations in the genes encoding the Mediator subunits Sin4 and Rox3 share a majority of the phenotypes caused by mutations in the C terminus of Rgr1.

Sin4

Sin4 is a member of the Rgr1 module of Mediator (see above) and is probably anchored by a direct interaction with the C terminus of Rgr1 (21, 31). Consistent with such interaction, a *sin4* deletion strain is viable and, as already indicated, exhibits essentially the same defects in repression, derepression, and cell morphology as an *rgr1* C-terminal truncation strain (33). It has been proposed that *SIN4* and *RGR1* influence these diverse processes by affecting chromatin organization (55), but the question remains of which phenotypes result from association with Mediator and which arise indirectly from impairment of expression of other transcriptional regulators. *SIN4* is one of several Mediator components that have been fused to heterologous DNA-binding domains and shown to stimulate transcription from promoters with appropriate binding sites (55). Mediator purified from a *sin4* deletion strain lacks not only Sin4 but also Pgd1 and Med2 (21). Genetic and biochemical studies have shown a functional relationship between *SIN4*, *PGD1*, and *MED2* that is reasonably consistent with this structural relationship (see below).

Pgd1, Med2, Gal11, Med1

Pgd1 and Med2, encoded by nonessential yeast genes, reside on the surface of the Rgr1/Sin4 module (21). A mutation in *PGD1* (*HRS1*) was initially isolated as an extragenic suppressor of the hyperrecombination phenotype of *hpr1*Δ cells (56). Since mutations in RNA polymerase II, TFIIB (57), and *SRB2* (58) also suppress the *hpr1*Δ phenotype, the participation of *PGD1* (*HRS1*) in direct repeat recombination probably reflects a general relationship between transcription and recombination. Prior to the full biochemical characterization of the Rgr1/Sin4 module, genetic analysis had revealed a striking similarity of the phenotypes of a *pgd1*Δ(*hrs1*Δ) strain and *sin4*Δ, *rgr1*Δ2, and *gal11*Δ strains (59). The *pgd1*Δ(*hrs1*Δ) strain is defective in both negative and positive transcriptional regulation, and its Spt-, Gal- and reduced α-factor production phenotypes are identical to those of *gal11* mutants (see below). A *pgd1*Δ(*hrs1*Δ) strain, however, was uniquely unable to derepress UAS-less promoters and uniquely able to suppress hyperrecombination in an *hpr1*Δ background to an extent about 100 times greater than either *sin4*Δ or *gal11*Δ strains. Recent genetic analysis has revealed defects of the *hpr1*Δ strain in transcriptional elongation, suggesting that *PGD1* (*HRS1*)-mediated events at initiation may effect the subsequent transcribing RNA polymerase II complex.

Purification of Mediator from a *pgd1*Δ strain yields a complex lacking Med2 as well as Pgd1 (21).¹ Conversely, Mediator purified from a *med2*Δ strain lacks Pgd1 as well as Med2. These two proteins probably interact with one another, and most likely associate with the Mediator complex through an interaction with Sin4, since

¹The presence of Gal11 in the *pgd1*Δ, *med2*Δ, and *sin4*Δ Mediators is difficult to confirm owing to overlapping bands in gels and the lack of highly specific antibodies for this subunit.

both proteins are absent from $\Delta sin4$ Mediator. A $med2\Delta1$ strain shares Gal- and defective mating phenotypes with a $pgd1\Delta1$ strain (24). As the genetic characterization of *MED2* progresses, additional genetic similarities with *PGD1* are likely to be revealed. Functional characterization of $\Delta med2$ and $\Delta pgd1$ Mediators in a purified transcription system and in vivo showed that these proteins play a key role in the stimulation of transcription by particular activators (21). Neither $\Delta med2$ nor $\Delta pgd1$ Mediators supported transcriptional activation in vitro by VP16, whereas both mutant Mediators enabled wild-type levels of activation by Gcn4 (activator of the yeast genes for histidine biosynthesis, *HIS3* and *HIS4*). Purified $\Delta sin4$ Mediator also failed to support activation by VP16, consistent with the absence of Med2 and Pgd1 from this mutant complex (see above). The $\Delta sin4$ Mediator did, however, exhibit an additional defect, the inability to support transcriptional activation by Gcn4. The defects in transcriptional activation in the purified system were consistent with defects in activation by VP16 and Gal4 (whose activation domain has been thought to resemble that of VP16) in $med2\Delta1$ and $pgd1\Delta1$ strains in vivo. The activator specificity with regard to Gcn4 seen in vitro is supported by induction of *HIS4* to wild-type levels in the $med2\Delta1$ strain in vivo, although recent evidence suggests that *MED2* does play a role in the Gcn4-activated transcription of *HIS3* in vivo (60). Although *HIS* gene expression is defective in a $sin4\Delta1$ strain (61), *GAL* gene expression is not, seemingly inconsistent with the absence of Med2 from $sin4\Delta$ Mediator and the Gal-phenotype of a $med2\Delta1$ strain (see below for possible resolution of this paradox). A whole-genome profile of a $med2\Delta1$ strain showed a greater than twofold defect in the expression of about 4% of all yeast genes (21). Many of the genes affected had previously been shown to require Gal11 for optimal expression.

Many Mediator genes have been identified in screens for either positive or negative regulators, but *GAL11* is the only Mediator gene that has been identified in both types of screen (for review see 33). Gal11 is also the only Mediator component that was identified in biochemical studies outside the context of Mediator. Gal11 was isolated along with several other proteins by affinity chromatography with an RNA polymerase II resin (see below).

Mediator purified from a $gal11\Delta1$ strain lacks both Pgd1 and Med2 subunits, and supports activation by Gcn4, but not VP16, just as do $\Delta pgd1$ and $\Delta med2$ Mediators (62). This similarity may be explained by interaction of Pgd1 and Med2 with Mediator through Gal11 as well as through Sin4. A contact with Gal11 strong enough to retain Pgd1 and Med2 in vivo could explain why a $sin4\Delta1$ strain fails to display some of the transcription defects, such as low *GAL* gene expression, associated with $pgd1\Delta1$ and $med2\Delta1$ strains. Fusions of Gal11 to heterologous DNA-binding domains have shown that artificial recruitment of Mediator to a promoter suffices to stimulate transcription in vivo (63), even in the case of *PHO5*, which requires chromatin remodeling for transcription (64). Several lines of evidence, however, indicate that natural activators function through Mediator by mechanisms other than or in addition to recruitment (65).

MED1 is not essential for yeast cell viability and encodes a stoichiometric member of the Mediator complex (34). Genetic analysis has shown that *MED1*, as well as *SIN4*, *ROX3*, and *SRBs 8–11*, are important for *SNF1*-dependent glucose repression. These same studies also showed that *MED1* is necessary for wild-type levels of *GAL* gene expression (34). The other phenotypes of a *med1Δ1* mutant seem to resemble those of *srb8–11* mutants (see below). It is intriguing, in this regard, that a fusion between Med1 and a heterologous DNA-binding domain (LexA-Med1) is only able to stimulate high levels of transcription in an *srb11Δ1* strain. This finding could indicate that *SRBs 8–11* physically interfere with LexA-Med1 function, or that phosphorylation by Srb10/11 is necessary for Med1 function.

Rox3

ROX3, an essential yeast gene, encodes a subunit of Mediator (66) originally identified in a search for mutants increasing aerobic expression of the *CYC7* gene (67). Recessive alleles of *ROX3* have since been found to relieve many of the expression defects relieved by mutations in *RGR1* (for review see 33). Despite the similarity of the *rox3* phenotype to those of *rgr1* and *sin4* mutants, the composition of mutant Mediators (21, 62) and the lethal phenotype of *rox3* deletions indicate that Rox3 is not a member of the Rgr1/Sin4 module. A further distinction is that the set of promoters affected by *rox3* mutations is overlapping but not identical with the set affected by *rgr1* and *sin4* mutations (33).

Med4, Med8, Med11, Cse2

Peptide sequencing of bands from the purified Mediator identified Med4, Med8 (24), Med11, and Cse2 (50). Deletion analysis of *MED4*, *MED8* (24), and *MED11* (52) demonstrated that each gene is essential for yeast cell viability. *CSE2* was originally identified by a screen for genes involved in chromosome segregation (68). A *cse2Δ1* strain is viable but exhibits slow-growth, temperature-sensitive, and cold-sensitive phenotypes. Differential display and Northern analysis showed that the *cse2Δ1* strain is defective in basal expression of the histidine biosynthesis genes, but not in the expression of genes known to be involved in chromosome segregation (52).

Little is known of the biochemistry of Med4, Med8, Med11, and Cse2. Med8 has putative leucine zipper motif (69) and has been shown to bind DNA directly at certain regulatory elements (70). Mediator purified from a *med11^{ts}* strain shows a slight defect in the stimulation of basal transcription in vitro at the restrictive temperature (52). *CSE2* putatively has a basic leucine zipper (68). Mediator purified from a *cse2Δ1* strain appears to be defective in the stimulation of basal transcription in vitro, but retains the ability to support transcriptional activation by both VP16 and Gcn4.

MEDIATOR-RELATED PROTEINS

The CTD and RNA Polymerase II Holoenzyme

The C-terminal domain (CTD) of the largest subunit of yeast RNA polymerase II and its reversible phosphorylation are central to the regulation of transcription through Mediator (33, 71, 72). The CTD also plays a key role in transcription elongation and mRNA processing. In *Saccharomyces cerevisiae*, the CTD consists of 26 or 27 repeats of a heptapeptide, whose consensus sequence is conserved across species, and the deletion of all repeats is inviable. Progressive truncation of the CTD leads to several phenotypes (39, 73, 74) resulting from defects in RNA polymerase II transcriptional regulation (22, 23). The CTD-binding subunit(s) of Mediator has not been identified. In any case, the CTD is unlikely to represent the sole point of contact between polymerase II and Mediator. Structural analysis indicates polymerase-Mediator interaction even in the absence of the CTD, and suggests multiple interaction sites (25).

Srb8, Srb9, Srb10, and Srb11

SRBs 8–11 were originally identified as recessive suppressors of CTD truncation mutations, and loss of function mutations in these genes cause derepression of certain promoters (see 33). Srb10 and Srb11 form a cyclin-kinase pair capable of phosphorylating serine 5 of the CTD heptapeptide (75). Srbs 8–11 have been found in large holoenzyme complexes isolated from yeast (76), and homologs of Srb10 and Srb11 are components of several Mediator complexes isolated from higher eukaryotes (Table 2). Although mutant alleles of *RGR1*, *SIN4*, *ROX3*, *GAL11*, and *SRBs 8–11* often exhibit similar defects in transcriptional repression, several findings have shown clear distinctions between these two groups of proteins (33). First, Srbs 8–11 are not detectable in the yeast Mediator isolated on the basis of its ability to support activated transcription (24). Second, no alleles of *RGR1*, *SIN4*, *ROX3*, or *GAL11* have been isolated as suppressors of CTD truncation mutations. Third, mutant alleles of *RGR1*, *SIN4*, *ROX3*, or *GAL11* may derepress UAS-less promoters, whereas mutant alleles of *SRBs 8–11* do not. Finally, the available data on genetic interactions are consistent with separate groupings of these proteins.

It has been suggested that Srbs 8–11 function in negative regulation by inappropriately phosphorylating the CTD of RNA polymerase II prior to initiation, thereby preventing initiation complex formation (75). The evidence for this hypothesis is twofold: first, recruitment of an *srb10* kinase mutant to a promoter (by fusion to a heterologous DNA binding domain) resulted in a much larger stimulation of transcription than the recruitment of wild-type *SRB10*; and second, preincubation of a holoenzyme isolated from a wild-type *SRB10* strain with ATP inhibited transcription, whereas preincubation of a holoenzyme from an *srb10* kinase mutant strain with ATP did not. A test of this hypothesis and definitive determination of

the functional role and mechanism of Srbs 8–11 awaits the establishment of an assay for transcriptional repression *in vitro*.

AN ALTERNATE RNA POLYMERASE II HOLOENZYME

RNA polymerase II immobilized on an anti-CTD antibody-resin has been used for affinity chromatography of polymerase-binding proteins (77). This procedure yielded the general transcription factors TFIIB and TFIIE and also Gal11, Ccr4, Cdc73, Hpr1, and Paf1. Presumably, Mediator was not obtained because the anti-CTD antibody prevented its interaction with the immobilized polymerase. Recovery of Gal11 raises the possibility that it is responsible for one of the contacts between Mediator and RNA polymerase II suggested by the low-resolution holoenzyme structure (25).

Two of the RNA polymerase II-binding proteins recovered from the affinity chromatography procedure are encoded by genes known to interact genetically with Mediator genes. Ccr4 forms a complex with products of the *NOT* genes, which have been shown to suppress the *srb4^{ts}* mutation (78). In addition to its role in the general negative regulation of transcription by the NOT complex, Ccr4 appears to play multiple roles in the negative and positive control of many genes (79). The *C. elegans* sequence database encodes a potential protein highly homologous to *S. cerevisiae* Ccr4 protein, but no homologs of the other RNA polymerase II-binding proteins.

Genetic interaction of *HPR1* with Mediator is shown by suppression of the *hpr1Δ1* hyperrecombination phenotype by alleles of *PGD1* (*HRS1*). Mutations in *PAF1* and *CDC73* also result in elevated levels of recombination between direct repeats. Comprehensive genetic interaction studies between the genes encoding the polymerase-binding proteins suggest that they serve as endpoints for certain signal transduction pathways (79). As with the Srb 8–11 proteins, further insight into the roles of the polymerase-binding proteins awaits the development of functional assays *in vitro*.

OTHER YEAST PROTEINS WITH MAMMALIAN MEDIATOR HOMOLOGS: Soh1 and Bdf1

Two protein components of mammalian Mediators have homologs in yeast that have not yet been reported to interact with the yeast complex. Murine Mediator contains a Ring-3-like protein (55) whose yeast homolog is *BDF1*. Human Ring-3 has been identified as a nuclear kinase that is activated by phosphorylation. Bdf1 is a nuclear protein that plays a role in sporulation (80).

The human Mediator complex SMCC contains a homolog of yeast Soh1 protein (53). The yeast *SOH1* gene encodes a positive regulator and interacts genetically with the genes for the two largest subunits of RNA polymerase II (*RPB1* and

RPB2), with the gene for TFIIB, and with the gene for polymerase-binding protein Hpr1 (57). SMCC does not require the RNA polymerase II CTD for function in transcriptional activation (53), and an interaction of Soh1 with RNA polymerase II may provide an alternative basis for communication of SMCC with the basal transcription apparatus. The use of this mechanism may be limited, however, as other mammalian Mediators do not appear to contain a Soh1 homolog, and there are no *SOH1* homologs in *C. elegans*.

PERSPECTIVES

The centrality and generality of Mediator are by now well established. Mediator is involved in the initiation of transcription at almost all eukaryotic promoters. It is important for transcription control, modulating the frequency of initiation in response to both positive and negative regulatory factors. It may play even more fundamental roles, as attested by the stimulation of basal transcription and CTD phosphorylation *in vitro*.

Issues for ongoing studies include the possible multiplicity of Mediator complexes, the mechanism of its effects on transcription, and its structure both in the free state and in RNA polymerase II holoenzyme. The variety of human Mediator complexes described in the relatively few reports available could reflect alteration during isolation or incomplete characterization, but the alternative has been suggested that Mediator occurs in multiple forms, subserving distinct regulatory roles. Cell-type-specificity might be investigated by the creation of transgenic animals expressing tagged variants of Mediator subunits for affinity isolation procedures. The possibility of multiple forms of yeast Mediator has also been raised, and the related issue of whole genome expression profiles for the many known Mediator subunits has only begun to be investigated. Analyses done so far have revealed two patterns, one of pervasiveness, such as the requirement of Srb4 for transcription of almost all yeast promoters, and the other of specificity, as in the involvement of Med2 in the transcription of about 5% of all promoters (under the particular culture conditions tested).

The Mediator mechanism has been more an object of conjecture than experimentation to date. Early enthusiasm for a recruitment model (7) has been tempered by evidence to the contrary (65), and incisive mechanistic studies remain to be done. Evidence against a simple recruitment model also includes the deleterious effect on activated transcription of the deletion of peripheral subunits such as Med2 and Pgd1 (21), despite indications that activator proteins do not directly contact these subunits. The importance of several Mediator subunits for both transcriptional activation and repression is also less consistent with recruitment than with alternatives, such as the involvement of Mediator at a “decision” point in the transcription initiation pathway (81).

Structural studies will doubtless prove instrumental, if not crucial, for understanding the Mediator mechanism. Structure determination has so far been limited

to electron microscopy of single particles, for want of crystalline specimens. Even at the low resolution of single particle analysis, insight into the evolutionary conservation of Mediator structure and conformational change upon interaction with RNA polymerase II has been obtained (25). Further single particle analysis, including complexes of Mediator with transcriptional activator proteins and additional components of the transcription machinery, should be informative about the activation mechanism. The well-defined structure and uniformity of Mediator and RNA polymerase II holoenzyme so far revealed also bode well for the prospects of crystallographic analysis in the future.

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