nature
structural &
molecular biology

Activator-specific recruitment of Mediator *in vivo*

Xiaochun Fan, Danny M Chou & Kevin Struhl

The Mediator complex associates with eukaryotic RNA polymerase (Pol) II and is recruited to transcriptional enhancers by activator proteins. It is believed that Mediator is a general component of the Pol II machinery that is crucial to connect enhancer-bound activators to basic transcription factors. However, we show that Mediator does not detectably associate with many highly active Pol II promoters in yeast cells. Furthermore, in response to stress conditions, Mediator association is not directly related to Pol II association and in some cases is not detectable at highly activated promoters. Thus, Mediator is recruited to enhancers in an activator-specific manner, and it does not seem to be a stoichiometric component of the basic Pol II machinery *in vivo*. Mediator is recruited by many activators involved in stress responses, but not by the major activators that function under optimal conditions.

Mediator, a highly conserved complex of approximately 25 proteins, associates with eukaryotic RNA polymerase II and is recruited to transcriptional enhancers by activator proteins *in vivo*[1–7]. Activator proteins directly interact with the Mediator complex, but the specific Mediator subunit that is contacted depends on the activator. Activator-dependent recruitment of Mediator *in vivo* can occur under conditions where the basic Pol II machinery is not associated with the promoter, indicating that Mediator recruitment is an early step in the transcription process that is important for preinitiation complex formation. Mediator stimulates basal Pol II transcription *in vitro*, and several subunits of Mediator are essential for general Pol II transcription in yeast cells[8,9]. These and other observations have led to the view that Mediator is a general and essential component of the Pol II machinery *in vivo* that is central to the transduction of activation signals from enhancer-bound activators to general transcription factors.

In yeast cells, the levels of association of individual components of the basic Pol II machinery (such as TBP, TFIIB and Pol II) are strongly correlated with each other and with transcriptional activity[10–12]. If Mediator is indeed a component of the general Pol II machinery *in vivo*, one would predict that the level of Mediator occupancy *in vivo* should correlate with Pol II association and transcriptional activity. With respect to this prediction, activator-mediated recruitment of Mediator has been observed[13–17], but such studies have been limited to a very small subset of genes and activators. We set out to test this hypothesis more generally. Here we show that Mediator occupancy does not strictly correlate with Pol II occupancy *in vivo* and hence that Mediator recruitment is activator-specific.

RESULTS

Mediator does not associate with some highly active genes

Mediator can be divided into three structural modules on the basis of biochemical, genetic and electron-microscopic evidence. We therefore analyzed association of the Med17, Med5 and Med15 subunits (also called Srb4, Nut1 and Gal11, respectively), which represent, respectively, the head, middle and tail of the complex. In the initial experiments, raffinose-grown cells were induced for 1 h with galactose, thereby permitting us to analyze Gal4-dependent recruitment of Mediator at the *GAL1,10* enhancer. As expected[15,17], galactose induction results in the recruitment of Med15, Med5 and Med17 to the *GAL1,10* enhancer (**Fig. 1a–c**) and Pol II association with the *GAL1* ORF (**Fig. 1d**). Furthermore, progressive reduction in the level of galactose induction (by changing the ratio of galactose and glucose in the medium) results in the progressive and corresponding decrease of Mediator, TBP and Pol II (**Fig. 1e–g**).

Unexpectedly, Med15, Med5 and Med17 do not seem to associate with promoters for three ribosomal protein genes (*RPS11B*, *RPS21B* and *RPL12A*) and two glycolytic genes (*CDC19* and *ENO2*) that are activated by Rap1 together with other proteins (**Fig. 1a–c** and **Supplementary Fig. 1** online). Importantly, Pol II associates with these ribosomal and glycolytic coding regions at levels roughly comparable to association with the *GAL1* ORF under inducing conditions (**Fig. 1d**). Thus, whereas Mediator is recruited to the *GAL1,10* enhancer under inducing conditions, Rap1-dependent activation of the ribosomal and glycolytic genes tested is not accompanied by detectable association of Mediator.

We extended these observations by analyzing Mediator association (Med15 subunit) on microarrays containing essentially all intergenic regions. Under optimal growth conditions (YPD medium), we did not detect any intergenic regions showing highly significant ($P < 10^{-5}$) Med15 association. Direct analysis of the 13 genomic regions with the highest log ratios or lowest *P* values for Med15 binding confirmed the lack of Med15 association. The ribosomal protein promoters were distributed randomly throughout a list of genomic regions that were ordered by their *P* values (**Supplementary Fig. 2** online). In contrast,

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115, USA. Correspondence should be addressed to K.S. (kevin@hms.harvard.edu).

Received 7 November 2005; accepted 8 December 2005; published online 22 January 2006; doi:10.1038/nsmb1049

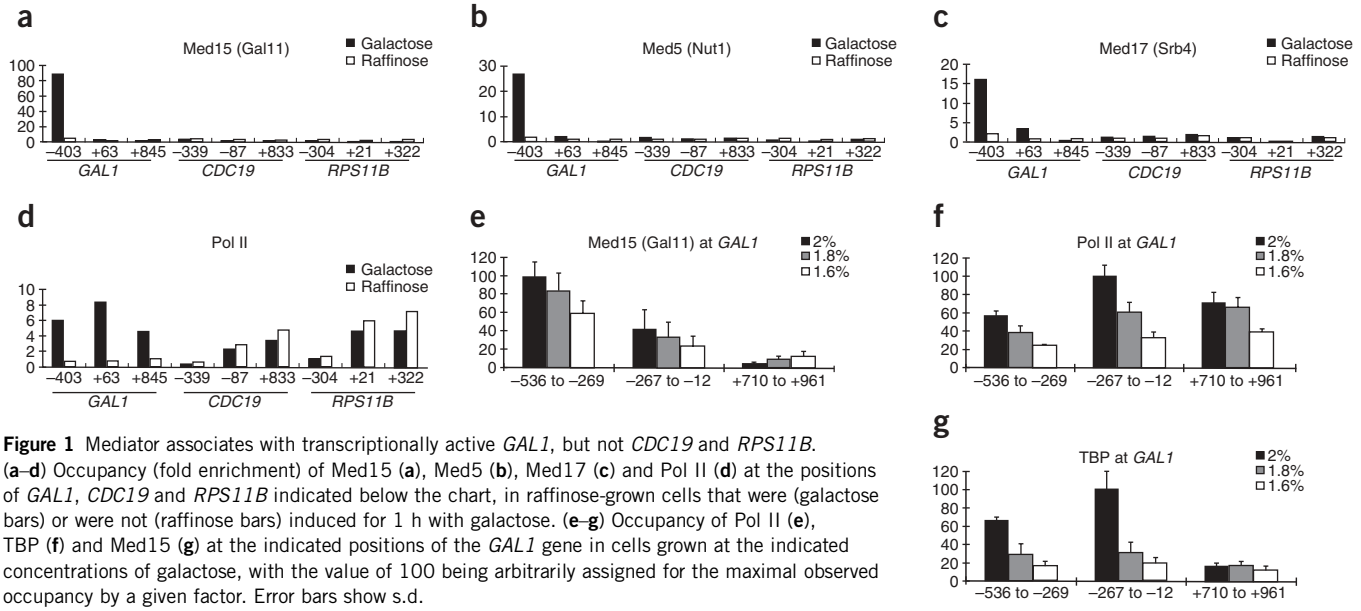

Figure 1 Mediator associates with transcriptionally active *GAL1*, but not *CDC19* and *RPS11B*. (**a**–**d**) Occupancy (fold enrichment) of Med15 (**a**), Med5 (**b**), Med17 (**c**) and Pol II (**d**) at the positions of *GAL1*, *CDC19* and *RPS11B* indicated below the chart, in raffinose-grown cells that were (galactose bars) or were not (raffinose bars) induced for 1 h with galactose. (**e**–**g**) Occupancy of Pol II (**e**), TBP (**f**) and Med15 (**g**) at the indicated positions of the *GAL1* gene in cells grown at the indicated concentrations of galactose, with the value of 100 being arbitrarily assigned for the maximal observed occupancy by a given factor. Error bars show s.d.

in cells subjected to heat shock, we detected 39 genomic regions with highly significant Med15 association ($P < 10^{-5}$) and 181 genomic regions at a less stringent cut-off ($P < 10^{-3}$). As expected, many of these Med15 targets are genes that are transcriptionally activated upon heat shock, although the correlation between Med15 association and transcriptional activity is only modest (see below). These observations indicate that Mediator associates extremely poorly, and perhaps not at all, with numerous highly active promoter regions in cells grown under optimal growth conditions.

Mediator and Pol II association are not strictly correlated

We directly addressed the relationship between Mediator recruitment and Pol II association by examining a variety of genes that are transcriptionally activated in response to heat shock. In all cases tested, levels of association for the Med15, Med17 and Med5 subunits are strongly correlated with one another (**Fig. 2a**–**c**), suggesting that recruitment involves the intact Mediator complex. Notably, the level of Mediator recruitment is not directly related to the level of Pol II and TBP association (**Fig. 2d**–**f**). Mediator is strongly recruited to all three enhancers (*SSA4*, *HSP104* and *HSP26*) strongly bound (**Fig. 2g**) and activated by Hsf1. In contrast, Mediator subunits are not recruited to the *CTT1* enhancer, which is specifically activated by the general stress activators Msn2 and Msn4. The failure to observe Mediator at *CTT1* was not due to the fact that Pol II and TBP levels at *CTT1* are somewhat below those at the Hsf1-activated promoters. Similar reductions in Gal4-mediated activation do not eliminate Mediator association (**Fig. 1e**–**g**), and the Mediator/Pol II occupancy ratio at *CTT1* is considerably lower than the ratios at Hsf1-activated genes (**Fig. 2f**). In addition, Mediator association at *HSP12*, a promoter activated primarily by Msn2 and Msn4, is relatively poor in comparison to TBP and Pol II association. These observations suggest that Hsf1, but not Msn2 and Msn4, can efficiently recruit Mediator.

Ace1-dependent activation of *CUP1* in response to copper and Sko1-dependent activation of *SED1* under conditions of osmotic stress

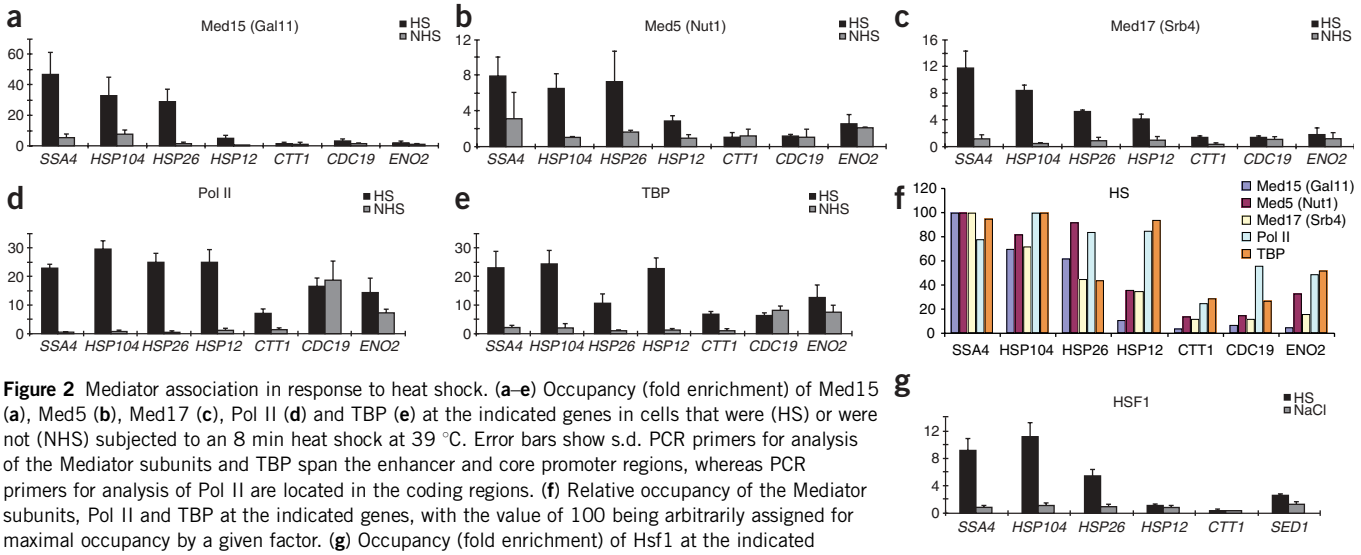

Figure 2 Mediator association in response to heat shock. (**a**–**e**) Occupancy (fold enrichment) of Med15 (**a**), Med5 (**b**), Med17 (**c**), Pol II (**d**) and TBP (**e**) at the indicated genes in cells that were (HS) or were not (NHS) subjected to an 8 min heat shock at 39 °C. Error bars show s.d. PCR primers for analysis of the Mediator subunits and TBP span the enhancer and core promoter regions, whereas PCR primers for analysis of Pol II are located in the coding regions. (**f**) Relative occupancy of the Mediator subunits, Pol II and TBP at the indicated genes, with the value of 100 being arbitrarily assigned for maximal occupancy by a given factor. (**g**) Occupancy (fold enrichment) of Hsf1 at the indicated enhancers in cells subjected to heat shock (HS) or osmotic stress (NaCl). Error bars show s.d.

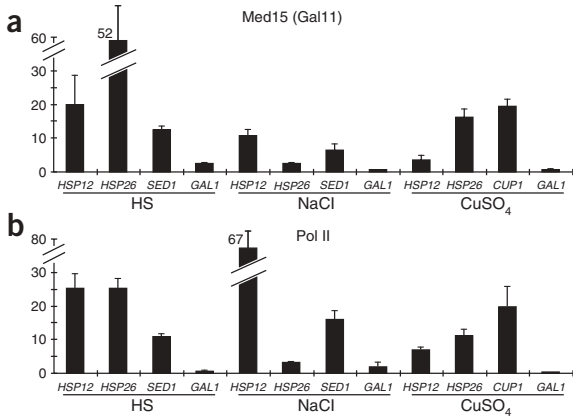

Figure 3 Mediator association in response to copper and osmotic stress. (**a**,**b**) Occupancy (fold enrichment) of Med15 (**a**) and Pol II (**b**) at the indicated genes in cells that were induced by heat shock (HS), osmotic stress (NaCl) or $CuSO_4$. Error bars show s.d. PCR primers for analysis of Med15 span the enhancer regions, whereas PCR primers for analysis of Pol II are located in the coding regions.

results in recruitment of Mediator (**Fig. 3**). However, the Mediator/Pol II ratio at the Sko1-activated *SED1* promoter is significantly lower than the ratios at promoters activated by Hsf1, Ace1 or Gal4. At *HSP12*, Mediator recruitment upon osmotic stress is lower than that upon heat shock, even though Pol II association is higher upon osmotic induction. Although the activators that stimulate *HSP12* expression under these two stress conditions are not fully identified (Hot1, Msn2 and Msn4 contribute to the osmotic response), the difference in Mediator recruitment is due to the activators and not the promoter. Together, these observations indicate that many, but not all, activators can recruit Mediator and that the level of Mediator recruitment can vary among different activators. Furthermore, unlike the recruitment of general transcriptional factors, the level of Mediator recruitment is not strictly correlated with the level of Pol II association and transcriptional activity.

DISCUSSION

Activator-specific recruitment of Mediator *in vivo*

Mediator has been presumed to be a general component of the Pol II machinery *in vivo* on the basis of the observation that thermal inactivation of either the Med17 or Med22 (also called Srb6) subunit causes a general decrease in Pol II transcription in a manner indistinguishable from thermal inactivation of Pol II itself[8,9]. Consistent with such a general function, Mediator forms stable complexes with Pol II, and it stimulates basal Pol II transcription *in vitro*. *In vivo*, Mediator associates with the enhancer region but not the core promoter[17], and transcriptional activator proteins bound at enhancers are required for transcriptional activity. Thus, the prevailing view is that Mediator serves as a universal bridge between activator proteins bound at the enhancer and the basic Pol II machinery[1–7].

In contrast to this general view, our results indicate that Mediator selectively associates with promoter regions of transcriptionally active genes. Specifically, Mediator strongly associates with promoters activated by Gal4, Hsf1, Ace1 and Sko1, but does not detectably associate with promoters activated by Msn2, Msn4 and Rap1, despite robust Pol II association at these promoters. Although it is experimental impossible to prove the absence of Mediator at ribosomal and glycolytic promoters, the Mediator/Pol II ratios are $<5\%$ of those observed at Gal4-, Hsf1- and Ace1-activated promoters. The fact that Mediator subunits in three distinct structural modules yield comparable results strongly supports the argument that our measurements reflect true association of Mediator *in vivo* and are not due to conformational effects that alter the efficiency of formaldehyde cross-linking.

Different behavior from basic Pol II initiation factors

Activator-specific recruitment and the apparent absence of Mediator from many highly active promoters strongly suggest that Mediator behaves differently from a general initiation factor and is not a stable component of the basic Pol II transcription machinery that associates with promoters *in vivo*. In accord with this view, Mediator does not detectably associate with core promoter regions, even when it is recruited to enhancers by activator proteins. Our results therefore argue against the idea that active promoters contain a Pol II holoenzyme consisting of Pol II and Mediator[8,9].

Several previous observations are consistent with the idea that Mediator is not absolutely required for transcription *in vivo*. First, Ace1- and Hsf1-dependent activation of natural and artificial genes is largely independent of Med17 and Med22, the essential subunits of Mediator[18–20]. In these cases, Ace1 and Hsf1 function through the tail (Gal11-containing) module of Mediator, consistent with our observation that these activators recruit Mediator to promoters in wild-type cells. Second, in a strain lacking the Med16 (also called Sin4) component of the tail module, the Gcn4 activator recruits the remaining components of this tail module in the absence of the other Mediator subunits, yet activates transcription[21]. In both examples, the activators recruit Mediator, but the 'essential' subunits of Mediator have a relatively small effect on transcriptional activation. Most importantly, our observation that Mediator associates extremely poorly, and perhaps not at all, with a considerable number of transcriptionally active promoters suggests the possibility that the intact Mediator complex may not be required for transcription for many genes in wild-type cells.

Requirement of Mediator for Pol II transcription *in vivo*

Our results prompt the question of why inactivation of Med17 or Med22 mimics the loss of RNA polymerase II[8,9]. One possibility is that Med17 and Med22 might have a general function in Pol II transcription that occurs outside the context of the Mediator complex. However, the lack of detectable Med17 association at several active promoters puts significant limitations on this explanation, particularly because numerous Pol II–associated factors are easily detected by chromatin immunoprecipitation. Alternatively, transient association of Mediator (or a novel subcomplex) with the promoter might be sufficient for the general function in Pol II transcription, acting in a manner mechanistically distinct from stable activator-specific recruitment of Mediator. Perhaps this general function reflects antagonism of Mediator with NC2, which can function as a general negative regulator of Pol II transcription[22]. Lastly, it is possible that the apparent general function of Med17 and Med22 is an artifact of mutant strains rather than a true function of the wild-type proteins. For example, *med17* and *med22* strains grow slowly under permissive conditions and hence might be physiologically altered such that a crucial component(s) of the basic Pol II machinery is limiting. This limitation might be exacerbated by shifting cells to the restrictive temperature, thereby causing a general transcriptional defect that does not reflect a direct function of Med17 or Med22.

Preferential recruitment by stress-response activators

Our results also define two mechanistically distinct kinds of activators on the basis of their ability to recruit Mediator. To date, all activators

shown to recruit Mediator are associated with genes that are transcriptionally activated in response to environmental stress or nonoptimal growth conditions (such as Gal4, Gcn4, Hsf1, Ace1 and Sko1). Mediator recruitment can be important for transcriptional activation of the target genes, but there are examples in which the intact Mediator complex is not essential for transcription. In such cases of 'Mediator-independent' transcription, it is presumed that the activator recruits (directly or indirectly) other coactivators that are functionally redundant with Mediator such as SWI/SNF and SAGA.

In contrast, Mediator is not detectably recruited to promoters representing the two major classes of highly expressed genes under optimal growth conditions, those encoding ribosomal proteins or glycolytic enzymes. Ribosomal protein genes are coordinately regulated by the combination of the Rap1, Fhl1, Ifh1 and perhaps Sfp1 activators[23–27], which recruit TFIID in an activator-specific manner[28,29]. Perhaps recruitment of TFIID (and other coactivators) is sufficient for high transcriptional activity in the absence of Mediator recruitment. The glycolytic genes are activated by the combination of Rap1, Gcr1 and Gcr2, but activation is not associated with TFIID recruitment[28,29]; presumably, recruitment of other coactivators is sufficient in the absence of Mediator. Whatever the specific activation mechanisms, recruitment of Mediator is a common but not universal property of activators that induce transcription in response to nonoptimal conditions. However, Mediator recruitment is not observed with activators that mediate high levels of transcription under optimal growth conditions.

METHODS

Yeast strains. Experiments in this study were performed in *S. cerevisiae* strain BY4742 containing C-terminally myc- or HA-tagged Mediator subunits or HA-tagged Hsf1 expressed from their natural promoters at their normal chromosomal locations. For *GAL* induction experiments (**Fig. 1a–d**), strains were grown initially at 30 °C in YP medium containing 2% raffinose (all sugar concentrations are w/v) and induced for 1 h by addition of 2% galactose. In other experiments related to Gal4-mediated activation (**Fig. 1e–g**), cells were grown in YP medium containing 2%, 1.8% or 1.6% galactose and an appropriate amount of glucose to maintain the total concentration of carbon source at 2%. For heat-shock stress, cells were grown initially at 30 °C in YP medium containing 2% glucose and then transferred to a 39 °C shaking water bath for 8 min. For osmotic stress, YPD-grown cells were treated with 0.4 M NaCl for 10 min, and for copper stress, cells grown in synthetic complete medium were treated with 1 mM copper sulfate for 10 min.

Chromatin immunoprecipitation. Chromatin immunoprecipitations were performed by standard methods[30] using antibodies to the Myc epitope (9E10), HA-1 epitope (F7), RNA polymerase II (8WG16) or TBP. Quantitative PCR was performed in real time using an Applied Biosystems 7000 sequence detection system. Relative occupancy values were calculated by determining the apparent immunoprecipitation efficiency (the amount of PCR product in the immunoprecipitated sample divided by the amount of PCR product in the input sample) and normalized to the level observed at an ORF-free region from chromosome V, which was defined as 1. All values represent averages from at least three independent experiments, and the error is approximately ±25%.

Microarray analysis. Microarrays containing duplicate spots of 6,528 PCR products corresponding to nearly all yeast intergenic regions were hybridized with a mixture of amplified immunoprecipitated (labeled with Cy5 fluorescent dye) and input (labeled with Cy3 dye) samples as described previously[31].

Note: Supplementary information is available on the Nature Structural & Molecular Biology website.

ACKNOWLEDGMENTS

We thank D. Hall for generating many of the tagged yeast strains, and Z. Moqtaderi and J. Wade for help with the microarray analysis. This work was supported by grants to K.S. from the US National Institutes of Health (GM30186).

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Published online at http://www.nature.com/nsmb/
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions/

1. Boube, M., Joulia, L., Cribbs, D.L. & Bourbon, H.M. Evidence for a mediator of RNA polymerase II transcriptional regulation conserved from yeast to man. *Cell* **110**, 143–151 (2002).
2. Kornberg, R.D. Mediator and the mechanism of transcriptional activation. *Trends Biochem. Sci.* **30**, 235–239 (2005).
3. Bjorklund, S. & Gustafsson, C.M. The yeast Mediator complex and its regulation. *Trends Biochem. Sci.* **30**, 240–244 (2005).
4. Kim, Y.J. & Lis, J.T. Interactions between subunits of *Drosophila* Mediator and activator proteins. *Trends Biochem. Sci.* **30**, 245–249 (2005).
5. Conaway, R.C., Sato, S., Tomomori-Sato, C., Yao, T. & Conaway, J.W. The mammalian Mediator complex and its role in transcriptional regulation. *Trends Biochem. Sci.* **30**, 250–255 (2005).
6. Malik, S. & Roeder, R.G. Dynamic regulation of Pol II transcription by the mammalian Mediator complex. *Trends Biochem. Sci.* **30**, 256–263 (2005).
7. Chadick, J.Z. & Asturias, F.J. Structure of eukaryotic Mediator complexes. *Trends Biochem. Sci.* **30**, 264–271 (2005).
8. Thompson, C.M. & Young, R.A. General requirement for RNA polymerase II holoenzymes *in vivo*. *Proc. Natl. Acad. Sci. USA* **92**, 4587–4590 (1995).
9. Holstege, F.C. *et al.* Dissecting the regulatory circuitry of a eukaryotic genome. *Cell* **95**, 717–728 (1998).
10. Kuras, L. & Struhl, K. Binding of TBP to promoters *in vivo* is stimulated by activators and requires Pol II holoenzyme. *Nature* **399**, 609–612 (1999).
11. Kuras, L., Kosa, P., Mencia, M. & Struhl, K. TAF-containing and TAF-independent forms of transcriptionally active TBP *in vivo*. *Science* **288**, 1244–1248 (2000).
12. Li, X.-Y., Virbasius, A., Zhu, X. & Green, M.R. Enhancement of TBP binding by activators and general transcription factors. *Nature* **399**, 605–609 (1999).
13. Cosma, M.P., Tanaka, T. & Nasmyth, K. Ordered recruitment of transcription and chromatin remodeling factors to a cell cycle- and developmentally regulated promoter. *Cell* **97**, 299–311 (1999).
14. Bhoite, L.T., Yu, Y. & Stillman, D.J. The Swi5 activator recruits the Mediator complex to the *HO* promoter without RNA polymerase II. *Genes Dev.* **15**, 2457–2469 (2001).
15. Bryant, G.O. & Ptashne, M. Independent recruitment *in vivo* by Gal4 of two complexes required for transcription. *Mol. Cell* **11**, 1301–1309 (2003).
16. Cosma, M.P., Panizza, S. & Nasmyth, K. Cdk1 triggers association of RNA polymerase to cell cycle promoters only after recruitment of the mediator by SBF. *Mol. Cell* **7**, 1213–1220 (2001).
17. Kuras, L., Borggrefe, T. & Kornberg, R.D. Association of the Mediator complex with enhancers of active genes. *Proc. Natl. Acad. Sci. USA* **100**, 13887–13891 (2003).
18. Lee, D. & Lis, J.T. Transcriptional activation independent of TFIIH kinase and the RNA polymerase II mediator *in vivo*. *Nature* **393**, 389–392 (1998).
19. Lee, D.K., Kim, S. & Lis, J.T. Different upstream transcriptional activators have distinct coactivator requirements. *Genes Dev.* **13**, 2934–2939 (1999).
20. McNeil, J.B., Agah, H. & Bentley, D. Activated transcription independent of the RNA polymerase II holoenzyme in budding yeast. *Genes Dev.* **12**, 2510–2521 (1998).
21. Zhang, F., Sumibcay, L., Hinnebusch, A.G. & Swanson, M.J. A triad of subunits from the Gal11/tail domain of Srb mediator is an in vivo target of transcriptional activator Gcn4. *Mol. Cell. Biol.* **24**, 6871–6886 (2004).
22. Gadbois, E.L., Chao, D.M., Reese, J.C., Green, M.R. & Young, R.A. Functional antagonism between RNA polymerase II holoenzyme and global negative regulator NC2 *in vivo*. *Proc. Natl. Acad. Sci. USA* **94**, 3145–3150 (1997).
23. Marion, R.M. *et al.* Sfp1 is a stress- and nutrient-sensitive regulator of ribosomal protein gene expression. *Proc. Natl. Acad. Sci. USA* **101**, 14315–14322 (2004).
24. Wade, J.T., Hall, D.B. & Struhl, K. The transcription factor Ifh1 is a key regulator of yeast ribosomal genes. *Nature* **432**, 1054–1058 (2004).
25. Schawalder, S.B. *et al.* Growth-regulated recruitment of Ifh1, an essential ribosomal protein gene activator. *Nature* **432**, 1058–1061 (2004).
26. Martin, D.E., Soulard, A. & Hall, M.N. TOR regulates ribosomal protein gene expression via PKA and the Forkhead transcription factor FHL1. *Cell* **119**, 969–979 (2004).
27. Rudra, D., Zhao, Y. & Warner, J.R. Central role of the Ifh1-Fhl1 interaction in the synthesis of yeast ribosomal proteins. *EMBO J.* **24**, 533–542 (2005).
28. Mencia, M., Moqtaderi, Z., Geisberg, J.V., Kuras, L. & Struhl, K. Activator-specific recruitment of TFIID and regulation of ribosomal protein genes in yeast. *Mol. Cell* **9**, 823–833 (2002).
29. Li, X.Y. *et al.* Selective recruitment of TAFs by yeast upstream activating sequences. Implications for eukaryotic promoter structure. *Curr. Biol.* **12**, 1240–1244 (2002).
30. Aparicio, O.M., Geisberg, J.V. & Struhl, K. in *Current Protocols in Molecular Biology* (eds. Ausubel, F.A. *et al.*) 21.3.1–21.3.17 (John Wiley & Sons, New York, USA, 2004).
31. Moqtaderi, Z. & Struhl, K. Genome-wide occupancy of the RNA polymerase III machinery in *Saccharomyces cerevisiae* reveals loci with incomplete transcription complexes. *Mol. Cell. Biol.* **24**, 4118–4127 (2004).