Kin28 regulates the transient association of Mediator with core promoters

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Mediator is an essential, broadly used eukaryotic transcriptional coactivator. How and what Mediator communicates from activators to RNA polymerase II (RNAPII) remains an open question. Here we performed genome-wide location profiling of *Saccharomyces cerevisiae* Mediator subunits. Mediator is not found at core promoters but rather occupies the upstream activating sequence, upstream of the pre-initiation complex. In the absence of Kin28 (CDK7) kinase activity or in cells in which the RNAPII C-terminal domain is mutated to replace Ser5 with alanine, however, Mediator accumulates at core promoters together with RNAPII. We propose that Mediator is released quickly from promoters after phosphorylation of Ser5 by Kin28 (CDK7), which also allows for RNAPII to escape from the promoter.

Regulation of transcription by RNAPII by sequence-specific transcription factors (TFs) requires coactivator proteins. Coactivators are usually large protein complexes carrying one or more enzymatic activities1. First identified in budding yeast (referred to hereafter as ‘yeast’) using both genetic and biochemical approaches, Mediator is one of the most widely studied coactivator complexes (recently reviewed in refs. 2–4). Conserved throughout eukaryotes, Mediator is thought to be an essential component for the expression of most if not all genes, at least in yeast.

The 25 (yeast) to 30 (human) proteins that comprise Mediator are organized into four distinct modules, but—given its size and complexity—Mediator can easily be envisioned to be highly multifunctional (reviewed in refs. 2,3). The tail module interacts with sequence-specific TFs that recruit Mediator to DNA. The head and middle modules make several interactions with RNAPII and the general transcription machinery. The kinase module, which is connected to the rest of Mediator by the middle module, contains a cyclin-dependent kinase (CDK) (CDK8, also called Srb10) that has been shown to have both positive and negative regulatory roles in gene expression. Biochemical and structural evidence suggests that the kinase module and RNAPII interact with Mediator in a mutually exclusive manner5–8, but so far, genome-wide location profiling of Mediator subunits has failed to detect differences in the location of the kinase module relative to the rest of Mediator8,10. Mediator purified from mammalian cells missing the kinase module generally contains an additional subunit called MED26. Interestingly, both CDK8 and MED26 have been shown to stimulate transcriptional elongation in different systems (reviewed in ref. 11).

The best-described function for Mediator, both in yeast and mammalian cells, is to promote pre-initiation complex (PIC) assembly12–20, although this function has not been previously investigated in vivo at the genomic scale. In addition, human Mediator has been shown to stimulate release from promoter-proximal pausing15, most likely by recruiting various elongation factors to the paused polymerase21,22. Mediator also participates in enhancer-promoter gene looping in mammalian cells and in promoter-terminator looping in yeast (reviewed in ref. 4). Mediator interacts with nucleosomes, histone tails and chromatin regulators (reviewed in ref. 3), although these interactions are less well understood. In addition, evidence also suggests a role for Mediator in mRNA processing23–25.

Despite two decades of intense research, several basic questions remain unanswered about Mediator function. Surprisingly, the genomic location of Mediator, although well understood in mammalian cells, remains a topic of intense debate in yeast. Indeed, Mediator has been proposed to bind to just about any kind of genomic region, including the upstream activating sequence (UAS), promoters and even coding regions (open reading frames or ORFs)9,10,26–28. Also under debate is the notion that Mediator may be recruited to specific genes rather than acting globally (reviewed in ref. 3). In order to address these issues, we performed a thorough investigation of the genomic location of Mediator in yeast and propose a model for how Mediator associates with genes in vivo. Our data provide an explanation for the divergent observations among previous studies. Furthermore, we identified a transient state of Mediator and RNAPII occupancy at promoters that is controlled by Kin28 (CDK7) phosphorylation at Ser5 of the C-terminal domain (CTD).

**RESULTS**

**Mediator associates with UAS elements rather than promoters**

To determine where in the yeast genome Mediator binds, we performed extensive genome-wide location profiling of Mediator subunits. After testing several antibodies against various Mediator subunits, we realized that the best signal-to-noise ratio is achieved using epitope-tagged proteins. Indeed, all the polyclonal antibodies

Received 29 January; accepted 11 March; published online 6 April 2014; doi:10.1038/nsmb.2810
against Mediator tested generated not only poor enrichment but also extensive enrichment in ORFs of highly expressed genes (Supplementary Fig. 1a,b). Although we cannot rule out completely the possibility that Mediator occupies ORFs, several lines of evidence argue against such a conclusion. First, the enrichment in ORFs was also present when IgG was used in control chromatin immunoprecipitation (ChIP) experiments (Supplementary Fig. 1a,b). Second, the enrichment in ORFs largely disappeared when we performed ChIPs using epitope-tagged subunits and normalized them against nontagged ChIP controls (Supplementary Fig. 1c) or mock IP samples using control IgGs (Supplementary Fig. 1d). Third, we failed to show that Mediator moves along a gene with RNAPII using the GAL1pr-YLR454W elongation assay developed by Mason and Struhl29 (data not shown). Thus, our experiments fail to support the model in which yeast Mediator travels with RNAPII during elongation9,10 (explained further in the Discussion section). Recent work from the Riné, van Oudenaarden and Iyer groups30,31 similarly reported artifactual ChIP enrichments in highly transcribed coding regions. Although the use of nontagged control ChIPs was not efficient at eliminating this systematic error in the previous studies, we found that using our ChIP protocol (which uses magnetic beads instead of agarose beads) and normalizing ChIP samples with nontagged controls reliably eliminated most of the signal in the coding regions of highly transcribed genes (Supplementary Fig. 1c, bottom). We therefore performed all Mediator ChIP experiments described in this study using strains with Myc-tagged Mediator subunits (head: MED19 (Rox3); tail: MED15 (Gal11) and MED16 (Sin4); and kinase: CDK8 (Srb10) and CycC (Srb11)). In all experiments, we hybridized against ChIP samples performed in isogenic nontagged strains, as we have done previously (an example is shown in ref. 32).

All Mediator subunits tested showed very similar binding profiles (Fig. 1a, green and blue traces, and a heat map representation is shown in Supplementary Fig. 1a,b). Notably, we did not detect Mediator on the highly transcribed gene PDC1 (Gal11)), Mediator (MED15 (Gal11)) and MED16 (Sin4); and kinase: CDK8 (Srb10) and CycC (Srb11)). In all experiments, we hybridized against ChIP samples performed in isogenic nontagged strains, as we have done previously (an example is shown in ref. 32).

The main proposed function of Mediator is to promote PIC assembly. Mediator occupancy correlates weakly but positively (Pearson r = 0.46) with RNAPII (Rpb3) occupancy (Fig. 1c). Quite strikingly, ribosomal protein (RP) genes appear to be an exception to this trend, as Mediator was barely detectable at these very highly transcribed genes (Fig. 1c, red dots, and Supplementary Fig. 2b–d). Although this observation might be used to argue that Mediator is not operating at all genes, further analyses suggest that this observation instead reflects different dynamics in the association of Mediator with different classes of genes (as discussed further below). The correlation between RNAPII and Mediator occupancy suggests a rather global role for Mediator. To further test this model, we examined whether Mediator occupancy is dynamically reorganized after remodeling of gene expression after a 30-min treatment with rapamycin, which is an inhibitor of the TOR pathway that triggers the induction of several nutrient response genes36. After treatment with rapamycin, we observed an increase in Mediator occupancy upstream of rapamycin-induced genes (Fig. 1d), which is consistent with a global role for Mediator in gene expression.

The main proposed function of Mediator is to promote PIC assembly. This function, however, was never directly tested in vivo at the genomic scale. We therefore took advantage of the recently developed anchor-away (AA) system37 to test for the effect of depleting Mediator components from the nucleus on PIC assembly. We tagged SRB4 and SRB5 with the FKBP12-rapamycin–binding (FRB) domain of human mTOR in the appropriate AA strain and assayed TFIIB occupancy...
Figure 2 Mediator is involved in PIC assembly in vivo. (a–c) Average TFIIB (purple) enrichment around the TSS in either WT cells (solid traces) or in cells in which Srb5 (srb5-FRB, dashed traces) or Srb4 (srb4-FRB, dotted traces) was depleted from the nucleus using the AA system for all genes with an Rpb3 average ORF occupancy >1 in (a) WT cells (n = 299), (b) RP genes (n = 76) and (c) a control group for the RP genes shown in b (n = 132). The genes in c are transcribed at similar level to the RP genes from b. (d) A scatter plot of Mediator occupancy (Gal11) versus the difference in TFIIB occupancy between WT and Srb5-depleted cells.

by ChIP-chip 90 min after rapamycin addition. Of note, these strains are immune to rapamycin stress, as they are defective for TOR1 and FPR1. TFIIB promoter occupancy was reduced after depletion of either MEDI7 (Srb4) or MED18 (Srb5) from the nucleus (Fig. 2a). Notably, TFIIB occupancy was also reduced at RP genes (Fig. 2bc), although to a smaller extent, even though Mediator was difficult to detect at these genes (Supplementary Fig. 2b–d). Overall, the effect of Mediator depletion on PIC assembly correlates with Mediator occupancy (Fig. 2d; Pearson r = 0.56). Together these data suggest that Mediator broadly promotes PIC assembly in vivo, although perhaps not to the same extent at all genes.

Transient association of Mediator with the PIC

Genes encoding many Mediator subunits were originally identified as suppressors of partial truncation of the RNAPII CTD5,39. Later, the association of Mediator with the CTD was shown to be disrupted by CTD phosphorylation in vitro40,41. In addition, Mediator’s ability to act as a coactivator in vitro requires the CTD5,39,42. For all these reasons, Mediator function is thought to be
Figure 4  The CAND system to study CTD function in vivo. (a) A schematic diagram of the experimental CAND system. The endogenous RPB1 gene is epitope tagged with the FRB (orange) domain of human mTOR in the AA-ready strain expressing the ribosomal protein anchor Rpl13A-FKBP12. An additional RPB1 allele carrying the desired CTD mutation is expressed ectopically from a CEN-HIS3 plasmid and under the RPB1 promoter, therefore ensuring physiological expression levels. This ectopic Rpb1 is Flag tagged (red). In the absence of rapamycin (−rapamycin), both RPB1 alleles coexist in the nucleus, and the cell is kept healthy because of the presence of the WT (endogenous) protein in the nucleus. After addition of rapamycin (+rapamycin; green), the WT (endogenous) protein is rapidly sequestered in the cytoplasm, whereas the mutant protein stays in the nucleus. Various assays can be applied to these cells shortly (60–90 min) after the addition of rapamycin. (b) Relative RNAPII (Rpb3) binding on the PMA1 gene after rapamycin addition. (c) Heat map representation of the occupancy of endogenous (FRB tagged, orange) and ectopically expressed (Flag tagged, red) Rpb1 proteins over the genes with an average Rpb1 (Flag) ORF occupancy >1 (n = 345) in the absence of rapamycin and 90 min after the addition of rapamycin. (d) Spotting assay of strains expressing RPB1 alleles carrying different CTD mutations. After growth in liquid medium lacking histidine (−HIS), cultures were washed, resuspended at equal optical density, serially diluted (five-fold series) and spotted on −HIS plates containing (right) or not containing (left) rapamycin.

...was accompanied by a shift in RNAPII toward the core promoter, leading to RNAPII accumulation in the 5′ end of genes at the expense of the 3′ region, as documented by us and others44–46 (Fig. 3a,b and Supplementary Fig. 3d). This result raises the possibility that Kin28 function in promoter escape by RNAPII could be Mediator dependent. Noteworthy is the fact that, like Mediator, RNAPII was not normally detected exactly coincidentally with TFIIB (a marker of the core promoter or transcription start site (TSS)). Instead, average RNAPII occupancy was higher further downstream (Figs. 1a and 3). Taken together, these data suggest that the association of RNAPII and Mediator in PICs is transient and that in both cases the release from the PIC is stimulated by Kin28-dependent phosphorylation.

Notably, Mediator occupancy upstream of genes is more reliably detected in kin28-as cells than in wild-type (WT) cells (Fig. 3a,b and Supplementary Fig. 3d). Indeed, when the activity of Kin28 was abrogated, Mediator, now trapped at promoters, became readily detectable even at genes where it is otherwise difficult to detect (Fig. 3c and Supplementary Fig. 3d), such as the RP genes (Fig. 3d). This finding has important implications, as it provides further support for a global role for Mediator in transcription. Indeed, our data are consistent with a model in which Mediator associates with all genes but is more difficult to detect at some of them (with RP genes being extreme cases) because of the transient nature of its association with promoters and probably also with UAS regions.

Phosphorylated Ser5 mediates Mediator release and RNAPII escape Although the experiments described above uncover a role for Kin28 in Mediator release and RNAPII escape, the functional target of Kin28 kinase activity has not been addressed. An obvious candidate that would fit with previous literature on Mediator would be the RNAPII CTD. However, Kin28 has also been shown to have non-CTD substrates41,47. Even if the CTD is presumed to be the relevant Kin28 target, two different serines in the CTD heptapeptide sequence (Ser5...
and Ser7) are substrates for Kin28 (reviewed in ref. 48). CTD kinase mutants, despite their utility, have serious limitations in dissecting CTD function. Complementation systems have been developed, using α-amanitin–resistant Rpb1 mutants49,50 or Rpb1 conditional knock-out in DT40 chicken cells51, to assay for various phenotypes of CTD mutants, but these systems require several hours or days to switch from the WT to the mutant allele, which can lead to secondary and toxicity effects. Here we took advantage of the recently developed AA system37 to rapidly deplete endogenous Rpb1 protein from the nucleus. By expressing various mutant alleles (such as CTD mutants) that are immune to nuclear depletion, we can assay for different phenotypes associated with these mutations (Fig. 4a). We call this system complementation after nuclear depletion (CAND). Rpb1 was acutely depleted from the highly expressed PMA1 gene in as short as 60 min after addition of rapamycin in this system (Fig. 4b). Notably, ChIP-chip for an exogenous RPB1 allele, here tagged with a Flag epitope, was unaffected by the addition of rapamycin, although the FRB-tagged endogenous RPB1 allele was completely wiped out from the genome (Fig. 4c). Consequently, the exogenous allele can functionally complement the endogenous allele. As expected, the WT allele or nonlethal mutations such as S7A could rescue growth in the presence of rapamycin, whereas lethal mutations such as S2A or S5A could not (Fig. 4d).

Using the CAND system, we tested whether specific CTD mutations could recapitulate the Mediator and RNAPII accumulation phenotypes observed in kin28-as cells. Because Kin28 is known to phosphorylate the CTD at Ser5 and Ser7, we tested both the S5A and S7A mutants separately, as well as a WT allele as a control. Notably, replacing the endogenous Rpb1 with a WT allele generated WT RNAPII and Mediator profiles (Fig. 5). Complementing Rpb1 depletion with an SSA mutant led to RNAPII and Mediator ChIP profiles similar to those from the kin28-as mutant. Indeed, after depletion of endogenous Rpb1, the SSA mutant caused Mediator to shift toward promoters and RNAPII to pile up in the 5’ end of genes (indicated by black arrows in specific examples shown in Fig. 5b). We did not observe this phenotype when complementing with the S7A mutant (dashed traces in Fig. 5a,b). Taken together, these observations are entirely consistent with the model that Kin28-dependent phosphorylation of the CTD leads to release of Mediator and RNAPII from core promoters or the TSS, leaving Mediator’s most stable chromatin association upstream toward presumptive activator recruitment sites and RNAPII’s downstream within transcription units.

DISCUSSION
We report an extensive investigation of Mediator occupancy across the yeast genome. The location of Mediator along yeast genes has a long history of debate. It has been reported to associate (or to not associate) with the UAS, promoters, and even transcribed regions9,10,26–28. Our examination of Mediator occupancy using a number of systems (antibodies and tagged proteins), approaches (normalization to input and to nontagged or mock controls) and subunits (we tested a total of 14 subunits), in addition to different CTD kinase mutants, allows us to more fully understand the nature of the previous discrepancies. We showed that Mediator stably associates with UAS regions but only transiently associates with core promoters (a schematic representation is shown in Fig. 6). The previous lack of clarity about whether Mediator is located at core promoters or not very likely has something to do with the resolution of the ChIP assays and the kinetics of the steps in the transcription cycle. The distance between the UAS and the core promoter can be as small as 60 base pairs and rarely exceeds 300 base pairs in yeast. Given the resolution of ChIP, a signal from a binding event at the UAS would ‘spill over’ the promoter to a considerable extent. When using a single PCR amplicon, this effect has certainly led some people to conclude that Mediator occupies core promoters. As for the binding of Mediator in coding regions, much

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**Figure 4c**

**Figure 5** Mediator occupancy shifts toward core promoters in the absence of CTD Ser5 phosphorylation. (a) Average Mediator (Gal11; green) and RNAPII (Rpb1-Flag; red) occupancy on all genes with an Rpb1-Flag average ORF occupancy >1 in the absence of rapamycin (n = 345) in cells expressing WT (solid traces), SSA (dotted traces) and S7A (dashed traces) Rpb1 proteins 90 min after nuclear depletion of the endogenous Rpb1 protein by CAND. TFIIB (purple) from WT cells is shown as a placeholder for core promoters. (b) Genome browser view of a region around the PMA1 gene. The vertical dashed line indicates the PIC position, and black arrows point to Mediator accumulation at the promoter and RNAPII pile up in the 5’ end in the S5A mutant.

**Figure 6** A schematic representation of the dynamic association of Mediator with yeast genes. Mediator (green) is recruited to the UAS by sequence-specific TFs (dark gray). In step 1, Mediator stimulates PIC (purple) assembly. GTFs, general transcription factors. RNAPII (red) and Mediator are the last components to join the PIC, creating a very unstable (transient) fully assembled PIC (step 2). Of note, our data do not allow the determination of whether promoter-bound Mediator preserves its contact with the UAS. Phosphorylation of the CTD at Ser5 by Kin28 promotes the eviction of Mediator and the concomitant or subsequent release of RNAPII from the promoter (Pr.) area (step 3).
of this binding might relate to nonspecific enrichment of highly transcribed regions in ChIP assays, as these regions are enriched using IgG control ChIPs or anti-Myc ChIP samples from nontagged strains. Consequently, most of this signal disappears when the signals from these controls are factored into the analyses. Previous ChIP-chip experiments reporting binding of Mediator in ORFs\(^6,10\) used input DNA as the baseline (as opposed to control ChIPs), which perhaps explains the discrepancies with other studies, including our current work. Another contentious issue about Mediator relates to whether it is ubiquitously used by all genes or is instead recruited by specific TFs at some but not all genes\(^26–28\). Here, the fact that Mediator is not detected as easily at some genes as compared to others in WT cells, coupled with the fact that it binds at variable distances from the TSS from gene to gene, most likely contributed to the divergent views. Because Mediator occupies promoters in a manner that correlates well with RNAPII in Kin28-mutant cells (conditions in which Mediator is proposed to be trapped at promoters), and because it affects PIC assembly at all genes, our data are consistent with the original view of Mediator as a general coactivator\(^28,52–54\). Our data also suggest that the variability in the ability to detect Mediator in WT cells has to do with the dynamics at which it goes from the nucleoplasm to the UAS to the promoter and to the nucleoplasm again rather than reflecting a gene-specific function.

Our data are consistent with a well-conserved Mediator mechanism between yeast and mammals. In mammalian cells, Mediator is detected mostly in enhancer regions\(^55–58\), which is reminiscent of the UAS binding described here. In addition to enhancers, a somewhat smaller Mediator peak is often observed at the core promoter in mammalian cells. Although this peak is generally considered to be the UAS binding described here. In addition to enhancers, a some smaller Mediator peak is often observed at the core promoter in mammalian cells. Although this peak is generally considered to be the consequence of the well-described enhancer-promoter looping, our data suggest that some of this promoter signal may be a consequence of a transient association of mammalian Mediator with promoters. Our data are also consistent with previous reports showing that the kinase module has a binding profile similar to that of core Mediator subunits\(^9,10,55\).

The short life of Mediator at core promoters is mediated by Kin28-dependent phosphorylation of the RNAPII CTD at Ser5. Indeed, phosphorylation of Ser5 promotes the eviction of Mediator, which is in agreement with previous in vitro studies\(^8,41\). In addition, Ser5 phosphorylation promotes the escape of RNAPII from the promoter. Our results therefore provide compelling in vivo support for diverse in vitro and structural studies regarding possible functions of TFIIH and Ser5 in promoter clearance\(^10,41,59,60\). We and others previously reported a pile-up of RNAPII in promoter regions in a Kin28 mutant\(^48–46\), but our current work is the first formal demonstration, to our knowledge, that this piling up is mediated by Ser5 phosphorylation as opposed to phosphorylation of Ser7 or some other Kin28 substrate. Indeed, Kin28 has at least two substrates in addition to the CTD. Interestingly, these two substrates are components of Mediator, namely MED14 (Rgr1) and MED4 (refs. 41,47). Abrogating phosphorylation of these two substrates, however, had no effect on Mediator or RNAPII occupancy (data not shown).

In this study we also describe a simple and powerful system to dissect the function of the RNAPII CTD in transcription and transcription-coupled processes. This assay, which we called CAND for complementation after nuclear depletion, consists of complementing the nuclear depletion of endogenous Rpb1 protein with various Rpb1 alleles that immune to the depletion. This allows for the replacement of WT Rpb1 with any mutant (including lethal mutants) in as little as 60 min. CAND can be used to test the growth properties of mutants but can also be combined with other assays such as RNA-Seq or, here, ChIP assays. CAND-ChIP will be a powerful method to test for the molecular function of the CTD in vivo.

**METHODS**

**METHODS**

Methods and any associated references are available in the online version of the paper.

**Accession codes.** Raw and normalized ChIP-chip data are available at the Gene Expression Omnibus (GEO) database under accession number GSE55402.

**Note: Any Supplementary Information and Source Data files are available in the online version of the paper.**

**ACKNOWLEDGMENTS**

We thank K. Struhl (Harvard Medical School) for discussing unpublished data and P. Collin (Robert laboratory) for assistance with the CTD mutant constructs, as well as R. Young (Whitehead Institute for Biomedical Research), S. Hahn (Fred Hutchinson Cancer Research Center) and L. Myers (Dartmouth-Hitchcock Medical Center) for sharing strains and antibodies. We are also grateful to C. Kaplan and N. Francis for their critical reading of the manuscript. This work was funded by a Canadian Institutes of Health Research grant (MOP-88291) to F.R.

**AUTHOR CONTRIBUTIONS**

C.J. and F.R. designed the project and wrote the manuscript. C.J. performed most of the experiments. F.R. performed most of the analyses.

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.

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ONLINE METHODS
Yeast strains and plasmids. Genotypes for the yeast strains used in this study are listed in Supplementary Table 1. The 9Myc (kiTRPI1) and 18Myc (kiURA3) tags on Mediator subunits (Gal11, Ro3x, Sin4, MED6, MED8, Srb10 and Srb11) were inserted by homologous recombination of PCR products amplified from the p3536 and p3747 plasmids (a gift from R. Young), respectively. The strain yFR1544 (Sr4-4FRB-kanMX6) was generated by homologous recombination of a PCR cassette obtained from plasmid pFA6a-FRB-kanMX6 (Euroscarf) with the appropriate primer pairs into the AA-ready strain yFR1321 (HHY168, Euroscarf).

Details about the construction of the plasmid expressing RPB1-3Flag without the CTD under its endogenous promoter (pRS313-RPB1-CTDless-3Flag-HIS3) are available upon request. To construct the final RPB1-3Flag-expressing plasmids, various CTD derivatives (either WT or in which all serines at position 2, 5 or 7 have been replaced by alanines (CTDS2A, CTDS5A and CTDS7A)) were synthesized as minigenes by Integrated DNA Technologies and cloned into the pRS313-RPB1-CTDless-3Flag-HIS3 plasmid. The sequence of each minigene was optimized in order to facilitate synthesis. All sequences are available upon request.

Growth conditions. Yeast cells were grown to an optical density at 600 nm (OD600) of 0.6–0.8 at 30 °C following standard procedures. For experiments involving inhibition of kin28 activity (Fig. 3), ATP analog–sensitive kin28Δ strains were precultured in yeast nitrogen base (YNB) medium lacking histidine (−HIS) before inoculation in yeast extract-peptone-dextrose (YPD) medium. kin28Δ strains and their controls were treated with 6 μM of NAP1 (Tocris Bioscience) for 15 min before cross-linking. Strains expressing the RPB1 CTD WT or CTD mutant plasmids were cultured in YNB medium lacking histidine (−HIS). For the AA experiments (Figs. 2, 4 and 5), all strains were treated with 1 μg/mL of rapamycin (Bio Basic Inc.) for 90 min before cross-linking. For the ChIP experiment involving rapamycin-induced genes (Fig. 1d), cells were treated with 200 ng/mL of rapamycin for 30 min before cross-linking.

Serial-dilution growth assay. Cells were grown to saturation in −HIS at 30 °C, washed and resuspended to an OD600 of 1.0 in water. Cells were then subjected to fivefold serial dilutions and spotted onto −HIS with or without rapamycin (1 μg/mL). Plates were incubated at 30 °C and inspected daily.

ChIP. ChIP experiments were performed at least in duplicates as previously described62 with minor modifications. In brief, yeast cultures were grown in 50 mL of the appropriate medium (described above) to an OD600 of 0.6–0.8 before cross-linking with 1% formaldehyde for 30 min and were quenched with 125 mM glycine. The following amounts of antibody per immunoprecipitation were used: from R. Young, 10 µL; TFIIB (a gift from R. Young, 2 µL); Myc (Euroscarf) with the appropriate primer pairs into the AA-ready strain yFR1321 (HHY168, Euroscarf).

Serial-dilution growth assay. Cells were grown to saturation in −HIS at 30 °C, washed and resuspended to an OD600 of 1.0 in water. Cells were then subjected to fivefold serial dilutions and spotted onto −HIS with or without rapamycin (1 μg/mL). Plates were incubated at 30 °C and inspected daily.

ChIP–quantitative PCR. To assess the relative RNAAPII (Rpb3) binding on the PMAI gene at different time points after rapamycin addition (Fig. 4b), ChIP DNA was analyzed by quantitative real-time PCR (qPCR) using SYBR Green. Primers directed against the ORF of the PMAI gene (forward: TCTTGTGTGTGGGTGGTGT; reverse: TCTTGTGACATGACACCATG) were used. For each time point n, the enrichment (relative to time 0) (Enrichment) was calculated using the ΔΔCt method as follows: 2^{-(ΔΔCt)}.

ChIP-chip. For epitope-tagged proteins, ChIP DNA was hybridized in competition with a control ChIP DNA prepared from an isogenic untagged strain. For ChIPs performed using rabbit polyclonal antibodies, the ChIP DNA was hybridized in competition with input DNA. The microarrays were custom designed by Agilent Technologies and contained a total of about 180,000 Tm-adjusted 60-mer probes covering the entire yeast genome with virtually no gaps between probes.

ChIP-chip data analysis. The ChIP-chip data were normalized using the Limma Loess method, and replicates were combined as described previously63. The data were subjected to one round of smoothing using a Gaussian sliding window with an s.d. of 100 bp to generate data points in 10-bp intervals as described before64.

Correction of systematic errors in ChIP-chip data. As indicated above, ChIP-chip samples performed using epitope-tagged proteins were hybridized against ChIP samples from isogenic nontagged strains. This reliably removes systematic errors, notably the enrichment in highly expressed ORFs, as shown in Supplementary Figure 1c. For ChIP experiments performed with polyclonal antibodies (TFIIB, FRB and various Mediator subunits shown in Supplementary Fig. 1a), however, the ChIP DNA was hybridized against the input DNA. In order to reduce the systematic error in these experiments, the log2 ratio from a mock ChIP-chip experiment performed using rabbit IgG was subtracted from the log2 ratio of these ChIP-chip experiments. As shown in Supplementary Figure 1d, this method eliminates most of the systematic error enrichment observed in highly transcribed ORFs.

Aggregate profiles. Aggregate profiles (as shown in Figs. 1a, 2a–c, 3b and 5a and Supplementary Figs. 2b and 3) were generated using the Versatile Aggregate Profiler (VAP), a stand-alone program described in a manuscript currently under review and based on methods used in our previous work32,44,56–57. In brief, for Figure 1, genes were virtually cut in the middle, and the first half was aligned on the TSS, whereas the second half was aligned on the poly(A) (pA) site. The TSS and pA sites were deduced using the untranslated region (UTR) sizes as determined by Xu and collaborators68. Genes for which the 5′ and 3′ UTRs have not been previously determined were therefore not included in these analyses. The aligned data were averaged over 10-bp bins (100 bins upstream from the TSS, 100 bins downstream from the TSS, 100 bins upstream of the pA and 100 bins downstream of the pA). For Figures 2a–c, 3b and 5a, genes were aligned on their TSS only and averaged over 100-bp bins (100 bins upstream of the TSS and 100 bins downstream of the TSS). In these analyses, only genes that are at least 1 kilobase long were regarded, as including shorter genes creates noise due to the resolution of the ChIP assay (signal from the 5′ end ‘contaminating’ the 3′ end and vice versa).

Transcription factor binding site frequency. The transcription factor binding site (TFBS) frequency was generated using the UCSC transReCode genome browser track representing data from Harbison et al.69. These data are associated with a quality score and information about whether or not there is ChIP evidence for binding (as opposed to computational prediction only). Using this information, we filtered the TFBSs to keep only those with a score above 300 and having ChIP evidence for DNA binding. Essentially, this keeps only high-quality binding site predictions and removes a large number of predictions that are likely false positives. The frequency of these TFBSs was computed relative to the TSS and pA sites in Figure 1a.

Heat map representation of ChIP-chip data. To generate heat maps (as shown in Figs. 1d and 4c), genes were aligned on their TSS, and the intensity was parsed into 10-bp bins. The heat map images were generated using ThreeView70.

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