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Activator-Specific Recruitment of TFIID and Regulation of Ribosomal Protein Genes in Yeast

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Summary

In yeast, TFIID strongly associates with nearly all ribosomal protein (RP) promoters, but a TAF-independent form of TBP preferentially associates with other active promoters. RP promoters are regulated in response to growth stimuli, in most cases by a Rap1-containing activator. This Rap1-dependent activator is necessary and sufficient for TFIID recruitment, whereas other activators do not efficiently recruit TFIID. TAFs are recruited to RP promoters even when TBP and other general transcription factors are not associated, suggesting that TFIID recruitment involves a direct activator-TAF interaction. Most RP promoters lack canonical TATA elements, and they are preferentially activated by the Rap1-containing activator. These results demonstrate activator-specific recruitment of TFIID in vivo, and they suggest that TFIID recruitment is important for coordinate expression of RP genes.

Introduction

TFIID is a multiprotein complex comprising the TATA binding protein (TBP) and approximately 14 associated factors (TAFs) (Green, 2000; Naar et al., 2001). TBP binds TATA elements, which are found in many promoters, and it nucleates the assembly of the RNA polymerase II (Pol II) machinery. In the context of TFIID, TAFs contact initiator and downstream promoter elements as well as sequences flanking the TATA element. In vitro, TAFs are required for transcription from promoters lacking TATA elements, and they play a role in the response to activator proteins; they are not required for basal TATA-dependent transcription. Some TAFs are also present in the SAGA histone acetylase complex; hence, elucidating physiological functions of TFIID requires the analysis of TFIID-specific TAFs.

In yeast, TBP association with promoters strongly correlates with preinitiation complex assembly and transcription (Kuras and Struhl, 1999; Li et al., 1999b; Kuras et al., 2000). In contrast, TAFs are required for transcription of only a subset of yeast genes (Moqtaderi et al., 1996, 1998; Walker et al., 1996, 1997; Holstege et al., 1998;), and such gene-specific effects are observed in other eukaryotes (Green, 2000; Naar et al., 2001). In yeast, TAFs are significantly underrepresented at many promoters, indicating that there are at least two forms of transcriptionally active TBP in vivo (Kuras et al., 2000; Li et al., 2000). One form is TFIID, while the TAF-deficient form corresponds to TBP itself or to another TBP complex. The distinct promoter selectivities of TFIID and the TAF-independent form of TBP explain why TAFs are required only for a subset of yeast genes in vivo.

Genetic analyses involving conditional inactivation of TFIID-specific TAFs indicate that TFIID has an important role as a core promoter selectivity factor. First, individual depletions of four TFIID-specific TAFs severely reduce *HIS3* transcription dependent on a nonconventional TATA element, but do not affect *HIS3* transcription dependent on a canonical TATA sequence (Moqtaderi et al., 1996, 1998). Second, chimeras between TAF-dependent and TAF-independent promoters indicate that TAF130-dependent transcription maps to the core region, although not specifically to the TATA element (Shen and Green, 1997). Third, TAF130 dependence of *TUB2* transcription is eliminated by creating a canonical TATA element in the *TUB2* promoter (Tsukihashi et al., 2000). These results are consistent with observations in vitro that TFIID is required for transcription from TATA-less promoters and that TAFs contact core promoters.

There are many biochemical experiments suggesting that individual TAFs interact with transcriptional activators (Naar et al., 2001), but there is virtually no evidence for activator-specific recruitment of TFIID in vivo. In yeast, TAF inactivation does not generally affect the response to activators (Moqtaderi et al., 1996; Walker et al., 1996), and as discussed above, TAF dependence of transcription often maps to the core promoter region. However, in some experiments involving chimeric promoters, TAF dependence does not map strictly to the core promoter (Tsukihashi et al., 2001). Similarly, an analysis of a hamster cell line containing a ts mutant of TAF250 showed that, for the two promoters tested, both the core and enhancer regions contributed to TAF dependence (O'Brien and Tjian, 2000; Weissman et al., 2000). Aside from the apparent inconsistencies, all in vivo analyses of TAF function have involved transcriptional analysis upon conditional inactivation of individual TFIID-specific TAFs, an approach that is not suited for addressing the issue of whether activator proteins recruit TFIID to promoters.

Ribosomal protein (RP) genes are coordinately regulated in response to growth stimuli and other environmental changes (Warner, 1999). In yeast, there are 137 RP genes, and they account for about 50% of the total Pol II transcripts in vivo (Warner, 1999). Growth regulation of RP genes requires protein kinase A (Klein and Struhl, 1994; Neuman-Silberberg et al., 1995) and is associated with recruitment of the Esa1 histone acetylase complex (Reid et al., 2000). Most RP promoters contain binding sites for the Rap1 activator (Lascaris et al., 1999; Warner, 1999), and Rap1 is indeed bound to essentially all such RP promoters in vivo (Lieb et al., 2001). Rap1 sites are important for growth-regulated expression

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(Moehle and Hinnebusch, 1991; Klein and Struhl, 1994; Li et al., 1999a) and Esa1 recruitment (Reid et al., 2000). Most of the remaining RP promoters (as well as some RP promoters with Rap1 sites) contain Abf1 sites, suggesting that Abf1 plays a similar role (Warner, 1999).

Several RP genes were initially identified as requiring TAF130 for transcription (Shen and Green, 1997), and the corresponding RP promoters have high levels of TFIID (Kuras et al., 2000; Li et al., 2000). Our analysis of published microarray experiments (Holstege et al., 1998) suggests that TAF130 dependence is a common property of RP genes. Under conditions of TAF130 inactivation, 60% of the RP genes but only 17% of all yeast genes show a transcriptional decrease that is at least 2-fold. These observations suggest the possibility that TFIID is specifically involved in the coordinate regulation of RP genes. Alternatively, the TAF130 dependence of many RP promoters might simply reflect an indirect response to growth limitation, particularly as TAF130 (and hence TFIID) is required for cell growth.

In this study, we provide clear evidence that TFIID is recruited to promoters in an activator-specific manner in vivo. We show that a Rap1-containing activator is necessary and sufficient for efficient TFIID recruitment, that the vast majority of RP promoters have high TFIID occupancy, that RP core promoters can be preferentially activated by the Rap1-containing activator, and that TAF dependence and growth regulation of RP transcription depends on both the Rap1-containing activator and on the RP core region. These results strongly suggest that activator-specific recruitment of TFIID is important for coordinate regulation of RP genes.

Results

The Vast Majority of RP Promoters Have High TFIID Occupancy

In yeast cells, TFIID and the TAF-independent form(s) of transcriptionally active TBP (Kuras et al., 2000; Li et al., 2000) are defined operationally by the TAF:TBP occupancy ratio at promoters. For TFIID-dependent promoters, such as the three RP promoters previously analyzed, we arbitrarily defined this ratio to be 1.0 (Kuras et al., 2000). For TAF-independent promoters such as *PGK1* and *PYK1*, this ratio was approximately 0.2. To determine whether high TFIID occupancy is a general feature of RP promoters, we examined the TAF:TBP occupancy ratio at nine additional RP promoters. Eight of these nine promoters represent typical RP promoters in that they contain Rap1 and/or Abf1 sites (Lascaris et al., 1999; Warner, 1999) and they are bound by Esa1 histone acetylase (Reid et al., 2000). The remaining RP promoter, *RPL18B*, is among the rare exceptions that lack binding sites for either activator protein and is not bound by Rap1, Abf1, or Esa1 in vivo (Reid et al., 2000). As shown in Figure 1, all eight of the newly tested RP promoters containing Rap1 and/or Abf1 sites have high TAF:TBP occupancy ratios (ranging from 0.8 to 1.3, except for *RPL19B*, which has an abnormally high ratio of 1.9). Thus, 11 out of 11 typical RP promoters have high TFIID occupancy, indicating that this property pertains to the vast majority of RP promoters. The exceptional RP promoter that is not bound by Rap1 or Abf1 shows a significantly lower TAF:TBP occupancy ratio (0.5), suggesting a role of Rap1 and Abf1 in TFIID recruitment.

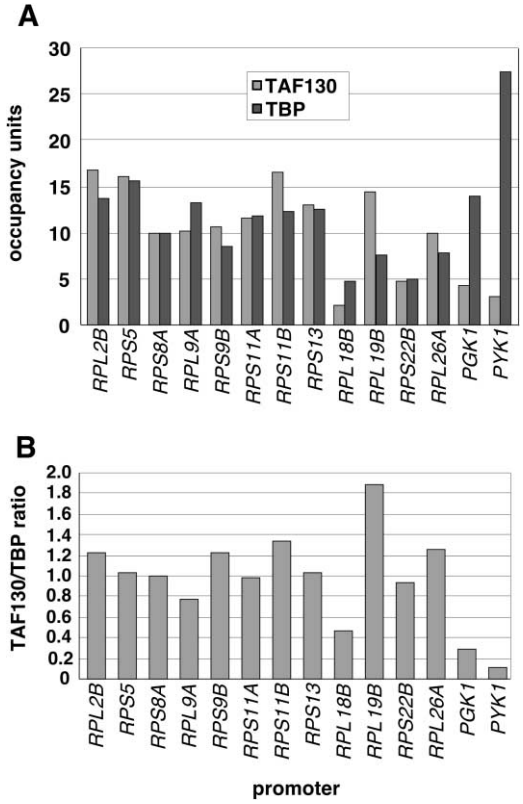


Figure 1. RP Promoters Have High TFIID Occupancy

(A) Crosslinked chromatin from a wild-type strain was immunoprecipitated with antibodies against the HA epitope or TBP, and immunoprecipitated and input material was analyzed by quantitative PCR with primers corresponding to the indicated promoters. TBP and TAF130 occupancy units were calculated as described in the Experimental Procedures, with the values for *RPS8A* being set arbitrarily to 10.

(B) TAF130:TBP occupancy ratios of the indicated promoters.

A Rap1-Containing Activator Is Sufficient to Recruit TFIID to Promoters

To determine whether TFIID association with RP promoters is determined by the upstream or core regions, we first analyzed chimeric promoters involving *RPL9A* (high-TAF) and *PGK1* (low-TAF). The promoter containing the *RPL9A* upstream region and the *PGK1* core results in a high TAF:TBP occupancy ratio (1.4), suggesting that the *RPL9A* upstream region can confer high TFIID occupancy on a core region from a low-TAF promoter (Figure 2A). Conversely, the promoter with the *PGK1* upstream region and *RPL9A* core is severely defective for transcription (see below), and hence cannot be assessed for TBP and TAF occupancy. A deleted version of this chimeric promoter, in which the distance between the *PGK1* upstream and *RPL9A* core region was significantly reduced, confers a TAF:TBP occupancy ratio (0.5) that is significantly lower than that of the *RPL9A* promoter, although above that of the *PGK1* promoter.

Two additional experiments confirm that RP upstream regions can recruit TFIID to core promoters (Figure 2A). First, in combination with the *PGK1* core promoter, the *RPS8A* upstream region confers high TFIID occupancy (TAF:TBP ratio of 1.0), whereas the upstream region from *PYK1* (a low-TAF promoter) confers low TFIID occupancy (TAF:TBP ratio of 0.2). Second, in combination with the *HIS3* core promoter, the *RPS8A* and *RPL9A* upstream regions confer high TAF occupancy, whereas the *PGK1* upstream region confers low TAF occupancy. Thus, the *PGK1* and *HIS3* core regions are permissive to TFIID recruitment, even though the natural promoters show low levels of TFIID occupancy.

Like many RP promoters (Lascaris et al., 1999; Lieb et al., 2001), the *RPS8A* upstream region contains two Rap1 sites that are closely spaced. To determine whether these Rap1 sites are sufficient to recruit TFIID, we fused various segments of the *RPS8A* upstream region with these two Rap1 sites (ranging from 41 to 209 bp) to the *PGK1* core promoter (Figure 2B). The shortest derivative tested (41 bp) is almost exclusively composed of the two Rap1 sites. All *RPS8A* segments support comparable levels of transcription (Figure 2C), and the 41 bp *RPS8A* segment with the two Rap1 sites confers a TAF:TBP occupancy ratio that is indistinguishable from that conferred by the intact *RPS8A* upstream region (Figure 2D). Mutation of either Rap1 site within the 41 bp fragment causes a 5-fold decrease in transcriptional activity, and mutation of both Rap1 sites abolishes transcription (Figure 2C). These results indicate that a short *RPS8A* segment containing two Rap1 sites is sufficient for TFIID recruitment. Furthermore, they indicate that a Rap1-containing activator is sufficient to recruit TFIID to many RP promoters.

Efficient TFIID Recruitment Is Specific to the Rap1-Containing Activator

To further address the specificity of TFIID recruitment, we analyzed TBP and TAF130 occupancy mediated by several LexA-based activator proteins at a promoter containing two LexA operators upstream of the *HIS3* core region (Figures 2E and 2F). In all three cases, transcription and TBP occupancy are very high (Figure 2E), but the TAF:TBP occupancy ratios are low and roughly comparable to those conferred by the *PGK1* and *PYK1* upstream regions (Figure 2F). Thus, unlike the Rap1-containing activator, all strong activators tested do not efficiently recruit TFIID to the promoter.

We could not address whether LexA-Rap1 can recruit TFIID, because in accord with previous results (Klein and Struhl, 1994), LexA-Rap1 is a very weak activator and thus confers unmeasurable levels of TBP and TAF130. The fact that LexA-Rap1 is a weak activator strongly suggests that efficient Rap1-dependent activation requires that Rap1 associate with promoters through its own DNA binding domain (see Discussion).

The Rap1 Sites in the *RPS11B* Promoter Are Required for TFIID Recruitment

To determine whether Rap1 sites are required for TFIID recruitment at a natural RP promoter, we analyzed a derivative of the *RPS11B* promoter in which a 25 bp region containing the two Rap1 sites is deleted (Reid et al., 2000). In comparison to the wild-type *RPS11B* promoter, the deleted promoter derivative shows a 2.5-fold decrease in TBP and a comparable 2.5-fold decrease in transcription (Figure 2G). These results are similar to a comparable derivative of the *RPS13* promoter lacking both Rap1 sites (Klein and Struhl, 1994), and they indicate that Rap1 contributes to, but is not essential for, *RPS11B* transcription. Strikingly, TAF130 and TAF61 association at the deleted *RPS11B* promoter is reduced to near background levels, and the TAF:TBP ratio is comparable to that of a low-TAF promoter. Thus, the Rap1 sites are necessary for efficient recruitment of TFIID to the *RPS11B* promoter.

As Esa1 histone acetylase is recruited to RP promoters (Reid et al., 2000), we examined whether Esa1 affects TFIID recruitment. Under conditions of Esa1 depletion, TAF130 occupancy at all four RP promoters tested is significantly reduced, whereas TBP occupancy is only slightly affected (Figure 2H). Thus, Esa1 affects TFIID recruitment to RP promoters, although the mechanistic basis for this effect remains to be determined.

TAF Association with RP Promoters Does Not Require TBP or Pol II

In a strain containing a temperature-sensitive mutant of TBP (ts-1), TBP and Pol II occupancy at several TAF-independent promoters (*PGK1*, *PYK1*, *ADH1*) is minimally affected at the restrictive temperature (Figure 3). In contrast, at the three RP promoters tested, occupancy by TBP and Pol II is reduced by a factor of 3–6, whereas occupancy by TAF130 and TAF61 is increased about 2-fold. The simplest interpretation of these results is that the ts-1 mutation disrupts the interaction of TBP with TAFs, thereby inactivating TFIID without affecting the TAF-independent form of TBP. In any event, Rap1-dependent recruitment of TAFs to RP promoters does not require TBP or Pol II and hence can occur in the absence of the preinitiation complex. These results are consistent with a direct interaction between the Rap1-containing activator and a TAF subunit(s) of TFIID. TBP- and Pol II-independent association of TAFs has been observed previously in a strain containing a different TBP derivative (ts-2), although in this case, thermal inactivation resulted in loss of TBP and Pol II occupancy at TAF-dependent and TAF-independent promoters (Li et al., 2000).

TFIID Appears to Confer Less Transcriptional Activity Than the TAF-Independent Form of TBP

Although the level of TBP occupancy strongly correlates with transcription (Kuras and Struhl, 1999; Li et al., 1999b), there is a minority class of promoters where this is not the case (Kuras and Struhl, 1999). The results below provide three new examples. First, TBP occupancy at the truncated *PGK1-RPL9A* promoter is 2-fold below that observed at the *RPL9A* promoter (Figure 2), despite the fact that these promoters support comparable levels of transcription (Figure 4A). Second, TBP occupancy at the *RPL9A* and *PGK1* promoters is comparable (Figure 1), even though the *PGK1* promoter is 3-fold more active (Figure 4A). Third, in promoters involving the *PGK1* core, the *PGK1* and *PYK1* upstream regions

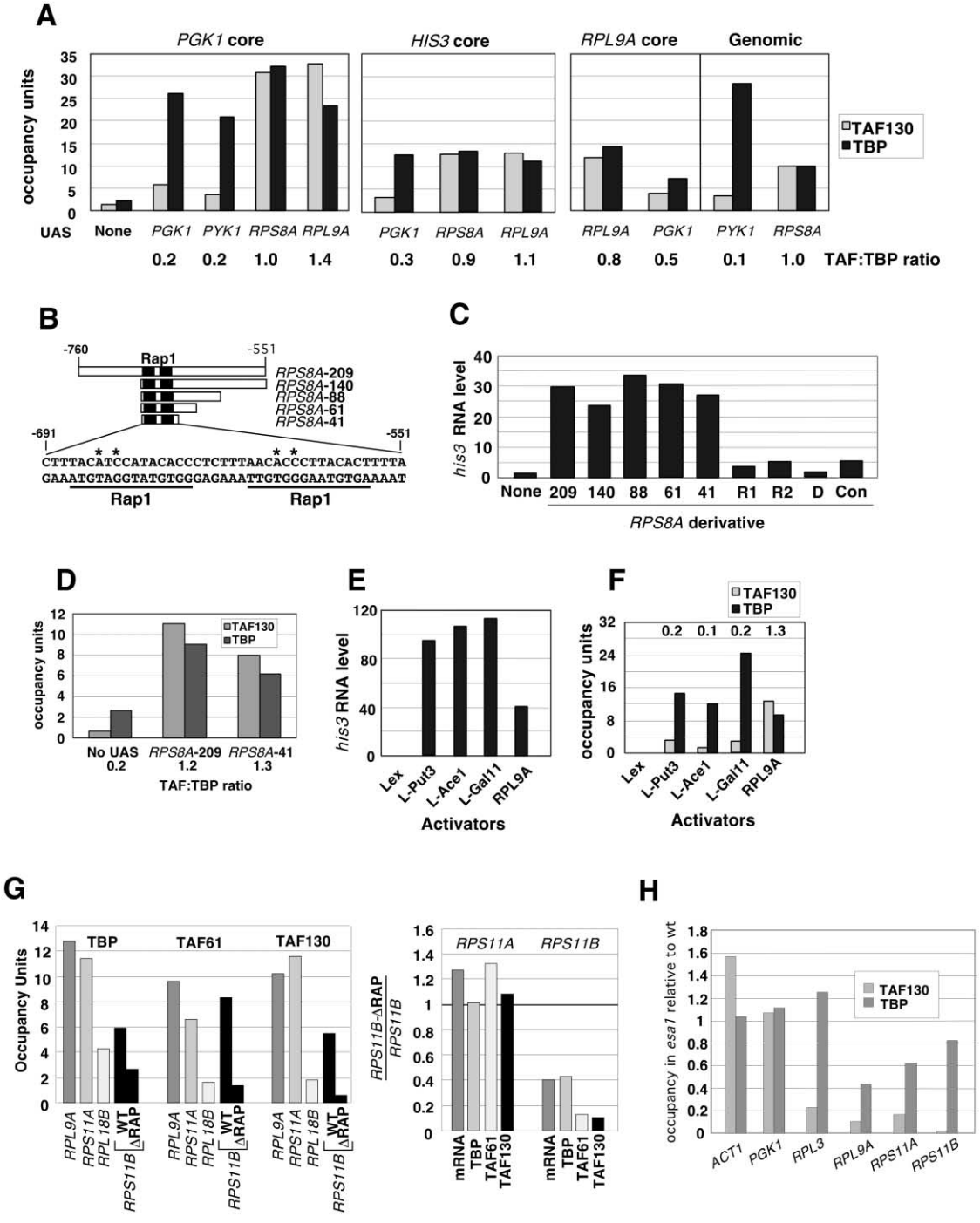


Figure 2. A Rap1-Containing Activator Is Necessary and Sufficient for TFIID Recruitment

(A) TBP and TAF130 occupancies in strains containing promoters with the indicated upstream (UAS) and core region promoters were calculated as described in Figure 1, and TAF130:TBP occupancy ratios are indicated.

(B) Diagram of the *RPS8A* promoter indicating the location of Rap1 sites and the regions used to make chimeric promoters with the *PGK1* core. The sequence of the minimal 41 bp region is shown with Rap1 sites underlined and mutated residues indicated by asterisks.

(C) *HIS3* RNA levels in strains containing the indicated *RPS8A* region fused to the *PGK1* core promoter and *HIS3* structural gene. For derivatives of *RPS8A*-41, "R1" and "R2" represent mutations of individual Rap1 sites, "D" represents the mutations at both Rap1 sites, and "CON" represents a consensus Rap1 site.

(D) TBP and TAF130 occupancies in strains containing chimeric promoters with the indicated regions of *RPS8A* were calculated as described in Figure 1, and TAF130:TBP occupancy ratios are indicated.

(E) *HIS3* RNA levels in strains containing the indicated LexA-based activator protein and a promoter with two LexA operators upstream of the *HIS3* core region.

(F) TBP and TAF130 occupancies in strains shown in (E) were calculated as described in Figure 1, and TAF130:TBP occupancy ratios are indicated.

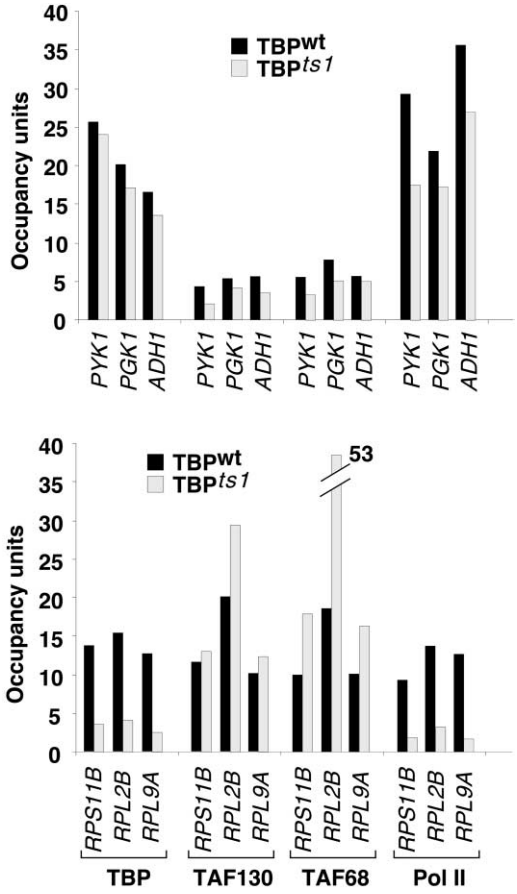


Figure 3. TAF Association with Promoters Can Occur in the Absence of the Preinitiation Complex

Crosslinked chromatin from isogenic wild-type (dark bars) and TBP temperature sensitive (ts-1 allele; light bars) strains shifted to 37°C for 45 min was immunoprecipitated with antibodies against the HA epitope, TBP, TAF61, and Pol II. Immunoprecipitated and input material was analyzed by quantitative PCR using primers to the indicated TAF-independent (top panel) and RP (bottom panel) promoters, and occupancy units were calculated as described in Figure 1.

confer 2-fold higher transcription than the *RPS8A* and *RPL9A* upstream regions (Figure 4B), despite the fact that TBP, TFIIB, and Pol II associate with these promoters to a comparable extent (Figure 4C). In all these cases, unexpectedly low transcription for a given amount of TBP is associated with high TAF130 occupancy, suggesting the possibility that preinitiation complexes containing TFIID are less active than those containing the TAF-independent form of TBP.

The Rap1-Containing Activator Acts in Concert with RP Core Promoters

The observation that a Rap1-containing activator is necessary and sufficient for TFIID recruitment to RP promoters suggests that RP upstream regions should be responsible for the TFIID dependence of RP transcription. However, one analysis of chimeric promoters indicated that TAF dependence is determined by the core promoter region (Shen and Green, 1997), whereas another analysis using different chimeras and different TAF mutations indicated that TAF130 dependency was determined by both the core and upstream regions of RP promoters (Tsukihashi et al., 2001). To address this issue independently, we analyzed chimeric and control promoters involving *PGK1* and *RPL9A* in strains depleted of TAF130 (Moqtaderi et al., 1996). As expected, transcription from the *PGK1* promoter is unaffected by TAF depletion, while transcription from the *RPL9A* promoter is reduced by a factor of nine (Figure 4D). For the two chimeric promoters, transcription decreases 3-fold under conditions of TAF depletion, indicating that TAF130 dependency of the *RPL9A* promoter is influenced by both the upstream and core promoter regions.

One explanation for the above observation is that RP core promoter regions require TFIID for efficient transcription, and hence are ideally suited for responding to an activator(s) that recruits TFIID. A prediction of this model is that strong activators that do not recruit TFIID would be relatively less active in combination with RP core promoters than at TAF-independent core promoters. We therefore examined transcriptional activity mediated by various activators in the context of the *PGK1*, *HIS3*, *RPL8A*, and *RPL9A* core promoter regions linked to the *HIS3* structural gene (Figure 5A). For strong activators (the *PGK1* upstream region, LexA-Put3, LexA-Ace1, LexA-Gal11), transcriptional activity in combination with the two RP core regions was approximately 10%–20% of that obtained in combination with the *PGK1* and *HIS3* core regions. By this criterion, the RP core regions behave similarly to weak TATA elements that function poorly in combination with standard activators (Iyer and Struhl, 1995). In contrast, the *RPS8A* and *RPL9A* core regions are 50%–60% as effective as the *PGK1* and *HIS3* core regions when activated by the two RP upstream regions tested. Thus, the RP core regions can respond to all activators tested, but they are 2- to 4-fold more active in combination with RP upstream regions.

The Rap1-Containing Activator Is Unusually Effective at a Heterologous TFIID-Dependent Core Promoter

The *HIS3* core contains a noncanonical TATA-like element (T_C) that mediates initiation from +1 and a consensus TATA element (T_R) that mediates initiation from +13 (Iyer and Struhl, 1995). TFIID-specific TAFs are required for T_C-dependent transcription from +1, but not for T_R-dependent transcription from +13, presumably because TAF-promoter interactions are particularly important at promoters where specific TBP-TATA contacts are minimal (Moqtaderi et al., 1996, 1998). Strong activators (e.g., Gcn4, Gal4, Hsf, Ace1) display a 10-fold preference

(G) TBP, TAF130, and TAF61 occupancies in strains containing the wild-type or deleted version of *RPS11B* lacking the Rap1 sites were calculated as described in Figure 1. For the control promoters, occupancy values for the two strains were very similar and are presented as an average; for *RPS11B*, the values of the wild-type and deleted promoter are presented separately.

(H) TBP and TAF130 occupancies at the indicated promoters in an Esa1-depleted strain relative to the parental wild-type strain 4 hr after copper addition.

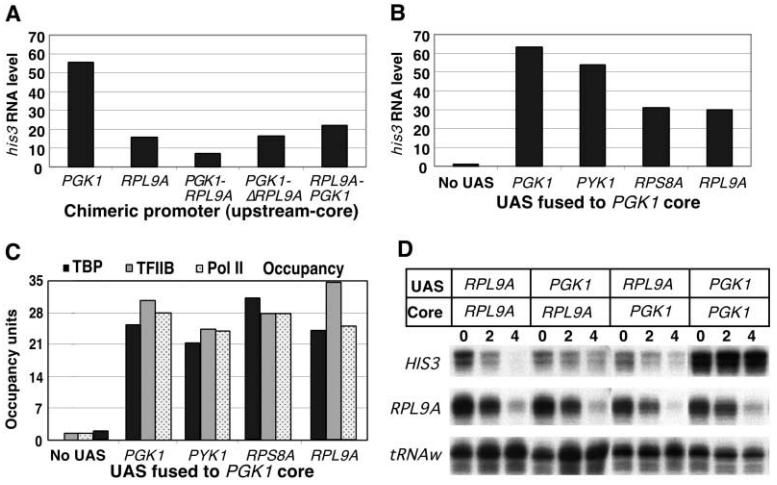


Figure 4. TFIID Appears to Be Less Transcriptionally Active Than the TAF-Independent Form of TBP

(A) *HIS3* RNA levels in strains containing the indicated natural or chimeric promoter region fused to the *HIS3* RNA coding sequences.

(B) *HIS3* RNA levels in strains containing the indicated upstream region (UAS) and the *PGK1* core.

(C) TBP, TFIIB, and Pol II occupancies in the above strains were calculated as described in Figure 1.

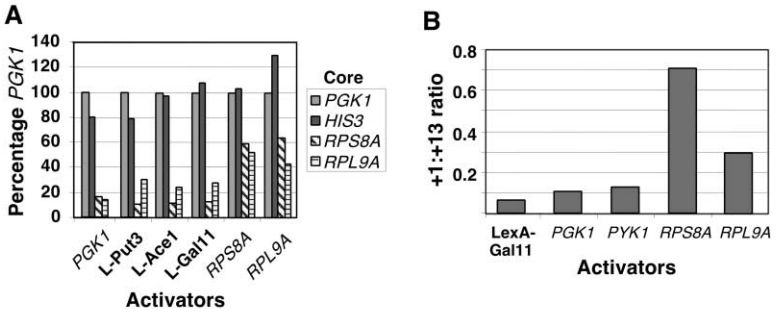
(D) Both the Rap1-containing activator and RP core regions contribute to TAF dependence of transcription. *HIS3*, *RPL9A*, and tRNA^{w} RNA levels in TAF130 depletion strains containing the indicated promoters fused to the *HIS3* structural gene at 0, 2, and 4 hr after copper addition are shown.

for T_R-dependent transcription from +13 due to the limited ability of T_C to respond to a strong activator (Iyer and Struhl, 1995). In contrast, artificial recruitment of TBP or TAFs results in strong T_C-dependent transcription from +1 (Gonzalez-Couto et al., 1997).

As expected, +1 transcription mediated by LexA-Gal11 and the *PGK1* and *PYK1* upstream regions occurs at only 10% the level of +13 transcription (Figure 5B). In contrast, the *RPS8A* and *RPL9A* upstream regions activate transcription from the +1 site to 70% and 30% of the level of that of the +13 site, respectively (Figure 5B). Importantly, the levels of +1 transcription observed in the *RPS8A* and (to a lesser extent) *RPL9A* derivatives are higher than those observed for any of the numerous derivatives containing the *HIS3* core region examined previously (Iyer and Struhl, 1995). Furthermore, the *HIS3* pattern generated by the *RPS8A* and *RPL9A* derivatives resembles the pattern that occurs upon artificial recruitment of TFIID (Gonzalez-Couto et al., 1997). Thus, the RP upstream regions (and presumably the Rap1-containing activator) are unusually effective at a heterologous TFIID-dependent core promoter.

RP Promoters Typically Lack Canonical TATA Elements

The observation that the Rap1-dependent activator is particularly effective in supporting transcription in combination with the TFIID-dependent core regions suggests that RP core regions might differ from core regions of non-RP promoters. Analysis of 121 RP promoters, 120 highly active non-RP promoters, and the near complete set of 6222 promoters indicates that canonical TATA elements are dramatically underrepresented in RP promoters (Figure 5C). In the region between −50 and



C

	DNA Element	RP [121 Genes]	Highly Expressed (Non-RP) [120 Genes]	All Pol II Promoters [6222 Genes]	P-Values RP vs. Non-RP	P-Values RP vs. All Pol II
Consensus	TATA$^{A}_{T}$A	17.4%	59.2%	45.8%	1.6×10^{-11}	1.1×10^{-10}
	TATA$^{A}_{T}A^{A}_{T}$	9.1%	53.3%	33.6%	2.8×10^{-14}	5.3×10^{-10}
Random $(A/T)_{5-6}$ Sequences	TTTAAA	9.9%	11.7%	14.4%	0.68	0.19
	TTAAAT	12.4%	14.2%	10.7%	0.71	0.55
	TTAAT	32.2%	40.0%	32.7%	0.22	0.58
	TTAATT	14.0%	20.0%	11.4%	0.23	0.38
	TAAATT	14.9%	15.0%	11.3%	1.0	0.24
	TTAAAA	10.7%	16.7%	15.6%	0.19	0.16
	ATATTT	14.0%	18.3%	20.8%	0.39	0.08

Figure 5. The Rap1-Containing Activator Acts in Concert with RP and Heterologous TAF-Dependent Core Regions

(A) *HIS3* RNA levels in strains containing the indicated LexA-based activators or RP or *PGK1* upstream regions and the indicated core regions (differently shaded bars). For each activator or upstream region, the level of transcription is expressed as the percentage of the level mediated by the promoter containing the *PGK1* core.

(B) Ratio of +1:13 *HIS3* transcription in strains containing LexA-Gal11 or the indicated upstream regions fused to the *HIS3* core region and structural gene.

(C) Canonical TATA elements are very underrepresented in RP promoters. For each DNA sequence, the frequencies of occurrence between −50 and −200 in the indicated samples are shown along with the probability (p values) that underrepresentation of canonical TATA elements in RP promoters occurs by chance.

−200, only 9% of RP promoters have canonical TATA elements, in contrast to 53% of the highly active promoters and 34% of all yeast promoters. Similar results are obtained when the search for canonical TATA elements is restricted to the region between −50 and −150. The probability that underrepresentation of canonical TATA elements in RP promoters occurs by chance is remote (about 10^{-10}). This striking underrepresentation of canonical TATA elements in RP promoters is not due to a difference in overall AT content. The frequencies in RP promoters of six randomly selected hexamers and one pentamer exclusively composed of AT residues are comparable to those of both control promoter groups.

The Rap1-Containing Activator Is Important for Growth Regulation of *RPS11B*

RP genes are coordinately regulated in response to growth stimuli and other environmental changes (Warner, 1999), and in the few cases tested, Rap1 sites are important for regulation (Moehle and Hinnebusch, 1991; Klein and Struhl, 1994; Li et al., 1999a). As expected, transcription of the *RPS11B*, *RPS11A*, and *RPL9A* genes increases 4-fold upon glucose upshift (Figure 6). The Rap1-containing activator is important for this regulatory response, because the *RPS11B* derivative lacking the two Rap1 sites shows reduced induction upon glucose upshift (2-fold). In addition, the Rap1-containing activator is partially sufficient for this regulatory response, because the chimeric promoter containing the minimal 41 bp fragment with the two Rap1 sites upstream of the *PGK1* core region is induced 2-fold upon glucose upshift. Thus, the response to glucose upshift is mediated partly by the Rap1-containing activator and partly by some other feature of the *RPS11B* promoter. This result is analogous to the observation that TAF dependence of RP transcription depends both on the Rap1-containing activator and on RP core promoter regions.

Discussion

A Rap-Containing Activator Recruits TFIID to RP Promoters

In yeast cells, activators function primarily by increasing the association of the general Pol II machinery to promoters (Kuras and Struhl, 1999; Li et al., 1999b). In principle, activators could increase TFIID association with promoters by three distinct, but not mutually exclusive, mechanisms. First, activators could directly recruit TFIID to promoters. Second, activators could directly recruit chromatin-modifying activities to promoters, thereby generating an accessible chromatin structure that passively facilitates the binding of TFIID. Third, activators could directly recruit other general transcription factors (e.g., TFIIA, TFIIB) or Pol II holoenzyme (loosely defined as the mediator plus Pol II core enzyme) to promoters, which would then stabilize the association of TFIID with the promoter. It is very difficult to distinguish between these mechanisms in the context of a living cell, and all three mechanisms predict an activator-dependent increase in TFIID occupancy. However, the facts that yeast cells contain at least two forms of transcriptionally active TBP (TFIID and a TAF-independent form) and that TAFs are not obligate components of the preinitiation complex in vivo (Kuras et al., 2000; Li et al., 2000) provide the basis for assessing whether an activator can directly recruit TFIID to promoters. In particular, the ability of a given activator to preferentially recruit TFIID over the TAF-independent form of TBP constitutes a strong argument for direct recruitment of TFIID by the activator in vivo.

Here, we demonstrate such activator-specific recruitment of TFIID in vivo, thereby providing clear evidence that TFIID can be a physiological target for activators. The Rap1-containing activator that functions at RP promoters is necessary and sufficient for TFIID recruitment. In contrast, the other three strong activators tested as

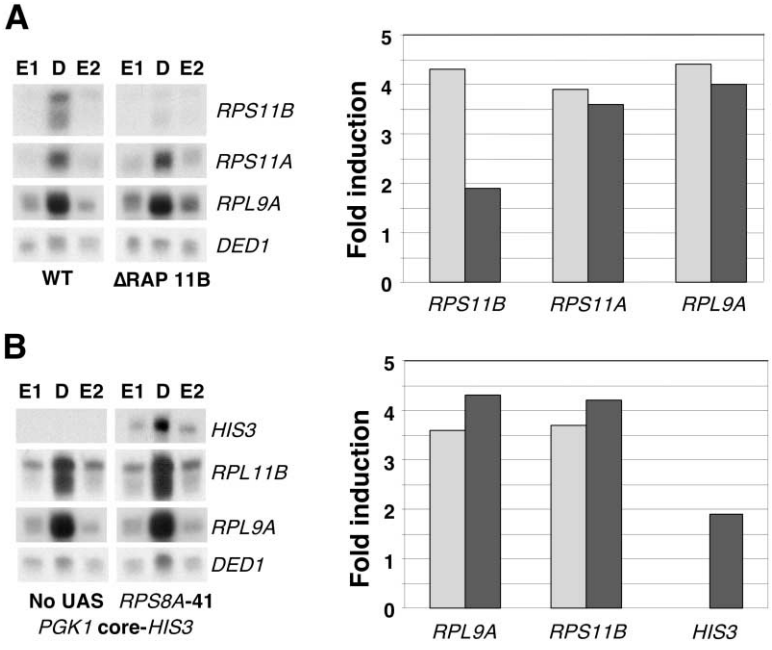


Figure 6. The Rap1-Containing Activator and the RP Core Contribute to Increased RP Transcription in Response to Glucose Upshift

(A) Strains containing the wild-type or the deleted version of *RPL11B* lacking the Rap1 sites were grown in ethanol medium (E1), shifted to glucose medium for 30 min (D), and then returned to ethanol medium for 2 hr (E2). RNA levels for the indicated transcripts were determined by S1 nuclease protection. The results are quantitated using *DED1* RNA as an internal control.

(B) Same as above, except that the *HIS3* gene is driven by the *PGK1* core promoter in the presence or absence of the minimal 41 bp region of the *RPS8A* promoter that contains two Rap1 sites.

well as the *PGK1* and *PYK1* upstream regions do not efficiently recruit TFIID to the *PGK1* or *HIS3* core regions. In addition, the *RPS8A* and (to a lesser extent) *RPL9A* upstream regions efficiently activate transcription from the TFIID-dependent T_C element in the *HIS3* core region, a pattern observed upon artificial recruitment of TFIID (Gonzalez-Couto et al., 1997) but not for any other activator tested here or elsewhere (Iyer and Struhl, 1995). These observations suggest that, in vivo, most yeast activators do not efficiently recruit TFIID, and hence must function through other targets, such as the SAGA histone acetylase complex (Bhaumik and Green, 2001; Larschan and Winston, 2001) and/or the mediator (Han et al., 1999; Park et al., 2000).

In all cases tested here and elsewhere (Kuras et al., 2000), the lowest TAF:TBP occupancy ratio is about 0.2, and this low-level TFIID association requires a functional activator. These results suggest that TFIID associates with most (and perhaps all) promoters at a detectable level, even though the TAF-independent form of TBP predominates in many cases. Such low-level TFIID association could be due to inefficient, but direct, recruitment by the activator, or it could reflect passive association that occurs after recruitment of Pol II holoenzyme and/or chromatin modifying activities. As low-level TFIID association occurs even when activation occurs by artificial recruitment of Pol II holoenzyme (Figure 2F), a considerable portion of the TFIID occupancy observed at many active promoters is not due to direct TFIID recruitment by activators. However, our results cannot exclude inefficient TFIID recruitment by an activator in any individual case.

TFIID recruitment by the Rap1-containing activator can occur, and is even improved, in the absence of TBP and Pol II (Li et al., 2000). Given the strict relationship between TBP, TFIIA, TFIIB, and Pol II occupancy (Kuras and Struhl, 1999; Li et al., 1999b, 2000; Kuras et al., 2000), it follows that association of TFIID-specific TAFs with RP promoters can occur in the absence of the basal Pol II machinery. This strongly suggests that the Rap1-containing activator recruits TFIID through a direct interaction with TAFs. Our results do not address whether TAFs interact directly with Rap1 or with a Rap1-associated protein.

Rap1 functions as an activator or silencer, depending on the promoter (Shore, 1994; Morse, 2000). Moreover, there are distinct Rap1-dependent activators that direct transcription of different classes of genes (Deminoff and Santangelo, 2001; Idrissi et al., 2001). For example, Rap1 is critical for growth-regulated expression of RP genes (Moehle and Hinnebusch, 1991; Klein and Struhl, 1994; Neuman-Silberberg et al., 1995; Li et al., 1999a), but Rap1-dependent activation of glycolytic and other genes is not regulated in this manner. The decision about which Rap1 function(s) will be utilized at a particular gene ultimately depends on the specific sequences at the promoter. The molecular bases for such decisions are poorly understood, although specific nucleotides within the Rap1 sites, sequences immediately adjacent to the Rap1 sites, and the spacing and arrangement of multiple Rap1 sites appear to play a role (Zeng et al., 1997; Idrissi et al., 1998; Idrissi and Pina, 1999). The critical role of the specific sequences at Rap1-controlled promoters explains why LexA-Rap1 is a very weak activator (Klein and Struhl, 1994).

Our observations indicate that TFIID recruitment is mediated by the Rap1-containing activator that functions primarily at RP promoters. Other promoters containing Rap1 sites (e.g., *PGK1*, *PYK1*) show low levels of TAF occupancy and do not require TFIID-specific TAFs for transcription. Two models for the molecular nature of the Rap1-containing activator have been proposed. In one model, the activator consists of Rap1 and a Gcr1 heterodimer that does not make specific contacts to DNA (Deminoff and Santangelo, 2001). In the other model, Rap1 structure is allosterically affected by the specific DNA sequences at RP promoters to expose an activation surface (Idrissi et al., 2001).

Functional Cooperation between the Rap1-Containing Activator and RP Core Promoters

Several lines of evidence indicate that transcription of many RP genes is enhanced by a functional interaction between the Rap1-containing activator and RP core promoters. First, RP core regions contribute to the TFIID dependence of RP transcription in all cases tested (Shen and Green, 1997; Tsukihashi et al., 2001) (Figure 4D), even though the Rap1-containing activator is necessary and sufficient for TFIID recruitment. Second, the RP core promoter regions tested respond poorly to conventional activators, yet they function efficiently in combination with the Rap1-containing activator (Figure 5A). Third, RP upstream regions (and presumably the Rap1-containing activator) are more effective in combination with T_C, a heterologous TFIID-dependent core promoter, than all other upstream regions and activators tested here (Figure 5B) and elsewhere (Iyer and Struhl, 1995).

These observations indicate that many RP promoters are designed to have a binding site for a Rap1-containing activator that recruits TFIID and a core region that requires TFIID for functional activity (Figure 7). Such an arrangement assures a functional synergy between the activator and the core region. Biochemical and genetic experiments indicate that TFIID is required for transcription from promoters with weak or nonexistent TATA elements, whereas TAFs are often not required for transcription from TATA-containing promoters. The RP core regions tested are functionally analogous to weak TATA elements in that they lack canonical TATA sequences, are TFIID-dependent for transcription, and respond poorly to typical activators. Canonical TATA elements are usually absent in RP core regions (Figure 5C), suggesting that the functional cooperation between the Rap1-containing activator and RP core promoters is a common (although probably not universal) phenomenon.

The organization of most RP promoters, in which an activator that recruits TFIID functions with a core region that requires TFIID for transcription, is different from the organization of promoters that respond to typical strong activators (Figure 7). All strong activators tested differ from the Rap1-containing activator in their ability to recruit TFIID to heterologous promoters (Figure 2F). Moreover, typical strong activators require a canonical TATA element for efficient activation (Iyer and Struhl, 1995), and TBP derivatives that reduce the TBP-TATA interac-

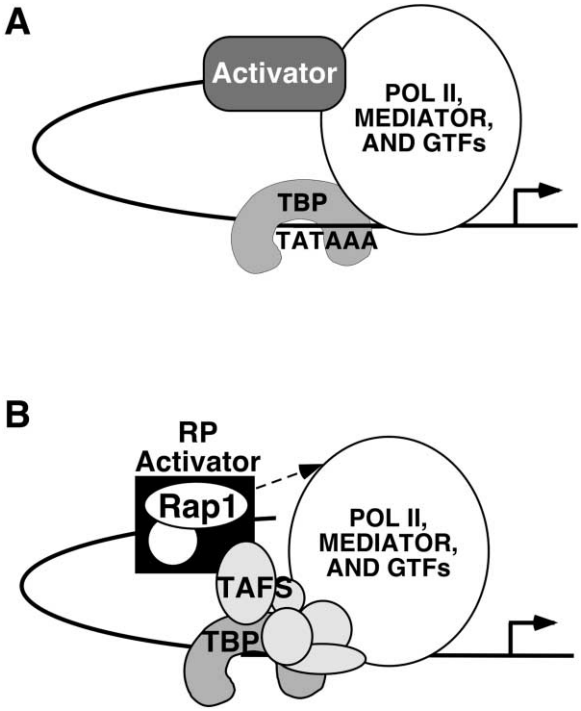


Figure 7. Model for the Predominant Transcriptional Mechanisms at RP and Strongly Activated Non-RP Promoters

(A) Typical strong activators function through the Pol II holoenzyme (loosely defined as containing Pol II subunits, mediator, and general transcription factors other than TBP) or chromatin modifying activities (not shown). The predominant form of TBP does not contain TAFs and is bound to a conventional TATA element.

(B) At typical RP promoters, the Rap1-containing activator (which may or may not contain Rap1-associated proteins) interacts with TAFs, thereby recruiting TFIID to a core promoter that typically lacks a canonical TATA element. This model does not exclude interactions of the Rap1-containing activator with the Pol II holoenzyme (indicated by dashed arrow) or with chromatin modifying activities (not shown). Hypothetical interactions of activators with TBP (not shown) are not excluded by either model.

tion are defective in their response to strong activators but are unaffected for RP transcription (Arndt et al., 1995; Lee and Struhl, 1995). Interestingly, RP promoters in fission yeast (Gross and Kaufer, 1998) and mouse (Hariharan and Perry, 1990) appear to have an unusual organization in that they contain a conserved sequence element(s) in place of a conventional TATA element. Perhaps RP promoters are organized in an atypical manner because they are highly active under essentially all conditions, whereas promoters responding to typical strong activators function only under particular environmental circumstances.

TFIID Recruitment and Coordinate Regulation of RP Genes

RP genes are coordinately regulated in response to growth stimuli and other environmental changes (Warner, 1999), and our results indicate that TFIID associates with the vast majority of RP promoters. The Rap1-containing activator involved in growth-regulated RP transcription is sufficient to recruit TFIID to heterologous promoters, and it is required for TFIID recruitment in the context of a natural RP promoter (Figure 2). These observations, and the fact that Rap1 association with promoters is unaffected by growth conditions (Reid et al., 2000), suggest that growth-regulated transcription of most RP genes involves recruitment of TFIID by the Rap1-containing activator.

TFIID recruitment by the Rap1-containing activator cannot be the sole mechanism for growth-regulated transcription of RP genes. The high TFIID occupancy at RP promoters containing Abf1, but not Rap1, sites suggests that growth-regulated transcription of some RP promoters involves recruitment of TFIID by an Abf1-containing activator. However, eight exceptional RP promoters lack Rap1 and Abf1 sites, yet these have regulatory profiles typical of RP promoters. In the one case tested here (*RPL18B*), the TAF:TBP occupancy ratio is considerably below those of other RP promoters, although it is above that of TAF-independent promoters such as *PGK1* and *PYK1*. These exceptional RP promoters may be analogous to the *RPS11B* promoter derivative lacking Rap1 sites, which is partially regulated in response to the glucose upshift (Figure 6). Thus, as is the case for TAF dependence of RP transcription, the glucose-upshift response depends both on the Rap1-containing activator and on the RP core regions.

Growth regulation of RP genes is also associated with recruitment of Esa1 histone acetylase (Reid et al., 2000). Esa1 affects TFIID recruitment to RP promoters (Figure 2H), although the mechanism by which this occurs is unknown. However, Esa1 recruitment is not specific to the Rap1-containing activator. Esa1 is recruited to promoters by the Hsf1 and Msn activators (Reid et al., 2000), and probably by Gcn4 (Deckert and Struhl, 2001). In contrast, the Rap1-containing activator is the only activator tested that recruits TFIID to promoters. This activator specificity, the TAF dependence of all RP core promoters tested, the striking underrepresentation of canonical TATA elements at RP promoters, and the requirement of both the activator and core region for the glucose-upshift response suggest that TFIID may be a key regulatory target for growth stimuli.

Experimental Procedures

Yeast Strains and DNAs

DNAs containing chimeric promoters were derived from HPIpV4, a vector designed by Elmar vomBaur that contains a modified version of a 6 kb SpeI-SalI fragment encompassing the *HIS3* locus cloned into pUC19. The *TRP1* gene is inserted at the XhoI site in the intergenic region between *HIS3* and *DED1*, and the *HIS3* promoter region (−668 to +11) is replaced with a polylinker (NcoI-FseI-XmaI-BspEI-EcoRI). In most cases, chimeric promoters were cloned between the NcoI and EcoRI sites of HPIpV4, and swapping was facilitated by a SacII site between most upstream and core promoter regions. Core promoters were fused to the HPIpV4 EcoRI site just upstream of their corresponding ATG codons. The relevant promoter fragments (defined with respect to the ATG initiation codon) are as follows: *PGK1* promoter (−653 to −7); *RPL9A* promoter (−588 to −7); *PYK1* promoter (−844 to −586 and −264 to −7; repression region deleted); *RPS8A* promoter (−761 to −215; intron deleted); *PGK1* upstream region (−653 to −246); *PGK1* core (−245 to −7); *RPL9A* upstream region (−588 to −220); *RPL9A* core (−219 to −7); truncated *RPL9A* core (−130 to −7); *PYK1* upstream region (−844 to −586); *RPS8A* upstream region (−761 to −550); *RPS8A* core (−549 to −215); *RPS8A*-141 (−692 to −550); *RPS8A*-89 (−692 to −602); *RPS8A*-62 (−692 to −630); *RPS8A*-42 (−692 to −650); *HIS3*

core (−98 to −1). The upstream (TACATCCATACACC) and downstream (AACACCCTTACACT) Rap1 sites in the *RPS8A* upstream region were mutated by introducing G residues at the underlined residues. LexA fusions were cloned in Ycp91 (Tzamarias and Struhl, 1994) and contained Put3 residues 889–979, Ace1 residues 109–225, or Gal11 residues 799–1081.

DNAs containing the chimeric promoters were cleaved with SpeI and SalI and integrated at the *HIS3* locus of SPY-ADE (*MATa*; *ade2::hisG*; *his3::ADE2*; *leu2::PET56*; *met17-Δ0*; *trp1-Δ63*; *ura3-Δ0*), a S288C-based yeast strain designed by Elmar vomBaur in which the *HIS3* locus (−668 to +881, which correspond to NcoI and XhoI sites) is replaced by *ADE2*. All the strains contained a version of TAF130 with three copies of the HA epitope at the N terminus (Kuras et al., 2000). Yeast strains that contain the deleted version of *RPL11B* that lacks Rap1 sites (Reid et al., 2000) or that contain the ts-1 mutation of TBP (Cormack and Struhl, 1992) have been described previously. In general, yeast strains were grown to early exponential phase (OD_{600} = 0.7) at 30°C in YPD medium unless otherwise indicated.

Chromatin Immunoprecipitation

Formaldehyde-crosslinked chromatin was immunoprecipitated with antibodies against the HA-1 epitope, TBP, TFIIB, TAF61, and Pol II as described previously (Kuras and Struhl, 1999; Kuras et al., 2000). Quantitative PCR analyses were performed in real time using an Applied Biosystems 7700 sequence detector, except for the experiments in Figures 2A and 2F, in which PCR products were separated on polyacrylamide gels and quantified using a Fujix PhosphorImager. To calculate the levels of TAF130 and TBP occupancy at an individual promoter, we first determined the apparent crosslinking efficiency by dividing the amount of PCR product from the immunoprecipitated sample by the amount of PCR product in the input sample prior to immunoprecipitation and subtracting the apparent crosslinking efficiency of a control DNA segment (an internal fragment of the *POL1* structural gene and/or an inactive promoter). The TAF:TBP ratio was calculated by dividing background-subtracted TAF130 binding by background-subtracted TBP binding. Typical background values are 1–2 units, except for the case of TFIIB, where the background was approximately 0.5–1 unit. To compare TAF130 and TBP occupancy levels across many experiments, we arbitrarily defined the levels of TAF130 and TBP occupancy at the *RPS8A* promoter to be 10 units and the TAF130:TBP ratio as 1.0. For the relevant experiments (Figures 3, 4C, and 6), we also arbitrarily defined TAF61, TFIIB, and Pol II occupancy levels at the *RPS8A* promoter to be 10. For all individual experiments, occupancy values were determined relative to the *RPS8A* promoter in the same samples. Each value represents the average of at least three independent experiments, and the error is approximately ±20%, except in cases of low occupancy, where the error is greater.

Transcriptional Analysis

Total RNAs were hybridized to completion with a mixture of oligonucleotide probes, and the resulting RNA:DNA hybrids were treated with S1 nuclease (Iyer and Struhl, 1996). In the glucose-upshift experiment (Figure 6), levels of the *DED1* and other control RNAs increase approximately 2-fold upon addition of glucose, in accord with previous observations (Klein and Struhl, 1994). In this case, the transcriptional levels for the various RP-related genes were normalized to the *DED1* internal control in the same sample. The error in these experiments is approximately ±20%.

Computer Analysis

Computer analysis was performed using tools from http://embnet.cifn.unam.mx/~jvanheld/rsa-tools/RSA_home.cgi. We retrieved sequences upstream (−200 to −50 or −150 to −50) of 121 RP coding regions, a sample of 120 nonribosomal yeast genes with highest rates of transcription (Holstege et al., 1998), and all the genes from the yeast genome. These sequences were searched for exact matches to the indicated sequences. p values were calculated using a two tailed Fisher's exact test.

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