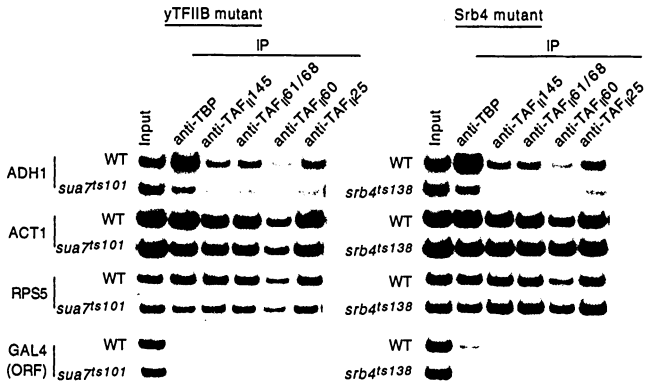

Fig. 4. Requirement of TFIIB and Srb4 for recruitment of TBP and TAFs to TAF_{dep} promoters. TFIIB (*11*) and Srb4 (*18*) ts mutant strains were grown at 23°C and shifted to 37°C for 1 hour; association of TBP and TAFs was analyzed as in Fig. 3.

but had little effect on binding of TBP to the TAF_{dep} promoters of the *ACT1* and *RPS5* genes (Fig. 4).

In summary, we have identified two distinct classes of yeast promoters based on their requirement for TAFs. At TAF_{dep} promoters, TAFs are recruited and are required for delivery of TBP (*19*). Recruitment of TAFs to TAF_{dep} promoters is activator dependent but appears to be relatively independent of other GTFs—surprisingly, even TBP. These data are consistent with the possibility that, at TAF_{dep} promoters, TAFs are directly targeted by activators, which results in recruitment of TAFs and TBP. The notion that TAFs may be direct targets of some activators is consistent with a variety of biochemical studies (*1*).

For TAF_{ind} promoters, TAFs are not required for transcriptional activity or for TBP recruitment (*19*). The same assay that revealed the approximate stoichiometric association of TAFs and TBP with TAF_{dep} promoters showed that the level of TAF association with these promoters is close to background. These results strongly suggest that TBP is recruited to TAF_{ind} promoters in the absence of TAFs, perhaps alone or in a complex with other proteins (*20*). The absence of TAFs on TAF_{ind} promoters in wild-type cells confirms their dispensability for transcription of certain genes, a conclusion independently derived from yeast TAF inactivation studies (*2–6, 21*).

Although recruitment of TBP to TAF_{ind} promoters does not require TAFs, there is a strong dependence on GTFs, such as TFIIB and Srb4. These results are consistent with the possibility that, at TAF_{ind} promoters, the activator targets one of these GTFs, either directly or through another component, which through cooperative interactions ultimately promotes TBP binding and transcription.

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8. The ts mutants of $yTAF_{II}60$ and $yTAF_{II}61/68$ were those used in (*7*). Although these TAFs were reported to be generally required for transcription, our results (Fig. 1) and results of genome-wide expression analysis (*6*) indicate that they are highly selective. Transcription of the *ADH1*, *SED1*, *PGK1*, *GAL1* (+Gal), and *CUP1* ($+Cu^{2+}$) genes also was not affected after inactivation of any of the other known yeast TAFs (data not shown).
9. In brief, the crosslinking procedure is as follows. Live yeast cells were treated with 1% formaldehyde to induce crosslinks and disrupted with glass beads; the resultant extract was sonicated to generate DNA fragments with an average size of about 500 base pairs. Crosslinked complexes were immunoprecipitated with the specified antibodies, and proteinase K was used to release immunoprecipitated DNA from protein A–agarose beads. DNA samples were incubated at 65°C for 6 hours to reverse the crosslinks, purified, and used for quantitative polymerase chain reaction (PCR) analysis. Under these conditions, the crosslinking assay provides a quantitative measure of factor association with the promoter (*11*).
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13. Quantitation of the data in Fig. 2A (using the NIH image 1.62 program after scanning the autoradiogram) indicates that $TAFs_{average}$/TBP ratios were as follows: TAF_{dep} promoters—*RPS5*, 0.94; *RPS30*, 0.91; *RPL25*, 0.89; *ACT1*, 0.80; *SSB1*, 0.86; TAF_{ind} promoters—*GAL1*, 0.11; *CUP1*, 0.16; *ADH1*, 0.14; *SED1*, 0.16; *PGK1*, 0.09.
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19. Inactivation of TAFs substantially decreased binding of TBP to TAF_{dep} but not to TAF_{ind} promoters (data not shown).
20. An alternative possibility, that TAFs are bound but not crosslinked to TAF_{ind} promoters, is unlikely for several reasons. First, the crosslinking procedure has been successfully used to detect the association of a wide variety of factors, some of which bind DNA and others do not, with the promoter. Second, and more importantly, the crosslinking data are consistent with the dispensability of TAFs for TAF_{ind} promoters.
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TAF-Containing and TAF-Independent Forms of Transcriptionally Active TBP in Vivo

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Transcriptional activity in yeast strongly correlates with promoter occupancy by general factors such as TATA binding protein (TBP), TFIIA, and TFIIB, but not with occupancy by TBP-associated factors (TAFs). Thus, TBP exists in at least two transcriptionally active forms in vivo. The TAF-containing form corresponds to the TFIID complex, whereas the form lacking TAFs corresponds to TBP itself or to some other TBP complex. Heat shock treatment altered the relative utilization of these TBP forms, with TFIID being favored. Promoter-specific variations in the association of these distinct forms of TBP may explain why only some yeast genes require TFIID for transcriptional activity in vivo.

Eukaryotic RNA polymerase II (Pol II) requires auxiliary factors to recognize promoters. The primary promoter recognition factor is TFIID, a complex that consists of TBP and about 10 TAFs (*1*). TBP binds TATA elements, which are found in most promoters, and it interacts with general transcription factors TFIIA and TFIIB (*2*). In yeast, TBP is generally required for Pol II transcription (*3*), and the level of TBP occupancy of promoters is correlated with transcriptional activity (*4*, *5*). In the context of TFIID, certain TAFs directly contact initiator or downstream promoter elements (*6*). For this reason, TAFs are

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important for transcription in vitro from promoters lacking TATA elements, although they are dispensable for basal TATA-dependent transcription.

Analysis of the physiological functions of the TAF subunits of TFIID is complicated by the presence of certain TAFs in the SAGA histone acetylase complex (*7*). Many studies suggest that, in the context of TFIID, TAF subunits are not generally required for transcription because depletion or inactivation of individual TFIID-specific TAFs affects only a subset of genes (*8*–*12*). These results suggest that, with respect to functions within TFIID, there are distinct TAF-dependent and TAF-independent promoters, although this view has been challenged (*13*). In contrast to the TFIID-specific TAFs, TAFs that are present in both the TFIID and SAGA complexes are broadly required for transcription (*12*, *14*).

If TFIID is present at both TAF-dependent and TAF-independent promoters, the TAF/TBP occupancy ratio should be constant at all promoters. To investigate this issue, we used chromatin immunoprecipitation to measure promoter occupancy by three TFIID-specific TAFs (TAF130, TAF150, and TAF40); TAF17, which is present in both TFIID and SAGA (*15*); and TBP. This approach permits analysis of TAFs in wild-type cells under physiological conditions.

The TAF/TBP occupancy ratio varies considerably among promoters (Fig. 1). When normalized to levels of TBP occupancy, the TAF-dependent *TRP3* (*8*, *12*) and ribosomal protein gene promoters *RPS8A*, *RPL9A*, and *RPL5* (*9*, *16*) had six to seven times higher levels of TAFs than TAF-independent promoters such as *ADH1*, *PGK*, and *PYK*. TAF occupancy at the *ACT1* and *EFT2* promoters was half that observed on the ribosomal protein gene promoters. When normalized to TBP occupancy levels, the relative levels of all four TAFs tested were similar. Thus, these TAFs, and hence TFIID, were underrepresented at TAF-independent promoters. Similar observations for these and three additional TAFs have been obtained independently (*17*).

Because TBP occupancy is correlated with transcriptional activity (*4*, *5*), the underrepresentation of TAFs at certain promoters suggests that there is a TAF-independent form of transcriptionally active TBP in vivo. To test this, we examined the occupancy of TFIIB and TFIIA at the same promoters (Fig. 2) and found that the TFIIA/TBP occupancy ratios were constant (within an experimental error of ±30%). Thus, associations of TBP, TFIIA, and TFIIB were very strongly correlated with each other, whereas the relationship with TAF association was much more variable. This observation indicates that recruitment of TAFs to promoters does not necessarily coincide with recruitment of TFIIA and TFIIB.

We next examined activator-dependent recruitment of TFIID by monitoring TAF occupancy at promoters whose transcription is induced by heat shock factor or the Msn2 and Msn4 activators (Fig. 3, A to C). In all cases tested, TAF occupancy increased upon heat shock, which indicates that these activators increase recruitment of TFIID. The TAF/TBP occupancy ratios suggest that TFIID is underrepresented at heat shock–inducible promoters (*18*). Occupancy by TAF17 was two times higher than occupancy by the other TAFs, which suggests the possibility of activator-dependent recruitment of the SAGA complex (*19*). Interestingly, heat shock caused a two- to threefold increase in the TAF/TBP occupancy ratio at several promoters whose levels of transcription were unaffected (Fig. 3, D to F). This increased TAF occupancy was not due to more efficient crosslinking at high temperature because it was not observed at the uninducible *TRP3* and *ARF1* promoters, which have inherently high TAF/TBP occupancy ratios (*18*).

Several lines of evidence indicate that our experiments provide quantitative measurements of promoter occupancy and are not influenced by conformational changes in pro-

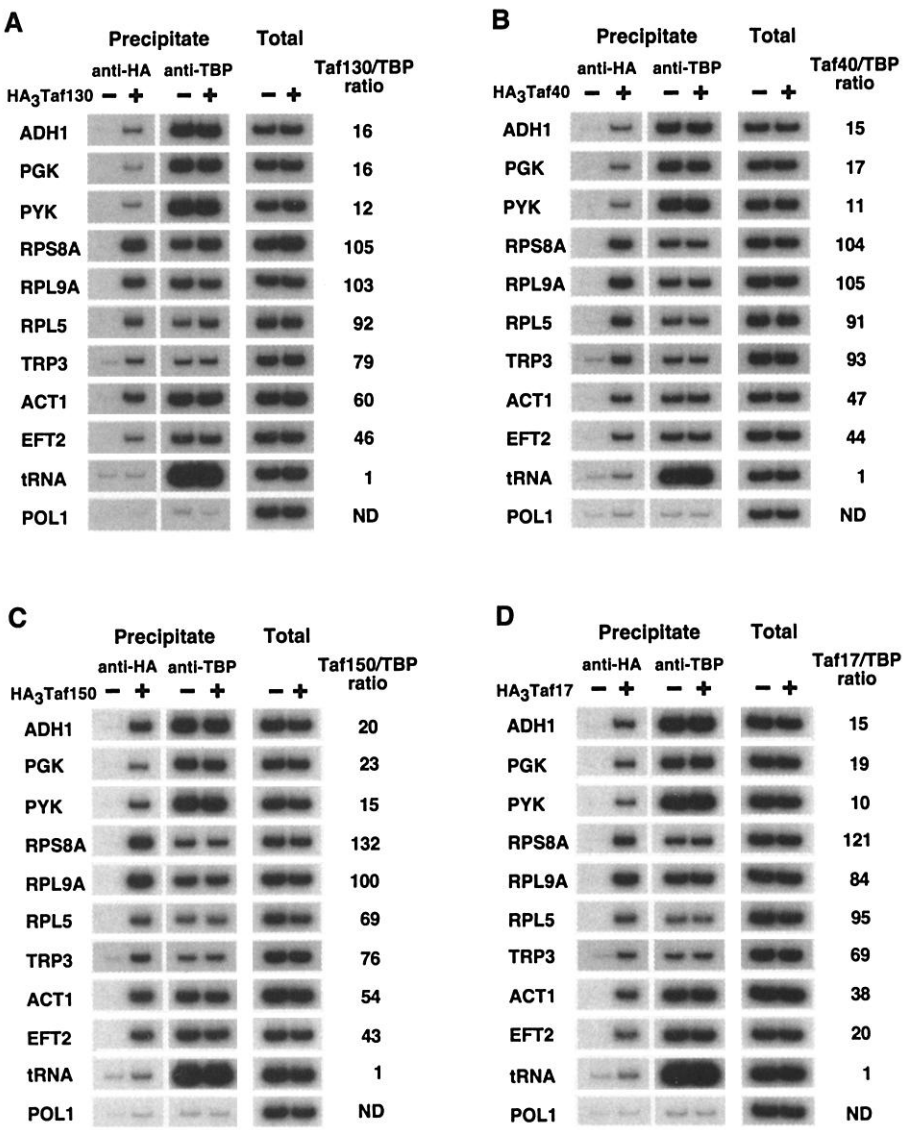

Fig. 1. TAF and TBP occupancy at selected promoters. Crosslinked chromatin preparations from strains containing triple hemagglutinin (HA_3)-tagged or untagged TAF130 (**A**), TAF40 (**B**), TAF150 (**C**), and TAF17 (**D**) were immunoprecipitated with monoclonal antibody to HA or polyclonal antibody to TBP (*4*). Polymerase chain reaction products corresponding to the indicated Pol II promoters, the $tRNA^{Arg}$ (ACG) Pol III promoter, and the *POL1* structural gene were generated from total chromatin or immunoprecipitated DNA. For each promoter, the relative TAF/TBP occupancy ratio is indicated in terms of the percent of the maximal observed ratio, which is arbitrarily defined as 1.0 (*21*).

teins or DNA that affect crosslinking efficiency. First, associations of TBP, TFIIB, and TFIIA with promoters are remarkably well correlated with each other, even though individual promoter sequences are typically unrelated. Second, the relative occupancies of the four TAFs tested are strongly correlated with each other. Thus, any promoter-specific conformational difference that affects TAF crosslinking would have to affect all TAFs in a quantitatively similar manner. Third, the absolute level of crosslinking is comparable for all four TAFs, even though homologs of TAF17, TAF130, and TAF150 contact promoter DNA to various extents (*6*), and there is no evidence that homologs of TAF40 contact DNA. Similarly, chromatin immunoprecipitation has been applied successfully to many proteins that do not directly contact DNA (*20*). Thus, protein-protein crosslinks that occur in the vicinity of the promoter contribute significantly (and perhaps predominantly) to the observed crosslinking to DNA, and it is unlikely that protein-protein interactions within TFIID will change when it is bound to different promoters. For these reasons, the reduced levels of multiple TAFs at certain promoters is almost certainly due to the absence of these TAFs, and hence TFIID, at these promoters.

Our results provide strong evidence for at least two transcriptionally active forms of TBP in vivo (Fig. 4). One form corresponds to TFIID and is defined here by the association of the four TAFs tested with promoters and by a TAF/TBP ratio of 1.0 (*21*). The other form lacks the four TAFs tested (and probably other TAFs present in the TFIID complex) and is defined by promoters whose TAF/TBP occupancy ratio is significantly less than 1. This TAF-independent form might correspond to TBP itself, or it might be another TBP complex. For simplicity, we consider the TAF-independent form to be a single entity, although multiple TAF-independent forms of transcriptionally active TBP are possible. In addition, our results do not exclude the possibility of transcriptionally active forms of TBP containing a subset of TAFs.

TFIID and the TAF-independent form(s) of TBP have distinct promoter selectivities. At one extreme, TFIID is the predominant (and perhaps exclusive) form of TBP at the TAF-dependent promoters. At the other extreme, the TAF-independent form predominates at the TAF-independent promoters, although TFIID may represent 10 to 20% of the transcriptionally active TBP. For the other promoters tested, the relative association of the two forms falls along a continuum between these extremes. The fact that heat shock–inducible promoters have intermediate TAF/TBP occupancy ratios suggests that heat shock factor and the Msn2 and Msn4 activators increase recruitment of both TFIID and the TAF-independent form of TBP.

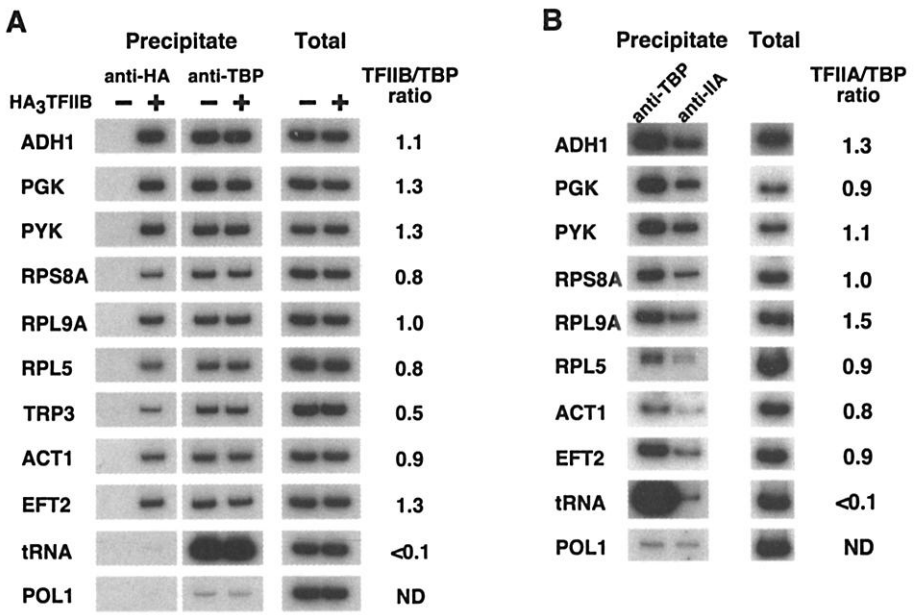

Fig. 2. TFIIB, TFIIA, and TBP occupancy at selected promoters. Crosslinked chromatin preparations from strains containing HA_3-tagged or untagged TFIIB were immunoprecipitated with monoclonal antibody to HA or polyclonal antibody to TBP or TFIIA. Experiments were done as in Fig. 1. For each promoter, the relative TFIIB/TBP (**A**) or TFIIA/TBP (**B**) occupancy ratio is indicated; a value of 1.0 is arbitrarily defined as the average ratio. ND = not determined.

The relative association of TFIID and the TAF-independent form of TBP is also affected by environmental conditions. Specifically, heat shock causes an increased TAF/TBP occupancy ratio at five of seven promoters that are transcriptionally unaffected; the two exceptions, *TRP3* and *ARF1*, have the maximal TAF/TBP occupancy ratio of 1 even under normal growth conditions. The simplest interpretation of these results is that heat shock differentially affects the activity or amount of the two TBP forms, so that the relative utilization of TFIID is increased genome-wide, except for promoters where TFIID already predominates (Fig. 4).

The existence of a TAF-independent form of TBP in wild-type strains provides a simple explanation for the observation that transcription of many genes is unaffected upon destruction of TFIID by depletion of TFIID-specific TAFs (*8*–*12*). The low TAF occupancy at many promoters argues that the broad transcriptional effects reported to occur upon inactivation of the TFIID-specific TAF40 are indirect (*13*). In contrast, TAF-dependent promoters have high levels of TAF (and hence TFIID) occupancy, presumably because the TAF-independent form of TBP is not stably associated and hence not transcriptionally active. Because TFIID-specific TAFs contact DNA in the core promoter region (*6*) and have core-specific functions in vivo (*8*, *10*, *12*), differential occupancy by the distinct forms of TBP may reflect promoter-specific variations in the requirement for TAFs to stabilize TBP association.

The presence of TFIID-specific TAFs at all promoters tested suggests that TFIID contributes to transcription of most, and perhaps all, genes. However, at many promoters, this contribution is small compared with that of the TAF-independent form of TBP. Thus, the broad decrease in transcription observed upon depletion of TAFs present in TFIID and SAGA (*12*, *14*) cannot be explained simply by destruction or inactivation of TFIID. Because SAGA-specific components are not essential for growth (*22*), widespread effects caused by depletion of certain TAFs are likely due to the simultaneous inactivation of TFIID and SAGA (and perhaps other TAF-containing complexes). Interestingly, TAF17, which is present in TFIID and SAGA and is broadly required for transcription, is underrepresented at many promoters to the same extent as the TFIID-specific TAFs. We suspect that, in the context of SAGA, TAF17 associates only transiently with promoters and/or is crosslinked with low efficiency.

The core RNA polymerases from bacteria and eukaryotes do not bind specific sequences. In bacteria, promoter recognition is provided by multiple σ factors that interact with the core RNA polymerase and direct the enzyme to specific classes of promoters. Many eukaryotes have distinct TBP-like proteins that show promoter specificity in vivo (*23*). In contrast to these structurally distinct promoter-recognition factors, yeast TBP exists in at least two distinct forms that differentially associate with promoters in vivo. These two forms may have distinct sequence recognition properties per se, and they may differ with

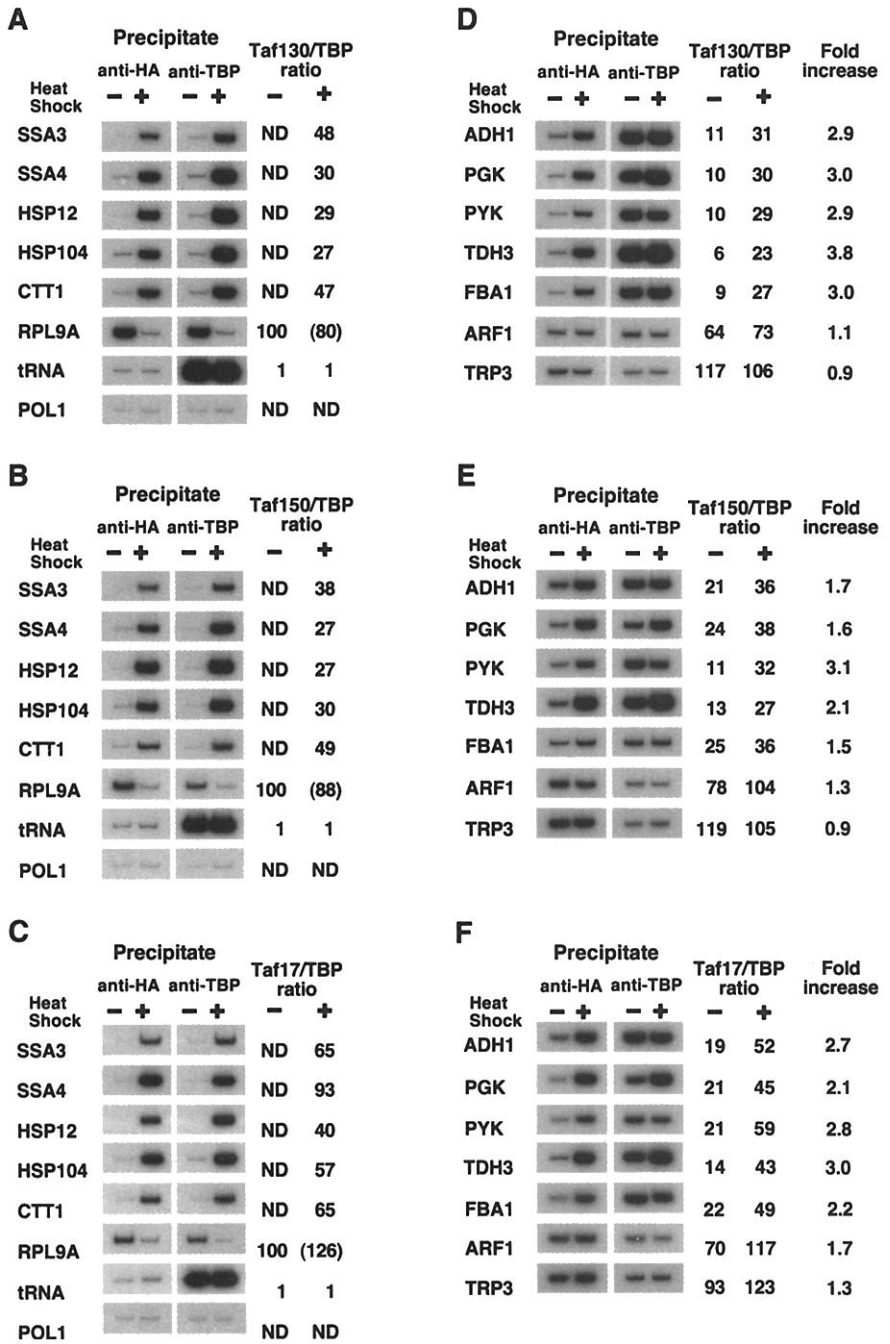

Fig. 3. TAF and TBP occupancy at heat shock–inducible and uninducible promoters in response to a transient heat shock. Crosslinked chromatin preparations from normally growing or heat shocked (15 min at 39°C) cells containing HA_3-tagged or untagged TAF130 (**A** and **D**), TAF150 (**B** and **E**), and TAF17 (**C** and **F**) were immunoprecipitated with monoclonal antibody to HA or polyclonal antibody to TBP. Experiments were performed as in Fig. 1. (A to C) Heat shock–inducible promoters. Values for *RPL9* under heat shock conditions are in parentheses to indicate very low occupancy levels due to transcriptional inhibition (*24*). (D to F) Promoters not inducible by heat shock. The fold increases in the TAF/TBP ratio as a consequence of heat shock are also indicated. ND = not determined.

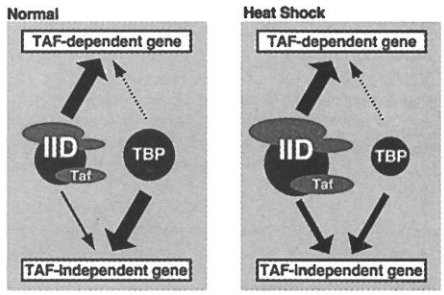

Fig. 4. Distinct forms of transcriptionally active TBP. Two forms of TBP are indicated, a TAF-containing form (presumably TFIID) and a TAF-independent form (depicted as free TBP, but it could contain non-TAF subunits). As indicated by the thickness of the arrows, TFIID is the predominant form at TAF-dependent promoters (e.g., *TRP3* and ribosomal protein gene promoters), whereas the non-TAF form predominates at TAF-independent promoters (e.g., *PGK*, *PYK*, *ADH1*). Heat shock results in preferential utilization of TFIID (free TBP shown by smaller thinner arrows) even at promoters whose transcriptional activity and TBP occupancy are unaffected by heat shock. Heat shock could directly affect the relative activities or levels of TFIID and the TAF-independent form of TBP, or it could affect some other component(s) of the Pol II machinery that indirectly influences utilization of these TBP forms.

respect to their ability to functionally interact with activators, repressors, or other transcriptional regulatory proteins.

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15. We generated isogenic yeast strains in which the genes encoding these TAFs were replaced by epitope-tagged versions containing three copies of the HA-1 epitope and performed chromatin immunoprecipitation and quantitative analysis (*4*). For all four TAFs tested, we observed occupancy of various Pol II promoters in strains containing epitope-tagged TAFs but not in control cells containing untagged TAFs. TAFs were not associated with a tRNA gene promoter, which is transcribed by Pol III, or with a centrally located segment of the *POL1* structural gene. When compared on the *RPL9A* promoter, crosslinking efficiencies of the various TAFs were about the same (0.2% to 0.4%). TBP occupancy on the same samples was determined by immunoprecipitation with TBP antibodies.

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21. Theoretically, all promoters that interact exclusively with TFIID should have the maximal TAF/TBP occupancy ratio. The ratio of 1.0 used here is arbitrarily defined, and it represents the maximal TAF/TBP occupancy ratio observed in a variety of promoters. It seems likely that promoters with a ratio of 1.0 interact exclusively with TFIID, although we cannot exclude the possibility that other promoters have higher TAF/TBP occupancy ratios.
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A Mutation in *PRKAG3* Associated with Excess Glycogen Content in Pig Skeletal Muscle

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A high proportion of purebred Hampshire pigs carries the dominant RN^- mutation, which causes high glycogen content in skeletal muscle. The mutation has beneficial effects on meat content but detrimental effects on processing yield. Here, it is shown that the mutation is a nonconservative substitution (R200Q) in the *PRKAG3* gene, which encodes a muscle-specific isoform of the regulatory γ subunit of adenosine monophosphate–activated protein kinase (AMPK). Loss-of-function mutations in the homologous gene in yeast (*SNF4*) cause defects in glucose metabolism, including glycogen storage. Further analysis of the PRKAG3 signaling pathway may provide insights into muscle physiology as well as the pathogenesis of noninsulin-dependent diabetes mellitus in humans, a metabolic disorder associated with impaired glycogen synthesis.

The presence of a dominant mutation (denoted RN^-) in Hampshire pigs with large effects on meat quality and processing yield was first recognized by segregation analysis of phenotypic data (*1*). Meat from RN^- pigs has a low ultimate pH (measured 24 hours after slaughter), a reduced water-holding capacity, and gives a reduced yield of cured cooked ham (*2*, *3*). These effects are due to a ~ 70% increase in muscle glycogen content in RN^- (RN^-/rn^+ or RN^-/RN^-) animals. No pathological effects of the RN^- mutation have been reported, and it does not cause a glycogen storage disease. Because the RN^- allele has been found only in Hampshire pigs, it is likely that the mutation arose in this breed and has increased in frequency due to its favorable effects on growth rate and meat content in the carcass (*3*). The RN^- allele is of considerable economic significance in the pig breeding industry, and most breeding companies would like to eliminate the mutation because of its negative effects on processing yield.

To identify the RN^- mutation, which resides on pig chromosome 15 (*4*–*6*), we screened a porcine Bacterial Artificial Chromosome (BAC) library (*7*) and constructed a 2.5 megabase pair (Mbp) contig of the *RN* region (Fig. 1C). The BAC clones were used to develop new genetic markers in the form of microsatellites (MS) and single nucleotide polymorphisms (SNPs). The markers were used to construct a high-resolution linkage map based on 1019 informative meioses (*8*) (Fig. 1A). We could exclude *RN* from the region proximal to *SLC11A1* and distal to SNP *S1010*. A porcine radiation hybrid panel was exploited for high-resolution mapping of genetic markers and coding sequences (*9*) (Fig. 1B). The corresponding region on human chromosome 2q and mouse chromosome 1 did not contain any obvious candidate genes for *RN*. Linkage disequilibrium analysis indicated complete association between RN^- and marker alleles at *S1006* and *S1007* (Fig. 1D). These marker alleles most likely define the haplotype in which the RN^- mutation arose. The two markers are present on the overlapping BAC clones 127G6 and 134C9, suggesting that *RN* may reside on the same clone or on one of the neighboring clones.

A shotgun library of the BAC clone 127G6 was constructed and more than 1000 individual sequences were determined and assembled into contigs (*10*). BLAST (*11*) searches of the National Center for Biotechnology Information (NCBI) nucleotide database (*12*) yielded three convincing matches of coding sequences. Two of these were matched to human cDNA sequences or genes (*KIAA0173* and *CYP27A1*) but did not appear to be plausible candidate genes for *RN*. The third coding sequence in BAC 127G6 showed significant sequence similarity to AMP-activated protein kinase (AMPK) γ subunits, including Snf4 in yeast. AMPK has a key role in regulating energy metabolism in eukaryotic cells and is homologous to the SNF1 kinase in yeast (*13*, *14*). AMPK (SNF1) is composed of three subunits (the analogous designations in yeast are given in parentheses): the catalytic α chain (Snf1) and the two regulatory subunits β (Sip1, Sip2, and Gal83) and γ (Snf4). AMPK is activated by an increase in the ratio of AMP to adenosine triphosphate (AMP:ATP). Activated AMPK turns on ATP-producing pathways and inhibits ATP-consuming pathways. AMPK can also inactivate glycogen synthase, the key regulatory enzyme of glycogen synthesis, by phosphorylation (*13*). Several isoforms of the three different AMPK subunits are present in

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