Requirement for Yeast TAF145 Function in Transcriptional Activation of the RPS5 Promoter That Depends on Both Core Promoter Structure and Upstream Activating Sequences

Yoshihiro Tsukihashi, Masashi Kawaichi, and Tetsuro Kokubo‡

From the Division of Gene Function in Animals, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0101, Japan

The general transcription factor TFIID has been shown to be involved in both core promoter recognition and the transcriptional activation of eukaryotic genes. We recently isolated TAF145 (one of TFIID subunits) temperature-sensitive mutants in yeast, in which transcription of the TUB2 gene is impaired at restrictive temperatures due to a defect in core promoter recognition. Here, we show in these mutants that the transcription of the RPS5 gene is impaired, mostly due to a defect in transcriptional activation rather than to a defect in core promoter recognition, although the latter is slightly affected as well. Surprisingly, the RPS5 core promoter can be activated by various activation domains fused to a GAL4 DNA binding domain, but not by the original upstream activating sequence (UAS) of the RPS5 gene. In addition, a heterologous CYC1 core promoter can be activated by RPS5-UAS at normal levels even in these mutants. These observations indicate that a distinct combination of core promoters and activators may exploit alternative activation pathways that vary in their requirement for TAF145 function. In addition, a particular function of TAF145 that is deleted in our mutants appears to be involved in both core promoter recognition and transcriptional activation.

In eukaryotes, transcriptional initiation by RNA polymerase II requires a set of general transcriptional factors (reviewed in Refs. 1–3). These factors are assembled in a stepwise manner to form a preinitiation complex on the core promoter (1) or are recruited as a few preassembled units (4–6). In either case, the first step in preinitiation complex assembly is the binding of a protein complex called TFIID to the core promoter, which in turn provides a structural platform for the remainder of the general transcriptional factors to be incorporated (6). Previous studies have shown that TFIID-promoter interactions are a major rate-limiting step during transcriptional initiation and therefore are one of the most important molecular targets for transcriptional activators (7–9).

TFIID is a multiprotein complex composed of the TATA-binding protein (TBP)1 and ~10–12 phylogenetically conserved TBP-associated factors (TAFs) (reviewed in Refs. 9 and 10). A number of biochemical studies have revealed coactivator and core promoter recognition activities to be two important functions for TAFs (reviewed in Refs. 9–11). Earlier experiments using in vitro transcription systems demonstrated that TBP can mediate basal transcription but is unable to support activated transcription by itself. In contrast, TFIID, even when reconstituted with recombinant TBP and TAFs (12), mediates both basal and activated transcription, supporting the idea that TAFs are essential cofactors for transcriptional activation (reviewed in Refs. 9 and 10). More recent studies have begun to address how core promoters of eukaryotic genes are recognized by TFIID (reviewed in Refs. 13 and 14). The three classes of core promoter elements that are currently known are the TATA element, the initiator, and the downstream promoter element, each of which may serve as a recognition site for distinct TFIID subunits (reviewed in Refs. 13 and 14). In addition to the extensively characterized TBP-TATA element interactions (15), the initiator and downstream promoter element have been shown to be recognized by TAF250-TAF150 and TAF60-TAF40 heterodimers, respectively (13, 16). These TAF-DNA and TBP-DNA interactions are important for the ability of TFIID to bind to the core promoter specifically and to mediate transcription efficiently (reviewed in Refs. 13 and 14). In addition, other cofactors such as TFIIA (17), TAF17, and initiator-dependent cofactors (17), and NC2 (18), appear to modulate the recognition by TFIID of a wide range of core promoter structures.

These principal functions of TAFs have also been evaluated in living cells (reviewed in Refs. 10 and 11). Genetic depletion or inactivation analysis of yeast TAF145, a subunit that is orthologous to human TAF250, revealed that it was not required for transcription generally but was essential for a subset of genes in vivo (reviewed in Ref. 11). Promoter swapping experiments demonstrated that TAF145 function is demanded by the core promoter region rather than by upstream activating sequences (UASs) as examined for the CLN2, RPS5, and TUB2 genes (19, 20). Thus, it appears that TAF145 function is tightly connected to core promoter recognition, in accordance with human TAF250, which directly recognizes an initiator element as described above (13). On the other hand, TAF145 has been shown to be required for other transcriptional activities, such as activation of the ADH2 gene by Adr1 (21) and derepression

1 The abbreviations used are: TBP, TATA-binding protein; TAF, TBP-associated factor; UAS, upstream activating sequence; DBD, DNA binding domain; PCR, polymerase chain reaction; bp, base pair(s).

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† To whom correspondence should be addressed: Division of Molecular and Cellular Biology, Science of Biological Supramolecular Systems, Graduate School of Integrated Science, Yokohama City University, 1-7-29, Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 222-0045, Japan. Tel.: 045-508-7237; Fax: 045-508-7369; E-mail: kokubo@tsurumi.yokohama-cu.ac.jp.

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of RNR genes by DNA damage, that are normally repressed by the Crt1 and Tup1-Ssn6 corepressor complex (22). Consistent with such apparently broad roles in transcription, yeast TAF145 and/or human TAF250 possess multiple activities (e.g. TAF N-terminal domain activity, which inhibits TBP function (23, 24); serine/threonine kinase that autophosphorylates and transphosphorylates TFIIF (25); histone acetyl transferase, which acetylates histones and TFIIE (26, 27); two bromodomains, which bind acetylated histones (28); and a ubiquitin-activating/conjugating activity for histone H1 (29)). Some of these activities have been shown to be required for gene expression in vivo (29–31).

How broadly TAF functions are required for gene expression has been extensively studied in yeast (reviewed in Refs. 11, 32, and 33). Genome-wide expression analysis suggests that any TAFs thus far examined are not universally required for transcription, in contrast to other general transcriptional factors, such as Srb4, Kin28, and the largest subunit of RNA polymerase II, which are required for almost all genes (reviewed in Refs. 11, 32, and 33). More puzzling, mutations in different TAFs affect different sets of genes, ranging from 3% (tum1) to 67% (taf17) of the whole genome (33). It has been proposed that TAFs shared by TFIID and SAGA, another crucial histone acetyl transferase complex regulating a subset of genes distinct from TFIID (34), tend to affect a broader range of genes. This is probably because mutations in the TAFs that they have in common should decrease the activities of TFIID and SAGA simultaneously (reviewed in Ref. 35). However, this supposition has not yet been firmly established, because TFIID-specific TAFs such as TAF40 (36) and TAF48/TSG2 (37, 38) have been shown to be generally required for transcription. The wide range of affected genes (3–67% of the genome) may be partly explained by the allele specificities because, for example, the tighter allele of TAF17 caused much more dramatic loss of transcription than the milder one (39). The recent finding that chick TAF31 can be deleted genetically without affecting the expression of most genes, although it is an ortholog of the TAF (TAF17) that has the most universal function among yeast TAFs, indicates that the requirement for TAFs is evolutionarily divergent (40).

To further clarify how TAF function is involved in gene expression in vivo, we believe it is important to isolate a wide range of conditional taf alleles and to inspect the transcriptional defect at the molecular level in each taf mutant. We recently isolated two novel temperature-sensitive taf145 mutants in which the expression profiles of some genes were not identical to those in previously reported taf145 mutants (20). In our mutants, the core promoter of the TUB2 gene failed to mediate basal transcription, but the function was restored by inserting a consensus TATA element (20). Consistent with this, we show here that the TATA element is important for transcription from the CLN2 and CYC1 promoters. Interestingly, however, the creation of a consensus TATA element cannot restore transcription from the RPS5 promoter. We demonstrate that the RPS5 promoter is mostly impaired in activated transcription and only slightly impaired in basal transcription and that the creation of a consensus TATA element was able to rescue the latter defect. Most importantly, we find that the requirement for TAF145 function in activated transcription of the RPS5 promoter depends on both core promoter structure and UASs. These results imply that a specific function of TAF145 is involved in both core promoter recognition and core promoter and UAS-specific activated transcription.

EXPERIMENTAL PROCEDURES

Yeast Strains and Media—Standard techniques were used for yeast growth and transformation (41, 42). The yeast strains used in this study, YTK3010 (wild type), YTK3002 (Y568Δ), and YTK3005 (T657K) were generated by plasmid shuffle techniques from the parental strain Y22.1 (20). They carry a deletion of the chromosomal TAF145 coding region and the wild type or mutant TAF145 gene on a low copy number vector (20).

Construction of Mini-CLN2 Hybrid Gene Reporters—pM1452 (shown as ∆CLN2-GAAG in Fig. 1) and pM1591 (shown as UASgal-CYC1TATAA–174 in Fig. 1 and UASgal-CYC1I–174 in Fig. 5) were described previously (20). pM1452 and pM1591 were subjected to site-specific mutagenesis (43) to create pM3226 (shown as ∆CLN2GAAG in Fig. 1) and pM3227 (shown as UASgal+CYC1TATAA–174 in Fig. 1) using oligonucleotide primers TK1298 and TK1299, respectively. The oligonucleotides used in this study are listed in Table I. pM3228 (shown as RPS55–593 TAAAT in Fig. 1) was constructed by replacing the 765-bp SphI/XhoI fragment of pM1452 (encompassing the CLN2 promoter) with a 732-bp DNA fragment containing the RPS5 promoter, which was amplified by PCR using the primer pair TK1291 (+SphI)-TK1292 (+XhoI) and genomic DNA as a template. pM3228 was subsequently subjected to site-specific mutagenesis to create pM3229 (TATAAAA), pM3230 (TATAAA), pM3231 (TATATATAA), and pM3232 (TATATATATAMAAA), using the oligonucleotides TK1293, TK1466, TK1467, and TK1468, respectively.

To enable the construction of CLN2-RPS5 hybrid promoters, a SpeI site was created at the −68 bp position of pM1452 by site-specific mutagenesis using the TAF N-terminal domain activity, which inhibits TBP function (23, 24); serine/threonine kinase that autophosphorylates and transphosphorylates TFIIF (25); histone acetyl transferase, which acetylates histones and TFIIE (26, 27); two bromodomains, which bind acetylated histones (28); and a ubiquitin-activating/conjugating activity for histone H1 (29)). Some of these activities have been shown to be required for gene expression in vivo (29–31).

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Transcriptional Activation Defects in taf145 Mutants

RESULTS

The TATA Element Is Important for Transcription from the CLN2 and CYC1 Promoters in taf145 Mutants—We previously demonstrated that transcription of a subset of genes at 37 °C is drastically impaired in the taf145-N568 and T657K mutants but only slightly impaired in the taf145-Y570N mutant, despite the much slower growth phenotypes of all of these mutants at 37 °C (20). Interestingly, the impaired transcription from the TUB2 promoter in the former two mutants can be rescued by creating a consensus TATA element, indicating that the TATA element compensates for the loss of TAF145 function (20). Therefore, we assume that the TATA element will become more crucial to the transcription of a subset of genes once TAF145 is mutated. To examine this possibility further by reciprocal experiments, we tested whether the TATA element is required for transcription from the CLN2 and CYC1 promoters, both of which were not affected in our taf145 mutants (20). To monitor the transcriptional activities of these promoters, we employed the mini-CLN2 hybrid gene reporter constructs detected by the 32P-labeled 411-bp XhoI/HindIII fragment isolated from pM1452.

| No.      | Sequence                                         |
|----------|--------------------------------------------------|
| TK1136   | 5'-CAC ACT AGT CAG ATC GGC CAG GGG TGT-3'         |
| TK1137   | 5'-CAC CTC GAG CCT TAT GTG GGC CAC CCT-3'        |
| TK1289   | 5'-GAT TGC TAT TTT TTT CTC TAT GAA AGA GAT AGT-3'|
| TK1290   | 5'-GAA AAC AAG AGT TTT CTC TAC ATA CAG AGC ACA-3'|
| TK1291   | 5'-CAC GCA TGG CAT TCC ACG AAA ACA CCT-3'        |
| TK1292   | 5'-CAC CTC GAG GSA CTT CTT CTG GAA TGG-3'        |
| TK1293   | 5'-TAA AAA GTA CAA AAT TTT ATA GAA CTA ATG GGC A-3'|
| TK1321   | 5'-CAC ACT AGT ACT TTT TAT CAA TAC TTA-3'        |
| TK1322   | 5'-AAC AGT TAC CAG AGT ACT CTA TTT TTT TAT ATA-3'|
| TK1345   | 5'-CAC GCA TGG ACT TCT TTT ATA TAA-3'            |
| TK1436   | 5'-CAC GCA TGG CCA AGC AGC CTC TGG CTA-3'        |
| TK1466   | 5'-GA TAA AAA GTA CAA ATT TTT ATA GAA CTA ATG GGC A-3'|
| TK1467   | 5'-TAA AAA GTA CAA AAT TTT ATA TA GAA CTA ATG GGC ACA-3'|


| Table I                                                                 |
|------------------------------------------------------------------------|
| No. | Sequence                                                                 |
|-----|--------------------------------------------------------------------------|
| TK1136 | 5'-CAC ACT AGT CAG ATC GGC CAG GGG TGT-3'                                |
| TK1137 | 5'-CAC CTC GAG CCT TAT GTG GGC CAC CCT-3'                                |
| TK1289 | 5'-GAT TGC TAT TTT TTT CTC TAT GAA AGA GAT AGT-3'                        |
| TK1290 | 5'-GAA AAC AAG AGT TTT CTC TAC ATA CAG AGC ACA-3'                        |
| TK1291 | 5'-CAC GCA TGG CAT TCC ACG AAA ACA CCT-3'                                |
| TK1292 | 5'-CAC CTC GAG GSA CTT CTT CTG GAA TGG-3'                                |
| TK1293 | 5'-TAA AAA GTA CAA AAT TTT ATA GAA CTA ATG GGC A-3'                      |
| TK1321 | 5'-CAC ACT AGT ACT TTT TAT CAA TAC TTA-3'                                |
| TK1322 | 5'-AAC AGT TAC CAG AGT ACT CTA TTT TTT TAT ATA-3'                        |
| TK1345 | 5'-CAC GCA TGG ACT TCT TTT ATA TAA-3'                                    |
| TK1436 | 5'-CAC GCA TGG CCA AGC AGC CTC TGG CTA-3'                                |
| TK1466 | 5'-GA TAA AAA GTA CAA ATT TTT ATA GAA CTA ATG GGC A-3'                    |
| TK1467 | 5'-TAA AAA GTA CAA AAT TTT ATA TA GAA CTA ATG GGC ACA-3'                  |

Plasmids Encoding Activation Domains Fused with the GAL4 DNA Binding Domain—The plasmids expressing activators in yeast cells, pM3254 (RPS5–200) and pM3244 (RPS5–87C), respectively. To construct pM3259 (UASgala+CYC1–174 in Fig. 5), pM1588 was created first by replacing the 260-bp SpeI/XhoI fragment of pM1585 (20) with the 344-bp PCR fragment containing the CYC1 promoter amplified by the primer pair TK1734-TK1735 into the SpbI site of pM1585. pM3259 was subsequently created by ligating the 150-bp (–450–300 bp of RPS5–UAS) DNA fragment amplified by the primer pair TK1734-TK1735 into the SphI site of pM1585. pM3259 was created by ligating the DNA fragment containing four repeats of the GAL4 binding site, which was amplified by PCR using the primer pair TK1625-TK1628 and pM2190 as a template, into the SpbI site of pM3244 (RPS5–87C). The PetII fragment of pM3266 containing the entire reporter gene was moved into pRS316 (45) to change the auxotrophic marker from LEU2 to URA3. The resulting plasmid pM3279 is shown as UASgala+RPS5–87C in Fig. 5.

Plasmids Encoding Activation Domains Fused with the GAL4 DNA Binding Domain—The plasmids expressing activators in yeast cells, pM254 (GAL4BD-Vp16C (amino acids 457–490)), pM1570 (GAL4BD-EBNA2 (amino acids 426–462)), pM967 (GAL4BD-GCN4 (amino acids 107–144)), and pM471 (GAL4BD) have been described previously (20). pM3261 (GAL4BD-RAP1 (amino acids 630–690)) was similarly constructed by ligating the DNA fragment encoding the RAP1 activation domain (amino acids 630–690) (46) that was amplified using the primer pair TK1694-TK1695 into the EcoRIPstI sites of pM471.

Northern blot Analyses—Northern blot analyses were performed as described previously (20). All mRNAs derived from mini-CLN2 hybrid gene reporter constructs were detected by the 32P-labeled 411-bp XhoI/HindIII fragment isolated from pM1452.
**FIG. 1.** Promoter-specific TATA dependence in *taf145* mutants. A, schematic representation of the reporter plasmids used in this experiment. The positions of the UAS, TATA element, transcriptional initiation site, open reading frame of the *CLN2* gene, and probe for Northern analysis are shown in the top row. The *ΔCLN2*TATA reporter construct was generated by removing an internal SpeI/NcoI fragment from the intact *CLN2* gene, as shown in the second row. Arrows indicate the initiation site and direction of transcription. Sequences derived from the *CYC1* and *RPS5* genes are indicated by striped and shaded boxes, respectively. Synthetic binding sites for GAL4 fusion activators were linked upstream of the *CYC1* promoter to generate the UAS<sub>Gal</sub><sup>+</sup>*CYC1*TATA/–174 reporter plasmid, although GAL4 fusion activators were not coexpressed in this experiment. TATA elements (open squares) of the *ΔCLN2*TATA and UAS<sub>Gal</sub><sup>+</sup>*CYC1*TATA/–174 reporter plasmids were replaced with GAGA elements (closed squares) by site-specific mutagenesis to generate *ΔCLN2*GAGA and UAS<sub>Gal</sub><sup>+</sup>*CYC1*GAGA/–174 reporter plasmids, respectively. Conversely, the TAAAAT sequence of the RPS5 promoter (RPS5/–593) was replaced with various TATA elements as indicated in the bottom row. B, Northern blot analysis of mRNA with a *CLN2* specific probe to test the requirement for the TATA element for transcription from the *CLN2*, *CYC1*, and RPS5 promoters. Total RNA was isolated from wild type or mutant strains (Y570N, N568<sup>D</sup>, and T657K) harboring the indicated reporter plasmids 2 h after a temperature shift to 37 °C (lanes 5–8) or after being continuously incubated at 25 °C over the same time period (lanes 1–4) and were blotted for hybridization with a radioactive *CLN2* probe. The upper band, marked with a white arrow, corresponds to mRNA derived from the endogenous *CLN2* gene, whereas the lower band, marked with a black arrow, corresponds to mRNA derived from the mini-*CLN2* gene on the reporter plasmid. The middle band, marked with an asterisk in the third (UAS<sub>Gal</sub><sup>+</sup>*CYC1*TATA/–174) and fourth (UAS<sub>Gal</sub><sup>+</sup>*CYC1*GAGA/–174) rows presumably corresponds to transcripts initiated from an unknown promoter on the reporter plasmid under these particular culture conditions.
with a black arrow in Northern blot analyses, as shown in Fig. 1B) and from the endogenous CLN2 gene (Fig. 1B, white arrow). The transcript derived from the former is ∼1 kilobase pair shorter than that derived from the latter because the SpeI-NcoI fragment encoding an essential carboxyl-terminal region of CLN2 was excised to generate the mini-CLN2 gene (Fig. 1A).

We constructed two parental reporter plasmids: ΔCLN2-TATA, which contains upstream sequences up to 332 bp from the transcriptional start site of the CLN2 promoter, and UAS_GAL+CYC1-TATA−174, which contains upstream sequences 174 bp from the transcriptional start site of the CYC1 promoter (Fig. 1A). In our mutants, these reporter plasmids were expressed normally at 37 °C as previously demonstrated (Fig. 1B, top section). The consensus TATA elements of both reporter plasmids were changed to GAGA elements by two-nucleotide substitutions to generate two novel TATA-less reporter plasmids, ΔCLN2_GAGA and UAS_GAL+CYC1_GAGA−174 (Fig. 1A). When these two plasmids were introduced into yeast cells, they produced much lower amounts of transcript at 37 °C in the Y5683 and T6575K mutants (Fig. 1B, top section). Together with the previous reciprocal results showing the stimulatory effect of the TATA element in the TUB2 promoter, this shows that the TBP-TATA element interaction is necessary in order to sustain normal levels of transcription in a broad set of genes when a particular function of TAF145 is lost.

**Impaired Transcription from the RPS5 Promoter Is Partially Restored by Creating a Canonical TATA Element**—Green and co-workers (19, 47, 48) initially isolated taf145 mutants (ts1 and ts2) in which a set of genes is affected that overlaps with, but is distinct from, the set of genes affected in our mutants (20). For instance, the CLN2 gene ceases its expression promptly after being shifted to the restrictive temperature in the ts1 and ts2 mutants (48), whereas it is continuously expressed in our mutants (20). On the other hand, ribosomal protein genes such as RPS5 are shut off in both groups of mutants (19, 20). Intriguingly, the creation of a consensus TATA element was able to restore the transcription from the TUB2 promoter in our mutants (20) but was not able to restore transcription from the RPS5 promoter in the ts2 mutant (19), showing that the TATA requirement is different in these two mutants. Thus, we next wished to test whether a consensus TATA element has a stimulatory effect on transcription from the RPS5 promoter in our mutants. First, we introduced the same two-nucleotide substitution, i.e. TAAAT to TATAA, in an RPS5−593 reporter plasmid (Fig. 1A), as previously tested by Green and Shen (19). Unexpectedly, this substitution increased RPS5 promoter activity in our mutants as well, although only slightly (Fig. 1B, bottom section). These observations suggest that the difference in the TATA requirement between these two mutants depends on the sort of promoter tested but not on allelic differences. Interestingly, in the ts2 mutant, the impaired transcription of the RPS5 promoter was restored by introducing both the TATA-surrounding and upstream sequences of the ADH1 promoter, but not by introducing only the upstream sequences of the ADH1 promoter (19). These observations strongly suggest that the TATA-surrounding sequences (but not the TATA element itself) are important in determining TAF145 dependence. We wondered whether the RPS5 promoter might lack this unknown DNA element(s) surrounding the TATAAA sequence that is required for TAF145 independence and therefore would be impaired in our mutants as well. Inspection of the TATA-surrounding sequences of the CLN2 and CYC1 promoters showing TAF145 independence in our mutants, as described above (Fig. 1B, top section), revealed that they were TATATAAAAA and TATATAAAAAAC, respectively. We next tested five consecutive A residues, such as CLN2−TATA (TATAAAAA), or the reiterated TA sequences found in both CLN2-TATA and CYC1-TATA (TATATAAA), and the combination effect of these two elements (TATATAAAAAAC), for the ability to restore transcription from the RPS5 promoter in our mutants (Fig. 1B, bottom section). We found that these constructs could not rescue the transcription significantly more than when they were changed to the simplest TATAAA element. The failure of the CLN2- or CYC1-type consensus TATA elements to confer TAF145 independence to the RPS5 promoter suggests that an unknown determinant of TAF145 dependence surrounds the TATA element but may not overlap the TATA element itself. More simply, another possibility is that the consensus TATA element may function well when combined with the TUB2, CLN2, and CYC1 initiators but not with the RPS5 initiator, at least in our mutants.

**The Initiator Region of the RPS5 Promoter Supports a Normal Level of Transcription When Combined with the TATA Region of the CLN2 Promoter**—In the ts2 mutant, as described above, a hybrid promoter connecting −600 bp upstream of the ADH1 promoter, including the TATA element, to the initiator region of the RPS5 promoter, was shown to be expressed independently of TAF145 function (19). This indicates that the RPS5 initiator does not require TAF145 function, at least in the ts2 mutant, when combined with an appropriate TATA element and UAS derived from a heterologous promoter. Therefore, we tested analogously whether upstream sequences including the TATA element of the CLN2 promoter could provide a similar TAF145-independent function to the initiator region of the RPS5 promoter in our mutants. The −332- to −68 bp region of the CLN2 promoter amplified by PCR as a Sphi-SpeI fragment was fused to the −73 bp position of the RPS5 promoter, resulting in the hybrid reporter plasmid CLN2(−332)−RPS5 (Fig. 2A). This construct was expressed normally in our mutants (Fig. 2B, top panel), attesting to the fact that the RPS5 initiator is fully functional when combined with the TATA element and UAS of the CLN2 promoter, even in our mutants.

To delineate the region of the CLN2 promoter that is responsible for conferring TAF145 independence to the RPS5 initiator, we successively deleted portions of the CLN2(−332)−RPS5 hybrid promoter from the 5′-terminus (Fig. 2A). Unexpectedly, even CLN2(−82)−RPS5, which retains only 1 bp upstream of the TATA element, was normally expressed in our mutants (Fig. 2B). The consensus TATA element was essential for supporting normal levels of transcription from this hybrid promoter, because CLN2(−80)−RPS5 lacking the TATA element significantly decreased its expression specifically at 37 °C in our mutants (Fig. 2B, bottom panel). Consistent with this, the substitution of the TATA element with a GAGA element in CLN2(−126)−RPS5 decreased transcription by a similar amount (data not shown). It is also noteworthy that the reiterated TA sequence overlapped with TATA element is not a determinant of TAF145 independence (compare CLN2(−84)−RPS5 and CLN2(−82)−RPS5 in Fig. 2B), which is consistent with the results described above (Fig. 1B, bottom section). These observations are in agreement with the importance of a consensus TATA element for transcription from the CLN2 and CYC1 promoters (Fig. 1B, top section) but appear to contradict the slight stimulatory effect of consensus TATA sequences on transcription from the RPS5 promoter (Fig. 1B, bottom section).

**The RPS5 Core Promoter Activity Is Slightly Affected in taf145 Mutants, but Can Be Restored by Creating a Consensus TATA Element**—It is surprising that a very short sequence derived from the CLN2 promoter, i.e. ATATAAAAAATAG flanked by Sphi and SpeI sites, was enough to confer TAF145
Fig. 2. Deletion analysis of CLN2-RPS5 hybrid promoters to delineate the region that confers TAF145 independence on the CLN2 promoter. A, schematic representation of the reporter plasmids used in this experiment. The −68 to +438 bp region of the CLN2 promoter of ΔCLN2TATA (as shown in Fig. 1A) was replaced with the region from −73 to +139 bp of the RPS5 promoter (shaded) to generate the CLN2(−332)-RPS5 reporter plasmid (top row). The region containing the CLN2 promoter was deleted successively from the 5′-end as described. SphI was originally located at the −332 bp position of the CLN2 promoter, whereas SpeI was created by site-specific mutagenesis to enable the fusion of the CLN2 and RPS5 promoters. Both restriction sites were utilized here for the constructions. B, Northern blot analysis of mRNA with a CLN2-specific probe. Wild type or mutant strains carrying the indicated reporter plasmids were cultured at 25 °C or 37 °C. Total RNA isolated from these cultures was analyzed as described in Fig. 1.
independence to the RPS5 initiator (Fig. 2B). Given that creation of several TATA elements could not restore the impaired transcription from the RPS5 promoter (Fig. 1), only a few candidates can be listed as possible determinants of TAF145 independence. First, the CLN2(–82)-RPS5 hybrid promoter has an A residue at just 1 bp upstream of the TATA element, but the original (i.e. TAF145-dependent) RPS5 promoter and its variants have a C residue at the corresponding position. Note that the residue 1 bp upstream of the TATA sequences in CLN2(–84)-RPS5S is A (i.e. ATATATA), whereas the corresponding position of RPS5/–593 variants is C (i.e. CTATATA).

Second, the distance between the TATA element and the transcriptional initiation site of CLN2(–82)-RPS5 is different from that of TAF145-dependent RPS5 promoters. Third, the region just downstream from the TATA element of CLN2(–82)-RPS5 promoter is A-rich (Fig. 2A), whereas the corresponding region of RPS5 promoters is T-rich (Fig. 3A). To determine which these elements are responsible for providing TAF145 independence, we first constructed a parental plasmid, RPS5/–87C, by removing the upstream sequences of the RPS5 promoter but...
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...TATAAA sequence completely restored the partial loss of transcription from the RPS5 promoter (Fig. 2). More surprisingly, the conversion of the TATAAT sequence in RPS5 to the TATAAA sequence restored the partial loss of transcription from the RPS5 promoter (see RPS5(TATAAA/-87C) in Fig. 3B). This stimulatory effect on transcription by creating a consensus TATA element parallels the case of the TUB2 core promoter (20), as well as that of the CLN2(–80)-RPS5 hybrid promoters (compare CLN2(–82)-RPS5 and CLN2(–80)-RPS5 in Fig. 2B). It is again puzzling that the same substitution creating a consensus TATA sequence was not as effective in the longer RPS5 promoters (Fig. 1B, bottom section).

The slightly impaired transcription in our mutants could not be restored by the adjustment of the distance between the TATAAA sequence and the transcriptional start site through the introduction of a Spel site (RPS5/-87C+SpeI), nor by the conversion of a C residue to an A residue at 1 bp upstream of the TATAAA sequence (RPS5/-87A), nor by the combination of these two changes (RPS5/-87A+SpeI) (Fig. 3B), indicating that these elements are not determinants of TAF145 independence. In contrast, the creation of a consensus TATA element (RPS5(TATAAA/-87C, RPS5(TATAAA/-87C+SpeI, RPS5(TATAAA/-87A, and RPS5(TATAAA/-87A+SpeI) in Fig. 3B) always restored the transcription levels, indicating that the TATA element is a major determinant of TAF145 independence as observed for the TUB2, CLN2, and CYC1 promoters. Such an apparent contradiction between the RPS5 core promoter (Fig. 3) and its longer version (Fig. 1) prompted us to reinspect and interpret these results more carefully. We immediately became aware of a difference between the two versions of the promoter, in that the ratios of the lower band (black arrows) to the upper band (white arrows) are much higher in Fig. 1 than in Fig. 3. The upper band, which represents the expression of the endogenous CLN2 gene, should be constant in any of the strains we tested. Therefore, if the intensity of the lower bands was normalized by that of the upper bands, it is evident that the expression of the longer promoters is much higher than that of the core promoters (e.g., compare RPS5/-593 in Fig. 1B with RPS5/-87C in Fig. 3B). These observations strongly suggest that the longer promoter represents the sum of the effects of the basal transcription supported by the core promoter and the activated transcription mediated by UASs located somewhere between the –593 and –87 bp positions of the RPS5 promoter. Only the basal transcription is represented in the core promoter RPS5/-87C. Hence, we are inclined to think that transcriptional activation of the longer promoter is significantly impaired in our mutants, whereas basal transcription of either the longer or the core promoter is only partially affected, leading to much larger transcriptional defects for the longer promoter than for the core promoter. Importantly, only the defect in core promoter recognition appears to be restored by creating a consensus TATA sequence (Fig. 3B). Careful inspection revealed that the creation of various TATA sequences in the longer promoter also appeared to restore basal transcription as they increased the ratio of the lower bands (reporter transcripts) to the upper bands (endogenous CLN2 transcripts) slightly but reproducibly (Fig. 1B, bottom section, lanes 7 and 8).

UAS Function of the RPS5 Promoter Is Significantly Impaired in taf145 Mutants—To verify the assumption described above, we deleted upstream promoter sequences of RPS5/-593 successively to generate a series of truncated promoter constructs, RPS5/-450, RPS5/-300, and RPS5/-200 (Fig. 4A), and compared the promoter activities of these constructs in wild type and taf145 mutants (Fig. 4B). In our mutants, a significant decrease in transcription was observed at 37 °C for RPS5/-593 and RPS5/-450, whereas only a slight decrease was observed for RPS5/-300 and RPS5/-200 (Fig. 4B). These observations indicate that transcriptional activation by the UAS located between –450 and –300 bp is significantly impaired in our mutants. Previous computational analysis predicted that the region from –415 to –403 bp might be the binding site for RAP1 (44), a transcription factor that appears to regulate the expression of many ribosomal protein genes (49). To examine whether RAP1 is responsible for such UAS activity, we constructed RPS5/-593ΔUAS RAP1 by removing the region from –415 to –403 bp from RPS5/-593. The expression profile of RPS5/-593ΔUAS RAP1 was found to be quite similar to that of RPS5/-593 (Fig. 4). Taken together, these observations suggest that the RPS5 promoter may be activated by an unknown transcription factor(s) other than RAP1, which binds to the region between –450 and –300 bp and requires TAF145 function to activate the transcription.

Functional Interactions between UAS and Core Promoters Are Selectively Impaired in taf145 Mutants—Our observations described above indicate that TAF145 function is required not only for core promoter recognition but also for transcriptional activation. However, our previous study concluded that the CYC1 core promoter could mediate the normal level of activated transcription by various activators, such as GAL4, GCN4, EBNA2, and VP16, even in the same taf145 mutants (20). Therefore, we next wished to ask whether the defect in transcriptional activation observed for the RPS5 promoter is confined to a specific UAS or core promoter structure or both. The fragments containing the RPS5-UAS, i.e. –450–361 bp (90 bp) and –450–300 bp (150 bp), were fused with either the RPS5/-87 or the RPS5/-200 core promoter to generate three reporter plasmids, UAS90bp+RPS5/-87, UAS150bp+RPS5/-200, and UAS150bp+RPS5/-87 (Fig. 5A). They showed quite similar expression profiles to that of RPS5/-593 (Fig. 5B), indicating that the sequences intervening between the UAS and the core promoter are not required to reconstitute TAF145-dependent transcriptional activation and that the UAS and the core promoter of the RPS5 promoter can be manipulated independently. We therefore fused UAS150bp to a heterologous CYC1 core promoter to generate the hybrid reporter plasmid UAS150bp+CYC1/-174 (Fig. 5A) and tested for TAF145 dependence (Fig. 5B). Interestingly, UAS150bp can activate the CYC1 core promoter at almost the same levels as the original RPS5 core promoter (Fig. 5B, bottom panel). More importantly, this activation is not affected in our mutants (Fig. 5B), suggesting that the RPS5 core promoter requires TAF145 function for activated transcription by UAS150bp, whereas any activator bound to UAS150bp does not require the same TAF145 function, at least when it stimulates the CYC1 core promoter. Next, we tested reciprocally whether the RPS5 core promoter could be activated by other activators in our mutants. To compare the effects of various activation domains in the same system, we fused GAL4 binding sites to the RPS5 core promoter to generate a reporter plasmid UAS GAL4+RPS5/-87C (Fig. 5A). The effector plasmids, expressing various activation domains connected to the GAL4 DNA binding domain (i.e. GAL4-RAP1, -VP16, -EBNA2, and -GCN4), were introduced into yeast cells together with UAS GAL4+RPS5/-87C to test for TAF145 dependence in transcription (Fig. 5C, bottom section). Surprisingly, all of these activation domains were able to stimulate transcription...
tion, even for the RPS5 core promoter. Although RPS5-UAS150bp (Fig. 5B, bottom section) and GAL4-RAP1 (Fig. 5C, top section) could activate the CYC1 core promoter at comparable levels, only the latter could activate the RPS5 core promoter in our mutants at the nonpermissive temperature (Fig. 5, B and C). These observations indicate that certain combinations of UAS and core promoters are selectively impaired in their functional interactions in our mutants, although each component appears to be normal if combined with a different partner. Thus, we conclude that there are several activation pathways, even for a single activator or a single core promoter, which vary in their requirement for TAF145 function.

**DISCUSSION**

Here, we report that TAF145 function is critically important for activated transcription from the RPS5 promoter. However, earlier studies done by Shen and Green (19) concluded differently, that TAF145 function was required only for the core promoter recognition of the RPS5 gene. They examined in the ts2 mutant whether ADH1-UAS or (dG:dC)_{42} could activate the RPS5 core promoter (–135 to +39), which lacks binding sites for any known yeast activators and therefore cannot mediate efficient transcription by itself. It was suggested that a homopolymorphic sequence such as (dG:dC)_{42} disrupts nucleosome structures so that it can activate a core promoter even in the absence of an activator. Both chimeric promoters, ADH1_UAS-RPS5core and (dG:dC)_{42}-RPS5core, produced much lower amounts of mRNA in the ts2 mutant when the temperature was raised to 37 °C. In addition, reciprocal experiments demonstrated that RPS5-UAS could activate the ADH1 core promoter. These observations allowed them to conclude that TAF145 function is not involved in activating the RPS5 gene. However, we think that the same observations could be interpreted differently. ADH1-UAS and (dG:dC)_{42} may have failed to activate the RPS5 core promoter even though the basal activity of the core promoter was only slightly impaired. If this is the case, then the impaired transcription from the RPS5 promoter in the ts2 mutant might be due to the core promoter-specific activation defect, i.e. RPS5-UAS could activate the ADH1 core promoter but could not activate its own core promoter, as we found in our experiments using different taf145 mutants. To distinguish these two possibilities, it will be necessary to examine the transcriptional activity at 37 °C of an RPS5 core promoter in...
Fig. 5. TAF145 dependence was observed only when the RPS5 UAS was combined with the RPS5 core promoter in taf145 mutants. A, schematic representation of the reporter plasmids used in this experiment. The regions derived from the CLN2, RPS5, and CYC1 genes are shown by open, shaded, and striped boxes, respectively. RPS5-UAS was fused with the RPS5 and CYC1 core promoters in its two forms, i.e. UAS$_{90\text{bp}}$ (~450 to 361 bp) or UAS$_{150\text{bp}}$ (~450 to 300 bp), to generate UAS$_{90\text{bp}}$+RPS5/-87, UAS$_{150\text{bp}}$+RPS5/-200, UAS$_{150\text{bp}}$+RPS5/-87, and UAS$_{150\text{bp}}$+CYC1/-174. Synthetic binding sites for GAL4 fusion activators were linked upstream of the CYC1 and RPS5 core promoters to generate UAS$_{GAL}$+CYC1/-174 and UAS$_{GAL}$+RPS5/-87C, respectively. B and C, Northern blot analysis of mRNA with a CLN2-specific probe. Wild type or mutant strains carrying the indicated reporter plasmids were cultured at 25 or 37°C. Total RNA isolated from these cultures was analyzed as described in Fig. 1. In C, activator expression plasmids (GAL4DBD-RAP1, -VP16C, -EBNA2, and -GCN4, or GAL4DBD alone as a negative control) were introduced into yeast cells with the reporter plasmid as indicated to measure the activation efficiencies.
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the ts2 mutant that is not connected to any UAS. If the original interpretation of Shen and Green (19) is correct, it would be intriguing to know the reason why these two classes of taf145 mutants (ts2 and ours) show distinct phenotypes in transcription from the RPS5 promoter and whether it might be related to the differential effect of these taf145 alleles on CLN2 transcription as described previously (20, 48).

Our results indicate that transcription factors bound to the RPS5-UAS require a particular TAF145 function to activate the RPS5 core promoter but not to activate the CYC1 core promoter. Furthermore, other activators that we tested here do not require the same TAF145 function to activate the RPS5 core promoter. Thus, the functional interaction between the RPS5-UAS and the RPS5 core promoter is impaired selectively in our mutants, implying that a single activator may exploit several alternative activation pathways in response to core promoterstructures. Consistent with this supposition, there have been several observations regarding core promoter-specific activation domains. For instance, GAL4-VP16 activates the TATA and initiator-containing core promoter more strongly than the TATA-less but initiator-containing core promoter, whereas Sp1 activates these two promoters almost comparably (50). USF-specific region, another activation domain that is found in mouse USF2, activates the adenovirus major late minimal promoter, but not the E1b minimal promoter (51). Insertion of initiator rescues USF-specific region function on the E1b minimal promoter, suggesting that the USF-specific region is an initiator-specific activation domain (51). Transcription factors derived from mammalian viruses (e.g. ICP4 and Zta) are also reported to carry core promoter-specific activation domains (52, 53). Although it is still unclear how such specificity between the activator and the core promoter is established, the difference in the requirement of activators for general transcription factors, coactivators, and/or cofactors might be related to this phenomenon. In this respect, it is notable that different classes of mammalian transcription factors, such as retinoid X receptor-retinoic acid receptor, cAMP response element-binding protein, and signal transducer and activator of transcription 1, require different configurations and different enzymatic components of the large histone acetyltransferase coactivator complex, which includes CBP/p300, NCoAs, and PCAF, in order to activate their target promoters properly (54). In yeast, similar activation domain-specific cofactor requirements have been observed. For instance, the VP16 and GAL4 activation domains require Srb4 function for activation, but the Ace1 and Hsf1 activation domains do not (55). Similarly, the GCN4 activation domain requires TAF17 function for activation, but the Hsf1 and Hsp104 activation domains do not (55). Interestingly, the Ace1 and Hsf1 activation domains do show an intricate requirement for TFIIE in CUP1 gene activation (56). Activation of the CUP1 promoter by either Ace1 or Hsf1 requires TFIIE function, but a CUP1-UAS-containing binding site for both activators circumvents the requirement for TFIIE (56). Thus, there might be a certain combinatorial effect of different activators for general transcriptional factor and/or cofactor requirements. Given that the core promoter structures are intimately linked to the activator function in prokaryotes (reviewed in Refs. 57–59), it should be more commonly observed in eukaryotes as well that a single activator takes multiple activation pathways depending on the structure of the core promoter. We believe, as a next step, it will be important to identify an activator or activators that show a core promoter-specific TAF145 requirement in our mutants and to characterize the impaired functional interactions between such an activator and TFID at the molecular level.

Our taf145 mutants showed defects in both core promoter recognition and transcriptional activation. Similarly, in the ts2 mutant, not only core promoter recognition (19) but also activation (21) and derepression (22) were affected on apparently different target genes. It is not easy to determine whether such multiple defects occur on all of the target genes, or whether each target gene has a specific defect. For instance, given that the integrity of core promoter recognition is a prerequisite for efficient activation, it is impossible to examine the effect of taf145 mutation on these two molecular events separately. Our observation that a creation of a TATA element restored the basal transcription from the RPS5 core promoter but not the activated transcription from the RPS5-UAS implies that activated transcription is not simply an enhanced form of basal transcription. In this respect, we propose that these two molecular events, i.e. core promoter recognition and activation, are functionally separate and independent processes, at least in terms of their TAF145 requirements. Consistent with this, the impaired transcription from the core promoter of a major histocompatibility complex class I gene was shown to be partially rescued by the presence of its own UAS or a heterologous SV40 enhancer in mammalian taf250/ccg1 mutant cells (60). This suggests that the same taf250/ccg1 mutation affects these two molecular events differently, in that the core promoter recognition is more severely damaged than the activation step in this particular case. In addition, it should be noted that the activator and core promoter specificities were also observed for mammalian cells; the cyclin D1 core promoter cannot be activated by its own UAS, although it can be activated well by GAL-p65, -p53, and -VP16, as shown by an in vitro transcription assay using the nuclear extract prepared from a taf250/ccg1 mutant (61). Therefore, the function of this particular TAF (TAF145/TAF250), which is involved in both core promoter recognition and activation of at least a subset of genes, must be conserved from yeast to human. It would be of special interest to know the molecular mechanisms of how a specific combination of core promoter and activation domains determines the degree of TAF145/TAF250 requirement in these two organisms.

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Transcriptional Activation Defects in taf145 Mutants
Requirement for Yeast TAF145 Function in Transcriptional Activation of the RPS5 Promoter That Depends on Both Core Promoter Structure and Upstream Activating Sequences

Yoshihiro Tsukihashi, Masashi Kawaichi and Tetsuro Kokubo

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