Srb7p is a physical and physiological target of Tup1p

Anne Gromöller and Norbert Lehming
Max-Delbrück-Laboratorium der Max-Planck-Gesellschaft, Carl-von-Linné-Weg 10, 50829 Köln, Germany
1Corresponding author
e-mail: lehming@mpiz-koeln.mpg.de

The holoenzyme of transcription integrates the positive and negative signals from the promoters of eukaryotic genes. We demonstrate that the essential holoenzyme component Srb7p is a physiologically relevant target of the global repressor Tup1p in Saccharomyces cerevisiae. Tup1p binds Srb7p in vivo and in vitro, and all genes tested that are repressed by Tup1p are derepressed when wild-type Srb7p is replaced by a mutant derivative of Srb7p that is no longer capable of interacting with Tup1p. Therefore, Srb7p is the first holoenzyme component essential for repression by Tup1p for which a physical interaction with Tup1p has been demonstrated. Furthermore, we find that Srb7p also binds Med6p and that this interaction is necessary for full transcriptional activation by different activators. Our finding that Med6p and Tup1p compete for the interaction with Srb7p suggests a model for Tup1p-mediated repression.

Keywords: repression/split-ubiquitin system/Srb7p/transcription/Tup1p

Introduction
The holoenzyme of transcription integrates the positive and negative signals from the promoters of eukaryotic genes (Koleske and Young, 1995). Positive signals emanate from activators bound to enhancers and negative signals from repressors bound to silencers. Both activators and repressors can also influence transcription by modulating the chromatin structure (Struhl, 1999). Activators can be associated with histone acetylases, leading to a chromatin structure favourable for transcription (Petterson and Workman, 2000), and repressors can bind chromatin components (Edmondson et al., 1996; Lehming et al., 1998; Laser et al., 2000) or be associated with histone deacetylases, resulting in a condensed chromatin structure hindering transcription (Ng and Bird, 2000). But both activators and repressors can also influence the holoenzyme of transcription directly. For activators, physiologically relevant interactions with specific components of the holoenzyme have been demonstrated (Lee et al., 1999). Various signals from different activators to different subunits of the holoenzyme are thought to be transmitted to Med6p, a holoenzyme component required for transcriptional activation processes (Han et al., 1999). The Saccharomyces cerevisiae transcriptional activator Gal4p, for example, binds Srb4p, and mutations in Gal4p can be compensated for by mutations in Srb4p (Koh et al., 1998). Srb4p, on the other hand, binds Med6p, and this interaction is necessary for the transmission of the signal to the polymerase. Mutations in Med6 can be suppressed by mutations in Srb4 in an allele-specific manner (Lee and Kim, 1998). For transcriptional repressors, the situation is less clear. Deletions of specific components of the holoenzyme result in derepression (Lee et al., 2000); for example, deletion of Srb10 leads to derepression of MFA2, a gene repressed by α2 with the help of the global repressor complex Sn6p–Tup1p (Wahi and Johnson, 1995). Sn6p is thought to interact directly with various repressor proteins bound to different promoters and to recruit the global repressor Tup1p to those promoters (Keleher et al., 1992). However, a physical association between Tup1p and Srb10p or any other Srb subunit has not yet been reported. Tup1p binds to the holoenzyme component Med3p, but deletion of MED3 does not result in upregulation of any of the genes repressed by Tup1p, demonstrating that Med3p does not mediate repression by Tup1p (Papamichos-Chronakis et al., 2000).

Here we show that the essential holoenzyme component Srb7p is a physiologically relevant target of Tup1p. Tup1p binds Srb7p in vivo and in vitro, and all genes tested that are repressed by Tup1p are derepressed when wild-type Srb7p is replaced by a mutant derivative of Srb7p that is no longer capable of interacting with Tup1p. Furthermore, we find that Srb7p also binds Med6p, and that this interaction is necessary for full transcriptional activation by different activators. Our finding that Med6p and Tup1p compete for the interaction with Srb7p suggests a model for Tup1p-mediated repression.

Results
Tup1p physically interacts with Srb7p in vivo
In order to identify potential targets of Tup1p in the holoenzyme of transcription, we tested 15 of its known components in the split-ubiquitin assay. The split-ubiquitin assay is an alternative two-hybrid system suited to analysing transcription factors (Johnsson and Varshavsky, 1994; Wellhausen and Lehming, 1999; Rojo-Niersbach et al., 2000). One of the variants of the split-ubiquitin system used here is outlined in Figure 1A. Ubiquitin fused to a green fluorescent protein (GFP) reporter with an arginine in position 1 (RGFP) is cleaved off by the ubiquitin-specific proteases (Ubps). Arginine is a destabilizing residue in the N-end rule pathway of protein degradation (Varshavsky, 1996), and therefore RGFP is rapidly degraded. No cleavage occurs if a fusion contains only the C-terminal half of ubiquitin (C_{ub}, Figure 1A, left). However, two interacting proteins, X and Y, can be fused to the N-terminal half of ubiquitin (N_{ub}) and the C_{ub}-RGFP reporter, respectively. If X and Y
form a complex inside the cell, the resulting increase in local concentration leads to the re-association of the two halves of ubiquitin. This native-like ubiquitin is now recognized by the Ubps and cleaved. Subsequently, the RGFP reporter is rapidly degraded by the enzymes of the N-end rule (Figure 1A, right). The following holoenzyme components were tested for interaction with Tup1p in the split-ubiquitin system: Rbp1p, Srb2p, Srb4p, Srb5p, Srb6p, Srb7p, Srb8p, Srb9p, Srb10p, Med6p, Gal11p, Toa1p, Toa2p, Ssu7p and Spt15p. Out of these 15 proteins, only Srb7p interacted with Tup1p in vivo. Figure 1B shows that the expression of an Srb7–Cub–RGFP fusion resulted in localized nuclear staining in the presence of Nub. As a consequence of the interaction between Tup1p and Srb7p, the staining disappeared when Nub was replaced by Nub–Tup1p. Srb7p is essential, and the Srb7–Cub–RGFP fusion was able to complement an Srb7 deletion (data not shown). Apparently, Srb7–Cub–RGFP was able to replace Srb7p in the holoenzyme, making it possible for Nub–Tup1p to interact with Srb7–Cub–RGFP in the context of the holoenzyme. Figure 1B further shows that coexpression of Nub–Tup1p and Srb7–Cub–RGFP resulted in delocalization of the staining when an S. cerevisiae strain deficient for the recognition component of the N-end rule pathway (ΔUBR1) was used. After cleavage, RGFP was no longer fused to the nuclear localized Srb7p. In the absence of a functional N-end rule pathway, RGFP was stable and evenly distributed throughout the cytosol of the cell. For Srb10p, a holoenzyme component necessary for transcriptional repression by Tup1p, no interaction was observed in the split-ubiquitin system (data not shown). The interaction between Srb7p and Tup1p was analysed in more detail with the help of the C ub–RURa3p reporter (Wittke et al., 1999). Here, the RURa3p moiety is subject to rapid degradation upon release from the C ub part of the fusion. Therefore, interaction between the two proteins is indicated by uracil auxotrophy and resistance to the drug 5-fluoroorotic acid (FOA). Cells coexpressing Nub–Tup1p and Srb7–Cub–RURa3p do not grow on plates lacking uracil but on plates containing FOA, confirming the interaction observed with the C ub–RGFP reporter (Figure 2A, compare lines 1 and 2). A deletion analysis of Tup1p revealed that the N-terminal half of the protein contains two non-overlapping Srb7p-interacting regions, located between residues 111 and 221, and between residues 222 and 331, respectively (Figure 2A, compare lines 3–6). The C-terminal half of Tup1p, containing the WD repeats, did not interact with Srb7p (Figure 2A, line 7). The same results were obtained when the fusions were expressed in a strain lacking endogenous Tup1p (data not shown). The Nub part of the fusions contained the haemagglutinin (HA) epitope, which was used to confirm correct protein sizes and equal expression by western analysis (data not shown). Fusions to the red fluorescent protein DsRed1 (Clontech) revealed that all Nub–Tup1p derivatives were located in the nucleus (data not shown). Surprisingly, when we switched the fusions, coexpressing Nub–Srbd7p and Tup1–Cub–RURa3p, the interaction was abolished (Figure 2A, compare lines 10 and 11). A deletion mutant lacking the first seven amino acids of Srb7p (Nub–Srb7Δ7p) also showed no interaction with Tup1p (Figure 2A, line 12). The integrity of the Tup1–Cub–RURa3p fusion was demonstrated in a control experiment, coexpressing Nub–Sn6p and Tup1–Cub–RURa3p (data not shown), and Nub–Srbd7p and Nub–Srb7Δ7p were both able to complement an Srb7 deletion. One possible explanation is that the interaction between Tup1p and Srb7p involves the N-terminus of Srb7p, and that the Nub part of the Nub–Srb7p fusion interferes with this interaction. Indeed, we found that the first 31 amino acids of Srb7p were sufficient for the interaction with Tup1p, as shown in Figure 2A, lines 8 and 9. When we tested the Nub–Tup1p deletion derivatives, we found that both Srb7p-interacting regions in Tup1p interacted independently with the N-terminal 31 amino acids of Srb7p (data not shown).

In order to demonstrate that the observed in vivo interaction between Tup1p and Srb7p was direct, both proteins were expressed in Escherichia coli. Tup1p was fused to a tag containing His 6 and the HA epitope, and Srb7p and its derivatives were fused either N- or C-terminally to glutathione S-transferase (GST). Figure 2B shows that Srb7p and Tup1p interacted in a GST-pulldown assay in vitro, and that the N-terminus of Srb7p was necessary and sufficient for this interaction (compare lanes 2 and 3). The figure further shows that Nub seemed to play an active role in the destruction of the Srb7p–Tup1p interaction. The addition of GST to the N-terminus of Srb7p had only a small effect on the interaction with Tup1p (Figure 2B, compare lanes 3 and 5), and the deletion of the first seven amino acids of Srb7p reduced the interaction with Tup1p ~3-fold (Figure 2B, compare lanes 5 and 6). However, substitution of the first seven amino acids of Srb7p for Nub eliminated the interaction.
(Figure 2B, compare lanes 3 and 4). For Srb10p, no interaction with Tup1p was observed (Figure 2B, lane 7).

**Disrupting the Tup1p–Srb7p interaction causes derepression**

SRB7 is an essential gene and cannot be deleted. However, as mentioned above, Nab–Srb7Δ7p was able to complement an Srb7 deletion. This enabled us to test whether the disruption of the interaction between Srb7p and Tup1p had physiological consequences. We noticed that independent *S. cerevisiae* strains expressing Nab–Srb7Δ7p instead of wild-type Srb7p grew clumpy, as did strains in which Tup1 had been deleted. Consistently, the FLO11 gene was derepressed in both Tup1-deleted and Nab–Srb7Δ7p strains, as revealed by northern blot analysis (Figure 3A, compare lanes 1 and 4 with 5). Deletion of the holoenzyme component Srb10 also resulted in a clumpy growth phenotype and derepression of the FLO11 gene (Figure 3A, lane 2). Re-integration of a PCR fragment containing the entire wild-type Srb7 gene into the disrupted Srb7 locus (Srb7pwt) complemented the clumpy phenotype and the loss of FLO11 repression (Figure 3A, lane 3). We performed mating assays and observed that the mating efficiency of the Nab–Srb7Δ7p MATα strain was decreased, as it was for the isogenic strain deleted for Tup1 (Figure 3B). We tested four other genes known to be derepressed by Tup1p (Trumbly, 1986; Mukai et al., 1991; Zhang et al., 1991; DeRisi et al., 1997) by fusing their promoters to the LacZ gene and integrating these promoter fusions into different strain backgrounds. Figure 4 shows that MFA1, STE2, SUC2 and GAL1, which were derepressed in strains deleted for Tup1 (columns 2), were also derepressed in the Nab–Srb7Δ7p strains (columns 5). The deletion of Srb10 also resulted in derepression, but to a lesser extent (data not shown). Disruption of the Tup1p–Srb7p interaction had a weaker effect than deletion of the entire Tup1 gene. This probably reflected the repressive effect Tup1p has on chromatin (Huang et al., 1997). The Nab–Srb7p strains also showed derepression, but with an intermediate phenotype (columns 4), consistent with the data presented in Figure 2B, where deletion of the first seven amino acids of Srb7p decreased the interaction between Srb7 and Tup1p in vitro. The derepression was readily complemented upon re-integration of wild-type Srb7 into the disrupted Srb7 locus (columns 3). Northern blot analysis with a LacZ probe revealed that the derepression took place at the level of transcription (data not shown).

**Srb7p is a physiological target of Med6p**

When we tested the strains with the integrated GAL1–LacZ fusion in galactose, we noticed that the Nab–Srb7Δ7p
strain was not only defective for transcriptional repression but also for transcriptional activation (Figure 4E, column 5). But whereas the N_{Δhr}–Srb7Δ7p strain showed a 10-fold decrease in transcriptional activation by Gal4p, the N_{Δhr}–Srb7p strain gave results similar to wild type (Figure 4E, column 4). We wanted to know how Srb7p might be involved in transcriptional activation, and so we tested the 14 other holoenzyme components mentioned above for interaction with Srb7p in the split-ubiquitin assay. Only Med6p gave the expected result. Figure 5A shows that Srb7p interacted with Med6p in vivo, as revealed by the FOA resistance of cells coexpressing N_{Δhr}–Med6p and Srb7–C_{Δhr}–Rura3p (Figure 5A, lines 1 and 2). The figure further shows that the N-terminal 31 amino acids of Srb7p were sufficient for the interaction.

Fig. 3. Srb7p is a physiological target of Tup1p. (A) Disruption of the Tup1p–Srb7p interaction leads to derepression of {FLO11}. Total RNA was isolated from JD53ΔTUP1 (ΔTUP1), JD53ΔSRB10 (ΔSRB10), JD53ΔSRB7 + Srb7p (Srb7pwt), JD53ΔSRB7 + N_{Δhr}–Srb7Δ7p (N_{Δhr}–Srb7Δ7p) and JD53 (wt), and the northern blot was probed with probes derived from the {FLO11} and {ACT1} genes. (B) Disruption of the Tup1p–Srb7p interaction leads to a decreased mating efficiency. JD53ΔSRB7 + Srb7p (Srb7pwt) and JD53ΔSRB7 + N_{Δhr}–Srb7Δ7p (N_{Δhr}–Srb7Δ7p), which are prototrophic for lysine, as well as JD53 (wt) and JD53ΔTUP1 (ΔTUP1), which are prototrophic for adenine, were mated with JD52 + HIS3, which is prototrophic for histidine. Serial dilutions of the crosses were spotted onto the indicated plates and only diploids were able to grow on the double selection plates.

Fig. 4. Srb7p affects transcriptional repression and activation. (A) An {MFA1}–LacZ fusion was integrated into the {MFA1} locus of the strains JD53 (MATα; wt), JD53ΔTUP1 (MATα; ΔTUP1), JD53ΔSRB7 + Srb7p (MATα; Srb7pwt), JD53ΔSRB7 + N_{Δhr}–Srb7Δ7p (MATα; N_{Δhr}–Srb7Δ7p), JD53ΔSRB7 + N_{Δhr}–Srb7Δ7p (MATα; N_{Δhr}–Srb7Δ7p) and JD52 (MATα; wt). The cells were grown in liquid culture and β-galactosidase activity was determined. (B) A {STE2}–LacZ fusion was integrated into the {URA3} locus of the same strains as in (A). The cells were grown in liquid culture and β-galactosidase activity was determined. (C) A {SUC2}–LacZ fusion was integrated into the {URA3} locus of the strains described in (A), with the exception of JD52. The cells were grown in liquid culture and β-galactosidase activity was determined. (D) A {GAL1}–LacZ fusion was integrated into the {GAL1} locus of the strains described in (A), with the exception of JD52. The cells were grown on YPAD plates, washed off the plates and β-galactosidase activity was determined. (E) The strains from (D) were grown in liquid medium with 2% galactose, and β-galactosidase activity was determined. All experiments were performed in triplicate and the standard deviation was <20%.
Fig. 5. Med6p interacts with Srb7p in vivo and in vitro. (A) Med6p interacts with Srb7p in vivo. Serial dilutions of cells expressing the depicted fusions were spotted onto control plates selecting for the presence of these fusions and onto plates also containing FOA. Interactions are revealed by growth on the FOA plates. (B) Med6p interacts with Srb7p in vitro. GST-pulldown assays with the depicted proteins were loaded onto an SDS gel and HA-Med6p was detected in a western blot with the help of an α-HA antibody.

(Figure 5A, lines 3 and 4). And whereas N<sub>ab</sub>-Srb7p interacted with Med6p-C<sub>ah</sub>-Rura3p, N<sub>ab</sub>-Srb7Δ7p showed no interaction (Figure 5A, lines 5–7). GST-pulldown assays with proteins expressed in E. coli demonstrated that the interaction between Srb7p and Med6p was direct (Figure 5B, compare lanes 2 and 3), and that an intact N-terminus of Srb7p was needed for the interaction (Figure 5B, compare lanes 3 and 4). The decrease in transcriptional activation by Gal4p that had been seen in Figure 4E was reflected by a GAL phenotype of the N<sub>ab</sub>-Srb7Δ7p strain when the cells were grown on galactose plates in the presence of the respiration inhibitor antimycin A (Figure 6A, line 6). Consistently, the N<sub>ab</sub>-Srb7p strain grew like wild type on galactose even in the presence of antimycin A (Figure 6A, compare lines 2 and 4). The activation of the MFac1 gene in α cells expressing N<sub>ab</sub>-Srb7Δ7p instead of wild-type Srb7p was decreased too, as can be seen in the northern blot presented in Figure 6B (compare lanes 1 and 2 with 3).

**Tup1p and Med6p compete for Srb7p**

Med6p is involved in the transmission of positive signals from activators to the polymerase (Han et al., 1999). We propose that this process involves the interaction with the N-terminus of Srb7p. Apparently, Med6p and Tup1p bind the same part of Srb7p, suggesting a model for how Tup1p could inhibit the holoenzyme directly. Tup1p binds the N-terminus of Srb7p, hindering Med6p transmitting the signal (Figure 7A). In order to test this hypothesis, we performed a competition experiment. Cells coexpressing N<sub>ab</sub>-Med6p and Srb7(1-31)-C<sub>ah</sub>-Rura3p were FOA resistant due to the interaction between Med6p and Srb7p (Figure 7B, lines 5–7). Overexpression of Tup1p resulted in a decreased interaction between Med6p and Srb7p, consistent with our model (Figure 7B, line 8).

Finally, we have observed that overexpression of Tup1p reduced activated levels of GAL1 transcription to 50% (data not shown).

**Discussion**

We have shown that Srb7p is involved in both transcriptional activation and repression. Our experiments suggest that Srb7p is a physiological target of the global repressor Tup1p, and that it also mediates positive signals obtained from Med6p. Tup1p and Med6p compete for binding to Srb7p, and we propose that this is how Tup1p exerts its direct effect on the holoenzyme of transcription.

The deletion analysis presented above revealed that Tup1p contains two independent Srb7p-interacting regions within its N-terminal half, both of them interacting with the N-terminal 31 amino acids of Srb7p. These two Srb7p-interacting regions map to the two non-overlapping transcriptional repression regions of Tup1p described previously (Tzamaris and Struhl, 1994). This is fully consistent with our model that Tup1p works by targeting Srb7p. Tup1p might bind Srb7p and block Med6p binding to Srb7p directly, but a change in conformation of Srb7p or the holoenzyme upon Tup1p binding is plausible too.

A comparison of the available Srb7p sequences from *Drosophila melanogaster* (DmSrb7p), *Homo sapiens* (HsSrb7p), *S. cerevisiae* (ScSrb7p) and *Schizosaccharomyces pombe* (SpSrb7p) revealed that the N-terminal 10 amino acids are extremely well conserved among all species (Figure 8). In this region, human Srb7p is 100%
Fig. 7. Tup1p works by blocking the access of Med6p to Srb7p. (A) A model for Tup1p action. The interaction between Med6p and Srb7p is needed to transmit positive signals emanating from transcriptional activators to the polymerase, and Tup1p interferes with this interaction. (B) Overexpression of Tup1p inhibits interaction between Med6p and Srb7p. Cells expressing Nub or Nub-Med6p together with the Srb7p(1-31)-CubIR3p fusion were transformed with a plasmid expressing Tup1p under the control of the GAL1 promoter, or the empty expression vector. Serial dilutions of the transformants were spotted onto plates containing the depicted medium. The presence of the interaction is revealed by the growth on the FOA plate.

Fig. 8. Sequence alignment of Srb7 proteins from *D. melanogaster* (DmSrb7p), *H. sapiens* (HsSrb7p) and *S. pombe* (SpSrb7p) to Srb7p of *S. cerevisiae* (ScSrb7p). Residues identical to the consensus sequence shown on top are shaded black. The sequences were derived from the DDBJ/EMBL/GenBank and the alignment was made with the program MegAlign.

identical to fly Srb7p and 80% identical to the two yeasts. Also, the first 30 amino acids represent a region with a high degree of homology (50% identity between man and *S. cerevisiae*), whereas the next 30 amino acids display only a low degree of homology (13% identity). The central 30 amino acids are again better conserved (37% identity). We tried to affect the interaction between Tup1p and Srb7p by mutating single amino acids in the N-terminus of Srb7p, but all point mutants tested failed to disrupt the protein interaction and displayed no obvious phenotype. The most likely explanation is that because two different regions of Tup1p interact independently with the N-terminus of Srb7p, single point mutations affecting one interaction are compensated for by the other interaction. Future experiments will involve testing the Srb7p mutants with the two different Tup1p regions separately. Mutations affecting one interaction will be combined with mutations affecting the other interaction and double mutants will be tested for phenotypes. Also, we have tried to see whether there is an interaction between HsSrb7p and HirAp, a human Tup1p homologue. In the split-ubiquitin assay, Tup1p interacted very strongly with itself, probably reflecting the higher order chromatin complexes described previously (Ducker and Simpson, 2000), and HirAp interacted strongly with Tup1p. However, HirAp was not able to complement a *TUP1* deletion, and no interaction between HirAp and HsSrb7p was observed (E. Rojo-Niersbach and N. Lehming, unpublished). This is not surprising, since the homology between Tup1p and HirAp is found in the C-terminal WD repeats and not in the N-terminal halves of the proteins.

Tup1p causes a decreased occupancy of the TATA-binding protein (TBP) at the promoters of Tup1p-repressed genes like *SUC2* and *MPA1* (Kuras and Struhl, 1999). How does the Tup1p–Srb7p interaction cause a repressive condition at Tup1p-repressed promoters if TBP (and presumably the rest of the holoenzyme) is not present? Tup1p causes a complex chromatin structure (Ducker and Simpson, 2000), and we suggest that this structure hinders TBP binding. However, even in the presence of Tup1p, binding of TBP to the Tup1p-repressed promoters can still be detected, and the differences in the occupancies of these promoters by TBP are much smaller than the differences in transcription (Kuras and Struhl, 1999). Apparently, the holoenzyme of transcription still binds to these promoters to some extent despite the complex structure, and transcription is repressed because Tup1p blocks the holoenzyme of transcription. If the block is removed by the use of Srb7p derivatives that Tup1p cannot bind, transcription can take place, but the level of transcription is reduced to ~50%, reflecting the influence of the decreased TBP occupancy. Alternatively, the
interaction between Tup1p and Srb7p might be important to stop transcription at any given moment, but it might not prevent recruitment of subsequent holoenzymes to the promoter. Perhaps the interaction halts transcription initiation long enough to facilitate chromatin remodelling, which would then provide a stable repressed state. That would also explain why neither histone mutations nor SRB mutations give full derepression of Tup1p-regulated genes.

Tup1p causes a complex chromatin structure along the entire repressed gene (Ducker and Simpson, 2000), and this structure cannot easily be reconstructed in vitro. This is presumably the reason why repression by Tup1p-dependent repressors in vitro is ~2% of that observed in vivo (Herschbach et al., 1994). A factor of four has been measured for repression by α2 in vitro, and since the effect we have observed for disrupting the Tup1p–Srb7p interaction is less than that observed for deleting the entire TUP1 gene, we cannot expect more than a factor of two for currently available in vitro systems. Therefore, it is currently not possible to confirm by in vitro transcription the effects of Srb7p on transcriptional repression that we have observed in vivo.

Other members of the holoenzyme of transcription are necessary for transcriptional repression by Tup1p in vivo too. For example, the deletion of SRF10 leads to the derepression of genes repressed by Tup1p (Wahi and Johnson, 1995). Srb10p is a kinase, and its function is needed for Tup1p to repress its target genes (Kuchin and Carlson, 1998). However, we have seen no interaction between Srb10p and Tup1p, either in vivo or in vitro. And Med3p, a holoenzyme component that interacts with Tup1p in vivo and in vitro (Papamichos-Chronakis et al., 2000), is not necessary for transcriptional repression by Tup1p. The deletion of MED3 does not lead to the derepression of any of the genes repressed by Tup1p (Papamichos-Chronakis et al., 2000). MED3 is not essential and the effects of deleting MED3 on transcription are not dramatic (Pinut et al., 1997). Therefore, it is not possible that no derepression was observed because transcription was completely abolished in the MED3 deletion strain. Hence, deleting MED3 reduces transcriptional activation of a GALI-LacZ reporter 5-fold (Pinut et al., 1997), whereas activation of GALI is eliminated in an Srb7ts strain at the restrictive temperature (Gromoller and Lehming, 2000). Thus, if Med3p were a physiological target of Tup1p, the deletion of MED3 should lead to the derepression of Tup1p-repressed genes, as observed for the NAB–Srb7Δp7 strain. Interestingly, overexpression of Med3p causes flocculence and derepression of FLO11 (Papamichos-Chronakis et al., 2000). Therefore, even though Med3p does not mediate repression by Tup1p, the interaction between Tup1p and Med3p is biologically relevant, and Med3p might be involved in counteracting Tup1p, similar to the role we are suggesting for Med6p here. Sfl1p, a Tup1p-dependent repressor that binds and represses the SUC2 promoter, co-immunoprecipitates with holoenzyme components like Srb9p (Song and Carlson, 1998). However, neither an involvement of Tup1p in the co-immunoprecipitation nor a direct protein interaction between Sfl1p, Tup1p and Srb9p has been demonstrated, and we have not seen an interaction between Tup1p and Srb9p in our assays. We conclude that Srb7p is the first holoenzyme component necessary for Tup1p-dependent repression for which a physical interaction with Tup1p has been demonstrated.

Materials and methods

Parental S. cerevisiae strains used were JD52 and JD53 (Dohmen et al., 1995). The split-ubiquitin system has been described previously (Wittke et al., 1999). The Cα-RGFP reporter was constructed by replacing the UR43 open reading frame of the Cα-RuRa3p reporter by GFP with the help of the oligonucleotides GCCAGGCTCATGAGTAAGG-AGAAGAACT and GCCATGATTGTTATGATCTACCA. The respective genes were cloned as PCR fragments into the NcoI and EcoRI fusion vectors and the constructs were sequenced by the AID Services unit of the MPIZ. GST-pull-down experiments were performed as described previously (Weißhausen and Lehming, 1999). Expression vectors used were GEX5X1 (Pharmacia) and pET11a (Invitrogen). The α-HA antibody used for the detection of bound proteins in western blots was obtained from Babco. Srb7p was deleted with the help of the LIS2 marker from JD53 in the presence of the URA3-marked vector YCP535 (Gietz and Sugino, 1988) containing the entire Srb7p gene, and TUP1 and Srb10 were deleted from JD53 with the help of the previously deleted ADE2 marker. Srb7p was also deleted in the unrelated strain NLY2 (Saha et al., 1993), with the same results as presented here. Northern blots were performed following standard procedures (Ausabel et al., 1998). For the mating assay, the HIS3 gene of the JD52 strain was repaired and diploids were selected on plates lacking histidine and lysine (for the Δsrb7 strains) or on plates lacking histidine and adenine (for the Δtup1 and wild-type strains). β-galactosidase assays were performed as described previously (Ausabel et al., 1998). The strains containing the GALI-LacZ fusion were assayed after the cells were grown on YPDA plates instead of in liquid glucose medium, because under these conditions derepression by the deletion of TUP1 was more pronounced.

The following oligonucleotides were used for cloning of fusions containing Srb7: 5′-GCCGAATTCATGACGAGATGATACA-CAAT-3′, 5′-GCCGCTGACATTGTCGTCTTGGTTT-3′, 5′-GGCGGCTGACCATGCTTCTTAATGTTG-3′ and 5′-GCCGCTGACATTGTCCTTGGTTT-3′. 5′-GCCGACATCCCACTAGCTACGGTTG-G3′ and 5′-GCGGTCGAACATGTAGTGGTTGATGC-3′; Tup1p: 5′-GGCGGCGACATGTAGTGGTTGATGC-3′ and 5′-GCCGCGACATGTAGTGGTTGATGC-3′; Med3p: 5′-GCCGACATCCCACTAGCTACGGTTG-G3′ and 5′-GCGGTCGAACATGTAGTGGTTGATGC-3′; FLO11: 5′-GCCGCGACATCCCACTAGCTACGGTTG-G3′ and 5′-GCGGTCGAACATGTAGTGGTTGATGC-3′; WC2: 5′-GCCGCGACATCCCACTAGCTACGGTTG-G3′ and 5′-GCGGTCGAACATGTAGTGGTTGATGC-3′; STE2: 5′-GCCGCGACATCCCACTAGCTACGGTTG-G3′ and 5′-GCGGTCGAACATGTAGTGGTTGATGC-3′; WC2: 5′-GCCGCGACATCCCACTAGCTACGGTTG-G3′ and 5′-GCGGTCGAACATGTAGTGGTTGATGC-3′; WC2: 5′-GCCGCGACATCCCACTAGCTACGGTTG-G3′ and 5′-GCGGTCGAACATGTAGTGGTTGATGC-3′; WC2: 5′-GCCGCGACATCCCACTAGCTACGGTTG-G3′ and 5′-GCGGTCGAACATGTAGTGGTTGATGC-3′.

Acknowledgements

We thank Stephanie Heck for excellent technical help, Nils Johnson for providing helpful suggestions and reagents, and Benno Müller-Hill for critical comments on the manuscript. This work was supported by grants from the Bundesministerium für Forschung und Bildung and the Max-Planck-Gesellschaft to N.L.

References


of the repressed yeast a cell-specific gene STE6 contains two molecules of the corepressor Tup1p per nucleosome. EMBO J., 19, 400–409.


