FEBS Letters 494 (2001) 64–68 FEBS 24746

Why Ppr1p is a weak activator of transcription

Andreas J. Pätzold, Norbert Lehming*

Max-Delbrück-Laboratorium in der Max-Planck-Gesellschaft, Carl-von-Linné-Weg 10, 50829 Cologne, Germany

Received 14 February 2001; accepted 11 March 2001

First published online 23 March 2001

Edited by Horst Feldmann

Abstract Upon uracil depletion, the transcriptional activator Ppr1p stimulates expression of the *Saccharomyces cerevisiae URA3* gene only four-fold. We performed a split-ubiquitin screen with Tup1p as bait, and we found that the global repressor Tup1p interacts with the transcriptional activator Ppr1p both in vivo and in vitro. The interaction is biologically significant, since the deletion of the *TUP1* gene as well as the removal of the Tup1p-binding domain from Ppr1p results in an increased expression of the *URA3* gene. Our results suggest that Tup1p blocks Ppr1p directly, and that Ppr1p is a weak activator of transcription because of its interaction with Tup1p. Thus we were able to demonstrate that the global repressor Tup1p can modulate transcription by interacting with an activator.

Key words: Ppr1p; Split-ubiquitin; Transcription; Tup1p; URA3; *Saccharomyces cerevisiae*

1. Introduction

Tup1p is a general repressor of RNA polymerase II transcription that is directed to its target promoters by specific DNA-binding proteins such as α2p, Mig1p, or Rox1p [1]. Thus Tup1p is involved in the repression of the mating type-specific genes, the glucose-repressed genes, and the anaerobic genes, amongst others. Tup1p contains seven WD repeats [2], and a direct protein interaction between the C-terminal WD repeat and α2p has been demonstrated [3]. Tup1p binds to the N-terminal tails of the histones H3 and H4 [4], and the deletion of these tails results in the derepression of some, but not all, Tup1p-repressed genes [5–7]. Ssn6p, which is known to form a complex with Tup1p [8], interacts with the histone deacetylases Rpd3p and Hos2p, and the triple deletion of Rpd3p, Hos1p, and Hos2p leads to the derepression of the Tup1p-repressed genes *MFA2* and *SUC2* [9]. Therefore, Tup1p could function by forcing its target genes into a chromatin structure unfavorable for transcription. However, Tup1p also interacts with the holoenzyme component Srb7p. Srb7p is an essential gene involved in both transcriptional activation and repression [10], and the disruption of the Tup1p–Srb7p interaction leads to the derepression of Tup1p-repressed genes as well [11]. Taken together, these observations suggest that Tup1p can function by influencing both the holoenzyme of transcription and the chromatin structure of the target gene.

The *Saccharomyces cerevisiae URA3* gene encodes orotidine-5′-phosphate decarboxylase (Ura3p), an enzyme required for uracil biosynthesis. The *URA3* gene contains six positioned nucleosomes flanked by nuclease-sensitive regions at the 5′ and 3′ ends [12]. The *ura3-52* allele, which is commonly used in laboratory strains, contains a Ty insertion in the coding region of the *URA3* gene [13]. The transcriptional activator of the *URA3* gene is Ppr1p. The N-terminal 123 amino acids of Ppr1p contain a Zn_2Cys_6 binuclear cluster domain responsible for binding specifically to a single DNA-binding site in the *URA3* promoter [14]. The activation domain is located within the C-terminal 134 amino acids [15]. Ppr1p (904 aa) is homologous to Gal4p (881 aa), but whereas Gal4 activates transcription more than 1000-fold from a single site [16], Ppr1p activates only four-fold from its site in the *URA3* promoter [17].

The split-ubiquitin assay is an alternative yeast two-hybrid assay whose readout is not based upon transcription but upon proteolytic stability [18]. The split-ubiquitin assay is therefore suited for the analysis of transcription factors [19,20]. A fusion containing ubiquitin and Ura3p with an arginine in position 1 (RUra3p) is cleaved by the ubiquitin-specific proteases (UBPs). The free RUra3p is degraded so rapidly by the enzymes of the N-end rule [21] that the cells are uracil-auxotrophic and resistant to the drug 5-fluoro-orotic acid (FOA; Fig. 1A, line 1). If only the C-terminal half of ubiquitin (C_{ub}) is used in a fusion, no cleavage is observed (Fig. 1A, line 2). The RUra3p part of the fusion is enzymatically active, and cells expressing the fusion are phenotypically uracil-prototrophic and FOA-sensitive. If the N-terminal half of ubiquitin (N_{ub}) and C_{ub} are fused to two interacting proteins X and Y respectively, the local concentration of N_{ub} and C_{ub} is raised, and a native-like ubiquitin is formed. The native-like ubiquitin is recognized by the UBPs and cleaved. Subsequently, the free RUra3p is rapidly degraded by the enzymes of the N-end rule (Fig. 1A, line 3). The cells have thus become uracil-auxotrophic and FOA-resistant as a consequence of the protein interaction between X and Y inside the cell.

A library of genomic *S. cerevisiae* DNA fragments has been fused to N_{ub} and screened for proteins binding to the general transcriptional repressor Tup1p in vivo [22]. Here we report that the transcriptional activator Ppr1p binds the transcriptional repressor Tup1p. The interaction between Tup1p and Ppr1p was confirmed in vivo with the help of a Tup1-C_{ub}-RGFP reporter and in vitro with purified proteins. The interaction is biologically relevant, as both the deletion of the *TUP1* gene and the removal of the Tup1p-interacting domain

*Corresponding author. Fax: (49)-221-5062 613.
E-mail: lehming@mpiz-koeln.mpg.de

Abbreviations: C_{ub}, C-terminal half of ubiquitin; FOA, 5-fluoro-orotic acid; GFP, green fluorescent protein; HA, hemagglutinin; N_{ub}, N-terminal half of ubiquitin; UBPs, ubiquitin-specific proteases

PII: S0014-5793(01)02312-2

from Ppr1p raised the level of the *URA3* transcript. Our results suggest that Ppr1p is a weak activator of transcription because Tup1p blocks its activation function.

2. Materials and methods

2.1. Strains and plasmids

The *S. cerevisiae* strains used are JD52 and JD53 [23]. For the experiments presented in Fig. 4, the wild-type *URA3* locus was reconstructed by homologous recombination of a functional *URA3* gene into the *URA3* locus carrying the *ura3-52* Ty insertion. The reconstruction was confirmed by Southern blotting. The *TUP1* deletion strains have been described [22]. The *PPR1* deletion strain was generated by deleting the entire open reading frame (ORF) with a *HIS3*-based knockout vector containing 500 bp from promoter and terminator. Genomic DNA was isolated of all strains, and the deletions of the respective genes were verified by PCR and Southern blotting. The *Escherichia coli* strain used for protein purifications was BL21(DE3)-LysS (Stratagene). The single-copy N_{ub} and C_{ub} fusion vectors have been described [22]. The GST-Ppr1p fusions were made by cloning the ORFs into GEX-5X-1 (Pharmacia). H_6hemagglutinin (HA)-Tup1p was made by cloning a PCR fragment containing the *TUP1* ORF, six histidines and an HA tag into pET11a (Novagen).

2.2. The split-ubiquitin screen

The split-ubiquitin screen with Tup1p as bait has been described [22]. One of the clones isolated contained a *Sau*3A fragment fusing amino acid residues 97–208 of Ppr1p in frame to N_{ub}.

2.3. In vitro binding assays

The GST fusion proteins were purified according the protocol of the manufacturer (Pharmacia, Freiburg). The H_6HA-Tup1 protein was loaded onto a Ni-column (Pharmacia, Freiburg) and eluted with stepwise increasing concentrations of imidazole. The peak fraction appeared at 250 mM imidazole. Purified H_6HA-Tup1p was incubated together with the glutathione matrix containing the purified GSTp (fusions) in phosphate-buffered saline. After washing, the pellets were resuspended in SDS sample buffer, loaded onto an 8% protein gel and analyzed by Western blot.

2.4. Western blots

Western blot analysis was performed according to [24]. Proteins were detected with the anti-HA or anti-GST antibodies from Babco (Berkeley, CA, USA). The secondary antibody (Bio-Rad, Hercules, CA, USA) was visualized using the ECL™ Western Blotting-Detection kit (Amersham, Freiburg) following the manufacturer's protocol.

2.5. Northern blots

Northern blots were performed as described [22].

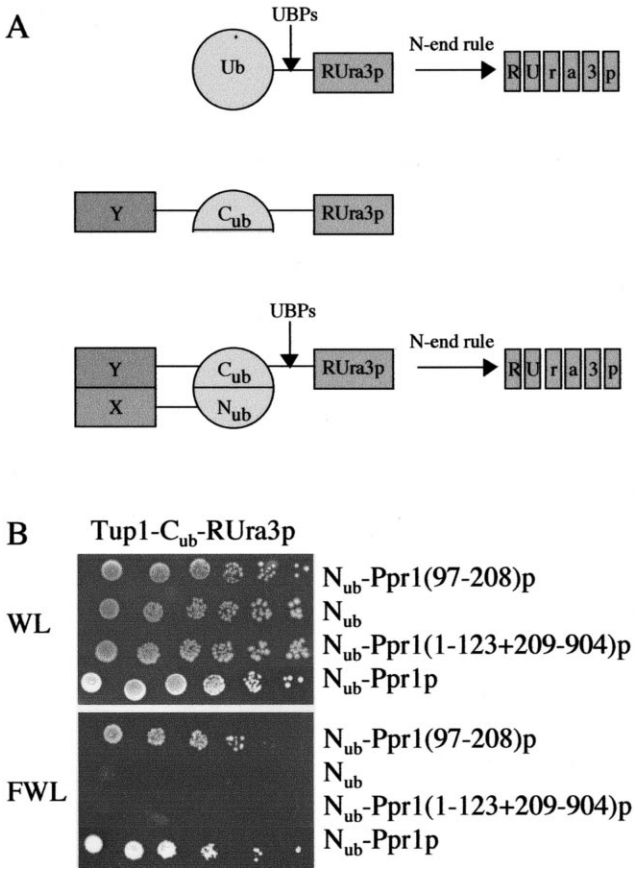

Fig. 1. A split-ubiquitin screen revealed that Tup1p interacts with Ppr1p in vivo. A: The split-ubiquitin system. A fusion protein containing ubiquitin fused to Ura3p with an arginine as first amino acid (RUra3p) is cleaved by the UBPs. The free RUra3p is rapidly degraded by the enzymes of the N-end rule (line 1). If only C_{ub} is fused between a protein Y and RUra3p, no cleavage is observed. Cells expressing this fusion are uracil-prototrophic and FOA-sensitive (line 2). A protein X is fused to the N-terminal half of ubiquitin. If X interacts with Y, a ubiquitin-like molecule is formed and the fusion is cleaved by the UBPs. The free RUra3p is rapidly degraded by the enzymes of the N-end rule, resulting in uracil auxotrophy and FOA resistance (line 3). B: Tup1p interacted with Ppr1p in vivo. Ten-fold serial dilutions of cells coexpressing the depicted fusions were grown on plates lacking tryptophan and leucine (WL) and on plates containing additionally FOA (FWL). Interaction was revealed by the growth on the FWL plate.

3. Results

We have performed a split-ubiquitin screen with Tup1-C_{ub}-RUra3p as bait [22]. One of the clones isolated by its ability to confer FOA resistance to an *S. cerevisiae* strain expressing Tup1-C_{ub}-RUra3p contained N_{ub} fused in frame to residues 97–208 of Ppr1p (Fig. 1B, compare lines 1 and 2). We fused N_{ub} to full-length Ppr1p and found that full-length Ppr1p also interacted with Tup1p (line 4). Deleting residues 124–208 of Ppr1p eliminated the interaction (line 3), even though N_{ub}-Ppr1(1–123+209–904)p was expressed at levels comparable to N_{ub}-Ppr1p (Fig. 2A). We had used a Ura3p fusion as reporter, and we had isolated Ppr1p, the activator of the *URA3* gene. Tup1-C_{ub}-RUra3p was not expressed from the *URA3* promoter but from the *CUP1* promoter. However, to exclude that Ppr1p influenced the growth on the FOA plates by a mechanism not based on the interaction between Ppr1p and Tup1p, we confirmed the interaction with the help of a green fluorescent protein (GFP)-based reporter. We found that cells expressing Tup1-C_{ub}-RGFP displayed green nuclear fluorescence in the presence of N_{ub} (Fig. 2B, left). Coexpression with N_{ub}-Ppr1p led to the disappearance of the fluorescence, thereby reflecting the interaction between N_{ub}-Ppr1p and Tup1-C_{ub}-RGFP in the nucleus of the cell, the cleavage of the Tup1-C_{ub}-RGFP fusion by the UBPs, and the subsequent degradation of the RGFP moiety by the enzymes of the N-end rule (Fig. 2B, middle). Again, deleting the Tup1p-interacting domain from Ppr1p eliminated the interaction and cells coexpressing N_{ub}-Ppr1(1–123+209–904)p and Tup1-C_{ub}-RGFP showed strong nuclear green fluorescence (Fig. 2B, right). The in vivo interaction between Ppr1p and Tup1p was therefore confirmed by an assay not based upon Ura3p.

The split-ubiquitin assay detects close proximity of two proteins inside the cell, but not necessarily a direct protein contact. In order to find out if Ppr1p and Tup1p interacted directly, we purified both proteins from *E. coli*. Tup1p was

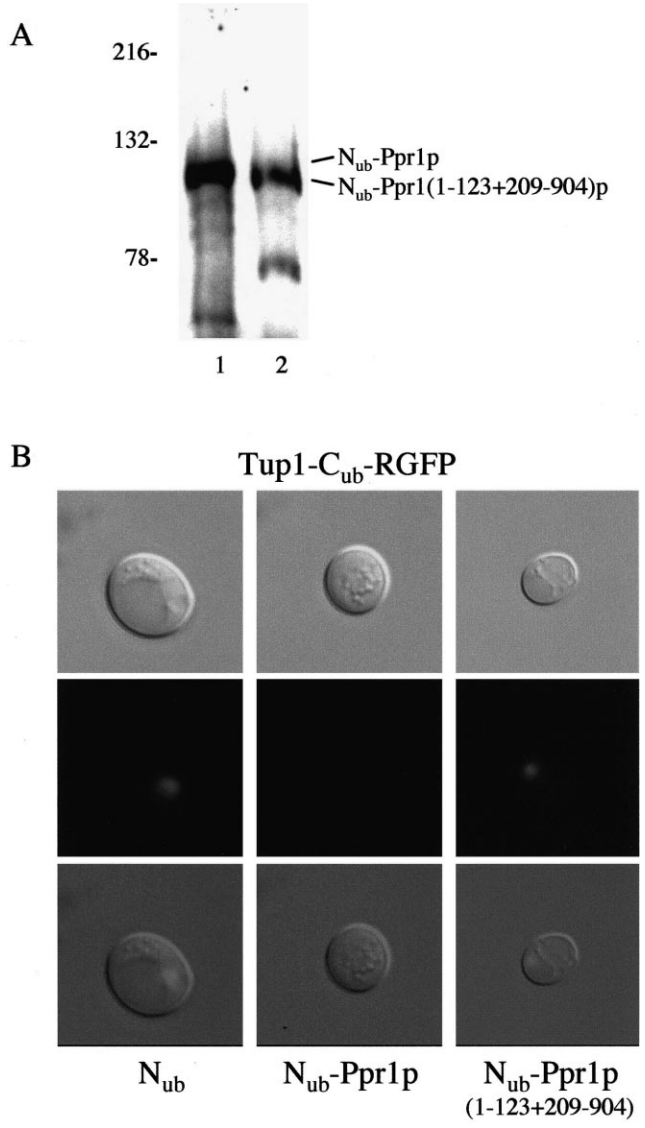

Fig. 2. N_{ub}-Ppr1p, but not a derivative lacking the interaction domain, interacted with Tup1-C_{ub}-RGFP in the nucleus of the cell. A: N_{ub}-Ppr1p (109 kDa, lane 1) and N_{ub}-Ppr1(1–123+209–904)p (100 kDa, lane 2) were expressed at comparable levels. Yeast cells were suspended in loading buffer and proteins were separated on an 8% SDS gel. After blotting onto nitrocellulose, the Ppr1p derivatives were detected by an HA tag present in the fusions. B: A GFP-based reporter for the split-ubiquitin system confirmed the interaction between Ppr1p and Tup1p. Cells expressing the depicted fusions were analyzed under a Leitz fluorescence microscope. Phase contrast (top line), fluorescence (middle line), and merged pictures (bottom line) are shown.

expressed as a fusion to six histidines and an HA tag, and Ppr1p and Ppr1(1–123+209–904)p were expressed as GSTp fusions. H_6HA-Tup1p was purified via a Ni-column, and GSTp, GST-Ppr1p, and GST-Ppr1(1–123+209–904)p were bound to glutathione beads (Fig. 3A). Fig. 3B shows that GST-Ppr1p, but not GST-Ppr1(1–123+209–904)p or GSTp alone, was able to bind and retain H_6HA-Tup1p on the matrix. Since both interacting proteins were purified from a heterologous system, a direct interaction between Ppr1p and Tup1p can be deduced. A degradation product of H_6HA-Tup1p consisting of approximately the N-terminal 300 residues was retained as well, suggesting that Ppr1p interacts with the N-terminal half of Tup1p. Our finding that the deletion of the Tup1p-interacting domain from Ppr1p eliminated the interaction both in vivo and in vitro showed that the interaction observed in vitro was specific and that it truly reflected what occurred in vivo.

Fig. 4A shows that the deletion of *TUP1* from the chromosome of *S. cerevisiae* increased transcription of the *URA3* gene. Consistent with previous reports [17], we found that the depletion of uracil from the medium upregulated the *URA3* mRNA four-fold only (Fig. 4A, compare lanes 1 and 3). However, deleting *TUP1* from this strain resulted in a 14-fold increase in the expression of the *URA3* gene in medium containing uracil (compare lanes 3 and 4 or lanes 5 and 6) and a seven-fold increase in medium lacking uracil (compare lanes 1 and 2). We deleted *PPR1* from the chromosome and complemented the deletion by expressing N_{ub}-Ppr1p or N_{ub}-Ppr1(1–123+209–904)p. Fig. 4B shows that the Ppr1p derivative lacking the Tup1p-interacting domain activated transcription three times stronger than the one containing the domain. Thus we conclude that the transcriptional activator Ppr1p was weakened by its interaction with Tup1p.

The N_{ub}-Ppr1p derivatives were expressed from the strong *ADH1* promoter. Also, the constructs did not contain the

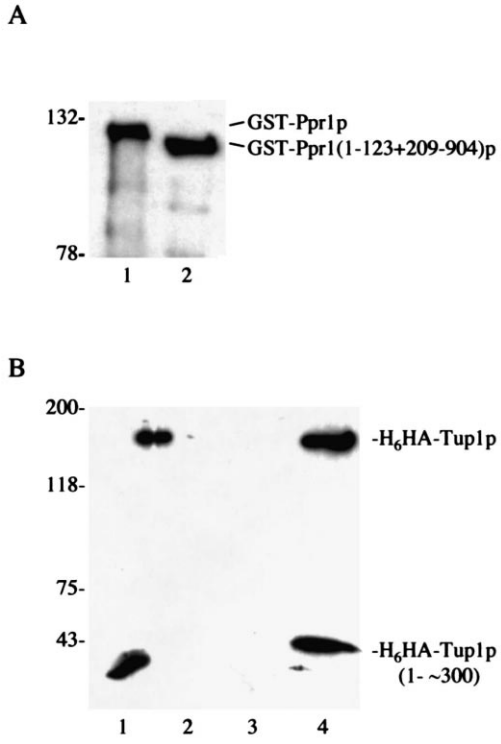

Fig. 3. GST-Ppr1p, but neither GSTp nor GST-Ppr1(1–123+209–904)p, interacted with H_6HA-Tup1p in vitro. A: Purification of GST-Ppr1p (129 kDa, lane 1) and GST-Ppr1(1–123+209–904)p (120 kDa, lane 2) from *E. coli*. The proteins were purified with a glutathione matrix and an aliquot was analyzed by Western blotting with the help of an anti-GSTp antibody (Babco). B: In vitro interaction assay. Purified H_6HA-Tup1p (81 kDa, lane 1) was incubated with equal amounts of GSTp (lane 2), GST-Ppr1(1–123+209–904)p (lane 3), and GST-Ppr1p (lane 4) bound to the glutathione matrix. The matrix was washed, eluted with SDS loading buffer and proteins were separated on an SDS gel. After blotting onto nitrocellulose, bound H_6HA-Tup1p was detected with the help of an anti-HA antibody (Babco). A degradation product consisting of approximately the N-terminal 300 amino acids of H_6HA-Tup1p is indicated.

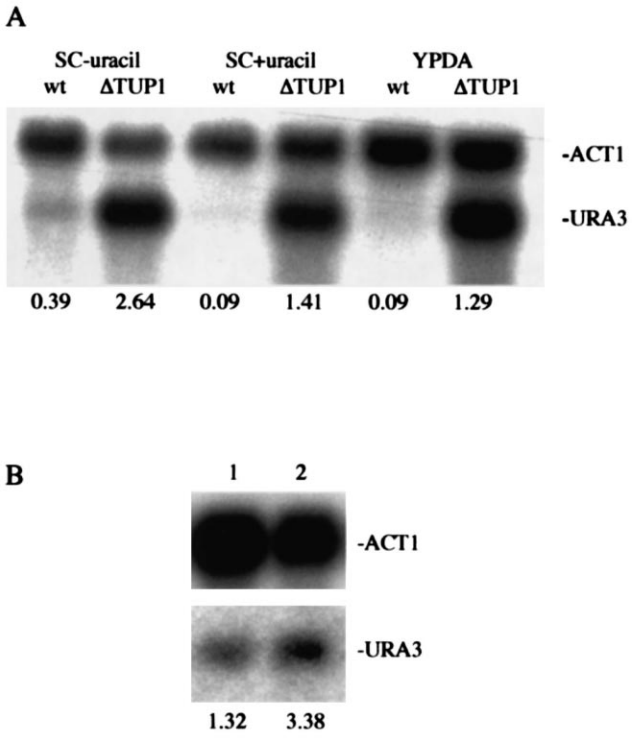

Fig. 4. The interaction between Ppr1p and Tup1p is biologically relevant. A: Tup1p repressed transcription of the *URA3* promoter. Northern blot analysis of cells grown in synthetic complete medium lacking uracil (SC−uracil), in synthetic complete medium containing uracil (SC+uracil), or in rich medium also containing uracil (YPDA). Total RNA was isolated, separated on a denaturing agarose gel, blotted onto nitrocellulose and hybridized simultaneously with a probe derived from the *ACT1* gene and a probe derived from the *URA3* gene. The bands were quantitated on a PhosphorImager, and the numbers given below each lane represent the amount of radioactivity measured for the URA3 band normalized to the ACT1 band in the same lane. B: N_{ub}-Ppr1(1–123+209–904)p (lane 2), which lacks the Tup1p-interacting domain, activated transcription more strongly than N_{ub}-Ppr1p (lane 1), which contains the Tup1p-interacting domain. Total RNA of cells grown in synthetic complete medium lacking uracil and leucine was isolated, blotted, and probed with probes derived from the *URA3* gene and from the *ACT1* gene. The numbers given below each lane represent the amount of radioactivity measured for the URA3 band normalized to the ACT1 band in the same lane.

degradation signal located in the 5′ untranslated region of the *PPR1* mRNA. Apparently, the overexpression of N_{ub}-Ppr1p resulted in approximately three times more *URA3* mRNA as compared to wild-type Ppr1p expressed from its own promoter (compare Fig. 4A, lane 1 with Fig. 4B, lane 1).

4. Discussion

We have shown that Tup1p represses transcription of the *URA3* gene. Our experiments suggest that Ppr1p is a weak activator of transcription because of its interaction with Tup1p. The experiments further suggest that the global repressor Tup1p is recruited to the *URA3* promoter by the transcriptional activator Ppr1p, and that Tup1p blocks transcriptional activation by direct interference. A genome-wide expression profile of a *TUP1* deletion has been published [25], but a significant increase in transcription of the *URA3* gene had not been observed. The most likely explanation for this discrepancy is that the profile was made with an *S. cerevisiae* strain carrying a Ty insertion in the *URA3* gene and that the insertion of this retrotransposon deregulates the *URA3* gene with respect to the repression by Tup1p.

An alternative explanation for the upregulation of the *URA3* gene upon deletion of *TUP1* is that the deletions positively increase the transcription of the *PPR1* gene and that the observed effects are therefore secondary. However, the genome-wide expression profile of the *TUP1* deletion demonstrated that this is not the case. We conclude that the observed effect is most likely to be a direct one.

The deletion of the *TUP1* gene resulted in higher amounts of *URA3* transcript than the deletion of the Tup1p-interacting domain in Ppr1p. There are two possible explanations for this difference. First, Ppr1(1–123+209–904)p might still have interacted with Tup1p, but so weakly that we did not detect this interaction with our methods. Second, Tup1p could have been recruited by a second protein binding to the *URA3* promoter. One candidate for such a protein is Bas2p, another weak activator with a putative binding site in the *URA3* promoter that has been shown to stimulate *URA3* transcription in the absence of adenine [26]. Hence this homeobox protein Bas2p might have been able to recruit Tup1p to some extent, even if Ppr1(1–123+209–904)p was not able to do so.

So far, only transcriptional repressors like α2p, Mig1p, or Rox1p have been described to interact with Tup1p [1]. Our findings demonstrate that the global repressor Tup1p is capable of interacting with transcriptional activators as well, and this increases the possibilities for Tup1p to modulate transcription. But what are the advantages for the cell to combine an activator with a repressor? We suggest two different reasons for this. First, the interaction may be regulated in order to give the cell the opportunity to produce a burst of Ura3p when it is needed – for example during mRNA production in the cell cycle. Changes in transcript level during the cell cycle have been determined on a genome-wide scale [27], but since the strains used carried the Ty insertion, the question of wild-type *URA3* mRNA levels changing during the cell cycle remains open. Interestingly, the abundance of the *TUP1* mRNA was found to fluctuate during the cell cycle, peaking in G2 and M phases. Second, an inefficient preform of the Ura3p enzyme might have existed previously and high concentrations of this preform were needed to produce sufficient amounts of uracil. The preform improved during evolution and the cell lowered the amount of Ura3p enzyme by attaching an interaction domain with a repressor to its activator, Ppr1p.

Acknowledgements: We thank Nils Johnsson for providing reagents and ideas, Renate Schmidt, Richard Reece, Eileen Rojo-Niersbach, and Debra Morley for critical comments on the manuscript, and Stephanie Heck for excellent technical help. This work was supported by grants from the BMBF and the MPG to N.L.

References

[1] Smith, R.L. and Johnson, A.D. (2000) Trends Biochem. Sci. 25, 325–330.
[2] Sprague, E.R., Redd, M.J., Johnson, A.D. and Wolberger, C. (2000) EMBO J. 19, 3016–3027.
[3] Komachi, K., Redd, M.J. and Johnson, A.D. (1994) Genes Dev. 8, 2857–2867.
[4] Edmondson, D.G., Smith, M.M. and Roth, S.Y. (1996) Genes Dev. 10, 1247–1259.

[5] Huang, L., Zhang, W. and Roth, S.Y. (1997) Mol. Cell. Biol. 17, 6555–6562.
[6] Deckert, J., Torres, A.M., Hwang, S.M., Kastaniotis, A.J. and Zitomer, R.S. (1998) Genetics 150, 1429–1441.
[7] Lee, M., Chatterjee, S. and Struhl, K. (2000) Genetics 155, 1535–1542.
[8] Varanasi, U.S., Klis, M., Mikesell, P.B. and Trumbly, R.J. (1996) Mol. Cell. Biol. 16, 6707–6714.
[9] Watson, A.D., Edmondson, D.G., Bone, J.R., Mukai, Y., Yu, Y., Du, W., Stillman, D.J. and Roth, S.Y. (2000) Genes Dev. 14, 2737–2744.
[10] Gromöller, A. and Lehming, N. (2000) FEBS Lett. 484, 48–54.
[11] Gromöller, A. and Lehming, N. (2000) EMBO J. 19, 6845–6852.
[12] Bernardi, F., Zatchej, M. and Thoma, F. (1992) EMBO J. 11, 1177–1185.
[13] Rose, M. and Winston, F. (1984) Mol. Gen. Genet. 193, 557–560.
[14] Marmorstein, R. and Harrison, S.C. (1994) Genes Dev. 8, 2504–2512.
[15] Flynn, P.J. and Reece, R.J. (1999) Mol. Cell. Biol. 19, 882–888.
[16] Liang, S.D., Marmorstein, R., Harrison, S.C. and Ptashne, M. (1996) Mol. Cell. Biol. 16, 3773–3780.
[17] Roy, A., Exinger, F. and Losson, R. (1990) Mol. Cell. Biol. 10, 5257–5270.
[18] Johnsson, N. and Varshavsky, A. (1994) Proc. Natl. Acad. Sci. USA 91, 10340–10344.
[19] Wellhausen, A. and Lehming, N. (1999) FEBS Lett. 453, 299–304.
[20] Rojo-Niersbach, E., Morley, D., Heck, S. and Lehming, N. (2000) Biochem J. 348, 585–590.
[21] Varshavsky, A. (1996) Proc. Natl. Acad. Sci. USA 93, 12142–12149.
[22] Laser, H., Bongards, C., Schüller, J., Heck, S., Johnsson, N. and Lehming, N. (2000) Proc. Natl. Acad. Sci. USA 97, 13732–13737.
[23] Dohmen, R.J., Stappen, R., McGrath, J.P., Forrova, H., Kolarov, J., Goffeau, A. and Varshavsky, A. (1995) J. Biol. Chem. 270, 18099–18109.
[24] Ausubel, F., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. (1998) in: (Chanda, V.B., Ed.) John Wiley and Sons, New York.
[25] DeRisi, J.L., Iyer, V.R. and Brown, P.O. (1997) Science 278, 680–686.
[26] Denis, V., Boucherie, H., Monribot, C. and Daignan-Fornier, B. (1998) Mol. Microbiol. 30, 557–566.
[27] Cho, R.J. et al. (1998) Mol. Cell 2, 65–73.