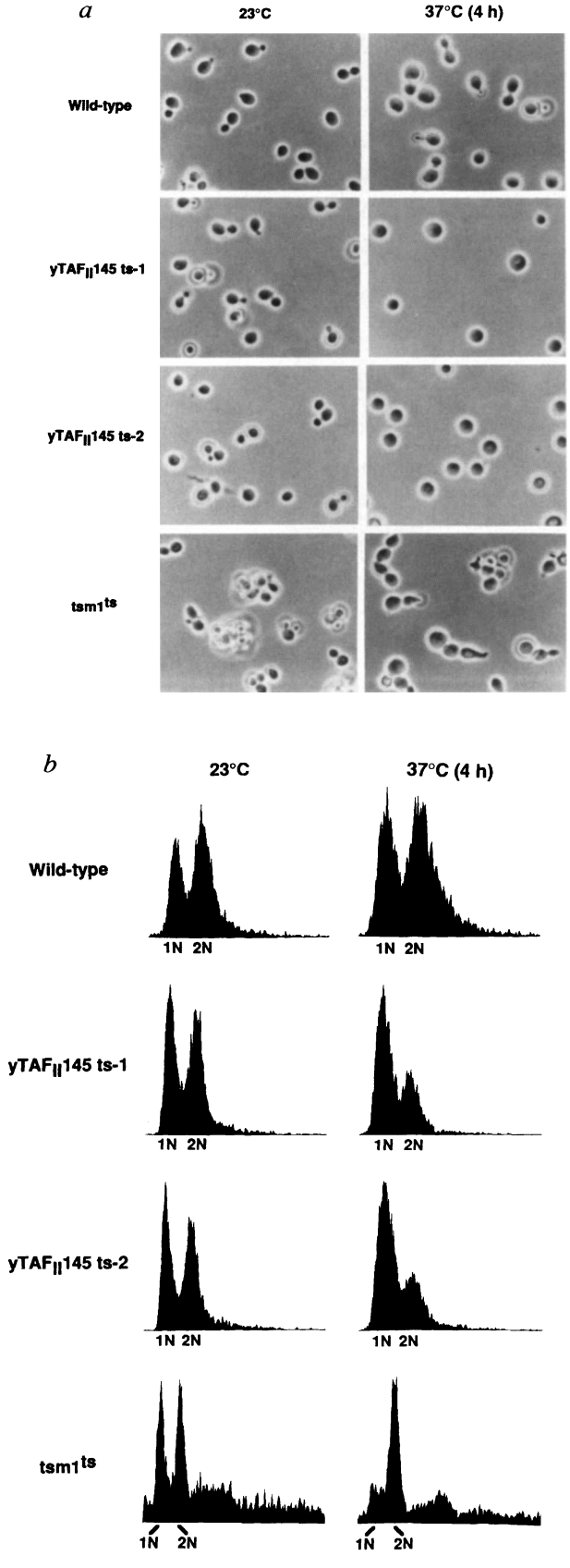

FIG. 4 Inactivation of yTAF$_{II}$145 and TSM1 leads to distinct cell-cycle phenotypes. *a*, Phase-contrast micrographs of wild type (YSW87), the two yTAF$_{II}$145 temperature-sensitive mutants, and the tsm1^{ts} mutant at 23 °C, and after 4 h at the non-permissive temperature (37 °C). All micrographs were taken at a magnification of ×1,000. *b*, Flow cytometric (FACS) analysis, by propidium iodide staining, of the DNA content of wild-type, ytaf$_{II}$145^{ts} and tsm1^{ts} strains at 23 °C and after 4 h at 37 °C.

to 37 °C, and a sample of each was taken after 4 h. Cell samples were fixed in formaldehyde and mounted on poly-L-lysine-coated slides. For FACS analysis, cells were grown at 23 °C to 0.1–0.2 A_{660}, a sample was taken, and the culture was shifted to 37 °C for 4 h. Cells were prepared for FACS quantification of DNA content as previously described[27].

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1. Burley, S. D. & Roeder, R. B. *Annu. Rev. Biochem.* **65,** 769–799 (1996).
2. Goodrich, J. A. & Tjian, R. *Curr. Opin. Cell Biol.* **6,** 403–409 (1994).
3. Reese, J. C., Apone, L., Walker, S. S., Griffin, L. A. & Green, M. R. *Nature* **371,** 523–527 (1994).
4. Poon, D. *et al. Proc. Natl Acad. Sci. USA* **92,** 8224–8228 (1995).
5. Chen, J.-L., Attardi, L. D., Verrijzer, C. P., Yokomori, K. & Tjian, R. *Cell* **79,** 93–105 (1994).
6. Verijzer, C. P., Yokomori, K., Chen, J.-L. & Tjian, R. *Science* **264,** 933–941 (1994).
7. Welch, M. D., Vinh, D. B. N., Okamura, H. H. & Drubin, D. G. *Genetics* **135,** 265–274 (1993).
8. Buchman, A. R. & Kornberg, R. D. *Mol. Cell. Biol.* **10,** 887–897 (1990).
9. Hamil, K. G., Nam, H. G. & Fried, H. M. *Mol. Cell. Biol.* **8,** 4328–4341 (1988).
10. Johnston, M. & Carlson, M. *The Molecular and Cellular Biology of the Yeast* Saccharomyces Vol. 2, Ch. 5 (eds Jones, W. W. *et al.*) (Cold Spring Harbor Laboratory Press, NY, 1992).
11. Kuo, M.-H. & Grayhack, E. *Mol. Cell. Biol.* **14,** 348–359 (1994).
12. Capieaux, E., Vignais, M. L., Sentenac, A. & Goffeau, A. *J. Biol. Chem.* **264,** 7437–7446 (1989).
13. Thompson, C. M. & Young, R. A. *Proc. Natl Acad. Sci. USA* **92,** 4587–4590 (1995).
14. Butt, T. R. *et al. Proc. Natl Acad. Sci. USA* **81,** 3332–3336 (1984).
15. Fürst, P., Hu, S., Hackett, R. & Hamer, D. *Cell* **55,** 705–717 (1988).
16. Lewin, B. *Genes* Vol. 4 (Cell, Cambridge, MA, 1990).
17. Bienz, M. & Pelham, H. R. B. *Adv. Genet.* **24,** 31–72 (1987).
18. Boorstein, W. R. & Craig, E. A. *J. Biol. Chem.* **256,** 18912–18921 (1990).
19. Mortimer, R. K. & Schild, D. *Microbiol. Rev.* **44,** 519–571 (1980).
20. Herskowitz, I. *Microbiol. Rev.* **52,** 536–553 (1988).
21. Nishimoto, T. *et al. Somat. Cell Genet.* **8,** 811–824 (1982).
22. Zawel, L. & Reinberg, D. *Annu. Rev. Biochem.* **64,** 533–561 (1995).
23. Koch, C. & Nasmyth, K. *Curr. Opin. Cell Biol.* **6,** 451–459 (1994).
24. Sikorski, R. S. & Hieter, P. *Genetics* **122,** 19–27 (1989).
25. Guthrie, C. & Fink, G. R. (eds) *Guide to Yeast Genetics and Molecular Biology* (Academic, New York, 1991).
26. Cormack, B. P. & Struhl, K. *Cell* **69,** 685–696 (1992).
27. Lew, D. J., Marinin, M. J. & Reed, S. I. *Cell* **69,** 317–327 (1992).

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CORRESPONDENCE and requests for materials should be addressed to M.R.G. (e-mail: michael.green@ummed.edu). Oligonucleotide sequences used are available from the authors on request. The sequences of yTAF$_{II}$47 (EMBL accession no. Z48483, ORF no. YP8132.02c) and yTAF$_{II}$68 (EMBL accession no. Z50046, ORF no. YD8358.02) are available with the completion of the yeast genome sequencing project.

TBP-associated factors are not generally required for transcriptional activation in yeast

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THE transcription factor TFIID, a central component of the eukaryotic RNA polymerase II (Pol II) transcription apparatus, comprises the TATA-binding protein (TBP) and approximately ten TBP-associated factors (TAFs)[1]. Although the essential role of TBP in all eukaryotic transcription has been extensively analysed *in vivo* and *in vitro*[2,3], the function of the TAFs is less clear. *In vitro*, TAFs are dispensable for basal transcription but are required for the response to activators[1]. In addition, specific TAFs may act as molecular bridges between particular activators and the general transcription machinery[4,5]. *In vivo*, TAFs are

required for yeast[6,7] and mammalian[8] cell growth, but little is known about their specific transcriptional functions. Using conditional alleles created by a new double-shutoff method, we show here that TAF depletion in yeast cells can reduce transcription from some promoters lacking conventional TATA elements. However, TAF depletion has surprisingly little effect on transcriptional enhancement by several activators, indicating that TAFs are not generally required for transcriptional activation in yeast.

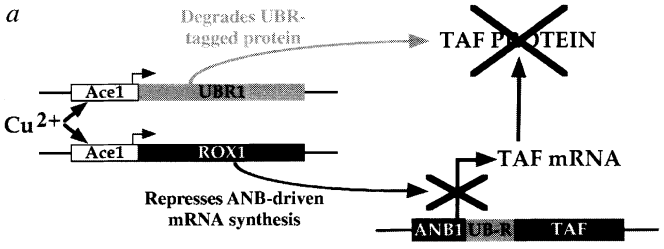
We used a two-pronged approach to create strains with conditional TAF alleles in which the addition of copper ion leads to the simultaneous cessation of TAF messenger RNA synthesis and destruction of any TAF protein present in the cell (Fig. 1*a*). We generated strains with conditional alleles of TAF130(TAF145), TAF90, TAF60 and TAF19(Fun81), which are homologous to human TAF250, *Drosophila* TAF80, *Drosophila* TAF60 and human TAF18, respectively. Of these, TAF130 is particularly interesting, as it appears to be the scaffold on which the remaining TAFs assemble into the TFIID complex[4]. As controls, we generated strains containing conditional alleles of TFIIB and TBP. In all cases, the conditional knockout strains fail to grow on copper-containing medium (Fig. 1*b*), and the addition of copper ion causes cells to stop growing within about six hours (Fig. 1*c*). TAF90-depleted cells arrest frequently as large, budded cells, whereas cells depleted of other TAFs display variable and abnormal morphologies.

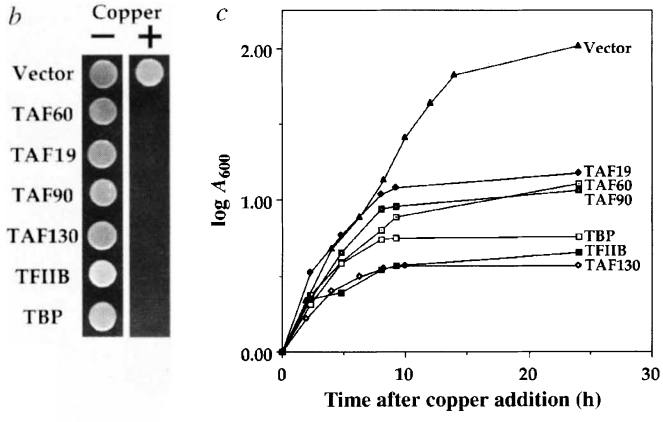
In general, transcription was analysed 8 hours after copper ion addition, when more than 95% of the cells are dead (they are unable to grow when returned to medium lacking copper). At this time, western blotting reveals that levels of TAF130, TAF90 and TBP are less than 5% of wild type (Fig. 1*d*). Although TAFs may not be completely eliminated by this procedure, they are reduced to less than 100–200 molecules per cell (data not shown; ref. 9), which is considerably less than the number of Pol II promoters per cell (~6,000).

When cells are grown under standard conditions, TAF depletion affects transcription of selected Pol II promoters (Fig. 2). Depletion of TAF130, TAF60, TAF90 and TAF19 does not significantly affect transcription of *ded1* or *his3* + 13, promoters with canonical TATA elements[10,11]. However, depletion of TAF130 significantly reduces the level of the *trp3* and his3 + 1 transcripts, which arise from promoters with suboptimal, non-consensus TATA elements[11,12]. This preferential effect on transcription from promoters containing weak TATA elements is also observed when TAF19 is depleted, albeit to a lesser extent and with slower kinetics, but it does not occur upon depletion of TAF90 or TAF60. Interestingly, the transcriptional pattern resulting from TAF130 or TAF19 depletion is similar to that mediated in yeast by human TBP, which has been suggested to interact inefficiently with yeast TAFs[13]. As expected, depletion of TBP or TFIIB results in a rapid and large reduction of all mRNA species tested. At a late time point (11 hours), depletion of TAF90 confers a moderate decrease of all transcripts. It is unclear whether this effect reflects a specific function of TAF90 or arises indirectly from cell death.

Surprisingly, when the conditional knockout strains are grown under conditions that support activation by Gcn4 or Ace1, TAF depletion does not significantly affect the level of activated transcription (Fig. 3*a*, *b*; Ace1-dependent activation appears slightly reduced upon TAF90 depletion). In contrast, depletion of TFIIB causes the loss of activated transcription in both situations. The observed Gcn4- and Ace1-activated transcription reflects initiation events that occur under conditions of TAF depletion, because mRNA half-lives are very short in comparison to the timescale of the experiment.

One explanation for the maintenance of Gcn4 and Ace1 activation after TAF depletion is that TAFs present in active transcription complexes might be preferentially sequestered from Ubr1-dependent degradation. To examine whether active transcription complexes could be assembled after TAF depletion, the





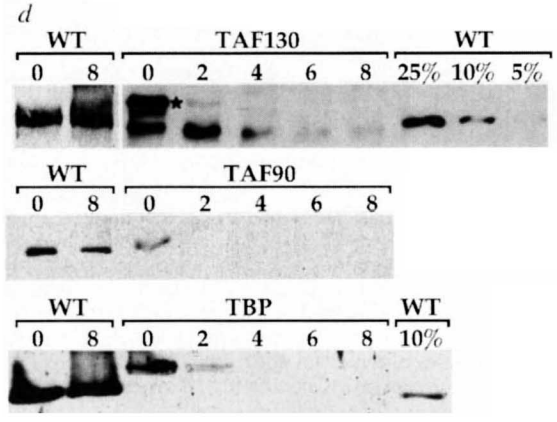


FIG. 1 Creation and characterization of strains with conditional TAF alleles. *a*, Strain construction. In the parent strain, ROX1[28], a transcriptional repressor, and UBR1[29], the N-end recognition protein involved in the ubiquitin degradation pathway, are expressed from a tightly regulated copper-inducible promoter[26]. The TAF gene of interest is replaced by a derivative that contains an N-end recognition signal for rapid ubiquitin-dependent degradation and is driven by the *ANB1* promoter, a target of Rox1. In the absence of copper ion, neither the repressor nor the protein degradation system is active, and the TAF is expressed. Upon copper addition, both the repressor and the ubiquitin degradation system are rapidly activated, leading to the simultaneous cessation of TAF mRNA synthesis and destruction of TAF protein. *b*, Growth of 10^4 cells from each strain on synthetic complete medium either lacking (left) or containing (right) 500 μM copper sulphate for two days at 30 °C. *c*, Growth curves of strains on synthetic complete medium to which 500 μM copper sulphate was added at time zero. *d*, Western blot analysis of TAF depletion. Protein (30 μg) from parental, TAF, and TBP depletion strains at various times after addition of 500 μM $CuSO_4$ were probed with the relevant antibodies; dilutions of protein from the parental strain are indicated for TAF130 and TBP. Although full-length TAF130 (star) is very rapidly degraded, a truncated product (lacking a fragment of M_r ~ 25K), which may or may not have functional TAF activity, is degraded more slowly so that ~5% remains 8 h after copper addition.

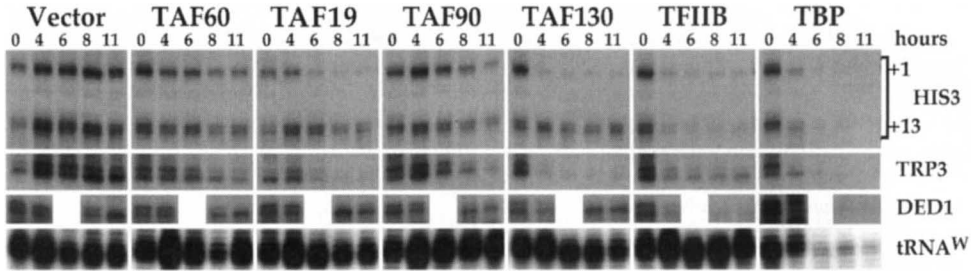


FIG. 2 Differential effects of TAF depletion on Pol II transcripts. Parental and TAF-depletion strains at the indicated time (hours) after the addition of copper were analysed for *his3*, *trp3*, *ded1* and tRNA^W transcription.

conditional knockout strains were grown in non-inducing conditions, treated with copper for 8 hours, and then tested for the ability to mediate activator-dependent transcription *de novo* (Fig. 4*a*, *b*). All the TAF-depleted strains show significant activation by Gal4 and heat-shock factor upon exposure to the relevant inducer, whereas activation is not observed in the TFIIB-depleted strain. Similarly, we observe efficient Ace1-dependent activation after TAFs were depleted either by placing the TAF genes under the control of the *GAL1,10* promoter and shifting cells to glucose (data not shown) or by shifting a *tsm1* (dTAF150 homologue) strain to the restrictive temperature (Fig. 4*c*). The heat-shock and Ace1 activation responses in the TAF-depleted strains are comparable to the parental strain; Gal4 activation is reduced 3–4 fold. However, as very small fluctuations in Gal4 levels or changes in growth potential can have pronounced effects on Gal4-dependent transcription[14], it is unclear whether the decrease reflects a mild activation defect or whether Gal4 expression is slightly perturbed for other reasons. Previously described activation-defective yeast strains are considerably more impaired for Gal4-dependent activation, and they are defective in the response to other acidic activators[15–17].

From the observation that TAF depletion does not significantly affect activation by Gcn4, Ace1, Gal4, Hsf, and unidentified activators involved in *ded1* and *his3* + 13 transcription, we conclude that the TAFs are not generally required for transcriptional activation in yeast cells. This conclusion was reached independently in experiments where TAF depletion was obtained using temperature-sensitive mutants or a glucose shutoff procedure[9]. It is particularly striking that this is true of TAF130, which provides the scaffold for TAF assembly and without which TFIID is likely to be disrupted. Although TAFs are not generally required for transcriptional activation, they are essential for cell growth. One possibility is that TAFs are required for the response to a subset of activators that affect one or more essential genes. Alternatively, TAFs could subtly affect activation of many genes, such that the cumulative effects lead to cell inviability. Finally, as suggested by the effects on *trp3* and *his3* + 1 transcription, TAFs may be important for transcription from promoters with weak TATA elements.

Our conclusion is in apparent contrast to numerous experiments *in vitro*, which indicate that TAFs are crucial in all activated transcription. We do not favour the hypothesis that yeast TAFs

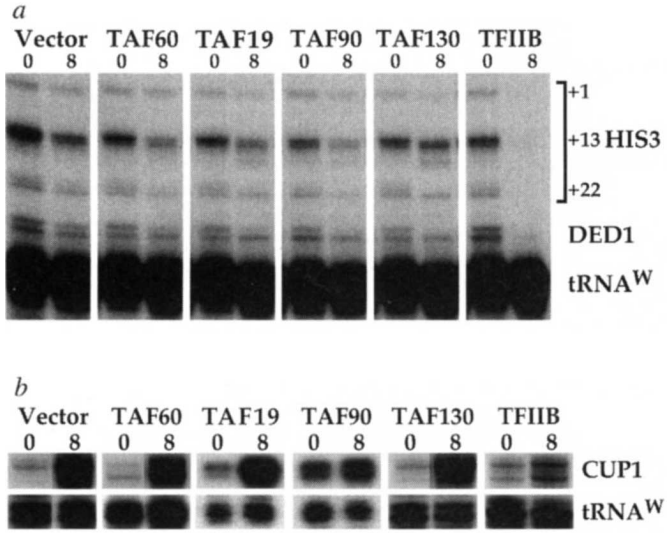


FIG. 3 Maintenance of Gcn4- and Ace1-activated transcription after TAF depletion. *a*, Parental and TAF-depletion strains cultured in glucose synthetic complete medium containing 10 mM 3-aminotriazole (a condition that induces Gcn4 synthesis) at 0 and 8 h after copper addition were analysed for *his3* and *ded1* transcription. Similar results were obtained using strains containing a plasmid that constitutively expresses Gcn4. *b*, Ace1 activation. Parental and TAF-depletion strains at 0 and 8 h after copper addition were analysed for *cup1* and tRNA^W transcription. Ace1 activation of *cup1* occurs simultaneously with the induction of TAF depletion.

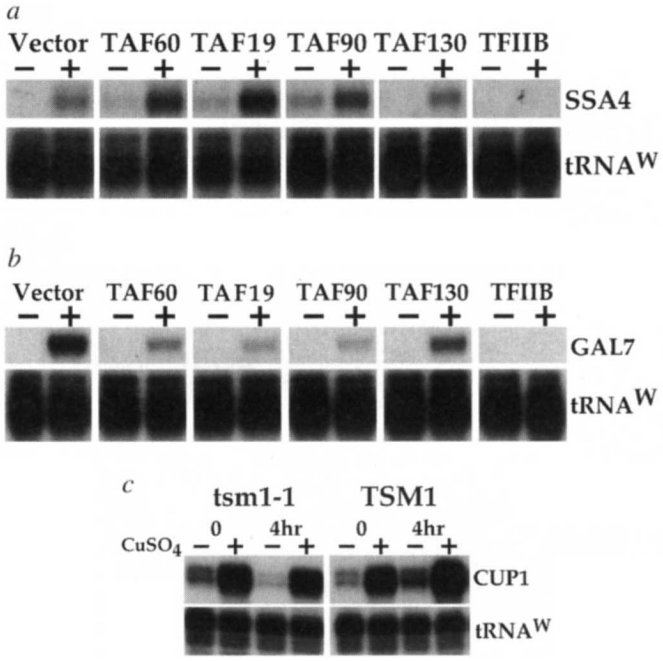


FIG. 4 Activation can be induced after TAF depletion. *a*, Activation by heat-shock factor. Parental and TAF-depletion strains at 8 h after copper addition that were (+ lanes) or were not (− lanes) subjected to a 12-min heat shock at 39.5 °C were analysed for *ssa4* and tRNA^W transcription. *b*, Activation by Gal4. Parental and TAF-depletion strains cultured in raffinose synthetic complete medium at 8 h after copper addition, which were or were not induced for 30 min with 2% galactose, were analysed for *gal7* and tRNA^W transcription. *c*, Activation by Ace1 after thermal inactivation of *TSM1*. Strain AW41[30] and a derivative containing a plasmid-borne *TSM1* allele at 0 and 4 h after temperature shift which were or were not induced for 30 min with 500 μM copper sulphate were analysed for *cup1* and tRNA^W transcription. Growth of the *tsm1*-1 strain ceases within 4 h after temperature shift.

are less important than their mammalian and *Drosophila* counterparts because: (1) TAFs are strongly conserved among eukaryotes; (2) TAF-dependent activation *in vitro* can be achieved with yeast components[6,7]; and (3) activation can occur in a hamster cell line in which TAF250 (yeast TAF130 homologue) has been thermally inactivated (Fos transcription occurs normally, and it is unclear whether the reduction of cyclin A transcription is an indirect effect of cell-cycle arrest or a direct effect of TAF250)[8]. A more likely explanation is that TAFs are functionally redundant with other factors that are absent in typical *in vitro* reactions. Indeed, activated transcription in the apparent absence of TAFs can occur *in vitro* when reactions either contain Pol II holoenzyme[18,19] or are performed on chromatin templates[20]. Moreover, most *in vitro* transcription reactions are reconstituted with core Pol II, and hence may lack components of the Pol II holoenzyme (for example, Srb proteins, Gal11) that are functionally important *in vivo*[21,22].

A common view of the transcriptional activation process is that activator proteins stabilize the Pol II machinery at the promoter, thereby permitting increased transcriptional initiation[22]. In principle, activator proteins can interact with individual components of the Pol II machinery, and indeed, artificial connection of enhancer-bound proteins to TBP[23,24], TAFs (M. Keaveney and K.S., unpublished results) and components of the Pol II holoenzyme[25] can bypass the need for an activation domain. If natural activators interact with multiple components, individual components such as TAFs are likely to be non-essential for activation, even if they are potential targets. Thus, although it is possible to generate conditions in which TAFs are required for activation *in vitro*, they do not appear to be generally required *in vivo*. However, at promoters lacking conventional TATA elements, which are inherently weak targets for TFIID, interactions of TAFs with basic transcription factors or with promoter DNA may be important for stabilizing the Pol II machinery. □

Methods

The parent strain ZMY60, containing copper-inducible alleles of *UBR1* and *ROX1*, was created as follows. A cassette containing the copper-inducible derivative of the *HIS3* promoter[26] and 2 kb of upstream flanking sequence was inserted at the initial ATG of a plasmid-borne genomic fragment of *ROX1* to create the *URA3* integrating plasmid ZM195. The same cassette was inserted at the initial ATG of *UBR1* to create ZM197. Both of these copper-driven alleles were introduced into yeast strain KY320[11] in successive two-step gene replacements. To create TAF disruption molecules, another cassette comprising an in-frame fusion of ubiquitin, arginine, LacI and the HA epitope driven by the *ANB1* promoter, was fused in-frame to a short 5′ fragment of TAF coding sequence beginning at the initial TAF ATG. To create a given conditional knockout strain, the relevant TAF knockout molecule on a *URA3* integrating plasmid was linearized within the TAF coding sequence fragment and transformed into ZMY60. This integration results in homologous recombination at the TAF locus to yield a short, non-functional 5′ TAF piece under its normal promoter, followed immediately downstream by a full-length copy of the tagged TAF under the *ANB1* promoter. Selection for uracil prototrophy was maintained in all experiments to avoid loss of the integrated plasmid. Details of the constructs and methods will be provided upon request.

RNA levels were determined by quantitative S1 analysis as described[13,27]. All hybridization reactions contained at least two probes, such that the relative levels of all transcripts are internally controlled. The error for any particular RNA determination is ±30%. TAF levels were determined by western blotting, with bands being detected by chemiluminescence (ECL). Relative levels of TAFs at various times were determined by comparing band intensities to serially diluted samples from wild-type cells.

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1. Tjian, R. & Maniatis, T. *Cell* **77,** 5–8 (1994).
2. Hernandez, N. *Genes Dev.* **7,** 1291–1308 (1993).
3. Struhl, K. *Annu. Rev. Genet.* **29,** 651–674 (1995).
4. Chen, J.-L., Attardi, L. D., Verrijzer, C. P., Yokomori, K. & Tjian, R. *Cell* **79,** 93–105 (1994).
5. Sauer, F., Hansen, S. K. & Tjian, R. *Science* **270,** 1783–1788 (1995).
6. Reese, J. C., Apone, L., Walker, S. S., Griffin, L. A. & Green, M. R. *Nature* **371,** 523–527 (1994).
7. Poon, D. *et al. Proc. Natl Acad. Sci. USA* **92,** 8224–8228 (1995).
8. Wang, E. H. & Tjian, R. *Science* **263,** 811–814 (1994).
9. Walker, S. S., Reese, J. C., Apone, L. M. & Green, M. R. *Nature* **385,** 185–188 (1996).
10. Chen, W. & Struhl, K. *EMBO J.* **4,** 3272–3280 (1985).
11. Iyer, V. & Struhl, K. *Mol. Cell. Biol.* **15,** 7059–7066 (1995).
12. Martens, J. A. & Brandl, C. J. *J. Biol. Chem.* **269,** 15661–15667 (1994).
13. Cormack, B. P., Strubin, M., Stargell, L. A. & Struhl, K. *Genes Dev.* **8,** 1335–1343 (1994).
14. Griggs, D. W. & Johnston, M. *Proc. Natl Acad. Sci. USA* **88,** 8597–8601 (1991).
15. Arndt, K. M., Ricupero-Hovasse, S. & Winston, F. *EMBO J.* **14,** 1490–1497 (1995).
16. Lee, M. & Struhl, K. *Mol. Cell. Biol.* **15,** 5461–5469 (1995).
17. Stargell, L. A. & Struhl, K. *Science* **269,** 75–78 (1995).
18. Koleske, A. J. & Young, R. A. *Nature* **368,** 466–469 (1994).
19. Kim, Y.-J., Bjorklund, S., Li, Y., Sayre, M. H. & Kornberg, R. D. *Cell* **77,** 599–608 (1994).
20. Workman, J. L., Taylor, I. C. A. & Kingston, R. E. *Cell* **64,** 533–544 (1991).
21. Koleske, A. J. & Young, R. A. *Trends Biochem. Sci.* **20,** 113–116 (1995).
22. Struhl, K. *Cell* **84,** 179–182 (1996).
23. Chatterjee, S. & Struhl, K. *Nature* **374,** 820–822 (1995).
24. Klages, N. & Strubin, M. *Nature* **374,** 822–823 (1995).
25. Barberis, A. *et al. Cell* **81,** 359–368 (1995).
26. Klein, C. & Struhl, K. *Science* **266,** 280–282 (1994).
27. Iyer, V. & Struhl, K. *Proc. Natl Acad. Sci. USA* **93,** 5208–5212 (1996).
28. Balasubramanian, B., Lowry, C. V. & Zitomer, R. S. *Mol. Cell. Biol.* **13,** 6071–6078 (1993).
29. Bartel, B., Wunning, I. & Varshavsky, A. *EMBO J.* **9,** 3179–3189 (1990).
30. Ray, B. L., White, C. I. & Haber, J. E. *Curr. Genet.* **20,** 25–31 (1991).

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