Balancing transcriptional interference and initiation on the *GAL7* promoter of *Saccharomyces cerevisiae*

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Transcriptional termination of the *GAL10* gene in *Saccharomyces cerevisiae* depends on the efficiency of polyadenylation. Either *cis* mutations in the poly(A) signal or *trans* mutations of mRNA 3′ end cleavage factors result in *GAL10* read-through transcripts into the adjacent *GAL7* gene and inactivation (occlusion) of the *GAL7* promoter. Herein, we present a molecular explanation of this transcriptional interference phenomenon. *In vivo* footprinting data reveal that *GAL7* promoter occlusion is associated with the displacement of Gal4p transcription factors from the promoter. Interestingly, overexpression of Gal4p restores promoter occupancy, activates *GAL7* expression, and rescues growth on the otherwise toxic galactose substrate. Our data therefore demonstrate a precise balance between transcriptional interference and initiation.

Transcriptional termination of RNA polymerase II (Pol II) is linked to mRNA 3′ processing, which is a two-step reaction consisting of endonucleolytic cleavage of the RNA precursor and subsequent polyadenylation (1). Mutation of poly(A) signals results in increased transcription beyond the poly(A) site of a gene (1) and recently has been shown to depend on the activity of 3′ end processing factors (2). Some RNA processing factors are associated with elongating Pol II, indicating a tight link between transcription and RNA processing (3, 4). In contrast to Pol I (5) and Pol III, termination of Pol II occurs at variable, ill-defined positions downstream of the poly(A) site of a gene (1). These findings suggest that transcriptional termination can be a random process.

In some instances, however, termination of transcription must occur efficiently, because enhanced transcriptional read-through can result in inhibition of an adjacent, downstream promoter and also perturbs origin of replication and centromere function (6–9). Closely spaced genes are particularly prone to promoter occlusion, especially when they are expressed at the same time, as in the case of the *GAL10* and *GAL7* genes of *Saccharomyces cerevisiae*. These two genes are separated by 600 bp and are activated simultaneously to high levels by galactose (gal). We have shown previously that deletion of the *GAL10* poly(A) signal leads to enhanced *GAL10* read-through transcription and formation of *GAL10-7* bicistronic transcripts, which in turn results in inhibition of the downstream *GAL7* promoter (ref. 10; Fig. 1*A*). Gal7p (gal uridyl transferase) catalyzes conversion of the metabolic intermediate gal 1-phosphate, which is otherwise toxic. Inhibition of Gal7p expression by transcriptional read-through into the *GAL7* promoter renders cells gal sensitive and results in a Gal$^-$ phenotype, emphasizing the importance of termination in this system.

Transcription of the *GAL* genes depends on the transcription factor Gal4p, which binds to 17-bp sequence elements within the promoter regions (11, 12). The binding affinity of Gal4p for these sites depends on both the sequence and spacing between the critical outer nucleotide triplets (CGG. . . GGC), which are contacted specifically by the Gal4p N-terminal domain (13, 14). Gal4p interacts directly with various components of the basal transcriptional machinery and is also capable of recruiting the Pol II holoenzyme to the promoter (15–18). Gal4p is expressed at very low levels in the cell because of low promoter activity (19).

Herein, we show that overexpression of Gal4p in cells harboring *GAL10* read-through mutations (which are thus Gal$^-$) restores growth on gal medium. RNA analysis of these strains shows that *GAL7* transcription is partially regained and that Gal7 protein is reexpressed at low levels. *In vivo* footprinting of the *GAL7* promoter reveals that transcriptional interference leads to a disruption of Gal4p–*GAL7* promoter contacts, which are reestablished on Gal4p overexpression, suggesting a balance between interference and the concentration of the respective DNA-binding protein in the cell. In agreement with previous data, we find that mutations in mRNA 3′ end processing factors inhibit termination, leading to *GAL7* promoter occlusion in *trans*.

Materials and Methods

Plasmids, Strains, and Growth Conditions. pYC10-7, pΔ-55, and pΔ-75 are based on YCplac22 (CEN4-TRP1; ref. 20) and have been described (10). pRJR197 and pRJR216 harbor the *GAL4* gene (driven by the *GAL4* promoter) and were kindly provided by R. Reece (University of Manchester, Manchester, U.K.; ref. 21). A 1,945-bp *Xba*I–*Eco*RV fragment containing the *GAL7* gene was cloned into pRS303 (CEN6-HIS3; ref. 22) and cut with *Xba*I/*Eco*RV to generate the *GAL7* plasmid pRSG7. Strains were grown and maintained on synthetic complete medium (SC) or on SC lacking tryptophan, supplemented with 2% (vol/vol) glucose, raffinose (SC-raf), or gal (SC-gal; ref. 23). Before induction, cells were grown in SC-raf and induced with 2% (vol/vol) gal for 1–2 h at early log phase. Temperature-sensitive (ts) mutant strains for cleavage and polyadenylation were incubated at 37°C for 45 min after gal induction, and then RNA was harvested. Yeast extract/peptone (YP)-gal + ethidium bromide contained 1% (vol/vol) yeast extract, 2% (vol/vol) peptone, 2% (vol/vol) gal, and 20 μg/ml ethidium bromide (24). Transformations were performed according to the method of Gietz *et al.* (25). Strains were kindly provided by W. Keller (University of Basel, Basel; *rna14*, *rna15*, *yth11*, and *fip1*), F. Lacroute (Centre National de la Recherche Scientifique, Gif sur Yvette, France; *pcf11*), A. Sachs (University of California, Berkeley, CA; *pap1*), and C. Moore (Tufts University, Medford, MA; *hrp1*).

Northern Blots. Northern blotting was performed as described (10). The *GAL7*-specific probe (Fig. 2*B*) is a 775-bp *Bgl*II–*Sal*I restriction fragment; the *GAL10-7* probe (Fig. 2*C*; see also Fig. 5) is a 1,530-bp *Bgl*II–*Sal*I fragment containing 506 bp of the

Abbreviations: gal, galactose; Pol II, RNA polymerase II; SC, synthetic complete medium; SC-raf, SC supplemented with raffinose; SC-gal, SC supplemented with gal; ts, temperature sensitive; DMS, dimethyl sulfate; YP, yeast extract/peptone; wt, wild-type; G *n*, guanine at position *n*; CF, cleavage factor.

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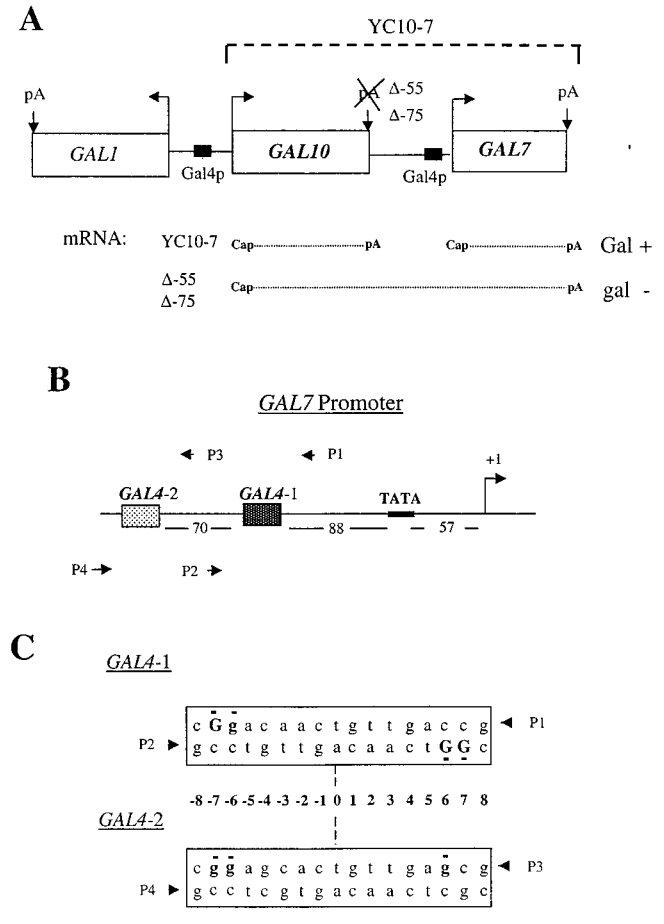

Fig. 1. Diagram of the *GAL* cluster and the *GAL7* promoter. (*A*) Diagram showing the arrangement of the structural *GAL* genes. *GAL10* and *GAL7* are contained within YC10-7. Deletions of the *GAL10* poly(A) site result in Δ-55 and Δ-75, respectively. YC10-7 gives rise to monocistronic *GAL10* and *GAL7* mRNA, respectively (dotted lines below), and is viable on gal medium (Gal+). Δ-55 and Δ-75 give rise to bicistronic mRNA, which does not produce *GAL7* mRNA, and are therefore gal sensitive (Gal−). Black boxes represent Gal4p-binding sites. (*B*) Schematic of the region 260 bp upstream of the *GAL7* transcription initiation site (+1). The TATA box and the two Gal4p binding sites are shown (dark boxes); the distances between these elements are indicated below. Also shown are the positions of primers used for *in vivo* footprinting (P1–P4). (*C*) Sequences of the two Gal4p binding sites (GAL4-1 and GAL4-2). The center of the roughly symmetrical 17-bp sites is indicated by a vertical, dashed line. The base pairs are numbered sequentially from the center; the central base pair has been assigned the number 0. The protected guanines of the highly conserved outer triplet are in bold and indicated by horizontal bars. They are shown in uppercase and lowercase, depending on the degree of protection. Also indicated are the primers (P1–P4), which were used to visualize the particular strand.

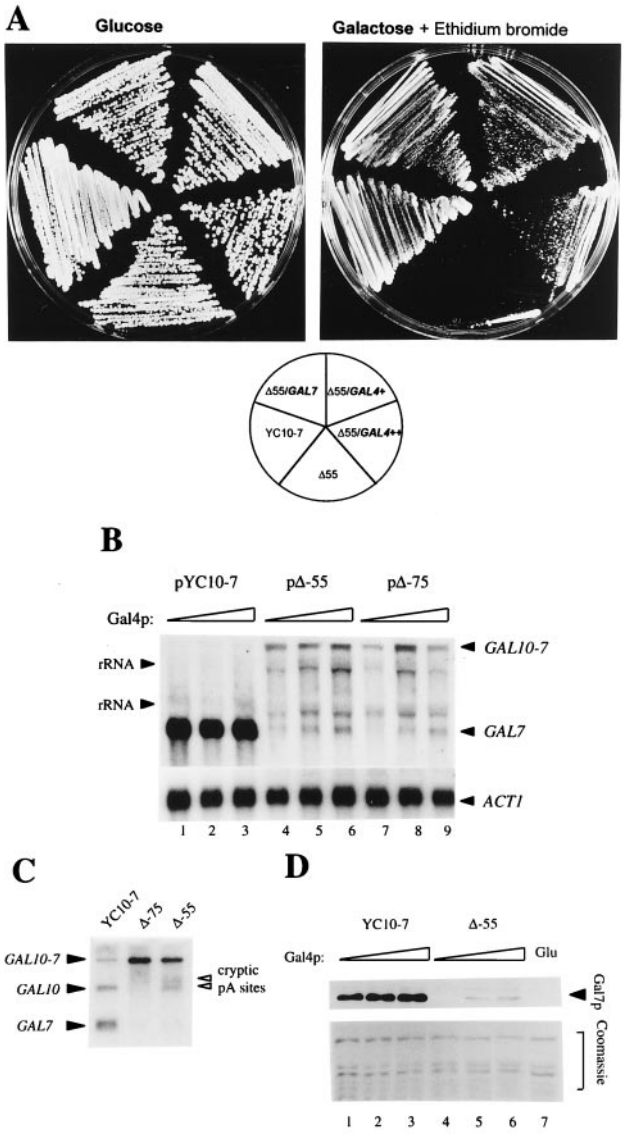

Fig. 2. Overexpression of Gal4p rescues Gal7p expression. (*A*) Overexpression of Gal4p restores growth of Δ-55 on gal + ethidium bromide medium. Strains, indicated below in the key diagram, were grown on YP/dextrose and YP-gal + ethidium bromide for 4 days at 30°C. Cells were streaked onto YP-gal + ethidium bromide first then onto YP/dextrose. (*B*) Northern blot of total RNA from gal-induced cells. (*Upper*) *GAL10-7* and *GAL7* mRNA was detected with a *GAL7*-specific probe. Gal4p was overexpressed to different levels in each strain as shown on the top. Lanes 1, 4, and 7, Gal4p at physiological levels; lanes 2, 5, and 8, cells transformed with Gal4p expressed from the autonomous replication sequence plasmid pRJR197; lanes 3, 6, and 9, cells transformed with Gal4p expressed from the 2-μ plasmid pRJR216. Crosshybridization to rRNA is indicated on the side. (*Lower*) An actin loading control (*ACT1*). (*C*) Northern blot of poly(A)$^+$ mRNA. *GAL* transcripts were detected with a probe recognizing *GAL10* and *GAL7*. Cryptic *GAL10* poly(A) sites (cryptic pA sites), formed by Δ-55, are indicated by empty arrowheads on the side. (*D*) Western blot of Gal7p. Yeast extracts from strains, indicated on the top, were separated by SDS/10% PAGE. The gel was stained with Coomassie brilliant blue after the transfer and serves as a loading control (Coomassie). The blot was probed with Gal7p antibody. Gal4p was overexpressed to different levels in each strain as shown on the top. Lanes 1 and 4, Gal4p at physiological levels; lanes 2 and 5, cells transformed with Gal4p expressed from the autonomous replication sequence plasmid; lanes 3 and 6, cells transformed with Gal4p expressed from the 2-μ plasmid.

GAL10 3′ end, the intergenic region, and 428-bp of *GAL7* the 5′ end. Poly(A)$^+$ mRNA was selected with oligo(dT) cellulose by using a commercial kit (MicroFast Track, Invitrogen), which was modified as described by Pritlove *et al.* (26).

Western Blots. Yeast protein extracts were prepared as described (23). Cell cultures were grown and induced as described in the section above; the cell density was determined spectrophotometrically and equalized for all cultures. Approximately 2 OD_{600} units of culture was mixed with 2 ml of 50 mM Tris (pH 7.5) and 10 mM NaN_3 on ice. Cells were collected, resuspended in 50 μl of ESB [2% (vol/vol) SDS/80 mM Tris, pH 6.8/10% (vol/vol) glycerol/1.5% (vol/vol) DTT/0.1 mg/ml bromophenol blue/5 mM PMSF], and heated for 3 min at 100°C. Approximately 0.1 g of 0.2-mm glass beads was added, and the mixture was vortexed for 2 min, diluted with 50 μl of ESB, and heated for 1 min at

100°C. A 15-μl extract was loaded onto SDS/10% polyacrylamide gels. Loading was equalized further relative to a Coomassie-stained gel of the respective extracts. Gal proteins were detected with antibodies kindly provided by T. Fukasawa (Kazusa DNA Research Institute, Kisarazu, Japan; refs. 27 and 28).

Dimethyl Sulfate (DMS) *in Vivo* Footprinting. Strains were grown in 5 ml of SC-raf overnight, and the cultures were diluted into 50 ml of YP-raf or SC-raf and grown to mid log phase. Cultures were expanded into 600 ml of YP-gal or SC-gal and grown to late log phase ($OD_{600} \approx 2.0$). DMS treatment and subsequent chromosomal DNA preparations were performed as described by Koch *et al.* (29). Chromosomal DNA was digested with *Eco*RI. Primer extensions were carried out with ≈15 μg of chromosomal DNA and ≈1 ng of end-labeled (gel-purified) primer. The reaction mixture was heated to 95°C for 5 min before addition of 8 units of *Taq* DNA polymerase (Promega) and 10 mM dNTPs (Roche Molecular Biochemicals). Cycling parameters were 37 cycles at 92.5°C for 1 min, annealing for 2 min, and extension at 72°C for 3 min, followed by a 10-min extension at 72°C. The sequences of the primers were as follows: P1, (−88 to −98 upstream of the *GAL7* start site at +1) 5′-ACATAGCTACACATTATTTTCAGCTTGGCT (annealing temperature = 59.4°C); P2, (−250 to −220) 5′-GATATATTTTCTGTCATTTTCCTTAACCCA (annealing temperature = 56.7°C); P3, (−184 to −214) 5′-CCCTTTCCCTTATTTTTGGGTTAAGGAAAA (annealing temperature = 59°C); and P4, (−332 to −301) 5′-GGGTAATTTTTCCCCTTTATTTTGTTCATAC (annealing temperature = 58.4°C).

PCRs were taken up in 300 μl of Tris/EDTA buffer supplemented with 190 mM NaOAc (pH 7.0), 2.5 mM EDTA, and 20 μg of tRNA (Sigma). Samples were phenol/chloroform extracted and ethanol precipitated. The final DNA pellets were taken up in 10 μl of formaldehyde loading dye and electrophoresed on 8% sequencing gels.

Results

Overexpression of Gal4p Restores Growth of Δ-55 on Gal Medium and Partially Restores Gal7p Expression. Our previous data emphasize the role of the *GAL10* poly(A) signal in modulating the efficiency of the downstream-positioned *GAL7* promoter by restricting transcriptional interference (10). We reasoned that *GAL7* promoter occlusion might be caused by a disruption of the *GAL7* promoter architecture (30) and therefore tested the effect of overexpressing Gal4p in strains harboring *GAL10* poly(A) site deletions. Gal4p binds to two sites in the *GAL7* promoter region (Fig. 1*B*). Gal4p was overexpressed in both the wild-type (wt) strain YC10-7 and in strains carrying *GAL10* alleles with poly(A) site deletions (Δ-55 and Δ-75), which are Gal^- (Fig. 1*A*). Gal4p was overexpressed to different levels: either from an autonomous replication sequence plasmid (in Δ-55/*GAL4*+; ref. 21), resulting in up to 10 additional *GAL4* copies per cell (expected copy number for an 2-μm plasmid), or from a 2-μ plasmid (in Δ-55/*GAL4*++; ref. 21), resulting in up to 50 additional *GAL4* copies per cell. Fig. 2*A* shows that overexpression of Gal4p rescues growth of Δ-55 on plates containing gal as the sole carbon source and ethidium bromide to block respiration (Fig. 2*A*, Δ-55/*GAL4*+ and Δ-55/*GAL4*++). In contrast, Δ-55 expressing normal levels of Gal4p was unable to grow under these conditions but grew well on plates containing glucose. These growth phenotypes suggest that interaction of Gal4p with the *GAL7* promoter is limiting in the *GAL10* read-through mutants and can be restored by increasing amounts of Gal4p transcription factor in the cell. Fig. 2*A* also shows that expression of *GAL7* on a single-copy centromeric plasmid, transformed into Δ-55 (Δ-55/*GAL7*), restores growth on gal to levels similar to that of the wt strain YC10-7. This finding confirms that the growth defect of Δ-55 is caused solely by inhibition of *GAL7* through *GAL10* transcriptional interference and can be restored by expression of *GAL7* in *trans*.

We next analyzed *GAL7* mRNA from the Δ-55 and Δ-75 strains overexpressing Gal4p. As shown in Fig. 2*B*, overexpression of Gal4p results in low *GAL7* mRNA levels in the Δ-55 and Δ-75 strains, demonstrating that high amounts of Gal4p partially restore transcription from the occluded *GAL7* promoter. Compared with YC10-7 mRNA (Fig. 2*B*, lanes 1–3), levels in the mutants were still ≈20-fold lower, indicating that low levels of *GAL7* mRNA are sufficient to restore growth of *S. cerevisiae* on gal medium. We also note that residual levels of *GAL7* mRNA are produced by Δ-55 (Fig. 2*B*, lane 4), but they are not detected in Δ-75 (Fig. 2*B*, lane 7). Analysis of $poly(A)^+$ RNA from these strains revealed that cryptic poly(A) sites are used in Δ-55, resulting in extended and shortened *GAL10* transcripts (Fig. 2*C*), which were also detected by S1-nuclease mapping (data not shown). These cryptic sites are absent in Δ-75, indicating that the Δ-75 deletion removes all essential *GAL10* poly(A) signals, probably resulting in more complete read-through and consequently *GAL7* promoter occlusion.

We finally analyzed Gal7p protein levels in the Δ-55 strain. As shown in Fig. 2*D*, Gal7p protein, which is undetectable in Δ-55, was present at low levels when Gal4p is overexpressed (Fig. 2*D*, lanes 5 and 6). In contrast to Gal7p, Gal1p and Gal10p were both expressed in Δ-55 (data not shown). As expected, no Gal7p signal was detected in glucose-grown YC10-7 (Fig. 2*D*, lane 7). Even though residual amounts of *GAL7* mRNA are present in Δ-55, no detectable Gal7p is expressed in this strain. Overall, these results demonstrate that expression of *GAL7* is partially restored when Gal4p is overexpressed in Δ-55 and Δ-75.

GAL10 Read-Through Transcription Disrupts Binding of Gal4p to the GAL7 Promoter. We next directly analyzed the interaction of Gal4p with the *GAL7* promoter by *in vivo* footprinting. The *GAL7* promoter contains a TATA box and two Gal4p-binding sites within 250 bp of the transcriptional start site (Fig. 1*B*; ref. 31). As shown in Fig. 1*C*, the proximal binding site, GAL4-1, closely matches the 17-bp consensus sequence and contains the critical outer 6 bp, 5′-CGG. . . CCG-3′, which are recognized specifically by Gal4p dimers (13). The distal binding site, GAL4-2, slightly diverges from the consensus, having the sequence 5′-CGG. . . GCG-3′, similar to the weak Gal4p binding site 4 in the *GAL10*/*GAL1* promoter (32, 33). Gal4p binding was analyzed by DMS *in vivo* footprinting. Gal4p was overexpressed from the 2-μ plasmid.

Upper Strand. The upper strand was analyzed with primers P1 and P3 (Fig. 1*B*). Analysis of the proximal binding site, GAL4-1, in the wt YC10-7 strain revealed protection of mainly guanine at position −7 (G −7) and, to a lesser extent, G −6 (Fig. 3*A*, lanes 1 and 2, and *C*). Protection at position −7 by Gal4p has been reported in a previous *in vivo* footprinting study on the *GAL10*/*GAL1* promoter (32) and is predicted according to a crystallographic analysis of Gal4p bound to DNA (13). Quantitation reveals that these protections were reduced 3-fold in Δ-55 (Fig. 3*C*) where the *GAL7* promoter is inhibited by transcriptional read-through, resulting in enhanced methylation of G −7 and −6 (Fig. 3*A*, lane 3). Importantly, transcription factor binding to this site is restored on overexpression of Gal4p (Fig. 3*A*, lane 4).

These findings hold true for the upstream binding site GAL4-2, although to a lesser extent. Gal4p binding to this site was reduced consistently ≈2-fold in Δ-55. Binding of Gal4p resulted in protection of G −7, −6, and 6; these protections were enhanced in YC10-7, when Gal4p was overexpressed, at G −6 and 6 (Fig. 3 *A* and *B*). Position G 4 appeared hypersensitive, particularly in YC10-7/*GAL4*++ (Fig. 3*A*, lane 2), and this hypersensitivity was reduced in YC10-7 and Δ-55/*GAL4*++ (Fig. 3*A*, lanes 1 and 4) and completely absent in Δ-55 (Fig. 3*A*,

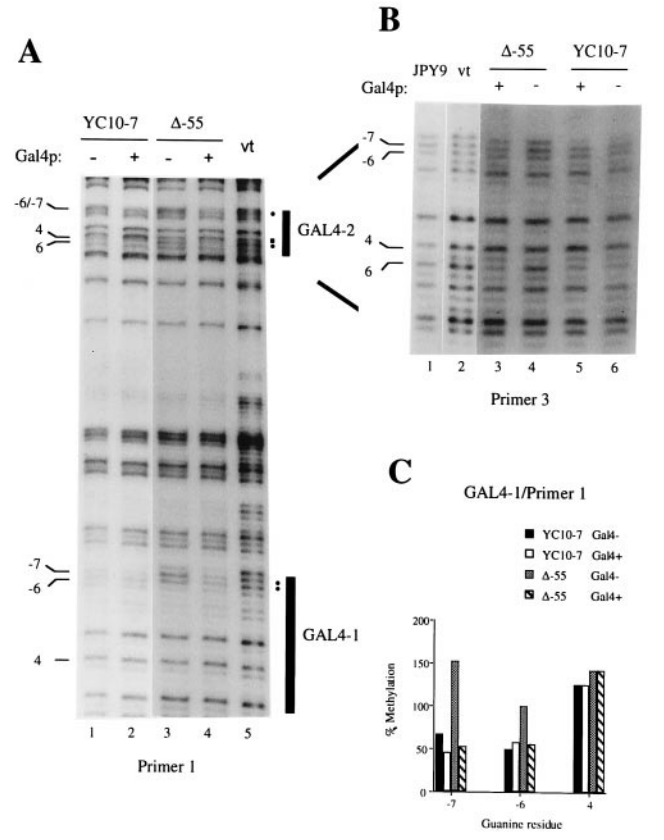

Fig. 3. *In vivo* footprinting of Gal4p sites on the upper strand. (*A*) Primer 1: both binding sites GAL4-1 and GAL4-2 are detected and are indicated by black vertical bars. DMS protections are denoted by filled circles on the right side; the respective protected guanines are shown on the left. The hypermethylated G 4 in GAL4-2 is indicated by a filled square. Lanes 1 and 2, YC10-7 in the absence and presence of extra Gal4p, respectively; lanes 3 and 4, Δ-55 in the absence and presence of extra Gal4p, respectively; lane 5, *in vitro* (vt) control (purified DNA treated with DMS *in vitro*). (*B*) Primer 3: detection of GAL4-2. Symbols used are the same as in *A*. Lane 1, footprint on DNA from the Gal4$^-$ strain JPY9; lane 2, *in vitro* (vt) control; lanes 3 and 4, Δ-55 in the presence and absence of Gal4p, respectively; lanes 5 and 6, YC10-7 in the presence and absence of Gal4p, respectively. (*C*) Quantitation of the GAL4-1 site shown in Fig. 1*A*. Shown are residues G −7, −6, and 4 (no changes were observed at position 4). The gel was scanned in a PhosphorImager. Lanes were equalized relative to a ''neutral'' guanine outside the footprinted region; values were calculated relative to the corresponding residue in the *in vitro* lane.

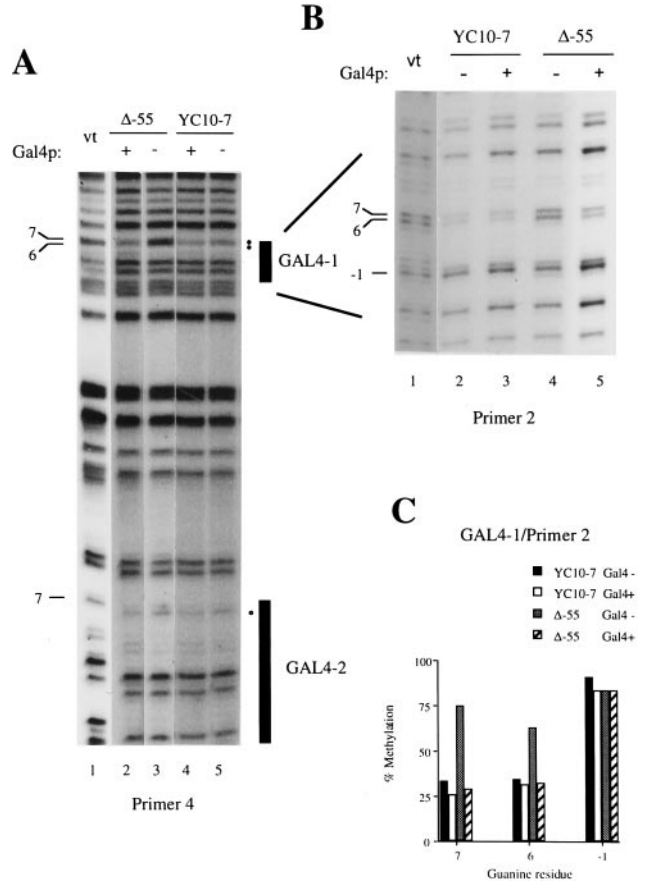

Fig. 4. *In vivo* footprinting of Gal4p sites on the lower strand. (*A*) Primer 4: both binding sites GAL4-2 and GAL4-1 are detected. Symbols used are the same as in Fig. 3*A*. The empty circle in GAL4-2 indicates that residue G 7 is unchanged in all lanes. Protected guanines are shown on the left side. Lane 1, *in vitro* (vt) control; lanes 2 and 3, Δ-55 in the presence and absence of extra Gal4p, respectively; lanes 4 and 5, YC10-7 in the presence and absence of Gal4p, respectively. (*B*) Primer 2: detection of GAL4-1. Lane 1, *in vitro* (vt) control; lanes 2 and 3, YC10-7 in the absence and presence of extra Gal4p, respectively; lanes 4 and 5, Δ-55 in the absence and presence of extra Gal4p, respectively. Note that lane 5 is more than 2-fold overrepresented (relative to lane 4). (*C*) Quantitation of the GAL4-1 site shown in *B*. Shown are residues G 7, 6, and −1 (no changes were observed at position −1). Quantitation was performed as described for Fig. 3*C*.

lane 3). Together, these results indicate that GAL4-2 binds Gal4p more weakly. The fact that footprints are also enhanced in the parental strain YC10-7 when Gal4p is overexpressed indicates that this site is fully occupied only at higher concentrations of Gal4p *in vivo*. The different binding patterns between GAL4-1 and GAL4-2 are most likely due to the deviation from the consensus in GAL4-2, resulting in a weaker binding site (see below). These *in vivo* footprinting data demonstrate that binding of Gal4p to the *GAL7* promoter in Δ-55 is disrupted by transcriptional read-through and can be restored on Gal4p overexpression.

Lower Strand. The lower strand was analyzed with primers P2 and P4 (Fig. 1*B*). Correlating with the pattern observed for the upper strand, there were clear protections over the proximal binding site GAL4-1 (Fig. 4). Again, the outer guanines G 6 and 7 were protected from methylation. Importantly, these protections were also clearly reduced in the *GAL10* read-through mutant Δ-55 (Fig. 4*A*, lane 3) and were restored when Gal4p was overexpressed in this strain (Fig. 4*A*, lane 2; see also Fig. 4*B*). In contrast, we did not detect different methylation patterns over the GAL4-2 site on this strand (Fig. 4*A*). According to previous data, we would have expected protections at residue G 7 (Fig. 1*C*; 13). As mentioned above, this half of GAL4-2 does not match the consensus sequence, which most likely results in reduced binding affinity of Gal4p to GAL4-2. Also, according to the crystal structure, contacts to C 8 are probable, which would not be apparent with DMS methylation (13). DMS methylates the N7 of guanine, the same position that is contacted by a lysine of Gal4p (Lys-18); the position of this methylation correlates with our protection patterns. Together, these data confirm the results obtained from the upper strand, showing that GAL4-1 is protected more tightly than GAL4-2; footprints are lost when the *GAL7* promoter is occluded (in Δ-55) and are restored when Gal4p is overexpressed. These results explain why overexpression of Gal4p restores growth of Δ-55 on gal (Fig. 2*A*) and how *GAL7* expression is partially restored in this strain (Fig. 2 *B* and *D*).

Although Gal4p overexpression facilitates Gal4p binding in Δ-55, full *GAL7* expression is not restored. We therefore also

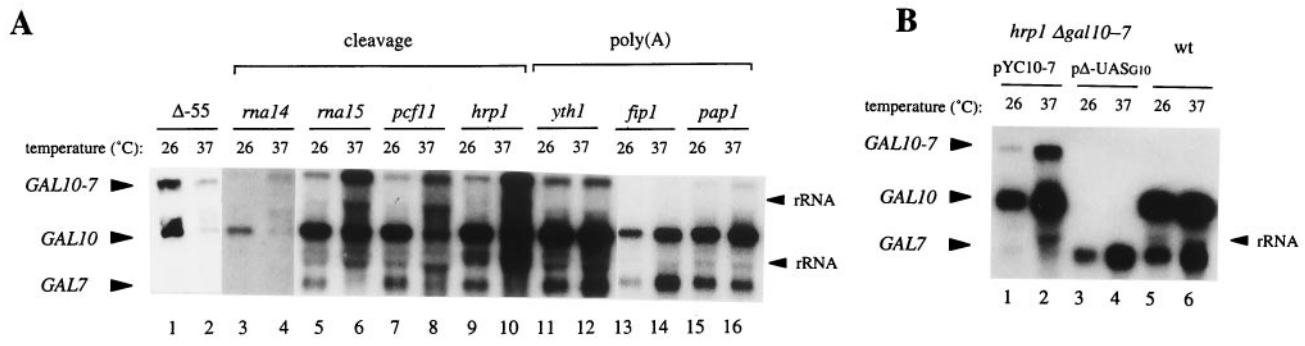

Fig. 5. The ts mRNA 3′ end CFs affect *GAL10* transcription termination. (*A*) Northern blot of total RNA from gal-induced ts strains (indicated on the top). Strains were grown at the permissive temperature (26°C), induced with gal for 1 h, and then shifted to the nonpermissive temperature (37°C) for 45 min. Because mRNA levels are overall reduced at 37°C, twice the amount of RNA was loaded for all samples at the restrictive temperature (except for Δ-55, where equal amounts were loaded; lanes 1 and 2). *GAL* transcripts were detected with a probe recognizing *GAL10* and *GAL7*. The filter was prehybridized with total RNA (10 μg/ml hybridization solution) extracted from cells grown in glucose to reduce crosshybridization to rRNA. (*B*) Northern blot of total RNA from the *hpr1 Δgal10-7* strain, transformed with *GAL* gene plasmids as indicated (lanes 1–4) versus wt (strain N222; lanes 5 and 6).

examined the basal *GAL7* promoter region by *in vivo* footprinting. Although footprints were detected over the TATA box region, no differences were observed between the occluded and functional *GAL7* promoters (data not shown). Basal transcription factors may interact more tightly with the promoter. Alternatively, because the concentration of basal factors is higher than Gal4p concentrations, displacement and rebinding might occur too fast to be detected by *in vivo* footprinting.

Mutations in mRNA 3′ End Processing Factors Affect GAL10 Termination and Result in GAL7 Promoter Occlusion in *trans*. Birse *et al.* (2) previously demonstrated that inactivation of mRNA 3′ processing factors results in impaired transcriptional termination of the *S. cerevisiae* CYC1 gene. In particular, ts mutants of mRNA 3′ end cleavage factors (CFs) affected termination at the restrictive temperature, whereas inactivation of polyadenylation factors did not (2). We therefore tested whether these ts mutations also affect termination of the *GAL* genes and analyzed the same mutations used previously as well as additional 3′ end processing factors.

As shown in Fig. 5*A*, Northern blot analysis of *GAL10* and *GAL7* mRNA in these various mutant strains shows altered mRNA profiles consistent with effects on *GAL10* termination. We previously showed that inactivation of the CFs Rna14p, Rna15p, and Pcf11p, which are all components of CF 1A (34), results in impaired *CYC1* transcriptional termination (2). Comparable to *cis*-deletion mutations of the *GAL10* poly(A) site (Δ-55; Fig. 5*A*, lanes 1 and 2), inactivation of Rna14p, Rna15p, and Pcf11p each results in loss of *GAL7* mRNA and a reduction in *GAL10* mRNA signals. Furthermore, enhanced read-through generating bicistronic *GAL10-7* mRNAs is observed. We obtained similar results for Hrp1p, which is the sole component of CF 1B. This protein, also referred to as Nab4p, has been shown to bind RNA and to participate in the cleavage reaction (35, 36). It should be noted that the ts mutations are leaky, resulting in increased read-through even at the permissive temperature. Thus, lower levels of *GAL7* mRNA are observed in these strains at 26°C (Fig. 5*A*, lanes 5, 7, 9, 11, and 15). In particular, the *rna14* ts strain grew poorly, and no *GAL7* mRNA was detectable even at the lower temperature.

The above results show that the *GAL10* and *GAL7* poly(A) sites are affected differently by the various CF 1A and CF 1B mutations. We further tested whether *GAL7* expression is affected directly by the ts mutations or indirectly by inefficient *GAL10* termination. The *GAL10-7* chromosomal locus was deleted from strains ts for CFs and then transformed with either wt pYC10-7 or pΔ-UAS_{G10} (pYC10-7 plasmid lacking the *GAL10* UAS_{G10}; ref. 10). As shown for the *hrp1* strain in Fig. 5*B*, the intact pYC10-7 plasmid gives similar results to the endogenous *GAL10-7* genes (compare Fig. 5*A*, lanes 9 and 10, with Fig. 5*B*, lanes 1 and 2). However, in the absence of *GAL10* transcription, *GAL7* signal is also present at the restrictive temperature, similar to *GAL7* mRNA levels in the wt strain (Fig. 5*B*, lanes 3–6). These results demonstrate that the *hrp1* mutation has no direct effect on the *GAL7* poly(A) site and that the loss of *GAL7* mRNA is caused by transcriptional interference. The fact that *GAL7* mRNA levels seem not to be affected by these ts mutations indicates that this poly(A) site is more robust than the *GAL10* and *CYC1* poly(A) sites.

In agreement with previous data (2), we show that ts mutations in subunits of polyadenylation factor 1, namely Yth1p and Fip1p, do not result in markedly increased transcription beyond the *GAL10* poly(A) site at the restrictive temperature. Thus, there was no significant difference in mRNA patterns at 26°C and 37°C. The *GAL10*:*GAL7* ratio of *yth1* ts did not change at 37°C, whereas the ratio even decreased in the *fip1* ts strain (2.0 at 26°C; 1.1 at 37°C). Read-through was slightly increased in the strain carrying the *pap1* ts allele at 37°C, with the *GAL10*:*GAL7* ratio shifting from 2.3 to 3.9 (Fig. 5*A*, lanes 15 and 16). Because strains carrying *yth1* ts and *pap1* ts showed some *GAL10* read-through, reduced *GAL7* mRNA levels were observed at both temperatures. Both of these factors have been suggested to play a role in transcript cleavage (L. Minvielle-Sebastia, S. Barabino, and W. Keller, personal communication).

Overall, these results confirm and extend previous data that link transcript cleavage at the poly(A) site to termination. In particular, they underline the role of CFs in transcriptional termination and show that their inactivation can result in transcriptional interference.

Discussion

In this paper, we have analyzed transcriptional interactions between the *GAL10* and *GAL7* genes of *S. cerevisiae*. This gene cluster emphasizes the need for regulated transcriptional termination and provides a genetic system for the delineation of this process. Using the Gal$^-$ phenotype of strains harboring defective *GAL10* poly(A) sites (in which expression of *GAL7* is impaired), we find that overexpression of Gal4p transcription factor restores growth of these mutants on the otherwise toxic gal substrate. Because Gal4p is expressed at very low levels (19), simply increasing the concentration of this transcription factor suffices to regain *GAL7* promoter activity and fermentation of gal. Analysis of *GAL7* mRNA and Gal7 protein reveals that Gal4p overexpression restores *GAL7* expression only partially, which is

still 20-fold lower compared with the wt strain. This finding suggests that only a fraction of Gal7p is required by the yeast cell to metabolize gal. It is possible that the excessive concentrations of Gal7p found in wt cells serve to ensure the efficient conversion of the toxic gal 1-phosphate intermediate.

In vivo footprinting of functional and occluded *GAL7* promoters reveals that *GAL10* read-through transcription impairs Gal4p–*GAL7* promoter interactions. These contacts are reestablished in mutant strains overexpressing Gal4p, explaining the Gal^+ phenotype of these cells. Because restoring Gal4p promoter occupancy does not result in wt *GAL7* levels, interference must affect other aspects of *GAL7* promoter function. However *in vivo* footprinting of the basal promoter region did not reveal major differences between YC10-7 and Δ-55 *GAL7* promoters, suggesting that interference may not affect binding of basal transcription factors. It is also known that Gal4p binds to its recognition sequence only with moderate affinity ($K_d = 2 \times 10^{-9}$ M; ref. 12). Alternatively, because basal factors are likely to be more abundant than Gal4p, the displacement/binding kinetics of these factors may not be detectable by DMS methylation. Whereas this "basal footprint" is detected only in gal-grown cells, we find that Gal4p contacts the promoter also in the uninduced state in raffinose medium (I.H.G. and N.P., unpublished results). Weak Gal4p–DNA interactions in glycerol/lactate medium have been proposed to occur on the *GAL1/GAL10* promoter (32) and were also detected by DNase I *in vivo* footprinting on the *GAL2* promoter (37). This finding suggests that prebound Gal4p recruits the basal transcription machinery on induction.

We have also extended the findings of Birse *et al.* (2) by showing that mRNA 3′ end CFs affect transcriptional termination of the *GAL10* gene. All ts CF mutants produce increased levels of bicistronic *GAL10-7* mRNA at the restrictive temperature and are impaired in *GAL7* expression. This effect is caused by the reduced activity of the *GAL10* poly(A) signal under these conditions, which results in *GAL7* promoter occlusion in *trans*. These experiments also reveal a functional difference between the *GAL7* and the *GAL10* and *CYC1* poly(A) sites. Whereas the later ones are affected strongly by mutant CFs, the *GAL7* processing element is still functional. In further agreement (2), inactivation of polyadenylation factors has only a slight effect on transcriptional termination of *GAL10*.

We find that Δ-55 reverts on gal + ethidium bromide medium and regains the ability to grow after extended incubation (ethidium bromide forces the cells to ferment gal by blocking respiration). Based on this observation, we have initiated a genetic screen to identify factors or elements involved in transcriptional termination (I.G., B. Lee, and N.P., unpublished work). The transcriptional interactions between *GAL10* and *GAL7* will allow the further characterization of transcriptional termination by RNA Pol II.

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