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# Poly(A) signals control both transcriptional termination and initiation between the tandem *GAL10* and *GAL7* genes of *Saccharomyces cerevisiae*

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**We have investigated transcriptional interactions between the *GAL10* and *GAL7* genes of *Saccharomyces cerevisiae*. Both genes are part of the galactose (*GAL*) gene cluster which is transcriptionally activated to high levels in the presence of galactose. Since *GAL7* is positioned downstream of *GAL10* and both genes are expressed co-ordinately at high levels, the possibility that *GAL10* transcription influences *GAL7* was analysed. Using transcriptional run-on assays, we show that high levels of polymerase are found in the 600 bp *GAL10–7* intergenic region that accumulate over the *GAL7* promoter. Furthermore, *GAL7* transcription is enhanced when the *GAL10* upstream activating sequence ($UAS_G$) is deleted, indicating that interference between *GAL10* and *GAL7* is likely to occur in the chromosomal locus. Deletions in the *GAL10* poly(A) signal result in complete inactivation of the *GAL7* promoter and cause a dramatic increase in bi-cistronic *GAL10–7* mRNA, predominantly utilizing the downstream, *GAL7* poly(A) site. These data demonstrate a pivotal role for the *GAL10* poly(A) site in allowing the simultaneous expression of *GAL10* and *GAL7*. In effect, this RNA processing signal has a direct influence on both transcriptional termination and initiation.**

*Keywords*: *GAL7*/*GAL10*/poly(A)/*Saccharomyces cerevisiae*/transcription/interference

## Introduction

Transcriptional control is determined largely by the promoter region of a gene. Activators and basal transcription factors assemble in an ordered fashion on the promoter to direct gene expression (reviewed in Tjian and Maniatis, 1994; Orphanides *et al.*, 1996). However, this critical process can also be influenced by the activity of an adjacent gene if transcriptional termination is impaired (Cullen *et al.*, 1984; Proudfoot, 1986; Henderson *et al.*, 1989; Greger *et al.*, 1998). This is especially true for the genome of *Saccharomyces cerevisiae* which is highly compressed, containing only short intergenic sequences (reviewed in Dujon, 1996; Springer *et al.*, 1997). In particular, the tandem arrangement of the *GAL10* and *GAL7* genes, which are induced co-ordinately to high levels, raises the possibility that termination of the upstream *GAL10* gene is required for full expression of *GAL7*. Inhibition of the *GAL7* promoter by *GAL10* interference would be lethal to the cell since *GAL7* is essential in the presence of galactose (Douglas and Hawthorne, 1964).

*GAL10* and *GAL7* are part of the structural *GAL* gene cluster of *S.cerevisiae* which also contains *GAL1* (Figure 1A; St. John and Davies, 1981). These three genes are required for metabolic conversion of galactose into glucose-6-phosphate, which is then funnelled into glycolysis (Figure 1B). The importance of this metabolic pathway is underlined both by its evolutionary conservation (Bajwa *et al.*, 1988; Webster and Dickson, 1988; Fridovich-Keil and Jinks-Robertson, 1993) and by the extreme severity of the human genetic disease galactosaemia, which is associated with mutations in these genes (reviewed in Petry and Reichardt, 1998). The regulatory pathway which leads to *GAL* gene expression is well understood (reviewed in Johnston, 1987; Johnston and Carlson, 1992; Lohr *et al.*, 1995). The key player is Gal4p, a transcriptional activator, which co-operatively

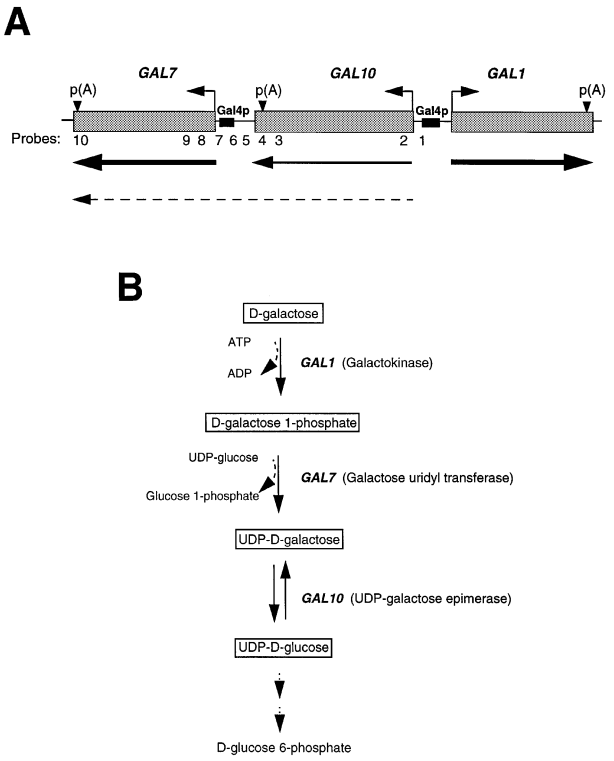


**Fig. 1.** Diagram of the *GAL* gene cluster in *S.cerevisiae*. (**A**) The three *GAL* genes (grey boxes) and their transcriptional orientation (small arrows) are indicated. Large arrows below represent the respective *GAL* transcripts, with the thickness indicating mRNA levels. The dashed arrow represents the bi-cistronic *GAL10–7* transcript. The black boxes are Gal4p-binding sites, p(A) the poly(A) sites (black triangles) and the numbers (1–10) show the position of the single-stranded M13 TRO probes. (**B**) Metabolic pathway illustrating the conversion of galactose to glucose 6-phosphate. The three enzymatic steps catalysed by the *GAL1*, *7* and *10* gene products are indicated.

binds as a dimer to sites in the promoter regions of the *GAL* genes (to $UAS_G$; Giniger and Ptashne, 1988; Xu *et al*., 1995) and activates transcription, through its C-terminus, at least 1000-fold (Giniger *et al.*, 1985). Gal4p is prevented from activating transcription by the repressor Gal80p which binds to Gal4p and blocks the activation domain in the absence of galactose (Ma and Ptashne, 1987; Leuther and Johnston, 1992).

Here we investigate potential transcriptional interactions between *GAL10* and *GAL7*. Using transcriptional run-on (TRO) analysis of permeabilized yeast cells, a high level of polymerases is detected in the *GAL10–7* intergenic region on both the chromosomal locus and on an episomal plasmid carrying *GAL10* and *GAL7*. To map the *GAL10* termination region, *GAL7* transcription was abolished by deleting the *GAL7* TATA box, thus allowing a distinction between *GAL10*- and *GAL7*-derived TRO signals. This deletion reveals that although a large fraction of polymerases terminates ~200 bp downstream of the *GAL10* poly(A) site and over the *GAL7* promoter, a fraction of polymerases continues transcription across the entire *GAL7* gene. These processive read-through polymerases produce a stable, bi-cistronic *GAL10–7* transcript which is also detected in steady-state RNA at very low levels (Figure 2A; St. John and Davies, 1981). Importantly, deletions in the *GAL10* poly(A) site region increase the fraction of processive polymerases forming the bi-cistronic transcript, which results in complete inhibition of the *GAL7* promoter. These data demonstrate a regulatory role for the *GAL10* poly(A) site which not only directs processing and termination of the *GAL10* transcript but is also required to allow initiation of transcription at the *GAL7* promoter.

## Results

### *Run-on analysis demonstrates a high level of polymerases in the GAL10–7 intergenic region*

We first analysed the induction kinetics of the three *GAL* genes (*GAL1*, *GAL10* and *GAL7*) following addition of galactose to the medium. A previous study, in which the kinetics of *GAL10* and *GAL7* mRNA accumulation were determined, showed that *GAL7* is induced before *GAL10* mRNA (St. John and Davies, 1981). Our data confirm and extend these earlier results. As shown in the Northern blot analysis presented in Figure 2A, *GAL7* mRNA is detected ~5 min after galactose induction, whereas *GAL1* and *GAL10* mRNAs appear ~10 min after induction. A low level of bi-cistronic *GAL10–7* mRNA is also detectable at later times of induction, as has been reported previously (St. John and Davies, 1981). *GAL7* mRNA is detected at ~2-fold higher levels than *GAL10* mRNA, throughout the time course. These higher levels are likely to be due to a more active *GAL7* promoter and not differences in mRNA stability, as *GAL7* mRNA actually decays more rapidly in a glucose shut-off experiment than does *GAL10* mRNA (Greger, 1998).

To investigate possible transcriptional interactions between *GAL10* and *GAL7*, we determined the polymerase distribution over these genes by TRO analysis of the endogenous *GAL* genes. This was performed on whole yeast cells, permeabilized with the anionic detergent Sarkosyl (Elion and Warner, 1986; Akhtar *et al.*, 1996; Birse *et al.*, 1997; Birse *et al.*, 1998). Prior to galactose induction, cells were grown in a raffinose-containing medium, since under these conditions the *GAL* genes are neither repressed nor transcriptionally active. Induction was then carried out by addition of 2% galactose. Nascent transcription was detected by hybridization to single-stranded M13 DNA probes of similar length (Figure 1A; Table I), covering the entire intergenic region, as well as the 5′ and 3′ ends of *GAL10* and *GAL7*, respectively. The endogenous TRO data are shown in Figure 2B. Surprisingly, following galactose induction, signals were detected not only over the *GAL10* and *GAL7* structural genes but also over the entire intergenic region (probes 5–7), indicating the presence of polymerases in this region. Indeed, the signals obtained with the two promoter proximal probes (probes 2 and 8) were relatively low compared with these intergenic signals. Some of the extended *GAL10* transcripts may read through the entire *GAL7* gene and so generate the bi-cistronic *GAL10–7* mRNA detected in the Northern blot analysis shown in Figure 2A.

To control for signal specificity, cells grown in 2%

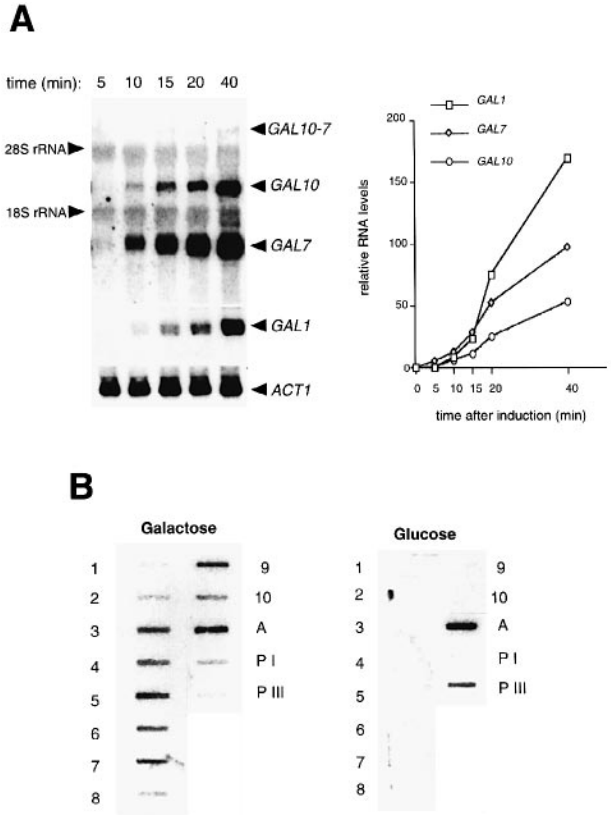


**Fig. 2.** Induction kinetics and TRO analysis of the chromosomal *GAL* gene cluster. (**A**) Northern blot of RNA from galactose-induced cells at various times after induction. *GAL10* and *GAL7* RNA was detected with a 1.42 kb probe for both genes and the intergenic region. The same RNA sample was probed with a separate 1.32 kb *GAL1–10*-specific probe as well as a probe hybridizing to actin (*ACT1*). Cross-hybridization to rRNA is indicated. In the graphic representation, the length of the region hybridizing to the labelled probe was taken into account. *GAL1* signals were normalized to *GAL10* signals present in both blots, and signals were also normalized to the *ACT1* loading control. (**B**) TRO of the chromosomal *GAL* locus. The left panel shows a filter probed with RNA from galactose-induced cells while the right panel shows RNA from the same culture grown in glucose. The numbers refer to the probes shown in Figure 1A. 'A' denotes actin-, 'PI' Pol I- and 'PIII' Pol III-specific probes.

**Table I.** TRO M13 probes

| Probes | Location[a] | U content (%) | G/C content (%) |
|---|---|---|---|
| 1 | –383/–200 (183) | 25 | 54 |
| 2 | 139/316 (177) | 32 | 39 |
| 3 | 1870/2055 (185) | 33 | 38 |
| 4 | 2042/2231 (189) | 38 | 33 |
| 5 | –574/–401 (173) | 27 | 42 |
| 6 | –422/–233 (189) | 35 | 36 |
| 7 | –246/–71 (175) | 27 | 41 |
| 8 | 55/232 (177) | 24 | 43 |
| 9 | 294/474 (180) | 30 | 37 |
| 10 | 1090/1271 (181) | 41 | 26 |

[a]The positions of the TRO probes are given relative to the transcriptional start sites, with their length in nucleotides in parentheses. Probes 1–4 are relative to the *GAL10* start site, probes 5–10 are relative to the *GAL7* start site.

glucose, which tightly represses the *GAL* genes (reviewed in Johnston and Carlson, 1992), were also analysed. No *GAL*-specific signals (probes 2–10) were obtained, whereas signals hybridizing to actin (A), rRNA (PI) and tRNA (PIII) probes were all detected (Figure 2B). The higher tRNA signal for the glucose TRO was consistent and may reflect different metabolic conditions of the cell. These data demonstrate the presence of transcriptionally active polymerases in the *GAL10–7* intergenic region, dependent on induction of the *GAL* gene cluster by galactose. We also note that the levels of nascent transcript over the *GAL7* gene appear to be similar to those for *GAL10*, even though the concentration of steady-state *GAL7* mRNA is higher than *GAL10* mRNA (Figure 2A). This difference may reflect the galactose induction time employed for the TRO analysis (12 h) since we have observed that shorter galactose induction times (10 min) result in predominant *GAL7* TRO signals (Greger, 1998). A similar discrepancy between steady-state and nascent RNA levels has been reported previously (Akhtar *et al.*, 1996).

### *Accumulation of polymerases in the GAL10–7 intergenic region is enhanced when the GAL7 promoter is active*

To allow a detailed analysis of nascent transcription between the *GAL10* and *GAL7* genes, they were cloned into plasmids and transformed into a *gal10*$^-$/*gal7*$^-$ deletion strain. Since the Gal4p transactivator is present in limiting amounts in the cell (Griggs and Johnston, 1993), centromeric (pYC) plasmids (Sikorski and Hieter, 1989), which are stably maintained at one or two copies per cell, were employed. As we detect nascent transcription in the *GAL10–7* intergenic region, overlapping transcription that initiates on the *GAL7* promoter, we first investigated *GAL10*-derived transcription in a construct that has the *GAL7* promoter inactivated by deletion of its TATA box. Deletion of the *GAL7* TATA box, 64 bp upstream of the initiation site, is known to abolish expression of a *GAL7–lacZ* reporter construct (Tajima *et al.*, 1986). Furthermore, a strain carrying this *GAL7* allele (pΔ-TATA$_{G7}$) in a *gal10*$^-$/*gal7*$^-$ genetic background is not viable on galactose since the metabolic intermediate galactose 1-phosphate (the substrate of Gal7p) is toxic to the cell (Douglas and Hawthorne, 1964).

Figure 3A shows TRO analysis of yeast transformed

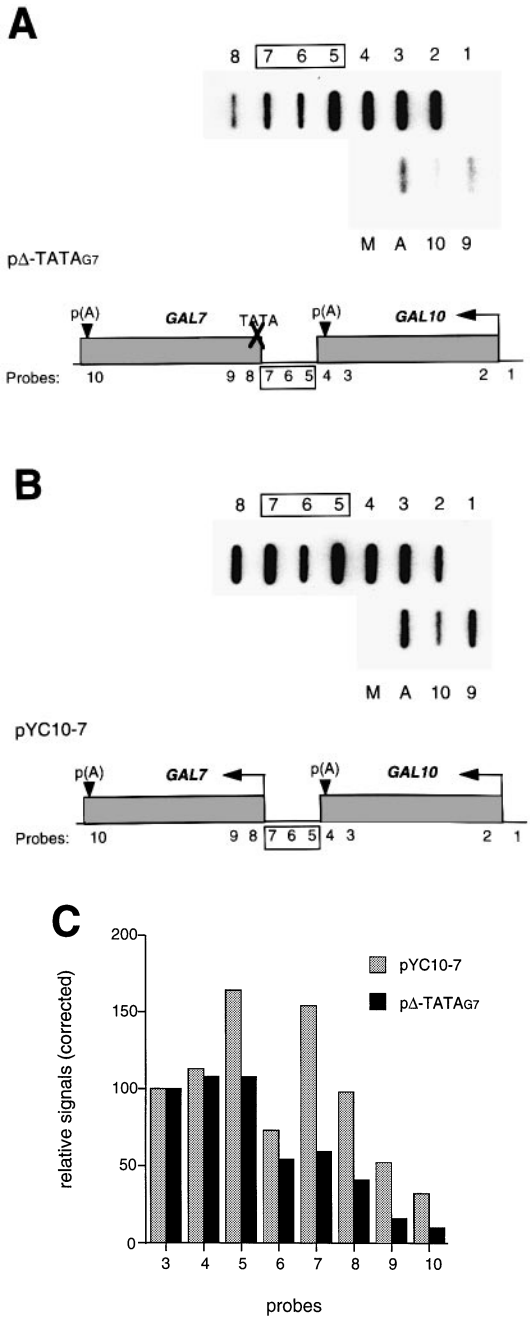


**Fig. 3.** TRO analysis of transformed *GAL10–7* plasmids. (**A**) TRO of pΔ-TATA$_{G7}$-transformed cells. The probes are as described in Figure 1A. 'M' is an M13 probe without insert serving as a negative hybridization control. The boxed numbers (5–7) represent probes spanning the intergenic region. The diagram below shows the *GAL10* and *7* genes (grey boxes) and their orientation of transcription in the cluster. The black cross denotes deletion of the *GAL7* TATA box. (**B**) TRO of pYC10-7-transformed cells. Details are as for (A). (**C**) Bar graph of the data shown in (A) and (B). The signals were quantified in a PhosphorImager (Molecular Dynamics). The values were corrected for background hybridizations (detected by the 'M' probe). Signals were also corrected for their G/C and their U content. For a direct comparison, signals were plotted as the percentage of probe 3 (set at 100%).

with pΔ-TATA$_{G7}$. As indicated, strong signals are detected over the three probes (probes 2–4) that cover the *GAL10* structural gene, but not over probe 1 (covering the *GAL10* promoter) that gives only background signals, at a similar level to the M13 control probe (M). Beyond the *GAL10*

gene, full signals are detected by the first intergenic probe (probe 5) but then they drop off initially by 50–55% over probes 6 and 7 and then to lower levels through the *GAL7* promoter and structural gene (probes 8–10). Quantitation of these data (Figure 3C) indicates that ~50% of polymerases initiated on the *GAL10* promoter terminate ~200 bp downstream of the *GAL10* poly(A) site, in the intergenic region. However, surprisingly, a significant fraction of polymerase reads past this termination region into the *GAL7* gene, apparently terminating heterogeneously.

TRO analysis of pYC10-7, which contains intact *GAL10* and *GAL7* genes, confirms the endogenous data (Figure 2B), with polymerases localized in the intergenic region (Figure 3B). However, in contrast to pΔ-$TATA_{G7}$, transcripts that escape termination immediately following the *GAL10* poly(A) signal now clearly accumulate over the active *GAL7* promoter. Thus probe 7, which contains the *GAL7* $UAS_G$, gives an enhanced signal. A slight accumulation in signal is also apparent over this probe with pΔ-$TATA_{G7}$, which still contains an intact $UAS_G$. We speculate that physical contact between Gal4p activators and the basal transcription machinery (Melcher and Johnston, 1995) might cause an impediment to elongating polymerases and thus result in the enhanced accumulation in pYC10-7. The *GAL7* structural gene probes 8–10 also give significant signals, although there is a marked polarity, with less transcript detectable at the 3′ end of this gene. Another reproducible difference between the pYC10-7 and pΔ-$TATA_{G7}$ profiles is that the first *GAL10* probe 2 gives a lower signal when both *GAL* genes are active. We also observe a similar lower signal for probe 2 in the endogenous TRO analysis presented in Figure 2B. This may result from competition effects between the two *GAL* gene promoters for limiting amounts of Gal4p transcription factor.

As described above, *GAL7* mRNA levels are ~2-fold higher than those of *GAL10* (Figure 2). However, we are surprised to note that the TRO signals in Figure 3B are lower for *GAL7* than *GAL10*. It is possible that a 'hand-over' of polymerases from the intergenic region, rather than *de novo* recruitment to the promoter, might operate in this system and explain this apparent difference.

### *GAL10 transcription reduces initiation from the GAL7 promoter*

To analyse the effect of *GAL10* transcription on the *GAL7* promoter and to confirm the origin of the intergenic TRO signals, the *GAL10* $UAS_G$ containing four Gal4p-binding sites was deleted. As expected, this deletion drastically reduces *GAL10* transcription (Figure 4A). The nascent transcription profile obtained from pΔ-$UAS_{G10}$ clearly shows that all intergenic signals derived from the *GAL10* promoter, since they are virtually abolished in the $UAS_{G10}$ mutant (Figure 4A and B). A low level of polymerases was detected mainly over the *GAL10* gene (probes 2, 3 and 4), which is due to basal (enhancer-independent) transcription. A strain carrying this allele was viable on galactose, although growth rates are reduced (Greger, 1998). Interestingly, on longer exposure of the TRO analysis (Figure 4B), it is clear that a decrease in signal occurs immediately after the poly(A) site (over probe 4), rather than after probe 5 as seen when the *GAL10* promoter is fully active (Figure 3A). This suggests that the site of *GAL10* termination depends on the efficiency of the *GAL10* promoter. Possibly a more active promoter generates more processive Pol II elongation complexes and thus termination occurs further downstream of the gene's poly(A) signals.

Analysis of steady-state RNA from pΔ-$UAS_{G10}$ revealed that *GAL7* mRNA levels increased up to 3-fold when compared with pYC10-7 (and normalized to *ACT1*). As predicted, *GAL10* mRNA levels drop ~20-fold due to inactivation of the $UAS_{G10}$. These data indicate that transcription initiated on the *GAL10* promoter, which traverses the *GAL7* gene to form the bi-cistronic *GAL10–7* mRNA (Figure 2A), significantly reduces initiation from the *GAL7* promoter (see below). These results therefore demonstrate that *GAL10* transcription can interfere directly with the *GAL7* promoter.

### *Analysis of the GAL10 poly(A) site*

We next analysed the *GAL10* poly(A) site since a role for poly(A) signals in the transcriptional termination process has been demonstrated repeatedly in higher eukaryotes (Citron *et al.*, 1984; Whitelaw and Proudfoot, 1986; Logan *et al.*, 1987; Connelly and Manley, 1988) and in yeast (Russo and Sherman, 1989; Russo, 1995; Birse *et al.*, 1997, 1998). We initially mapped the *GAL10* poly(A) site by RT–PCR analysis and identified a major site positioned ~100 bp downstream of the *GAL10* translational stop codon (Figure 5A) with three other minor sites positioned close by. These results have been confirmed by S1 nuclease mapping (Greger, 1998). The sequence around the *GAL10* poly(A) sites is particularly AT rich, as is often the case for *S.cerevisiae* poly(A) signals (reviewed in Guo and Sherman, 1996).

To determine the sequence requirements of the *GAL10* poly(A) site, competition with the *GAL7* poly(A) site was increased by deleting sequences between these two poly(A) sites in pYC10-7 (Figure 5A). As indicated, most of the *GAL7* gene has been removed in Δ-1160, so that the 3′ endpoint of the deletion is located only 167 bp upstream of the *GAL7* poly(A) site, while the 5′ endpoint is 534 bp downstream of the *GAL10* poly(A) site. Therefore, a space of 687 bp remains between the two poly(A) sites, mainly comprising the *GAL10–7* intergenic region. As shown in Figure 5B (lane 5), the *GAL10* poly(A) site is used exclusively in Δ-1160. No read-through to the *GAL7* poly(A) site can be detected. In Δ-1674, all the downstream region of the *GAL10* poly(A) site is deleted so that the two poly(A) sites are only 167 bp apart (Figure 5A). Even in this construct, the upstream *GAL10* poly(A) site was still used exclusively (Figure 5B, lane 4). These data demonstrate that there are no sequence requirements downstream of the *GAL10* poly(A) site for its efficient utilization *in vivo*. In contrast, processing at this poly(A) site required downstream sequences *in vitro* (Sadhale and Platt, 1992).

Since elements directing mRNA 3′-processing and transcriptional termination are positioned upstream of the cleavage site in the majority of *S.cerevisiae* genes (reviewed in Guo and Sherman, 1996), deletions were also introduced into the *GAL10* 3′-untranslated region (3′-UTR) in pΔ-1160, as shown in Figure 5A. All three *GAL10* 3′-UTR deletions dramatically enhanced read-

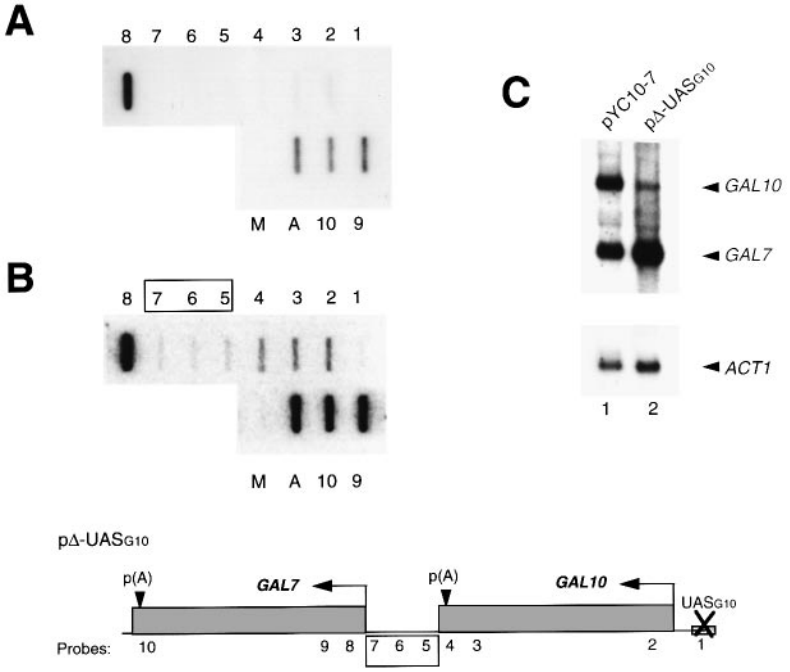


**Fig. 4.** Transcriptional interference between *GAL10* and *GAL7* genes. (**A**) TRO on pΔ-$UAS_{G10}$-transformed cells. Details are as for Figure 3A. (**B**) A longer exposure of (A). The diagram below shows *GAL10–7* as in Figure 3A. The black cross denotes deletion of the *GAL10* $UAS_G$. (**C**) Northern blot of pYC10-7 (lane 1) and pΔ-$UAS_{G10}$ (lane 2) total RNA. The *GAL10–7* probe was as in Figure 2A. The membrane was stripped and reprobed with an actin-specific probe (*ACT1*). *GAL7* mRNA levels in lane 2 were >3-fold increased relative to lane 1. The *GAL10* signal in lane 2 was ~20-fold reduced relative to the signal in lane 1, but could not be quantitated accurately due to high background.

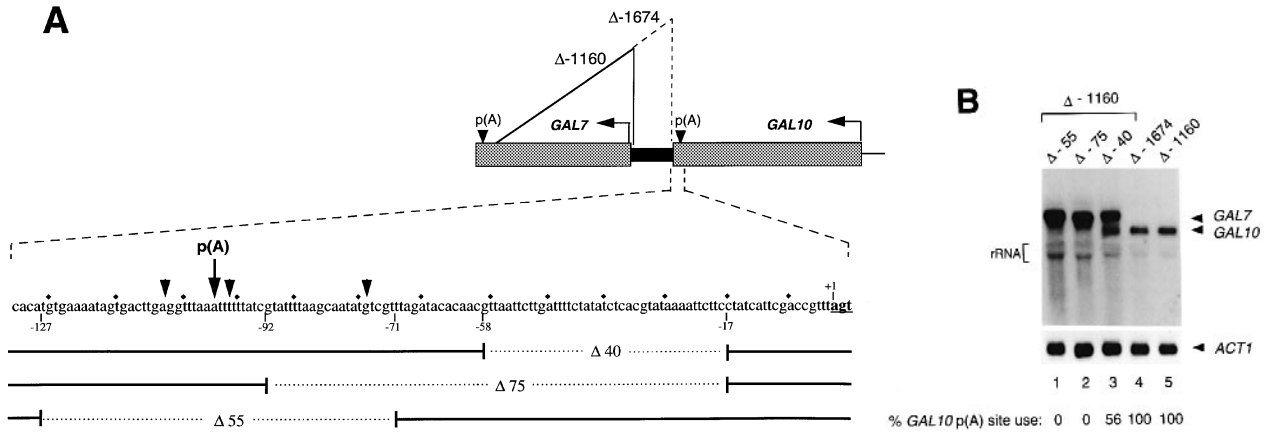


**Fig. 5.** Effect of mutating the *GAL10* poly(A) signal. (**A**) Sequence of the *GAL10* 3′-UTR. The diagram above shows the position of the 3′-UTR relative to the *GAL10–7* genes. The downstream deletions to the *GAL10* poly(A) site are indicated above the diagram. The exact positions of the upstream deletions are shown below, with the stippled lines representing the region deleted. The arrowheads point to minor poly(A) sites and the arrow to the major poly(A) site. The numbers below the sequence denote the distance from the *GAL10* UGA stop codon, with the A taken as +1. (**B**) Northern blot of RNAs from cells transfected with the *GAL10* poly(A) site deletion plasmids shown in (A). Δ-40, Δ-55 and Δ-75 are based on Δ-1160. The *GAL10–7* probe used was as in Figure 2A. The membrane was stripped and reprobed with an actin-specific probe (*ACT1*). The percentage 3′ end formation (in lane 3) was calculated as the percentage of the lower band to the total amount of RNA detected, and normalized to the length of the region hybridizing to the probe.

through to the *GAL7* poly(A) site (Figure 5B). Δ-55 and Δ-75 resulted in 100% use of the downstream poly(A) site (lanes 1 and 2), while Δ-40 had an intermediate effect, with the *GAL10* poly(A) site still functioning at 56% (lane 3).

### *GAL10* poly(A) signal deletions generate *GAL10–7* bi-cistronic mRNA and abolish *GAL7* promoter activity

The effect of the *GAL10* 3′-UTR poly(A) signal deletions was investigated further in the context of the otherwise intact *GAL10* and *GAL7* genes. Analysis of these *GAL10* poly(A) site deletions, incorporated into pYC10-7 by Northern blotting, revealed greatly increased levels of *GAL10–7* bi-cistronic mRNA. Most importantly, formation of these transcripts resulted in complete inhibition of *GAL7* transcription (Figure 6A). In Δ-55 and Δ-75, where the level of read-through transcripts increased ~40-fold relative to pYC10-7 (normalized to *ACT1*), *GAL7* mRNA signal was virtually abolished (Figure 6A, lanes 2 and 3). Δ-40, as before, had an intermediate effect, with *GAL7* mRNA levels decreasing ~3-fold (relative to pYC10-7;

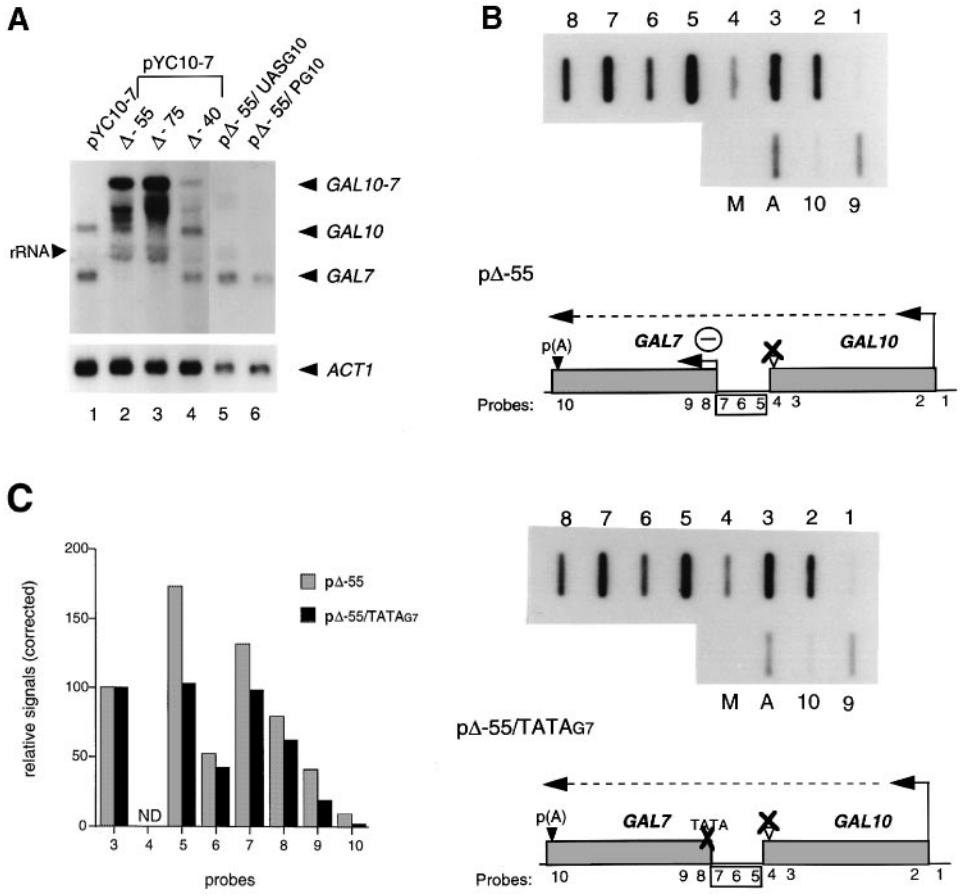


**Fig. 6.** The *GAL10* poly(A) signal regulates GAL7 promoter activity. (**A**) Effect of the *GAL10* 3′-UTR deletions on *GAL7* transcription. Northern blot of pYC10-7 (lane 1), pΔ-55 (lane 2), pΔ-75 (lane 3), pΔ-40 (lane 4), pΔ-55/$UAS_{G10}$ (lane 5) and pΔ-55/$P_{G10}$ (lane 6) RNA. Lanes 5 and 6 derive from a separate experiments and the signals are lower overall (compare *ACT1* signal). The *GAL10–7* probe was described in Figure 2A. The membrane was stripped and reprobed with the actin-specific probe (*ACT1*). (**B**) TRO analysis of pΔ-55 and pΔ-55/$TATA_{G7}$. The long dashed arrows indicate the bi-cistronic *GAL10–7* transcript, the minus sign inhibition of the *GAL7* promoter, and the black crosses deletions of the *GAL10* poly(A) signal and *GAL7* TATA box, respectively. (**C**) Bar graph of the data shown in (B). The signals were quantified in a PhosphorImager (Molecular Dynamics). The values were corrected for background hybridizations (detected by the 'M' probe). Signals were also corrected for their G/C and U content. For a direct comparison, signals were plotted as the percentage of probe 3 (set at 100%).

compare lanes 1 and 4) while the read-through transcript increased ~5-fold. In effect, the *GAL7:GAL10* ratio in Δ-40 was reversed, compared with pYC10-7, due to the reduction in *GAL7* RNA levels. These data demonstrate that the effect of inactivating the *GAL10* poly(A) signal is to cause polymerase complexes to remain highly processive, reading through the *GAL7* gene and so generating *GAL10–7* bi-cistronic mRNA. This has the added effect of completely inactivating the *GAL7* promoter by transcriptional interference.

It should also be noted that in each of the pYC10-7 plasmids with the *GAL10* poly(A) signal deletions, shorter mRNA species as well as the bi-cistronic *GAL10–7* mRNA are generated. In particular, an RNA 3′ end close to the deleted major *GAL10* poly(A) site was formed in Δ-55 (Figure 6A, lane 2) as well as longer transcripts in both Δ-55 and Δ-75 using different cryptic poly(A) sites further downstream. The lowest bands detected in lanes 2 and 3 are, as before, due to cross-hybridization to rRNA. Presumably the DNA probes used to detect these *GAL* mRNAs have weak homology to rRNA.

It was necessary finally to prove that inhibition of the *GAL7* promoter by deletions to the *GAL10* poly(A) signal was caused by *GAL10* transcription *per se*, rather than by deletion of an essential *GAL7* promoter element. Elements important for full *GAL7* promoter activity have been mapped previously (Tajima *et al.*, 1986). The 5′ border delineated in this study mapped to a position ~260 bp upstream of the *GAL7* start site. Since the *GAL10* poly(A) site deletions are positioned ~600 bp upstream of the *GAL7* start site, no direct influence on promoter activity was expected to arise from these deletions. The *GAL10* promoter was deleted in pΔ-55 and the *GAL* mRNA produced was analysed (Figure 6A). Two deletions were generated, Δ-55/$UAS_{G10}$ and Δ-55/$P_{G10}$, which remove the *GAL10* $UAS_G$ or the entire *GAL10* promoter, respectively. As expected, both deletions abolished *GAL10* mRNA but at the same time restored *GAL7* mRNA synthesis (lanes 5 and 6). These data demonstrate a direct interaction between the *GAL10* and *GAL7* promoters.

### *The occluded GAL7 promoter in pΔ-55 resembles a non-functional promoter at the TRO level*

We also carried out TRO analysis on pΔ-55 (Figure 6B) to demonstrate directly the effect of the poly(A) site deletion on *GAL10* termination. As predicted, pΔ-55 resulted in read-through nascent transcription. While probe 4 gave a reduced signal, consistent with the deletion to a

part of this sequence (Δ-55), probes 5–10 gave signals at levels similar to the parent pYC10-7 plasmid. We presume that the read-through transcripts that derive from the *GAL10* gene [lacking a poly(A) signal] give a polymerase profile similar to transcripts that initiate on the *GAL7* promoter. Interestingly, a comparative TRO analysis of the double mutant plasmid, pΔ-55/$TATA_{G7}$ gives a nearly identical pattern, although signal intensities over probes 7 and particularly 5 were less pronounced in pΔ-55/$TATA_{G7}$ (Figure 6B and C). This similarity was surprising since signals over the *GAL7* promoter region were clearly reduced in pΔ-$TATA_{G7}$ (Figure 3A and C). A possible explanation is the increased concentration of polymerases in the intergenic region in pΔ-55/$TATA_{G7}$. The fact that no signal over probe 7 is seen in pΔ-$UAS_{G10}$ strengthens this observation, since the low level of read-through polymerases in this construct results in virtually no accumulation (Figure 4A and B). The similarity between the pΔ-55 and the pΔ-55/$TATA_{G7}$ profile suggests that the occluded *GAL7* promoter in pΔ-55 resembles a non-functional promoter with a deleted TATA box, at the TRO level.

We also note the low level of polymerases over probes 9 and 10 (Figure 6B and C), which is surprising considering the increased level of read-through at the steady-state mRNA level (Figure 6A). RNA half-life analysis revealed that the bi-cistronic transcript is relatively stable (compared with the *GAL10* and *GAL7* monocistronic transcripts), which may partly explain this discrepancy (Greger, 1998). The polymerase profiles also demonstrate that termination of *GAL10* occurs to a large extent in the intergenic region and over the *GAL7* promoter. We conclude that inactivation of the *GAL10* poly(A) signal has a direct inhibitory effect on the *GAL7* promoter at both nascent and steady-state levels.

## Discussion

The highly compressed genomes of lower eukaryotes pose a particular problem for gene transcription. It seems plausible that there must be a tight requirement for efficient transcriptional termination following a gene's poly(A) signal to prevent transcriptional overlap between adjacent genes. Indeed, we recently have demonstrated such an efficient termination process for the *CYC1* gene of *S.cerevisiae* and the *ura4* gene of *Schizosaccharomyces pombe* (Birse *et al.*, 1997, 1998). The consequences of inefficient termination may vary depending on gene positions. As revealed by the *S.cerevisiae* genome sequence, genes may be positioned either divergently or convergently (reviewed in Dujon, 1996). In the former case, they may share promoter and enhancer elements (such as *GAL1* and *GAL10*), while in the latter case failure to terminate transcription may result in the generation of overlapping antisense transcripts. We recently have discovered examples of such antisense transcripts in the *S.pombe* genes *nmt1* and *nmt2*, which both transcribe across downstream, antisense genes (Hansen *et al.*, 1998). Surprisingly, neither downstream gene is affected significantly, which may indicate that mechanisms exist to unravel such transcriptional overlap (Hansen *et al.*, 1998). Where adjacent genes transcribe in the same direction, lack of transcriptional termination by the upstream gene may result in inhibition of the downstream gene's promoter, as shown in both prokaryotes and eukaryotes (Adhya and Gottesman, 1982; Cullen *et al.*, 1984; Bateman and Paule, 1988; Henderson *et al.*, 1989; Eggermont and Proudfoot, 1993). We have demonstrated previously that this inhibition may be caused by read-through transcripts, perturbing the association of transcription factors to a downstream positioned promoter (Greger *et al.*, 1998). A recent study in *S.cerevisiae* has shown that placing a strong promoter (derived from the *ACT1* gene) upstream of the *ARO4* poly(A) signal resulted in inhibition of the downstream positioned *HIS7* gene, especially when the poly(A) site was inactivated by deletion mutation (Springer *et al.*, 1997). This raised the possibility that such promoter inhibition may occur in a physiological gene context. In the present study, transcriptional termination of the *GAL10* gene and the possible effect of *GAL10* transcription on the adjacent *GAL7* promoter have been investigated. Since these genes are induced at very high levels, they provide a clear physiological case for transcriptional interaction between adjacent genes

We have detected polymerases in the 600 bp *GAL10–7* intergenic region by TRO analysis in the chromosomal locus and on the centromeric plasmid pYC10-7. To map the *GAL10* termination region, *GAL7*-derived TRO signals were abolished by deleting the *GAL7* TATA box in pYC10-7. This deletion completely inactivates *GAL7* transcription, based both on genetic analysis and on the absence of detectable *GAL7* mRNA (Greger, 1998). Although >50% of *GAL10* transcription terminates ~200 bp downstream of its poly(A) site and another fraction in the *GAL7* promoter region (Figures 3A and C, and 6B and C), a small fraction of Pol II elongation complexes continue transcription and traverse the entire *GAL7* gene. This class of polymerases produced a *GAL10–7* bi-cistronic transcript, which can be detected in steady-state mRNA at very low levels (Figure 2A; St. John and Davies, 1981). Importantly, the region of termination appeared to be determined by promoter strength, since in pΔ-$UAS_{G10}$ (the *GAL10* $UAS_G$ deletion), signals decreased immediately after probe 4, directly downstream of the *GAL10* poly(A) site. The level of read-through polymerases is also reduced drastically in this construct (Figure 4B).

We also found that accumulation of *GAL10*-initiated polymerases in the *GAL7* promoter region (especially over probe 7) was, to some extent, dependent on a functional *GAL7* promoter, since it was markedly reduced in pΔ-$TATA_{G7}$, when compared with pYC10-7 (Figure 3). The TATA deletion removed eight nucleotides (5′-$ATATA_4$-3′) outside of probe 7, and thus cannot have affected the hybridization efficiency of the probe. Since probe 7 is positioned upstream of the *GAL7* coding region, the enhanced signal in pYC10-7 does not reflect *GAL7* transcription. Moreover, the conditions used for the TRO (0.5% Sarkosyl) should inhibit re-initiation during the transcriptional pulse (Hawley and Roeder, 1985). The polymerases that accumulate over the *GAL7* promoter region might be stalled by Gal4p activators contacting the basal *GAL7* transcription apparatus (Melcher and Johnston, 1995).

*GAL7* mRNA is more abundant than *GAL10* RNA at the steady-state level, which is not due to RNA stability. Since polymerases are localized in the *GAL7* promoter

region, *de novo* recruitment to the *GAL7* promoter might not be as critical as for the *GAL10* promoter, which may explain the higher *GAL7* steady-state mRNA levels, analogous to the 'hand-on' mechanism suggested for the Pol I gene system (Mitchelson and Moss, 1987). It should also be noted that *GAL10* mRNA was up to 2-fold more abundant (relative to *GAL7*) when transcribed from pYC10-7 than from the endogenous *GAL* locus. We suspect that the adjacent *GAL1* promoter negatively affects *GAL10* transcription in the endogenous *GAL* locus, which in turn would reduce the effect of *GAL10* impinging on *GAL7*. It is probable that in the endogenous *GAL* cluster, transcription is well balanced, thus allowing full expression of the essential *GAL7* gene. We currently are testing this model.

That the *GAL10* poly(A) site contributes a crucial role to this balance is demonstrated in Figure 6A. All deletions in the *GAL10* poly(A) region dramatically reduced *GAL7* expression. A mechanism whereby the highly processive *GAL10*-initiated Pol II complex displaces transcription factors from the *GAL7* promoter is possible (Greger *et al.*, 1998). It should be noted that the *GAL10* poly(A) site deletion (Δ-55) did not result in increased levels of polymerases at the 3′ end of the *GAL7* gene (Figure 6B and C). Termination of *GAL10* transcription still occured, to a large extent, in the intergenic region and over the *GAL7* promoter, similar to the transcription profiles shown in Figure 3. This may be partly explained by the utilization of cryptic poly(A) sites in pΔ-55 (Figure 6A). The effect of the poly(A) signal deletions described here clearly demonstrates the central role of this poly(A) signal in controlling both initiation and termination of transcription.

## Materials and methods

### Strains and plasmids

The yeast strain used in all experiments was BY4732, a S288C derivative, which carried a *ura3* Δ0 deletion (Brachmann *et al.*, 1998). The *GAL10* and *GAL7* genes from this strain were replaced with a linear *URA3* cassette, containing the *URA3* gene flanked on the 5′ side by 170 bp from the 3′ end of *GAL7* (32 bp upstream of the *GAL7* stop codon and extending 170 bp further 3′ of *URA3*) and on the 3′ side by 190 bp of the *GAL10* promoter (208 bp upstream of the *GAL10* start codon, extending 190 bp further 5′ of *URA3*). This gene replacement was verified by Southern blot analysis.

pYC10-7 was constructed by inserting a 4814 bp *GAL10–7* fragment (generated by PCR with *Bam*HI linker-containing primers, hybridizing 397 bp upstream of the *GAL10* start codon and 491 bp downstream of the *GAL7* stop codon; DDBJ/EMBL/GenBank accession No. X81324) into the *Bam*HI site of YCplac22 (Sikorski and Hieter, 1989). pΔ-$TATA_{G7}$ was constructed by long-range PCR of pYC10-7, precisely deleting 5′-ATATAAAA-3′, 87 bp upstream of the *GAL7* start codon. pΔ-$UAS_{G10}$ was constructed by inserting a 4556 bp *Bam*HI–*Ban*II *GAL10–7* fragment into the *Bam*HI site of YCplac22. The *Ban*II site lies 136 bp upstream of the *GAL10* start codon and so excludes all Gal4p-binding sites. Δ-40, Δ-55 and Δ-75 are pYC10-7 derivatives, constructed by long-range PCR round the plasmid. The extent of the deletions is indicated in Figure 5A. The poly(A) competition construct, Δ-1160, was constructed by long-range PCR of pYC10-7, with the *GAL10–7* region from 92 bp upstream of the *GAL7* start codon to 1067 bp downstream of the *GAL7* start codon deleted. Δ-1674 is a Δ-1160 derivative, where the *GAL10–7* intergenic region (606 bp upstream of the *GAL7* start codon) was deleted. Δ-1160/Δ-40, Δ-55 and Δ-75 are Δ-1160 derivatives with the deletions shown in Figure 5.

### Northern blots

Total RNA was extracted from exponentially growing cells ($OD_{600}$ = 0.5) by the hot phenol method (Köhrer and Domdey, 1991). Total RNA (8–10 μg) was separated on 1.5% formaldehyde agarose gels at 30 V o/n, hybridized to nylon membranes (Hybond-NX; Amersham) in 20× SSC and probed with random-primed (Boehringer Mannheim) [$^{32}$P]DNA fragments. *GAL10–7* transcripts were detected with a 1.42 kb probe containing 343 bp of *GAL10* 3′ sequence and 484 bp of *GAL7* 5′ sequence (including the *GAL10–7* intergenic region). *GAL1*- and *GAL10*-specific transcripts were detected using a 1.33 kb probe containing 415 bp of *GAL1* 5′ sequence and 318 bp of *GAL10* 5′ sequence (including the *GAL1–10* intergenic region). Blots were stripped and reprobed with an *ACT1*-specific probe. The *ACT1* probe was a 567 bp fragment containing 277–844 bp 3′ of the *ACT1* start codon. For quantitation, blots were scanned in a PhosphorImager (Molecular Dynamics) and the bands quantified, taking into account the length of the region hybridizing to the labelled probe.

### Transcription run-on analysis

The lengths and position of single-stranded M13 probes 1–10 are shown in Table I. These were isolated from pYC10-7 by PCR (using *Pfu* DNA polymerase; Stratagene) and cloned into the *Hin*cII site of M13mp18 or 19 (RF) (Boehringer Mannheim). The M13 control probe (M) carried no insert; the *ACT1* M13 probe has an insert as described above. 'PI' and 'PIII' have inserts of 300 bp from the 18S rDNA (chromosome XII) and a 225 bp fragment containing the *SUP11* gene, respectively. All of these control M13 probes were cloned into the *Hin*cII site of M13mp19 (RF). All M13 probes were verified by sequence analysis.

The TRO procedure was as described (Birse *et al.*, 1997), except that 50 ml cultures at an $OD_{600}$ of ~0.12, induced with 2% galactose (Sigma), were used. The transcriptional pulse was with 160 μCi of [α-$^{32}$P]UTP (800 Ci/mmol; Amersham) for 5 min at 30°C. After partial hydrolysis, RNA was hybridized directly to immobilized single-stranded M13 probes. After two stringent washes (0.2× SSC, 0.1% SDS at 42°C for 25 min), filters finally were washed with 2 μg/ml RNase A (Boehringer Mannheim) in 5× SSC for 20 min at room temperature. Signals were quantitated in a PhosphorImager (Molecular Dynamics). For quantitation, values obtained with probe 'M' were subtracted and then corrected for their U and their G/C content (which was necessary since the probes are relatively short).

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