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A novel yeast gene, *THO2*, is involved in RNA pol II transcription and provides new evidence for transcriptional elongation-associated recombination

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We have identified two novel yeast genes, *THO1* and *THO2*, that partially suppress the transcription defects of *hpr1*Δ mutants by overexpression. We show by *in vivo* transcriptional and recombinational analysis of *tho2*Δ cells that *THO2* plays a role in RNA polymerase II (RNA pol II)-dependent transcription and is required for the stability of DNA repeats, as previously shown for *HPR1*. The *tho2*Δ mutation reduces the transcriptional efficiency of yeast DNA sequences down to 25% of the wild-type levels and abolishes transcription of the *lacZ* sequence. In addition, *tho2*Δ causes a strong increase in the frequency of recombination between direct repeats (>2000-fold above wild-type levels). Some DNA repeats cannot even be maintained in the cell. This hyper-recombination phenotype is dependent on transcription and is not observed in DNA repeats that are not transcribed. The higher the impairment of transcription caused by *tho2*Δ, the higher the frequency of recombination of a particular DNA region. The *tho2*Δ mutation also increases the frequency of plasmid loss. Our work not only identifies a novel yeast gene, *THO2*, with similar function to *HPR1*, but also provides new evidence for transcriptional blocks as a source of recombination. We propose that there is a set of proteins including Hpr1p and Tho2p, in the absence of which RNA pol II transcription is stalled or blocked, causing genetic instability.

Keywords: DNA repeats/genetic instability/*THO1* and *THO2*/transcription elongation/transcription-induced recombination

Introduction

Transcription, in addition to its essential and unique role in gene function, may be intimately related with other DNA transactions. A paradigm of this relationship is the eukaryotic transcription factor TFIIH, which contains proteins of the nucleotide excision repair (NER) machinery and has a functional role in both transcription and excision repair (Bhatia *et al*., 1996; Friedberg, 1996; Hoeijmakers *et al*., 1996). Although the putative dual function of this factor in transcription and NER is not obvious, it clearly seems to play an important role in transcription-coupled repair. Transcriptional activity has also been shown to be related to mutagenesis. Thus, high rates of mutation have been found in actively transcribed genes in yeast (Datta and Jinks-Robertson, 1995). In addition, the hypermutation mechanism of immunoglobulin genes, essential in the origin of antibody diversity, seems to be associated with transcription (Goyenechea *et al*., 1997). Although the molecular basis of this association is not yet understood, it clearly indicates an important role of transcription in mutation and repair.

A very intriguing relationship has also been observed between transcription and recombination. Recombination has been shown to be stimulated by transcriptional activity both in prokaryotes (Dul and Drexler, 1988; Villette *et al*., 1992), and eukaryotes from yeast to mammals. In yeast it has been shown that recombination leading to deletions between direct repeats is stimulated by activation of transcription occurring via the RNA pol I enhancer *HOT1* (Voelkel-Meiman *et al*., 1987; Stewart and Roeder, 1989), RNA pol II-dependent promoters (Thomas and Rothstein, 1989; Grimm *et al*., 1991; Bratty *et al*., 1996) or Ty-expression (Nevo-Caspi and Kupiec, 1994). A connection between transcription and recombination has also been provided in mammalian cells for homologous genes (Nickoloff, 1992; Thygarajam *et al*., 1995) and for immunoglobulin gene rearrangements (Blackwell *et al*., 1986; Lauster *et al*., 1993). It is certainly likely that unwinding of the DNA duplex, changes in local supercoiling or remodelling of the chromatin structure associated with transcription might facilitate recombination by improving (i) the accessibility of the recombination-repair proteins, (ii) the formation of transient DNA–protein structures with transcription-associated activities that could initiate recombination, or (iii) the strand exchange reaction (Thomas and Rothstein, 1989; McCormack and Thompson, 1990; Dröge, 1993; Kotani and Kmiec, 1994). Consistent with these hypotheses, it has been shown that mutations in the structural genes for DNA topoisomerases *TOP1*, *TOP2* (Christman *et al*., 1988) and *TOP3* (Wallis *et al*., 1989), and the genes involved in chromatin structure *SIR2* (Gottlieb and Esposito, 1989) or *SPT4* and *SPT6* (Malagón and Aguilera, 1996) confer hyper-recombination between different types of DNA repeats in yeast. Site-specific recombination is stimulated *in vitro* by negative supercoiling transiently built by an advancing RNA polymerase (Dröge, 1993) and transcription stimulates *in vitro* RecA-promoted strand exchange of nucleosomal templates (Kotani and Kmiec, 1994). In addition, it has been shown that meiotic recombination initiates preferentially at promoter regions (Baudat and Nicolas, 1997) or is increased at the DNA binding sites of transcription factors in eukaryotes from yeast (White *et al*., 1991) to mammals (Shenkar *et al*., 1991). Altogether these results suggest that there may be different transcription-associated events triggering recombination.

We have recently shown that impairment of RNA pol II-dependent transcriptional elongation may induce mitotic recombination between direct repeats in the yeast

Saccharomyces cerevisiae. Hpr1p is a protein required for proper transcriptional elongation by RNA pol II. In the absence of Hpr1p, the elongating RNA pol II is presumably stalled or blocked at particular DNA regions (Chávez and Aguilera, 1997), triggering a deletion event by recombination between the flanking repeats (Chávez and Aguilera, 1997; Prado *et al*., 1997). The intimate relationship between recombination and transcription in *hpr1*Δ cells is strengthened by the observation that two components of the mediator of the RNA pol II holoenzyme, Srb2p (Koleske *et al*., 1992) and Hrs1p/Pgd1p (Piruat *et al*., 1997; Myers *et al*., 1998) are completely required for *hpr1*-induced deletions (Piruat and Aguilera, 1996; Santos-Rosa *et al*., 1996).

To gain further information on the mechanism of induction of recombination by transcription-elongation impairment, we have identified two new yeast genes, *THO1* and *THO2*, that suppress the *hpr1*Δ transcriptional defects by overexpression. *In vivo* genetic and molecular analyses of the effect of these genes and their corresponding null mutations reveals that Tho2p has similar effects on transcriptional and genetic stability to Hpr1p. Our work not only provides new evidence that transcriptional elongation may be associated with recombination, but suggests that there is a set of proteins required for RNA pol II-transcription, including Hpr1p and Tho2p, the absence of which may cause recombinogenic stalls. In addition, this work confirms our recombination-based approach as a powerful way to identify new genes participating in transcription.

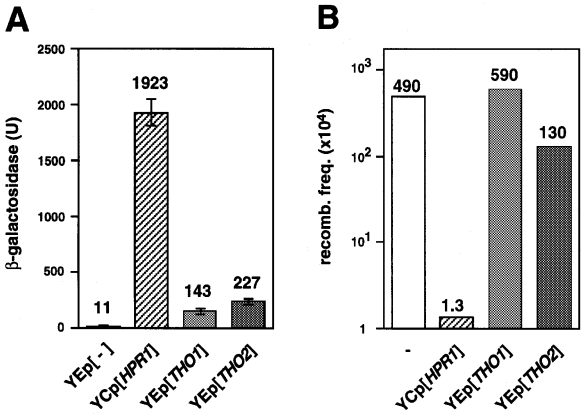

Fig. 1. Effect of the overexpression of *THO1* and *THO2* on *lacZ* gene expression and DNA repeat recombination. (**A**) β-galactosidase activities of the *hpr1*Δ strain AYW3-3D transformed with the multicopy plasmid pLGS5 carrying the *GAL1::lacZ* construct and either YEp351 (control, YEp[–]), YCpA13 (YCp[*HPR1*]), pSUP4 (YEp[*THO1*]) or pSUP38 (YEp[*THO2*]). Either 2% glucose or 2% galactose was added to 16 h mid-log phase cultures in glycerol-lactate synthetic medium, and enzymatic activities were assayed 8 h later. Only the data of induced expression (2% galactose) are given. Under repression conditions (2% glucose) β-galactosidase values were ~1 U in all cases (data not shown). Numbers represent the average value of two different transformants in 2% galactose. Standard deviations are indicated as vertical bars. (**B**) Recombination frequency of the chromosomal *leu2-k::ADE2-URA3::leu2-k* construct of the same transformants as before with the only exception of the control, that is the untransformed AYW3-3D strain. Recombinants were scored on SC+FOA. Numbers represent the median frequency value obtained from six independent cultures each.

Results

Isolation of THO1 and THO2 as multicopy suppressors of the ts and transcriptional phenotypes of hpr1Δ

To understand the function of *HPR1* in transcription and the mechanism by which transcriptional elongation is associated with genetic instability, we searched for genes that suppressed *hpr1*Δ by overexpression. Such genes might have functions partially related to *HPR1*. To isolate genes that suppressed the incapacity of *hpr1*Δ cells to express *lacZ*, we first selected clones that in multicopy suppressed the thermosensitivity (*ts*) phenotype of *hpr1*Δ cells, expecting that they would also suppress their incapacity to express *lacZ*. We first selected 19 yeast transformants with the MW90 multicopy library that were able to grow at 37°C and, subsequently, confirmed their capacity to express the *GAL1–lacZ* construct of plasmid p416GAL1lacZ. Deletion analysis and partial DNA sequencing of the 19 clones isolated (see Materials and methods) permitted us to define two previously uncharacterized open reading frames (ORFs), *THO1* (16 clones) and *THO2* (3 clones) (suppressors of the transcriptional defects of *hpr1*Δ by overexpression), as multicopy suppressors of *hpr1*Δ. *THO1* corresponds to the previously defined YER063w ORF of chromosome *V* (DDBJ/EMBL/GenBank accession No. U18813) and *THO2* to the YNL139c ORF of chromosome *XIV* (DDBJ/EMBL/GenBank accession No. Z71416; Mallet *et al*., 1995), also included in the DDBJ/EMBL/GenBank as *RLR1* (Required for LacZ RNA) by R.W.West (Ithaca, NY). *THO1* encodes a basic protein of 218 amino acids (theoretical mol. wt 24.1 kDa) with no relevant homology to any other yeast protein. No homologous gene has yet been detected in any other organism. *THO2* encodes a protein of 1597 amino acids (theoretical mol. wt 184 kDa), with no relevant homology to any other yeast protein. An homologous ORF of *THO2* exists in *Schizosaccharomyces pombe* (region SPAC22F3.14c). There is no relevant domain to be mentioned from either Tho1p or Tho2p that could give us a clue to their function.

As shown in Figure 1A, *hpr1*Δ cells show 13 and 21 times higher levels of *lacZ* expression when containing *THO1* or *THO2* in multicopy, respectively. This is 7.4 and 11.8% of the wild-type levels, respectively, indicating that overexpression of *THO1* and *THO2* partially suppresses the incapacity of *hpr1*Δ cells to transcribe through *lacZ*. However, this partial suppression of the transcriptional phenotype is not accompanied by suppression of the strong hyper-recombination phenotype of *hpr1*Δ. Recombination of *hpr1*Δ cells is not significantly changed by the presence of *THO1* or *THO2* in multicopy (Figure 1B), although it is noteworthy that the latter has levels of recombination 4.5 times below the *hpr1*Δ levels. These results are indeed consistent with the idea that transcription is defective in *hpr1*Δ cells transformed with *THO1* or *THO2* in multicopy.

The tho1Δ mutation has no effect on transcription and recombination; tho2Δ increases recombination and impedes GAL1–lacZ expression

To gain more insight into the biological processes in which *THO1* and *THO2* could participate *in vivo* we constructed *tho1Δ::TRP1* and *tho2Δ::LEU2* deletions by gene replacement (see Materials and methods). Both

deletion mutants were viable, implying that neither *THO1* nor *THO2* is essential. Whereas *tho1*Δ mutants grow with the same doubling time as the wild type on YEPD at 30°C (85 min), *tho2*Δ form small colonies and grow with twice the doubling time of the wild-type (180 min). In addition, *tho2*Δ cells are also thermosensitive for growth (and do not form colonies) at 37°C.

The most relevant phenotypes of *hpr1*Δ are impairment of transcription elongation through *lacZ* (Chávez and Aguilera, 1997) and increased recombination between repeats (Aguilera and Klein, 1990; Prado *et al*., 1997). Since the way *THO1* and *THO2* were isolated suggests that they might have functions partially related to *HPR1*, we decided to analyze expression of *GAL1–lacZ* and recombination in *tho1*Δ and *tho2*Δ mutants. As can be seen in Figure 2A, *lacZ* expression occurs at wild-type levels in *tho1*Δ cells, whereas it was abolished in *tho2*Δ. In addition, *tho1*Δ has wild-type levels of recombination in the chromosomal *leu2-k::ADE2-URA3::leu2-k* repeat construct, whereas *tho2*Δ shows a strong increase in recombination (2620 times above the wild-type levels), that is 7-fold above even the *hpr1*Δ levels (Figure 2B). Such a hyper-recombination phenotype is clearly observed as a strong red-sectoring phenotype of the *tho2*Δ cells containing the *leu2-k::ADE2-URA3::leu2-k* construct (Figure 2C).

These results suggest that Tho2p is functionally related to Hpr1p. Not only does overexpression of Tho2p partially overcome the transcription defects of *hpr1*Δ, but *tho2*Δ mutants show similar incapacity to express *GAL1–lacZ*, increase of DNA-repeat recombination and slow growth and ts phenotypes as *hpr1*Δ. In addition, *hpr1*Δ and *tho2*Δ double mutants do not show a synergistic effect on recombination (Figure 2B), indicating that *THO2* and *HPR1* act on the same biological process. Interestingly, overexpression of *HPR1* does not suppress the transcriptional defects of *tho2*Δ (data not shown), what might suggest that the Tho2p has a more important role on transcription than Hpr1p. Neither overexpression of *HPR1* nor that of *THO2* has any effect on *lacZ* transcription and recombination of wild-type cells (data not shown). *THO1* might also have a role in transcription that can partially substitute for that of Hpr1p. However, given the lack of phenotypes of *tho1*Δ, even in a *hpr1*Δ background, we decided to concentrate on the *in vivo* molecular analysis of *THO2*.

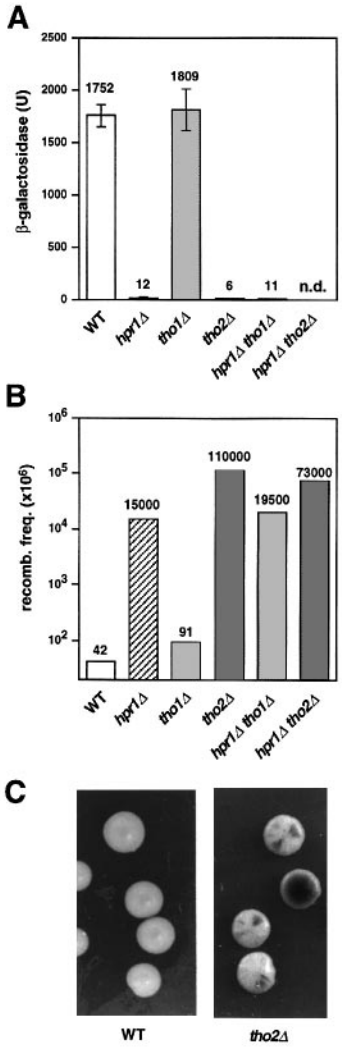

Fig. 2. Effect of the *tho1*Δ and *tho2*Δ mutations on *lacZ* gene expression and DNA repeat recombination. (**A**) β-galactosidase activities of the *GAL1–lacZ* fusion construct in the strains AW33-1B (WT), AW33-1C (*hpr1*Δ), AW33-2C (*tho1*Δ), WR-4B (*tho2*Δ) and AW33-8A (*hpr1*Δ *tho1*Δ) transformed with multicopy plasmid pLGSΔ5 under induced conditions of expression (2% galactose). No determination was made for *hpr1*Δ *tho2*Δ strains (n.d., not determined). Under repression conditions (2% glucose) β-galactosidase values were ~1 U in all cases (data not shown). For other details see legend to Figure 1A. (**B**) Recombination frequency of the chromosomal *leu2-k::ADE2-URA3::leu2-k* construct in the strains AW33-12A (WT), AW33-9D (*hpr1*Δ), AW33-1D (*tho1*Δ), WRA-4D (*tho2*Δ), AW33-2A (*hpr1*Δ *tho1*Δ) and WRA-31B (*hpr1*Δ *tho2*Δ). For other details see legend to Figure 1B. (**C**) Wild-type AW33-12A and *tho2*Δ WRA-7B strains growing on SC medium with 16 mg/l adenine and 75 mg/l FOA. A strong red-sectored phenotype is observed in the *tho2*Δ mutant as a consequence of the high frequency of deletions of the *ADE2* gene by recombination.

THO2 is required for transcription by RNA pol II

The incapacity of *hpr1*Δ strains to express *GAL1–lacZ* is due to their incapacity to transcribe through *lacZ*, since *hpr1*Δ strains are able to activate the *GAL1* promoter. Indeed, *hpr1*Δ cells are able to transcribe a *GAL1–PHO5* fusion construct (Chávez and Aguilera, 1997). In order to determine whether the lack of expression of *GAL1–lacZ* in *tho2*Δ strains is due to their incapacity to transcribe the *lacZ* sequence, we compared the expression levels of *GAL1–lacZ* with those of *GAL1–PHO5* located in single-copy centromeric plasmids. As seen in Figure 3, whereas β-galactosidase was very weakly expressed in *tho2*Δ strains, acid phosphatase reached 25% of the wild-type levels. This result indicates that transcription can initiate at the *GAL1* promoter in *tho2*Δ strains. Therefore, *THO2* is required for transcription of the *lacZ* sequence. However, our result also indicates that *tho2*Δ confers a significant reduction in the expression of *GAL1–PHO5*. As expected, no effect of *tho2*Δ on transcription was detected under repression conditions (data not shown).

Similar levels of expression of *GAL1–lacZ* and *GAL1–PHO5* were observed in *tho2*Δ and *tho2*Δ *hpr1*Δ cells (Figure 3), confirming a lack of synergism of both mutations.

To confirm that the absence of *lacZ* expression and the reduced expression levels of *PHO5* was caused by

transcriptional rather than post-transcriptional defects, we determined the kinetics of activation of both the *lacZ* and the *PHO5* mRNA by Northern analysis. Figure 4 shows that *GAL1*-driven *lacZ* mRNA was not accumulated at all in *tho2*Δ cells after galactose induction, whereas *PHO5* was accumulated up to 18% of the wild-type levels (Figure 4B). These data are consistent with the enzymatic assays (Figure 3), and confirm that *tho2*Δ cells, as previously shown for *hpr1*Δ, cannot transcribe through *lacZ*. In addition, even though transcription occurs through the *PHO5* ORF, there is a significant reduction in the level of *PHO5* mRNA, which suggests that Tho2p might also be required for transcription of *PHO5*. Nevertheless, an additional role for Tho2p in initiation at the *GAL1* promoter cannot be dismissed in order to explain such reduction in *PHO5* mRNA levels.

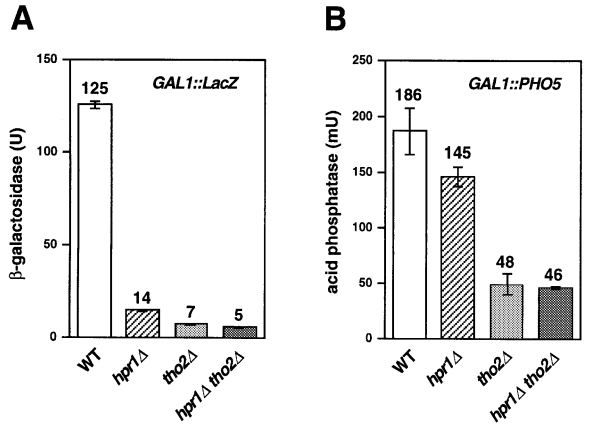

Fig. 3. Expression of *lacZ* and *PHO5* fused to the *GAL1* promoter. (**A**) β-galactosidase activity of isogenic strains WR-4A (WT), U768–4C (*hpr1*Δ), WR-4B (*tho2*Δ) and UR-1A (*hpr1*Δ *tho2*Δ) transformed with centromeric plasmid p416GAL1lacZ carrying the *GAL1–lacZ* fusion construct. (**B**) Acid phosphatase activity of the same strains as before, transformed with centromeric plasmid pSCh202 carrying the *GAL1–PHO5* fusion construct. The average value and standard deviation of two different transformants are given. Only the data of induced expression are given. Under repression conditions values were ~1 U and 5 mU for β-galactosidase and acid phosphatase, respectively. Other details are the same as in Figure 1.

Since all results previously shown refer to *GAL1* fusion constructs located in centromeric plasmids, it was important to show that *tho2*Δ had similar effects on transcription of chromosomal endogenous genes, whether constitutive or regulated. The mRNA driven from the endogenous chromosomal *GAL1* gene is also accumulated in *tho2*Δ cells to 16% of the wild-type levels after induction with galactose (Figure 5), a value similar to those obtained with the episomal *GAL1–PHO5* construct. On the other hand, the mRNA driven from the constitutive *ACT1* endogenous gene is also significantly reduced, as it only reaches 32–53% of the wild-type levels. These results suggest a general role for Tho2p in transcription of yeast genes, regardless of whether they are located in plasmids or chromosomes, and whether they are constitutive or regulated.

Promoter-independent defects of transcription in tho2Δ cells

Known regulatory block- or pausing-sites of transcription in eukaryotic genes are near their 5′ ends and require transcriptional activators to be bypassed by RNA pol II (for review see Eick *et al*., 1994; Bentley, 1995). Transcriptional elongation impairment in *hpr1*Δ cells, however, is promoter-independent (Chávez and Aguilera, 1997). To

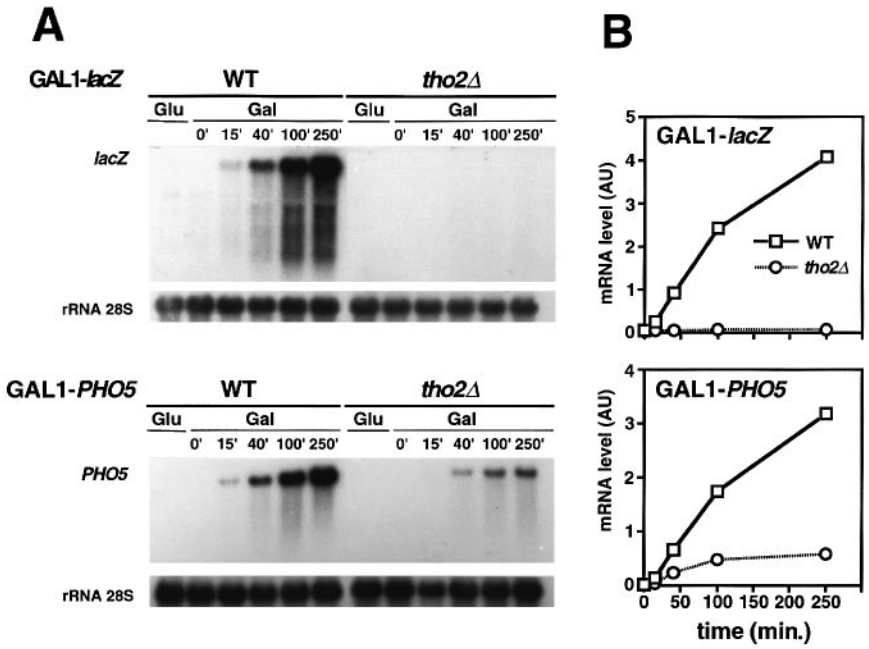

Fig. 4. Transcription analysis of *GAL1–lacZ* and *GAL1–PHO5*. (**A**) Northern analysis of *lacZ* and *PHO5* mRNAs driven from the *GAL1* promoter in the strains WR-4A (WT) and WR-4B (*tho2*Δ) transformed with p416GALlacZ and pSCh202. Transformants were obtained from overnight cultures in glycerol-lactate synthetic media lacking uracil and diluted in identical fresh media to an OD_{600} of 0.5 for wild type and 1.0 for *tho2*Δ. Galactose (Gal) was then added and samples were taken for Northern analysis after different times, as specified. For repression conditions (Glu), total RNA was isolated from mid-log phase cultures in 2% glucose synthetic media lacking uracil. The DNA probes used were the 0.5 kb *Bam*HI–*Hpa*I 5′ end fragment of *lacZ* (*lacZ*), a 1.5 kb *Eco*RI–*Pst*I internal *PHO5* fragment (*PHO5*) and a 589 bp 28S rRNA internal fragment obtained by PCR (rRNA). (**B**) Kinetics of induction of *GAL1*-promoter driven expression of full-length *lacZ* and *PHO5* mRNAs as determined by quantification of the Northern blots. The mRNA values are given with respect to the rRNA levels. AU, arbitrary units.

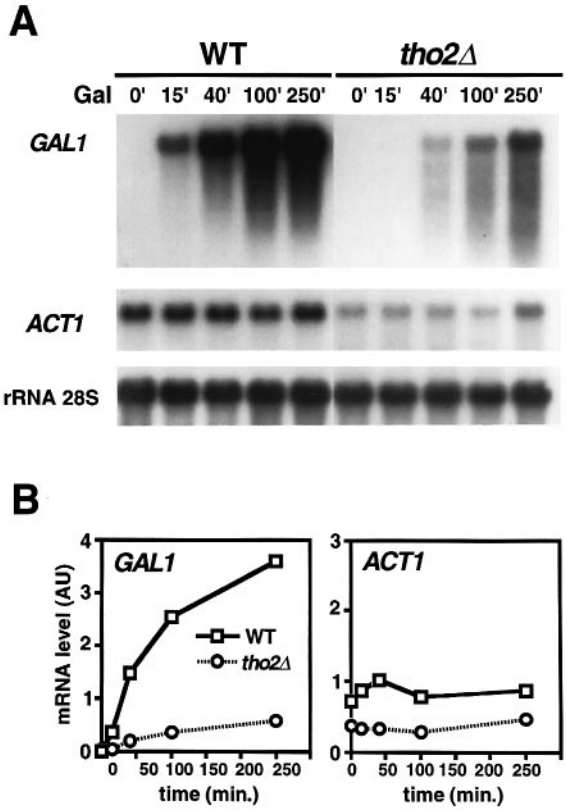

Fig. 5. Transcription analysis of the endogenous *GAL1* and *ACT1* genes. (**A**) Northern analysis of endogenous *GAL1* and *ACT1* mRNA levels in wild-type WR-4A and *tho2*Δ WR-4B strains after different times of addition of 2% galactose to 2% glycerol-3% lactate medium. (**B**) Kinetics of expression as determined by quantification of the previous Northern blots. The DNA probes used were a 0.75 kb *Pvu*II–*Ava*I GAL1 internal fragment (*GAL1*) and a 0.55 kb *Cla*I–*Cla*I *ACT1* internal fragment (*ACT1*). Other details are the same as in Figure 4.

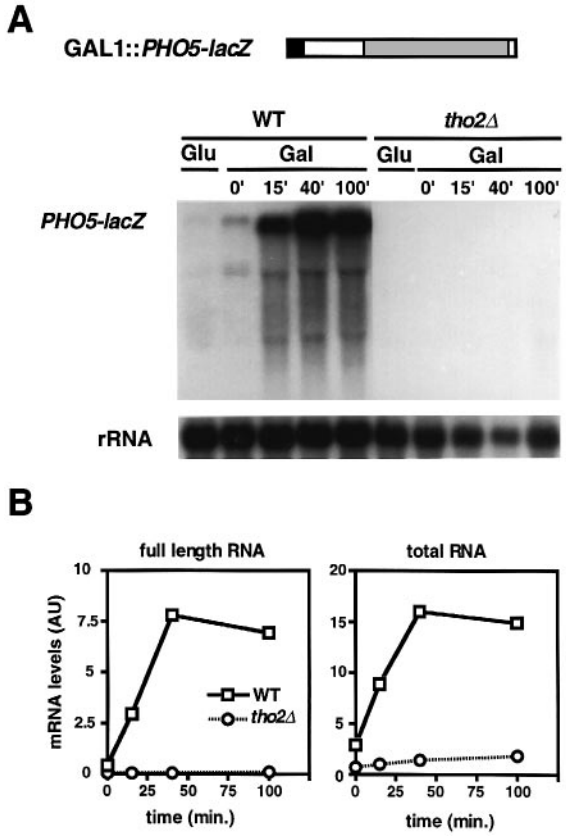

Fig. 6. Transcription analysis of the *GAL1–PHO5–lacZ* fusion construct in wild-type and *tho2*Δ strains. (**A**) Northern analysis of the yeast strains RK2-6A (WT)and RK2-6C (*tho2*Δ) transformed with the centromeric plasmid pSCh212 carrying the *GAL1–PHO5–lacZ* construct in which the *lacZ* ORF is inserted at the 3′ UTR of *PHO5*. (**B**) Kinetics of induction of expression of full-length *PHO5–lacZ* and total mRNA as determined by quantification of the Northern blots. The mRNA values are given with respect to the rRNA levels. Other details are the same as in Figure 4.

assess whether transcription elongation is defective in *tho2*Δ cells we determined the effect of *tho2*Δ on transcription of a *GAL1–PHO5–lacZ* fusion construct identical to the previously characterized *GAL1–PHO5*, but with the *lacZ* coding sequence inserted at the untranslated region (UTR) of *PHO5*. This means that the RNA pol II has to elongate 1.5 kb of *PHO5* sequences before entering *lacZ*. We have already observed that RNA pol II can elongate through *PHO5* to some extent in *tho2*Δ cells (Figures 3 and 4). However, as can be seen in Figure 6, when *lacZ* is inserted downstream of *PHO5* (Figure 4) full-length *PHO5–lacZ* mRNA is accumulated in wild-type cells but not in *tho2*Δ cells after galactose-induction. Since in the *GAL1–PHO5* construct *PHO5* transcripts could be clearly detected (Figure 4), the incapacity of *GAL1–PHO5–lacZ* to produce *PHO5–lacZ* mRNA must be caused by a transcriptional elongation defect at *lacZ*. Quantification of total mRNA from the *GAL1–PHO5–lacZ* construct, indeed, shows that *tho2*Δ cells accumulate up to 12% of the levels of the wild-type (Figure 6B), confirming that transcription proceeds through the *PHO5* coding sequence, as expected, but not through *lacZ*. Therefore, the reduction in mRNA levels observed in *tho2*Δ cells can be explained by the incapacity of the RNA pol II to transcribe through *lacZ*, regardless of the distance to the promoter from which it is transcribed.

To confirm whether transcription elongation was impaired in *tho2*Δ cells at the *GAL1–PHO5–lacZ*, we performed run-on analysis in permeabilized cells. We found very low levels of RNA synthesis at any given place along the *PHO5–lacZ* fragment, including the 5′ end (Figure 7). Therefore, the strong effect of *tho2*Δ on transcription under the conditions used is observed even at initiation. It is probable that the negative effect of *tho2*Δ on transcription initiation impedes detection of any possible effect on elongation. However, given the complete lack of transcript acumulation in the *GAL1–PHO5–lacZ* construct in *tho2*Δ cells (Figure 6), it is also possible that elongation through *PHO5–lacZ* is blocked in the absence of Tho2p. As a consequence, transcription may not be able to reinitiate or to resume upstream of potential stall regions, explaining the very low levels of RNA pol II activity along the whole *PHO5–lacZ* region observed in the run-on analysis (Figure 7). Therefore, the run-on analysis clearly confirms an important role of Tho2p in transcription, but cannot answer the question of whether Tho2p has a role in transcription elongation and/or initiation.

Transcriptional elongation impairment causes hyper-recombination in tho2Δ cells

We have recently shown that the impairment of transcriptional elongation causes genome instability (high frequen-

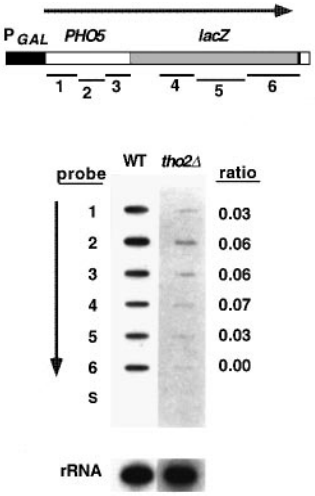

Fig. 7. Transcriptional run-on analysis in wild-type and *tho2*Δ cells. Total RNA was isolated from wild-type RK2-6A and *tho2*Δ RK2-6C strains transformed with single copy plasmid pSCh212 carrying the *GAL1–PHO5–lacZ* construct. Two percent galactose was added to yeast cultures in glycerol-lactate synthetic medium at an OD_{600} of 0.1, 1 h prior to the run-on analysis. A 589 bp 28S rRNA internal fragment, three fragments of *PHO5* (lanes 1–3) and three of *lacZ* (lanes 4–6) were immobilized in hybond-N+ filters. The *PHO5–lacZ* DNA region covering each of the six DNA fragments used is shown. In all cases, the percentage of radiolabelled mRNA bound to each fragment was normalized with respect to their corresponding levels in wild-type cells, taken as 100%. The orientation of the *PHO5–lacZ* arrow indicates the direction of transcription. As negative control we used DNA from *Salmonella typhimurium* (lane S). Experiments using 2% glucose (repression conditions) instead of galactose gave no signal for any of the DNA fragments used in both wild-type and *tho2*Δ cells (data not shown).

cies of recombination and plasmid-loss), as shown in *hpr1*Δ cells (Chávez and Aguilera, 1997). Since *tho2*Δ cells show similar hyper-recombination and transcription defects as *hpr1*Δ, we assessed whether or not both phenotypes were also linked in *tho2*Δ cells.

We determined the effects of *tho2*Δ on recombination between two 0.6 kb direct repeats. We used three direct-repeat constructs, all of them based on the same 0.6 kb *leu2* internal fragment (Chávez and Aguilera, 1997). In these constructs, either the *lacZ* or *PHO5* coding sequences have been inserted between the two direct repeats, inmediately downstream from a 3′-end truncated copy of *LEU2* and immediately upstream of a 5′-end truncated copy of *LEU2*. In the three repeats, transcription is initiated at the unique *LEU2* promoter located outside of the repeats, and has to traverse 760 bp of *LEU2* before proceeding through *lacZ* or *PHO5*. The *lacZ* is inserted in the same transcriptional orientation as *LEU2* (L-*lacZ* construct), whereas *PHO5* is inserted in either the same (L-*PHO5*) or the opposite (L-*PHO5r*) orientation. In the latter case, transcription terminates exactly downstream of the *LEU2* 3′-end truncated repeat, at the terminator of the *PHO5* gene (Chávez and Aguilera, 1997). If the strong hyper-recombination phenotype of *tho2*Δ is associated with defective transcription elongation through *lacZ* or *PHO5*, we predict a very strong hyper-recombination phenotype at L-*lacZ*, weaker at L-*PHO5* and much weaker, if any, at L-*PHO5r*.

As can be seen in Figure 8, the results confirmed our predictions. The frequency of recombination in the L-*lacZ* construct is so high that these strains cannot maintain the duplication of the *leu2* fragment (Figure 8B). All cells (100%) lost the duplication. As a consequence, the *LEU2*-driven mRNA, clearly observed in wild-type cells, is undetectable in *tho2*Δ cells. In the L-*PHO5* construct, the levels of mRNA covering the first *leu2* repeat and *PHO5* in *tho2*Δ cells were 6–7% of the wild-type levels, whereas recombination frequencies reach 700 times the wild-type levels (38% of the cells lost the construct). This result confirms that, indeed, transcription elongation is impaired at the *PHO5* sequence causing the deletion of the repeat construct, as in L-*lacZ*. The higher recombination frequencies of L-*lacZ* versus L-*PHO5* in *tho2*Δ strains (Figure 8B) are consistent with the observation that transcription does not proceed through *lacZ*, but does it through *PHO5* with 25% of the wild-type efficiency (Figures 3 and 4). In the L-*PHO5r* construct, in which only the 3′-end truncated copy of the *LEU2* repeat is transcribed, recombination in *tho2*Δ strains is 48 times the wild-type levels. The transcript levels in *tho2*Δ cells is 12% of the wild-type levels. The lower recombination frequencies of L-*PHO5r* (3.7%) versus L-*PHO5* (38%) are consistent with the observation that transcription does not elongate properly through *PHO5*, causing stronger DNA repeat instability. Indeed, these results suggest that transcription of *LEU2* is also defective in *tho2*Δ cells and responsible for the increase of recombination observed in L-*PHO5r*. This is consistent with a general role for Tho2p in transcriptional elongation of RNA pol II-transcribed yeast genes. It is important to note that in the direct repeat constructs studied, recombination can initiate only inside the repeat or in the regions flanked by the repeats, but not outside such sequences (Prado and Aguilera, 1995). Thus, a putative defect of transcription at the externally located *LEU2* promoter would not have consequences on the stability of the repeats. Therefore, our recombination studies confirm a role of Tho2p in transcription elongation, even though they do not exclude an additional role in initiation.

To provide definitive evidence that the hyper-recombination phenotype conferred by *tho2*Δ is dependent on transcription we decided to change the external *LEU2* promoter of the three direct-repeat constructs studied by the regulated *GAL1* promoter, so that we could determine the effect of *tho2*Δ on recombination under conditions of no transcription. No transcripts were detected in glucose-repressed conditions (Figure 8C), and the frequency of recombination was very similar in the three repeat constructs in wild-type and *tho2*Δ cells, with the only significant exception of the 10-fold increase observed in the frequency of recombination of *tho2*Δ in the GL-*lacZ* construct. This 10-fold increase could be due to leaky transcription of the *GAL1* promoter-derived constructs, as could be detected in the Northern blot experiments when the radiolabelled filters were overexposed (data not shown). The low levels of recombination observed in the three constructs studied under non-transcription conditions implies that the presence of either *lacZ* or *PHO5* between the *leu2* flanking repeats has no effect on *tho2*Δ-induced recombination, unless both sequences are transcribed. Thus, whereas in wild-type cells the frequency of recombination of any of the repeat constructs tested

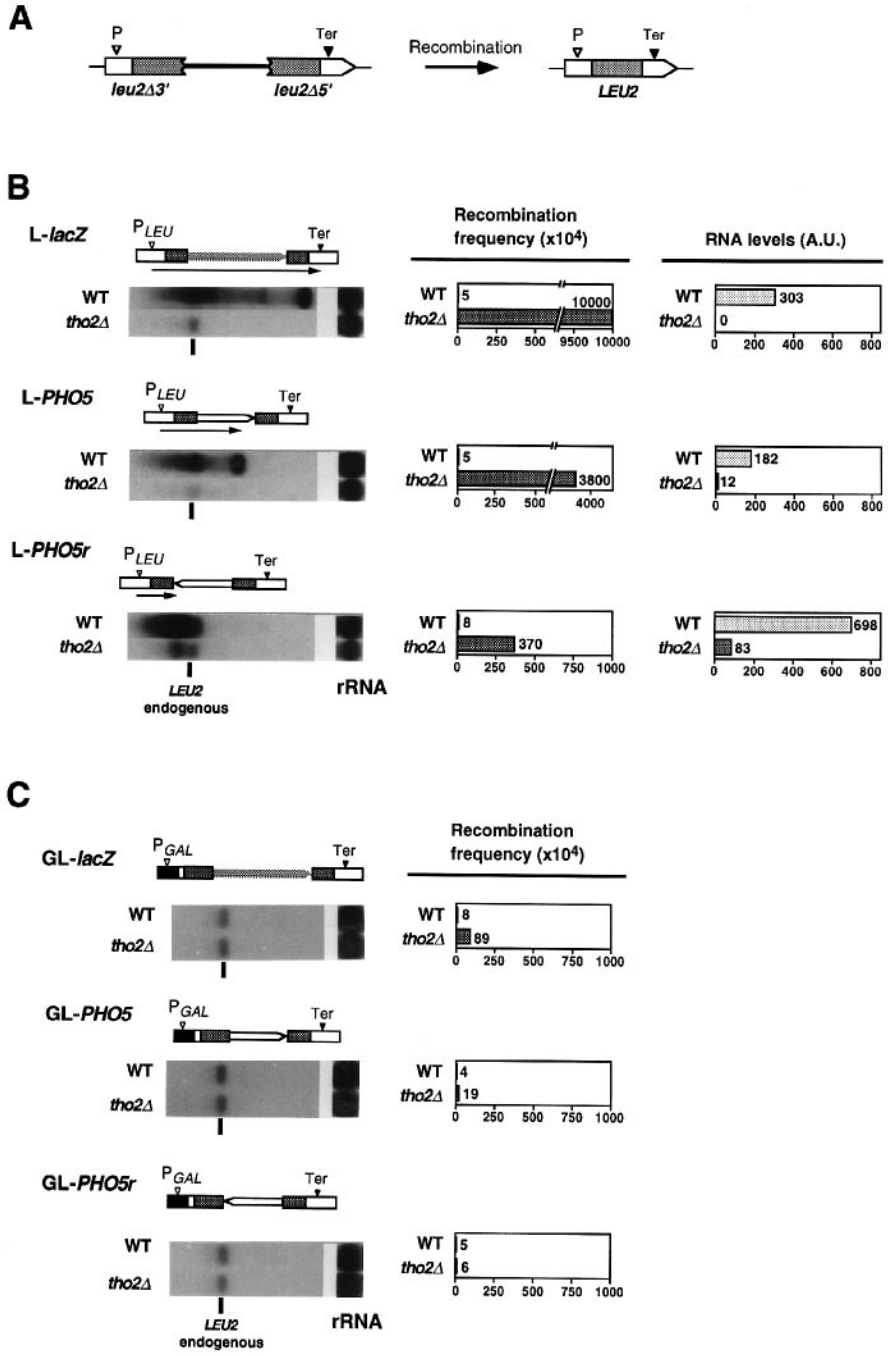

Fig. 8. Transcription and recombination analysis of direct repeat systems in wild-type and *tho2*Δ strains carrying *lacZ*- (3 kb) or *PHO5*- (1.5 kb) coding regions. (**A**) Scheme of the deletion resulting from recombination between the direct repeats used. The diagram shows the 0.6 kb internal *LEU2* repeated sequences (shaded boxes), the position of the promoters (P), whether the *LEU2* or *GAL1* promoter, and the *LEU2* transcription terminators (Ter). (**B**) Recombination frequencies and mRNA levels determined by Northern analysis in wild-type RK2-6A (WT) and RK2-6C (*tho2*Δ) transformed with the centromeric plasmids pSCh204, pSCh206 or pSCh207 carrying the L-*lacZ*, L-*PHO5* and L-*PHO5r* repeat constructs, respectively. The orientation of the *lacZ* and *PHO5* coding regions is shown. The transcripts driven from the *LEU2* promoter (P_{LEU}) are indicated as arrows, and their 3′ ends have been made to coincide with the position of the corresponding band in each Northern blot. Total RNA was isolated from overnight cultures in synthetic media lacking tryptophan. The *LEU2* probe used in the hybridization experiments were the 485 bp *Cla*I–*Eco*RI *LEU2* repeat. The rDNA probe used was the same as in Figure 4. The transcript corresponding to the *LEU2* endogenous chromosomal band is indicated. (**C**) Northern analysis and recombination frequencies of the GL-*lacZ*, GL-*PHO5* and GL-*PHO5r* repeat constructs in which the direct repeats have been placed under the control of an external *GAL1* promoter (PGAL). Wild-type RK2-6A (WT) and RK2-6C (*tho2*Δ) strains transformed with the centromeric plasmids p414GLlacZ, pSG206 and pSG207, respectively, were used. For the fluctuation tests, colonies were obtained from SC-trp (see Materials and methods). Recombinants were selected in SC-leu supplemented with 2% galactose.

was the same regardless of transcription, in *tho2*Δ cells recombination under transcription conditions (*LEU2* promoter) (Figure 8B) was up to 200-fold higher than under non-transcription conditions (*GAL1* promoter under repression conditions), in which case *tho2*Δ cells show wild-type levels of recombination (Figure 8C). These results confirm that the hyper-recombination phenotype of *tho2*Δ is associated with transcriptional elongation.

Table I. Mitotic stability of centromeric plasmids in *tho2*Δ mutants

Strain[a]	Cells carrying plasmid[b] p416GAL1lacZ (%)		Cells carrying plasmid[b] pSCh202 (%)	
	Glucose	Galactose	Glucose	Galactose
THO2	64.0	27.0	42.0	22.0
*tho2*Δ	4.9	2.1	5.4	1.3

[a]Strains used were RK2-6A (*THO2*) and RK2-6C (*tho2*Δ).
[b]Stability was determined after 23 generations on YEPD. Each value represent the median value of six independent determinations.

Plasmid-instability in tho2Δ cells

Recombination could not be assayed in the *GAL1*-derived repeats under induction conditions for transcription (galactose), because *tho2*Δ transformants carrying such constructs were very unstable in galactose-containing medium, suggesting that transcription was associated with plasmid instability in *tho2*Δ cells. Given these results and the increased levels of plasmid loss associated with transcription in *hpr1*Δ cells (Chávez and Aguilera, 1997), we assayed whether or not plasmids became unstable in *tho2*Δ cells in a transcription-dependent manner.

We determined the frequency of loss of the centromeric plasmids p416GAL1lacZ and pSCh202 carrying the *GAL1–lacZ* and *GAL1–PHO5* fusion constructs, respectively. As can be seen in Table I, both plasmids were very unstable under repression conditions (Glu) in *tho2*Δ mutants, and even more unstable under induction conditions of transcription (Gal). The differences observed between repression and induction conditions are not sufficient to conclude that *tho2*Δ also causes plasmid instability in a transcription-dependent manner, as was previously shown for *hpr1*Δ. However, since the shuttle vectors used in our study carry the yeast *URA3* gene as the selection marker, and transcription through *URA3* might be impaired in *tho2*Δ cells, we cannot exclude the possibility that the instability observed in glucose was also transcription-dependent.

Hyper-recombination in tho2Δ is completely suppressed by the hrs1Δ and srb2Δ mutations of the RNA pol II basal transcription machinery

If *hpr1*Δ and *tho2*Δ mutations cause the same transcriptional elongation defects responsible for hyper-recombination, the two previously identified suppressors of the hyper-recombination phenotype of *hpr1*Δ should also abolish hyper-recombination in *tho2*Δ cells. Consequently, we assayed whether hyper-recombination in *tho2*Δ requires a funtional Hrs1p and/or Srb2p components of the RNA pol II holoenzyme, as has been previously shown for *hpr1*Δ cells (Piruat and Aguilera, 1996; Santos-Rosa *et al*., 1996; Piruat *et al*., 1997). Both *hrs1*Δ and *srb2*Δ completely suppress the hyper-recombination phenotype of *tho2*Δ cells (Figure 9). These results are consistent with the conclusion that Hpr1p and Tho2p participate in the same biological process, their null mutations having identical consequences for the cell.

Discussion

We have identified two new yeast genes, *THO1* and *THO2*, that suppress the transcriptional defects of *hpr1*Δ by overexpression. We show genetic and molecular evidence that *THO2* (also named *RLR1* in the yeast genome database), is a gene with a similar effect as *HPR1* on RNA pol II-driven transcription. Putative stalling of transcription produced in *tho2*Δ null mutants is responsible for a very high frequency of deletions between repeats. We show that Tho2p is not only required for proper RNA pol II-dependent transcription, but also provides new evidence that transcriptional elongation is associated with recombination and genetic instability. In addition, our work confirms our recombination-based approach as a succesful way to identify new proteins involved in transcription *in vivo*.

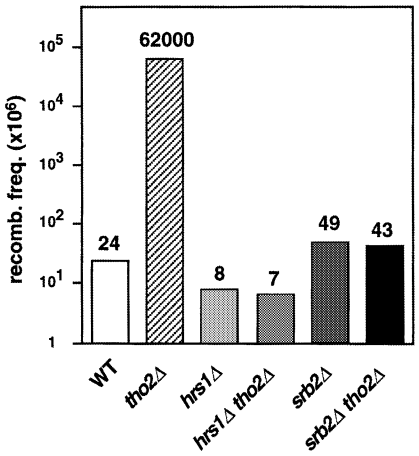

Fig. 9. Suppression of the hyper-recombination phenotype of *tho2*Δ by *hrs1*Δ and *srb2*Δ in the *leu2-k::ADE2-URA3::leu2-k* construct. Strains used were WRK5-3C (WT), WRK5-1C (*tho2*Δ), WRK5-7B (*hrs1*Δ), WRK5-2D (*tho2*Δ *hrs1*Δ), HDY3-3D (*srb2*Δ) and WDY3-3C (*tho2*Δ *srb2*Δ). Other details are the same as in Figure 1.

Tho2p has a role in transcription

Since overexpression of *THO1* and *THO2* partially suppresses the incapacity of *hpr1*Δ cells to express *lacZ* mRNA, it is likely that both genes encode functions partially related to *HPR1*. However, whereas no transcriptional or recombinational phenotype is associated with the *tho1*Δ mutation, *tho2*Δ confers the same phenotypes as *hpr1*Δ.

Our *in vivo* genetic and molecular analysis of *tho2*Δ cells suggests a role for Tho2p in transcription similar to Hpr1p. The *tho2*Δ mutants are unable to transcribe through *lacZ*, as previously observed for *hpr1*Δ (Chávez and Aguilera, 1997). Transcription through yeast genes is also impaired in *tho2*Δ cells, reaching 17–37% of the wild-type levels, a phenotype observed in *hpr1*Δ cells only under the presence of the transcriptional elongation inhibitor 6-azauracil. These results are independent as to whether the promoter lies in a plasmid or its natural chromosomal location (Figures 4, 5 and 6). Such results can be interpreted by an additional role of Tho2p at the initiation step of transcription, as the run-on analysis of the *GAL1–PHO5–lacZ* construct suggests (Figure 7). In any case, Northern analysis on different fusion constructs (Figures 4 and 6) reveals that Tho2p may have a role in elongation. Thus, when the *lacZ* sequence was inserted at the 3′ end UTR *PHO5* region, no accumulation of *PHO5–lacZ* full-length transcript could be observed, implying that the attempt of the RNA pol II to elongate transcription through

lacZ after having transcribed 1.5 kb of *PHO5r* causes a reduction in the accumulation of *PHO5* mRNA (Figure 6). The same result was obtained when either *lacZ* or *PHO5* was located downstream of the 760 bp of the *LEU2* 5′ end (Figure 8). Again, the attempt of the *LEU2* promoter-driven RNA pol II to elongate transcription through *lacZ* or *PHO5* strongly reduces the accumulation of *LEU2* mRNA. Thus, the strong reduction in the kinetics of transcript accumulation in *tho2*Δ cells is observed regardless of either the type of promoter used, whether constitutive or regulated, or the distance between the putative stall region and the promoter. This suggests that at least part of the reduction of transcript accumulation observed in *tho2*Δ cells is caused by transcriptional elongation defects. Indeed we cannot exclude the possibility that the low transcript-initiation levels observed in the run-on analysis (Figure 7) could be caused by an incapacity of *tho2*Δ cells to reinitiate transcription as a consequence of strong transcriptional blocks downstream of the promoter. In any case our work does not exclude a possible role for Tho2p in transcription initiation or promoter clearance.

The defective transcription of yeast genes observed in *tho2*Δ mutants (Figures 4 and 5), that was not observed in *hpr1*Δ mutants (Chávez and Aguilera, 1997), might suggest that the role of Tho2p in transcription is more important than that of Hpr1p. One possibility is that Tho2p had an active role in transcription, by either interacting with chromatin or the transcription machinery, and that Hpr1p was only required for such interaction or for full Tho2p function. In the absence of Hpr1p, Tho2p could still have some residual function as to allow transcription through yeast genes, but not through *lacZ*.

The role of *THO2* in transcription elongation is strengthened by the observation that the strong hyper-recombination phenotype of *tho2*Δ cells is only observed in DNA repeats in which transcription elongation takes place. The repeat constructs used in our studies are transcribed from an externally located promoter (*GAL1* or *LEU2*). As we have previously shown that deletion events can only be initiated at the *leu2* repeated sequences or at the intervening sequence located in between, but not outside of such sequences (Prado and Aguilera, 1995), a putative defect of *tho2*Δ cells in transcription initiation or promoter clearance, cannot be responsible for the hyper-recombination phenotype. Instead, hyper-recombination is only observed when RNA pol II attempt to elongate through either the repeats or the intervening sequences (Figure 8), consistent with the suggestion that *tho2*Δ is affected at the transcriptional elongation step. In this sense, it is noteworthy the high instability of the *PHO5* sequence located between the *leu2* repeats when transcribed from the external *LEU2* promoter (38% of the cells deleted *PHO5*; Figure 8B) as compared with its wild-type levels of instability (0.06%) when not transcribed (Figure 8C). This result confirms indeed that transcription elongation through the yeast *PHO5* gene is impaired, explaining both the low accumulation of *PHO5* mRNA (Figures 4, 6 and 8) and the hyper-recombination phenotype asscociated with *tho2*Δ.

The impairment of transcription through yeast genes may be among the reasons why *tho2*Δ strains grow so poorly, especially in synthetic medium. Transcription defects might be critical for some essential genes at high temperatures or might be overcome by other redundant functions at low temperatures, causing thermosensitivity for growth. It is important to note that the *hpr1*Δ *tho2*Δ double mutants do not show synergistic effects on either the recombinational or transcriptional phenotypes. This suggests that both *HPR1* and *THO2* play a functional role at a similar step in transcription, a conclusion also supported by the observation that hyper-recombination associated with transcriptional elongation in both *hpr1*Δ and *tho2*Δ are suppressed by the same mutations, *hrs1*Δ and *srb2*Δ (Figure 9). In any case, Tho2p seems to have a stronger effect on transcription and recombination than Hpr1p. Not only is transcription of yeast genes impaired in *tho2*Δ strains in the absence of the transcriptional elongation inhibitor 6-azauracil (Figures 3,4 and 5), but a strong hyper-recombination phenotype is observed in DNA repeat constructs in which *hpr1*Δ has no effect (Figure 8; Chávez and Aguilera, 1997).

As with Hpr1p, we do not know the function that Tho2p might have in transcription. Tho2p may be required for both initiation and elongation of transcription. However, its putative role in transcription elongation is the only one responsible for the hyper-recombination phenotype of *tho2*Δ cells, as previously shown for *hpr1*Δ (Chávez and Aguilera, 1997; Prado *et al*., 1997). We believe that the function of Tho2p is not related to TFIIS, because Tho2p seems to function at the same level as Hpr1p, which we have shown is distinct from TFIIS (Chávez and Aguilera, 1997). Consistent with this, *tho2*Δ does not confer sensitivity to 6-azauracil (S.Chávez and A.Aguilera, unpublished results), as expected if TFIIS activity was impaired in *tho2*Δ cells. Tho2p, like Hpr1p, might have a role in either facilitating elongation of RNA pol II through particular chromatin structures and supercoiled DNA or preventing arrest of RNA pol II by allowing reading through transient pauses. Interestingly, it has recently been identified a new factor from HeLa nuclear extracts, FACT, that facilitates transcription elongation through nucleosomes (Orphanides *et al*., 1998). Other proteins involved in transcriptional elongation that may act at this level are TFIIF, elongin, ELL (eleven-nineteen lysine-rich leukemia) (for review see Uptain *et al*., 1997) or the mammalian Cockayne syndrome group B protein CSB (van Gool *et al*., 1997a), which resides in an RNA pol II-containing complex (van Gool *et al*., 1997b) and has been shown to contact ternary complexes of elongating RNA pol II (Tantin *et al*., 1997) and to enhance elongation (Selby and Sancar, 1997).

We do not know whether there is a particular DNA or chromatin feature or topological constraint causing a putative stalling of the RNA pol II in the absence of a functional Tho2p. In this sense, it is noteworthy that the chromatin-related Spt4p and Spt5p proteins are involved in transcription elongation (Hartzog *et al*., 1998; Wada *et al*., 1998) and that pausing during transcriptional elongation in the human *hsp70* gene depends on nucleosome templates and requires active SWI/SNF complex and the HSF1 activator for its release (Brown *et al*., 1996; Brown and Kingston, 1997). However, we believe that the transcriptional defects of *tho2*Δ strains is different to pausing controlled by transactivators as an integral part of the initiation step (Yankulov *et al*. 1994), because they

can be caused by DNA regions located 1.5 kb downstream of the promoter (Figure 6). Tho2p might also be required for the processivity of the RNA pol II, since the longer the DNA sequence to be transcribed the stronger the effect of the null mutations (Figures 3–7).

Putative stalling of transcription elongation in tho2Δ cells induces recombination

The *tho2*Δ mutants show a very strong increase in the frequency of recombination between DNA repeats leading to deletions, that can be up to 2000-fold the wild-type levels (Figures 2 and 8). As discussed earlier, the hyper-recombination phenotype is only observed in repeat constructs that are transcribed. The stronger the effect of *tho2*Δ on transcription elongation of the region flanked by the repeats, the higher the frequency of deletions. Thus, the *lacZ* sequence, that cannot be transcribed in *tho2*Δ cells, cannot even be maintained between direct DNA repeats (Figure 8B). This is consistent with the idea that deletions in *tho2*Δ cells are caused by transcriptional elongation blocks. Tho2p is also important for the stability of centromeric plasmids (Table I). However, we cannot establish whether this instability is also transcription-dependent.

The linkage between transcriptional elongation and hyper-recombination between repeats suggests a likely explanation for the induction of recombination associated to *tho2*Δ similar to that proposed for *hpr1*Δ (Chávez and Aguilera, 1997). A stalled transcriptional elongation complex may promote either (i) the formation of double-strand breaks, or (ii) the arrest of the replication fork. In the first case, DNA breaks may be facilitated by a major accessibility of nucleases to the stall sequence, either because an open chromatin structure is formed around the putatively stalled ternary complex, or because proteins participating in elongation (i.e. topoisomerase I) caused nicks. In this context, we cannot dismiss the possibility that the stalled RNA pol II holoenzyme could participate in a recruitment of recombination proteins such as in transcription-coupled repair (for review see Bhatia *et al*., 1996; Friedberg, 1996; Hoeijmakers *et al*., 1996). Intriguingly, the recombinational-repair protein Rad51p has been found in the human RNA pol II holoenzyme (Maldonado *et al*., 1996). Also, hyper-recombination in *tho2*Δ cells is completely dependent on functional Srb2p and Hrs1p, two of the components of the mediator of transcriptional regulation of the RNA pol II holoenzyme (Koleske and Young, 1994; Piruat and Aguilera, 1996; Piruat *et al*., 1997; Myers *et al*., 1998).

The second hypothesis suggesting that recombination in *tho2*Δ cells is induced by an arrest of the replication fork after colliding with a stalled transcription complex is consistent with the observations that replication forks transiently arrest at transcribed DNA regions in yeast (Desphande and Newlon, 1996) and that high frequencies of illegitimate recombination are associated with converging replication and transcription machineries in *Escherichia coli* (Vilette *et al*., 1992). Such collisions could either cause double-strand breaks, as reported for replication arrests in *E.coli* (Michel *et al*., 1997) or could lead to the deletion of the DNA region containing the putative stalled complex by slippage of the DNA polymerase between flanking repeats.

Transcription elongation and its connection with DNA repeat recombination

Enhancement of recombination by transcription has been reported in different organisms from prokaryotes to higher eukaryotes (Blackwell *et al*., 1986; Dul and Drexler, 1988; Grimm *et al*., 1991; Nickoloff, 1992). In yeast it is well-established that recombination between repeats is stimulated by transcription driven from strong promoters that results in high transcript levels (Stewart and Roeder, 1989; Thomas and Rothstein, 1989; Nevo-Caspi and Kupiec, 1994). Interestingly, in *tho2*Δ mutants, DNA repeat recombination is also stimulated by transcription driven from strong promoters. However, it results in low rather than high transcript levels as a consequence of the incapacity of the RNA pol II to complete elongation, as was also shown for *hpr1*Δ mutants (Chávez and Aguilera, 1997; Prado *et al*., 1997). Whether this difference implies a distinct mechanism of induction of recombination is an open question. Hyper-recombination in *tho2*Δ cells requires that the RNA pol II attempt to elongate through the repeat construct. Interestingly, *HOT1*-stimulated recombination requires transcription through the repeats (Voelkel-Meimann *et al*., 1987), and the *GAL10* direct repeats used by Thomas and Rothstein (1989) express a weak transcript of the DNA sequence located between the repeats. Also, in all recombinational substrates used to show transcription-induced recombination, DNA sequences that are normally expressed at low levels, such as Ty and ORFs from genes involved in amino acid or nucleotide metabolism, were fused to a strong promoter (Grimm *et al*., 1991; Nevo-Caspi and Kupiec, 1994; Bratty *et al*., 1996). Therefore, it is still possible that an enhanced rate of transcription in such constructs could increase the frequency of elongation failures that could trigger recombination, consistent with our hypothesis for *hpr1*Δ- (Chávez and Aguilera, 1997) and *tho2*Δ- (this study) induced recombination events.

Finally, it is noteworthy the recent observation that recombination between a plasmid and a chromosome is enhanced by transcription if driven from the *GAL1* promoter (RNA pol II-dependent), but not if driven from the *ADH1* (RNA pol II), rDNA (RNA pol I) and *RPR1* (RNA pol III) promoters (Bratty *et al*., 1996). This argues against the possibility that the opening of the chromatin structure associated with transcriptional activity is sufficient to explain transcription-induced recombination. Chromatin changes are associated with transcription driven from any of these promoters, yet the recombination effects are very different.

In summary, we provide new evidence that transcription elongation can be a source of mitotic recombination between repeats. Our work suggests that the recombinogenic events caused during transcriptional elongation are not rare events produced uniquely under the absence of Hpr1p (Chávez and Aguilera, 1997). There is a subset of proteins, including Hpr1p and Tho2p in yeast, whose function might be to facilitate transcription by RNA pol II (Kane, 1994; Friedberg, 1996; Uptain *et al*., 1997). In the absence of such proteins the elongating RNA pol II could stall, triggering genetic instability by either interference with DNA replication or induction of recombinational repair. Our transcription elongation-dependent recombination assay is a powerful tool for the

Table II. Strains

Strain	Genotype	Source
AYW3-3D	*MAT***a** *ura3 ade2 his3 leu2-k::ADE2-URA3::leu2-k hpr1Δ3::HIS3*	Santos-Rosa and Aguilera (1995)
AYW3-1B	*MAT*α *ura3 ade2 his3 trp1 leu2-k::ADE2-URA3::leu2-k can1-100*	Piruat and Aguilera (1996)
HDY3-3D	*MAT*α *ura3 ade2 his3 trp1 leu2-k::ADE2-URA3::leu2-k srb2Δ102::HIS3 can1-100*	Piruat and Aguilera (1996)
W303-1A	*MAT***a** *ura3-1 ade2-1 his3-11,5 trp1-1 leu2-3,112 can1-100*	R.Rothstein
W303-1B	*MAT*α *ura3-1 ade2-1 his3-11,5 trp1-1 leu2-3,112 can1-100*	R.Rothstein
U768-4C	*MAT***a** *ura3-1 ade2-1 his3-11,5 trp1-1 leu2-3,112 hpr1Δ3::HIS3 can1-100*	R.Rothstein
AWY-3A	*MAT***a** *ura3 ade2 his3 trp1 leu2-k::ADE2-URA3::leu2-k can1-100*	this study
AW33-1B	*MAT***a** *ura3 ade2 his3 trp1 leu2*	this study
AW33-1C	*MAT*α *ura3 ade2 his3 trp1 hpr1Δ3::HIS3*	this study
AW33-2C	*MAT*α *ura3 ade2 his3 trp1 leu2 tho1Δ::TRP1*	this study
AW33-8A	*MAT***a** *ura3 ade2 his3 trp1 leu2 tho1Δ::TRP1 hpr1Δ3::HIS3*	this study
AW33-12A	*MAT*α *ura3 ade2 his3 trp1 leu2-k::ADE2-URA3::leu2-k*	this study
AW33-9D	*MAT***a** *ura3 ade2 his3 trp1 leu2-k::ADE2-URA3::leu2-k hpr1Δ3::HIS3*	this study
AW33-1D	*MAT*α *ura3 ade2 his3 trp1 leu2-k::ADE2-URA3::leu2-k tho1Δ::TRP1*	this study
AW33-2A	*MAT***a** *ura3 ade2 his3 trp1 leu2-k::ADE2-URA3::leu2-k tho1Δ::TRP1 hpr1Δ3::HIS3*	this study
WRA-4D	*MAT***a** *ura3 ade2 his3 trp1 leu2-k::ADE2-URA3::leu2-k tho2Δ::LEU2 can1-100*	this study
WRA-7B	*MAT*α *ura3 ade2 his3 trp1 leu2-k::ADE2-URA3::leu2-k tho2Δ::LEU2 can1-100*	this study
WRA-31B	*MAT***a** *ura3 ade2 his3 trp1 leu2-k::ADE2-URA3::leu2-k tho2Δ::LEU2 hpr1Δ3::HIS3*	this study
WR-4A	*MAT*α *ura3-1 ade2-1 his3-11,5 trp1-1 leu2-3,112 can1-100*	this study
WR-4B	*MAT***a** *ura3-1 ade2-1 his3-11,5 trp1-1 leu2-3,112 tho2Δ::LEU2 can1-100*	this study
UR-1A	*MAT*α *ura3-1 ade2-1 his3-11,5 trp1-1 leu2-3,112 tho2Δ::LEU2 hpr1Δ3::HIS3 can1-100*	this study
WRK5-3C	*MAT***a** *ura3 ade2 his3 trp1 leu2-k::ADE2-URA3::leu2-k can1-100*	this study
WRK5-1C	*MAT*α *ura3 ade2 his3 trp1 leu2-k::ADE2-URA3::leu2-k tho2Δ::LEU2 can1-100*	this study
WRK5-7B	*MAT*α *ura3 ade2 his3 trp1 leu2-k::ADE2-URA3::leu2-k hrs1Δ::KAN1 can1-100*	this study
WRK5-2D	*MAT***a** *ura3 ade2 his3 trp1 leu2-k::ADE2-URA3::leu2-k tho2Δ::LEU2 hrs1Δ::KAN1*	this study
WDY3-3C	*MAT*α *ura3 ade2 his3 trp1 leu2-k::ADE2-URA3::leu2-k tho2Δ::LEU2 srb2Δ102::HIS3*	this study
RK2-6A	*MAT*α *ura3-1 ade2-1 his3-11,5 trp1-1 leu2-3,112 can1-100*	this study
RK2-6C	*MAT***a** *ura3-1 ade2-1 his3-11,5 trp1-1 leu2-3,112 tho2Δ::KAN1 can1-100*	this study

identification of new genes involved in transcription. This is necessary not only to understand the transcription process itself, but to provide a molecular framework to explain transcriptional elongation-associated recombination and genetic instability.

Materials and methods

Strains and plasmids

The yeast strains used in this study, listed in Table II, are congenic to W303-1A and AYW3-3D, which are congenic strains. Plasmids are listed in Table III.

To obtain the 0.16 kb *Bam*HI–*Cla*I *LEU2* fragment used to construct the plasmid p414GLEU2 (Table III), 500 bp of the 5′ end of the *LEU2* ORF starting 6 bp upstream of the translation initiation site were PCR-amplified with the oligonucleotides CGCGGATCCATTCTAATGT-CTGCCCCTAA and CTTCTGGAACGGTGTATTGT. This procedure introduced a *Bam*HI site (underlined sequence) at the 5′ end of the *LEU2* ORF that was used for subcloning right after the *GAL1* promoter.

Genetic analysis and determination of recombination and plasmid-loss frequencies

Genetic analysis was performed as previously described, following standard procedures for media and growth conditions (Kaiser *et al*., 1994). Yeast strains were transformed with lithium acetate according to Schiestl and Gietz (1989). 5-FOA was added at the concentration of 500 mg/l in synthetic medium with 1 g/l proline as the nitrogen source. For the scoring of the bacterial KAN gene in yeast, G418-sulfate was used at the concentration of 200 mg/l in YEPD.

Recombination frequencies were calculated as the median frequency of six independent cultures as previously published (Prado and Aguilera, 1995). For direct-repeat constructs placed under the control of the *GAL1* promoter, recombination frequencies were calculated from colonies growing on selective synthetic medium supplemented with either 2% glucose (repressed conditions). Recombinants were scored in 2% galactose-containing media.

The frequency of plasmid loss was calculated as the median frequency of six independent cultures grown on non-selective rich medium for 23 generations, as previously described (Chávez and Aguilera, 1997). All tests were made in duplicate.

Enzymatic assays

For the analysis of *GAL1*-driven expression, mid-log phase cells were inoculated in 3% glycerol-2% lactate synthetic medium at a concentration of 1.5–2.0×10^7 cells/ml and incubated for 16 h. Afterward, either 2% glucose or 2% galactose was added. After 8 h at 30°C either β-galactosidase or acid phosphatase activity was assayed as described (Guarente *et al*., 1982; Haguenauer-Tsapis and Hinnen, 1984) in either permeabilized or whole cells, respectively.

DNA manipulation and analysis

DNA from *E.coli* and yeast was prepared as previously published (Prado *et al*., 1997). [^{32}P]dCTP-labelled DNA probes were prepared as described (Feinberg and Vogelstein, 1984). DNA hybridization was performed in 50% deionized formamide, 6× SSC, 5× Denhardt's solution, 25mM $NaPO_4$ pH 6.5, 0.5% SDS and 0.1 mg/ml salmon sperm DNA at 42°C for 18 h.

DNA amplification was made by PCR with Expand-High-Fidelity *Taq* polymerase (Boehringer Mannheim).

Double-chain DNA was sequenced by the dideoxy-chain termination method with T7 DNA polymerase (Sequenase) and 5′-[α-^{35}S]thiotriphosphate using the M13 universal primers.

RNA analysis

Yeast RNA was prepared from exponential cultures in the appropriate selective medium, subjected to electrophoresis on formaldehyde-agarose gels and hybridized with radiolabeled DNA probes following previously published procedures (Chávez and Aguilera, 1997). Filters were first hybridized with either the *lacZ*, *PHO5* or *LEU2* probe, and then rehybridized with the *ACT1*, *GAL1* or 28S rDNA probe after removal of the former signals. Quantification of mRNA levels was performed in a β-radiation Fujix Analizer and are given in arbitrary units. All values were normalized with respect to the 28S rRNA detected by hybridization with a ^{32}P-oligolabelled 589 bp rRNA internal fragment obtained by PCR as described (Chávez and Aguilera, 1997).

For the run-on analysis we used one microgram of DNA denatured with NaOH from each *lacZ* and *PHO5* fragment immobilized on Hybond-N+ filters with a pR600 Slot Blot (Hoefer, USA). Run-on was performed according to previously described protocols (Elion and Warner, 1986; Osborne and Guarente, 1989) following the modifications described by Chávez and Aguilera (1997), with the only difference that cells were harvested after 1 h of induction with galactose instead of 5 h.

Table III. Plasmids

Plasmid	Description	Source
YEp351	YEp vector based on the *LEU2* marker	Hill *et al*. (1986)
pRS316	YCp vector based on the *URA3* marker	Sikorski and Hieter (1989)
p314LB	YCp vector based on *TPR1* and carrying two tandem direct repeats of 600 bp of *LEU2*	Prado and Aguilera (1996)
YCpA13	YCp vector based on *LEU2* and carrying the *HPR1* gene	Aguilera and Klein (1990)
pFA6KANMX4	*E.coli* vector carrying a translational fusion of the *KAN* ORF of Tn*903* to the *TEF* gene of *A.gossypii*	Wach *et al*. (1994)
pSUP4	YEp351 with a 7.5 kb *Sau3A*[a]*THO1* genomic fragment inserted at *Sal*I	this study
pSUP6	YEp351 with a 7.4 kb *Sau*3A[a]*THO1* genomic fragment inserted at *Sal*I	this study
pSUP24	YEp351 with a 6.3 kb *Sau*3A[a]*THO1* genomic fragment inserted at *Sal*I	this study
pSUP38	YEp351 with a 7.3 kb *Sau*3A[a]*THO2* genomic fragment inserted at *Sal*I	this study
pSP401	YEp351 with the 1.6 kb *Pst*I–*Xba*I fragment of pSUP4 inserted at *Pst*I–*Xba*I	this study
pSP402	YEp351 with the 2.2 kb *Pst*I–*Xba*I fragment of pSUP4 inserted at *Pst*I–*Xba*I	this study
pSP381	YEp351 with the 3.0 kb *Xba*I–*Xba*I fragment of pSUP38 inserted at *Xba*I	this study
pSP383	YEp351 with the 4.0 kb *Xba*I–*Pst*I fragment of pSUP38 inserted at *Pst*I–*Xba*I	this study
pSTH5	pRS316 with the 0.75 kb *Spe*I–*Ksp*I fragment of pSUP4 inserted at *Spe*I–*Ksp*I	this study
pSTH35	pSTH5 with the 0.45 kb *Xho*I–*Dra*I *THO1* 3′ end fragment of pSP402 inserted at *Xho*I–*Cla*I[a]	this study
pSTH35ΔT	pSTH35 with the 0.85 kb *Bgl*II–*Eco*RI *TRP1* fragment inserted at *Eco*RI–*Bam*HI	this study
pSP38L	pSUP38 in which the 5.75 kb *Bam*HI–*Sal*I fragment has been substituted by the 2.68 kb *Bgl*II–*Sal*I *LEU2* fragment	this study
pSP38ΔL	pSP38L with the 1.9 kb *Hin*dIII–*Hin*dIII fragment of pSUP38 inserted at *Sma*I	this study
pBSTH02Δ	pBluescriptII SK- with the 3.78 kb *Sma*I–*Hin*dIII fragment of pSP38ΔL inserted at *Sma*I–*Hin*dIII	this study
pLGSΔ5	YEp plasmid based on the *URA3* marker and the *lacZ* gene under the yeast *CYC1–GAL1,10* promoter	Guarente *et al*. (1982)
p416GAL1lacZ	YCp plasmid based on the *URA3* marker and the *lacZ* coding region under the *GAL1* promoter	Mumberg *et al*. (1994)
pSCh202	identical to p416GAL1lacZ with the *PHO5* coding region, instead of *lacZ*, fused to the *GAL1* promoter	Chávez and Aguilera (1997)
pSCh204	YCp plasmid based on the *TRP1* marker and containing the L-*lacZ* construct	Chávez and Aguilera (1997)
pSCh206	identical to pSCh202 with the L-*PHO5* construct instead of L-*lacZ*	Chávez and Aguilera (1997)
pSCh207	identical to pSCh206 with the L-*PHO5r* construct instead of L-*PHO5*	Chávez and Aguilera (1997)
p414GAL1	YCp expression plasmid based on the *TRP1* marker and the *GAL1* promoter	Mumberg *et al*. (1994)
p414GLEUΔ	p414GAL1 with the 1.41 kb *Cla*I–*Sal*I *LEU2* fragment fused to the *GAL1* promoter inserted at *Cla*I–*Xho*I	this study
p414GLEU2	p414GLEUΔ with a 0.16 kb *Bam*HI–*Cla*I fragmentb containing the 5′ end of *LEU2* inserted at *Bam*HI–*Cla*I	this study
pSG206	pSCh206 in which the 1.22 kb *Sac*I–*Cla*I *LEU2* promoter region has been substituted by the 0.62 kb *Sac*I–*Cla*I fragment of p414GLEU2 containing the *GAL1* promoter and the 5′ end of *LEU2*	this study
pSG207	identical to pSG206 but based on the pSCh207	this study
p314GLB	p314LB in which the 1.22 kb *SacI–Cla*I *LEU2* promoter region has been substituted by the 0.62 kb *Sac*I–*Cla*I fragment of p414GLEU2 containing the *GAL1* promoter and the 5′ end of *LEU2*	S.González-Barrera
p314GLlacZ	p314GLB with the 3 kb *Bam*HI *lacZ* fragment from pPZ (Straka and Hörz, 1991) inserted at *Bgl*II between the *leu2* repeats	S.González-Barrera
pSCh212	YCp plasmid containing the *GAL1–PHO5–lacZ* fusion construct	Chávez and Aguilera (1997)

[a]Restriction sites blunt-ended with Klenow prior to ligation.
[b]Fragment obtained by PCR as described in text.

Isolation of the THO1 and THO2 genes

The *hpr1*Δ strain AYW3-3D was used to screen for yeast genes that, when placed in a multicopy vector, complemented the growth-thermosensitivity (*ts*) phenotype of *hpr1*Δ. From 2.5×10^5 transformants with the yeast MW90 library constructed in the YEp351 multicopy vector (Waldherr *et al*., 1993), we selected 24 for their capacity to grow on SC-leu at 37°C. From 19 of the transformants we isolated four different plasmids that defined two different genomic DNA regions. Three of the plasmids (pSUP4, pSUP6 and pSUP24) were found in 8, 2 and 6 independent transformants, respectively. These plasmids overlapped in an internal 4.75 kb DNA region (Figure 10). The fourth plasmid, pSUP38, was found in three independent transformants and contains a unique 7.3 kb fragment from a different DNA region from that of the first three plasmids. Deletion analysis of the two different DNA regions isolated indicated that the 2.27 kb *Xba*I–*Pst*I internal fragment of the inserts of the first three plasmids (Figure 10A) contains one gene and the central *Xba*I site of the insert of plasmid pSUP38 mapped in the ORF of a second gene (Figure 10B) that was able to complement the ts phenotype of *hpr1*Δ, as shown by back-transformation experiments. We named these genes *THO1* and *THO2*, respectively. DNA sequence analysis of 200 and 181 bp from the *Xba*I and *Pst*I sites of pSP402 (Figure 10A), respectively, and 170 bp from the left end of pSUP38 (Figure 10B) and subsequent comparison of these sequences with the yeast genome database revealed that *THO1* was the YER063w ORF from the right arm of chromosome *V* (DDBJ/EMBL/GenBank accession No. U18813), whereas *THO2* was the YNL139c ORF from the left arm of chromosome *XIV*, also named *RLR1* (DDBJ/EMBL/GenBank No. Z71416; Mallet *et al*., 1995).

Construction of tho1Δ and tho2Δ null mutants

The genomic *THO1* and *THO2* genes were deleted by gene replacement. To construct the *tho1Δ::TRP1* deletion we replaced the 0.7 kb *Spe*I–*Dra*I fragment of *THO1* with a 0.85 kb *Bgl*II–*Eco*RI fragment containing the entire *TRP1* gene. It was made by transforming the wild-type diploid strain AYW3-1B×AWY-3A with the linearized 2.3 kb *Xho*I–*Ksp*I fragment of plasmid pSTH35ΔT (Figure 1A). For the *tho2Δ::LEU2* deletion we replaced the 3.66 kb *Hin*dIII–*Sal*I internal fragment of *THO2* with a 2.62 kb *Sal*I–*Bgl*II fragment containing the entire *LEU2* gene. It was made by transforming the wild-type diploid strain W303-1A× W303-1B with the linearized 3.8 kb *Sma*I–*Hin*dIII fragment of plasmid pSP38ΔL (Figure 10B). For the *tho2Δ::KAN* deletion we replaced the entire 4.79 kb *THO2* ORF, with the exception of the 40 bp at each end, with a 1.53 kb fragment containing the bacterial *KAN* gene (Figure 10B). It was made by transforming the wild-type diploid strain W303-1A×W303-1B with a linear 1.6 kb fragment containing the *KAN* gene flanked on each side by 40 bp of the *THO2* end-regions. This linear DNA fragment was obtained by PCR of plasmid pFA6KANMX4 with the 60-mers: **ATG**GCAGAACAGACGCTACTTTCCAAATTGAACG-

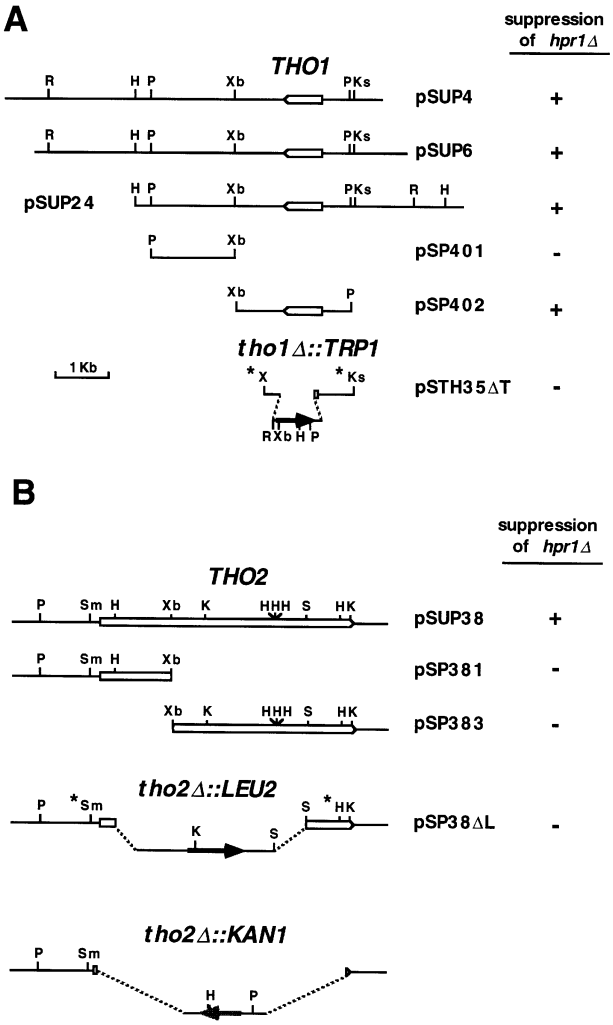

Fig. 10. Restriction maps of different *THO1* and *THO2* clones and the *tho1*Δ and *tho2*Δ mutations. (**A**) Restriction maps of the *THO1* DNA region contained in the plasmids isolated or constructed for this work. The ability of each construct to complement the ts phenotype of *hpr1*Δ in a multicopy vector is shown. (**B**) Idem for *THO2*. The structure of the *tho1*Δ*::TRP1*, *tho2*Δ*::LEU2* and *tho2*Δ*::KAN1* mutation allele is also shown. An (*) indicates the restriction sites used to obtain the linear DNA fragments used for the gene-replacement transformation. The linear *tho2*Δ*::KAN1* construct was obtained by PCR and used directly for transformation (see Materials and methods). The *THO1* and *THO2* genes are indicated as open boxes. Restriction sites shown are: H, *Hin*dIII; K, *Kpn*I; Ks, *Ksp*I; P, *Pst*I; R, *Eco*RI; S, *Sal*I; Sm, *Sma*I; X, *Xho*I; Xb, *Xba*I.

CTCTTTCTAGTGGATCTGATATCATC and **AAT**TACCTCTGGTA-CCTACTGACGTAATTCCCACCCTTGGCAGCTGAAGCTTCGT-ACGCT, which cor- respond to 20 bp of the sequences flanking the *KAN* gene (indicated as plain text) plus the (+1 to +40) and the (+4756 to +4796) sequences of the *THO2* gene (underlined; start and stop codons indicated in bold).

The resulting heterozygous *THO1*/*tho1*Δ and *THO2*/*tho2*Δ diploids were subjected to tetrad analysis. In all cases, 4-viable-spore tetrads were obtained, with a 2:2 segregation for either the *LEU2*, *TRP1* or *KAN* traits, according to the genotype of the strain analysed. Southern and DNA hybridization analysis of two complete tetrads of each of the three diploids constructed, revealed the expected cosegregation of the absence of *THO1* and *THO2* bands with the presence of *TRP1* and either *LEU2* or *KAN* bands, respectively (data not shown). Therefore, the three deletion alleles constructed had the expected genomic structure and both *THO1* and *THO2* are non-essential genes. The [^{32}P]dCTP-labelled DNA probes used in the DNA hybridization experiments were the 0.64 kb *Pst*I–*Xba*I TRP1 and 0.31 kb *Dra*I–*Spe*I *THO1* internal fragments for the *tho1*Δ*::TRP1* deletion, the 0.48 kb *Cla*I–*Eco*RI *LEU2* and 3.1 kb *Hin*dIII–*Hin*dIII *THO2* internal fragments for *tho2*Δ*::LEU2* and the 6.5 kb *Pst*I–*Pst*I DNA fragment covering the *THO2* gene for *tho2*Δ*::KAN*.

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References

Aguilera,A. and Klein,H.L. (1990) *HPR1*, a novel yeast gene that prevents intrachromosomal excision recombination, shows carboxy-terminal homology to the *Saccharomyces cerevisiae* TOP1 gene. *Mol. Cell. Biol.*, **10**, 1439–1451.

Baudat,F. and Nicolas,A. (1997) Clustering of meiotic double-strand breaks on yeast chromosome III. *Proc. Natl Acad. Sci. USA*, **94**, 5213–5218.

Bentley,D.L. (1995) Regulation of transcriptional elongation by RNA polymerase II. *Curr. Opin. Genet. Dev.*, **5**, 210–216.

Bhatia,P.K., Wang,Z. and Friedberg,E.C. (1996) DNA repair and transcription. *Curr. Opin. Genet. Dev.*, **6**, 146–150.

Blackwell,T.K., Moore,M.W., Yancopoulos,G.D., Suh,H., Lutzker,S., Selsing,E. and Alt,F.W. (1986) Recombination between immuno-globulin variable region gene segments is enhanced by transcription. *Nature*, **324**, 585–589.

Bratty,J., Ferbeyre,G., Molinaro,C. and Cedergren,R. (1996) Stimulation of mitotic recombination upon transcription from the yeast *GAL1* promoter but not from other RNA polymerase I, II and III promoters. *Curr. Genet.*, **30**, 381–388.

Brown,S.A. and Kingston,R.E. (1997) Disruption of downstream chromatin directed by a transcriptional activator. *Genes Dev.*, **11**, 3116–3121.

Brown,S.A., Imbalzano,A.N. and Kingston,R.E. (1996) Activator-dependent regulation of transcriptional pausing on nucleosomal templates. *Genes Dev.*, **10**, 1479–1490.

Chávez,S. and Aguilera,A. (1997) The yeast *HPR1* gene has a functional role in transcriptional elongation that uncovers a novel source of genome instability. *Genes Dev.*, **11**, 3459–3470.

Christman,M.F., Dietrich,F.S. and Fink,G.R. (1988) Mitotic recombination in the rDNA of *S. cerevisiae* is suppressed by the combined action of DNA topoisomerases I and II. *Cell*, **55**, 413–425.

Datta,A. and Jinks-Robertson,S. (1995) Association of increased spontaneous mutation rates with high levels of transcription in yeast. *Science*, **268**, 1616–1618.

Deshpande,A.M. and Newlon,C.S. (1996) DNA replication fork pause sites dependent on transcription. *Science*, **272**, 1030–1033.

Dröge,P. (1993) Transcription-driven site-specific DNA recombination *in vitro*. *Proc. Natl Acad. Sci. USA*, **90**, 2759–2763.

Dul,J.L. and Drexler,H. (1988) Transcription stimulates recombination. I. Specialized transduction of *Escherichia coli* λ*trp* phages. *Virology*, **162**, 466–470.

Eick,D., Wedel,A. and Heumann,H. (1994) From initiation to elongation: comparison of transcription by prokaryotic and eukaryotic RNA polymerases. *Trends Genet.*, **10**, 292–296.

Elion,E.A. and Warner,J.R. (1986) An RNA polymerase I enhancer in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.*, **6**, 2089–2097.

Feinberg,A.P. and Volgelstein,B. (1984) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.*, **137**, 266–267.

Friedberg,E.C. (1996) Relationships between DNA repair and transcription. *Annu. Rev. Biochem.*, **65**, 15–42.

Gottlieb,S. and Esposito,R.E. (1989) A new role for a yeast transcriptional silencer gene, *SIR2*, in regulation of recombination in ribosomal DNA. *Cell*, **56**, 771–776.

Goyenechea,B., Klix,N., Yelamos,J., Williams,G.T., Ridell,A., Neuberger,M.S. and Milstein,C. (1997) Cells strongly expressing Igκ transgenes show clonal recruitment of hypermutation: a role for both MAR and the enhancers. *EMBO J.*, **16**, 3987–3994.

Grimm,C., Schaer,P., Munz,P. and Kohli,J. (1991) The strong *ADH1* promoter stimulates mitotic and meiotic recombination at the *ADE6* gene of *Schizosaccharomyces pombe*. *Mol. Cell. Biol.*, **11**, 289–298.

Guarente,L., Rocum,R.R. and Gifford,P. (1982) A *GAL10–CYC1* hybrid yeast promoter identifies the *GAL4* regulatory region as an upstream site. *Proc. Natl Acad. Sci. USA*, **79**, 7410–7414.

Haguenauer-Tsapis,R. and Hinnen,A. (1984) A deletion that includes the signal peptidase cleavage sites impairs processing, glycosilation and secretion of cell surface yeast acid phosphatase. *Mol. Cell. Biol*., **4**, 2668–2675.

Hartzog,G.A., Wada,T., Handa,H. and Winston,F. (1998) Evidence that Spt4, Spt5 and Spt6 control transcription elongation by RNA polymerase II in *Saccharomyces cerevisiae*. *Genes Dev.*, **12**, 357–369.

Hill,J.E., Myers,A.M., Koerner,T.J. and Tzagaloff,A. (1986) Yeast/*E.coli* shuttle vectors with multiple unique restriction sites. *Yeast*, **2**, 163–167.

Hoeijmakers,J.H.J., Egly,J.-M. and Vermeulen,W. (1996) TFIIH: a key component in multiple DNA transactions. *Curr. Opin. Genet. Dev.*, **6**, 26–33.

Kaiser,C., Michaelis,M. and Mitchell,A. (1994) *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Kane,C.M. (1994) Transcript elongation and gene regulation in eukaryotes. In Conaway,R.C. and Conaway,J.W. (eds), *Transcription: Mechanisms and Regulation*. Raven Press, New York, NY, pp. 279–296.

Koleske,A.J., Buratowski,S., Nonet,M. and Young,R.A. (1992) A novel transcription factor reveals a functional link betwen the RNA polymerase II CTD and TFIID. *Cell*, **69**, 883–894.

Koleske,A.J. and Young,R.A. (1994) An RNA polymerase II holoenzyme responsive to activators. *Nature*, **368**, 466–469.

Kotani,H. and Kmiec,E.B. (1994) Transcription activates RecA-promoted homologous pairing of nucleosomal DNA. *Mol. Cell. Biol*., **14**, 1949–1955.

Lauster,R., Reynaud,C.-A., Martenson,I.-L., Peter,A., Bucchini,D., Jami,J. and Weill,J.-C.. (1993) Promoter, enhancer and silencer elements regulate rearrangement of an immuglobulin transgene. *EMBO J*., **12**, 4615–4623.

Malagón,F. and Aguilera,A. (1996) Differential intrachromosomal hyper-recombination phenotype of *spt4* and *spt6* mutants of *S.cerevisiae*. *Curr. Genet*., **30**, 101–106.

Maldonado,E. *et al*. (1996) A human RNA polymerase II complex associated with SRB and DNA-repair proteins. *Nature*, **381**, 86–89.

Mallet,L., Bussereau,F. and Jacquet,M. (1995) A 43.5 segment of yeast chromosome XIV, which contains *MFA2*, *MEP2*, *CAP/SRV2*, *NAM9*, *FKB1/FPR1/RPB1*, *MOM22* and *CPT1*, predicts an adenosine deaminase gene and 14 new open reading frames. *Yeast*, **11**, 1195–1209.

McCormack,W.T. and Thompson,C.B. (1990) Chicken IgL variable region gene conversions display pseudogene donor preference and 5′ to 3′ polarity. *Genes Dev.*, **4**, 548–558.

Michel,B., Ehrlich,S.D. and Uzest,M. (1997) DNA double-strand breaks caused by replication arrest. *EMBO J*., **16**, 430–438.

Mumberg,D., Müller,R. and Funk,M. (1994) Regulatable promoters of *Saccharomyces cerevisiae*: comparison of transcriptional activity and their use for heterologous expression. *Nucleic Acids Res*., **22**, 5767–5768.

Myers,L.C., Gustafsson,C.M., Bushnell,A.D., Lui,M., Erdjument-Bromage,H., Tempst,P. and Kornberg,R.D. (1988) The Med proteins of yeast and their function through the RNA polymerase II carboxiterminal domain. *Genes Dev.*, **12**, 45–54.

Nevo-Caspi,Y. and Kupiec,M. (1994) Transcriptional induction of Ty recombination in yeast. *Proc. Natl Acad. Sci. USA*, **88**, 12711–12715.

Nickoloff,J.A. (1992) Transcription enhances intrachromosomal homologous recombination in mammalian cells. *Mol. Cell. Biol*., **12**, 5311–5318.

Orphanides,G., LeRoy,G., Chang,C.-H. and Reinberg,D. (1998) FACT, a factor that facilitates transcript elongation through nucleosomes. *Cell*, **92**, 105–116.

Osborne,B.I. and Guarente,L. (1989) Mutational analysis of a yeast transcriptional terminator. *Proc. Natl Acad. Sci. USA*, **86**, 4097–4101.

Piruat,J.I. and Aguilera,A. (1996) Mutations in the yeast *SRB2* general transcription factor suppress *hpr1*-induced recombination and show defects in DNA repair. *Genetics*, **143**, 1533–1542.

Piruat,J.I., Chávez,S. and Aguilera,A. (1997) The yeast *HRS1* gene is involved in positive and negative regulation of transcription and shows genetic characteristics similar to *SIN4* and *GAL11*. *Genetics*, **147**, 1585–1594.

Prado,F. and Aguilera,A. (1995) Role of reciprocal exchange, one-ended invasion crossover and single-strand annealing on inverted and direct repeat recombination in yeast: different requirements for the *RAD1*, *RAD10* and *RAD52* genes. *Genetics*, **139**, 109–123.

Prado,F., Piruat,J.I. and Aguilera,A. (1997) Recombination between DNA repeats in yeast *hpr1*Δ cells is linked to transcription elongation. *EMBO J*., **16**, 2826–2835.

Santos-Rosa,H. and Aguilera,A. (1995) Isolation and genetic analysis of extragenic suppressors of the hyper-deletion phenotype of the *Saccharomyces cerevisiae hpr1*Δ mutation. *Genetics*, **139**, 57–66.

Santos-Rosa,H., Clever,B., Heyer,W.-D. and Aguilera,A. (1996) The yeast *HRS1* gene encodes a polyglutamine-rich nuclear protein required for spontaneous and *hpr1*-induced deletions between direct repeats. *Genetics*, **142**, 705–716.

Schiestl,R.S. and Gietz,R.D. (1989) High efficiency transformation of intact yeast cells using single stranded nucleic acids as a carrier. *Curr. Genet*., **16**, 339–346.

Selby,C.P. and Sancar,A. (1997) Cockayne syndrome group B protein enhances elongation by RNA polymerase II. *Proc. Natl Acad. Sci. USA*, **94**, 11205–11209.

Shenkar,R., Shen,M.H. and Arnheim,N. (1991) DNase I-hypersensitive sites and transcription factor-binding motifs within the mouse E beta meiotic recombination hot spot. *Mol. Cell. Biol*., **11**, 1813–1819.

Sikorski,R.S. and Hieter,P. (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics*, **122**, 19–27.

Stewart,S.E. and Roeder,G.S. (1989) Transcription by RNA polymerase I stimulates mitotic recombination in *Saccharomyces cerevisiae*. *Mol. Cell. Biol*., **9**, 3464–3472.

Tantin,D., Kansal,A. and Carey,M. (1997) Recruitment of the putative transcription-repair coupling factor CSB/ERCC6 to RNA polymerase II elongation complexes. *Mol. Cell. Biol*., **17**, 6803–6814.

Thomas,B.J. and Rothstein,R. (1989) Elevated recombination rates in transcriptionally active DNA. *Cell*, **56**, 619–630.

Thyagarajan,B., Johnson,B.L. and Campbel,C. (1995) The effect of target site transcription on gene targeting in human cells *in vitro*. *Nucleic Acids Res*., **23**, 2784–2790.

Uptain,S.M., Kane,C.M. and Chamberlin,M.J. (1997). Basic mechanisms of transcript elongation and its regulation. *Annu. Rev. Biochem.*, **66**, 117–172.

van Gool,A.J., Citterio,E., Rademakers,S., van Os,R., Vermeulen,W., Constantinou,A., Egly,J.-M., Bootsma,D. and Hoeijmakers,J.H.J. (1997a) The Cockayne syndrome B protein, involved in transcription-coupled DNA repair, resides in an RNA polymerase II-containing complex. *EMBO J*., **16**, 5955–5965.

van Gool,A.J., van der Horst,G.T.J., Citterio,E. and Hoeijmakers,J.H.J. (1997b) Cockayne syndrome: defective repair of transcription? *EMBO J*., **16**, 4155–4162.

Vilette,D., Uzest,M., Ehrlich,S.D. and Michel,B. (1992) DNA transcription and repressor binding affect deletion formation in *Escherichia coli* plasmids. *EMBO J*., **11**, 3629–3634.

Voelkel-Meiman,K., Keil,R.L. and Roeder,G.S. (1987) Recombination-stimulating sequences in yeast ribosomal DNA correspond to sequences regulating transcription by RNA polymerase I. *Cell*, **48**, 1071–1079.

Wach,A., Brachat,A., Pöhlmann,R. and Philippsen,P. (1994) New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. *Yeast*, **10**, 1793–1808.

Wada,T. *et al.* (1998) DSIF, a novel transcription elongation factor that regulates RNA polymerase II processivity, is composed of human Spt4 and Spt5 homologs. *Genes Dev.*, **12**, 343–356.

Waldherr,M., Ragnini,A., Jank,B., Teply,R., Wiesenbergern,G. and Schweyen,R.J. (1993) A multitude of suppressors of group II intron-splicing defects in yeasts. *Curr. Genet*., **24**, 301–306.

Wallis,J.W., Chrebet,G., Brodsky,G., Rolf,M. and Rothstein,R. (1989) A hyper-recombination mutation in *S. cerevisiae* identifies a novel eukaryotic topoisomerase. *Cell*, **58**, 409–419.

White,M.A., Wierdl,M., Detloff,P. and Petes,T.D. (1991) DNA-binding protein RAP1 stimulates meiotic recombination at the HIS4 locus in yeast. *Proc. Natl Acad. Sci. USA*, **88**, 9755–9759.

Yankulov,K., Blau,J., Purton,T., Roberts,S. and Bentley,D.L. (1994) Transcriptional elongation by RNA polymerase II is stimulated by transactivators. *Cell*, **77**, 749–759.

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