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Hpr1 Is Preferentially Required for Transcription of Either Long or G+C-Rich DNA Sequences in *Saccharomyces cerevisiae*

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Hpr1 forms, together with Tho2, Mft1, and Thp2, the THO complex, which controls transcription elongation and genome stability in *Saccharomyces cerevisiae*. Mutations in genes encoding the THO complex confer strong transcription-impairment and hyperrecombination phenotypes in the bacterial *lacZ* gene. In this work we demonstrate that Hpr1 is a factor required for transcription of long as well as G+C-rich DNA sequences. Using different *lacZ* segments fused to the *GAL1* promoter, we show that the negative effect of *lacZ* sequences on transcription depends on their distance from the promoter. In parallel, we show that transcription of either a long *LYS2* fragment or the *S. cerevisiae YAT1* G+C-rich open reading frame fused to the *GAL1* promoter is severely impaired in *hpr1* mutants, whereas transcription of *LAC4*, the *Kluyveromyces lactis* ortholog of *lacZ* but with a lower G+C content, is only slightly affected. The hyperrecombination behavior of the DNA sequences studied is consistent with the transcriptional defects observed in *hpr1* cells. These results indicate that both length and G+C content are important elements influencing transcription in vivo. We discuss their relevance for the understanding of the functional role of Hpr1 and, by extension, the THO complex.

The control of genome stability is essential to ensure maintenance of genetic information in all cells of a living organism. Dysfunction of this control causes mutations and chromosomal aberrations that can give rise to loss of gene function, cell death, or irreversible changes in the cell program.

Genetic recombination is required for mitotic DNA repair and for proper meiotic chromosome segregation. In addition, it may also be responsible for processes of genetic instability. A number of animal diseases, including cancer, originate by events of mitotic recombination between repeats that lead to chromosomal aberrations (34). Several elements have been described to enhance mitotic recombination, including DNA damage, replication defects, alteration of chromatin structure, and transcriptional activity (reviewed in reference 3). Ikeda and Matsumoto (26) first described the influence of transcription on recombination showing that recombination of phage λ was stimulated by transcription. In yeast, the first example of transcription-associated recombination was the finding that a hotspot of ribosomal DNA (rDNA) recombination, *HOT1*, was dependent on RNA polymerase I-driven transcription (55, 60). Thomas and Rothstein (56) extended transcription-induced recombination to sequences transcribed by RNA polymerase II (RNAPII). Additional examples of RNAPII-dependent recombination have been subsequently described in yeast (21, 36, 50) and mammalian cells (37, 57). Special mention must be made of the modulation of recombination at the immunoglobulin loci, as both V(D)J recombination (7, 31, 38) and class switching (15) are positively controlled by transcription.

A gene linking transcription and genome instability in *Saccharomyces cerevisae* is *HPR1*, as *hpr1* mutants show both increased levels of recombination between direct repeats and chromosome loss (2, 49) as well as strong transcriptional defects (11, 44, 67). Detailed characterization of these defects has shown that the absence of Hpr1 causes impairment of transcription elongation. The intensity of such a transcriptional impairment depends on the transcribed DNA sequence (11). There is a close correlation between the reluctance of a DNA sequence to be transcribed in *hpr1* cells and the ability of such a sequence to promote recombination when inserted between direct repeats (11, 44).

Biochemical and genetic analyses have contributed to identifying several factors that participate in RNAPII-mediated transcription elongation (reviewed in reference 14). According to their function in transcriptional elongation, these factors can be classified in different groups. TFIIS prevents RNAPII arrest and induces nascent transcript cleavage (reviewed in reference 64). Some other factors, like TFIIF, CSB, ELL, and elongin, influence elongation by suppressing the pausing of RNAPII (5, 46, 52, 53). P-TEFb stimulates transcription elongation in response to transactivators (reviewed in reference 45) by antagonizing negative factors like DSIF and NELF (22, 61, 65). Finally, some transcription elongation factors like FACT and Elongator play a role in facilitating RNAPII-driven transcription on chromatin templates (39, 40).

Although *hpr1*Δ cells are affected in transcription elongation in vivo, Hpr1 does not seem to be physically associated with any of the known elongation factors. It has been demonstrated that Hpr1 is physically present in a new form of RNAPII holoenzyme that has been proposed to respond to protein kinase C-mediated signal transduction (10). Hpr1 forms the THO complex in vivo together with the products of the *THO2*, *MFT1*, and *THP2* genes (12). The absence of any of the four proteins confers similar phenotypes of transcriptional elongation impairment and hyperrecombination, indicating that the THO complex is a functional unit in gene expression and genome stability (12). However, the way THO controls these processes remains obscure.

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TABLE 1. Plasmids

Plasmid	Description	Source or reference
pRS416	YCp vector based on the *URA3* gene	54
p416GALI-lacZ	pRS416 containing the *lacZ* region fused to the *GAL1* promoter	35
pSCh202	pRS416 containing the *PHO5* region fused to the *GAL1* promoter	11
pSCh212	pSCh202 with *lacZ* transcriptionally fused to the 3′ end UTR[a] of *PHO5*	11
pRS314-L	YCp vector pRS314, based on the *TRP1* gene and containing two 598-bp *LEU2* internal fragments repeated in direct orientation, separated by a polylinker	43
pSCh211	pSCh212 with *lacZ* in opposite orientation	This study
pSCh211Δ17-1	pSCh202 with a ~0.4-kb fragment of the 3′ end of *lacZ* fused to the 3′ end UTR of *PHO5*	This study
pSCh229	pSCh202 with the first 439 bp of *lacZ* ORF fused to the 3′ end UTR of *PHO5*	This study
pSCh226	pSCh202 with the *Hpa*I-*Bss*HII 447-bp fragment of *lacZ* inserted in the 3′ end UTR of *PHO5*	This study
pSCh251	pSCh202 with a 393-bp fragment of *GAL1* (from position 843 to 1236 of the ORF) inserted in the 3′ end UTR of *PHO5*	This study
pSCh215	pRS416 containing a 439-bp fragment of the 5′ end of *lacZ* fused to the *GAL1* promoter	This study
pSCh213	pRS416 containing the 447-bp *Hpa*I-*Bss*HII fragment of *lacZ* fused to the *GAL1* promoter	This study
pSCh216	pRS416 containing the 352-bp *Pvu*II-*Eco*RI fragment of *lacZ* fused to the *GAL1* promoter	This study
pSCh218	pSCh202 containing the first 439-bp fragment of the 5′ end of *lacZ* inserted between the *GAL1* promoter and the *PHO5* ORF	This study
pSCh219	pSCh202 containing the 447-bp *Hpa*I-*Bss*HII fragment of *lacZ* inserted between the *GAL1* promoter and the *PHO5* ORF	This study
pSCh220	pSCh202 containing the 352-bp *Pvu*II-*Eco*RI fragment of *lacZ* inserted between the *GAL1* promoter and the *PHO5* ORF	This study
pSCh221	pRS314-L containing the first 439 bp of *lacZ* ORF inserted between the repeats	This study
pSCh222	pRS314-L containing the 447-bp *Hpa*I-*Bss*HII fragment of *lacZ* inserted between the repeats	This study
pSCh223	pRS314-L containing the 352-bp *Pvu*II-*Eco*RI fragment of *lacZ* inserted between the repeats	This study
pSCh205	pRS314-L containing the entire *lacZ* gene inserted between the repeats	11
pSCh227	pRS416 containing the 3.7-kbp *Eco*RV fragment of *LYS2* fused to the *GAL1* promoter	This study
pSCh230	pRS314-L containing the 3.7-kbp *Eco*RV fragment of *LYS2* inserted between the repeats	This study
pSCh255	pRS416 containing the entire *LAC4* coding region fused to the *GAL1* promoter	This study
pSCh254	pRS314-L containing the entire *LAC4* coding region inserted between the repeats	This study
pSCh247	pRS416 containing the entire *YAT1* coding region fused to the *GAL1* promoter	This study
pSCh248	pRS314-L containing the entire *YAT1* coding region inserted between the repeats	This study

[a] UTR, untranslated region.

As relevance of the THO complex in transcription depends on the transcribed DNA sequence, investigation of the features that make transcription of a particular DNA sequence dependent on Hpr1 can provide some clues to understanding its precise function. We have found that transcriptional impairment in *hpr1* occurs primarily in long transcription units as well as in DNA sequences with a high G+C content fused to the *GAL1* promoter. The relevance of these results for understanding the functional role of the THO complex is discussed.

MATERIALS AND METHODS

Yeast strains and plasmids. The two isogenic yeast strains used in this study were W303-1A (*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*) and U768-4C (*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hpr1Δ::HIS3*). All plasmids used are monocopy *CEN*-based plasmids and are listed in Table 1.

Analysis of gene expression and recombination. For the analysis of *GAL1*-driven expression, mid-log phase cells were inoculated with 3% glycerol–2% lactate synthetic medium at an optical density at 600 nm (OD_{600}) of 0.1. After 16 h of incubation at 30°C, 2% galactose was added and incubation was continued for another 8 h at 30°C. Acid phosphatase activity and mRNA levels were determined as described previously (11). It is important that all transcription analyses shown in this study, with few exceptions, were made in monocopy *CEN*-based plasmids, which are lost at higher frequencies in *hpr1* versus wild-type strains (11). However, since in all experiments cells were grown under the selection conditions for the plasmid, more than 90% of the *hpr1* cells still contained plasmids. Therefore, all observed transcriptional effects are not caused by plasmid loss.

For expression analysis of *EGT2*, *CDC48*, *KAR2*, *OLE1*, and *GOG5* cells were grown in yeast extract-peptone-dextrose (YEPD)-rich medium to an OD_{600} of 1.0 and subsequently sampled. DNA probes for Northern experiments were obtained by PCR amplification using the following pairs of primers: TCATTTC GATACTCGGCCTAG and GCAGCATCAGAGCTAGTTGTG for *EGT2*; AAACCACTTTTGGACGCCTC and TCTTGTCTCTCTTTGGAGCT for *CDC48*; TTCAACAGACTAAGCGCTGG and CAATTTCAATACGGGTGG ACA for *KAR2*; ATGCCAACTTCTGGAACTAC and CCGAAAGTAACAAT GGCAGT for *OLE1*; and TTGAAAACAGGTCATGCAGG and TGGGCTT GTTGCTTCTTTTG for *GOG5*.

Recombination frequencies were calculated as the median of six independent cultures as previously published (43).

Mapping of MNase cleavage sites. Yeast spheroplasts and micrococcal nuclease (MNase) digestions were performed according to Fedor and Kornberg (19) with the modifications of Chávez et al. (13). Spheroplasts prepared from mid-log phase cultures transformed with p416GAL1lacZ and grown in the appropriate selective medium containing 2% glucose or 2% galactose were lysed and immediately digested with 6.25 to 800 mU of MNase. For naked DNA controls, genomic DNA was extracted as previously described (28) and digested with 0.003 to 1.6 mU of MNase under the same conditions.

MNase-cleaved genomic DNA was digested with either *Eco*RI (for the endogenous *GAL1* gene) or *Cla*I (for the *GAL::lacZ* fusion) and resolved in 1.5% agarose. As internal size markers, we used genomic DNA digested with *Sac*I or *Xba*I (for the *GAL1* promoter fused to *lacZ*). For the analysis of the endogenous *GAL1* gene, the probe used was the 196-bp *GAL1* fragment located immediately downstream of the *Eco*RI site and obtained by PCR with the oligonucleotides ATTCGACAGGTTATCAGCAAC and TTAAACTTCTTTGCGTCCATC. For the analysis of *GAL1::lacZ* the probe used was the 202-bp *lacZ* fragment immediately upstream of the *Cla*I site and obtained by PCR with the oligonucleotides TCGTTGCTGCATAAACCG and TCGATAATTTCACCGCCG.

Miscellaneous. Serial deletions of the *PHO5::lacZ* fusion constructs were constructed using a double-stranded nested deletion kit from Amersham Pharmacia. Published methods were used for RNA and DNA hybridizations (13, 44).

RESULTS

Transcription impairment through *lacZ* caused by *hpr1Δ* is not dependent on particular *lacZ* sequences but on their distance from the promoter. Transcription of the *Escherichia coli lacZ* gene in *S. cerevisiae* is severely impaired in *hpr1* mutants

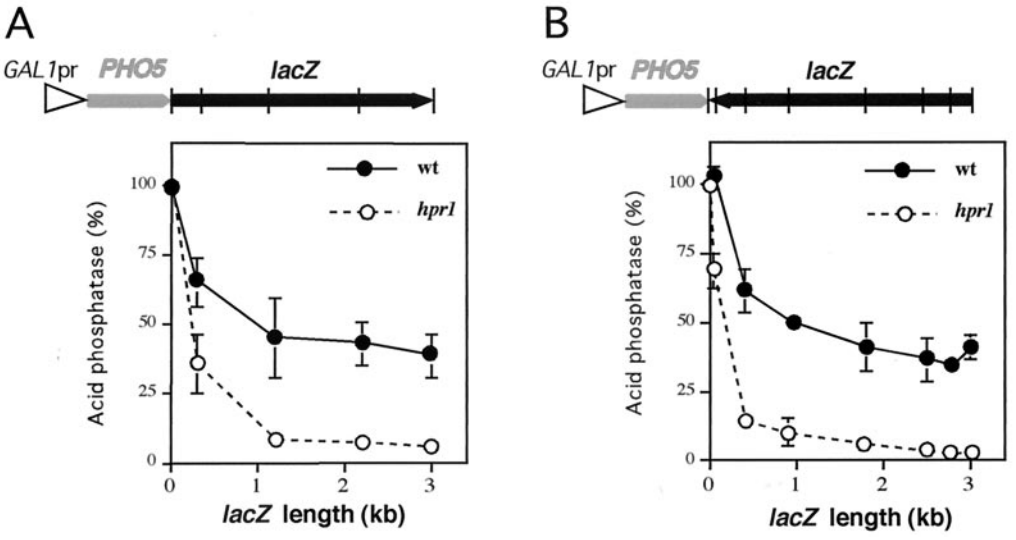

FIG. 1. Expression patterns of serial deletions of *GAL1pr::PHO5-lacZ* fusion constructs. Acid phosphatase activities under induced conditions of wild-type (W303-1A) and *hpr1* (U768-4C) strains transformed with *lacZ*-deleted variants of plasmids pSCh212 (A) or pSCh211 (B) that contain the entire *lacZ* coding sequence fused to *PHO5* in the same and opposite orientations, respectively, under the *GAL1* promoter. The average value and standard deviation of four different transformants is shown for each strain. Vertical lines across the *lacZ* sequences indicate the end points of the deletion constructs analyzed.

at the elongation level. Transcription through *PHO5* is not appreciably affected in *hpr1* cells, but it becomes sensitive to *hpr1* when *lacZ* is fused to *PHO5* in a single transcription unit (11). In order to find out which structural elements or sequence motifs present in *lacZ* are responsible for this transcriptional elongation impairment, we constructed serial deletions of the *lacZ* gene in the *PHO5::lacZ* transcriptional fusion under control of the *GAL1*-regulated promoter. The resulting deletions were introduced into both wild-type and *hpr1* cells. Yeast transformants grown in galactose-containing medium were then assayed for acid phosphatase activity. Expression was clearly lower in the transformants harboring *PHO5-lacZ* fusions than in those containing only *PHO5*, in both wild-type and *hpr1* cells (Fig. 1A). The presence of a *lacZ* fragment as short as 1 kb downstream of *PHO5* reduced *PHO5* expression to 40% in wild-type cells. However, the reduction was considerably stronger in *hpr1*, reaching transcription values below 10% of those of *PHO5* alone (Fig. 1A). Even the shortest fusion, encompassing ≈0.4 kb of the 5′ end of *lacZ*, showed reduced levels of phosphatase activity in the wild-type (65%) and, to a greater degree, *hpr1* (35%) cells (Fig. 1A).

Serial deletions were also made in a *PHO5::lacZ* fusion carrying *lacZ* in an opposite orientation. A similar profile of phosphatase activities was obtained (Fig. 1B). Although all fusions showed lower expression levels than *PHO5* alone, the negative transcriptional effect was clearly stronger in *hpr1* than in wild-type cells. A ≈0.4-kb *lacZ* fragment, for example, was enough to reduce the phosphatase activity to under 15% of the level shown by *PHO5* alone in *hpr1* strains (Fig. 1B). Thus, the two end fragments of *lacZ* were able to impair transcription in *hpr1* cells.

The previous results suggest that there is not a particular *lacZ* sequence responsible for the transcriptional elongation impairment caused by *hpr1*Δ. On the contrary, transcriptional impairment could occur through any *lacZ* region. To confirm this and to show that the negative effect of *hpr1*Δ on acid phosphatase expression really takes place at the transcriptional rather than posttranscriptional level, we performed Northern analyses of selected *PHO5::lacZ*-fragment constructs. We inserted three different ≈0.4-kb fragments of *lacZ* corresponding to the two ends and the center of the gene (plasmids pSCh229, pSCh226, and pSCh251; Table 1) immediately downstream of a *PHO5* gene under *GAL1* control. Galactose-induced transcription of the resulting fusion constructs was analyzed in wild-type and *hpr1* cells. The results shown in Fig. 2 indicate a substantial decrease in the accumulation of full-length mRNA of the three fusion constructs in *hpr1* cells (13 to 25% of the wild-type levels), whereas only a minor effect was observed with *PHO5* alone. In addition to the full-length *PHO5-lacZ* mRNA, a shorter transcript exhibiting the same size as *PHO5* was detected in *hpr1*. The presence of this shorter transcript suggests that *hpr1* cells transcribe poorly through *lacZ* sequences, downstream of the *PHO5* open reading frame (ORF). The same results were obtained when the *lacZ* fragments were located in the opposite orientation (data not shown). To confirm that this phenomenon was due to *lacZ* itself and not to the 3′ end of *PHO5*, we replaced the *lacZ* segment with a 416-bp fragment of the 5′ end of *GAL1*, a gene whose expression is not affected in *hpr1* cells (67) (see Fig. 9A). Northern analysis of the resulting *PHO5::GAL1*Δ fusion was carried out in wild-type and *hpr1* cells (Fig. 2). A weaker reduction in accumulation of full-length mRNA was measured in *hpr1* (60% of wild-type levels), and no short transcript was detected. This confirms that the transcriptional defects of the *PHO5-lacZ* fusion constructs were mainly due to the presence of *lacZ* fragments in the transcription units.

Altogether, these results suggest that the longer distance between the promoter and *lacZ* sequences the greater the transcriptional elongation impairment. To test this possibility, we inserted upstream of *PHO5* the same three *lacZ* fragments

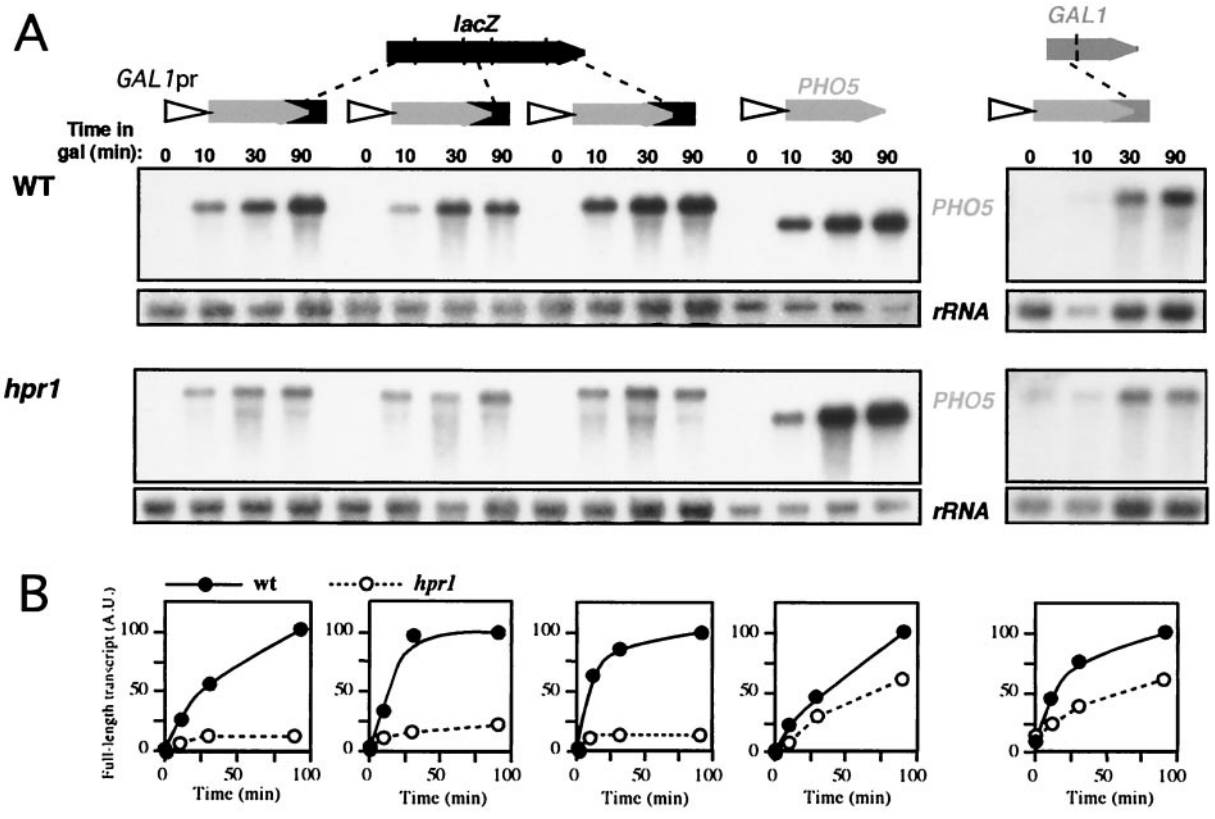

FIG. 2. Transcription analysis of *GAL1pr::PHO5*, three different *GAL1pr::PHO5-lacZ* fusion constructs, and a *GAL1pr::PHO5-GAL1* fusion in wild-type (W303-1A) and *hpr1* (U768-4C) cells. (A) Northern blot analyses of *PHO5*-containing mRNAs driven from the *GAL1* promoter. Plasmids used were pSCh229, pSCh226, and pSCh211Δ17-1 (carrying ≈0.4 kb of the 5′ end, middle part, and 3′ end of *lacZ* fused to *PHO5*, respectively), pSCh202 (carrying the *PHO5* gene), or pSCh251 (carrying ≈0.4 kb of the 5′ end of *GAL1* fused to *PHO5*). Mid-log phase cells were cultured in 3% glycerol–2% lactate synthetic complete (SC)-Ura medium and diluted into identical fresh media to an OD_{600} of 0.3 and incubated for 16 h. Galactose (Gal) was then added and samples were taken for Northern analysis at different times, as specified. A 0.9-kb *Eco*RV *PHO5* internal fragment and a 589-bp 28S rDNA internal fragment obtained by PCR (rRNA) were used as DNA probes. (B) Kinetics of induction of mRNAs as determined by quantification of Northern blots in a Fuji FLA3000. The mRNA values are given in arbitrary units (A.U.) with respects to rRNA levels. For any given construct, RNA levels are related to the wild-type (wt) levels at 90 min, which was set at 100 for each panel.

used previously. The resulting transcriptional fusions exhibited similar transcription levels and patterns in wild-type and *hpr1* cells (Fig. 3). Therefore, the distance between *lacZ* and the promoter can modulate the negative effect of the *lacZ* fragments on transcription in *hpr1* cells.

Transcription through long DNA sequences is negatively affected by *hpr1*Δ. The data shown in Fig. 1 indicate that the longer the constructs containing *lacZ* fragments are, the lower the transcriptional yield exhibited in both wild-type and *hpr1* cells. To evaluate the influence of transcript length on the transcriptional effect of *hpr1*, we put the same three above-mentioned *lacZ* fragments immediately downstream of the *GAL1* promoter. The kinetics of accumulation of these three short *lacZ* fragments was identical in wild-type and *hpr1* cells (Fig. 4A). Full-length mRNA accumulated shortly after induction in both strains and at similar levels in all three constructs, in contrast with the clear difference between the wild type and *hpr1* shown by the entire *lacZ* (Fig. 4A). The same results were obtained when the *lacZ* fragments were cloned in the opposite orientation (data not shown). These results strongly suggest that short transcription units are not impaired by *hpr1*, even if they contain DNA fragments that hinder transcription in a different context.

We have previously shown that in *hpr1* and other mutants affected in the THO complex, the ability of a given DNA segment, like *lacZ*, to impair transcriptional elongation correlates with hyperrecombination of a direct-repeat system containing that segment. This hyperrecombination is transcription dependent (11, 42, 44). We tested, therefore, the recombination frequency of direct-repeat systems containing either one of the three *lacZ* fragments used in the previous experiments flanked by two *leu2* repeats. In agreement with the absence of effect of the *hpr1* mutation on transcription of such *lacZ* fragments, we did not detect a significant stimulation of recombination when the fragments were located between the *leu2* repeats (Fig. 4B). Again, in this case there was a clear difference between the short *lacZ* fragments and the entire *lacZ*, which stimulates recombination between direct repeats up to 200-fold in *hpr1* cells (11) (Fig. 4B).

The previous results suggest that transcript length is an important feature in determining the requirement of Hpr1 in transcription. To confirm this, we constructed comparable transcription and recombination systems containing only yeast long DNA sequences. We randomly chose a fragment from a long yeast ORF, a 3.7-kb fragment of the *S. cerevisiae LYS2* gene. We either fused it to the *GAL1* promoter or inserted it

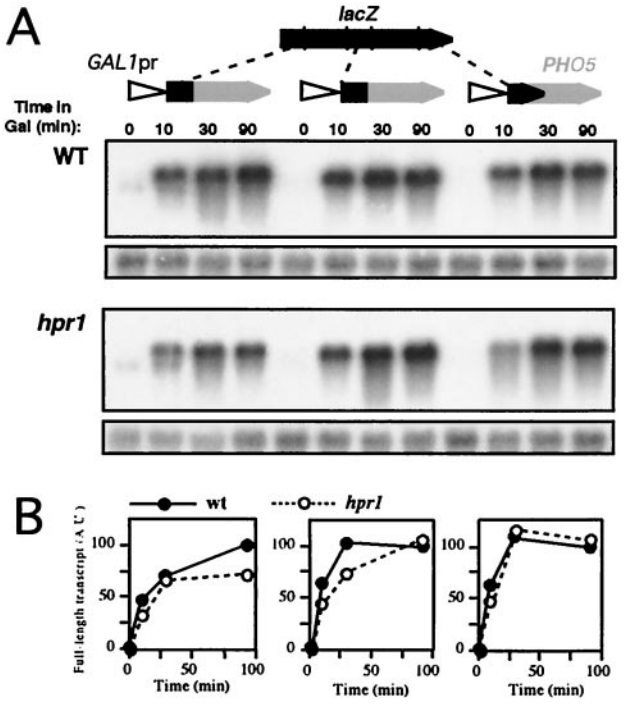

FIG. 3. Transcription analysis of three different *GAL1pr::lacZ*Δ-*PHO5* fusion constructs in wild-type (W303-1A) and *hpr1* (U768-4C) cells. (A) Northern blot analyses of *PHO5*-containing mRNAs driven from the *GAL1* promoter. Plasmids used were pSCh218, pSCh220, and pSCh219 (carrying ≈0.4 kb of the 5′ end, middle part, and 3′ end of *lacZ* fused to *PHO5*, respectively). As a control we used pSCh202 (carrying the *PHO5* gene; data not shown), which gave identical results as those shown in Fig. 2. The transcript levels of each construct with respect to *PHO5* were similar to those of the wild type shown in Fig. 2. Other details were as described for Fig. 2. (B) Quantification of Northern analyses.

between the *leu2* repeats for transcriptional and recombinational analyses, respectively. The new constructs were introduced in wild-type and *hpr1* cells (Fig. 5). Full-length mRNA from the *GAL1pr::LYS2* transcriptional fusion accumulated shortly after transferring wild-type cells to galactose. However, only a smear of incomplete transcript was detected in similar Northern experiments performed with *hpr1* samples (Fig. 5A), a very similar pattern to that obtained for the entire 3-kb-long *lacZ* in *hpr1* (11) (Fig. 4A). As expected for a DNA sequence that cannot be properly transcribed in *hpr1* cells, *LYS2* promoted a strong hyperrecombination in *hpr1* when located between *leu2* repeats (L-*LYS2* system). The recombination frequency reached in *hpr1* (5%) is 60 times higher than the wild-type levels, but still 3- to 10-fold lower than that of analogous systems containing *lacZ* (11) (Fig. 4B). This result supports our hypothesis for the influence of transcript length on *hpr1* sensitivity of transcription.

If the contribution of Hpr1 to the accumulation of long transcripts initiating at the *GAL1* promoter is not restricted to the artificial construct that we have analyzed, we should expect the genome-wide effect of *hpr1* to be more dramatic on long genes than on short ones in highly expressed genes. To test this idea we analyzed the effect of *hpr1*Δ on transcription of five endogenous chromosomal genes with sizes ranging from 0.5 to 3.1 kb. They were selected because they have high and comparable expression levels in YEPD-rich medium in wild-type cells (between 10 and 14 mRNAs per cell, according to Holstege et al. [24]). The longest genes, *EGT2*, *CDC48*, and *KAR2*, showed significantly lower expression levels in *hpr1* than in the wild type. The shortest ones, *OLE1* and *GOG5*, exhibited even higher expression levels in *hpr1* than in the wild type (Fig. 6). As we are not controlling transcription, such as with the regulatable *GAL1* promoter in these experiments, we cannot exclude the possibility that such higher expression levels are an indirect effect of *hpr1*. The correlation between transcript size and the *hpr1*:wild-type transcript ratio is not perfect. This may reflect the facts that (i) each ORF is under the control of a different promoter, (ii) the ORFs are in different chromosomal locations, and (iii) the different DNA sequence context of each gene may affect its transcription pattern. These results are consistent with transcript length being at least one feature partially responsible for impairing transcription driven from strong promoters in *hpr1* cells.

Transcription of G+C-rich DNA sequences is severely impaired by *hpr1*Δ. Although the length of a gene is an important feature influencing the sensitivity of its transcription to the *hpr1* mutation, there are several pieces of evidence indicating that it cannot be the only feature. First, replacement of the *lacZ* fragments by *GAL1* in the *PHO5* transcriptional fusion constructs largely suppressed the *hpr1* effect (Fig. 2). In addition, the two sets of *PHO5* fusions that we constructed, in which *lacZ* fragments were located either at the 3′ or the 5′ end, share the same length but behave differently in *hpr1* cells. Finally, although both *lacZ* and *LYS2* are hyperrecombinogenic when flanked by direct repeats, *lacZ* is significantly more recombinogenic than *LYS2* (Fig. 3B and 4B). Therefore, we decided to explore other features of *lacZ*, the most *hpr1*-sensitive sequence detected so far, in order to identify additional elements influencing transcriptional impairment by *hpr1*.

The most evident difference between *lacZ* and the bulk of *S. cerevisiae* genes is the G+C content. The majority of yeast genes show a G+C content of around 40%, whereas that of *lacZ* is 56.2%. In order to investigate whether the G+C content influences the transcriptional impairment of *lacZ* in *hpr1* cells, we used the *Kluyveromyces lactis LAC4* gene, a yeast homologue of *lacZ* with 40% G+C (1). We placed *LAC4* under *GAL1* control, creating a *GAL1pr::LAC4* fusion similar to those previously used in this work. Transformants of wild-type and *hpr1* isogenic strains were used to determine the kinetics of accumulation of mRNA. The results presented in Fig. 7A show that accumulation of *LAC4* full-length mRNA was only moderately diminished in *hpr1* cells (50% of the wild-type level after 90 min of induction). Nonetheless, in the same background and after an identical induction time, *lacZ* full-length mRNA was almost absent (Fig. 4A) (11). The overall comparison of *LAC4* and *lacZ* transcriptional behaviors showed that transcription through *LAC4* was at least fivefold more efficient than that through *lacZ* in *hpr1* cells. Thus, two transcription units, identical in length and differing in G+C content, were differentially affected by *hpr1*. This indicates that, in addition to transcript length, the G+C content of a DNA sequence may be an important feature influencing transcriptional impairment by *hpr1*.

To determine the effect of *LAC4* in recombination, we placed the entire *LAC4* ORF between the *leu2* direct repeats.

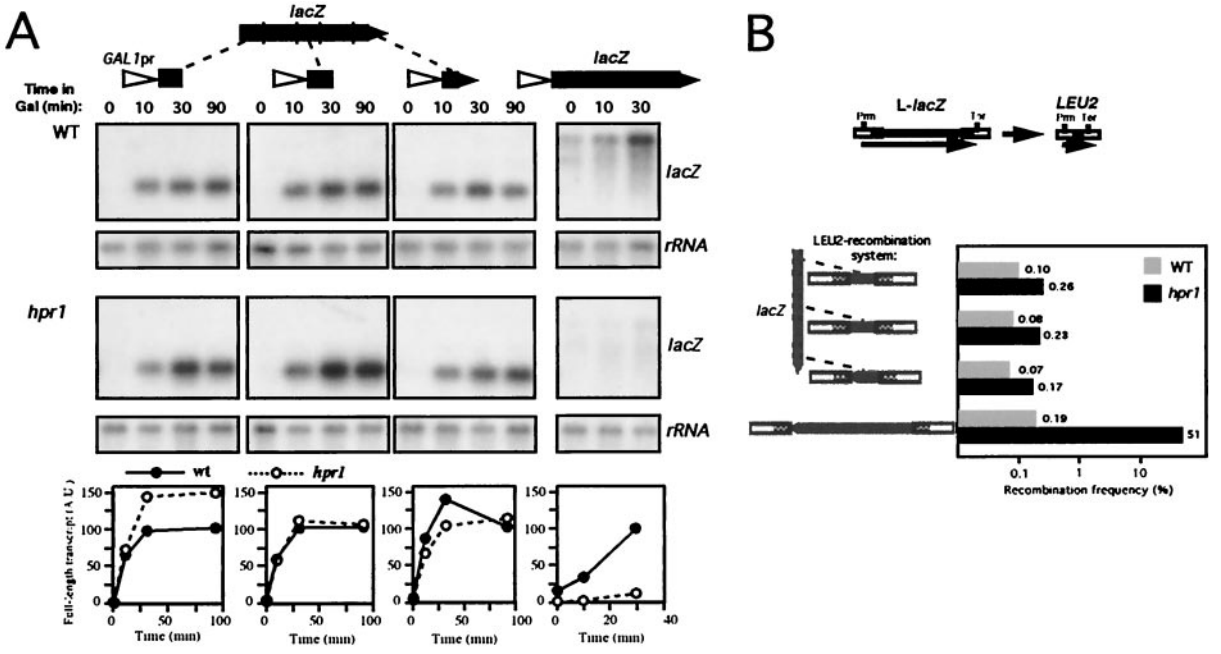

FIG. 4. Transcription and recombination analyses of several *GAL1pr::lacZ* fusion constructs in wild-type (W303-1A) and *hpr1* (U768-4C) cells. (A) Northern analyses of *lacZ*-containing mRNAs transcribed from the *GAL1* promoter. Plasmids used were pSCh215, pSCh213, and pSCh216 (carrying ≈0.4 kb of the 5′ end, middle part, and 3′ end of *lacZ,* respectively) or p416GAL1-lacZ (carrying the entire *lacZ* ORF). Other details were as described for Fig. 2. (B) Recombination frequencies of *leu2*-based direct-repeat systems containing the same short *lacZ* fragments used in the previous Northern experiments. Plasmids used were pSCh221, pSCh222, and pSCh223 (carrying ≈0.4 kb of the 5′ end, middle part, and 3′ end of *lacZ,* respectively) or pSCh205 (carrying the entire *lacZ* ORF). A schematic diagram of the recombination products obtained with the direct-repeat *LEU2* recombination systems used is shown at the top of panel B. The *LEU2* promoter (Prm) and transcriptional terminator (Ter) as well as the RNA (arrow) produced by the system are indicated. The median recombination frequency of six independent values is given in each case. All median frequencies were calculated in duplicate with two independent transformants. Recombinants were selected in SC-Leu-Trp. Data from the L-*lacZ* system containing the entire *lacZ* gene (bottom) are taken from Chávez and Aguilera (11).

The resulting L-*LAC4* system exhibited a high frequency of recombination in *hpr1* cells (90-fold above wild-type levels [Fig. 7B]). This frequency was eightfold lower than that shown by L-*lacZ* but was comparable to the frequency reached by L-*LYS2* (Fig. 3B and 4B). An increase in transcription efficiency is accompanied therefore by a lower recombination frequency. It is important that the L-*LAC4* system shows a hyperrecombination phenotype, because the size of the full transcript in this system is approximately 5 kb. Indeed, and consistent with our hypothesis, we have shown by Northern analysis (Fig. 7C) that transcription of the L-*LAC4* system is impaired in *hpr1* cells.

To further investigate the influence of G+C content on transcription of a DNA sequence, regardless of whether coming from bacteria or yeast, we decided to analyze a yeast gene with a G+C content comparable to that of *lacZ*. *YAT1*, a 2-kb long ORF, is the gene in the *S. cerevisiae* genome with the highest G+C content (58%). We placed *YAT1* under *GAL1* control in a transcriptional system similar to those used in the previous experiments. Northern analysis showed that high levels of *YAT1* full-length mRNA were reached after galactose induction in the wild type, but only a minimal accumulation was detected in *hpr1* cells (Fig. 8A). In agreement with this transcriptional impairment, a recombination system bearing *YAT1* as intervening sequence (L-*YAT1*) displayed an extremely high frequency of recombination in *hpr1* (12%) that was 173 times the frequency reached in the wild type (Fig. 8B).

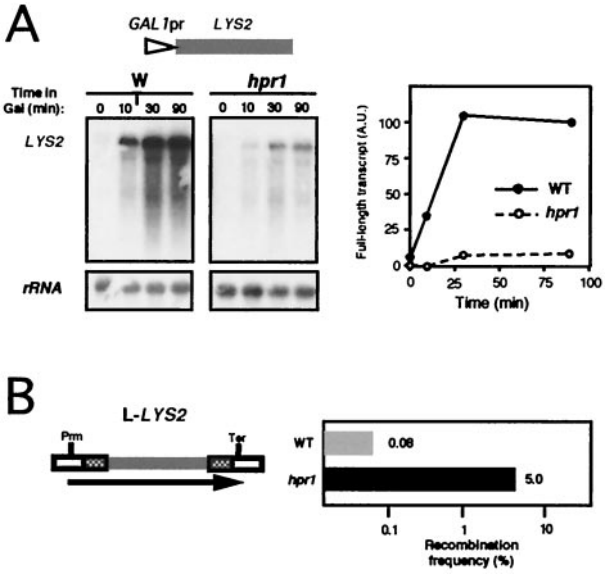

FIG. 5. Transcription and recombination analyses of *LYS2* sequences in wild-type and *hpr1* cells. (A) Northern blot analyses of *LYS2* mRNAs in strains transformed with plasmid pSCh227 containing a 3.7-kb fragment of the *LYS2* coding sequence under the control of the *GAL1* promoter. (B) Recombination frequencies of strains transformed with plasmid pSCh230 harboring a *leu2*-based direct repeat system containing as intervening sequence the same 3.7 kb fragment of *LYS2* used for the transcription assays. Other details are as described for Fig. 4.

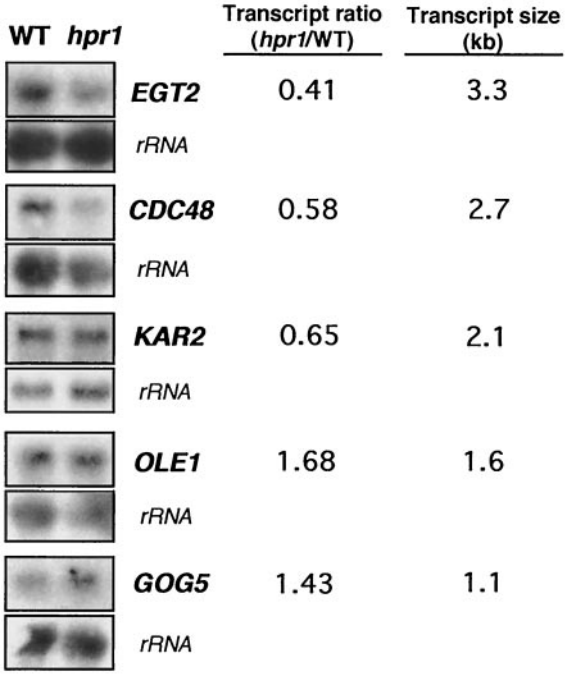

FIG. 6. Transcription analyses of five yeast endogenous genes, *EGT2*, *CDC48*, *KAR2*, *OLE1*, and *GOG5*, having high levels of expression and different transcript sizes, in wild-type and *hpr1* cells. Total RNA was isolated from mid-log phase cells, grown in YEPD broth, and used for Northern analyses. Internal fragments of each gene and of the 23S rDNA, obtained by PCR, were used as DNA probes. The *hpr1*:wild-type transcript ratio was obtained from the mRNA levels that were quantified in a Fuji FLA3000 and normalized with respect to the rRNA levels.

This level of hyperrecombination is comparable to that of L-*lacZ* (Fig. 4B) and higher than the levels of L-*LYS2* (Fig. 5B) and L-*LAC4* (Fig. 7B). Thus, transcription through a medium-size G+C-rich gene is clearly *hpr1* sensitive, indicating that G+C content can modulate the Hpr1 dependency of gene transcription.

Nucleosome positioning is lacking in *lacZ* sequences. The organization of DNA in a proper nucleosome-positioned chromatin structure has been shown to be favored by A+T-rich motifs (27) and prevented by G+C-rich sequences (62). In order to test whether there is a relationship between chromatin structure and transcriptional efficiency in *hpr1* cells, we determined whether the chromatin structure of G+C-rich sequences such as *lacZ* was different from that of low-G+C-content sequences. We performed MNase sensitivity assays of the *GAL1pr::lacZ* fusion construct and the *GAL1* endogenous genes, in which transcription was strongly and poorly impaired in *hpr1* cells, respectively (11, 17, 67) (Fig. 9A). As previously shown (19), clear and specific nucleosome positioning along the endogenous *GAL1* gene was observed (Fig. 9B). Such a pattern of MNase sensitivity was identical for both wild-type and *hpr1*Δ cells. The more diffuse pattern of MNase digestion under induced conditions in both wild-type and *hpr1* cells reflects the destabilization of chromatin structure caused by transcription (9). Interestingly, the pattern of MNase sensitivity of *lacZ* shows no nucleosome organization in either wild-type or *hpr1*Δ cells under both induced and repressed conditions of transcription. Nucleosome positioning is only limited to the *GAL1* promoter (Fig. 9C). Indeed, a lack of nucleosome positioning is also observed over the bacterial sequences upstream of the *GAL1* promoter, through which transcription has also been shown to be impaired in *hpr1* mutants (44). Our results, therefore, show that *lacZ* adopts a random nucleosomal organization in yeast and that *hpr1* has no effect on nucleosome positioning.

DISCUSSION

In this work we have investigated why transcription of DNA sequences like *E. coli lacZ* is especially sensitive to *hpr1*. We have shown that 0.4-kb *lacZ* fragments fused to *PHO5* under the *GAL1* promoter are sufficient to increase the Hpr1 dependency of transcription, but not when they are transcribed alone. Such an effect is position dependent: the longer the distance between the *lacZ* sequence and the *GAL1* promoter, the stronger the impairment of transcription caused by *hpr1*. In addition, we see transcription of long yeast DNA sequences like *LYS2* fused to the *GAL1* promoter is negatively affected by *hpr1*. We have also shown that transcription of *K. lactis LAC4*, a eukaryotic homologue of *lacZ* that is equal in length but with a much lower G+C content, exhibits a milder Hpr1 dependency in *S. cerevisiae*, whereas *YAT1*, an *S. cerevisiae* G+C-rich gene shorter than *lacZ*, is dramatically affected by *hpr1*. Taken

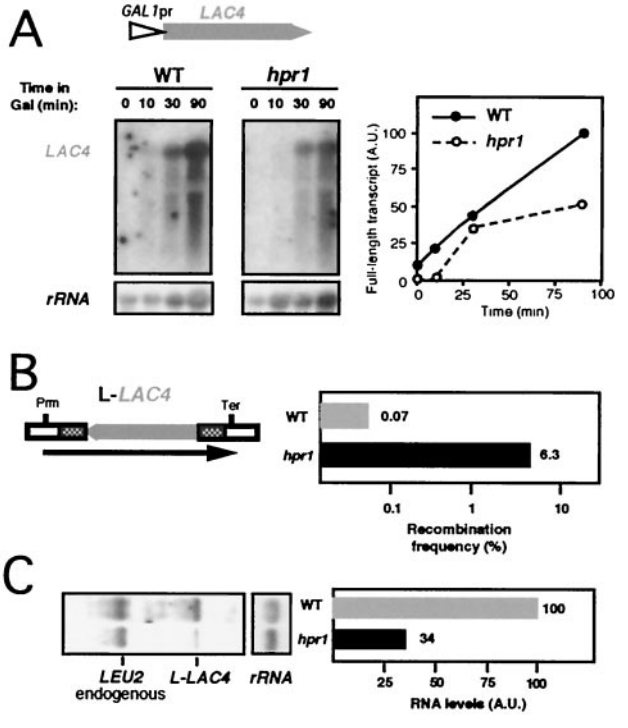

FIG. 7. Transcription and recombination analyses of *LAC4* in wild-type and *hpr1* cells. (A) Northern analyses of *LAC4* mRNAs in strains transformed with the plasmid pSCh255, which contains the entire *LAC4* coding sequence under the control of the *GAL1* promoter. (B) Recombination frequencies of cells transformed with plasmid pSCh254, which harbors the *leu2*-based direct-repeat L-*LAC4* construct containing *LAC4* as the intervening region. (C) Northern analyses of the L-*LAC4* repeat construct in wild-type and *hpr1* cells. Other details are as described for Fig. 4.

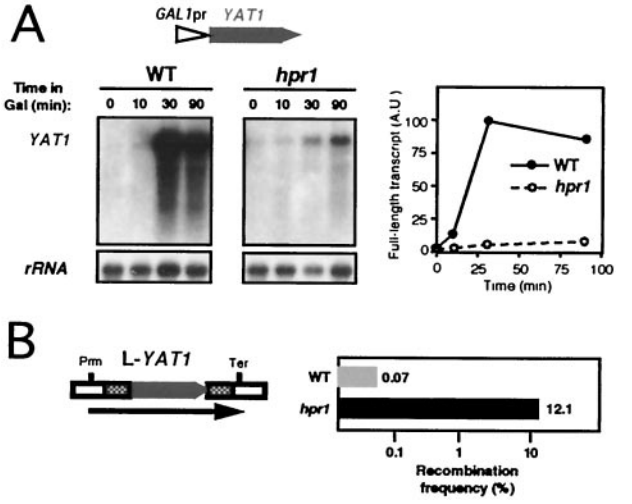

FIG. 8. Transcription and recombination analyses of *YAT1* in wild-type and *hpr1* cells. (A) Northern blot analyses of *YAT1* mRNAs in cells transformed with plasmid pSCh247 containing the entire *YAT1* coding sequence under the control of the *GAL1* promoter. (B) Recombination analyses of cells transformed with plasmid pSCh248, which harbors the *leu2*-based direct-repeat system containing the entire *YAT1* gene as intervening sequence. Other details are as described for Fig. 4.

together, these results indicate that both length and G+C content are important elements influencing gene transcription in vivo and that Hpr1 is an important factor controlling transcription of either long or G+C-rich DNA sequences fused to a strong promoter such as *GAL1pr*.

Hpr1 is required for proper transcription of long DNA sequences. We have shown that transcription of long DNA sequences is compromised in *hpr1* cells, whereas shorter sequences are either unaffected or mildly influenced. This conclusion is supported by the inappreciable effect of *hpr1* on transcription of short *lacZ* fragments directly fused to the *GAL1* promoter, whether or not upstream of *PHO5*, whereas the same *lacZ* fragments do confer *hpr1* dependency when fused downstream of *PHO5* (Fig. 2 and 3). This conclusion is also supported by the marked negative effect of *hpr1* on transcription of the 3.7-kb-long *LYS2* fragment under control of the *GAL1* promoter (Fig. 5). The transcriptional analysis of five highly transcribed chromosomal genes showed a negative effect of *hpr1* on transcription of the three longest ones, *EGT2* (3.3 kb), *CDC48* (2.7 kb), and *KAR2* (2.1 kb), but no negative effect on the other two, *OLE1* (1.6 kb) and *GOG5* (1.1 kb) (Fig. 6). Further experiments would be required to know whether these results are also valid for poorly expressed genes.

Processivity defects of an RNA polymerase can be more easily detected with transcription of long rather than short DNA templates. Comparison between long and short transcripts is in fact a common method to quantify the effect of transcription factors on RNAPII-mediated elongation (66). Consequently, a length-dependency effect of *hpr1* on transcription is expected if Hpr1 controls elongation. The longer the transcription unit, the higher the probability of RNAPII reaching a DNA region requiring the function of Hpr1. Even in the wild-type strain, long transcription units are less efficiently expressed than short ones, as we have observed by comparing

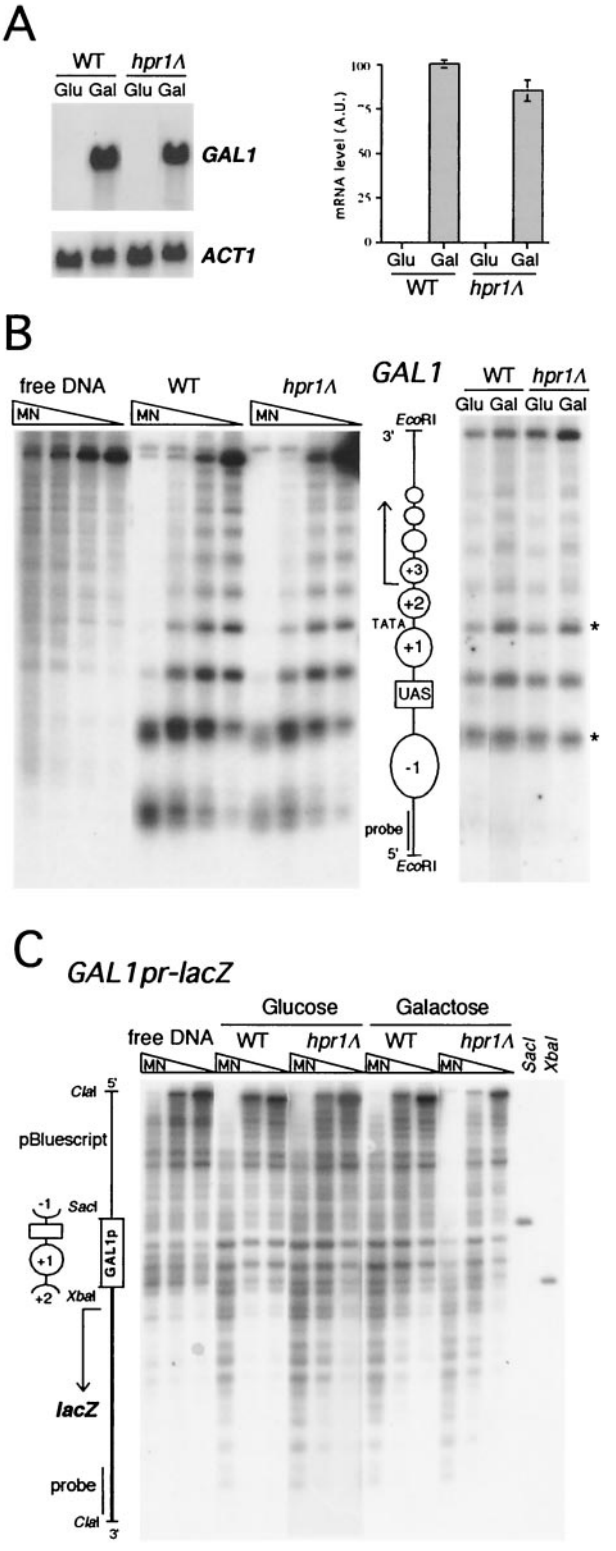

FIG. 9. MNase digestion pattern of the *GAL1* gene and the *GAL1pr::lacZ* fusion in wild-type and *hpr1* strains under repression and activation conditions. (A) Northern blot analysis of *GAL1* mRNAs. (B) Nucleosome positioning over the *GAL1* gene. (C) MNase digestion pattern of the *GAL1pr::lacZ* fusion. A scheme of the analyzed regions of *GAL1* and *lacZ* indicating the position of nucleosomes and the most relevant regulatory elements is shown. Asterisks indicate the MNase hypersensitive sites associated with the activation of transcription of *GAL1*.

the expression levels of several *PHO5::lacZ* fusion constructs that differ in the size of the *lacZ* fragment (Fig. 1). Thus, it is possible that the absence of Hpr1 enhances elongation defects already present in the wild type, the occurrence of such defects being more likely as the DNA sequence to be transcribed becomes longer.

Transcription of G+C-rich DNA sequences is Hpr1 dependent. Our results support a correlation between G+C content and *HPR1* function. This conclusion is based on three sets of data. First, although most tested genes are either slightly or not affected by *hpr1* (Fig. 7) (11, 67), transcription of *lacZ* (56% G+C) in *Saccharomyces* is strongly affected and direct-repeat systems containing *lacZ* sequences that are transcribed exhibit hyperrecombination in *hpr1* cells (11). Second, transcription of *LAC4*, a 40% G+C-rich *lacZ* orthologue of *K. lactis,* is at least five times more efficient than *lacZ* in *hpr1* cells (Fig. 7A), and direct repeats flanking *LAC4* recombine at frequencies eightfold lower than those containing *lacZ* (Fig. 7B). Finally, *YAT1*, a 2-kb-long gene from *Saccharomyces* with a 58% G+C content, is strongly affected by *hpr1* both in transcription and in recombination (Fig. 8).

As far as we know, neither a direct influence of the G+C content of the template on transcription elongation efficiency nor the requirement of auxiliary factors for transcription of G+C-rich genes has been described. The only sequence features that have been shown to affect elongation are those of specific transcriptional pausing sites (59). Some pause sites identified in bacteria are G+C rich, like the *ops* signals in *E. coli* (4), but others are not. It is, therefore, unlikely that G+C-rich genes exhibit, in general, a higher probability of containing a pause signal. Nonetheless, a high G+C content might affect transcriptional elongation by stabilizing secondary structures in the nascent RNA that can function as pausing signals. At least in the case of T7 phage, a lower number of hydrogen bonds in the RNA hairpins eliminates some pauses (32), suggesting that a high G+C content might contribute to stronger RNA-mediated elongation impairments.

A G+C-rich nascent RNA might also form more stable RNA-DNA hybrids within the template. Twelve-nucleotide-long RNA-DNA hybrids negatively affect RNAPII processivity in vitro (29). Another class of RNA-DNA hybrids are R-loops, produced by the association of nascent mRNA with upstream template DNA. It has been proposed that these R-loops are formed during transcription of the G+C-rich human immunoglobulin switch region in vivo (15), and they have been detected after in vitro transcription (58). The cleavage of R-loops by specific nucleases might initiate class-switch recombination (58), providing a mechanism to explain transcription-associated recombination. Nothing is known about the influence of RNA-DNA structures on RNAPII-dependent transcription (20). However, it has been proposed that R-loops formed during rRNA transcription elongation in *E. coli* constitute roadblocks for the next transcribing RNA polymerase (25).

Molecular features making transcription elongation Hpr1 dependent. Unless Hpr1 plays more than one function, we should expect a common mechanistic requirement during transcription of long versus G+C-rich genes requiring its action. It is not evident which kind of transcription-impairing signal might link long and G+C-rich DNA sequences. One possibility would be the existence of shorter G+C-rich regions hindering transcription within long genes. However, we can exclude this possibility with the results of this work. Considering a 300-bp-long window (the minimal *lacZ* fragment conferring an effect of *hpr1* in transcription), the maximum G+C content is 46% for *LYS2*, 45.5% for *LAC4*, and 59% for *lacZ*. Shorter or longer windows show similar results. In addition, no difference in the G or C content is found on the cDNA strands. As *LYS2* is no more G+C-rich than *LAC4*, length is the most likely reason the two genes behave differently in *hpr1* mutants. Consistently, when *LAC4* is located between the *leu2* repeats in the L-*LAC4* construct, the transcription unit containing *LAC4* becomes longer and transcription becomes significantly affected in *hpr1* cells (Fig. 7).

Alternatively, the link between long and G+C-rich sequences might be unrelated to the DNA sequence itself. Transcription of long and G+C-rich sequences may produce some kind of transcriptional event that would be overcome by the action of Hpr1. Genetic analyses have provided some hints as to the nature of this kind of event. Several mutants have been described that display synthetic phenotypes with *hpr1* (2, 67). The fact that topoisomerase mutants (*top1*, *top2*, and *top3*) become sick in an *hpr1* background may establish a link with DNA topology (2, 48). For example, the accumulation of negative supercoiling impairs transcriptional elongation of bacterial genes in vitro (30), and positive supercoiling diminishes RNAPII-dependent transcription in yeast cells. R-loops are formed in the absence of DNA topoisomerase I in *E. coli* (16), and they have been proposed to impair transcription elongation (25). Elongation by RNAPII alters template topology (8), producing an accumulation of positive and negative supercoiling ahead of and behind the RNAPII, respectively (33). It is, therefore, expected that positive supercoiling will be stronger at the 3′ region of long transcription units. Examples of DNA sequences that impair transcription elongation more efficiently in distal locations have been reported (63). The strong effect of *hpr1* on transcription of long DNA sequences agrees with this view.

Chromatin structure is another source of stress affecting RNAPII-mediated transcription elongation that requires the action of specific auxiliary factors (reviewed in reference 39). Some mutations resulting in a poor growth phenotype with *hpr1* are in fact related to chromatin structure, like *SIN1-2* or those causing histone imbalance (67). The organization of DNA in a proper, nucleosome-positioned chromatin structure is favored by some A+T-rich motifs (27) and is prevented by some G+C-rich sequences (62). A G+C-rich sequence might, therefore, be biased against a proper chromatin structure. The *lacZ* gene is in fact unable to support stably organized chromatin in *S. cerevisiae* (Fig. 9). Transcription of G+C-rich genes might then be impaired in *hpr1* due to an aberrant chromatin structure.

If these hypotheses are true, the location of a G+C-rich region in the 3′ region of a gene should produce a stronger effect, since it would combine two sources of transcriptional stress in the same place: superhelicity and aberrant chromatin structure. Indeed, negative supercoiling can induce changes of DNA structure in CG sequences, with this altered structure being able to produce transcriptional elongation blocks (41). Our results with the *PHO5::lacZ* fusion constructs support this view, since the location of the G+C-rich *lacZ* fragments at the

3′ region produced a clear transcriptional effect in *hpr1* mutants (Fig. 2), whereas their location at the 5′ region had no effect (Fig. 2 and 3).

Finally, we cannot exclude the possibility that the size and G+C content of the nascent RNA, and not of its DNA template, are what determines the requirement of Hpr1 in transcription. This would imply that Hpr1, and by extension the THO complex, might also control RNA metabolism beyond transcription elongation. Such a possibility is consistent with the observation that transcription and RNA processing are coupled (reviewed in references 6, 23, 47) and could explain the observed RNA export defects of *hpr1* mutants (51). In this respect, the recent observations that *hpr1* is suppressed by overexpression of the putative RNA helicase *SUB2* and that *sub2* mutants are also hyperrecombinant (18) are noteworthy.

Hpr1 is stably associated in the cell nucleus with Tho2, Mft1, and Thp2, forming the THO complex (12). Mutations affecting any of the four proteins cause the same phenotypes in transcription and transcription-associated recombination, although the quantitative effect of each mutation is different (12). Since the THO complex is a functional unit, the information obtained studying the gene spectrum affected by *hpr1* sheds light on the in vivo functional role of the complex. Characterization of the biochemical and functional properties of the THO complex in vitro will help in understanding why transcription of long as well as G+C-rich genes preferentially requires a specific cellular function. It remains to be seen whether these conclusions can be extended to endogenous yeast genes or to DNA sequences transcribed from poorly expressed promoters. The existence of Tho2 homologues as well as of long genes and G+C-rich DNA sequences in *Drosophila,* mice, and humans opens the possibility that this might be a general phenomenon in all eukaryotes.

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