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ORIGINAL PAPER

A reduction in RNA polymerase II initiation rate suppresses hyper-recombination and transcription-elongation impairment of *THO* mutants

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Abstract Hrs1/Med3, a component of the Mediator involved in transcription initiation, was previously isolated as a suppressor of *hpr1*Δ hyper-recombination linked to transcription elongation. Here we show that *hrs1*Δ-mediated suppression is specific of transcription-associated hyper-recombination (TAR). The decrease in recombination associated with *hrs1*Δ, either in wild-type or *hpr1*Δ cells is only observed in DNA repeats constructs in which transcription is Hrs1-dependent. We propose that the suppression of THO mutants by *hrs1*Δ is due to the specific effect of *hrs1*Δ on transcription initiation of the recombination system. In parallel we show that the higher the transcription of a gene the more important becomes the THO complex for its expression, implying that the in vivo relevance of this complex is dependent on the frequency of RNAPII transcription initiation. This study furthers the understanding of the importance of THO in transcription and the maintenance of genome stability.

Keywords Hrs1/Med3 · Transcription-associated recombination · THO complex · Hyper-recombination · Transcription

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Introduction

The Mediator of RNA polymerase II (RNAPII) is an essential factor in the regulation of transcription initiation, and is conserved from yeast to humans (Boube et al. 2002; Reeves and Hahn 2005). It is composed of ∼20 proteins grouped in different subcomplexes with a putative specialized function each. One of these proteins is Hrs1/Med3, which was isolated as a suppressor of the hyper-recombination phenotype of *hpr1*Δ (Santos-Rosa and Aguilera 1995). Hpr1 is part of THO/TREX, a conserved eukaryotic complex first identified in yeast (Chavez et al. 2000; Strasser et al. 2002), which functions at the interface between mRNP formation and RNA export during transcription elongation (Aguilera 2005). THO mutations were identified by their strong hyper-recombination phenotype, which is linked to defective transcription elongation of GC-rich and long DNA sequences (Chavez et al. 2001). Different studies have led to the proposal that THO functions during transcription elongation, consistent with the fact that THO immunoprecipitates with transcribed chromatin (Strasser et al. 2002; Kim et al. 2004), and their null mutations impair transcription elongation both in vivo and in vitro (Mason and Struhl 2005; Rondon et al. 2003). The physical and functional interaction of THO with RNA export factors have contributed to the present view that transcription and RNA export are coupled, the THO/TREX complex being a key player in this interconnection. Genome-wide analysis of Nuclear Pore Complex-tethered loci showed an association of highly transcribed genes with the nuclear periphery. This is the case of *Saccharomyces cerevisiae INO1* gene and genes induced by galactose or α-factor; they move towards the nuclear periphery after transcriptional activation (Brickner and Walter 2004; Casolari et al. 2004, 2005; Cabal et al. 2006).

The synthetic lethal phenotypes of THO mutants with mutations in transcription elongation factors such as *spt4*, exosome components such as *rrp6*, transcription termination/3′-end processing factors, such as *rna14* and *rna15*, or RNA export factors such as *mex67* or *yra1*, and also RNAPII recruitment determined by ChIP analysis (Jimeno et al. 2002; Strasser et al. 2002; Zenklusen et al. 2002; Kim et al. 2004; Luna et al. 2005) support the view that THO functions during elongation and affects later steps of transcription.

Interestingly, different screenings for suppressors of *hpr1* have identified several components of the yeast Mediator. One such component is Hrs1/Med3, whose null mutation suppresses the hyper-recombination phenotype of *hpr1*Δ (Santos-Rosa and Aguilera 1995; Piruat and Aguilera 1996; Santos-Rosa et al. 1996). *hrs1*Δ also reduces recombination between direct-repeats in wild-type cells (Santos-Rosa et al. 1996). These findings raise the question of how a mutation in a transcription-initiation factor can suppress a phenotype linked to a transcription elongation defect. In order to decipher the molecular basis for these phenotypes, we analysed the effect of *hrs1*Δ on recombination and found that the decrease in recombination caused by *hrs1*Δ, both in wild type and in THO mutants, is specific to transcription-associated recombination (TAR) and is only observed in recombination systems in which transcription is dependent on Hrs1. Importantly, we show that Hpr1 becomes critical for transcription driven from a strong promoter but of little importance for transcription driven from weak promoters, implying that the in vivo relevance of THO in the expression of one gene depends on the strength of transcription. Altogether, our analysis shows that suppression of TAR by *hrs1*Δ*/med3*Δ is caused by the abolishment of transcription initiation and enhances the understanding of in vivo relevance of THO in transcription, this being dependent on promoter strength.

Materials and methods

Strains

Strains used in this study are listed in Table 1.

Plasmids

Plasmids pRS416GY (pRS416 containing the *LYS2* gene under the control of the Gal1 promoter) and pCM184-tet-LYS (pCM184 containing the *LYS2* gene under the control of the tet promoter) were generated in this study. Plasmids pRS314LY, pRS314LYΔNS, pRS314LU, pRS314LNA, pRS314LNB, pRS314L-*lacZ*, pRS314GL-*lacZ* and pLAUR, used for the studies of recombination and transcription have been previously published (Prado et al. 1997; Jimeno et al. 2002).

Table 1 Strains

Strain	Genotype	References
AYW3-3C	*MATα ade2-1 can1-100 his3 ura3 leu2k::ADE2-URA3::leu2k*	Santos-Rosa and Aguilera (1994)
C2Y-4C	*MATα ade2 his3 trp1 ura3 cdc2Ts*	Conrad and Newlon (1983)
C9Y-1B	*MATα his3 leu2 cdc9Ts*	Hartwell et al. (1973)
UKH-1A	*MATα ade2 can1-100 his3 leu2 trp1 ura3 hpr1ΔHIS3*	This study
UKH-1B	*MAT***a** *ade2 can1-100 his3 leu2 trp1 ura3 hpr1ΔHIS3 hrs1ΔKAN*	This study
UKH-1C	*MATα ade2 can1-100 his3 leu2 trp1 ura3 hrs1ΔKAN*	This study
UKH-1D	*MATα ade2 can1-100 his3 leu2 trp1 ura3*	This study
SSL-2A	*MAT***a** *ade2 his3 leu2 ura3 leu2k::ADE2-URA3::leu2k hrs1-1-KAN*	This study
MC9-1D	*MAT***a** *ade2 his3 ura3 cdc9Ts leu2k::ADE2-URA3::leu2k*	This study
MC9-8B	*MAT***a** *ade2 ura3 leu2k::ADE2-URA3::leu2k hrs1-1-KAN cdc9Ts*	This study
MC2-7B	*MATα ade3 his3 ura3 leu2k::ADE2-URA3::leu2k cdc2Ts*	This study
MC2-7D	*MATα ade3 his3 ura3 leu2k::ADE2-URA3::leu2k hrs1-1-KAN cdc2Ts*	This study
SSAA-12B	*MATα ade2 his3Δ200 ura3 leu2k::ADE2-URA3::leu2k hrs1ΔLEU2*	Santos-Rosa et al. (1996)
SSAA-8B	*MAT***a** *ade2 his3Δ200 ura3 leu2k::ADE2-URA3::leu2k*	Santos-Rosa et al. (1996)
AYW3-1B	*MAT***a** *ade2 can1-100 his3 trp1 ura3 leu2k::ADE2-URA3::leu2k*	Santos-Rosa and Aguilera (1995)
HRN1-4A	*MATα his3 leu2 lys2Δ0 ura3*	This study
HRN1-2C	*MATα his3 leu2 lys2Δ0 trp1-1 ura3 hpr1ΔHIS3*	This study
W303-1A	*MAT***a** *ade2-1 can1-100 his3-11 trp1-1 ura3-1 leu2-3,112*	Thomas and Rothstein (1989)
RK2-6D	*MAT***a** *ade2-1 can1-100 his3-11 trp1-1 ura3-1 leu2-3,112 tho2ΔKAN*	Piruat and Aguilera (1998)
WMK-2A	*MAT***a** *ade2-1 can1-100 his3-11 trp1-1 ura3-1 leu2-3,112 mft1ΔKAN*	Chavez et al. (2000)
U678-1C	*MAT***a** *ade2-1 can1-100 his3-11 trp1-1 ura3-1 leu2-3,112 hpr1ΔHIS3*	Piruat and Aguilera (1998)

Recombination analyses

Recombination frequency was obtained from three different transformants for each genotype. It was determined as the average of 3 median frequencies of 6–12 independent values for each transformant, as previously reported (Santos-Rosa and Aguilera 1994).

Gene expression analyses

Northern analyses were performed according to standard procedures. ^{32}P-labelled DNA probes used in Northern assays were as follows: *lacZ*: 3-kb *Bam*HI fragment of pPZ plasmid (Straka and Horz 1991); *LEU2*: 0.6-kb *Cla*I *Eco*RV fragment of pRS415 plasmid; *pBR322:* pBR322 linearized with *Eco*RI*;* 5′*ADE2*: 1.2-kb *Hin*dIII fragment of pHSR1 (*ADE2* cloned into *Bam*HI *Eco*RV of pRS306 plasmid (Sikorski and Hieter 1989); 3′*ADE2*: 0.5-kb *Eco*RI-*Hin*dIII fragment of pHSR1 plasmid; *URA3*: 0.9-kb *Pst*I-*Sma*I fragment of the pRS315-U plasmid (pRS315 with a *URA3 Hin*dIII–*Pst*I fragment); *YOR129c*: 0.55-kb *Bam*HI–*Hin*dIII fragment of pHSR1; *LYS2*: 1.4-kb *Bst*EII fragment of pRS416GY, and the 589-bp internal 25S rDNA fragment obtained from genomic DNA by PCR.

Results

*hrs1*Δ*/med3*Δ only suppresses transcription-dependent hyper-recombination mutants

We have previously shown that the absence of Hrs1/Med3 suppresses the transcription-associated hyper-recombination phenotype of *hpr1*Δ, *tho2*Δ and *thp1*Δ mutants in the *leuk-2k::ADE2-URA3::leu2-k* chromosomal recombination construct, based on two 2.16-kb *leu2-k* direct repeats (Lk-AU system) (Santos-Rosa and Aguilera 1995; Piruat and Aguilera 1998; Gallardo and Aguilera 2001). We then wondered whether such a suppression was specific to hyper-recombination caused by mutations in the THO and Thp1–Sac3 complexes, or if it could also be observed in other hyper-recombination mutants whose effect was not related to transcription. Consequently, we analysed the effect of *hrs1*Δ on the hyper-recombination phenotypes of DNA polymerase III (*cdc2*) and DNA ligase (*cdc9*) mutants that are presumably caused by their intrinsic defects in DNA replication (Montelone et al. 1981; Kokoska et al. 2000). Recombination was assayed in the Lk-AU system. As can be seen in Fig. 1, *cdc2* and *cdc9* mutations caused an 800- and 32-fold increase above wild-type levels in the frequency of recombinants, respectively. However, hyper-recombination of neither mutant was suppressed by the strong *hrs1-1* mutation, suggesting that the suppression effect of *hrs1* is not general

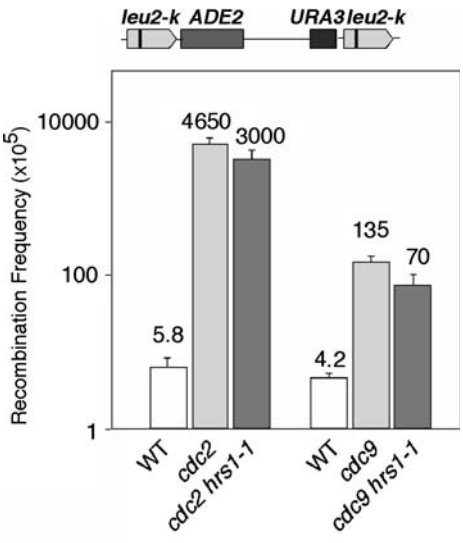

Fig. 1 Recombination analyses of *cdc2*, *cdc2 hrs1-1*, *cdc9 and cdc9 hrs1-1* mutants. Recombination frequencies of *cdc2 (*MC2-7B*)*, *cdc9 (*MC9-1D*)*, *cdc2 hrs1-1* (MC2-7D), *cdc9 hrs1-1* (MC9-8B) and wild type (AYW3-1B) congenic strains carrying the Lk-AU system are shown. A scheme of the system is shown at the *top*. For recombination analyses, independent colonies were obtained from SC and recombinants were selected in SC medium containing FOA

for any type of hyper-recombination, but presumably specific for hyper-recombination linked to transcription.

hrs1 only suppresses hyper-recombination linked to transcription

We next studied the ability of *hrs1*Δ to suppress hyper-recombination of *hpr1*Δ in systems such as L-lacZ and LY, both based on two 0.6-kb *LEU2* truncated repeats located in *CEN* (centromeric) plasmids (Chavez et al. 2001). In both systems transcription is driven from the *LEU2* promoter and the RNAPII traverses through a long and high G–C DNA fragment as a result of which a severe hyper-recombination phenotype is observed in *hpr1*Δ mutants. Unexpectedly, *hrs1*Δ did not suppress the *hpr1*Δ hyper-recombination phenotype in these systems (Fig. 2a). A slight reduction of recombination in the double mutant *hpr1*Δ *hrs1*Δ in the LY system has been reported in a previous study (Santos-Rosa and Aguilera 1995), but we found that this was due to a diminished capacity of the *hpr1*Δ *hrs1*Δ transformants to form colonies in the selective minimal medium in which recombinants were scored, independent of recombination (data not shown). Instead, hyper-recombination in the Lk-AU system was completely suppressed. Therefore, suppression of *hpr1*Δ hyper-recombination seems to be specific of recombination constructs.

It is important to notice that the specificity of the reduction of TAR by *hrs1*Δ mutation is not exclusive of THO mutants, but it is also observed in wild-type cells. Whereas *hrs1*Δ reduces the recombination frequency in the Lk-AU

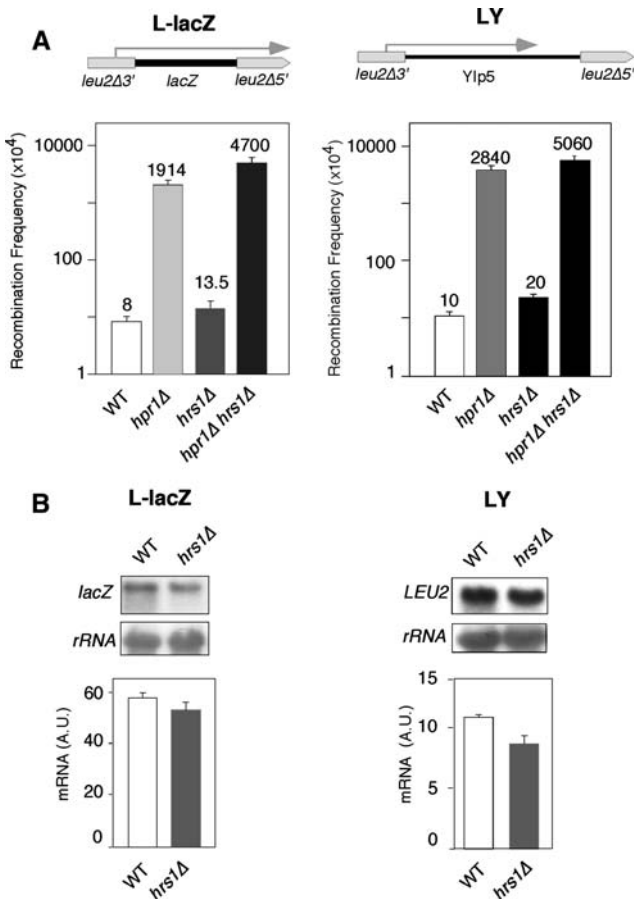

Fig. 2 Recombination and transcription analyses of *hrs*Δ, *hpr1*Δ and *hpr1*Δ *hrs1*Δ mutants. **a** Recombination frequencies of *hpr1*Δ (UKH-1A), *hrs1*Δ (UKH-1C), *hpr1*Δ *hrs1*Δ (UKH-1B) and wild type (UKH-1D) isogenic strains transformed with L-lacZ and LY systems are shown. The schemes of the systems are shown at the *top*. Independent colonies of three different transformants were obtained from SC-trp and recombinants were selected in SC-leu-trp. **b** Effect of *hrs1*Δ mutant on transcription. Northern analyses of L-lacZ and LY mRNAs driven from the *LEU2* promoter in *hrs1*Δ (UKH-1C) and the isogenic wild type (UKH-1D) are shown. The *LEU2*, *lacZ* and rDNA probes used are detailed in "Materials and methods". RNA levels in arbitrary units (*A.U.*) were obtained in a Fuji FLA 3000 and were normalized with respect to rRNA levels of each sample

system (Santos-Rosa et al. 1996), this is not the case for the LY and L-lacZ systems, in which we did not observe such a reduction (Fig. 2a) Thus, we can conclude that the effect of *hrs1* on recombination is general to TAR.

Suppression of TAR by *hrs1* is linked to its capacity to reduce transcription initiation

To understand the molecular basis of the difference between the recombination systems in which suppression was observed from those in which it was not, we analysed the effect of *hrs1*Δ on transcription of the different recombination systems used, as can be seen in Fig. 2b, *hrs1*Δ was not defective in transcription of either of the LY or L-lacZ systems, in which hyper-recombination was not suppressed. Both wild type and *hrs1*Δ strains accumulated the same RNA levels. Therefore, since the *hpr1*Δ hyper-recombination phenotype has only been observed with high levels of transcription, it was possible that the *hrs1*Δ-mediated suppression of *hpr1*Δ associated hyper-recombination as well as TAR reduction in the wild-type background could only be seen in recombination systems whose transcription was dependent on Hrs1/Med3. Therefore, we expected that at least some transcripts of the Lk-AU system should be dependent on Hrs1. To test this possibility, we carried out a detailed Northern study of this system. In Fig. 3a we show a scheme of the Lk-AU system, the probes used (marked as lines), and the transcripts obtained (marked as arrows).

We first observed that transcription of *leu2-k* and *URA3* was not affected in *hrs1*Δ mutants (data not shown). However, as can be seen in Fig. 3b, when we hybridized with a probe containing the *ADE2* region (probe a) we obtained two bands: a 1.7-kb band (transcript 1) that corresponds to the *ADE2* allele and a 3-kb band (transcript 2) present in the wild type and disappeared in *hrs1*Δ. This latter band corresponded to the fragment we were looking for. To confirm the identity of this band, we carried out a more detailed analysis of transcripts using probes against more specific regions. These were: 5′ *ADE2* (probe b), 3′ *ADE2* (probe c), 3′ *YOR129c* (probe d) and pBR322. With probe b (5′ *ADE2)* we only obtained a 1.7-kb band (transcript 1) that corresponds to the *ADE2* region. With probe c (3′ *ADE2*) we obtained three bands: a 1.7-kb band (transcript 1) that corresponds to *ADE2*, a 3-kb band (transcript 2) (which was the same hybridizing with probe a), plus a smaller 1.5-kb band (transcript 3) that presumably corresponds to the 3′ end of *ADE2* as it does not hybridize with probe b. Transcripts 2 and 3 were Hrs1-dependent. With probe d (*YOR129c*) we obtained two bands: a 2.8-kb band that corresponds to the endogenous *YOR129* gene (transcript E) and a 0.5-kb band that corresponds to the small fragment of this gene present in the Lk-AU system (transcript 4). Hybridization with *pBR322* led to a 3-kb band (transcript 2) plus a smaller 1.5-kb band (transcript 3), which are the same obtained with probe c (3′ ADE2) and indeed are Hrs1-dependent. Therefore, we can conclude that there are two Hrs1-dependent transcripts produced by the Lk-AU system. These correspond to the 3′ end of the *ADE2* and to pBR322. These data suggest that hyper-recombination in the Lk-AU system is dependent on pBR322-based transcripts, and that the abolishment of transcription through pBR322 by *hrs1*Δ is the cause of the suppression of hyper-recombination.

Confirmation of this hypothesis required the use of recombination assays lacking the pBR322 region covering transcripts 2 and 3. Since these pBR322 sequences in Lk-AU could only be manipulated in the yeast chromosome and because it implied removal of the unique bacterial *ori*

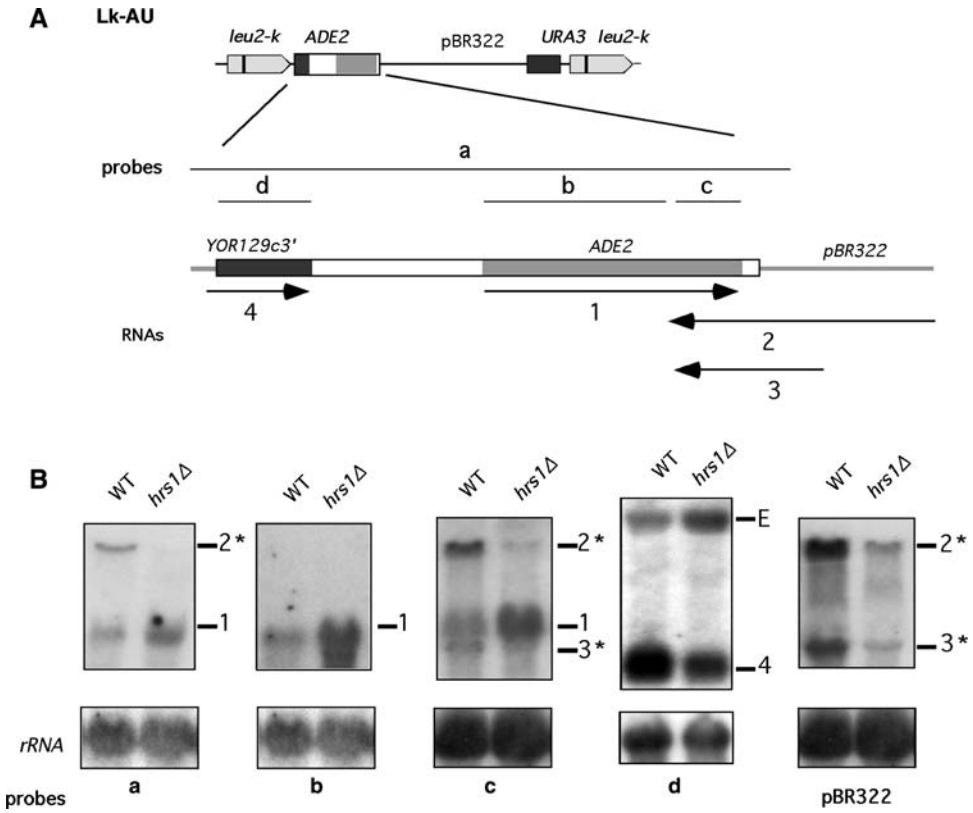

Fig. 3 Expression analysis of the Lk-AU system in *hrs1*Δ mutant. **a** Scheme of ^{32}P-labelled DNA probes used in the study (detailed in "Materials and methods"). **b** Northern analysis of *hrs1*Δ (SSAA-12B) and wild type (SSAA-8B) congenic strains carrying the Lk-AU system. Hrs1-dependent transcripts are marked with an asterisk. Probes used are indicated by letters (**a**–**d**). RNAs produced by the Lk-AU system are identified by numbers (*1*–*4*). *E* Indicates The RNA of the *YOR129c* endogenous gene. Other details as in Fig. 2

required for propagation in *E. coli*, it could not be modified directly without introducing selectable markers that would affect the transcription patterns. For this reason we used the plasmid-borne recombination systems LY, LYΔNS, LU and LA containing also two *leu2* repeats flanking different regions of Lk-AU, including pBR322 (Fig. 4a). In contrast to Lk-AU, in these constructs the pBR322 transcripts are originated at the external *LEU2* promoter which is independent of Hrs1, as it occurs in the LY system (see Fig. 2b; Prado et al. 1997).

As previously reported, whereas the LY and LYΔNS systems, in which the pBR322 sequences corresponding to Lk-AU transcripts 2 and 3 are actively transcribed, are hyper-recombinant in *hpr1* strains, this is not the case for LU and LA, which contain, respectively, the *URA3* and *ADE2* regions present in Lk-AU but not the pBR322 sequences (Fig. 4a, b). This confirms that, as it happens in Lk-AU, transcription of the region of pBR322 covering transcripts 2 and 3 is needed for *hpr1*Δ hyper-recombination to take place in these systems. *hpr1*Δ hyper-recombination is only suppressed when such transcripts are removed either by abolishing its transcription in Lk-AU with the *hrs1*Δ mutation, or by physically removing the DNA sequences, as in LU and LA. Consistently, and as shown with LY (Fig. 2a), *hrs1*Δ does not suppress *hpr1*Δ hyper-recombination of the LYΔNS system in agreement with the incapacity of *hrs1*Δ to abolish transcription through pBR322 that is initiated at the Hrs1-independent *LEU2* promoter (Fig. 4c).

Hyper-recombination in *hpr1*Δ depends on transcription levels

The previous results suggest that suppression of TAR is due to the reduction of transcription caused by *hrs1* in the systems tested, which is consistent with the fact that a modulation of transcription affects the levels of recombination in *hpr1*Δ strains. Thus, as we have previously reported, the increase in recombination of the *leu2* repeats under a repressed *GAL1* promoter (GL-*lacZ* system in glucose; low transcription) is much lower (sevenfold above wild type) than under the *LEU2* promoter (L-*lacZ*; medium transcription) (86-fold) (Huertas et al. 2006). However, as can be seen in Fig. 5, the increase in recombination became much higher (224-fold) when transcription from the *GAL1* promoter was induced with galactose (GL-*lacZ* system in galactose; high transcription).

The levels of transcription impairment in *hpr1*Δ are dependent on the rate of transcription initiation

Another hallmark phenotype of mutants of the THO complex is their gene expression defect. *hpr1*Δ mutants cannot properly express long and high-GC content genes that are driven from strong promoters or genes containing direct repeats (Chavez et al. 2001; Voynov et al. 2006). This is clearly observed in the pLAUR gene expression system

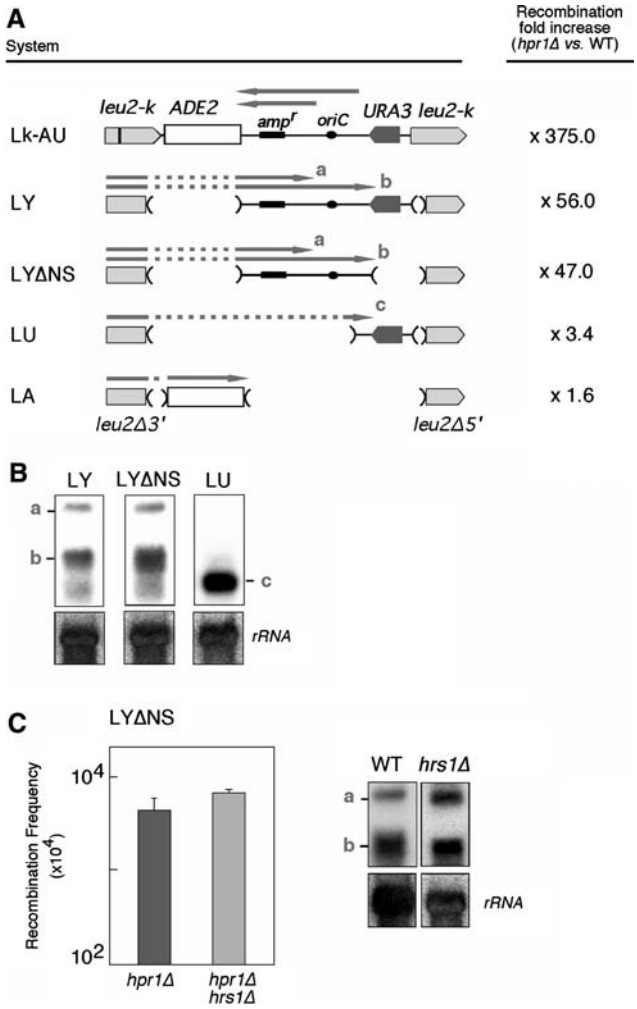

Fig. 4 Correlation between recombination and expression of pBR322 in different recombination constructs. **a** Schematic representation of the LY, LYΔNS, LU and LA plasmid-based recombination systems compared with Lk-AU. The fold-increase of the frequency of recombination of *hpr1*Δ above wild type of each system is shown (data taken from Prado et al. 1997). **b** Northern analysis of the LY, LYΔNS and LU plasmid-based constructs in wild-type cells using pBR322 as probe. **c** Northern analysis of LYΔNS system in wild type and *hrs1*Δ cells using pBR322 as probe. Median frequencies of recombination of *hpr1*Δ (UKH-1A) and *hpr1*Δ *hrs1*Δ (UKH-1B) isogenic strains are shown. pBR322 and rDNA probes used are detailed in "Materials and methods". Other details as in Fig. 2

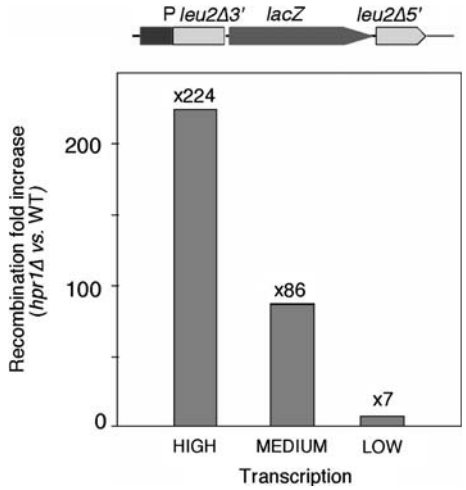

Fig. 5 Effect of the level of transcription on the *hpr1*Δ hyper-recombination phenotype. The increases in recombination of *hpr1*Δ (UKH-1A) above the isogenic wild type strain (UKH-1D) transformed with L-lacZ or GL-lacZ systems are shown. The data of recombination of the GL-lacZ system in glucose (transcription off; low transcription) and in galactose (transcription on; high transcription) are shown (low and medium transcription data are taken from Huertas et al. 2006). Other details as in Fig. 1

containing a *lacZ–URA3* translational fusion under the control of the *tet* promoter. Whereas wild-type cells express this fusion properly, this is not the case for THO mutants. Thus, *hpr1*Δ cells carrying the pLAUR system are unable to form colonies on SC-ura and do not show *β*-galactosidase activity (Jimeno et al. 2002). We wanted to know whether the *hrs1*-mediated hyper-recombination suppression could be due to the suppression of the gene expression defect. We, therefore, tested whether *hrs1*Δ suppressed *hpr1*Δ gene expression defect of pLAUR. As can be seen in Fig. 6, *hrs1*Δ could not suppress *hpr1*Δ transcription defect as measured either by its ability to grow on SC-ura (Fig. 6a), or to accumulate *LacZ–URA3* mRNA (Fig. 6b). It is important to notice that *hrs1*Δ mutant is not affected in the expression of the *tet* promoter as the mutant accumulates similar RNA levels as the wild type (Fig. 6b).

Since the *hrs1*Δ-mediated suppression of *hpr1*Δ hyper-recombination is due to the reduction of the transcription through the recombination system, we wanted to know if this was also the case for the gene expression defect of this mutant. To study whether the intensity of transcription could affect the levels of transcription impairment, we determined the effect of *hpr1*Δ on the transcription of *LYS2* as a function of the intensity of the promoter used. For this purpose we constructed a new plasmid-borne gene expression system in which the *LYS2* gene was placed under the control of the *tet* promoter (*tetpr::LYS2*). We compared the expression of this construct with a similar construct based on the *GAL1* promoter (*GAL1pr::LYS2*), instead of *tet*. The level of expression of the *GAL1* promoter is five times higher than the maximum level of the *tet* promoter in the wild type (Fig. 7a). As can be seen in Fig. 7a, transcription in the *tetpr::LYS2* system is reduced in *hpr1*Δ mutant (50% of the levels of the wild type). However, the reduction observed in the *GAL1pr::LYS2* construct is much stronger given that the mutant only accumulated about 5% of the transcript levels of the wild type (Fig. 7a). Moreover, we can see an actual increase in mRNA levels in *hpr1*Δ mutant when the promoter strength is decreased, given that the mutant shows a twofold increase in the amount of RNA in the *tetpr::LYS2* construct compared to the *GAL1pr::LYS2*

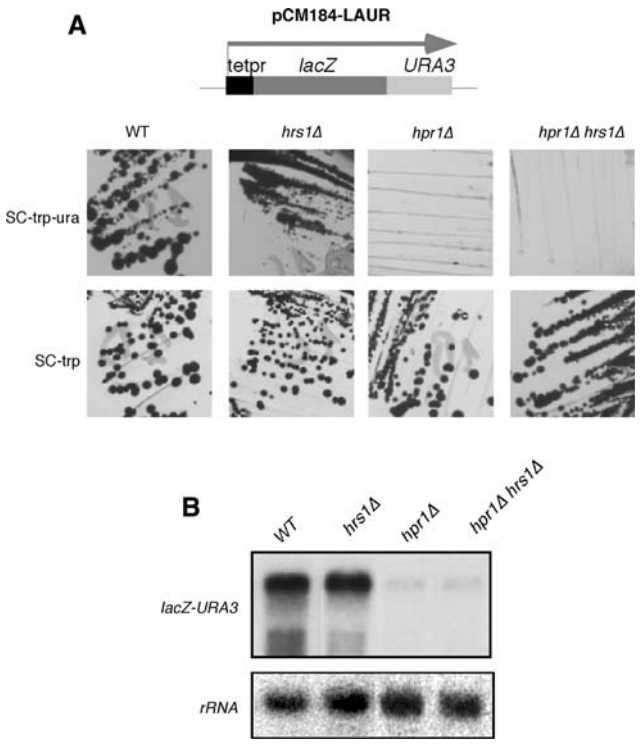

Fig. 6 Expression analysis of *hrs1*Δ and *hpr1*Δ *hrs1*Δ mutants in the pLAUR system. **a** Phenotypic analysis of *hpr1*Δ (UKH-1A), *hrs1*Δ (UKH-1C), *hpr1*Δ *hrs1*Δ (UKH-1B) and wild type (UKH-1D) isogenic strains transformed with pLAUR system. The scheme of the system is shown at the *top*. The capacity of each strain to form colonies on synthetic medium lacking tryptophan with or without uracil after four days at 30°C is shown. **b** Northern analysis of the transformants listed in **a**. The *lacZ* and rDNA probes used are detailed in "Materials and methods". Other details as in Fig. 2

one, while in the wild type there is a fivefold reduction (Fig. 7a). This result suggests that the strength of transcription could modulate the effect of *hpr1*Δ in gene expression. To asses this possibility we analysed the effect of *hpr1*Δ on transcription of the *tetpr::LYS2* system.

Transcription in the *tetpr::LYS2* construct was assayed with different doxycycline (dox) concentrations (0, 0.0025 and 0.005 mg/ml). The higher the concentration the weaker the promoter activation. Accordingly, the levels of *LYS2* mRNA decreased progressively in correlation with the amount of doxycycline either in wild-type or *hpr1*Δ cells (Fig. 7b). As expected, *hpr1*Δ mutant transcript accumulation was reduced with respect to the wild type. However, as the strength of transcription became lower the difference between mutant and wild-type cells became weaker. There was a progressive disappearance of the transcription impairment effect caused by *hpr1*Δ that correlated with the decreasing frequency of transcription initiation at the *tet* promoter until reaching a point in which no transcription defect was observed. As can be seen in Fig. 7b, at 0.005 mg/ml doxycycline the difference in transcription levels between wild type and *hpr1*Δ strains is not significant.

Similar results were obtained using the pLAUR system in *hpr1*Δ, *tho2*Δ and *mft1*Δ mutants. Figure 8 shows that, as for the *tetpr::LYS2* construct, the transcriptional defect of *tho* mutants became weaker when promoter firing was reduced. The *hpr1*Δ mutant accumulated 9.3% of the wild-type *LacZ-URA3* mRNA levels when transcription was high, but reached a 46% value in the presence of 0.0025 mg/ml of doxycycline. Under these conditions, *tho2*Δ and *mft1*Δ mutants behaved similarly, the increase going from 2.2 to 25% and from 15 to 41%, respectively (Fig. 8).

These results indicate that, together with the G + C content and the length of the DNA sequence, the frequency at which transcription is fired from the promoter influences the physiological relevance of the THO complex in gene expression.

Discussion

Hrs1 (Med3) is a component of the Mediator of RNAPII that was genetically identified as a suppressor of the hyper-recombination phenotype of *hpr1*Δ mutant. In this study we show that *hrs1*Δ does not suppress the hyper-recombination that occurs independent of transcription. The *hrs1*-mediated suppression of *hpr1*Δ hyper-recombination is not general and is only observed in recombination constructs in which transcription is Hrs1-dependent. In addition we demonstrate that *hpr1*Δ gene expression defect can be alleviated reducing the frequency of transcription. Altogether, our results agree with the idea that gene expression and recombination phenotypes of *THO* mutants can be alleviated or suppressed by decreasing the transcription rate.

Suppression of hyper-recombination by *hrs1*Δ is the result of the abolishment of transcription initiation

We have studied the effect of *HRS1* deletion in *cdc2* and *cdc9* hyper-recombinant mutants, which recombination phenotype is linked to a defect in replication (Montelone et al. 1981; Kokoska et al. 2000). We have observed a small reduction in the recombination frequency in both *hrs1*Δ *cdc2* and *hrs1*Δ *cdc9* double mutants compared to the *cdc* simple mutants. However, this reduction was due to the decrease in recombination observed in the *hrs1*Δ simple mutant (Santos-Rosa and Aguilera 1995). *hrs1*Δ also suppresses the transcription-associated hyper-recombination phenotype observed in other mutants like *tho2*Δ and *thp1*Δ (Gallardo and Aguilera 2001). Therefore, *hrs1*-mediated suppression is not general to all types of hyper-recombination, but specific of transcription-associated hyper-recombination.

Several transcription mutants have been isolated as *hpr1*Δ suppressors in different screenings. Although all of them affect transcription they seem to suppress in different

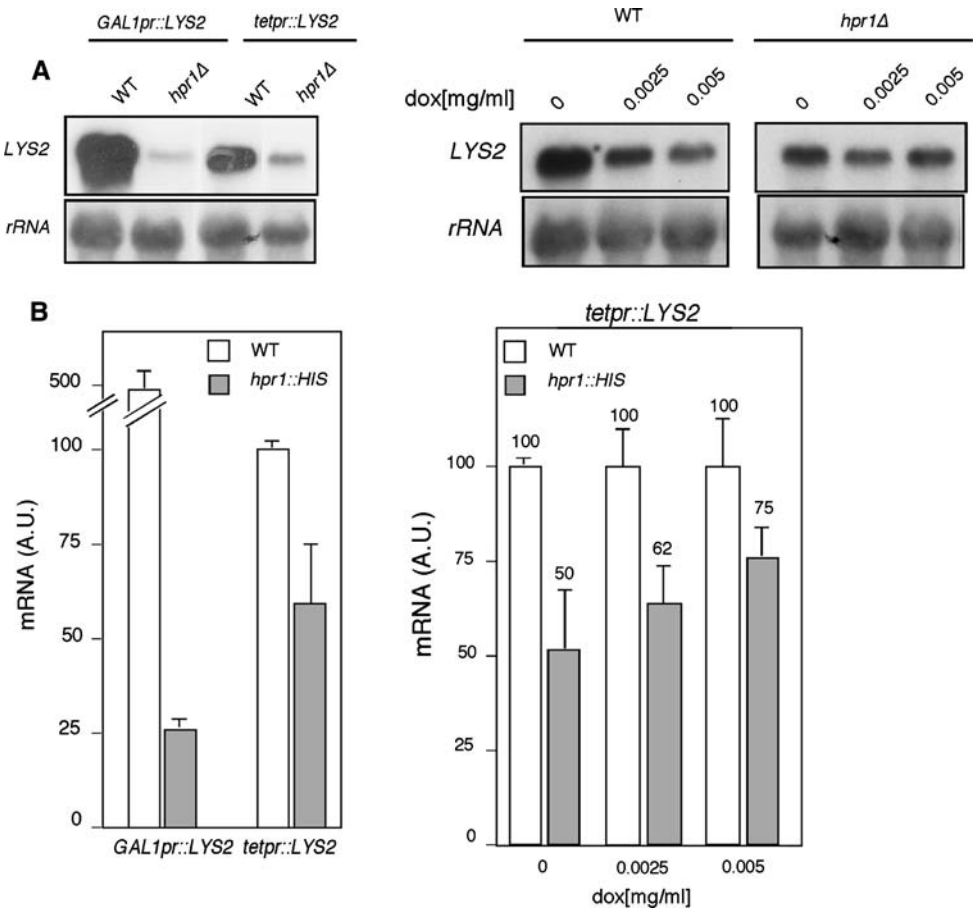

Fig. 7 Effect of the strength of the promoter on *hpr1*Δ transcription defect of *LYS2*-based constructs. **a** Northern analysis of the wild type (HRN1-4A) and *hpr1*Δ mutant (HRN1-2C) congenic strains transformed either with *GAL1pr::LYS2* or with *tetpr::LYS2* systems. The *LYS2* and rDNA probes used are detailed in "Materials and methods". Other details as in Fig. 2. **b** Northern analysis of wild type and *hpr1*Δ congenic strains carrying the *tetpr::LYS2* system using increasing amounts of doxycycline (*dox*). The percentage of RNA levels respect to the wild type is indicated on the *top* of each value. Media and the standard deviation of three independent experiments are shown. Other details as in **a**

ways. Thus, *soh1*, *soh2* and *soh4* (mutants in Med31, Rpb2 and TFIIB, respectively) were identified as suppressors of *hpr1*Δ thermosensitivity phenotype (Fan and Klein 1994). This observation together with the fact that *soh* mutants suppress the synthetic growth defects of *hpr1* Δ*top1*Δ and *hpr1*Δ *hht1-hhf1*Δ double mutants suggests that there is a genetic interaction between these factors. Besides, *soh* mutants and mutations in other mediator components genes as *GAL11/MED15* and *SIN4/MED16* have been shown to suppress *hpr1*Δ hyper-recombination phenotype, although to a lesser extent than *hrs1*Δ (Fan and Klein 1994; Piruat et al. 1997).

hrs1 and *srb2* mutants were identified as the suppressors of *hpr1*Δ hyper-recombination phenotype. However, while *hrs1*Δ mutation decreases TAR in the wild type, *srb2*Δ does not (Santos-Rosa and Aguilera 1995). Here we demonstrated that *hrs1*Δ-mediated suppression is specific of the recombination assay studied. *hrs1*Δ reduces TAR both in wild-type and *hpr1*Δ cells only in the Lk-AU repeat construct, consistent with the idea that in both cases recombination reduction by *hrs1*Δ occurs by similar mechanisms. These results indicate that the suppression of hyper-recombination by *hrs1*Δ is not due to a direct genetic interaction between Hrs1 and THO, but is the result of the abolishment of transcription of the DNA repeat construct.

A confirmation that transcription through the pBR322 region covering the *amp* gene and the *ori* sequence in Lk-AU is responsible for the hyper-recombination caused by *hpr1*Δ that is suppressed by *hrs1*Δ was obtained with recombination assays producing such transcripts from the *LEU2* promoter, which is Hrs1-independent. In these systems (LY and LYΔNS) hyper-recombination was not abolished by *hrs1*Δ. Consistently, identical assays lacking such pBR322 sequences, but containing *URA3* and *ADE2* (LU and LA) were not hyper-rec in *hpr1*Δ cells (Fig. 4; Prado et al. 1997). Therefore, we can conclude that the suppression by *hrs1*Δ is the consequence of the effect of *hrs1* on transcription abolishment. Although it is well established the relationship between hyper-recombination observed in *hpr1*Δ and transcription through the intervening region (Prado et al. 1997) we could still consider the possibility of additional transcription requirements (overlapping transcription, direction of transcription, transcription of the repeats, etc.) that could account for part of the *hrs1*Δ suppression. However, given the specificity of suppression of *hrs1*Δ for *hpr1*Δ and related mutants, we can basically rule out this possibility.

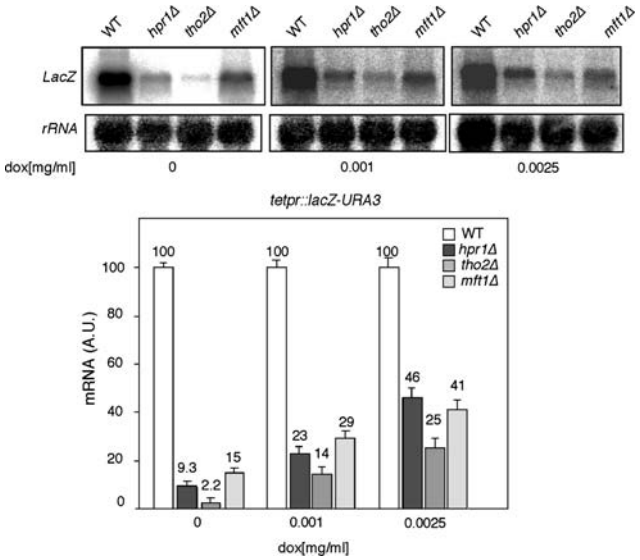

Fig. 8 Transcription impairment in *tho* mutants as a function of promoter strength. Northern analyses of wild-type (W303-1A), *hpr1*Δ (U678-1C), *tho2*Δ (RK2-6D) and *mft1*Δ (WMK-2A) isogenic strains harbouring the plasmid-based pLAUR system using increasing amounts of doxycycline (*dox*) are shown. The percentage of RNA levels respect to the wild type is indicated on the *top* of each value. Media and the standard deviation of three independent experiments are shown. The *lacZ* and rDNA probes used are detailed in "Materials and methods". Other details as in Fig. 2

The gene expression defect of *hpr1*Δ mutants is modulated by the transcription rate

Using the regulatable *tet* promoter, we see that the transcriptional defect of *hpr1*Δ mutant decreases with the transcription rate. This decrease is not due to a defect in the repression of the *tet* promoter in *hpr1*Δ mutant, because *LYS2* mRNA levels increase in this mutant when the strong *GAL1* promoter is replaced by the *tet* promoter. The same effect was observed in other THO mutants (*tho2*Δ and *mft1*Δ). Therefore, the stronger the transcription of a gene the more important becomes the THO complex for its transcription. The relevance of the transcription rate in the phenotypes of THO mutants is not restricted to gene expression and hyper-recombination phenotypes. Thus, it has been shown that mutations in the Rad3 component of the TFIIH transcription initiation factor and transcription elongation inhibition suppress the accumulation of the transcript at the site of transcription sites in *mft1*Δ mutants (Jensen et al. 2004). These observations are not exclusive of THO-complex mutants, since it has also been observed in other mutants defective in mRNA processing such as *dbp5* (Estruch and Cole 2003) and *ssu72* (Pappas and Hampsey 2000; Dichtl et al. 2002).

One important feature of most of the phenotypes associated to mutants of THO is the dependency on the RNA molecule (Huertas and Aguilera 2003). This, together with the fact that the recruitment of export factors Yra1, Sub2 and Mex67 to chromatin is dependent on the THO/TREX complex (Zenklusen et al. 2002; Gwizdek et al. 2006) opens the possibility that in THO/TREX mutants, at high transcription rates, such export factors are not properly loaded onto the nascent mRNA, while at low transcription rates, there could be an alternative loading mechanism independent of THO/TREX. Moreover, *hpr1*Δ cells show wild-type levels of transcription when the novel RNA-binding protein Tho1 or the RNA helicase involved in transport Sub2 are overexpressed (Jimeno et al. 2006), suggesting that in *hpr1*Δ background, the nascent mRNA would need additional RNA-binding factors to alleviate the expression defect. Our results agree with the view that at high transcription rates the demand for RNA packaging factors required for the formation of an export-competent mRNP is higher, in which case THO becomes crucial for the transcription process.

There are other data that may provide plausible explanations to these observations. Thus, genes containing short direct repeats require the THO complex for its transcription, a phenotype that is suppressed by topoisomerase I overexpression (Voynov et al. 2006). Also, *hpr1*Δ and *top1*Δ mutants are synthetically sick (Aguilera and Klein 1990; Sadoff et al. 1995). These data may indicate that the THO complex could be necessary to avoid aberrant DNA structures arising during transcription elongation. In this sense it would be plausible that those types of topological constraints become more frequent as the transcription frequency increases. In the absence of THO, a reduction in the firing of the promoter could allow the action of a topo I-dependent pathway that might remove such structures or directly decrease their formation. It is interesting that, as it occurs with the *tetpr::LYS2* assay, THO- dependency of transcription of the *TIR1* gene, containing short DNA repeats, is only observed under strong transcription conditions (Voynov et al. 2006).

We have previously reported in vivo and in vitro data that show that THO is important for transcription elongation of strongly transcribed constructs (Chavez et al. 2001; Rondon et al. 2003). Our results confirm that highly transcribed genes require the THO complex for transcription elongation. At low transcription rates, however, THO becomes dispensable.

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