Mol Gen Genomics (2005) 274: 528–538
DOI 10.1007/s00438-005-0042-3

ORIGINAL PAPER

C. E. Hepfer · S. Arnold-Croop · H. Fogell
K. G. Steudel · M. Moon · A. Roff
S. Zaikoski · A. Rickman · K. Komsisky
D. L. Harbaugh · G. I. Lang · R. L. Keil

DEG1, encoding the tRNA:pseudouridine synthase Pus3p, impacts *HOT1*-stimulated recombination in *Saccharomyces cerevisiae*

Received: 19 January 2005 / Accepted: 6 August 2005 / Published online: 18 October 2005
© Springer-Verlag 2005

Abstract In *Saccharomyces cerevisiae*, *HOT1*-stimulated recombination has been implicated in maintaining homology between repeated ribosomal RNA genes. The ability of *HOT1* to stimulate genetic exchange requires RNA polymerase I transcription across the recombining sequences. The *trans*-acting nuclear mutation *hrm3-1* specifically reduces *HOT1*-dependent recombination and prevents cell growth at 37°. The *HRM3* gene is identical to *DEG1*. Excisive, but not gene replacement, recombination is reduced in *HOT1*-adjacent sequences in *deg1*Δ mutants. Excisive recombination within the genomic rDNA repeats is also decreased. The hypo-recombination and temperature-sensitive phenotypes of *deg1*Δ mutants are recessive. Deletion of *DEG1* did not affect the rate of transcription from *HOT1* or rDNA suggesting that while transcription is necessary it is not sufficient for *HOT1* activity. Pseudouridine synthase 3 (Pus3p), the *DEG1* gene product, modifies the anticodon arm of transfer RNA at positions 38 and 39 by catalyzing the conversion of uridine to pseudouridine. Cells deficient in pseudouridine synthases encoded by *PUS1, PUS2* or *PUS4* displayed no recombination defects, indicating that Pus3p plays a specific role in *HOT1* activity. Pus3p is unique in its ability to modulate frameshifting and readthrough events during translation, and this aspect of its activity may be responsible for *HOT1* recombination phenotypes observed in *deg1* mutants.

Keywords *HOT1* · Recombination hotspot · *DEG1* · tRNA:pseudouridine synthase · Pus3p

Communicated by A. Aguilera

C. E. Hepfer (✉) · S. Arnold-Croop · H. Fogell · K. G. Steudel
M. Moon · A. Roff · S. Zaikoski · K. Komsisky
D. L. Harbaugh · G. I. Lang
Department of Biology, Millersville University,
50 East Frederick Street, PO Box 1002,
Millersville, PA 17551, USA
E-mail: carol.hepfer@millersville.edu
Tel.: +1-717-872-3791
Fax: +1-717-872-3905

A. Rickman · R. L. Keil
Department of Biochemistry and Molecular Biology,
The Milton S. Hershey Medical Center,
The Pennsylvania State University,
Hershey, PA 17033, USA

Introduction

HOT1 is a mitotic recombination hotspot isolated from the non-transcribed spacer (NTS) of the ribosomal DNA (rDNA) repeats in *Saccharomyces cerevisiae* (Keil and Roeder 1984). When inserted at ectopic locations within the genome, *HOT1* increases the rate of homologous recombination in adjacent repeated sequences (Keil and Roeder 1984; Roeder et al. 1986). Recombination is thought to play a role in maintaining the homogeneity of repeated genes (Amstutz et al. 1985), and *HOT1* activity may be important in preserving the sequence identity of the tandem rDNA repeats in yeast (Keil and Roeder 1984). The mechanisms involved in *HOT1*-stimulated exchange have not been elucidated.

Indispensable *HOT1* sequences correspond closely to the enhancer and initiation site required for maximal transcription from 35S rRNA sequences at ectopic sites (Elion and Warner 1984, 1986; Voelkel-Meiman et al. 1987; Wai et al. 2001). There is substantial evidence that the ability of *HOT1* to stimulate recombination relies on its capacity to promote high levels of transcription by RNA polymerase I (Pol I) (Voelkel-Meiman et al. 1987; Stewart and Roeder 1989; Huang and Keil 1995). Wai et al. (2001) demonstrated that the Pol I enhancer is not required for rDNA transcription within the normal tandem array of repeat units. They suggest that at ectopic locations the enhancer is needed to recruit Pol I transcription machinery from the nucleolus and enable sufficient transcription for recombination to occur. To stimulate exchange, *HOT1*

sequences must be oriented so that transcription proceeds across the recombining regions (Keil and Roeder 1984; Voelkel-Meiman and Roeder 1990). *HOT1* activity is abolished by deleting a gene encoding a subunit of RNA Pol I (Huang and Keil 1995) or by inserting a transcription termination site between *HOT1* and the recombining sequences (Voelkel-Meiman et al. 1987). In general, mutations in the enhancer and initiation site that decrease rDNA transcription also reduce levels of *HOT1*-stimulated exchange (Stewart and Roeder 1989; Huang and Keil 1995). However, several mutations that dramatically reduce *HOT1* activity only slightly affect transcription (Huang and Keil 1995).

To elucidate the mechanism by which *HOT1* stimulates recombination, Lin and Keil (1991) isolated and initiated characterization of five *trans*-acting nuclear genes that reduce *HOT1* activity. Mutations in *HRM1* [identical to *FOB1* (Defossez et al. 1999)], *HRM2* [identical to *SCH9* (Prusty and Keil 2004)], *HRM5* [identical to *RAD52* (Lin and Keil 1991)] and *HRM3* reduce *HOT1* activity at the ectopic *his4::URA3::his4* reporter sequence and also depress *HOT1*-stimulated rDNA exchange. Mutations in *FOB1*, *SCH9* and *HRM3* specifically affect *HOT1*-dependent exchange whereas *RAD52* also affects *HOT1*-independent recombination. The *hrm4-1* mutation is unique in that it affects *HOT1* activity at *his4::URA3::his4* but not exchange within rDNA.

Here we report further characterization of the *hrm3-1* mutant. The *HRM3* gene was cloned based on its ability to complement the temperature-sensitive phenotype of *hrm3-1* strains. Genetic analysis and sequencing revealed that *HRM3* is identical to *DEG1*, a *CEN6*-proximal gene that results in depressed growth when disrupted (Carbone et al. 1991). The product of the *DEG1* gene, pseudouridine synthase 3 (Pus3p), catalyzes pseudouridylation at positions 38 and 39 in the anticodon arm of transfer RNA (Lecointe et al. 1998). A relationship between modification of tRNAs and genetic recombination has not been previously identified. The effect of *deg1* mutations on *HOT1*-stimulated exchange may be related to altered recognition of termination codons during translation.

Materials and methods

Strains, media and growth conditions

Yeast strains used in this study are listed in Table 1. The *E. coli* strains MC1066 [*leuB trpC pyrF::*Tn5 (Kan^R) *araT lacX74 del strA hsdR hsdM* (obtained from M. Casadaban)] and DH5α (InVitrogen) were used to propagate plasmids. Yeast growth medium containing G418 was prepared as described by Prusty and Keil (2004). Other growth media for yeast (Lin and Keil 1991) and bacteria (Sambrook et al. 1989) were prepared as previously described.

DNA and RNA isolation and analysis

Restriction and modification enzymes were purchased from various manufacturers and used according to their recommendations. Isolation of plasmid (Sambrook et al. 1989) and yeast (Rose et al. 1990) DNA was done according to standard protocols.

Primer extension analysis was performed as described by Huang and Keil (1995) with the following modifications. The AMV Reverse Transcriptase - Primer Extension System (Promega) was used with 20 μg RNA and saturating amounts of primer in each reaction. Primers O-38 (GTTCGCGTTCTTTAGCCCACTTTG TCTTGTG) and O-40 (GAGACTAGGCAGATCTGA CGATCACCTAGC) (Huang and Keil 1995) were used at 1 picomole per reaction to quantify transcripts of 35S rRNA and *HIS4*, respectively. Primer CYH2EX2 (ATACCTCTACCACCGGGGTGCTTTCTGTGC) was used at 0.1 pmol per reaction to quantify *CYH2* transcripts (Schwindinger and Warner 1987). Transcript levels were quantified by using a Molecular Dynamic PhosphoImager 425E in the Macromolecular Core Facility of the M. S. Hershey Medical Center.

Cloning and sequencing of the *HRM3*(*DEG1*) gene

To clone the *HRM3* gene, an *hrm3-1* mutant strain was transformed with a single copy yeast genomic library (Rose et al. 1987) and temperature-resistant transformants were isolated. Plasmids were evaluated by restriction enzyme analysis and the boundaries of the *HRM3* gene were established by $\gamma\delta$ mutagenesis (Guyer 1983). The gene sequence was obtained by manual sequencing (Sanger et al. 1977) using primers to the ends of the transposons. Plasmid pL1615, shown to contain the *HRM3* gene by its ability to restore temperature resistance and normal levels of recombination to *hrm3-1* mutant cells, was used for subcloning and further manipulations.

Null mutant strains

The 2.9-kb *Eco*RV fragment of plasmid pL1615, containing *CEN6*, *HRM3*(*DEG1*) and part of *SPB4*, was ligated into the *Eco*RV site of pBR322. Following cleavage with *Kpn*I or *Kpn*I plus *Xho*I to remove portions of *DEG1*, a *Not*I linker was introduced. *TRP1* was then inserted in the *Not*I site. Restriction analysis showed that both deletion plasmids contained two copies of *TRP1* orientated opposite to the *HRM3(DEG1)* coding sequence. The deletions remove −49 to +1,238 (*deg1-Δ1*; *Kpn*I/*Xho*I deletion) or +56 to +1,238 (*deg1-Δ2*; *Kpn*I deletion) of the gene. Plasmids containing each deletion were linearized with *Hin*cII and then transformed into diploid HRM500 cells. Trp^+ transformants were evaluated by Southern analysis (Sambrook et al. 1989) to verify that one chromosomal copy of the *DEG1* gene had

Table 1 Yeast strains used

Strain	Genotype
HRM500	*MAT***a**/*MATα his4-260/his4-260 ade2-1/ade2-1 ade5/ade5 ura3-52/ura3-52 trp1-HIII/trp1-HIII LYS2/lys2-ΔBX can1/can1 DEG1/DEG1*
HRM574[a]	*MAT***a** *his4-Δ::URA3::his4-260 ade2-1 ade5 ura3-52 trp1-HIII lys2-ΔBX::CAN1::LYS2 can1 DEG1*
HRM577[a]	*MAT***a** *HOT1::* *his4-Δ::URA3::his4-260 ade2-1 ade5 ura3-52 trp1-HIII lys2-ΔBX::CAN1::LYS2 can1 DEG1*
HRM580[a]	*MAT***a** *his4-260 ade2-1 ade5 ura3-52 trp1-HIII lys2-ΔBX::CAN1::LYS2 can1 deg1(hrm3-1)*
HRM582[b]	*MAT***a** *his4-260 ade2-1 ura3-52 trp1-HIII leu2 lys2-ΔBX::CAN1::LYS2 can1* rDNA *::URA3 DEG1*
HRM592	*MAT***a** *HOT1::* *his4-Δ::URA3::his4-260 ade2-1 ade5 ura3-52 trp1-HIII lys2-ΔBX::CAN1::LYS2can1 DEG1*
HRM598	*deg1-Δ1* isogenic to HRM592
HRM595	*deg1-Δ2* isogenic to HRM592
HRM607	*deg1(hrm3-1)* isogenic to HRM592
HRM646	*MAT***a** *his4-Δ::URA3::his4-260 ade2-1 ade5 ura3-52 trp1-HIII lys2-ΔBX::CAN1::LYS2 can1 DEG1*
HRM649	*deg1-Δ1* isogenic to HRM646
HRM635	*deg1(hrm3-1)* isogenic to HRM646
HRM642	*MAT***a** *his4-260 ade2-1 ade5 ura3-52 trp1-HIII lys2-ΔBX::CAN1::LYS2 can1* rDNA*::URA3 DEG1*
HRM639	*deg1-Δ1* isogenic to HRM642
HRM631	*deg1(hrm3-1)* isogenic to HRM642
HRM552	*leu2* isogenic to HRM598
HRM750	*leu2* isogenic to HRM592
PUS1G418	*pus1-Δ1* isogenic to HRM 592
PUS2G418	*pus2-Δ1* isogenic to HRM 592
PUS3G418	*deg1(pus3)-Δ3* isogenic to HRM 592
PUS4G418	*pus4-Δ1* isogenic to HRM 592
G355-1[c]	*MAT***a** *his3-1ade2-1 ura3-1 1trp1-1 leu2-3.112 LYS2::CAN1::TRP1::LYS2can1 can1-100 DEG1*
G355-1-3a[c]	*MAT***a** *his3-1ade2-1 ura3-1 1trp1-1 leu2-3.112 LYS2::CAN1::TRP1::LYS2can1 can1-100 deg1-Δ3*
G359-1[c]	*MAT***a** *his3-1ade2-1 ura3-1 1trp1-1 leu2-3.112 HOT1::LYS2::CAN1::TRP1::LYS2can1 can1-100 DEG1*
G359-1-2a[c]	*MAT***a** *his3-1ade2-1 ura3-1 1trp1-1 leu2-3.112 HOT1::LYS2::CAN1::TRP1::LYS2can1 can1-100 deg1-Δ3*

[a]HRM574, HRM577 and HRM580 were derived from K2302, K2307 and *hrm3-1* (Lin and Keil 1991), respectively, by standard genetic methods
[b]HRM582 was derived from K1875 (Keil and McWilliams 1993)
[c]G355-1 and G359-1 are Ura$^-$ derivatives of *RPA135* (NOY408-1b-based) strains (Huang and Keil 1995)

been replaced correctly. Tetrad dissection yielded Trp$^+$ colonies that were backcrossed to appropriate strains to generate isogenic *HRM3*(*DEG1*), *hrm3-1*, *deg1-Δ1* and *deg1-Δ2* strains carrying various recombination assays (Fig. 1).

Strains containing deletions of other *PUS* genes were generated in HRM592 (Table 1) by PCR-mediated gene disruption using the *loxP–kanMX–loxP* cassette (Guldener et al. 1996) to precisely replace the open reading frame with a gene that confers resistance to G418. Disruptions of the *PUS1, PUS2, PUS4* and *DEG1(PUS3)* genes were confirmed by Southern-blot analysis (Sambrook et al. 1989) or PCR.

Centromeric plasmids containing *DEG1* or *deg1-D151A*, or the empty pR315 vector [(Lecointe et al. 2002); provided by H. Grosjean] were transformed into the *deg1-Δ1* strain HRM552 and transformants were evaluated for recombination.

Comparison of *hrm3-1* and *HRM3(DEG1)* sequences

PCR using primers HRM301 (GATGAAAA**GAATT**CTAGTCTTCAAG) and HRM302 (TTATTGCTGC**T**GA**ATTC**TTAATATTATC) [underlined bases were added to introduce *Eco*RI sites (bold)] and *Pfu*I polymerase (Stratagene) was employed to amplify *HRM3(DEG1)* and *hrm3-1* genomic sequences and introduce an *Eco*RI site on each side of the gene. After ligating the PCR product into the *Eco*RI site of YCplac111, plasmids containing the wild-type and mutant genes were isolated and sequenced at the Macromolecular Core Facility of the M. S. Hershey Medical Center using primers HRM301, HRM302, HRMINTA (TCGTTAGAAGTCCGTTCC), HRMINTB (GAGGCAGACAGCTGATATAC), HRMINTC (CCAAAGAGCAGCTAATAC) and HRMINTE (TTGATCTCGTTGGTTCAG).

Construction of epitope-tagged alleles and Western-blot analysis

To evaluate the effect of *HRM3(DEG1)* overexpression, an epitope-tagged allele of the gene was constructed. The 901 epitope (M.J. Tevethia and S.S. Tevethia, unpublished) coding for a portion of the large T antigen of SV40 (Fiers et al. 1978) was inserted just upstream of the stop codon using a two-step PCR approach. YCplac111 containing *HRM3(DEG1)* was used as template in separate PCR reactions with primers HRM301 and HRM3C or HRM302 and HRM3D. Primers HRM3C (*ATTTGTAGAGGTTTT*ACTTGCTTTAAAAAA**TAA**AATAATATATAAACCTGTATAATATAAC) and HRM3D (*AAAACCTCTACAAAT*GTGATATGGCTGATTCTTATTTTTGTTGTTCTTTTTCTTG) contain overlapping (italics) epitope (underlined) sequences as well as sequences flanking the

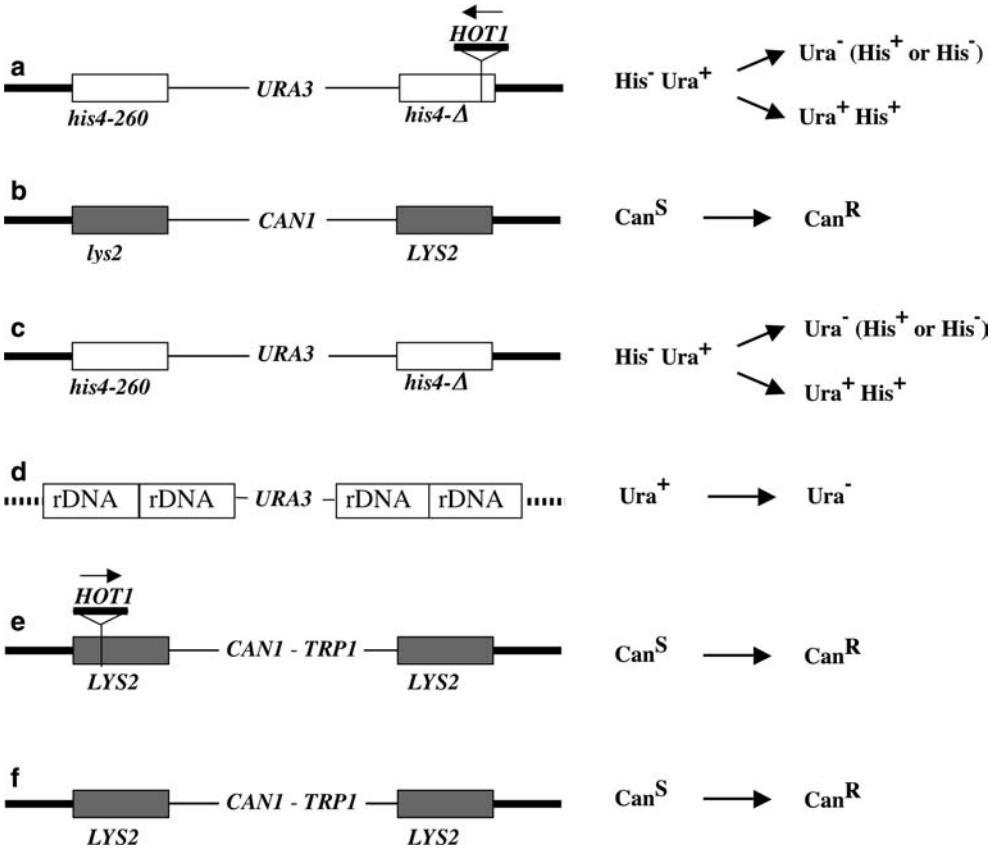

Fig. 1 Substrates used to determine recombination rates. Substrates measure (**a** and **e**) *HOT1*-dependent recombination, (**b, c** and **f**) *HOT1*-independent recombination, or (**d**) recombination within the ribosomal DNA repeats. In **a** and **c**, excisive recombination produces Ura$^-$ cells while gene replacement generates His$^+$ Ura$^+$ cells. In **d**, excisive recombination results in Ura$^-$ recombinants. In **b, e** and **f**, excisive recombination produces Can^R cells

HRM3(DEG1) stop codon (indicated in bold). The products of these two PCR reactions were combined in a second round of PCR to generate a complete epitope-tagged gene that is amplified with primers HRM301 and HRM302. The final PCR product was ligated into the *Eco*RI site of YCplac111. Correct insertion of the 901 epitope and conservation of wild-type *HRM3(DEG1)* sequences were verified by sequencing the entire gene. The tagged gene was cleaved from YCplac111 with *Eco*RI and inserted at the *Eco*RI site of YEplac181 (Gietz and Sugino 1988). Wild-type *HRM3(DEG1)* or *deg1-Δ1* yeast cells were transformed to isolate strains containing the vector backbones (YEp or YCp), YEp*DEG1::901* or YCp*DEG1::901*. Levels of *DEG1::901* expression were determined by Western blot analysis using an ECL chemiluminescent detection kit (Amersham). Mouse anti-PAB901 (Thompson et al. 1990) was used as primary antibody for detection of the epitope tag, and mouse anti-actin antibody was used as the control. Tagged protein in strains containing YCp*DEG1::901* was virtually undetectable while substantial levels (at least tenfold higher) were present in strains transformed with YEp*DEG1::901*.

Recombination assays and statistical analysis

Rates of recombination for duplications constructed at *HIS4* were quantified using fluctuation tests as described previously (Lin and Keil 1991). At least three cultures from each of three independent colonies of the same genotype were evaluated in each trial to determine rates. Cells were grown in SC to assess both gene replacement and excisive recombination (Lin and Keil 1991). All fluctuation tests were repeated at least twice. To establish proportions of His$^+$ recombinants that arose by gene replacement (His$^+$ Ura$^+$) as compared to excision (His$^+$ Ura$^-$), the Ura phenotype of at least 50 independent His$^+$ colonies from each of the appropriate strains was determined. Calculation of recombination rates and statistical analysis based on the medians obtained in the fluctuation tests were performed as described previously (Yuan and Keil 1990).

Fluctuation analysis was also used to determine the rates of recombination for duplications constructed at *LYS2*. Isolated colonies of each strain grown on SC were resuspended in water and serial dilutions were spotted on plates with or without canavanine to determine the frequency of Can^R recombinants. *HOT1* was lost at a very high rate in *DEG1* cells containing the *HOT1::LYS2::CAN1::TRP1::LYS2* substrate. Retention of *HOT1* was verified by PCR in all colonies used to calculate recombination rates. Each evaluation was replicated and rates were determined from at least ten independent samples for each genotype. Calculation of recombination rates and statistical analysis based on the medians obtained in the fluctuation tests were performed as described previously (Yuan and Keil 1990).

Exchange within rDNA was measured for strains grown in SC-ura as described previously (Lin and Keil 1991). The recombination rate per generation was determined by dividing the mean frequency of recombination events by two, and the *t*-test was used to determine statistical significance of differences. At least nine cultures were used for each genotype and each evaluation was replicated at least twice.

Results

HRM3 is identical to *DEG1*

Hrm3-1 was identified by Lin and Keil (1991) as a mutant that reduces *HOT1*-stimulated recombination. In addition, the *hrm3-1* mutation leads to decreased levels of rDNA exchange, slow growth at 30° that is more pronounced on YEPG medium, and an inability to grow at 37° . The wild-type *HRM3* gene was cloned from a single-copy yeast genomic DNA library (Rose et al. 1987) based on its ability to complement the temperature-sensitive phenotype of *hrm3-1* mutants. Fourteen temperature-resistant transformants were recovered. When plasmids containing the putative *HRM3* gene were lost from each of these transformants, the strains again became temperature sensitive indicating the gene essential for correcting this phenotype is on the plasmids. Plasmids containing the candidate *HRM3* gene also restored wild-type levels of *HOT1*-stimulated recombination in *hrm3-1* strains.

Restriction mapping showed that these 14 plasmids contain overlapping inserts. The identity of the gene complementing the growth defect at high temperature in one of these plasmids, pL1615, was established using $\gamma\delta$ mutagenesis (Guyer 1983). Seven plasmids with transposon insertions that destroyed the ability of pL1615 to complement the temperature-sensitive phenotype of *hrm3-1* mutants were isolated. In all seven cases, sequences adjacent to the transposons were identical to various portions of *DEG1* (Carbone et al. 1991), a gene reported to depress growth when disrupted or overexpressed. The product of the *DEG1* gene is a pseudouridine synthase (Pus3p) that catalyzes pseudouridylation at positions 38 and 39 in the anticodon arm of transfer RNA (Lecointe et al. 1998).

The proximity of *DEG1* to *CEN6* forced vector rearrangements in the fourteen *DEG1*(*HRM3*)-containing plasmids. All were completely missing the centromere DNA elements (CDEs) of *CEN4* (Wolfe and Lohan 1994, Rose et al. 1987), and, at the ends of the deletions, each plasmid had a single copy of 1–4 bp that was directly duplicated in the original YCp50 vector (data not shown) suggesting the involvement of nonhomologous recombination events (Kramer et al. 1994). Sequences encoding the carboxy-terminus of *DEG1(HRM3)* are only 50 bp from the CDEI element of *CEN6* (Brambilla et al. 1997), and insertion of this tightly linked centromere into intact YCp50 creates an unstable dicentric plasmid (Mann and Davis 1983; Haber and Thorburn 1984; Kramer et al. 1994). *DEG1* transcription termination sequences overlap *CEN6* (Carbone et al. 1991), and selection for *DEG1* activity would understandably result in specific loss of *CEN4* sequences to produce stable monocentric plasmids.

Deletions of the *DEG1* gene were generated in the diploid strain HRM500 by replacing the *Kpn*I–*Xho*I (*deg1-Δ1*) or the *Kpn*I–*Kpn*I (*deg1-Δ2*) regions with *TRP1*. The *deg1-Δ1* deletion removes the amino-terminus and 49 bp of sequence upstream of *DEG1*, while the *deg1-Δ2* deletion leaves 55 bp of the amino-terminus intact. In both deletions 133 bp of the *DEG1* carboxy-terminus remains after the *TRP1* insert. In tetrads derived from these transformants, Trp^+ spores containing either deletion were viable, and temperature sensitivity co-segregated (2:2) with the Trp^+ phenotype. *Hrm3-1*/*deg1-Δ1* or *hrm3-1*/*deg1-Δ2* diploids are temperature sensitive, and all spores produced from 34 tetrads were temperature sensitive with 50% being Trp^+. These results show that the *TRP1* marker is less than 1 cM from the *HRM3* locus and indicate that *HRM3* is likely to be identical to *DEG1*.

To confirm that *DEG1* contains a mutation in *hrm3-1* strains, sequences of the wild-type and *hrm3-1* genes were determined. The wild-type *DEG1* sequence is identical to that reported in the *Saccharomyces* Genome Database (http://www.yeastgenome.org/). In *hrm3-1* a CG to TA transition occurs at position +500 in the *DEG1* ORF. This results in an amino acid substitution where phenylalanine replaces serine at amino acid 167 (S167F). Although Ser^{167} lies outside the functional motifs common to pseudouridine synthases (Koonin 1996) and sequence motifs conserved in TruA-like proteins (Lecointe et al. 1998), only 15 amino acids separate the affected site from the invariant Asp^{151} that is essential for Pus3p activity (Koonin 1996).

deg1 deletions specifically reduce *HOT1*-associated recombination

Rates of *HOT1*-associated exchange were quantified initially using a *HOT1::his4-Δ::URA3::his4-260* duplication (Fig. 1a) that contains a *URA3* gene and pBR322 sequences flanked by distinct mutant *his4* genes (Lin and Keil 1991). *HOT1* activity is measured as the frequency of Ura^- excision or His^+ Ura^+ gene replacement recombinants. In *deg1-Δ1*, *deg1-Δ2* and *hrm3-1* strains, the rate of *URA3* excision in the *his4* substrate containing *HOT1* was significantly reduced (Table 2). The effect of *deg1* mutations is limited to excisive recombination as there is no significant change in *HOT1*-stimulated gene replacement (His^+ Ura^+ recombinants) for any of the mutants as compared to the wild-type cells.

Rates of *HOT1*-independent exchange in these strains were measured using two different recombination substrates. The *his4-Δ::URA3::his4-260* duplication lacking *HOT1* (Fig. 1c) generates Ura^- excision or

Ura$^+$ His$^+$ gene replacement recombinants, and a *lys2::CAN1::LYS2* substrate lacking *HOT1* (Fig. 1b) produces Can^R excision recombinants (Lin and Keil 1991). No differences were observed for any of these recombination events between *DEG1* and mutant cells (Table 2), showing that mutations in *DEG1* specifically impact *HOT1*-stimulated exchange.

Levels of recombination within the rDNA array were evaluated by assaying the loss of a *URA3* gene inserted within the genomic rDNA repeats (Fig. 1d) (Lin and Keil 1991). Significant decreases occurred in rDNA recombination rates in *deg1* strains as compared to wild-type controls (Table 2). These differences indicate that *DEG1* plays a role in recombination occurring in the normal array of rDNA repeats.

To verify that the effect of *deg1* mutations on *HOT1* activity is not unique to the *HOT1::his4-Δ::URA3::his4-260* substrate, the impact of *deg1* was investigated using *HOT1::LYS2::CAN1::TRP1::LYS2* (Fig.1e) and *LYS2::CAN1::TRP1::LYS2* (Fig. 1f) substrates (Huang and Keil 1995). The rate of excisive exchange (Can^R recombinants) was determined in *DEG1* and *deg1-Δ3* cells. *HOT1* activity was significantly reduced in *deg1-Δ3* strains as compared to *DEG1* strains (514.4×10^{-5} and $2{,}343.3\times10^{-5}$, respectively; $P < 0.02$). *HOT1*-independent recombination of this duplication was not significantly different between *DEG1* and *deg1-Δ3* strains (3.5×10^{-5} and 2.2×10^{-5}, respectively). These results demonstrate that *DEG1* has a general effect on *HOT1* activity in distinct substrates in different genetic backgrounds.

The recessiveness of the *hrm3-1* and *deg1Δ* mutations was tested by evaluating recombination and growth in diploid strains (Table 3). A significant eightfold decrease in *HOT1*-stimulated excision was observed for *hrm3-1/hrm3-1*diploids as compared to wild-type diploid cells. Recombination rates of heterozygotes (*DEG1/hrm3-1, DEG1/deg1-Δ1* or *DEG1/deg1-Δ2*) did not differ significantly from those of wild-type strains, indicating that the *hrm3-1, deg1-Δ1* and *deg1-Δ2* mutations are recessive with respect to their effect on *HOT1* activity. While diploids homozygous for *hrm3-1* were temperature-sensitive, all heterozygous diploids grew normally at 37°, demonstrating that this aspect of the phenotype is also recessive. These findings indicate the specific effects of *deg1* deletions and the *hrm3-1* mutation on growth, *HOT1*-stimulated excision and rDNA exchange result from loss of gene function.

Overexpression of *DEG1* does not alter *HOT1*-dependent recombination

It has been suggested that overexpression of *DEG1* causes the same slow growth defect as gene disruption (Carbone et al. 1991). Therefore, we tested the effect of overexpression on *HOT1*-stimulated recombination and growth at 37°. To measure the level of *DEG1* expression, an epitope-tagged wild-type allele of the gene was cloned into a single-copy (YCp) or multicopy (YEp) plasmid. Transformed *DEG1* cells were isolated that contained vector backbone with or without epitope-tagged *DEG1::901*. The level of gene expression and *HOT1* activity of these transformants was evaluated. Substantial Deg1p(Pus3p) was detectable by Western blots in strains containing the YEp*DEG1::901* construct. Barely discernible levels of protein in strains with the YCp*DEG1::901* construct support the idea that *DEG1* is a poorly expressed gene (Carbone et al. 1991). The difference in Deg1p(Pus3p) levels between the two strains was at least ten-fold. The YCp*DEG1::901* plasmid corrected the recombination and growth defects of *deg1-Δ1* mutants, proving that the epitope-tagged gene is functional. Contrary to expectations based on observations by Carbone et al. (1991), overexpression of *DEG1* did not impede cell growth at 30° or 37° and did not affect *HOT1*-stimulated exchange in our strains.

Table 3 *deg1*(*hrm3*) mutations are recessive

Genotype	Growth at 37°[a]	Recombination rate ($\times 10^5$)	
		HOT1	no *HOT1*
		Ura$^-$	Can^R
DEG1/DEG1	+ +	154.9	6.2
DEG1/deg1-Δ1	+ +	283.1	9.2
DEG1/deg1-Δ2	+ +	216.4	17.4
DEG1/hrm3-1	+ +	114.0	19.9
hrm3-1/hrm3-1	−	20.1**	6.2

**$P \le 0.01$ as compared to *DEG1/DEG1*
[a] + + indicates dense growth, −indicates no growth

Table 2 Effect of *deg1*(*hrm3*) mutations on recombination

Genotype	Recombination rate ($\times 10^5$)					
	HOT1		no *HOT1*			rDNA
	Ura$^-$	His$^+$ Ura$^+$	Ura$^-$	His$^+$ Ura$^+$	Can^R	Ura$^-$
DEG1(HRM3)	201.1 (100)[a]	3.8	1.2	1.3	6.1	4.4 (100)[a]
hrm3-1	19.7* (10)	1.9	0.4	1.7	4.2	0.9* (20)
deg1-Δ1	68.3* (34)	4.5	1.1	1.1	5.2	1.1* (25)
deg1-Δ2	49.9* (25)	3.8	ND	ND	ND	ND

*$P \le 0.05$as compared to wild type; differences between *hrm3-1* and *deg1Δ* strains are not statistically significant
[a]Percent recombination relative to wild-type *DEG1* strain

Deletion of *PUS1, PUS2* or *PUS4* does not reduce *HOT1*-stimulated recombination

The product of the *DEG1* gene, Pus3p, is a pseudouridine (Ψ) synthase that specifically modifies the anticodon arm of several tRNAs at positions 38 and 39 (Lecointe et al. 1998). Other *S. cerevisiae* tRNA:Ψ -synthases include Pus1p, which introduces Ψ s at positions 26–28, 34–36, 65 and 67 (Simos et al. 1996; Motorin et al. 1998), and Pus4p, which catalyzes the formation of Ψ at position 55 in the TΨG loop (Becker et al. 1997) of many tRNAs. Although Pus2p activity has not been fully characterized, it is potentially a member of the Pus1 family (Hellmuth et al. 2000). Modifications of tRNA are important to many physiological functions, including codon recognition, aminoacylation and wobble base pairing (Björk 1995; Yarian et al. 2002). To evaluate whether reduced *HOT1* activity in *deg1* strains reflects a general physiological change that occurs when tRNA modification is disrupted, we evaluated *HOT1*-stimulated recombination in yeast cells with deletions in *PUS1, PUS2* and *PUS4*. Deletion of *PUS1, PUS2* or *PUS4* did not significantly alter *HOT1*-dependent or *HOT1*-independent mitotic exchange (Table 4). Only the *deg1*(*pus3*)−Δ*3* deletion caused a decrease in *HOT1* activity and inhibited growth at elevated temperatures. These findings indicate that some activity specific to Pus3p is critical for *HOT1*-stimulated exchange.

tRNA:Ψ 38/39-synthase activity is required for optimal *HOT1* activity

To determine whether the loss of tRNA:Ψ 38/39-synthase activity is specifically responsible for reduced *HOT1*-stimulated exchange, we tested *deg1-D151A* (Lecointe et al. 2002) for its ability to restore wild-type levels of excisive recombination in *deg1*-Δ*1* mutants. Deg1p-D151A is defective at the active site (Asp^{151}) for Pus3p (Koonin 1996), which is essential for pseudouridylation at positions 38 and 39 as well as restoration of normal cell growth in *deg1*Δ cells (Lecointe et al. 2002). When transformed into *deg1*-Δ*1* mutants, *deg1-D151A* did not restore *HOT1*-dependent excisive (Ura^-) recombination to the same levels as *DEG1* carried in the equivalent vector backbone (Table 5). The recombination rates in strains containing the *deg1-D151A* plasmid did not differ significantly from those of *deg1*-Δ*1* mutants with the vector backbone alone. These results support the idea that tRNA:Ψ 38/39-synthase activity is essential for optimal *HOT1*-stimulated exchange.

Deleting *DEG1* does not reduce the levels of *HOT1*-initiated transcription

Previous work demonstrated that stimulation of recombination relies on the ability of *HOT1* to promote high levels of RNA Pol I transcription (Voelkel-Meiman et al. 1987; Stewart and Roeder 1989; Huang and Keil 1995). While transcription is necessary, it is not sufficient for optimal *HOT1* activity (Huang and Keil 1995; Prusty and Keil 2004). To determine if *DEG1* affects *HOT1* activity by altering Pol I transcription, we evaluated levels of transcripts initiated within *HOT1* sequences at *his4* (*HOT1*::*his4*) and within the 35S rRNA genes. Primer extension analysis (Stewart and Roeder 1989; Huang and Keil 1995) was used to compare quantities of these transcripts in *DEG1* and *deg1*-Δ*1* strains (Fig. 2). Levels of *HOT1::his4* and rRNA transcripts in *deg1*-Δ*1* strains were not significantly different from the levels in *DEG1* cells. These results indicate that as in *sch9* mutants (Prusty and Keil 2004) changes in *HOT1*-stimulated transcription are not responsible for the reduced recombination rates observed in *deg1*-Δ*1* mutants.

Table 4 Effect of *pus* mutations on *HOT1*-stimulated recombination and growth at 37°

Genotype	Growth at 37°[a]	Relative recombination[b]	
		HOT1	no *HOT1*
		Ura^-	Can^R
Wild type	+ +	100	100
hrm3-1	−	17**	82
deg1(pus3) −Δ*3*[c]	−	16**	72
pus1-Δ*1*[c]	+ +	159	104
pus2-Δ*1*[c]	+ +	117	124
pus4-Δ*1*[c]	+ +	107	133

** $P \leq 0.01$ as compared to wild type
[a] + + indicates dense growth, −indicates no growth
[b] Percent recombination relative to wild-type strain
[c] Precise deletions of respective genes that contain *loxP–kanMX–loxP* module (Guldener et al. 1996)

Discussion

In *Saccharomyces cerevisia*e, *HOT1*-stimulated mitotic recombination has been suggested to be important for maintaining homology among the repeat units encoding ribosomal RNA genes (Keil and Roeder 1984). To

Table 5 *deg1−D151A* does not restore the recombination or growth defects of the *deg1−*Δ*1* mutant

Plasmid construct[a]	Growth at 37°[b]	Relative recombination[c]		
		HOT1		no *HOT1*
		Ura^-	His^+Ura^+	Can^R
YCp*DEG1*	+ +	100.0	100.0	100.0
YCp*deg1-D151A*	−	41.8*	110.2	137.0
YCp	−	24.8*	121.3	124.0

* $P \leq 0.05$ as compared to YCp*DEG1* strain
[a] Plasmids [(Lecointe et al. 2002); provided by H. Grosjean] were transformed into the *deg1*-Δ*1* strain HRM552
[b] + + indicates dense growth, −indicates no growth
[c] Percent recombination relative to YCp*DEG1* strain

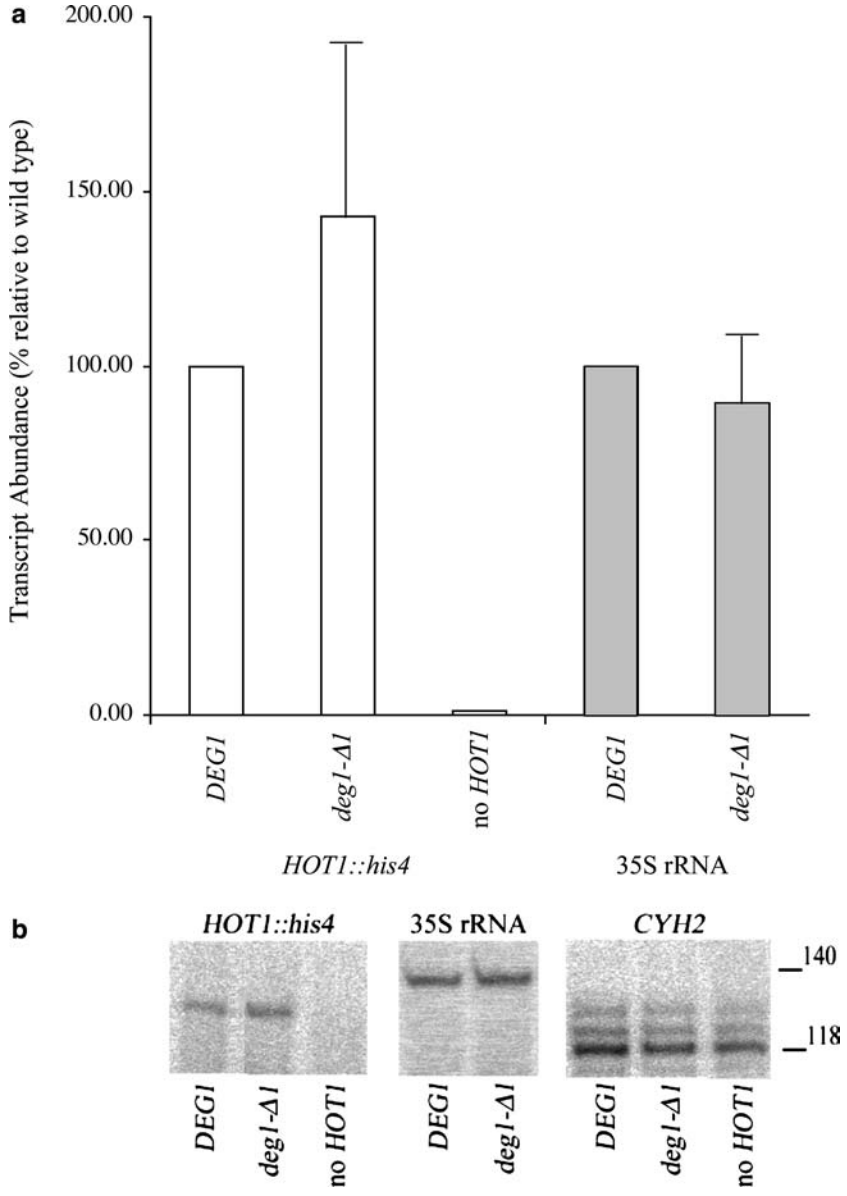

Fig. 2 Relative transcript abundance for *HOT1*-adjacent sequences in wild-type *DEG1* and *deg1-Δ1* deletion strains. **a** Mean transcript abundance (± SEM) for at least three separate trials using primer extension analysis. Transcript abundance = (Mt−Nt)/(Wt−Nt), where Mt, Nt and Wt represent the normalized transcriptional activities for *deg1*, no-*HOT1* and *DEG1* strains, respectively. Normalized transcriptional activity for each trial was obtained by dividing counts for the *HOT1::his4* or 35S rRNA primer extension product by the counts for the corresponding control *CYH2* product after the background counts were subtracted (Huang and Keil 1995). **b** Phosphoimage of a representative gel for primer extension analysis. Four doublets ranging in size from 113 to 126 nucleotides represent mature mRNAs transcribed from *CYH2* (Schwindinger and Warner 1987), which serves as internal control. Expected sizes are 129 nucleotides for the transcript from *HOT1* at *his4* (Huang and Keil 1995) and 139 nucleotides for the 35S rDNA precursor (Bayev et al. 1980; Klemenz and Geiduschek 1980). Size standards were supplied with the primer extension kit (Promega) and positions of migration of the 118 and 140 nucleotide standards are indicated

elucidate mechanisms involved in *HOT1* activity, *trans*-acting mutations that affect *HOT1*-stimulated exchange were isolated (Lin and Keil 1991). We cloned *HRM3* and established its identity as *DEG1* (Carbone et al. 1991), which encodes the pseudouridine (Ψ) synthase, Pus3p (Lecointe et al. 1998). Mutants of *DEG1* specifically decrease excision events associated with *HOT1* activity, but do not reduce *HOT1*-stimulated gene replacement or recombination that is independent of *HOT1*. These effects are observed in distinct recombination substrates containing *HOT1* in different contexts. *Deg1* mutants also have reduced levels of rDNA recombination. Our results indicate that among the four tRNA:Ψ -synthases tested, only *DEG1* affects *HOT1*-dependent recombination and that the effect of *DEG1* on *HOT1* activity requires the active site Asp^{151}, which is essential for the pseudouridine synthase activity of Pus3p.

Recombination defects are specific to *DEG1* and not a general consequence of reduced tRNA modification

Pus3p is the yeast homolog of *E. coli* TruA and contains the conserved aspartic acid (Asp^{151}) proposed to be the enzymatic catalyst (Koonin 1996). In *hrm3-1* strains, substitution of Phe for Ser^{167} in *DEG1* reduces *HOT1* activity and impacts cell growth as drastically as *deg1* deletions. The *deg1-D151A* mutation, which targets the active site for Pus3p activity, does not restore *HOT1* activity or the growth defects of *deg1* deletion strains. These results support the conclusion that loss of tRNA:Ψ 38/39-synthase function is responsible for both the recombination and growth defects. To our knowledge, connections between tRNA modification and genetic recombination have not been reported previously.

Pseudouridylation of the anticodon loop and stem of tRNAs plays an important role in protein synthesis and cellular metabolism. In prokaryotes, the absence of pseudouridines at positions 38/39/40 affects mRNA decoding and leads to a slow growth phenotype associated with attenuation-related derepression of several operons (Barnes 1978). It has been suggested that the growth defects of Pus3p-deficient yeast cells may result from either a general decrease in mRNA translation or an induction of some prokaryotic-like attenuation event that impacts mitochondrial functions (Lecointe et al. 1998). Perturbed mitochondrial activity could result in the growth defects observed in *deg1* cells and it could also explain our observation that loss of *DEG1* function reduces growth on media containing glycerol as a carbon source. However, it seems unlikely that there is a relationship between mitochondrial activity and *HOT1*-dependent recombination as *DEG1* petite mutants lacking mitochondrial activity do not exhibit altered *HOT1* activity (data not shown; Prusty and Keil 2004).

Other pseudouridine synthases, including *PUS1, PUS2* and *PUS4*, also modify tRNAs in *Saccharomyces cerevisiae*. Pus1p modifies cytoplasmic tRNAs and is primarily found in the nucleus (Simos et al. 1996). Like Pus3p, Pus4p is found in both the nucleus and cytoplasm and modifies both mitochondrial and cytoplasmic tRNAs (Becker et al. 1997). The location and function of Pus2p are not fully characterized (Hellmuth et al. 2000). Null mutants of these pseudouridine synthases are viable and show no apparent growth defects (Simos et al. 1996; Becker et al. 1997). To test the hypothesis that changes in *HOT1* activity reflect general changes in levels of tRNA modification, we compared recombination rates for cells deficient in each of the four pseudouridine synthases. Loss of Pus3p activity was unique in reducing *HOT1*-stimulated recombination. None of the Pus mutants significantly altered *HOT1*-independent mitotic recombination. It appears that Pus3p activity plays a unique role in some mechanism that specifically affects *HOT1*-dependent mitotic exchange.

Translation recoding specifically attributed to Pus3p may influence *HOT1* activity

When Lecointe et al. (2002) tested Pus1p, Pus3p and Pus4p tRNA-modifying enzymes to evaluate effects on stop codon readthrough and +1 frameshift events, termed recoding (Gesteland et al. 1992), they found that only loss of Pus3p activity detectably changes these aspects of translation. A substantial reduction in stop codon readthrough and +1 frameshift efficiency was observed in Pus3p-deficient cells (Lecointe et al. 2002). Recoding mechanisms are known to control the activity of several *S. cerevisiae* genes: *EST3* and *ABP140* both require a +1 frameshift to be expressed (Morris and Lundblad 1997; Asakura et al. 1998); expression of *PDE2* is substantially reduced when stop codon readthrough in *DEG1(PUS3)* cells generates an elongated, unstable protein product (Namy et al. 2002); and *RCK2*, *CST6* (Ouspenski et al. 1999) and five uncharacterized genes (Namy et al. 2002) have high levels of stop codon readthrough. Pus3p may indirectly affect *HOT1*-stimulated exchange by modulating expression of another protein or proteins more directly involved in recombination or chromatin organization. Further investigation is necessary to identify potential targets and to determine if *DEG1* regulation of any of these proteins affects *HOT1* activity.

Preliminary investigations indicate that overexpression of *SCH9* or deletion of *PDE2* partially restores *HOT1* activity but not normal growth or temperature tolerance to *deg1-Δ3* cells (unpublished results). These preliminary results indicate that the recombination and growth defects of *deg1* mutants are separable and suggest that *DEG1*'s effect, like *SCH9*'s (Prusty and Keil 2004), may involve signal transduction pathways that regulate target proteins essential for *HOT1* activity. Additional experimentation is needed to confirm this connection.

DEG1 affects excision but not gene replacement

Substantial evidence indicates that gene replacement (conversion) and gene excision (crossing over) are mechanistically linked but mutationally separable aspects of genetic recombination (Rong et al. 1991; Pâques and Haber 1999; Sung et al. 2000). Different genetic requirements exist for gene conversion and crossing over (Freedman and Jinks-Roberson 2002), and Aguilera and Klein (1988) identified three distinct classes of mutants that primarily increase excision, gene replacement, or both types of intrachromosomal exchange events. *HOT1*-stimulated gene excision and rDNA exchange are both decreased by mutations in *DEG1*, while *HOT1*-stimulated gene replacement is not impacted. This is the first *HOT1*-specific mutant characterized that differentially affects gene replacement and excision. These results indicate that each type of recombination likely requires some distinct

proteins. In contrast, deletion of *SCH9* affects both excision and gene replacement (Prusty and Keil 2004) indicating that some proteins play roles in both types of events. Pus3p must participate in a *HOT1*-dependent pathway that specifically facilitates excisive recombination. In the absence of *HOT1*, the reduction in excision is even more substantial than observed in *deg1* deletion strains (Table 2). This suggests that more than one mechanism is involved in *HOT1*-dependent intrachromosomal excision, and that Pus3p plays a role in only some of these pathways.

Reduced *HOT1* exchange does not result from decreased transcription in *deg1* strains

Substantial evidence supports the idea that high levels of transcription are required for *HOT1* activity (Voelkel-Meiman et al. 1987; Stewart and Roeder 1989; Huang and Keil 1995). Mutations in *DEG1* that substantially decrease *HOT1*-stimulated recombination have no detectable effect on *HOT1*-dependent transcription or transcription of the 35S rRNA. This finding is identical to the recent report by Prusty and Keil (2004) that *SCH9*(*HRM2*) decreases *HOT1*-dependent recombination without affecting transcription from this element. *DEG1* provides a second example showing that, although necessary, increased transcription is not sufficient for optimal *HOT1* exchange.

Pseudouridylation and genome stability

In addition to being present in tRNAs, pseudouridines are also found in ribosomal RNAs, small nuclear RNAs and small nucleolar RNAs (reviewed in Charette and Gray 2000). In some instances, deficiency of modification has readily observable phenotypic consequences such as in the genetically inherited disease dyskeratosis congenita (Ruggero et al. 2003). In other cases, slight or no phenotypic effects are apparent. For example, growth defects of *E. coli* containing a mutated RluA pseudouridine synthase only become apparent when grown in competition with a wild-type strain (Raychaudhuri et al. 1999). While *deg1* mutants of yeast have a readily observable growth defect (Carbone et al. 1991 and this work), they also have a less apparent effect that alters stability and potentially homogeneity of repeated DNA sequences. These findings expand the realm of phenotypic effects imparted by altered RNA modification.

Acknowledgements We thank Susan DiBartolomeis, Tom Reiner, Laura Palmer and Reeta Prusty for sharing their valuable technical expertise, and Anita Hopper for critical reading of the manuscript. Technical assistance from Jonathan Morgan and Paul Lewis is also appreciated. Plasmids containing *deg1-D151A* and controls were kindly provided by Henri Grosjean (CNRS, Laboratoire d'Enzymologie et de Biochimie Structurales, France). This research was supported by funding from the Pennsylvania State System of Higher Education Faculty Professional Development Council and the Millersville University Faculty Grants Committee to CEH, a Sigma Xi Grant-in-Aid of Research to KGS, Millersville University Alumni Association Neimeyer-Hodgson Student Research Grants to MM, AR, GIL and DLH, and by National Institutes of Health grant GM-36422 to RLK.

References

Aguilera A, Klein HL (1988) Genetic control of intrachromosomal recombination in *Saccharomyces cerevisiae*. I. Isolation and genetic characterization of hyper-recombination mutations. Genetics 119:779–790

Amstutz H, Munz P, Heyer WD, Leupoid U, Kohli J (1985) Concerted evolution of tRNA genes: intergenic conversion among three unlinked serine tRNA genes in *S. pombe*. Cell 40:879–886

Asakura T, Sasaki T, Nagano F, Satoh A, Obaishi H, Nishioka H, Imomura H, Hotta K, Tanaka K, Nakanishi H, Takai Y (1998) Isolation and characterization of a novel actin filament-binding protein from *Saccharomycescerevisiae*. Oncogene 16:121–130

Barnes WM (1978) DNA sequence from the histidine operon control region: seven histidine codons in a row. Proc Natl Acad Sci USA 75:4281–4285

Bayev AA, Georgiev OI, Hadjiolov AA, Kermekchiev MB, Nikolaev N, Skryabin KG, Zakharyev VM (1980) The structure of the yeast ribosomal RNA genes. 2. The nucleotide sequence of the initiation site for ribosomal RNA transcription. Nucleic Acids Res 8:4919–4926

Becker HF, Motorin Y, Planta RJ, Grosjean H (1997) The yeast gene YNL292w encodes a pseudouridine synthase (Pus4) catalyzing the formation of psi55 in both mitochondrial and cytoplasmic tRNAs. Nucleic Acids Res 25:4493–4499

Björk GR (1995) Biosynthesis and function of modified nucleosides in tRNA. In: Söll D, RajBhandary UL (eds) tRNA: structure, biosynthesis, and function. ASM Press, Washington, pp 165–205

Brambilla A, Mainieri D, Carbone MLA (1997) A simple signal element mediates transcription termination and mRNA 3′ end formation in the *DEG1* gene of *Saccharomyces cerevisiae*. Mol Gen Genet 254:681–688

Carbone MLA, Solinas M, Sora S, Panzeri L (1991) A gene linked to *CEN6* is important for growth of *Saccharomyces cerevisiae*. Curr Genet 19:1–8

Charette M, Gray MW (2000) Pseudouridine in RNA: what, where, how, and why. IUBMB Life 49:341–351

Defossez PA, Prusty R, Kaeberlein M, Lin SJ, Ferrigno P, Silver PA, Keil RL, Guarente L (1999) Elimination of replication block protein Fob1 extends the life span of yeast mother cells. Mol Cell 3:447–455

Elion EA, Warner JR (1984) The major promoter element of rRNA transcription in yeast lies 2 kb upstream. Cell 39:663–673

Elion EA, Warner JR (1986) An RNA polymerase I enhancer in *Saccharomyces cerevisiae*. Mol Cell Biol 6:2089–2097

Fiers W, Contreras RR, Haegemann G, Rogiers R, Van de Voorde A, Van Heuverswyn H, Van Herreweghe J, Volckaert G, Ysbaert M (1978) Complete nucleotide sequence of SV40 DNA. Nature 273:113–120

Freedman JA, Jinks-Robertson S (2002) Genetic requirements for spontaneous and transcription-stimulated mitotic recombination in *Saccharomyces cerevisiae*. Genetics 162:15–27

Gesteland RF, Weiss RB, Atkins JF (1992) Recoding: reprogrammed genetic decoding. Science 257:1640–1641

Gietz RD, Sugino A (1988) New yeast—*Escherichia coli* shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair restriction sites. Gene 74:527–534

Guldener U, Heck S, Fielder T, Beinhauer J, Hegemann JH (1996) A new efficient gene disruption cassette for repeated use in budding yeast. Nucleic Acids Res 24:2519–2524

Guyer MS (1983) Uses of the transposon $\gamma\delta$ in the analysis of cloned genes. Methods Enzymol 101:362–369

Haber JE, Thorburn PC (1984) Healing of broken linear dicentric chromosomes in yeast. Genetics 106:207–226

Hellmuth K, Grosjean H, Motorin Y, Deinert K, Hurt E, Simos G (2000) Cloning and characterization of the *Schizosaccharomyces pombe* tRNA: pseudouridine synthase Pus1p. Nucleic Acids Res 28:4604–4610

Huang GS, Keil RL (1995) Requirements for activity of the yeast mitotic recombination hotspot *HOT1*: RNA polymerase I and multiple cis-acting sequences. Genetics 141:845–855

Keil RL, McWilliams AD (1993) A gene with specific and global effects on recombination of sequences from tandemly repeated genes in *Saccharomyces cerevisiae*. Genetics 135:711–718

Keil RL, Roeder GS (1984) *Cis*-acting, recombination-stimulating activity in a fragment of the ribosomal DNA of *S. cerevisiae*. Cell 39:377–386

Klemenz R, Geiduschek EP (1980) The 5′ terminus of the precursor ribosomal RNA of *Saccharomyces cerevisiae*. Nucleic Acids Res 8:2679–2689

Koonin EV (1996) Pseudouridine synthases: four families of enzymes containing a putative uridine-binding motif also conserved in dUTPases and dCTP deaminases. Nucleic Acids Res 24:2411–2415

Kramer KM, Brock JA, Bloom K, Moore JK, Haber JE (1994) Two different types of double-strand breaks in *Saccharomyces cerevisiae* are repaired by similar *RAD52*-independent, nonhomologous recombination events. Mol Cell Biol 14:1293–1301

Lecointe F, Simos G, Sauer A, Hurt EC, Motorin Y, Grosjean H (1998) Characterization of yeast protein Deg1 as pseudouridine synthase (Pus3) catalyzing the formation of psi38 and psi39 in tRNA anticodon loop. J Biol Chem 273:1316–1323

Lecointe F, Namy O, Hatin I, Simos G, Rousset JP, Grosjean H (2002) Lack of pseudouridine 38/39 in the anticodon arm of yeast cytoplasmic tRNA decreases in vivo recoding efficiency. J Biol Chem 277:30445–30453

Lin Y-H, Keil RL (1991) Mutations affecting RNA polymerase I-stimulated exchange and rDNA recombination in yeast. Genetics 127:31–38

Mann C, Davis RW (1983) Instability of dicentric plasmids in yeast. Proc Natl Acad Sci USA 80:228–232

Morris DK, Lundblad V (1997) Programmed translational frameshifting in a gene required for yeast telomere replication. Curr Biol 7:969–976

Motorin Y, Keith G, Simon C, Foiret D, Simos G, Hurt E, Grosjean H (1998) The yeast tRNA: pseudouridine synthase Pus1p displays a multisite substrate specificity. RNA 4:856–869

Namy O, Duchateau-Nguyen G, Rousset JP (2002) Translational readthrough of the *PDE2* stop codon modulates cAMP levels in *Saccharomyces cerevisiae*. Mol Microbiol 43:641–652

Ouspenski S II, Elledge SJ, Brinkley BR (1999) New yeast genes important for chromosome integrity and segregation identified by dosage effects on genome stability. Nucleic Acids Res 27:3001–3008

Pâques F, Haber JE (1999) Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. Microbiol Mol Biol Rev 63:349–404

Prusty R, Keil RL (2004) *SCH9*, a putative protein kinase from *Saccharomyces cerevisiae*, affects *HOT1*-stimulated recombination. Mol Gen Genomics 272:264–274

Raychaudhuri S, Niu L, Conrad J, Lane BG, Ofengand J (1999) Functional effect of deletion and mutation of the *Escherichia coli* ribosomal RNA and tRNA pseudouridine synthase RluA. J Biol Chem 274:18880–18886

Roeder GS, Keil RL, Voelkel-Meiman KA (1986) A recombination-stimulating sequence in the ribosomal RNA gene cluster of yeast. In: Klar A, Strathern JN (eds) Mechanisms of yeast recombination. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 29–33

Rong L, Palladino F, Aguilera A, Klein H (1991) The hyper-gene conversion *hrp5-1* mutation of *Saccharomyces cerevisiae* is an allele of the *SRS2/RADH* gene. Genetics 127:75–85

Rose MD, Novick P, Thomas JH, Botstein D, Fink GR (1987) A *Saccharomyces cerevisiae* genomic plasmid bank based on a centromere-containing shuttle vector. Gene 60:237–243

Rose MD, Winston F, Hieter P (1990) Methods in Yeast genetics: a laboratory course manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor

Ruggero D, Grisendi S, Piazza F, Rego E, Mari F, Rao PH, Cordon-Cardo C, Pandolfi PP (2003) Dyskeratosis congenita and cancer in mice deficient in ribosomal RNA modification. Science 299:259–262

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467

Schwindinger WF, Warner JR (1987) Transcriptional elements of the yeast ribosomal protein gene *CYH2*. J Biol Chem 262:5690–5695

Simos G, Tekotte H, Grosjean H, Segref A, Sharma K, Tollervey D, Hurt EC (1996) Nuclear pore proteins are involved in the biogenesis of functional tRNA. EMBO J 15:2270–2284

Stewart SE, Roeder GS (1989) Transcription by RNA polymerase I stimulates mitotic recombination in *Saccharomyces cerevisiae*. Mol Cell Biol 9:3464–3472

Sung P, Trujillo KM, Van Komen S (2000) Recombination factors of *Saccharomyces cerevisiae*. Mutat Res 451:257–275

Thompson DL, Kalderon D, Smith AE, Tevethia MJ (1990) Dissociation of Rb-binding and anchorage-independent growth from immortalization and tumorigenicity using SV40 mutants producing N-terminally truncated large T antigens. Virology 178:15–34

Voelkel-Meiman K, Roeder GS (1990) A chromosome containing *HOT1* preferentially receives information during mitotic interchromosomal gene conversion. Genetics 124:561–572

Voelkel-Meiman K, Keil RL, Roeder GS (1987) Recombination-stimulating sequences in yeast ribosomal DNA correspond to sequences regulating transcription by RNA polymerase I. Cell 48:1071–1079

Wai H, Johzuka K, Vu L, Eliason K, Kobayashi T, Horiuchi T, Nomura M (2001) Yeast RNA polymerase I enhancer is dispensable for transcription of the chromosomal rRNA gene and cell growth, and its apparent transcription enhancement from ectopic promoters requires Fob1 protein. Mol Cell Biol 21:5541–5553

Wolfe KH, Lohan AJ (1994) Sequence around the centromere of *Saccharomyces cerevisiae* chromosome II: similarity of *CEN2* to *CEN4*. Yeast 10:S41–S46

Yarian C, Townsend H, Czestkowski W, Sochacka E, Malkiewicz AJ, Guenther R, Miskiewicz A, Agris PF (2002) Accurate translation of the genetic code depends on tRNA modified nucleotides. J Biol Chem 277:16391–16395

Yuan LW, Keil RL (1990) Distance-independence of mitotic intrachromosomal recombination in *Saccharomyces cerevisiae*. Genetics 124:263–273