

Note

Telomeric Silencing of an Open Reading Frame in *Saccharomyces cerevisiae*

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Manuscript received March 21, 2006
Accepted for publication March 24, 2006

ABSTRACT

The endmost chromosome *I* ORF is silenced by a natural telomere position effect. YAR073W/*IMD1* was found to be transcribed at much higher levels in *sir3* mutants and when its adjacent telomere was removed from it. These results suggest that telomeres play a role in silencing actual genes.

IN the yeast *Saccharomyces cerevisiae* subtelomeric sequences are distinctly different from the rest of the chromosomal DNA because they are mostly repetitive and contain a relatively low density of ORFs. Subtelomeric sequences compose ~7% (~25 kb × 32 ends/12,000 kb) of the genome and are largely made up of sequences termed W', Y', or X. The number, distribution, and arrangement of these sequences vary on each chromosome and in different strains. W' and Y' sequences contain some short ORFs but they are likely to be pseudogenes. Other subtelomeric ORFs belong to repetitive gene families and appear to be nonessential for routine growth in the laboratory (PRYDE and LOUIS 1997). Subtelomeric sequences from *S. cerevisiae* have been proposed to play a role in preventing nonfunctional meiotic crossovers near chromosome ends (BARTON *et al.* 2003). Otherwise, their precise function is not known. Subtelomeric DNA from *S. cerevisiae* has been compared to the telomeric heterochromatin of higher organisms, which also contains repetitive sequences and has few active genes.

Most subtelomeric genes or ORFs either are not expressed at all or are expressed at relatively low levels during routine growth in the laboratory. A few such as those from the *MAL*, *SUC*, and *PHO* gene families are abundantly expressed but only under certain conditions (PRYDE and LOUIS 1997). When these genes are repressed, the amount of RNA produced from any individual gene is probably quite low. It is possible that low transcript levels from subtelomeric genes are in some part due to the well-characterized telomere position

effect (TPE) that was first revealed by an epigenetic repression of genes inserted within or near telomeres (GOTTSCHLING *et al.* 1990; PIRROTTA and GROSS 2005). About 60 genes show increased transcript levels in the presence of mutations that alleviate TPEs such as *sir2*, *sir3*, and *rap1* and many of these genes are relatively close to a telomere (WYRICK *et al.* 1999; VEGA-PALAS *et al.* 2000; HALME *et al.* 2004). Nevertheless, a true TPE dependent on actual telomere proximity has never been demonstrated for a naturally occurring yeast gene other than a small effect on *HMR*, the silenced copy of the mating-type locus on the right arm of chromosome *III* (THOMPSON *et al.* 1994).

The three endmost ORFs on the right arm of chromosome *I* are paralogous to three ORFs near the right end of chromosome *VIII* and have almost identical DNA sequences. The most prominent differences between the two gene sets are the chromosome *VIII* sequences are ~6 kb farther from the telomere than the chromosome *I* sequences and the chromosome *VIII* sequences are transcribed while the chromosome *I* genes appear silent. These observations led to the suggestion that the chromosome *I* genes could be repressed by a TPE (BARTON *et al.* 1997). The endmost chromosome *I* ORFs are YAR073W/*IMD1*, encoding an IMP dehydrogenase ortholog (HYLE *et al.* 2003); YAR071W/*PHO11*, encoding an inducible acid phosphatase (KABACK *et al.* 1989; SHNYREVA *et al.* 1996); and YAR068W, encoding a nonessential putative membrane protein of unknown function that when overexpressed produces a drug-resistance phenotype (BARTON *et al.* 1997; LAUNHARDT *et al.* 1998). In this report we demonstrate that the endmost chromosome *I* ORF, YAR073W/*IMD1*, is indeed silenced by its telomere, suggesting that telomeres play a role in silencing actual genes.

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TABLE 1
Yeast strains

Strain	Genotype	Source
D139-11B	<i>MATα ade1 his3-11,15 leu2-3,112 trp1 can1</i>	J. Szostak
DK330-2B	<i>MATα his3-11,15 leu2-3,112 ura3-1 can1</i>	Congenetic to D139-11B
DK392	<i>MATα/MATα ADE1/ade1 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 TRP1/trp1 URA3/ura3-1 can1/can1</i>	DK330-2B \times D139-11B (BARTON and KABACK 1994)
DK420	<i>MATα/MATα ADE1/ade1 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 TRP1/trp1 URA3/ura3-1 can1/can1 $\Delta I(LEU2)/WT$</i>	DK392 with ΔI
DK420-1D	<i>MATα ade1 his3-11,15 leu2-3,112 $\Delta I(LEU2)$</i>	
DK421	<i>MATα/MATα ADE1/ade1 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 TRP1/trp1 URA3/ura3-1 can1/can1 $\Delta I(LEU2)/WT \Delta VIII(HIS3)/WT$</i>	DK420 with $\Delta VIII$
DK421-1A	<i>MATα his3-11,15 leu2-3,112 trp1 ura3-1 can1 $\Delta VIII(HIS3)$</i>	
DK421-1Asir3	<i>MATα his3-11,15 leu2-3,112 trp1 ura3-1 can1 $\Delta VIII(HIS3) sir3::TRP1$</i>	
DK421-2D	<i>MATα ade1 his3-11,15 leu2-3,112 can1 $\Delta I(LEU2) \Delta VIII(HIS3)$</i>	
DK423	<i>MATα/MATα ade1/ADE1 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 TRP1/trp1 ura3-1/ura3-1 can1/can1 WT/$\Delta VIII(HIS3)$</i>	DK392-1D \times DK421-1A
DK424	<i>MATα/MATα ade1/ADE1 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1/TRP1 ura3-1/ura3-1 can1/can1 $\Delta VIII(HIS3)/\Delta VIII(HIS3)$</i>	DK423-1A \times DK423-2B
DK425-14A	<i>MATα his3-11,15 leu2-3,112 ade1 can1 sir3::TRP1 $\Delta I(LEU2) \Delta VIII(HIS3)$</i>	Haploid spore from DK421-2D \times DK421-1Asir3

S. cerevisiae strains were constructed, grown, sporulated, and transformed with DNA using standard procedures (BURKE *et al.* 2000). All recombinant DNA procedures were carried out using *Escherichia coli* strain DH5 α (Invitrogen, Carlsbad, CA) and plasmid pBluescriptII (Stratagene, San Diego) using standard procedures (SAMBROOK *et al.* 1989). All gene replacements and insertions were verified by either Southern blot hybridization (SOUTHERN 1975) or PCR (MULLIS and FALOONA 1987). Construction of $\Delta 2LEU2$, herein labeled ΔI for the chromosome *I* right-end deletion, and $\Delta 4HIS3$, herein labeled $\Delta VIII$ for the chromosome *VIII* right-end deletion, were previously described (BARTON *et al.* 1997). These constructs were introduced sequentially into strain DK392, yielding strains DK420 and DK421, respectively, which were used to breed strains with appropriate genotypes. *SIR3* was deleted by one-step gene replacement (ROTHSTEIN 1983) using plasmid pKL12 provided by R. Sternglanz (STONE *et al.* 1991) as described.

To determine whether the three endmost chromosome *I* ORFs were subject to a TPE, their expression was examined in *sir3* mutant and wild-type control strains that contained a deletion of the paralogous chromosome *VIII* genes (Table 1; BARTON *et al.* 1997). Experiments using blot hybridization revealed a YAR073W/*IMD1* RNA species only in *sir3* mutant strains. Quantitation of the autoradiogram using *FUN31* RNA as an internal standard (BARTON and KABACK 1994) indicated that this RNA was present at a rate of approximately one copy per cell. Interestingly, this RNA was ~ 50 nucleotides larger than the paralogous YHR216 transcript (Figure 1B). A low level of the smaller RNA species was still present in the chromosome *VIII* deletions. This previously observed species (BARTON *et al.* 1997) does not appear to be affected by *sir3* and is probably transcribed from the additional orthologous copies of this ORF found on chromosomes *XII* and *XIII*. A quantitative RT-PCR assay specific for YAR073W/*IMD1* (BARTON *et al.* 1993) was used to show that *sir3* mutant strains contained approximately sevenfold more YAR073W/*IMD1* RNA than the wild-type controls (Figure 1C). The controls also show that this transcript could not be detected when chromosome *I* sequences were deleted. Similar assays were carried out for YAR071/*PHO11* or YAR068W (data not shown) using the same chromo-

some *VIII* deletion strains but there was no evidence for any *SIR3*-dependent effects. However, results for YAR071/*PHO11* were inconclusive since multiple remaining acid phosphatase genes could have obscured any effect. Therefore, at present there is evidence that only the endmost ORF, YAR073W/*IMD1*, is repressed by *SIR3*.

To examine whether YAR073W/*IMD1* was controlled by a *bona fide* TPE, two different homeologous fragments from the middle of *Saccharomyces carlsbergensis* chromosome *III*, 7.0 and 8.4 kb in length, were inserted between YAR073W/*IMD1* and its proximal telomere and transcript levels were determined by quantitative RT-PCR as above. *LEU2*, the selectable marker was introduced in both orientations in each insert to better ensure that its presence would not affect YAR073W/*IMD1* expression and all strains lacked the paralogous chromosome *VIII* genes. The results showed that, when YAR073W/*IMD1* was separated from its telomere, it produced three- to ninefold more RNA (Figure 2), an effect that was similar in magnitude to that observed in *sir3* mutants. Quantitation of the results indicated that cells containing the *S. carlsbergensis* insert contained ~ 0.3 – 0.6 copies of the YAR073W/*IMD1* RNA/cell or slightly less than was observed in *sir3* cells. However, the controls that did not contain inserts also were slightly lower than in the

previous experiment. Consistent with these observations was a report that YAR073W/IMD1 RNA could only be detected when this gene was contained on a plasmid (HYLE *et al.* 2003). Thus, telomere proximity appears to be involved in the silencing of YAR073W/IMD1.

YAR068W and YAR071/PHO11 were also examined but produced the same low level of transcript with and without the *S. carlsbergensis* insert (data not shown). Thus, the TPE appeared specific for YAR073W/IMD1.

On the basis of its apparent lack of expression and a sequence that either truncates or uses frameshifting to change the reading frame of the last 20% of the protein, it has been suggested that YAR073W/IMD1 is a pseudogene and cannot encode functional IMP dehydrogenase (HYLE *et al.* 2003). A functional *IMD* gene makes cells resistant to the antifungal mycophenolic acid, providing a simple assay that was carried out on strains where an *IMD1* transcript could be detected (HYLE *et al.* 2003). We found that *sir3* and *SIR3*⁺ control strains deleted for the end of chromosome VIII (Δ VIII), where the functional *IMD2* gene is located, were equally sensitive to mycophenolic acid. Furthermore, all *S. carlsbergensis* insertion strains that removed YAR073W/IMD1 from its proximal telomere also were equally sensitive. Control strains containing the end of chromosome VIII were fully resistant as previously described (HYLE *et al.* 2003; data not shown). These results are consistent with previous studies that demonstrate that YAR073W/IMD1 does not encode this enzyme (HYLE *et al.* 2003). Nevertheless, it is possible that this sequence has a different but unknown function, especially in light of the finding that

both the mRNA and its frameshifted open coding region are longer than that found for the active *IMD2* gene.

Genome-array expression studies with different *SIR* mutants and histone depletion revealed that telomere-proximal genes were more likely than telomere-distal genes to be affected (WYRICK *et al.* 1999). In these studies, Wyrick *et al.* reported only a twofold *sir3*-dependent effect on YAR073W/IMD1, which was not considered significant. This discrepancy is likely due to

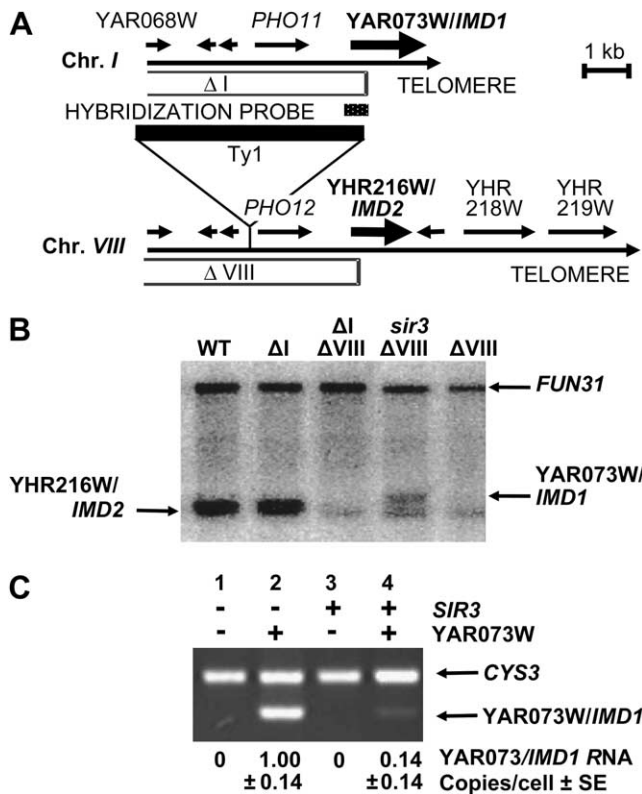


FIGURE 1.—YAR073W/IMD1 is repressed by *SIR3*. (A) Physical maps of the right end of chromosomes I and VIII. Arrows indicate open reading frames. Extent of deletion constructs that eliminate cross-hybridizing genes are noted (Δ I and Δ VIII). (B) YAR073W/IMD1 expression analyzed by blot hybridization of poly(A⁺) RNA extracted from cells carrying noted deletions and *sir3*. WT, wild-type strain D139-11B; Δ I, chromosome I deletion strain DK420-1D; Δ I Δ VIII, chromosomes I and VIII double-deletion strain DK421-2D; *sir3* Δ VIII, *sir3::trp1* chromosome VIII deletion strain DK421-1A*sir3* strain; Δ VIII, chromosome VIII deletion strain DK421-1A. The ~5-kb *FUN31* transcript, present at approximately one copy per cell (BARTON *et al.* 1997), was used as an abundance standard to quantitate YAR073W/IMD1 RNA. (C) RT-PCR analysis of YAR073W/IMD1 RNA in haploid strains deleted for the end of chromosome VIII. Relevant genotypes are shown above each lane. Lanes: 1, DK425-14A; 2, DK421-1A*sir3*; 3, DK421-2D; 4, DK421-1A. YAR073W/IMD1 transcript abundance (RNA copies per cell) was determined by RT-PCR using simultaneously amplified *CYS3* RNA as a loading control (BARTON *et al.* 1993). Nucleic acid isolation and manipulation were as described (SAMBROOK *et al.* 1989). Transcript analysis using blot hybridization was carried out as described (BARTON and KABACK 1994). The *FUN31* probe was a cloned 1.4-kb *Hind*III fragment (SGD chromosome I coordinates 122,392–122,832 and the YAR073W/IMD1 probe was a 0.4-kb *Kpn*I–*Bgl*II fragment from λ 4599 (RILES *et al.* 1993) [Saccharomyces Genome Database (SGD, <http://www.yeastgenome.org>) chromosome I coordinates 227,378–227,767]. RT-PCR was performed using the Superscript first strand synthesis system (Life Technologies, Rockville, MD) using poly(A⁺) RNA and AmpliTaq Gold (Roche Molecular Systems, Branchburg, NJ) according to the manufacturer's directions. Oligonucleotide primers were 5'-AGACTACAAGACCGCACTAGAT and 5'-TGGCACCCACCAACTTTGCAT for YAR073W/IMD1 and 5'-GGAAAACACCAAATTGGTCTG and 5'-CAAAAACACCAGAGGCCTCTC for *CYS3*. To provide specificity, the forward YAR073W/IMD1 primer sequence was absent from chromosome VIII due to the Δ VIII deletion and the reverse primer was from a part of the YAR073W ORF with the least homology to the orthologs on chromosomes XII and XIII. Reactions were run in a GeneAmp PCR System 2400 (Perkin-Elmer, Norwalk, CT) utilizing 24 cycles of 94° for 30 sec, 55° for 30 sec, and 72° for 1 min. Quantitation of blots and gels was carried out using a phosphorimager and Image Quant Software (Molecular Dynamics, Sunnyvale, CA) and serial dilutions to ensure that band intensities were in the linear range of the photo scanner. Four measurements were carried out on each band and the average \pm the standard error is shown. *CYS3* RNA showed virtually constant levels relative to template RNA added in all strains examined and was used as a loading control (BARTON *et al.* 1993). RNA copies per cell are calculated from the amount of the YAR073W/IMD1 RNA level measured in the *sir3* strain by blot hybridization (one copy/cell).

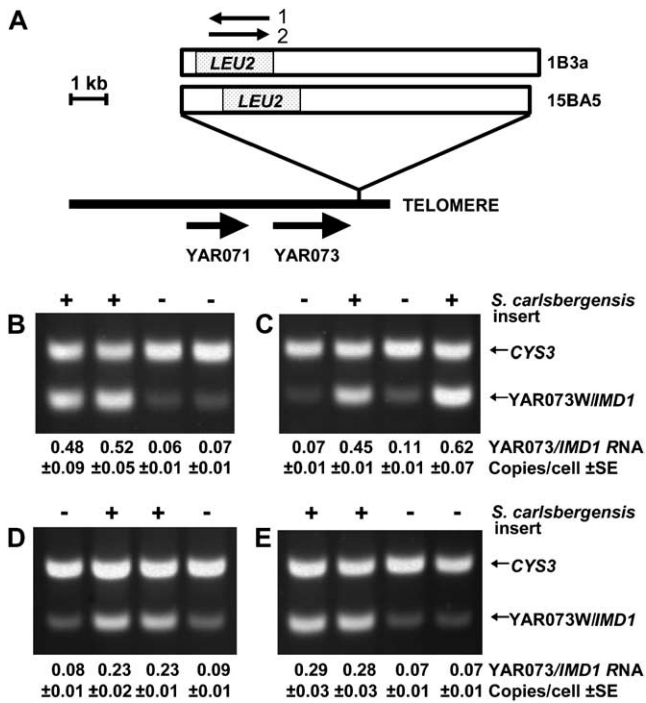


FIGURE 2.—Removal of the telomere from *YAR073/IMD1* relieves repression. (A) Structure of the right end of chromosome III showing the location of the *S. carlsbergensis* DNA inserts 1B3a and 15BA5 that moved *YAR073W/IMD1* from its proximal telomere. Arrows above indicate the *LEU2* transcript direction for each numbered construct. (B–E) Analysis of *YAR073W/IMD1* expression in haploid spores from strain DK424 transformants. Relative transcript abundance was determined by RT-PCR using *CYS3* RNA as a loading control. B–E each show four spores from a single ascus from each heterozygous *S. carlsbergensis* DNA insert; two spores contain the insert (+) and two spores lack it (–). (B) Insert 1B3a-1. (C) Insert 1B3a-2. (D) Insert 15BA5-1. (E) Insert 15BA5-2. 1 and 2 indicate *LEU2* orientation. Two *Bam*HI fragments from chromosome III of *S. carlsbergensis*, 1B3a (7.3 kb) and 15BA5 (8.3 kb), provided by C. Newlon (YANG *et al.* 1999) were inserted adjacent to the telomere at position 229,850 (coordinates from SGD, <http://www.yeastgenome.org>) by one-step gene replacement (ROTHSTEIN 1983) using the chromosome I insert from plasmid pEL56H2 provided by Ed Louis as the target and a PCR-amplified *S. cerevisiae* (MULLIS and FALOONA 1987) 1.6-kb *LEU2* gene with primer-encoded *Bg*III ends as the selectable marker inserted at *Bg*III sites within each *S. carlsbergensis* sequence. Fragment 15BA5 had a 1.6-kb *Bg*III fragment deleted where *LEU2* was inserted. In spite of repeated attempts and for unknown reasons, one-step gene replacements involving sequences near telomeres were unsuccessful using haploids. Accordingly, all constructs were introduced successfully in diploids and haploid segregants containing the inserted DNA obtained by sporulation and tetrad dissection. Quantitation of the PCR products was carried out as described in Figure 1. “Copies/cell RNA” was estimated from the *CYS3* RNA normalized relative intensity of the PCR products using RT-PCR-amplified RNA from strain DK421-1Asir3 as an abundance standard that was run in parallel using the same gel (not shown).

either strain variations or uncertainties in measuring relatively low levels of RNA using arrays.

While it has been suggested that TPEs play a role in gene expression, it is also likely that many telomere-proximal genes are unaffected due to insulator elements that prevent the effects from spreading (LOUIS 1995). Nevertheless, *HMR* showed a modest telomere proximity effect and now *YAR073W/IMD1* has been shown to have *bona fide* TPE. As there are now ~60 telomere-proximal genes that are affected by various *SIR* gene mutants, it is likely that some of these genes are directly regulated by their nearby telomeres.

The function of subtelomeric DNA is still not entirely understood. In addition to its suggested structural role preventing nonfunctional crossing over during meiosis, these sequences could also provide an area of the genome where rarely needed genes and gene progenitors are efficiently prevented from being expressed, especially if their expression could be deleterious.

This work was supported by grants from the National Institutes of Health (GM053924) and the National Science Foundation (MCB0136278).

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Communicating editor: F. WINSTON