Yeast hnRNP K-Like Genes Are Involved in Regulation of the Telomeric Position Effect and Telomere Length

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Mammalian heterogeneous nuclear ribonucleoprotein K (hnRNP K) is an RNA- and DNA-binding protein implicated in the regulation of gene expression processes. To better understand its function, we studied two *Saccharomyces cerevisiae* homologues of the human hnRNP K, *PBP2* and *HEK2* (heterogeneous nuclear RNP K-like gene). *pbp2Δ* and *hek2Δ* mutations inhibited expression of a marker gene that was inserted near telomere but not at internal chromosomal locations. The telomere proximal to the ectopic marker gene became longer, while most of the other telomeres were not altered in the double mutant cells. We provide evidence that telomere elongation might be the primary event that causes enhanced silencing of an adjacent reporter gene. The telomere lengthening could, in part, be explained by the inhibitory effect of *hek2Δ* mutation on the telomeric rapid deletion pathway. Hek2p was detected in a complex with chromosome regions proximal to the affected telomere, suggesting a direct involvement of this protein in telomere maintenance. These results identify a role for hnRNP K-like genes in the structural and functional organization of telomeric chromatin in yeast.

Heterogeneous nuclear RNPs (hnRNPs) bind to primary transcripts in the nucleus and along with small RNA ribonucleoprotein complexes mediate RNA maturation and transport to the cytoplasm (5). Much progress has been made in the identification and elucidation function of these components. It is becoming clear that hnRNPs have a broader role than was previously thought. For instance, yeast Rfflp and the mammalian A1 hnRNPs were shown to directly bind telomeric DNA sequences and alter the metabolism of telomeres (21, 23). Mammalian hnRNP K was shown to bind CT-rich elements sequences and alter the metabolism of telomeres (21, 23). These data imply that hnRNP K and other RNA-binding proteins are able to bind and regulate both DNA- and RNA-dependent processes.

K protein contains three evolutionarily conserved KH domains; similar domains were also found in RNA- and DNA-binding proteins derived from organisms as diverse as *Escherichia coli* and mammals (54). The structure of the K protein KH3 domain has recently been determined (2). It contains a three-stranded β-sheet stacked against three α-helices, β3αββ0α, a structural fold found in other RNA-binding proteins unrelated to K protein in primary sequence (5). K protein contains a cluster of three proline-rich SH3-binding segments (16, 57, 59) that reside within the K-interactive domain (3). The K-interactive domain mediates the interaction of K protein with a number of its protein partners (6, 12, 18, 39, 47, 59). K protein also contains both the nuclear localization signal and nuclear shuttle domain (38). A general model is emerging where K protein may serve to link signal transduction pathways to nuclear acid-directed processes (42).

We have recently shown that the mammalian K protein interacts with the Polycomb Group protein Eed (11). K protein also binds DNA-methyltransferase (50). Involvement of these K protein partners in chromatin rearrangements suggested a role for K protein in chromatin function. Consistent with this notion is the observation that K protein binds telomeric repeat DNA in vitro (24). Here we identified two *Saccharomyces cerevisiae* hnRNP K-like proteins, Pbp2p (Hek1p) and Hek2p, as suppressors of the telomeric position effect (TPE). Our data provide evidence for a direct role of these genes in chromatin-dependent processes.

**MATERIALS AND METHODS**

**Yeast strains, plasmids, and methods.** Media used for the growth of *S. cerevisiae* were as previously described (13); cells were grown at 30°C. All strains used in this study (Table 1) except UCC3505, UCC3515, and AVH2.45 were isogenic with YPH250 (51). Yeast transformation was performed by the lithium acetate procedure as described in Technical Tips Online (http://ttt.trends.com). 5-Fluoroorotic acid resistance (5-FOA) was determined as described in reference 1. 5-FOA was obtained from Zymo Research (Orange, Calif.). PCR-mediated gene disruption was performed as described in reference 4.

The BamHI-MspAI fragment of the pHs6-23 plasmid (44) containing the *SIR3* gene with the upstream putative regulatory elements was cloned into a BamHI-EcoRI cut derivative of the pVP16 plasmid (2am LEU2) into which the *ADH* promoter and VP16 open reading frame (ORF) were deleted (SphI fragment). The final plasmid pSI3 was used to overexpress *SIR3* in yeast strains.

**Northern, Southern, and Western blot analysis.** RNA was purified from mid-log-phase yeast cultures (5 ml) as described in reference 46. RNA samples were analyzed as described previously (12). After first being denatured in a buffer containing formamide at 65°C for 15 min, the RNA samples were cooled on ice. Five micrograms of the total RNA per lane was resolved by electrophoresis in a 1.2% agarose gel containing 2.2 M formaldehyde. RNA then was transferred to nylon membrane (Schleicher & Schuell, Keene, N.H.) and UV irradiated. The
membranes were prehydrated for 2 h at 42°C in prehybridization buffer (50% formamide, 5× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 0.5% sodium dodecyl sulfate [SDS], 0.1 mg of denatured salmon sperm DNA/ml, and 0.1 mg of E. coli tRNA/ml). After prehybridization, 32P-labeled cDNA probe (2 × 10^6 cpm/ml) was added and hybridization was carried out overnight at 42°C. Following hybridization, the membranes were washed twice in 2× SSC with 0.1% SDS at 22°C for 10 min, washed twice in 0.1× SSC with 0.1% SDS at 50°C for 30 min, and exposed to X-ray film.

DNA from yeast cells was purified by the phenol/glass bead method as described in reference 17. Cells from 2-ml overnight cultures were collected by centrifugation, frozen in liquid nitrogen, and kept at −70°C. After two rounds of phenol deproteinization, nucleic acids were precipitated with ethanol, collected by centrifugation, and dissolved in 100 μl of Tris-EDTA buffer. The samples were treated with RNase A (100 μg/ml, 20 min at 37°C) and then extracted with phenol/chloroform, precipitated with 3 volumes of ethanol, washed once with 70% ethanol, air-dried, and dissolved in 50 μl of water. One or two micrograms of DNA was used for Southern blot analysis. DNA samples were resolved in a 1% agarose gel (Tris-acetate-EDTA buffer), and after electrophoresis nucleic acids were transferred from the gel onto a Nitran membrane (Schleicher & Schuell). Prehybridization and hybridization conditions were identical to those described in the Northern blot analysis protocol.

The following DNA fragments were used as probes: (i) URA3, a Pat-Nael fragment of the URA3 gene that was excised from pRS306, (ii) Y′, a fragment of the Y′ subtelomeric element neighboring the conserved Xhol site at the end of the element, was amplified by PCR. The primers were designed to the regions located 390 bp upstream (forward primer) and 360 bp downstream (reverse primer) of the Xhol site. (iii) ACT1 ORF was amplified by PCR. (iv) VR, a fragment of the VR chromosomal end neighboring the EcoRI site located upstream of the Y′ element, was amplified by PCR. The primers were designed to the regions with coordinates 567023 (forward) and 567576 (reverse) on chromosome V. PCR products were used directly as probes. (v) The EcoRI-BamHI fragment of pYTC2-2 (13) containing the Tg3 repeat was used as a probe for telomere repeat sequences.

Reverse transcriptase PCR (RT-PCR). RNA samples were treated with RNase-free DNase I (1 U/10 μg of RNA; Epicentre Technologies, Madison, Wis.) for 15 min at 37°C and then phenol deproteinized. One microgram of DNA-free RNA was reverse transcribed by SuperscriptII (200 U; Gibco BRL, Gaithersburg, Md.) with a random hexanucleotide mixture (1 μM) for 1 h at 42°C. One-tenth of the reaction mixture was then amplified by PCR with the following sets of primers: (i) primers specific to the beginning and end of ACT1 or URA3 ORFs and (ii) primers specific to the regions with coordinates 567023 (forward) and 567576 (reverse) on chromosome V. PCR was performed in a 25-μl final volume with 1 U of Taq DNA polymerase (Gibco BRL) for 30 to 32 cycles. Five microliters of the reaction mixtures was analyzed by agarose gel electrophoresis.

### RESULTS

**Yeast homologues of mammalian hnRNP K.** Blast search analysis of the S. cerevisiae genome revealed two ORFs similar to the mammalian K protein, YBR233w and YBL032w. Like K protein, each of the deduced yeast proteins contains three KH domains that appear as the most conserved regions of the yeast and mammalian proteins (Fig. 1A and B). We have also found other KH-containing proteins, but those proteins shared much lower similarity with the mammalian K protein. We have designated the YBR233w ORF as **HEK1** (heterogeneous nuclear RNP K-like gene) and the YBL032w ORF as **HEK2**. The **HEK1** gene was recently isolated as one of the clones, PBP2, interacting in a two-hybrid screen with yeast poly(A)-binding protein (34). PBP2 was also described as a gene conferring resistance to the antimalarial drug mefloquine in yeast (10). In this assay, mammalian hnRNP K can fully replace the PBP2 function, providing evidence that the yeast and mammalian proteins are functional homologues (10). Until now no functional studies of the **HEK2** gene have been described.

The **PBP2** and **HEK2** genes were cloned and transcribed and translated in a cell-free system (Fig. 1C). Both of the cDNAs gave a single translational product of the predicted size. K protein binds avidly to poly(C) RNA, in contrast to most other cellular RNA-binding proteins, which prefer poly(U) (36, 37). Therefore, we compared the RNA-binding specificity of the murine K and yeast Hek proteins (Fig. 1D). K protein binds poly(C) and poly(U) but does not bind poly(A). Both Pbp2p and Hek2p bind poly(C) and poly(U) well, which is consistent with the sequence analysis evidence that these yeast proteins are homologues of K protein. To define the function of K-like proteins in yeast, we generated single (pfb2Δ and hek2Δ) and double (pfb2Δ hek2Δ) mutants. These strains were viable and had the same growth rates as the parental wild-type strains, and the pattern of 32P pulse-labeled cellular proteins observed by two-dimensional gel electrophoresis was not detectably altered by the mutations (not shown). These results show that PBP2 and HEK2 are nonessential genes under the conditions used.

PBP2 and HEK2 act as modifiers of TPE. Recently we identified the Polycomb group protein Eed as one of the part-

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**TABLE 1. Yeast strains**

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ners of the mammalian K protein (11). This finding suggested a direct role for K protein in chromatin-dependent processes. We wondered if Pbp2/Hek2 proteins could play such a role. To test this possibility we examined the effect of PBP2 and HEK2 disruption on TPE, a well-described reporter system for studying chromatin-mediated processes in yeast (13). This system is based on the finding that a marker gene placed near the telomere is a subject of heritable silencing. In the first experiment we used a strain where URA3 was introduced to the end of chromosome VR (44) (Fig. 2A). Disruption of both PBP2 and HEK2 resulted in a substantial increase in the fraction of cells with repressed URA3, compared to the wild-type strain, the amount of which was measured by counting 5-FOA R cells present in exponentially growing cultures. Double mutation
(pbp2Δ hek2Δ) further increased the percentage of 5-FOA<sup>R</sup> cells. Thus, these mutations enhance TPE. A similar effect of pbp2Δ hek2Δ mutation on TPE was observed in a strain where URA3 was introduced to the end of chromosome VIII (not shown), indicating that this effect is not chromosome specific.

To exclude the possibility that these effects were specific to the URA3 gene, we next tested another strain where the ADE2 gene was introduced near the VR telomere (52). The wild-type strain colonies have several white (ade<sup>−</sup>/H11001) and fewer pink (ade<sup>−</sup>/H11002) sectors, while the pbp2Δ hek2Δ colonies have more red than white sectors (Fig. 2B). This result shows that the pbp2Δ hek2Δ mutation enhances repression of subtelomeric ADE2, indicating that the observed effects of PBP2 and HEK2 deletion on TPE are not specific to one marker gene.
cells on uracil-lacking medium, indicating that silencing of the hml::URA3 construct was increased (Fig. 2C; see also reference 53). We similarly found no effect of hek mutations on URA3 gene expression at the HMR locus (data not shown). To increase the sensitivity of this assay, we performed experiments employing 6-azauracil (6-AU), a competitive inhibitor of the URA3-encoded enzyme orotidine 5'-phosphate decarboxylase (28, 53). Likewise, these experiments revealed no difference between the wild-type and hek mutant strains in the growth rates on uracil-lacking media supplemented with 6-azauracil (data not shown). In agreement with these results, hek mutations had no effect on the mating efficiency of these strains (data not shown). Thus, unlike the enhanced TPE observed in hek mutants, there were no changes in silencing of the mating loci.

To discriminate between transcriptional and translational effects, the level of URA3 mRNA was measured in these strains. The results of Northern blot analysis are shown in Fig. 2D. The level of URA3 mRNA was substantially decreased in the mutant pbp2Δ hek2Δ strain, compared to the wild-type strain (Fig. 2D, compare lanes 4 to 6 and 1 to 3). Expression of the URA3 gene that was localized farther from the telomere (Fig. 2D, lanes 7 and 8) and expression of the internal ura3-52 gene were changed little in the mutant strains. These data suggest that PBP2/HEK2 genes modulate URA3 transcription, a process that depends on the chromosomal location of the URA3 gene. Alternatively, Pbp2 and Hek2 proteins could modulate the stability of URA3 mRNA, depending on the position of this gene within the genome, but this possibility is less likely.

The effect of pbp2Δ hek2Δ mutation on the length of telomeres. In S. cerevisiae, telomeres contain ~350 bp of (TG1-3)n, (49, 62). Some mutations of genes that modify TPE also alter the length of telomeres (14, 30, 63), implying that components of telomeric chromatin are involved in the maintenance of chromosomal ends. Therefore, we measured the length of telomeres in our strains. The pbp2Δ hek2Δ mutation resulted in a substantial increase in the length of the telomere neighboring the URA3 gene (Fig. 3A, lanes 1 and 2, and B, lanes 1 to 4). This increase was not dependent on the chromosomal end location.

Next we tested the effect of pbp2Δ and hek2Δ mutations on silencing of the URA3 gene inserted at the HML locus. The fractions of cells surviving on uracil-lacking media or 5-FOA-supplemented media were similar for the wild-type and hek mutant strains (Fig. 2C). In contrast to pbp2Δ and hek2Δ mutations, deletion of the PPR1 transcription factor for the URA3 gene (ppr1Δ mutation) substantially decreased growth of these

![FIG. 3. The effect of PBP2 and HEK2 disruption on the length of telomeres. (A) UCC519 (PBP2 HEK2 [44]) and DY25 (pbp2Δ hek2Δ) strains were grown on yeast-peptone-dextrose agar media; separate colonies were grown overnight in the same liquid media. Cells were collected by centrifugation; DNA was extracted by the phenol/glass bead method (Materials and Methods). One microgram of DNA was digested with either HindIII (upper panel, lanes 1 and 2), HindIII and BamHI (lanes 3 and 4), or XhoI (lower panel, lanes 1 and 2). The products were analyzed by the Southern method with either WT, wild type; mut, mutant. (B) UCC513 (PBP2 HEK2 [44]) and DY22 (PBP2 hek2Δ) strains were grown and analyzed as described for panel A. DNA was digested with XhoI (lanes 1 and 2) and was analyzed by the Southern method with the Y′ probe. For lanes 3 and 4, genomic DNA was cut with Smal (this site is introduced with URA3 to the telomeric repeat either proximal to the telomeric repeat (INT)). Arrowhead and arrow mark the position of DNA fragments bearing the telomeric repeat either proximal to the telomeric repeat (INT). Arrowhead and arrow mark the position of DNA fragments corresponding to Y′ probe. All other chromosomal ends recognized by the Y′ probe do not contain a Smal site within 25 kb adjacent to telomeres; the 11-kb zone was purified from gel, cut with XhoI, and then analyzed by the Southern method with the Y′ probe. WT, PBP2 HEK2 cells; mut, PBP2 hek2Δ cells; asterisk, position of DNA fragment specific to the sample purified from hek2Δ cells. (C) DNA samples shown in panel B, lanes 1 and 2, were cut with XhoI and analyzed by Southern blotting with a probe specific to the TG1,3 repeat. Position of fragments corresponding to the X- and Y′-type chromosomal ends is marked. The asterisk indicates the position of DNA fragment specific to the sample purified from hek2Δ (mut) cells. (D) Chromosomal constructs and probes (bold lines) used to measure telomere length. The diffuse dark end represents the telomeric (TG1,3), repeat and is not drawn to scale.

![FIG. 4. RT-PCR analysis of transcription of the subtelomeric region in pbp2Δ hek2Δ cells. After DNase treatment, RNA samples (1 μg each, same as shown in Fig. 2D) were reverse transcribed with random hexanucleotide mixture as a primer and then amplified by PCR with primers specific to ACT1, URA3, and the chromosomal region localized between the URA3 gene and the neighboring TG1,3 repeat (INT). –RT, no RT added; DNA, 1 μg of total DNA was used as a template in PCR. Strains used: 1 and 2, UCC509 (WT, PBP2 HEK2); and 3 and 4, DY16 (mut, pbp2Δ hek2Δ).]
FIG. 5. Telomere length in 5-FOA-resistant cells. (A) The UCC523 strain (PBP2 HEK2 ppr1−/− [44]) that was used in these experiments contains the URA3 gene at the end of the VR chromosome. Cells were grown on either complete medium (Complete, lanes 1 to 3) or complete medium supplemented with 5-FOA (5FOA, lanes 4 to 6), and the individual colonies were then grown overnight in corresponding liquid media. DNA purified from the overnight cultures was cut with HindIII (upper panel) or XhoI (lower panel) and was analyzed by the Southern method with VR (upper panel) or Y′ (lower panel) probes. Arrowheads mark fragments corresponding to the probes. (B) Cells (UCC523, PBP2 HEK2 ppr1−/−) were grown on either complete medium (Complete, lane 1) or complete medium supplemented with 5-FOA. Individual colonies from the 5-FOA plate were then consecutively passed on two plates without selection. Several colonies from the final plate were grown overnight in liquid medium (After 5FOA, lanes 2 to 5). To assess silencing of subtelomeric URA3, overnight cultures were plated as 10-fold serial dilutions of either complete medium without (lower panel, Complete) or with (middle panel, 5FOA) 5-FOA. DNA purified from the same cultures was cut with HindIII and was analyzed by the Southern method with VR probe (upper panel) to estimate the length of the VR telomere. (C) Wild-type (WT) (UCC509, PBP2 HEK2 PPR1 RAD52) and rad52Δ mutant (DY1000, PBP2 HEK2 PPR1 rad52Δ) strains were grown on either complete (Complete, lanes 1 and 5) or 5-FOA-supplemented (5FOA, lanes 2 to 4 and 6 to 8) media, and the length of the VR telomere was analyzed as done for panel A. In contrast to RAD52 colonies, rad52Δ colonies were small and grew slowly on 5-FOA media. Chromosomal constructs and VR probe (bold line) are shown below.
Lengthening of a telomere is associated with enhanced TPE in cis. The telomeric TG₁₋₃ repeat region contains binding sites for Rap1p and other TPE modifiers. Thus, lengthening of a single telomere could render it more competitive in recruiting limiting silencing complexes and could enhance TPE (22). To explore the relationship between the telomere length and silencing in cis, we applied the following approach. Cells were selected for the repressed state of the UR₃₃ gene (5-FOA<sup>R</sup>), and their telomeres were measured. The UR₃₃-proximal telomere was significantly longer in 5-FOA<sup>R</sup> cells than in unselected cells (Fig. 5A, compare lanes 1 to 3 and 4 to 6), and vice versa, in cells with the derepressed UR₃₃ gene (selected on an uracil-lacking agar plate), the neighboring telomere was shorter (not shown). The length of Y'-type telomeres was not changed in the cells selected for the repressed state of UR₃₃ (Fig. 5A, compare upper and lower panels), showing that the observed effects were specific to the UR₃₃-modified telomere. These results reveal an association between the length of the telomere and the efficiency of silencing in cis. Similar correlation was reported for ADE₂ inserted near telomere VIII (27). After 5-FOA withdrawal, cells preselected on 5-FOA-supplemented medium could maintain a high frequency of UR₃₃ silencing and an elongated telomere proximal to the UR₃₃ gene (Fig. 5B). In some colonies, this telomere was shortened to nearly normal size, an alteration correlated with the decreased ability of cells to grow on a 5-FOA-supplemented plate (Fig. 5B, middle and lower panels). Shortening of the affected telomere was likely mediated by the telomeric rapid deletion (TRD) pathway (27).

The relationship between gene silencing and telomere length in cis (Fig. 5) is further supported by the positive correlation between the extent of telomere lengthening in cells selected for the silenced UR₃₃ (5-FOA<sup>R</sup>) and the distance between UR₃₃ and the telomere (Fig. 6).

The RAD52-dependent system of homologous recombination is involved in telomere length control (19, 20, 25, 32, 40). We wondered if long telomeres found in 5-FOA<sup>R</sup> cells were generated through homologous recombination. Because we were not able to find the elongated VR telomere in 5-FOA<sup>R</sup> rad52A cells (Fig. 5C), the dramatic elongation of the UR₃₃-proximal telomere is likely to represent a recombination-dependent event. Cells with a modestly elongated VR telomere could still be found in the 5-FOA<sup>R</sup> rad52A strain (Fig. 5C, lanes 7 and 8), supporting a previous observation that short-range telomere length variations are RAD52 independent (48).

The observed lengthening of the UR₃₃-proximal telomere in 5-FOA<sup>R</sup> cells could result from the enhanced silencing of UR₃₃, or vice versa, lengthening of this telomere could cause enhanced silencing of the marker gene. To distinguish between the two possibilities, we increased the efficiency of UR₃₃ silencing in the wild-type strain by overexpressing Sir3p (44) and measured the length of the neighboring telomere. The results...
show that the dramatically improved efficiency of \textit{URA3} silencing in the strain overexpressing Sir3p (Fig. 7A) was not associated with changes in the length of the \textit{URA3}-proximal telomere (Fig. 7B, compare lanes 1 to 3 to lanes 4 to 6). Moreover, cells overexpressing Sir3p that were selected for the repressed state of \textit{URA3} (5-FOA\textsuperscript{R}) have a nearly normal size for the \textit{URA3}-proximal telomere (Fig. 7B, compare lanes 1 to 6 to lanes 11 to 13). In the control 5-FOA\textsuperscript{W} cells, this telomere is 8

![Diagram](https://example.com/diagram.png)
A triple HA epitope was introduced to the 3′ coprecipitated with telomeric chromatin from cellular extracts. Telomere length control, we tested if these proteins can be compared to that in the single hek2Δ mutant, indicating a contribution of the hek2Δ mutation. This observation is consistent with the finding that the double mutants display the greatest increase in telomeric silencing (Fig. 2). Thus, the hek2Δ mutation inhibits TRD at the UR43-proximal telomere, an observation that could, at least in part, explain the mechanism of telomere lengthening in the mutant cells.

In vivo association of Hek2p with subtelomeric DNA. To test the possibility that Pbp2p and Hek2p play a direct role in telomere length control, we tested if these proteins can be coprecipitated with telomeric chromatin from cellular extracts. A triple HA epitope was introduced to the 3′ ends of both ORFs. Tagging procedure abolished PBP2 function but had little effect on HEK2; therefore, we used only the HEK2-3HA construct in the chromatin immunoprecipitation assay (15, 41). We precipitated HEK2-3HA from cells cross-linked with formaldehyde in situ and analyzed coprecipitated DNA by PCR (Fig. 9). These studies demonstrate that HEK2-3HA is associated with VR subtelomeric sequences (Fig. 9A, lane 4). Other genomic loci, the Y′ box and TY1 transposable element, are bound only weakly to HEK2-3HA. The DNA-binding pattern of HEK2-3HA is clearly different from that of SIR3HA. For example, SIR3HA but not HEK2-3HA bound the subtelomeric Y′ box (Fig. 9A, compare lanes 4 and 6). This result suggests that Hek2p is not a structural constituent of telomeric chromatin. However, we found that the sir3Δ mutation decreased binding of HEK2-3HA to the VR telomere-proximal region (VR in Fig. 9A) but had little effect on binding to the telomere-distal region (VR-22kb in Fig. 9A). Densitometric analysis (Opti-Quant; Packard) showed that the VR/VR-22kb probe (bold line) are shown below.

**DISCUSSION**

In this study we exploited the *S. cerevisiae* model system to better understand the function of the hnRNP K family of
proteins. We show that two yeast K-like genes, \textit{PBP2} and \textit{HEK2}, act as suppressors of TPE and regulate telomere length. These observations identify the family of K-like proteins as regulators (modifiers) of chromatin-dependent processes.

**HEK genes and telomere length control.** In yeast, the length of telomeres is maintained within the narrow distribution of sizes around ~350 bp (48, 62). The median size of the telomeric TG$_1^\text{GO}$-repeat region is, at least in part, defined by the Rap1p-dependent system (35). There are several known systems of factors that elongate or shorten telomeres to maintain their size around this median. As the cells divide, telomeres are prone to shortening because of incomplete replication of DNA ends but their shortening is prevented by the specialized telomerase complex (7, 31, 52). Cells lacking telomerase activity show gradual telomere shortening and loss of viability after ~70 cell divisions (26, 52). In telomerase-null survivors, telomeres are maintained by the \textit{RAD52}-dependent system of homologous recombination and double-strand break repair (19, 20, 25, 32, 40). While shortened telomeres are corrected by the telomerase complex and the recombination system, elongated telomeres are processed down to median sizes by the TRD pathway (27).

We have found that disruption of \textit{HEK} genes increased the length of the telomeric repeat proximal to the \textit{URA3} marker gene (Fig. 3). This increase was unique to the chromosomal end that contained the marker gene, because other Y'-type or X-type telomeres were not significantly altered (Fig. 3C). Regulation of telomeric processes by Hek2p most likely reflects the direct physical interaction of this protein with subtelomeric chromatin (Fig. 9). We have further shown that the \textit{hek2}$\Delta$ mutation inhibited the TRD pathway (27) at the \textit{URA3}-modified telomere (Fig. 8). Telomere lengths of individual chromosomes vary among clonal populations, and telomere length heterogeneity increases with additional rounds of cell division (48). Thus, blocking the pathway (TRD) responsible for shortening of elongated telomeres at the \textit{URA3}-modified telomere will result in lengthening of this telomere. The reason why the \textit{URA3}-modified telomere is more sensitive than the other telomeres to the deletion of \textit{HEK} genes remains to be defined. This type of chromosome-specific effects are not unique to \textit{hek} mutations, since other mutations specifically altered the length of either Y'- or X-type telomeres (9), suggesting that there are telomere maintenance systems able to discriminate between telomeres, depending on subtelomeric sequences.

TRD likely involves intra- and interchromatid interactions. This assumption is based on two observations: (i) TRD is stimulated by the \textit{hpr1} mutation, which is known to enhance intrachromatid excision events and (ii) the efficiency of TRD at one telomere depends on the length of other telomeres (27). We propose that the yeast K-like protein Hek2p, along with other chromatin factors, binds subtelomeric regions and facilitates long-range interactions within and/or between telomeres. Similarly, it was recently reported that the mammalian K protein increased the frequency of interaction between two nonadjacent chromosomal loci if they were separated by an array of K-binding sites (58). It was suggested that the two loci were brought together because K protein bound and facilitated bending of the CT-enriched DNA region that separates these loci. Thus, it is conceivable that the role of Hek2p in TRD is to facilitate intrachromatid long-range interactions. It is possible that Pbp2p assists Hek2p action, because the effect of the \textit{pbp2}$\Delta$\textit{hek2}$\Delta$ mutation on TPE, telomere length, and TRD was reproducibly stronger than that of the single \textit{hek2}$\Delta$ mutation (Fig. 2 and 8; data not shown).

**Association between length of telomere and silencing in \textit{cis}**. Known TPE modifiers could either increase or decrease the average length of telomeric TG$_1^\text{GO}$ repeats at most chromosomal ends (19, 63). These data suggest that telomere length reflects changes in the structure of telomeric chromatin. In contrast, in the experiments utilizing an alternative approach to elongate a fraction of telomeres in otherwise wild-type cells, it was concluded that an elongated telomere increased the frequency of inheritance of the repressed state in \textit{cis} (22, 43). Similarly, long internal tracts of TG$_1^\text{GO}$ repeat were more efficient silencers than short tracts (55). Our observations also support an association between the length of a telomere and silencing in \textit{cis}. (i) Cells selected for the repressed or derepressed state of subtelomeric \textit{URA3} contain elongated or shortened adjacent telomere respectively (Fig. 5 and data not shown). These results suggest that cells with a certain length of an individual telomere could be selected from the entire cell population, where the length of telomeres varies from cell to cell (48, 62). Long telomeres were generated through \textit{RAD52}-dependent events (Fig. 5C). (ii) Elongation of the \textit{URA3}-proximal telomere in 5-FOA$^\text{R}$ cells is proportional to the distance from the \textit{URA3} gene to the telomere (Fig. 6). (iii) In agree-
ment with the observation that the URA3 transcription factor Ppr1p suppresses TPE (44), we found that elongation of the URA3-proximal telomere was more dramatic in ppr1Δ 5-FOAΔ than in otherwise isogenic ppr1Δ 5-FOAΔ strains (compare Fig. 5A and B, 6, and 7). (iv) Importantly, the telomere elongation in 5-FOAΔ cells was alleviated by overexpressed SIR3 (Fig. 7). This result suggests that the elongated telomere is more competitive for the limiting Sir3p. Taken together with the studies published by others (22, 43), our data indicate that there is a direct link between the length of telomeric TG1-3 repeat and the efficiency of silencing of neighboring genes.

According to this view, the hckΔ2 mutation inhibits TRD at the URA3-modified telomere; this telomere becomes longer and enhances efficiency of silencing of the adjacent URA3. In addition, there might be other telomeric processes involved where PBP2 exerts its action.

In summary, the above studies identified two yeast hnRNP K-like genes, PBP2 and HEK2. We show that these genes are involved in regulation of TPE, telomere length, and TRD. We suggest that the yeast and mammalian K proteins play a direct role in chromatin-dependent gene-silencing processes.

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