PERSPECTIVES: CELL BIOLOGY

Telomeres—Unsticky Ends

David Shore

Telomeres—the physical ends of eukaryotic chromosomes—consist of tracts of short repeated nucleotide sequences that are characteristic of all telomeric DNA, from yeast to human. Telomeres are replicated by a special reverse transcriptase, called telomerase, which can synthesize the tracts of short repeat sequences (TG_{1-3} in yeast, T_2AG_3 in most higher eukaryotes) that mark the ends of eukaryotic chromosomes.

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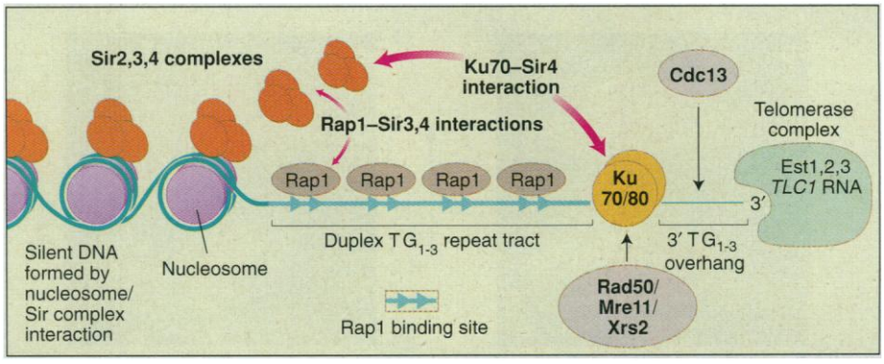
Telomeres are especially difficult for the cell to maintain. Sophisticated surveillance systems constantly monitor the integrity of DNA, and DNA repair enzymes move in to fix any breaks that are found. This machinery might easily mistake the ends of chromosomes for broken DNA in need of repair (*1*). Telomeres thus run the risk of being joined together (or fused to genuine chromosomal breaks) by recombinational or end-joining mechanisms, with the disastrous consequence of unstable, dicentric chromosomes. This year, a flurry of reports has begun to explain how the cell protects the chromosome ends from being party to such unwanted joining reactions.

Although much of the new work has been done in *Saccharomyces cerevisiae* (baker's yeast), its roots are in biochemical studies of animal cells. Several years ago the human protein Ku70, which was originally identified as an autoimmune antigen, was shown to be part of a heterodimer (~70/85 kD) that binds with high affinity to DNA ends, whether blunt, overhanging, or hairpin in structure (*2*). Cells without Ku cannot repair double-strand breaks or perform recombination of the immunoglobulin V(D)J region and are hypersensitive to ionizing radiation. The yeast homologs of these proteins (Yku70p and Yku80p) were subsequently identified and also shown to be critical for joining ends of DNA (nonhomologous end-joining), consistent with the results from mammalian cells (*3*–*5*).

Even though cells must avoid Ku-mediated end-joining of telomeres, telomeric DNA is in principle an excellent substrate for Ku binding. Therefore, the yeast Ku homologs (whose genes go by the names *YKU70* or *HDF1* and *YKU80* or *HDF2*) might play some role in telomere metabolism. And in fact two independent groups found that *yku70* and *yku80* mutants have abnormally short telomeres (*3*, *6*), implying that yeast Ku helps to maintain normal telomere structure. However, cells lacking Ku are also inviable at high temperatures and appear to be defective in DNA replication control (*7*), so this effect of Ku on telomere length could have been indirect.

An important hint that Ku protein does in fact contribute directly to telomere function came from the unexpected identification of Sir4p in a two-hybrid screen for factors that interact with Hdf1p (Ku70) (*8*). Sir4p, together with Sir2p and Sir3p, are required for telomeric silencing (or telomere position effect), a phenomenon in which genes placed immediately adjacent to telomere repeat tracts in yeast are subject to a variegated form of repression, analogous to the repression conferred by heterochromatin in higher eukaryotes. In support of the biological significance of this observation, *yku70* mutant cells display severe defects in telomeric silencing, as first shown by Boulton and Jackson (*9*). Furthermore, mutations in *SIR2*, *SIR3*, or *SIR4* all lead to severe defects in nonhomologous end-joining, suggesting a completely new function for the Sir proteins (*8*, *9*). Although it is still unclear whether this effect of *sir* mutations is direct, rather than a consequence of their effect on cell mating type, an interesting possibility is that Sir proteins are normally recruited to double-strand DNA breaks as part of a mechanism to facilitate end-joining, perhaps through local repression of transcription. Likewise, recruitment of the Sir protein complex to telomeres by Yku70p, in conjunction with the telomere repeat-binding protein Rap1p, may explain the role of Ku in telomeric silencing. A specific role for an end-binding factor in telomeric silencing had been predicted some years ago when it was noted that extra telomeres added to cells disrupted silencing at native telomeres, whereas the equivalent amount of telomere repeat sequences present on circular molecules did not have this effect (*10*).

Several recent reports reinforce the view that Ku is an active player at telomeres in yeast, and suggest that the protein participates in telomere replication as well as silencing. In the first of these, Wellinger's group at Sherbrooke University in Quebec identified an allele of the *YKU80* gene by screening a collection of temperature-sensitive yeast strains for those that arrest in G_2/M phase with an abnormal telomere DNA structure (*11*). In previous studies, Wellinger and colleagues had shown that telomeres in wild-type yeast cells acquire a G-rich 3′ overhang during S phase (*12*). Biochemical studies suggest that this overhang may be an essential substrate for telomerase, which cannot act on blunt ends of DNA. Furthermore, two essential telomere proteins, Cdc13p and Est1p, bind preferentially in vitro to G-rich single-stranded DNA (*13*–*15*). Although the generation of the G-rich overhang in vivo does not require telomerase itself, the timing of its appearance strongly suggests that it is an integral part of the telomere replication mechanism. Gravel *et al.* showed that *Yku80* mutants display a G-rich overhang throughout the cell cycle, suggesting that Ku might be an important regulator of this process. They also reported two other important findings (*11*). Using a chromatin immunoprecipitation assay, they provided direct evidence that the Ku complex is actually physically bound to telomeres in living cells, strongly supporting the notion that it



The telomere. Emerging evidence is beginning to paint a picture of the proteins that bind to and regulate telomeric function.

The author is in the Département de Biologie Moléculaire, Université de Genève, Genève 4, CH-1211 Switzerland. E-mail: david.shore@molbio.unige.ch

acts directly at telomeres. In addition, they showed that mutation of *YKU80* leads to accelerated cell death in strains lacking telomerase function.

In a related study, Nugent *et al.* (*16*) searched for additional factors that affect telomere replication by screening for mutations that would exacerbate the phenotypes of either of two mutants defective in telomere maintenance: *cdc13-1*^{ts} and *est1-*Δ. Cdc13p is a single-strand telomere binding protein that contributes to both telomere end protection and telomerase activity (*14*), whereas Est1p has a similar binding activity but appears to be specifically required for telomerase function (*13, 15*). These complementary genetic screens both identified the *YKU80* gene, and in addition the first screen identified the *RAD50* gene. Significantly, Rad50p appears to be part of a complex with Xrs2p and Mre11p in the nonhomologous end-joining pathway. Through genetic epistasis (double mutant) analysis, Nugent *et al.* (*16*) provided evidence that the Ku end-binding complex provides a novel telomere function, independent from that of either the telomerase complex (defined by three *EST* genes and the telomerase template RNA gene, *TLC1*) or the end-protecting protein Cdc13p (Est4p). In contrast, their results suggest that the activity of the Rad50/Mre11/Xrs2 protein complex, required together with Ku for repair of double-strand breaks, is specifically required for the telomerase pathway of end maintenance. Because the Rad50/Mre11/Xrs2 protein complex may be an exonuclease, these proteins might generate the single-strand substrate required for telomerase activity. Although *rad50*, *mre11*, and *xrs2* mutants all display a telomere-shortening phenotype similar to that of Ku mutants (*9, 16*), these three mutants are not defective in telomeric silencing (*9*). These (and other) results indicate that Ku has distinct functions at telomeres that clearly differ from its role in nonhomologous end-joining at internal chromosome breaks.

The idea that yeast Ku proteins carry out a special function at telomeres is further supported by two additional studies. In the first of these, Laroche *et al.* (*17*) showed that *yku70* and *yku80* mutants, in addition to displaying a loss of telomeric silencing, exhibit altered (less peripheral) nuclear localization of telomeres, and reduced telomere clustering. In cytological studies of mutants with altered telomere function, Ku mutants are the only ones that show an altered spatial distribution of telomere clusters within the nucleus (*18*). (It was not reported whether the *RAD50* group mutants also show this unusual phenotype.) A *yku80* mutant was also isolated in a screen for increased recombination between subtelomeric and internal chromosomal regions, further supporting a role for Ku in nuclear localization and regulation of telomeric recombination (*17*). In another report, Lustig and his co-workers reported that mutations in either Ku subunit lead to enhanced instability of elongated telomeres, by increasing their sensitivity to either degradation or recombination reactions (*19*). This is the most direct evidence that Ku protects telomeres from nucleases and recombinases, perhaps by controlling telomere structure, and in particular the reaction that leads to 3′ overhang formation.

Taken together, this new work places Ku firmly at the yeast telomere and suggests that it is a central player in processes that regulate telomere structure, replication, recombination, and telomeric silencing. Apart from the considerable challenge of understanding the molecular basis of these multiple functions, this work raises a serious paradox that needs to be resolved: How does the cell subvert the "normal" function of Ku in DNA end-joining at the telomere? In mammalian cells, Ku is a DNA binding subunit of a large enzyme, DNA-dependent protein kinase (DNA-PK_{CS}), a member of the *ATM* gene family required for normal DNA repair function and V(D)J recombination (*2*). Given this paradigm, one might expect that a related kinase in yeast helps to distinguish telomeres from broken DNA ends, which stimulate a *RAD9*-dependent cell cycle arrest (*20*). Possible candidates are Mec1p and Tel1p. Significantly, absence of Tel1p results in a telomere shortening phenotype similar to that of Ku mutants (*6*). Nonetheless, genetic studies suggest that Ku and Tel1p act in different pathways to affect telomere structure. It remains to be determined which kinase (or kinases) interact with Ku in yeast and how such interactions may be blocked or modified at functional telomeres.

What about the function of Ku at telomeres in mammalian cells, where Ku was first detected and characterized? Here the jury is still out: Cell lines and animals lacking Ku activity exist, yet no reports have emerged that would indicate that these exhibit altered telomere structure or function. Recent studies, however, of the newly identified mammalian telomere repeat tract binding protein TRF2 suggest that it is critical in protection of telomeres from end-to-end joining (*21*). TRF2 and its close relative TRF1 are Myb-domain proteins that bind to the T_2AG_3 repeats of telomeres as homodimers. Although TRF1 has been implicated as a negative regulator of telomere elongation (analogous to the budding yeast repeat binding protein Rap1p), TRF2 acts quite differently. Expression of TRF2 truncation alleles that block DNA binding of the endogenous wild-type protein leads to a marked increase in telomere end-joining events, strongly suggesting that TRF2 usually either directly or indirectly protects DNA ends. Whether this effect is mediated at least in part by Ku is still unclear. Nonhomologous end-joining is a much more active pathway in mammalian cells than in yeast, where homology-based repair pathways predominate, so it is possible that mammalian cells have evolved a mechanism of telomere protection that prevents Ku from ever binding to the chromosome ends.

The replication and protection of telomeres is carried out as a complex, integrated process. Remarkably, the Ku protein is emerging as a key player, at least in yeast, despite the fact that its function in DNA end-joining would argue against such a role. Ku's participation also suggests that recombination may play an important role in maintenance of telomere structure, a suggestion that fell somewhat into disfavor after the discovery of telomerase enzyme. These and many other questions remain to be sorted out, not least of which are the role of Sir proteins at telomeres and the mechanisms underlying telomere length regulation. With the accelerating pace of work in this field, it is safe to assume that new surprises are forthcoming.

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