the chance that K10 will be passed on to the next generation. More recent examples have been found with *Schizosaccharomyces pombe* plasmids carrying portions of a canonical centromere and human marker chromosomes (*51*). In all three cases, epigenetic modifications may control the centromeric activity of these regions.
16. M. T. Fuller, *Cell* **81**, 6 (1995)
17. P. Zhang, B. Knowles, L. S. B. Goldstein, R. S. Hawley, *ibid.* **62**, 1053 (1990); K. Afshar, N. R. Barton, R. S. Hawley, L. S. B. Goldstein, *ibid.* **81**, 129 (1995).
18. I. Vernos *et al.*, *ibid.*, p. 117.
19. A. Dernberg and R. S. Hawley, unpublished data.
20. G. Ostergren, *Hereditas* **37**, 85 (1951); R. B. Nicklas and C. A. Stachly, *Chromosoma* **21**, 1 (1967); J. C. Waters, R. W. Cole, C. L. Rieder, *J. Cell Biol.* **122**, 361 (1993).
21. R. B. Nicklas, *Genetics* **78**, 205 (1974); J. G. Ault and R. B. Nicklas, *Chromosoma* **98**, 33 (1989); R. B. Nicklas and S. C. Ward, *J. Cell. Biol.* **126**, 1241 (1994).
22. C. L. Rieder and E. D. Salmon, *J. Cell Biol.* **124**, 223 (1994).
23. R. V. Skibbens, C. L. Rieder, E. D. Salmon, *J. Cell Sci.* **108**, 2537 (1995).
24. K. S. McKim, J. K. Jang, W. E. Theurkauf, R. S. Hawley, *Nature* **362**, 364 (1993).
25. A. A. Hyman and T. J. Mitchison, *ibid.* **351**, 206 (1991); J. R. McIntosh and C. M. Pfarr, *J. Cell Biol.* **115**, 577 (1991); C. L. Rieder, *Curr. Opin. Cell Biol.* **3**, 59 (1991).
26. R. B. Nicklas, *Philos. Trans. R. Soc. London Ser. B* **277**, 267 (1977).
27. G. Simchen and Y. Hugerat, *Bioessays* **15**, 1 (1993).
28. M. J. D White, *Animal Cytology and Evolution* (Cambridge Univ. Press, Cambridge, ed. 3, 1973).
29. R. B. Nicklas and P. Arana, *J. Cell Sci.* **102**, 681 (1992).
30. D. L. Lindsley and E. Novitski, *Genetics* **43**, 790 (1958).
31. L. H. Hartwell and T. A. Weinert, *Science* **246**, 629 (1989); A. W. Murray, *Nature* **359**, 599 (1992); T. Weinert and D. Lydall, *Semin. Cancer Biol.* **4**, 129 (1993).
32. P. de Boer and J. H. de Jong, in *Fertility and Chromosome Pairing: Recent Studies in Plants and Animals*, C. B. Gillies, Ed. (CRC Press, Boca Raton, FL, 1989); P. S. Burgoyne, S. K. Mahadevaiah, M. J. Sutcliffe, S. J. Palmer, *Cell* **71**, 391 (1992); S. M. Baker *et al.*, *ibid.* **82**, 309 (1995).
33. D. K. Bishop, D. Park, L. Xu, N. Kleckner, *Cell* **69**, 439 (1992); M. Sym, J. Engebrecht, G. S. Roeder, *ibid.* **72**, 365 (1993). The *zip-1* mutants do not arrest meiosis in all yeast cell types; see M. Sym and G. S. Roeder, *ibid.* **79**, 283 (1994).
34. W. C. Earnshaw, *J. Cell Sci.* **99**, 1 (1991).
35. C. L. Rieder, A. Schultz, R. Cole, G. Sluder, *J. Cell Biol.* **127**, 1301 (1994).
36. X. Li and R. B. Nicklas, *Nature* **373**, 630 (1995).
37. F. Spencer and P. Hieter, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 8908 (1992).
38. W. C. Earnshaw, R. L. Bernat, C. A. Cooke, N. F. Rothfield, *Cold Spring Harbor Symp. Quant. Biol.* **56**, 675 (1991); K. Bloom, *Cell* **73**, 621 (1993), J. Tomkiel, C. A. Cooke, H. Saitoh, R. L. Bernat W. C. Earnshaw, *J. Cell Biol* **125**, 531 (1994).
39. C. L. Rieder, R. W. Cole, A. Khodjakov, G. Sluder, *J. Cell. Biol.* **130**, 941 (1995).
40. G. J. Gorbsky and W. A. Ricketts, *ibid.* **122**, 1311 (1993).
41. R. B. Nicklas, S. C. Ward, G. J. Gorbsky, *ibid.* **130**, 929 (1995).
42. M. S. Campbell and G. J. Gorbsky, *ibid.* **129**, 1195 (1995).
43. S. Hughes-Schrader, *Chromosoma* **3**, 258 (1948).
44. E. R. Sears, *ibid.* **4** (suppl.), 535 (1952); L. Sandler and G. Braver, *Genetics* **39**, 365 (1954); O. Miller, *ibid.* **48**, 1445 (1963); J. A. Hodgkin, H. R. Horvitz, S. Brenner, *ibid.* **91**, 67 (1979); J. A. Hodgkin, *ibid.* **96**, 649 (1980).
45. J. K. Jang, L. Messina, M. B. Erdman, T. Arbel, R. S. Hawley, *Science* **268**, 1917 (1995).
46. J. R. McIntosh, *Cold Spring Harbor Symp. Quant. Biol.* **56**, 613 (1991).
47. P. Hunt, R. LeMaire, P. Embury, L. Sheean, S. Mroz, *Hum. Mol. Genet.*, in press.
48. G. Sluder, F. J. Miller, E. A. Thompson, D. E. Wolf, *J. Cell. Biol.* **126**, 189 (1994).
49. D. G. Albertson and J. N. Thomson, *Chromosoma* **86**, 409 (1982).
50. M. M. Rhodes, in *Heterosis*, J. W. Gowen, Ed. (Iowa State College Press, Ames, IA, 1952), pp. 66–80.
51. W. Brown and C. Tyler-Smith, *Trends Genet.* **11**, 337 (1995).
52. K. S. McKim, J. K. Jang, R. S. Hawley, unpublished data.
53. D. L. Lindsley, and G. G. Zimm, *The Genome of* Drosophila melanogaster (Academic Press, San Diego, CA, 1992).
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Telomeres: Beginning to Understand the End

Virginia A. Zakian

Telomeres are the protein-DNA structures at the ends of eukaryotic chromosomes. In yeast, and probably most other eukaryotes, telomeres are essential. They allow the cell to distinguish intact from broken chromosomes, protect chromosomes from degradation, and are substrates for novel replication mechanisms. Telomeres are usually replicated by telomerase, a telomere-specific reverse transcriptase, although telomerase-independent mechanisms of telomere maintenance exist. Telomere replication is both cell cycle– and developmentally regulated, and its control is likely to be complex. Because telomere loss causes the kinds of chromosomal changes associated with cancer and aging, an understanding of telomere biology has medical relevance.

Eukaryotes have linear chromosomes, and the ends of these linear chromosomes are composed of protein-DNA structures called telomeres. Telomeres were first characterized in ciliated protozoans such as *Tetrahymena* (*1*) and *Oxytricha* (*2*, *3*). After meiosis, in a developmentally regulated process, ciliate chromosomes are broken up into subchromosomal sized fragments. These fragments are replicated to generate a polyploid nucleus, the macronucleus, which can contain literally millions of telomeres (reviewed in *4*). Thus, compared to the modest number of chromosomes, and hence telomeres, in most organisms, the ciliate macronucleus is a rich source of both telomeric DNA and the structural proteins and enzymes that protect and replicate this DNA. In spite of the structural novelty of the ciliate macronucleus, many features of telomeres first discovered in ciliates are also true of telomeres in organisms like *Saccharomyces* and humans, both of which have conventional chromosomes. Indeed, telomeres display considerable conservation of both structure and function from single-celled organisms to higher plants and animals, as well as some intriguing interspecies differences.

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Telomeric DNA

In most organisms, telomeric DNA consists of a tandem array of very simple sequence DNA (Table 1) (*1–3*, *5–28*). Most telomeric repeat sequences are short and precise. For example, telomeric DNA in *Tetrahymena* is comprised of the 6-bp (base pair) sequence C_4A_2/T_2G_4. However, some telomeric sequences are heterogeneous (for example, $C_{1\text{-}3}A/TG_{1\text{-}3}$ in *Saccharomyces*) and in some, the repeat unit is considerably longer (for example, 25 bp in *Kluyveromyces lactis*). Moreover, *Drosophila* has a completely different and so far novel telomere structure. Rather than simple repeats, the DNA at the ends of *Drosophila* chromosomes is composed of a transposable element (*29–31*).

In most organisms, the subtelomeric regions immediately internal to the simple repeats consist of middle repetitive sequences, called telomere-associated (TA) DNA, which bear a superficial similarity to the transposons at the ends of *Drosophila* chromosomes. In *Saccharomyces*, there are two classes of TA elements, X and Y′ (*32*), one or both of which are found on most or all telomeres (Fig. 1). The array of TA DNA at a given chromosome end can expand and contract. However, in those or-

ganisms in which these changes have been studied, they occur by recombination, not transposition (*33, 34*). Because a yeast chromosome without TA DNA replicates, segregates, and recombines in mitosis and meiosis in a manner indistinguishable from that of the same chromosome with TA DNA at both ends, yeast TA DNA is dispensable under normal growth conditions (*35*). Here, telomeric DNA refers only to the repeats at the very ends of chromosomes.

The sequences of telomeric DNA from a wide variety of organisms have been determined (Table 1). Because multiple telomeric sequences are found, telomere function does not require a unique DNA sequence. Moreover, in many organisms, telomeric DNA is also found at internal sites on the chromosome (for example, *36–38*), which demonstrates that sequence alone does not make a telomere. Although there is considerable diversity among the different telomeric DNAs, even very distantly related organisms can have the same telomeric sequence. For example, C_3TA_2/T_2AG_3 is the sequence of telomeric DNA in all vertebrates, the protozoan *Trypanosoma*, and several slime molds and fungi (Table 1). In other cases, different organisms have a different telomeric sequence, but the two sequences are clearly related, like the telomeric sequences of the distantly related ciliates *Tetrahymena* and *Oxytricha*, which are, respectively, C_4A_2/T_2G_4 and C_4A_4/T_4G_4. As more telomeric sequences become known, it becomes more difficult to identify even a loose consensus sequence to describe them. For all known telomeric DNA sequences except that of *Parascaris*, there is a strand bias in the various telomeric DNAs such that the strand running 5′ to 3′ from the center toward the end of the DNA molecule has more G residues than its complement and, further, the G's in this strand are clustered.

In the macronuclear DNA of ciliated protozoans, the G-rich strand is extended to form a 12- to 16-base single-strand G tail that exists throughout most or all of the cell cycle (*2, 3*). G tails can also be detected on *Saccharomyces* chromosomes, but they are longer and transient, appearing late in the S phase at the same time that telomeric DNA is replicated (*39, 40*). In vitro, the G strand of telomeric DNA can form a variety of non–Watson-Crick base-paired structures, including four-stranded helices or G quartets held together by multiple G–G base pairs (*41–43*). Although it is not clear if these stable DNA structures exist in vivo, the single-strand G tails on *Saccharomyces* chromosomes allow two telomeres to interact, apparently by non–Watson-Crick base pairing (*40*). If DNA structures based on G–G base pairs are important for some aspect of telomere function, it would explain the prevalence of tandem G residues in telomeric DNA.

The average amount of telomeric DNA at a chromosome end varies from organism to organism. For example, mice have as much as 150 kb of telomeric DNA per telomere (*44, 45*), whereas telomeres on *Oxytricha* macronuclear DNA molecules are only 20 bp in length (*3*). Moreover, in contrast to *Oxytricha* and related ciliates, in which all macronuclear telomeres are the same discrete size, in all other organisms the amount of telomeric DNA per telomere fluctuates. For example, the amount of $C_{1\text{-}3}A/TG_{1\text{-}3}$ DNA at individual yeast telomeres in a wild-type strain ranges from ~200 to ~400 bp, and this amount increases and decreases stochastically (*46*). Heterogeneity and spontaneous changes in telomere length probably reflect a complex balance between processes that lead to degradation and those that lengthen telomeric tracts. In addition to this inherent length heterogeneity, the average telomere length in yeast and other organisms can increase or decrease in response to genetic (*47*) or nutritional (*48*) changes. In some organisms, like *Tetrahymena* (*49*) and *Trypanosoma* (*50*), the average telomere length continuously increases during log-phase growth. In contrast, in human somatic cells, telomeres slowly shrink during successive cell divisions (*51, 52*). The inherent heterogeneity characteristic of virtually all telomeric DNAs suggested that telomeres are not maintained by a conventional replication process. Further, the fluid nature of telomeric and subtelomeric regions suggests

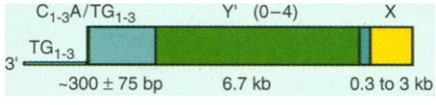

Fig. 1. Structure of a *Saccharomyces* telomere (not drawn to scale). Each yeast chromosome begins and ends with ~300 bp of duplex $C_{1\text{-}3}A/TG_{1\text{-}3}$ telomeric DNA with the exact length varying from telomere to telomere. Single-strand $TG_{1\text{-}3}$ tails of >30 bases are detected transiently at the end of the S phase (*40*). It is not known if shorter $TG_{1\text{-}3}$ tails exist at other times in the cell cycle. About two-thirds of the telomeres bear one to four copies of the 6.7-kb Y′ element or a 5.2-kb deletion derivative of Y′ (*32*). X, the middle repetitive DNA element internal to Y′, is heterogeneous and comprised of several small repeats, some of which are found on most chromosomes (*32, 147*). Internal stretches of $C_{1\text{-}3}A/TG_{1\text{-}3}$ DNA of ~50 to 130 bp are often found between tandem Y′ elements or between X and Y′ (*36*).

Table 1. Sequences of telomeric DNAs. The sequence of the strand running 5′ to 3′ from the end of the molecule forward to its center is presented first.

Organism	Sequence	Reference
Protozoa		
Tetrahymena	C_4A_2/T_2G_4	(*1*)
Paramecium	$C_{3A}^{C}A_2/T_{2T}^{G}G_3$	(*5*)
Oxytricha	C_4A_4/T_4G_4	(*2, 3*)
Plasmodium	$C_3T_G^AA_2/T_{2C}^TAG_3$	(*6*)
Trypanosoma	C_3TA_2/T_2AG_3	(*7, 8*)
Giardia	C_3TA/TAG_3	(*9*)
Slime molds		
Physarum	C_3TA_2/T_2AG_3	(*10*)
Didymium	C_3TA_2/T_2AG_3	(*10*)
Dictyostelium	$C_{1\text{-}8}T/AG_{1\text{-}8}$	(*11*)
Fungi		
Saccharomyces	$C_{2\text{-}3}ACA_{1\text{-}6}/T_{1\text{-}6}GTG_{2\text{-}3}$	(*12, 13*)
Kluyveromyces	$ACAC_2ACATAC_2TA_2TCA_3TC_2GA/TCG_2AT_3GAT_2AG_2TATGTG_2TGT$	(*14*)
Candida	$ACAC_2A_2GA_2GT_2AGACATC_2GT/ACG_2ATGTCTA_2CT_2CT_2G_2TGT$	(*15*)
Schizosaccharomyces	$C_{1\text{-}6}G_{0\text{-}1}T_{0\text{-}1}GTA_{1\text{-}2}/T_{1\text{-}2}ACA_{0\text{-}1}C_{0\text{-}1}G_{1\text{-}6}$	(*16*)
Neurospora	C_3TA_2/T_2AG_3	(*17*)
Podospora	C_3TA_2/T_2AG_3	(*18*)
Cryptococcus	$A_2C_{3\text{-}5}T/AG_{3\text{-}5}T_2$	(*19*)
Cladosporium	C_3TA_2/T_2AG_3	(*20*)
Invertebrates		
Caenorhabditis	GC_2TA_2/T_2AG_2C	
Ascaris	GC_2TA_2/T_2AG_2C	(*21*)
Parascaris	$TGCA_2/T_2GCA$	(*22*)
Bombyx; other insects	C_2TA_2/T_2AG_2	(*23*)
Vertebrates		
	C_3TA_2/T_2AG_3	(*24*)
Plants		
Chlamydomonas	C_3TA_4/T_4AG_3	(*25*)
Chlorella	C_3TA_3/T_3AG_3	(*26*)
Arabidopsis	C_3TA_3/T_3AG_3	(*27*)
Tomato	$C_3A_A^TT_2/A_{2T}^ATG_3$	(*28*)

that telomere function does not require a precise protein-DNA structure. For example, *Saccharomyces tel1* strains have telomeres about a third of the size of those in wild-type cells (*47*), although they have chromosome loss rates close to those of the wild type (*53*) and presumably relatively normal telomere function.

Telomere Structural Proteins

Much less is known about the structural proteins that interact with telomeric DNA than about the DNA itself. The telomeric DNA at the ends of *Saccharomyces* chromosomes (*54*) and on ciliate macronuclear DNA molecules (*55*, *56*) is found in a non-nucleosomal but discrete chromatin structure, called the telosome, which encompasses the entire terminal array of telomeric repeats. Although the DNA adjacent to the *Saccharomyces* telosome is packaged in nucleosomes (*54*), these nucleosomes differ from those in most other regions of the yeast genome, having features (such as hypoacetylated histones) that are characteristic of transcriptionally inactive chromatin (*57*). In mammals, whose telomeres are much longer than those of yeasts or ciliates, most of the simple repeated telomeric DNA is packaged in closely spaced nucleosomes (*58*, *59*). However, the telomeric repeats at the very ends of human chromosomes are found in a telosome-like structure (*59*). Thus, at least some features of the chromatin structure of telomeric regions are conserved between lower and higher eukaryotes.

The major structural protein in the *Saccharomyces* telosome is the product of the essential gene *RAP1* (*60*, *61*). In vitro the *RAP1* protein, Rap1p, binds with high affinity (*62*) to the many recognition sites distributed in tandem throughout the length of a telomere (*63*). Both in vivo and in vitro Rap1p binding is not limited to telomeres. When this multifunctional protein binds at an internal site on the chromosome, it can act either as a transcriptional activator or as a transcriptional repressor (for example, *64*, *65*). Rap1p also binds to single-strand $TG_{1\text{-}3}$ DNA in vitro but with an affinity several orders of magnitude less than that for duplex telomeric DNA (*66*).

Telosomal proteins have also been identified in *Oxytricha* (*67*). The genes encoding the *Oxytricha* proteins have no sequence similarity to *RAP1* nor to any of the other proteins thought to be minor constituents of the yeast telosome (*68*, *69*). Binding of the *Oxytricha* proteins requires the single-strand $T_4G_4T_4G_4$ tail found at each end of all macronuclear DNA molecules (*67*). Thus, unlike Rap1p, the *Oxytricha* proteins will not bind to internal tracts of telomeric sequence. Because the binding of the *Oxytricha* telosomal proteins is limited to the very end of the chromosome, these proteins are terminus-specific DNA binding proteins. One of the two *Oxytricha* telosomal proteins facilitates the formation of G quartet DNA in vitro (*70*), providing strong circumstantial support for the presence of four-stranded DNA in vivo. Although G strand–specific binding proteins have been identified in other organisms, including yeast (*71*), the *Oxytricha* protein is the only one known to be localized to telomeres in vivo. Indeed, many of the abundant proteins that display G strand–specific binding in vitro are probably RNA binding proteins in vivo (*71*–*73*). However, genetic data suggest that a terminus-specific binding activity is present in *Saccharomyces* (*74*). In addition, an activity has been detected in *Xenopus* extracts that, like that of the *Oxytricha* proteins, displays terminus-specific binding in vitro (*75*). Thus, terminus-limited binding proteins may be a general feature of telomeric chromatin.

Telomere Functions

Telomeres were first defined functionally in *Drosophila*. Muller argued that the inability to recover *Drosophila* chromosomes without an end after x-ray–induced chromosome breakage indicated that the telomere was essential (*76*). McClintock reported that broken chromosomes in maize frequently fuse with other broken chromosomes to generate dicentric chromosomes, destined for breakage in a subsequent cell division. Because chromosomes with telomeres did not fuse with one another, she hypothesized that the telomere's essential function can be explained by its role in protecting chromosomes from end-to-end fusions (*77*, *78*).

The biochemical properties of DNA polymerases suggested another essential function for telomeres (*79*) (Fig. 2). Conventional DNA polymerases replicate DNA only in the 5′ to 3′ direction and cannot initiate synthesis of a DNA chain de novo. The DNA polymerases that replicate eukaryotic chromosomes use an 8- to 12-base stretch of RNA to prime DNA synthesis. As a consequence, after DNA replication one end of a linear chromosome will be replicated to the very end, whereas the other end will have a short 8- to 12-base gap generated by removal of the RNA primer (Fig. 2). Because this 5′ gap cannot be filled in by a conventional DNA polymerase, in every other cell division a given DNA end will be incompletely replicated. Hence, the end of a linear chromosome will shorten by an average of 4 to 6 bases per cell division unless telomeres act as substrates for an alternative replication mechanism.

By removing the telomeric $C_{1\text{-}3}A/TG_{1\text{-}3}$ tract from one end of a dispensable chromosome, it was possible to determine directly the fate of a yeast chromosome after telomere loss (*80*). A chromosome end without a telomere is progressively lost (*80*, *81*). Because this loss occurs in the absence of cell division and at a rate much faster than that expected for incomplete replication, it must be the result of degradation (*80*, *82*). Thus, yeast telomeres are essential for chromosome integrity. The ability of telomeres to protect DNA ends from degradation can be mimicked in vitro. The presence of the *Oxytricha* telosomal proteins on otherwise naked macronuclear DNA molecules prevents their exonucleolytic degradation (*67*). The importance of telomeres in preventing degradation can also

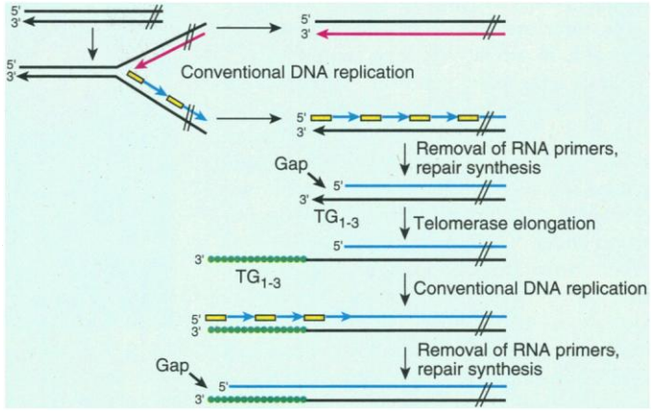

Fig. 2. Replication of the end of a *Saccharomyces* chromosome. Replication of one end of a chromosome is shown. The parental strands are in black; the newly synthesized strands generated by a standard DNA polymerase are in pink or blue. The top or leading strand can be replicated to its very end by a conventional DNA polymerase, represented by the pink arrow. The lower strand DNA, which runs 5′ to 3′ from the center to the end of the DNA molecule, is the $TG_{1\text{-}3}$ strand. The $TG_{1\text{-}3}$ strand must be copied discontinuously, represented in blue. Discontinuous DNA synthesis is RNA-primed: the 8- to 12-base-long RNA primers are represented by yellow boxes. After removal of the RNA primers, the internal gaps can be repaired by a conventional DNA polymerase. However, an 8- to 12-base gap is left at the end of the discontinuously synthesized strand. This gap leaves an 8- to 12-base G tail that can be extended by telomerase (where telomerase-synthesized $TG_{1\text{-}3}$ DNA is in green). A conventional DNA polymerase can synthesize the complementary $C_{1\text{-}3}A$ strand, but again a short gap will be left after removal of the terminal RNA primer. There is no information on the identity of the polymerase that generates the $C_{1\text{-}3}A$ complement of the telomerase-generated DNA.

explain the essential function of *RAP1*. Cells carrying alleles that generate Rap1p with reduced DNA binding activity in vitro have shorter telomeres in vivo (*60*, *83*), which suggests that Rap1p prevents degradation of telomeric DNA.

A yeast chromosome without a telomere causes a transient cell division arrest. This arrest is mediated by the *RAD9* checkpoint (*80*), which detects damaged DNA (*84*, *85*). Thus, another critical function of yeast telomeres is to help the cell distinguish intact from broken chromosomes. Telosomal proteins like those from *Oxytricha*, whose binding is terminus-limited (*67*), provide a biochemical explanation for the cell's ability to distinguish a true telomere from a broken end or an internal stretch of telomeric DNA.

Although a dispensable chromosome without a telomere causes a prolonged cell cycle arrest, a yeast cell with a single chromosome lacking a telomere ultimately resumes division without any loss of viability, even if the broken chromosome is not repaired (*80*). In these cells, the chromosome without a telomere is transcribed, replicated, and segregated for as many as 10 cell divisions before its loss. Thus, at least in yeast, telomeres serve no function that must be carried out in cis in every cell cycle. Moreover, *Drosophila* chromosomes that totally lack telomeric DNA have been isolated and maintained in fly stocks for over a decade (*86*, *87*). *Drosophila* chromosomes without telomeric DNA shorten at the very slow rate expected for incomplete replication (*86*, *88*), and therefore they are not degraded nor do they appear to cause a cell cycle arrest. Thus, these functions of yeast telomeric DNA are probably not universal. Indeed, the only essential function of *Drosophila* telomeric DNA is to compensate for incomplete replication, and even that function is necessary only in a relatively long term sense.

In at least some organisms, telomeres are specialized sites for gene expression. In *Drosophila* (*89*), *Saccharomyces* (*90*), and fission yeast (*91*), the transcription of a gene placed near a telomere is reversibly repressed, a phenomenon called telomere position effect or TPE. This repression and its reversibility are easily seen when a gene that affects eye (*Drosophila*) or colony (yeast) color is affected (Fig. 3). Because internal tracts of telomeric sequence can cause transcriptional repression, even on a circular chromosome (*92*), position effects on transcription must not require any novel aspect of telomere structure, such as a free, single-strand G tail. TPE can be eliminated in cis at a single telomere without affecting chromosome loss (*48*), which demonstrates that the structural requirements for TPE are separable from those required for chromosome stability. Because many genes required for TPE are nonessential (*93*), the ability of the telomere to affect transcription is a dispensable function of yeast telomeres.

Evidence from a variety of organisms suggests that mitotic telomeres often associate both with each other and with the nuclear periphery (*94*–*97*). Such associations are widespread, perhaps ubiquitous, during early stages of meiosis, which suggests that these interactions may be important for homolog pairing, recombination, or some other aspect of meiotic chromosome behavior. In mitotic yeast cells, telomere clustering and a localization at the nuclear periphery may be prerequisites for TPE (*98*, *99*).

Mechanisms for the Maintenance of DNA Ends

Biochemical considerations led to the realization that replication of the very ends of linear DNA molecules requires a different mechanism than replication of the rest of the genome (*79*) (Fig. 2). Different solutions to this dilemma are seen in viruses, plasmids, and organelle DNA (reviewed in *100*). One evolutionarily widespread solution is protein-nucleotide priming of replication, which generates a linear molecule covalently bound to protein at a 5′ terminal nucleotide, not templated by the genome (for example, as in ϕ29 and adenoviruses). Another solution is to have a sequence at the end of the molecule that allows formation of a hairpin, concatamer, or circle that eliminates ends at the time of replication (for example, in vaccinia, T7, and lambdoid viruses). Alternatively, the ends of a linear DNA molecule can be maintained by recombination, as in T4 bacteriophage (*101*) or *Tetrahymena* mitochondrial DNA (*102*).

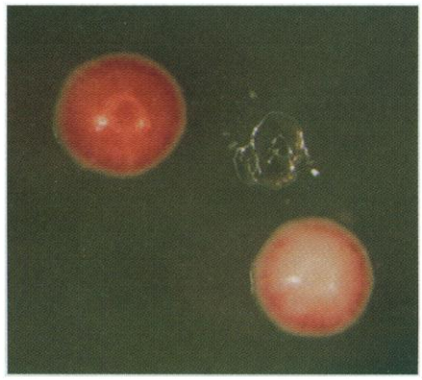

Fig. 3. Telomeres repress transcription of adjacent genes (taken with permission from *74*). Two colonies are shown from a strain in which the *ADE2* gene is next to the right telomere of chromosome V in *Saccharomyces*. About half of the cells produce red colonies, the Ade2$^-$ phenotype, and half produce white colonies, the Ade2$^+$ phenotype. Because the colonies are mostly red or mostly white, both the "off" and "on" transcriptional states are stable. Sectors of opposite color are seen within both red and white colonies, which demonstrates that both states are reversible.

Several different mechanisms also exist to maintain the ends of eukaryotic chromosomes. The termini of *Drosophila* chromosomes usually bear middle repetitive elements that transpose to chromosome ends (reviewed in *103*). Although these terminal transposons are slowly lost by incomplete replication (*104*), their rate of transposition is sufficient to compensate for this loss, and their presence protects internal, single-copy DNA from erosion.

The lengthening of *Drosophila* chromosome ends by transposition is a relatively rare event (*104*). Likewise, for all organisms telomere replication need only be frequent enough to compensate for the very slow loss of DNA that results from incomplete replication. In contrast to the regulated, once-per–cell cycle replication required for the rest of the chromosome, telomere replication could be accomplished as a repair function and need not a priori be regulated by the cell cycle.

In most organisms, telomere replication involves a specialized reverse transcriptase called telomerase. Telomerase is a ribonucleoprotein, its activity depending on both RNA and protein components (*105*). Telomerase circumvents the problem of end replication by using RNA, not DNA, to template the synthesis of telomeric DNA. Before telomerase was discovered, work with ciliates and yeast suggested that telomeric DNA was not templated in a conventional manner. During the formation of the ciliate macronucleus, new telomeres are generated at sites that were previously internal on the chromosome (*4*). Virtually all of these sites have no telomere-like DNA to serve as a template for telomere addition. In contrast, during transformation, yeast telomeres are added to the ends of a linear plasmid, if and only if the end has at least a short stretch of telomeric or telomere-like DNA (*106*). In the yeast transformation assay, *Tetrahymena* and *Oxytricha* telomeres are suitable substrates for telomere addition (*107*–*109*). However, yeast adds its own telomeric sequence to these foreign DNAs, which demonstrates that the newly synthesized telomere is not templated by the preexisting sequence (*12*, *109*, *110*).

Telomerase was first detected in extracts prepared from *Tetrahymena* cells at a stage in macronuclear development when many new telomeres are formed (*111*). Telomerase was discovered by its ability to extend a G strand telomeric oligonucleotide in the absence of a DNA template. Like the activity responsible for forming telomeres during transformation in yeast, *Tetrahymena* telomerase will extend different telomeric G strand oligonucleotides, but the sequence added by the *Tetrahymena* telomerase al-

ways consists of *Tetrahymena* T_2G_4 telomeric repeats, not the sequence used as a primer (*111*). In vitro, *Tetrahymena* telomerase is extremely processive, adding many residues (an average of ~500 bases) to the G strand primer before the enzyme disassociates (*112*).

Telomerase was later identified in extracts prepared from other ciliated protozoans (*113, 114*), humans (*115*), mice (*116*), *Xenopus* (*117*), and very recently from *Saccharomyces* (*118–120*). In each case, telomerase extends the G strand of telomeric DNA. Thus, the expected product of telomerase-mediated replication is a duplex molecule with a single-strand G tail (Fig. 2). A conventional DNA polymerase could theoretically complete telomere replication by synthesizing the complementary C strand (Fig. 2). C strand synthesis by a conventional DNA polymerase that uses an RNA primer will generate a largely duplex molecule with an 8- to 12-base gap at its 5′ end; that is, this molecule will have an 8- to 12-base G tail. Thus, the end product of telomerase replication is also a suitable substrate for telomerase. As long as telomerase adds, on average, more telomeric DNA than that lost by removal of the RNA primer, there will be a net gain of telomeric DNA and the substrate for the next round of telomerase replication will be generated. Whether telomeres grow, shrink, or hover about an average length will depend on a combination of factors, including the telomerase's processivity, its frequency of action at individual telomeres, and the rate of degradation of telomeric DNA.

Genes encoding telomerase RNA have been cloned from ciliates (*121–124*), yeasts (*125, 126*), humans (*127*), and mice (*128*). The RNAs encoded by these genes contain an 8- to 30-base stretch with 1.2 to 1.9 copies of the C strand form of the telomeric repeat of the particular organism from which it was isolated. In *Tetrahymena* (*129*), yeasts, (*125, 126*), humans (*127*), and mice (*128*), the sequence of this region was altered, and the mutant genes were introduced back into the cells. In each case, the altered sequence was either incorporated into telomeric DNA in vivo (*125, 126*) or into telomerase products in vitro (*127, 128*), which indicates that telomerase RNA determines the sequence of telomeric DNA. When the single-copy telomerase RNA gene is deleted in *Saccharomyces* or *K. lactis*, telomeres shorten at exactly the rate expected for incomplete replication (*125, 126*). Thus, in these organisms telomerase must be the major pathway for telomere maintenance. The discovery of telomerase in an organism like *Saccharomyces*, with a heterogeneous telomeric repeat, and in *K. lactis*, with a 25-bp repeat, demonstrates that a telomerase replication mechanism can account for virtually all of the known telomeric DNA sequences.

The protein components of telomerase have been much more elusive than telomerase RNAs. Deletion of the *Saccharomyces EST1* gene, which encodes a 77-kD polypeptide (*130*), yields a phenotype indistinguishable from that of cells lacking telomerase RNA (*125*). Thus, Est1p is required for telomere length maintenance in vivo (*130*). Est1p is specifically associated with telomerase RNA in cell-free extracts (*119*). Moreover, a processive telomerase activity detected by a polymerase chain reaction assay in *Saccharomyces* cell-free extracts is Est1p-dependent (*119*). In contrast, a nonprocessive telomerase detected by a conventional primer extension assay in fractionated *Saccharomyces* extracts is Est1p-independent (*118*). These data can be reconciled if Est1p makes yeast telomerase more processive either by acting directly as a processivity factor or by inactivating a telomerase inhibitor. The genes encoding an 80-kD and a 95-kD protein that co-purify with *Tetrahymena* telomerase activity and telomerase RNA have been isolated (*131*). The 80-kD protein binds specifically to telomerase RNA, whereas the 95-kD protein can be cross-linked to G strand primers. Like Est1p, neither of the *Tetrahymena* proteins has appreciable sequence similarity to RNA or DNA polymerases nor to any known replication accessory proteins, and as yet, neither protein has been shown to be essential for telomerase activity in vivo or in vitro.

Even organisms that normally rely on a telomerase mechanism of replication have alternative pathways for telomere maintenance. Telomeres in *Saccharomyces* strains that lack either *TLC1*, the gene encoding telomerase RNA (*125*), or *EST1* (*130*) slowly shorten. After ~50 to 100 divisions, most of the cells in these cultures stop dividing (*125, 130*). Failure to divide might be a consequence of a *RAD9* arrest more prolonged than that seen when a single telomere is lost (*80*). Alternatively, once telomeric DNA and hence telomere function is gone, degradation may eliminate essential genes, resulting in cell death. Although most *tlc1*Δ and *est1*Δ cells stop dividing, survivors appear spontaneously in all such cultures (*125, 132*). Most survivors have very short telomeres but in addition acquire multiple copies of Y′ or deletion derivatives of Y′ immediately internal to the $C_{1\text{-}3}A/TG_{1\text{-}3}$ terminal repeats (*132*). These tandem Y′ elements, like the *Drosophila* telomeric transposons, might serve as a passive buffer to protect internal sequences from loss. The generation of these survivors requires *RAD52*, a gene needed for most mitotic recombination events. Thus, Y′ acquisition occurs by recombination, not by transposition (*132*).

A *RAD52*-independent recombination process that could theoretically contribute to telomere maintenance has also been detected in *Saccharomyces* (*106*). In contrast to Y′-Y′ recombination, this recombination occurs between two terminal tracts of telomeric repeats. Telomere-telomere recombination proceeds by gene conversion and results in a net increase in telomeric DNA (*133*). It is not known if telomere-telomere recombination contributes to telomere maintenance in mutant or wild-type cells nor if similar pathways can be activated in other organisms. However, in some human cell lines, immortalization and telomere length increases occur even in the absence of detectable telomerase activity (*134–136*). These data suggest that telomerase-independent mechanisms for telomere maintenance also exist in higher cells.

Regulation of Telomere Replication

There are many intriguing aspects of telomere biology that deserve further attention. Of considerable interest is the regulation of telomere replication. In *Saccharomyces*, generation of the single-strand $TG_{1\text{-}3}$ tail is controlled by the cell cycle. The $TG_{1\text{-}3}$ tails are detectable at the very end of the S phase but disappear before the next cell cycle (*39, 40*). Although there is no inherent reason for telomere replication itself to be regulated by the cell cycle, the substrate or product of telomere replication might serve a cell cycle function. Any proposed cell cycle function for telomeres must be reconciled with the fact that a cell with a chromosome lacking one telomere is viable (*80*). One role of telomeres that is consistent with the viability of cells that have a broken chromosome is their function in a cell cycle checkpoint, such as the *RAD9* checkpoint, which is dispensable in the absence of DNA damage (*85, 137*). For example, the transient single-strand G tails that are intermediates in telomere replication may allow the formation of inter- or intratelomeric non–Watson-Crick base-paired structures or may provide a substrate for a terminus-binding protein. Either the DNA structures or the protein-DNA complexes could serve as signals to the *RAD9* cell cycle checkpoint that chromosome replication is complete (*39*).

In addition to the cell cycle regulation of telomere replication seen in yeast, mammalian telomerase is developmentally regulated (*138*). In humans, telomerase activity is not found in most somatic tissues (*139*). In contrast, telomerase is detected in many human tumors (*139*). These data have led to the hypothesis that telomere length may serve as a mitotic clock, which acts to limit the replication potential of human cells both in vivo and in vitro (*52, 140*). The

expression of telomerase in tumor cells may provide a selective advantage that allows these cells to divide indefinitely (*141*).

Telomerase activity and telomere length could be and almost surely are controlled at many levels. Tissue- or stage-specific expression of an essential telomerase subunit is an obvious possibility. However, telomerase RNA is detected in many human somatic cells that lack detectable telomerase activity, which suggests that control of its synthesis is unlikely to be a general mechanism for modulating telomerase activity (*127*). Alternatively, the processivity of telomerase might be regulated. In *Tetrahymena* and in humans, telomerase activity in vitro is very processive, whereas in mice and *Xenopus*, the activity adds only a few bases at a time to G strand primers (*116, 117*). Because mouse telomeres are exceptionally long (*44*), in vivo there may be accessory factors that increase the processivity of mouse telomerase. In *Saccharomyces*, the Pif1p helicase reduces telomere length and de novo telomere formation in vivo (*142*). Thus, Pif1p acts as if it inhibits telomere replication, although it is not known if this inhibition is on telomerase or on telomerase-independent pathway of telomere maintenance, such as telomere-telomere recombination. The 5′ to 3′ helicase activity of Pif1p could reduce telomerase processivity by dissociating telomerase RNA from its G strand substrate, or it could inhibit telomere-telomere recombination by dissociating recombination intermediates.

Telomerase might also be regulated by telomere accessibility. For example, several Rap1p-interacting proteins in *Saccharomyces* appear to limit telomere replication because depletion of these proteins results in telomere lengthening (*60, 74, 143*). Alternatively, DNA structure could affect telomere replication. When G strands assume a G quartet, this structure inhibits their ability to serve as primers for telomerase as well as reduces the extent of telomerase elongation (*144*). Hence, the formation of G quartets might affect both telomere accessibility to telomerase and telomerase processivity. Accessibility to nucleases will also influence telomere length: for example, telomeres in human cells lacking telomerase shorten at a rate ~10 times faster than that expected from incomplete replication (*145, 146*); this result suggests that in these cells telomeric DNA is degraded, as well as incompletely replicated. Unexpectedly and still inexplicably, overexpression of truncated forms of the *Saccharomyces* telomerase RNA gene causes telomere shortening and loss of telomere position effect (*125*). These results indicate that there is considerable interplay between telomere structure, function, and replication.

In *Saccharomyces*, a surprisingly large number of proteins and conditions affect telomeres. Moreover, even in yeasts and in ciliates our understanding of telomere biology is relatively modest, with new information still emerging at a rapid rate. Telomerase is often proposed as an ideal target for cancer therapy because its activity is widespread among human tumors but absent from most somatic cells. A detailed understanding of telomere replication, including information on telomerase-independent mechanisms of telomere maintenance, will be crucial if telomerase is to become a realistic target for cancer therapy.

REFERENCES AND NOTES

1. E. H. Blackburn and J. G. Gall, *J. Mol. Biol.* **120**, 33 (1978).
2. Y. Oka, S. Shiota, S. Nakai, Y. Nishida, S. Okubo, *Gene* **10**, 301 (1980).
3. L. A. Klobutcher, M. T. Swanton, P. Donini, D. M. Prescott, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3015 (1981).
4. D. M. Prescott, *Microbiol. Rev.* **58**, 233 (1994).
5. A. Baroin, A. Prat, F. Caron, *Nucleic Acids Res.* **15**, 1717 (1987).
6. M. Ponzi, T. Pace, E. Dore, C. Frontali, *EMBO J.* **4**, 2991 (1985).
7. E. H. Blackburn and P. B. Challoner, *Cell* **36**, 447 (1984).
8. L. H. T. Van der Ploeg, A. U. C. Liu, P. Borst, *ibid.*, p. 459.
9. S. M. Le Blancq, R. S. Kase, L. H. T. Van der Ploeg, *Nucleic Acids Res.* **19**, 5790 (1991).
10. J. Forney, E. R. Henderson, E. H. Blackburn, *ibid.* **15**, 9143 (1987).
11. H. S. Emery and A. M. Weiner, *Cell* **26**, 411 (1981).
12. J. Shampay, J. W. Szostak, E. H. Blackburn, *Nature* **310**, 154 (1984).
13. S.-S. Wang and V. A. Zakian, *Mol. Cell. Biol.* **10**, 4415 (1990).
14. M. J. McEachern and E. H. Blackburn, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 3453 (1994).
15. M. J. McEachern and J. B. Hicks, *Mol. Cell. Biol.* **13**, 551 (1993).
16. T. Matsumoto *et al.*, *ibid.* **7**, 4424 (1987).
17. M. G. Schectman, *Gene* **88**, 159 (1990).
18. J. P. Javerzat, V. Bhattacherjee, C. Barreau, *Nucleic Acids Res.* **21**, 497 (1993).
19. J. C. Edman, *Mol. Cell. Biol.* **12**, 2777 (1992).
20. M. J. Coleman, M. T. McHale, J. Arnau, A. Watson, R. P. Oliver, *Gene* **132**, 67 (1993).
21. F. Muller, C. Wicky, A. Spicker, H. Tobler, *Cell* **67**, 815 (1991).
22. C. Teschke, G. Solleder, K. B. Moritz, *Nucleic Acids Res.* **19**, 2677 (1991).
23. S. Okazaki, K. Tsuchida, H. Maekawa, H. Ishikawa, H. Fujiwara, *Mol. Cell. Biol.* **13**, 1424 (1993).
24. J. Meyne, R. L. Ratliff, R. K. Moyzis, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 7049 (1989).
25. M. E. Petracek, P. A. Lefebvre, C. D. Silflow, J. Berman, *ibid.* **87**, 8222 (1990).
26. T. Higashiyama, S. Maki, T. Yamada, *Mol. Gen. Genet.* **249**, 29 (1995).
27. E. J. Richards and F. M. Ausubel, *Cell* **53**, 127 (1988).
28. M. W. Ganal, N. L. Lapitan, S. D. Tanksley, *Plant Cell* **3**, 87 (1991).
29. H. Biessman *et al.*, *Cell* **61**, 663 (1990).
30. R. W. Levis, R. Ganesan, K. Houtchens, L. A. Tolar, F.-m. Sheen, *ibid.* **75**, 1083 (1993).
31. F.-m. Sheen and R. Levis, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 12510 (1994).
32. C. S. M. Chan and B.-K. Tye, *Cell* **33**, 563 (1983).
33. E. J. Louis and J. E. Haber, *Genetics* **131**, 559 (1992).
34. L. M. Corcoran, J. K. Thompson, D. Walliker, D. J. Kemp, *Cell* **53**, 807 (1988).
35. S.-S. Wang, S. Balakumaran, V. A. Zakian, unpublished results.
36. R. M. Walmsley, C. S. M. Chan, B.-K. Tye, T. D. Petes, *Nature* **310**, 157 (1984).
37. J. Meyne *et al.*, *Chromos.* **99**, 3 (1990).
38. R. A. Wells, G. G. Germino, S. Krishna, V. J. Buckle, S. T. Reeders, *Genomics* **8**, 699 (1990).
39. R. J. Wellinger, A. J. Wolf, V. A. Zakian, *Mol. Cell. Biol.* **13**, 4057 (1993).
40. ———, *Cell* **72**, 51 (1993).
41. D. Sen and W. Gilbert, *Nature* **344**, 410 (1990).
42. J. R. Williamson, M. K. Raghuraman, T. R. Cech, *Cell* **59**, 871 (1989).
43. W. I. Sundquist and A. Klug, *Nature* **342**, 825 (1989).
44. D. Kipling and H. J. Cooke, *ibid.* **347**, 400 (1990).
45. J. A. Starling, J. Maule, N. D. Hastie, R. C. Allshire, *Nucleic Acids Res.* **18**, 6881 (1990).
46. J. Shampay and E. H. Blackburn, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 534 (1988).
47. A. J. Lustig and T. D. Petes, *ibid.* **83**, 1398 (1986).
48. L. L. Sandell, D. E. Gottschling, V. A. Zakian, *ibid.* **91**, 12061 (1994).
49. D. D. Larson, E. A. Spangler, E. H. Blackburn, *Cell* **50**, 477 (1987).
50. A. Bernards, P. A. M. Michels, C. R. Lincke, P. Borst, *Nature* **303**, 592 (1983).
51. N. D. Hastie *et al.*, *ibid.* **346**, 866 (1990).
52. C. B. Harley, A. B. Futcher, C. W. Greider, *ibid.* **345**, 458 (1990).
53. P. W. Greenwell *et al.*, *Cell* **82**, 823 (1995).
54. J. H. Wright, D. E. Gottschling, V. A. Zakian, *Genes Dev.* **6**, 197 (1992).
55. D. E. Gottschling and T. R. Cech, *Cell* **38**, 501 (1984).
56. E. H. Blackburn and S.-S. Chiou, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2263 (1981).
57. M. Braunstein, A. B. Rose, S. G. Holmes, C. D. Allis, J. R. Broach, *Genes Dev.* **7**, 592 (1993).
58. V. L. Makarov, S. Lejnine, J. Bedoyan, J. P. Langmore, *Cell* **73**, 775 (1993).
59. H. Tommerup, A. Dousmanis, T. de Lange, *Mol. Cell. Biol.* **14**, 5777 (1994).
60. M. N. Conrad, J. H. Wright, A. J. Wolf, V. A. Zakian, *Cell* **63**, 739 (1990).
61. J. H. Wright and V. A. Zakian, *Nucleic Acids Res.* **23**, 1454 (1995).
62. A. R. Buchman, W. J. Kimmerly, J. Rine, R. D. Kornberg, *Mol. Cell. Biol.* **8**, 210 (1988).
63. E. Gilson, M. Roberge, R. Giraldo, D. Rhodes, S. M. Gasser, *J. Mol. Biol.* **231**, 293 (1993).
64. W. Kimmerly, A. Buchman, R. Kornberg, J. Rine, *EMBO J.* **7**, 2241 (1988).
65. P. K. Brindle, J. P. Holland, C. E. Willett, M. A. Innis, M. J. Holland, *Mol. Cell. Biol.* **10**, 4872 (1990).
66. R. Giraldo and D. Rhodes, *EMBO J.* **13**, 2411 (1994).
67. D. E. Gottschling and V. A. Zakian, *Cell* **47**, 195 (1986).
68. B. J. Hicke, D. W. Celander, G. H. MacDonald, C. M. Price, T. R. Cech, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 1481 (1990).
69. J. T. Gray, D. W. Celander, C. M. Price, T. R. Cech, *Cell* **67**, 807 (1991).
70. G. Fang and T. R. Cech, *ibid.* **74**, 875 (1993).
71. J. J. Lin and V. A. Zakian, *Nucleic Acids Res.* **22**, 4906 (1994).
72. F. Ishikawa, M. J. Matunis, G. Dreyfuss, T. R. Cech, *Mol. Cell. Biol.* **13**, 4301 (1993).
73. S. J. McKay and H. Cooke, *Nucleic Acids Res.* **20**, 6461 (1992).
74. E. Wiley and V. A. Zakian, *Genetics* **139**, 67 (1995).
75. M. E. Cardenas, A. Bianchi, T. de Lange, *Genes Dev.* **7**, 870 (1993).
76. H. J. Muller, *The Collecting Net* **13**, 181 (1938).
77. B. McClintock, *Proc. Natl. Acad. Sci. U.S.A.* **25**, 405 (1939).
78. ———, *Genetics* **26**, 234 (1941).
79. J. D. Watson, *Nature* **239**, 197 (1972).
80. L. L. Sandell and V. A. Zakian, *Cell* **75**, 729 (1993).
81. C. I. White and J. E. Haber, *EMBO J.* **9**, 633 (1990).
82. L. Sandell, J. Mangahas, V. Schulz, V. A. Zakian, unpublished results.
83. A. J. Lustig, S. Kurtz, D. Shore, *Science* **250**, 549 (1990).
84. T. A. Weinert and L. H. Hartwell, *ibid.* **241**, 317 (1988).
85. R. H. Schiestl, P. Reynolds, S. Prakash, L. Prakash,

Mol. Cell. Biol. **9**, 1882 (1989).
86. R. W. Levis, *Cell* **58**, 791 (1989).
87. H. Biessmann, S. B. Carter, J. M. Mason, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 1758 (1990).
88. H. Biessmann and J. M. Mason, *EMBO J.* **7**, 1081 (1988).
89. R. Levis, T. Hazelrigg, G. M. Rubin, *Science* **229**, 558 (1985).
90. D. E. Gottschling, O. M. Aparicio, B. L. Billington, V. A. Zakian, *Cell* **63**, 751 (1990).
91. E. R. Nimmo, G. Cranston, R. C. Allshire, *EMBO J.* **13**, 3801 (1994).
92. J. B. S. Stavenhagen and V. A. Zakian, *Genes Dev.* **8**, 1411 (1994).
93. O. M. Aparicio, B. L. Billington, D. E. Gottschling, *Cell* **66**, 1279 (1991).
94. C. L. Walker, C. B. Cargile, K. M. Floy, M. Delannoy, B. R. Migeon, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 6191 (1991).
95. D. Mathog, M. Hochstrasser, Y. Gruenbaum, H. Saumweber, J. Sedat, *Nature* **308**, 414 (1984).
96. H. M. Chung *et al.*, *EMBO J.* **9**, 2611 (1990).
97. H. Funabiki, I. Hagan, S. Uzawa, M. Yanagida, *J. Cell Biol.* **121**, 961 (1993).
98. F. Palladino *et al.*, *Cell* **75**, 543 (1993).
99. M. Cockell *et al.*, *J. Cell Biol.* **129**, 909 (1995).
100. V. A. Zakian, *Annu. Rev. Genet.* **23**, 579 (1989).
101. G. Mosig, *ibid.* **21**, 347 (1987).
102. G. B. Morin and T. R. Cech, *Cell* **52**, 367 (1988).
103. J. M. Mason and H. Biessmann, *Trends Genet.* **11**, 58 (1995).
104. H. Biessmann *et al.*, *EMBO J.* **11**, 4459 (1992).
105. C. W. Greider and E. H. Blackburn, *Cell* **51**, 887 (1987).
106. A. F. Pluta and V. A. Zakian, *Nature* **337**, 429 (1989).
107. J. W. Szostak and E. H. Blackburn, *Cell* **29**, 245 (1982).
108. G. M. Dani and V. A. Zakian, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3406 (1983).
109. A. F. Pluta, G. M. Dani, B. B. Spear, V. A. Zakian, *ibid.* **81**, 1475 (1984).
110. R. M. Walmsley, J. W. Szostak, T. D. Petes, *Nature* **302**, 84 (1983).
111. C. W. Greider and E. H. Blackburn, *Cell* **43**, 405 (1985).
112. C. W. Greider, *Mol. Cell. Biol.* **11**, 4572 (1991).
113. A. M. Zahler and D. M. Prescott, *Nucleic Acids Res.* **16**, 6953 (1988).
114. D. Shippen-Lentz and E. H. Blackburn, *Mol. Cell. Biol.* **9**, 2761 (1989).
115. G. B. Morin, *Cell* **59**, 521 (1989).
116. K. R. Prowse, A. A. Avilion, C. W. Greider, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 1493 (1993).
117. L. L. Mantell and C. W. Greider, *EMBO J.* **13**, 3211 (1994).
118. M. Cohn and E. H. Blackburn, *Science* **269**, 396 (1995).
119. J.-J. Lin and V. A. Zakian, *Cell* **81**, 1127 (1995).
120. N. F. Lue and J. C. Wang, *J. Biol. Chem.* **270**, 21453 (1995).
121. C. W. Greider and E. H. Blackburn, *Nature* **337**, 331 (1989).
122. D. Shippen-Lentz and E. H. Blackburn, *Science* **247**, 546 (1990).
123. D. P. Romero and E. H. Blackburn, *Cell* **67**, 343 (1991).
124. J. Lingner, L. L. Hendrick, T. R. Cech, *Genes Dev.* **8**, 1984 (1994).
125. M. S. Singer and D. E. Gottschling, *Science* **266**, 404 (1994).
126. M. J. McEachern and E. H. Blackburn, *Nature* **376**, 403 (1995).
127. J. Feng *et al.*, *Science* **269**, 1236 (1995).
128. M. A. Blasco, W. Funk, B. Villeponteau, C. W. Greider, *ibid.*, p. 1267.
129. G.-L. Yu, J. D. Bradley, L. D. Attardi, E. H. Blackburn, *Nature* **344**, 126 (1990).
130. V. Lundblad and J. W. Szostak, *Cell* **57**, 633 (1989).
131. K. Collins, R. Kobayashi, C. W. Greider, *ibid.* **81**, 677 (1995).
132. V. Lundblad and E. H. Blackburn, *ibid.* **73**, 347 (1993).
133. S.-S. Wang and V. A. Zakian, *Nature* **345**, 456 (1990).
134. E. M. Rogan *et al.*, *Mol. Cell. Biol.* **15**, 4745 (1995).
135. J. P. Murnane, L. Sabatier, B. A. Marder, W. F. Morgan, *EMBO J.* **13**, 4953 (1994).
136. T. M. Bryan, A. Anglezou, J. Gupta, S. Bacchetti, R. R. Reddel, *ibid.* **14**, 4340 (1995).
137. T. A. Weinert and L. H. Hartwell, *Mol. Cell. Biol.* **10**, 6554 (1990).
138. K. R. Prowse and C. W. Greider, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 4818 (1995).
139. N. W. Kim *et al.*, *Science* **266**, 2011 (1994).
140. R. C. Allsopp *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 10114 (1992).
141. C. M. Counter, H. W. Hirte, S. Bacchetti, C. B. Harley, *ibid.* **91**, 2900 (1994).
142. V. P. Schulz and V. A. Zakian, *Cell* **76**, 145 (1994).
143. C. F. J. Hardy, L. Sussel, D. Shore, *Genes Dev.* **6**, 801 (1992).
144. A. M. Zahler, J. R. Williamson, T. R. Cech, D. M. Prescott, *Nature* **350**, 718 (1991).
145. C. M. Counter *et al.*, *EMBO J.* **11**, 1921 (1992).
146. M. Z. Levy, R. C. Allsopp, A. B. Futcher, C. W. Greider, C. B. Harley, *J. Mol. Biol.* **225**, 951 (1992).
147. E. J. Louis, E. S. Naumova, A. Lee, G. Naumov, J. E. Haber, *Genetics* **136**, 789 (1994).
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Equality for X Chromosomes

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In many species, females possess two X chromosomes and males have one X chromosome. This difference is critical for the initial determination of sex. However, the X encodes many functions required equally in males and females; thus, X chromosome expression must be adjusted to compensate for the difference in dosage between the sexes. Distinct dosage compensation mechanisms have evolved in different species. A common theme in the *Drosophila melanogaster* and *Caenorhabditis elegans* systems is that a subtle alteration of chromatin structure may impose this modest, but vital adjustment of the X chromosome transcription level.

Dosage Compensation in *Drosophila*

The predominant dosage compensation mechanism in *Drosophila melanogaster* is hypertranscription of the single male X chromosome in order to achieve an activity equal to that of both female X's (reviewed in *1*). Genetic analyses have identified four genes that are required exclusively for male viability and whose products mediate hypertranscription of the male X chromosome. These genes—*male specific lethal-1*, *-2*, *-3*, and *maleless* (*msl-1*, *-2*, *-3*, and *mle*, collectively called the *msls*)—have been characterized at the molecular level. Antibodies to any one of the MSL proteins specifically recognize hundreds of sites along the male polytene X chromosome (*2*–*7*). The *msl* mutants display similar phenotypes, and the proteins colocalize on the X chromosome, suggesting that they act in a heteromeric complex. Furthermore, each of the MSL proteins must be functional in order to observe the wild-type chromatin-binding pattern of the remaining three (reviewed in *1*). Direct evidence for a physical interaction between the MSLs has been demonstrated by coimmunoprecipitation of MSL-1 and MSL-2 (*6*).

The biochemical function of the putative MSL protein complex is not understood, but it may function in histone modification. Male X chromatin is highly enriched for an isoform of histone H4 monoacetylated at lysine-16 (H4Ac16) (*8*). Mutation of the corresponding lysine of yeast histone H4 produces altered transcription of several genes (reviewed in *9*). Although the mechanism of altered gene expression is not understood, neutralizing a key positive charge on the NH_2-terminal histone H4 tail may allow greater access of the transcriptional machinery to DNA (*9*). The MSL banding pattern is highly similar to that of H4Ac16, and mutation in any of the *msl* genes prevents accumulation of H4Ac16 on the male X chromosome (*10*). Perhaps one component of the MSL complex is a histone acetyltransferase or an inhibitor of a histone deacetylase.

Sequence analysis shows that MSL-1 and -3 are unlike any previously reported protein (*3*, *4*); MSL-2 contains a zinc-binding motif called the RING finger (*5*–*7*), and MLE is closely related to human RNA helicase A (*11*). The finding that MSL-2 contains a RING finger present in several other chromatin-binding proteins has led to the suggestion that this subunit provides the recognition specificity to distinguish the X chromosome from the autosomes (*5*–*7*). Mutations in the RING finger destroy *msl-2* activity (*5*), but so far no RING finger protein has been shown to possess sequence-specific DNA-binding activity (*12*). Two members of this family from *Drosophila* are *Posterior sex combs (Psc)* and *suppressor of zeste 2 [su(z)2]*, which both function in the maintenance of repressive chromatin structure in discrete regions of the genome (*13*).

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