28. D. Gorlich, S. Prehn, R. A. Laskey, E. Hartmann, *Cell* **79**, 767 (1994).
29. J. Moroianu, G. Blobel, A. Radu, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 2008 (1995).
30. K. Weis, I. W. Mattaj, A. I. Lamond, *Science* **268**, 1049 (1995).
31. N. C. Chi, E. J. Adam, S. A. Adam, *J. Cell Biol.* **130**, 265 (1995).
32. D. Gorlich *et al.*, *Curr. Biol.* **5**, 383 (1995).
33. A. Radu, G. Blobel, M. S. Moore, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 1769 (1995).
34. N. Imamoto *et al.*, *FEBS Lett.* **368**, 415 (1995).
35. J. D. Aitchison, G. Blobel, M. P. Rout, *Science* **274**, 624 (1996).
36. M. Fornerod *et al.*, *EMBO J.* **16**, 807 (1997).
37. D. Gorlich *et al.*, *J. Cell Biol.* **138**, 65 (1997).
38. I. W. Mattaj and L. Englmeier, *Annu. Rev. Biochem.* **67**, 265 (1998).
39. L. F. Pemberton, G. Blobel, J. S. Rosenblum, *Curr. Opin. Cell Biol.* **10**, 392 (1998).
40. Y. M. Chook and G. Blobel, *Nature* **399**, 230 (1999).
41. G. Cingolani, C. Petosa, K. Weis, C. W. Muller, *Nature* **399**, 221 (1999).
42. M. Hetzer and I. W. Mattaj, *J. Cell Biol.* **148**, 293 (2000).
43. K. Straber and E. Hurt, *EMBO J.* **19**, 410 (2000).
44. F. Stutz *et al.*, *RNA* **6**, 638 (2000).
45. C. A. Hodge, H. V. Colot, P. Stafford, C. N. Cole, *EMBO J.* **18**, 5778 (1999).
46. S. I. Dworetzky and C. M. Feldherr, *J. Cell Biol.* **106**, 575 (1988).
47. O. Keminer, J.-P. Siebrasse, K. Zerf, R. Peters, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 11842 (1999).
48. D. R. Finlay, D. D. Newmeyer, T. M. Price, D. J. Forbes, *J. Cell Biol.* **104**, 189 (1987).
49. Y. Yoneda, N. Imamoto-Sonobe, M. Yamaizumi, T. Uchida, *Exp. Cell Res.* **173**, 586 (1987).
50. D. D. Newmeyer and D. J. Forbes, *Cell* **52**, 641 (1988).
51. K. J. Ryan and S. R. Wente, *Curr. Opin. Cell Biol.* **12**, 361 (2000).
52. N. R. Yaseen and G. Blobel, *J. Biol. Chem.* **274**, 26493 (1999).
53. R. H. Kehlenbach, A. Dickmanns, A. Kehlenbach, T. Guan, L. Gerace, *J. Cell Biol.* **145**, 645 (1999).
54. S. Shah, S. Tugendreich, D. Forbes, *J. Cell Biol.* **141**, 31 (1998).
55. B. Talcott and M. S. Moore, *Trends Cell Biol.* **9**, 312 (1999).
56. M. V. Nachury and K. Weis, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 9622 (1999).
57. M. K. Iovine, J. L. Watkins, S. R. Wente, *J. Cell Biol.* **131**, 1699 (1995).
58. S. Shah and D. J. Forbes, *Curr. Biol.* **8**, 1376 (1998).
59. S. Nakielny, S. Shailkh, B. Burke, G. Dreyfuss, *EMBO J.* **18**, 1982 (1999).
60. J. Rappsilber, S. Siniossoglou, E. C. Hurt, M. Mann, *Anal. Chem.* **72**, 267 (2000).
61. M. Damelin and P. A. Silver, *Mol. Cell* **5**, 133 (2000).
62. A. Kaffman and E. O'Shea, *Annu. Rev. Cell Dev. Biol.* **15**, 291 (1999).
63. B. R. Cullen, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 4 (2000).
64. S. L. Wolin and A. G. Matera, *Genes Dev.* **13**, 1 (1999).
65. J. D. York, A. R. Odom, R. Murphy, E. B. Ives, S. R. Wente, *Science* **285**, 96 (1999).
66. P. N. Unwin and R. A. Milligan, *J. Cell Biol.* **93**, 63 (1982).
67. C. W. Akey and M. Radermacher, *J. Cell Biol.* **122**, 1 (1993).
68. V. W. Pollard *et al.*, *Cell* **86**, 985 (1996).
69. U. Kutay *et al.*, *Mol. Cell* **1**, 359 (1998).
70. M. Fornerod, M. Ohno, M. Yoshida, I. W. Mattaj, *Cell* **90**, 1051 (1997).
71. I sincerely apologize to those whose primary references were omitted because of space limitations. I am most grateful to J. Gall, C. Hardy, M. Moore, K. Ryan, L. Strawn, and J. York for helpful discussions and comments on the manuscript and S. Johnson for the computer design of Fig. 2. S.W. is supported by grants from NIH.

VIEWPOINT

A Sense of the End

Susan M. Gasser

How a cell distinguishes a double-strand break from the end of a chromosome has long fascinated cell biologists. It was thought that the protection of chromosomal ends required either a telomere-specific complex or the looping back of the 3′ TG-rich overhang to anneal with a homologous double-stranded repeat. These models must now accommodate the findings that complexes involved in nonhomologous end joining play important roles in normal telomere length maintenance, and that subtelomeric chromatin changes in response to the DNA damage checkpoint. A hypothetical chromatin assembly checkpoint may help to explain why telomeres and the double-strand break repair machinery share essential components.

The telomere is a unique chromosomal structure consisting of repetitive DNA sequences bound by protein complexes that cooperate to protect the termini of linear chromosomes from fusion and degradation, as well as to promote chromosomal end replication [reviewed in (*1–3*)]. It was reasonable to expect that the double-strand break (DSB) repair machinery would be specifically excluded from telomeric chromatin, yet this is not the case. In yeast, as in mammals, several of the complexes directly involved in nonhomologous end joining (NHEJ) are telomere-bound and affect telomere length maintenance. For instance, yeast strains lacking either subunit of Ku, a heterodimer directly implicated in end-joining reactions, have abnormally short telomeres (*4–6*) and reduced levels of subtelomeric silencing (*7–9*). Immunofluorescence and cross-linking assays show that yeast Ku (yKu) associates with subtelomeric heterochromatin as well as telomeric repeats (*6, 10*). Consistently, loss of either subunit is lethal when combined with mutations in telomerase (*EST2*) or in Cdc13p, a single-strand binding protein that helps protect the C-rich telomeric strand from degradation (*11, 12*). Mutation of Mre11p—which forms a complex with Rad50p and Xrs2p to process breaks for repair either by end-joining or homologous recombination—also impairs telomere maintenance and is lethal in combination with telomerase mutations (*11, 13*). This sharing of telomere maintenance and DSB repair functions appears to be conserved from yeast to humans. The human Ku complex was shown to bind telomeric DNA (*14, 15*), and both *mre11*-deficient chicken cells (*16*) and *ku*-deficient mice (*17–19*) have high rates of end-to-end chromosomal fusions, in addition to a pronounced sensitivity to γ irradiation.

Telomere structure is affected not only by proteins involved in repair, but also by DNA damage checkpoint proteins. In *Caenorhabditis elegans*, the loss of *mrt2*—a gene encoding the homolog of the yeast DNA checkpoint factor Rad17p—results in short telomeres, end-to-end fusions, and chromosome loss, much like telomerase-deficient cells (*20*). Deletion of *MEC3*, which is a budding yeast checkpoint gene downstream of *RAD17*, induces the lengthening of telomeres and counteracts the derepression of telomeric silencing provoked by loss of *SET1*, a yeast member of the *trithorax* gene family (*21*). Moreover, the ataxia telangiectasia mutated (ATM) kinase homologs in fission yeast, $rad3^+$ and $tel1^+$, like *MEC1* and *TEL1* in budding yeast, affect both telomere maintenance and the DNA damage checkpoint response (*22*). In *Schizosaccharomyces pombe*, this pathway is clearly independent of the downstream checkpoint kinases, $cds1^+$ and $chk1^+$ (*22*), which suggests that the ATM homologs may directly modify telomere-associated factors.

The observation that yeast telomeric chromatin itself responds to a Mec1p-mediated checkpoint signal provides further evidence that ATM1-like kinases have telomeric targets (*10, 23, 24*). In budding yeast, the induction of a single DSB is sufficient to provoke the displacement from telomeric foci of yKu, Rap1p, and the silent information regulatory proteins Sir2p, Sir3p, and Sir4p; this event coincides with a drop in subtelomeric silencing. The delocalization is dependent on the DNA damage signaling components Rad9p (*10*), Ddc1p (*23*), and the yeast ATM-like kinase Mec1p (*23, 24*). Like the telomere effects of *rad3* mutants in *S. pombe*, however, the response is independent of Rad53p (the $cds1^+$ homolog).

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Both yKu and Sir proteins are then recruited to an induced DSB, although the simple hypothesis that the purpose of the telomere response is to release proteins required for NHEJ repair is probably wrong. *RAD9*, which is necessary for this release, is not necessary for the recruitment of yKu, and *rad9*-deficient cells are only slightly less efficient than wild-type cells in the end-joining reaction (*25, 26*). Moreover, the initial observation that *sir* mutants are deficient in DSB repair (*7, 13, 24*) has now been shown to reflect a controlled suppression of the NHEJ pathway in yeast that express both **a** and α mating type information, which occurs in *sir* mutants and in a normal diploid state (*26, 27*). Quite logically, diploid yeast favor repair through homologous recombination, and in *sir*-deficient diploids end joining is only one-third to one-half as efficient as in SIR^+ counterparts (*26*). This effect may not reflect a residual role for Sir proteins in DSB repair, but rather their ability to modulate gene expression elsewhere in the genome or to mediate the resumption of cell division after repair is achieved. It is intriguing, however, that other kinds of DNA damage (e.g., ultraviolet irradiation or replication fork arrest) do not provoke an equivalent change in subtelomeric chromatin, despite activation of the central checkpoint kinase Mec1p.

It was initially surprising to find that telomeres require and sequester repair proteins for their length maintenance, yet in retrospect it makes sense that the cell would treat the end of the chromosome as a specialized DSB, perhaps one exposed in S phase only. Telomeres, like DSBs, are able to be "repaired" through recombination events when telomerase is absent (*28*). Thus, the only essential differences between telomeres and DSBs may be that the religation complex, Xrcc4 and ligase IV (*29–31*), is efficiently suppressed at telomeres, and that telomerase is selectively recruited when the free end becomes exposed. A telomere-specific kinase, such as Tel1p, may serve a dual role in telomere maintenance: In addition to mediating the Rap1p-Rif1/2 "counting mechanism" that regulates telomerase activity to keep yeast telomeres at a given length (*32, 33*), it may inactivate ligase IV in a telomeric context, reducing the chance of end-to-end fusion events. Similarly, other factors may specifically recruit ligase IV and Mre11p to sites of NHEJ repair.

Could there be advantages in having telomere replication recognized by the checkpoint machinery as a form of DNA repair? For a haploid cell, entry into mitotic division with a single unrepaired DSB is potentially a lethal event; so is entry into mitotic division with a single unreplicated telomere. It is thus possible that the same checkpoint signal prevents cell cycle progression in the presence of either defect. In analogy to the spindle checkpoint, where the absence of kinetochore attachment keeps the checkpoint signal active (*34*), the absence of complete telomere replication may prevent mitotic entry in an analogous manner by preventing decay of a checkpoint-mediated signal. To date, it is unknown what mechanisms sense that telomere replication is complete, or whether damaged DNA is fully repaired. Yet it is striking that the complexes that assemble nucleosomes after repair-coupled DNA synthesis, like the DNA damage checkpoint machinery, are implicated in the proper maintenance of telomeric chromatin as well (*35–38*).

In budding yeast, two chromatin assembly complexes, chromatin assembly factor (CAF1) and anti-silencing factor (ASF), bind the histone H3-H4 heterodimer to promote nucleosome assembly, together with a protein related to Swi2 (imitation switch, or ISWI) and a loading factor (*35, 39*). Neither complex is essential for viability, although yeast deficient for *cac1* have reduced telomeric silencing and are hypersensitive to ultraviolet irradiation (*36–38*). Parallel effects on telomere structure and DNA repair are seen for Asf1p as well: Increased *ASF1* dosage provokes a loss of telomeric silencing, whereas mutant strains are hypersensitive to DSB-inducing agents such as bleomycin and γ irradiation (*35, 39*).

Although both CAF1 and ASF load nucleosomes after DNA replication in vitro (*35, 40*), in vivo they preferentially affect regions of silent chromatin. In mammalian cells, it has been shown that CAF1 stays associated with replicated DNA as the nucleosomal structure "matures" into a repressed state marked by the presence of underacetylated histone tails (*41*). Perhaps progression into metaphase awaits a signal that senses the completion of repair and of telomere synthesis. This could correlate with the release of CAF1 or with the unloading of the proliferating cell nuclear antigen, which is a processivity factor for DNA polymerase δ and a factor integrating signals in the S-phase replication checkpoint (*42*). In one scenario, this release would occur only when nucleosomes have matured into an ordered array. Alternatively, the assembly of a higher order nucleosomal array may sequester a factor that activates the checkpoint kinase Mec1p (or ATM1 in mammals), as long as chromatin remains in an "open" state. For instance, let us propose that "free" yKu is part of the signal; as SIR complexes assemble at sites of DSB repair, or as they assemble onto subtelomeric nucleosomes, the newly assembled heterochromatin itself may extinguish the checkpoint signal by sequestering yKu. An analogous event could occur at telomeres. Thus, cell cycle progression may require the assembly of an ordered nucleosomal state that can occur only if DNA repair and end-replication events are completed. This model would provide a function for the silent chromatin found in subtelomeric regions, and would help explain why cells lacking chromatin assembly factors are hypersensitive to agents that provoke DNA damage.

Do the available data support such a hypothesis? If a checkpoint signal can be sequestered by heterochromatin, extra telomeres might be expected to titrate out a limiting factor. In this context, it is noteworthy that extra copies of linear minichromosomes are highly deleterious for yeast cell survival (*43*), although it is not known why. Alternatively, the assembly of chromatin may send a positive signal, such as modification of a repair factor or of the nucleosome assembly machinery, that promotes cell cycle progression. Although the exact mechanism is not yet clear, it is attractive to imagine that the sharing of components by the DNA repair and telomere replication systems allows both events to be linked to controls over cell cycle progression.

References

1. D. Shore, *Science* **281**, 1818 (1998).
2. V. A. Zakian, *Annu. Rev. Genet.* **30**, 141 (1996).
3. F. E. Pryde, H. C. Gorham, E. J. Louis, *Curr. Opin. Genet. Dev.* **7**, 822 (1997).
4. S. J. Boulton and S. P. Jackson, *Nucleic Acids Res.* **24**, 4639 (1996).
5. S. E. Porter, P. W. Greenwell, K. B. Ritchie, T. D. Petes, *Nucleic Acids Res.* **24**, 582 (1996).
6. S. Gravel, M. Larrivee, P. Labrecque, R. J. Wellinger, *Science* **280**, 741 (1998).
7. Y. Tsukamoto, J. Kato, H. Ikeda, *Nature* **388**, 900 (1997).
8. T. Laroche *et al.*, *Curr. Biol.* **8**, 653 (1998).
9. K. Mishra and D. Shore, *Curr. Biol.* **9**, 1123 (1999).
10. S. G. Martin, T. Laroche, N. Suka, M. Grunstein, S. M. Gasser, *Cell* **97**, 621 (1999).
11. C. I. Nugent *et al.*, *Curr. Biol.* **8**, 657 (1998).
12. R. M. Polotnianka, J. Li, A. J. Lustig, *Curr. Biol.* **8**, 831 (1998).
13. S. J. Boulton and S. P. Jackson, *EMBO J.* **17**, 1819 (1998).
14. A. Bianchi and T. de Lange, *J. Biol. Chem.* **274**, 21223 (1999).
15. H. L. Hsu, D. Gilley, E. H. Blackburn, D. J. Chen, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 12454 (1999).
16. Y. Yamaguchi-Iwai *et al.*, *EMBO J.* **18**, 6619 (1999).
17. A. Nussenzweig *et al.*, *Nature* **382**, 551 (1996).
18. Y. Gu *et al.*, *Immunity* **7**, 653 (1997).
19. S. M. Bailey *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 14899 (1999).
20. S. Ahmed and J. Hodgkin, *Nature* **403**, 159 (2000).
21. Y. Corda *et al.*, *Nature Genet.* **21**, 204 (1999).
22. A. Matsuura, T. Naito, F. Ishikawa, *Genetics* **152**, 1512 (1999); K. B. Ritchie, J. C. Mallory, T. D. Petes, *Mol. Cell. Biol.* **19**, 6065 (1999).
23. A. D. McAinsh, S. Scott-Drew, J. A. Murray, S. P. Jackson, *Curr. Biol.* **9**, 963 (1999).
24. K. D. Mills, D. A. Sinclair, L. Guarente, *Cell* **97**, 609 (1999).
25. K. Dubrana, S. Martin, S. M. Gasser, unpublished data.
26. S. E. Lee, F. Paques, J. Sylvan, J. E. Haber, *Curr. Biol.* **9**, 767 (1999).
27. S. U. Astrom, S. M. Okamura, J. Rine, *Nature* **397**, 310 (1999).
28. V. Lundblad and E. H. Blackburn, *Cell* **73**, 347 (1993).
29. P. Schar, G. Herrmann, G. Daly, T. Lindahl, *Genes Dev.* **11**, 1912 (1997).

30. G. Herrmann, T. Lindahl, P. Schar, *EMBO J.* **17**, 4188 (1998).
31. W. Ramos, G. Liu, C. N. Giroux, A. E. Tomkinson, *Nucleic Acids Res.* **26**, 5676 (1998).
32. S. Marcand, E. Gilson, D. Shore, *Science* **275**, 986 (1997).
33. A. Ray and K. W. Runge, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 15044 (1999).
34. D. J. Burke, *Curr. Opin. Genet. Dev.* **10**, 26 (2000).
35. J. K. Tyler *et al.*, *Nature* **402**, 555 (1999).
36. S. Enomoto, P. D. McCune-Zierath, M. Gerami-Nejad, M. A. Sanders, J. Berman, *Genes Dev.* **11**, 358 (1997).
37. P. D. Kaufman, R. Kobayashi, B. Stillman, *Genes Dev.* **11**, 345 (1997).
38. E. K. Monson, D. de Bruin, V. A. Zakian, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 13081 (1997).
39. S. Le, C. Davis, J. B. Konopka, R. Sternglanz, *Yeast* **13**, 1029 (1997).
40. A. Verreault, P. D. Kaufman, R. Kobayashi, B. Stillman, *Cell* **87**, 95 (1996).
41. A. Taddei, D. Roche, J. B. Sibarita, B. M. Turner, G. Almouzni, *J. Cell Biol.* **147**, 1153 (1999).
42. J. G. Moggs *et al.*, *Mol. Cell. Biol.* **20**, 1206 (2000); Z. Kelman and J. Hurwitz, *Trends Biochem. Sci.* **23**, 236 (1998).
43. K. W. Runge, R. J. Wellinger, V. A. Zakian, *Mol. Cell. Biol.* **11**, 2919 (1991).

REVIEW

Splitting the Chromosome: Cutting the Ties That Bind Sister Chromatids

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In eukaryotic cells, sister DNA molecules remain physically connected from their production at S phase until their separation during anaphase. This cohesion is essential for the separation of sister chromatids to opposite poles of the cell at mitosis. It also permits chromosome segregation to take place long after duplication has been completed. Recent work has identified a multisubunit complex called cohesin that is essential for connecting sisters. Proteolytic cleavage of one of cohesin's subunits may trigger sister separation at the onset of anaphase.

Back to Basics: Chromosome Mechanics

Instructions for the behavior of every cell in the bodies of worms, flies, and humans will soon reside in public databases for all to read. A complete set of such instructions, packaged as chromosomes, is inherited by most cells in our body. Because of this, many if not most somatic nuclei in mammals are totipotent; that is, they are capable of programming all of mammalian development when injected into enucleated eggs (*1*). The cloning of Dolly had dramatic practical consequences, but its feasibility was never improbable on theoretical grounds. How cells inherit two complete packages of the genome at each cell division is one of the most fundamental questions in biology (Fig. 1A).

Recent studies of the chromosome cycle have concentrated on control mechanisms, such as the crucial part played by cyclin-dependent protein kinases in triggering chromosome duplication and segregation (*2*) and surveillance mechanisms (checkpoints) that monitor the fidelity of these two processes (*3*). This focus on "control" is, however, a recent phenomenon. Earlier studies, largely cytological in nature, concentrated on the mechanics of chromosome segregation (*4*–*7*). What, for example, was "the nature of the initial act of doubling of the spireme thread (chromosome)" (*5*, p. 109), and how were the sister threads moved to opposite poles of the cell during mitosis?

The elucidation of DNA's structure largely answered the first of these questions (*8*), and work on cytoskeletal proteins like tubulin and the spindle fibers assembled from it has gone a long way toward solving the mystery of chromosome movement. In contrast, until recently the mechanisms by which sister chromatids are tied together after chromosome duplication and then separated at the metaphase-to-anaphase transition was largely neglected, despite being equally crucial for the mitotic process (*9*).

Importance of Sister Cohesion

The ability of eukaryotic cells to delay segregation of chromosomes until long after their duplication distinguishes their cell cycle from that of bacteria, in which chromosome segregation starts soon after the initiation of DNA replication (*10*). This temporal separation forms the basis for the cell cycle's partition into four phases—G_1, S, G_2, and M—and it has played a central role in the evolution of eukaryotic organisms. Meiosis, during which two rounds of chromosome segregation follow a single round of duplication, requires separable S and M phases. Furthermore, mitotic chromosome condensation, without which large genomes cannot be partitioned between daughter cells at cell division, would not be possible if chromosome segregation coincided with DNA replication. A gap between S and M phases therefore made possible the evolution of large genomes. It is sister chromatid cohesion that permits chromosome segregation to take place long after duplication. Cohesion provides a memory of a duplication process that may have occurred long ago (up to 50 years in the case of human oocytes)—a memory that defines which chromatids within a nucleus are to be parted from each other at cell division. Were chromatids to drift apart before building a mitotic spindle, there would be no way for cells to determine whether chromatids were sisters (to be segregated to opposite poles) as opposed to being merely homologous chromosomes, a distinction that is crucial for all diploid organisms.

The structures holding sister chromatids together are responsible for generating bilaterally symmetrical chromosomes during mitotic divisions. The bilateral symmetry of chromosomes underlies the symmetry of the spindle apparatus and hence forms the basis for the exact and symmetrical partition of chromosomes and the roughly equal partition of most other cell constituents at cell division. In addition, tying sister chromatids together generates a centromere geometry that favors the attachment of sister kinetochores to spindles that extend to opposite poles. Only those kinetochore-spindle connections that result in tension are stabilized, which enables the chromosome alignment process to be proofread (*11*). Despite its importance, the mechanism by which sister chromatids are tied together is still poorly understood.

Chromatid Separation Independent of the Spindle Apparatus

The chromatid separation process has also remained mysterious. It is an autonomous process that does not directly depend on the mitotic spindle (*5*, *7*). This is most vividly seen in cells whose spindles have been destroyed by spindle poisons such as colchicine. In many organisms, in particular in plant cells, the cell cycle delay induced by colchicine is only transient, and chromatids eventually split apart in the complete absence of a mitotic spindle (*12*, *13*) (Fig. 2).

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