VIEWPOINT

Gatekeepers of the Nucleus

Susan R. Wente

Nuclear pore complexes (NPCs) form the site for entry and exit from the nucleus. A convergence of studies have defined the physical framework for the nuclear transport mechanism. This includes definition of the soluble transport machinery required for protein and RNA movement, x-ray structure analysis of transport factors, definitive compositional analysis of yeast NPCs, and documentation of the relative steady state arrangement of NPC components within the portal. With this information, researchers are now in the exciting position to examine the dynamic interplay between shuttling transport factors and the static pore complex.

The nuclear envelope has been referred to as the karyotheca (*1*), a coat or sheath protecting the genomic material. The NPCs are large proteinaceous structures that traverse this coat and serve as the gatekeepers for both spatially and temporally segregating the genomic material from all cytosolic processes. NPCs allow controlled nucleocytoplasmic exchange, which is essential for proper cell growth and progression through the cell cycle, responses to extracellular and developmental signals, and viral proliferation. Important advances have been made in the understanding of how protein and RNA macromolecules are recognized by the soluble transport machinery, and models for the mechanisms by which these complexes cross the NPC have been proposed. This has been largely achieved by a reductionist approach to the problem.

Higher Orders of Resolution for the Nuclear Transport Machinery

Fifty years ago, the first descriptions of "pores" and "annuli" in nuclear envelopes were reported in electron microscopy (EM) studies (*2*). The hallmark eightfold structural symmetry of the annuli was visualized shortly thereafter, and researchers proposed that the composition of the annulus was distinct from the membrane (*3–5*). By the end of the 1950s, the term "nuclear pore complex" was coined (*6*). However, over the past half century, researchers have struggled to analyze the structure and function of this organelle at a finer level. Today, it is clear that the NPC is a universal feature of the nuclear envelope in all eukaryotes, and the physical structure has been resolved.

Molecular aspects of NPC structure have been directly attacked with a variety of tools and strategies. The early EM view of an octagonal cylinder has evolved to an elaborate structure consisting of at least three separate structural elements (Fig. 1): (i) cytoplasmic fibrils, (ii) the central core, and (iii) the nuclear basket. The central core is formed from eight spokelike structures that encircle a central plug structure and are sandwiched between two rings. The NPC is built from protein components termed "nucleoporins" (Nups). The molecular characterization of at least 25 distinct Nups in *Saccharomyces cerevisiae* has occurred at a steady pace since 1990, on the basis of the combined successes of classic biochemical, cell biological, and genetic analyses in many laboratories [reviewed in (*7–9*)]. This compositional dissection culminated with a tour de force analysis of yeast *S. cerevisiae* NPCs (*10*), involving collaborative efforts of the Rout, Aitchison, and Chait laboratories to identify every polypeptide present in a purified NPC preparation (*10*). After a combination of protein sequencing and mass spectrometry, each candidate was epitope tagged and tested for cofractionation with NPCs and substructural localization. This work is remarkable, not so much for the identification of three new NPC-associated proteins, but rather for the comprehensiveness of the approach (*10*). From not knowing the number of yeast Nups with confidence, we now know the relative stoichiometries and steady state substructural localizations for the ~30 yeast Nups (Fig. 1D).

At least 65% of the yeast *S. cerevisiae* Nups have clear orthologs in vertebrate genomes (*9*). The vertebrate NPC is predicted to be larger than that of *S. cerevisiae* with ~50 components (*11–13*). A similar proteomics approach could be used to conclusively define vertebrate NPC composition. The studies of the *S. cerevisiae* NPC revealed four distinct surface-accessible Nup localization patterns (*10*): symmetrical, strictly cytoplasmic, strictly nuclear, or a biased asymmetric distribution to either the cytoplasmic or nuclear side. Symmetrical localization was most common with many Nups present in two or four copies per spoke. This substantially improves the molecular resolution of the NPC architecture (Fig. 1D). The existence of Nups localized exclusively to either the cytoplasmic fibrils or nuclear basket was previously well established, particularly in vertebrates [(*14–16*); reviewed in (*9*)]. However, the new analysis documents that such asymmetric localization is true for only a small subset of yeast Nups (9 of 29) (*10*). Three of these are highly homologous, functionally redundant, and likely the result of gene duplications (*17*). The unique localizations may allow for discrete functions at specific nuclear transport steps.

Roughly one-third of the yeast NPC mass is composed by members of the FG (F, phenylalanine; G, glycine) Nup family (*10*). Each FG Nup harbors a domain with multiple GLFG, FXFG, or FG amino acid repeats separated by polar spacer sequences (L, leucine; X, any amino acid) (*7*). In vertebrates and yeast, different FG Nups reside in each of the NPC substructures (Fig. 1D) (*9, 10*); 160 copies of FG repeat domains are present in each NPC, with half being symmetrically positioned and half being asymmetrically positioned (*10*). They comprise most asymmetrically localized Nups (seven of nine). Overall, this progress rekindles the excitement generated with the first EM visualization of the NPC and leaves us poised to determine how these structures operate as the nuclear gatekeeper.

Coupled Complexity and Simplicity for the Mechanism of Transport

The semipermeable barrier formed by the NPC allows small molecules to passively diffuse between the nucleus and cytoplasm, limited only by their molecular size. How protein and RNA macromolecules cross the NPC invokes a higher level of control. Early models suggested that compartment-specific localization in the nucleus was due simply to diffusion through the pore followed by binding and retention in the given compartment. However, with the discovery in the mid-1980s that a simple or bipartite basic amino acid sequence could serve as a specific nuclear localization signal [a classic nuclear localization signal (cNLS)] (*18, 19*), a series of experiments showed that nucleocytoplasmic transport reflects active movement against a concentration gradient (*20, 21*). Moreover, competition experiments with different types of import and export substrates indicated that distinct saturable pathways exist [reviewed in (*22, 23*)]. The import of U small nuclear ribonucleoprotein particles (Usn RNPs) does not compete with the cNLS pathway. The export of transfer RNA, 5*S* ribosomal RNA, Usn RNA, and messenger RNA (mRNA) are each largely independent. Indeed, recent find-

Department of Cell Biology and Physiology, Box 8228, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110, USA. E-mail: swente@cellbio.wustl.edu

ings have confirmed that each substrate class possesses unique transport signals and requires distinct transport mediators (*24*).

Combining a key in vitro protein import assay with subcellular fractionation showed that the receptor for cNLS-based import was physically distinct from the NPC (*25*). The first biochemically identified transport factor was the small guanosine triphosphatase (GTPase) Ran (*26, 27*), quickly followed by an adapter protein (karyopherin α/importin α/NLS receptor/Srp1/Kap60) (*28–30*) and a transport receptor (karyopherin β1/importin β/p97/PTAC97/Kap95/Rsl1) (*31–34*). Each protein performs a specific role in the import of the cNLS substrate (Fig. 2).

At this point, the nuclear transport field witnessed an explosion in knowledge. After molecular identification of the first receptor, genome comparisons revealed a superfamily of proteins with limited homology (*35–37*). The molecular mapping of other signals (for example, a nuclear export signal) and the biochemical demonstration that the distinct signal receptors were all members of this superfamily were exciting steps forward [reviewed in (*24, 38*)]. Each family member likely recognizes a different transport signal. Because of coincident discovery by multiple laboratories, the superfamily and family members are referred to by multiple names (including karyopherin, importin, exportin, and transportin). There are 14 different *S. cerevisiae* karyopherins that facilitate the entry and exit, and the number in human genome is likely more than 20 (*24, 39*).

Overall, the common properties that define a karyopherin directly delineate the transport mechanism: substrate binding, RanGTP binding, interaction with Nups, and nucleocytoplasmic shuttling (Fig. 2). The energy requirements for this process are unclear, and GTP hydrolysis by Ran is likely not required for movement [reviewed in (*24*)]. RanGTP binding to a karyopherin does play a critical role in modulating karyopherin-cargo interaction. The sharply contrasting distributions for the cytoplasmic GTPase–activating protein and the nuclear guanine-nucleotide exchange factor results in a nuclear RanGTP pool and rapid conversion of any cytoplasmic Ran to the guanosine diphosphate (GDP) form. During import, the karyopherin-cargo complex binds RanGTP after entering the nucleus, and this triggers release of the cargo. In contrast, during export, nuclear RanGTP binding stabilizes the karyopherin-cargo complex until the cytoplasm is reached, and the conversion to RanGDP results in cargo release. Thus, the Ran GTP-bound status confers directionality in transport (*24*). Precisely how RanGTP binding to the karyopherin influences cargo binding has been exquisitely detailed in recent x-ray crystallographic studies (*40, 41*). The structures of Ran and karyopherins provide key information for experimental tests of the transport mechanism.

Strikingly, there are transport pathways, such as mRNA export, that apparently do not require a karyopherin family member [summarized in (*42*)]. Insights from yeast and viral RNA export systems have identified Mex67/Tap as a central player [(*43, 44*); reviewed in (*24*)]. Mex67/Tap binds RNA, a heterogenous nuclear RNP (hnRNP) protein, and Nups, as well as undergoing nucleocytoplasmic shuttling. Other factors are also required (*45*), yet how their actions are coordinated to mediate mRNA export is unknown.

The complexity intrinsic to the nuclear transport mechanism arises from the large number of components and their many isoforms (including transport factors, signals, and Nups), the diversity of connections between these components, and the selectivity and specificity at each level of transport. This complexity provides a molecular explanation for the range of substrate types that a eukaryotic cell must transport. Despite the plethora of signals and receptors, all NPCs are thought to be of identical composition, and a single NPC can mediate both import and export (*46, 47*). Thus, the nuclear transport mechanism is also beautifully simplistic.

Moving Through the NPC

The common denominator for the mechanism of active transport is physical interaction with a Nup (Fig. 2). Pioneering experiments showed that the binding of the lectin wheat germ agglutinin to NPCs inhibited nuclear transport and suggested that particular components of the NPC were important (*48–50*). This was confirmed biochemically when the soluble factors and Nups became available.

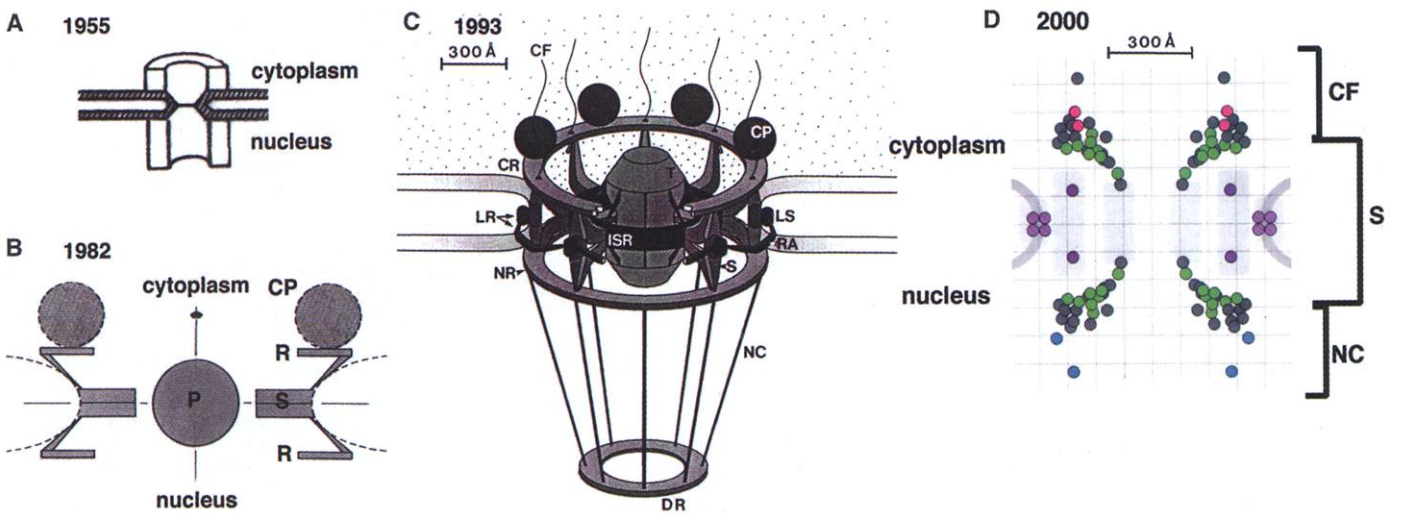

Fig. 1. The NPC at increasing structural resolution. Over the past 50 years, a series of EM studies have resolved the structural features of the vertebrate and yeast NPCs at an increasingly fine level. Illustrations based on representative structures are presented from different points in time. The models are shown in section perpendicular to the nuclear envelope and are reproduced from those in the original publications [reproduced from (*4*) by copyright permission of Academic Press and from (*10*), (*66*), and (*67*) by copyright permission of The Rockefeller University Press]. (**A**) From the work of Afzelius in 1955, a cylindrical mass was observed in the nuclear envelope of sea urchin oocytes (*4*). (**B**) EM studies by Unwin and Milligan resolved the structure of NPCs in *Xenopus* oocyte nuclear envelopes using Fourier averaging methods (*66*). CP, cytoplasmic particles; R, rings; S, spokes; P, central plug. (**C**) In 1993, Akey and Radermacher reported the three-dimensional reconstruction of *Xenopus* NPCs resolved by cryo-EM technology (*67*). CF, cytoplasmic filaments; CP, cytoplasmic particles; CR, cytoplasmic ring; NR, nucleoplasmic ring; LR, lumenal ring; LS, lumenal spoke; RA, radial arms; ISR, inner spoke ring; S, spokes; T, transporter; NC, nuclear cage (basket); DR, distal ring. (**D**) Rout and colleagues mapped the relative surface-accessible locations of the yeast *S. cerevisiae* Nups on the yeast NPC structure by immunoEM (*10*). Each circle represents a Nup position with green for symmetrical FG Nups, blue for strictly nuclear FG Nups, red for strictly cytoplasmic Nups, gray for non-FG Nups, and purple and purple stripes for different integral membrane proteins associated with the NPC.

Members of the karyopherin superfamily interact directly with the FG Nups [reviewed in (*51*)], many of which are modified on serine and threonine residues by the O-linked addition of monosaccharidic *N*-acetyl glucosamine in vertebrate cells. At least two of the factors involved in mRNA export also associate with FG Nups.

Biochemical and EM evidence strongly suggests that the first and last steps of transport are defined by interaction with asymmetrically localized FG Nups at the cytoplasmic fibrils and nuclear basket (*52–54*). How such docked complexes move between the NPC faces is unresolved (Fig. 2). Most models have invoked facilitated diffusion controlled by association and disassociation with FG Nups (*10, 55*), and the wealth of NPC architectural information will now allow direct tests of such models (Fig. 1, C and D). Roles for classic motor proteins may not be essential because of their notable absence in yeast NPCs (*10*). A series of binding reactions with graded affinities along FG Nup "tracks" also seems unlikely, given the symmetrical localization for many FG Nups (*10*). The possibility that RanGTP-based vectorality is sufficient for directionality has been considered (*56*); however, when not bound to cargo, some karyopherins traverse the NPC in a Ran-independent manner [reviewed in (*24*)]. A direct role for Ran is also unclear for non-karyopherin transport pathways.

Does the NPC itself exert any control on nucleocytoplasmic exchange, influencing selectivity, rate, and/or directionality? There is evidence that NPC architecture is important. The direction of transport across an NPC is not reversible in vitro (*47*), and the distinct FG repeat domains are not necessarily functionally interchangeable (*57*). In addition, different transport factors show binding preferences for different subsets of Nups (*51*), and two karyopherins that use the same Nup bind to discrete domains (*58, 59*). To further resolve the mechanism, we must rigorously determine exactly how many transport factor–Nup binding events are required to propel the cargo through the central NPC channel. Overall, a notable missing link is the structure of a karyopherin docked at an FG Nup. Does karyopherin binding change the structure of the NPC or does the Nup influence the karyopherin? The elegant NPC architecture provides fuel for the debate on how Nups facilitate macromolecular movement.

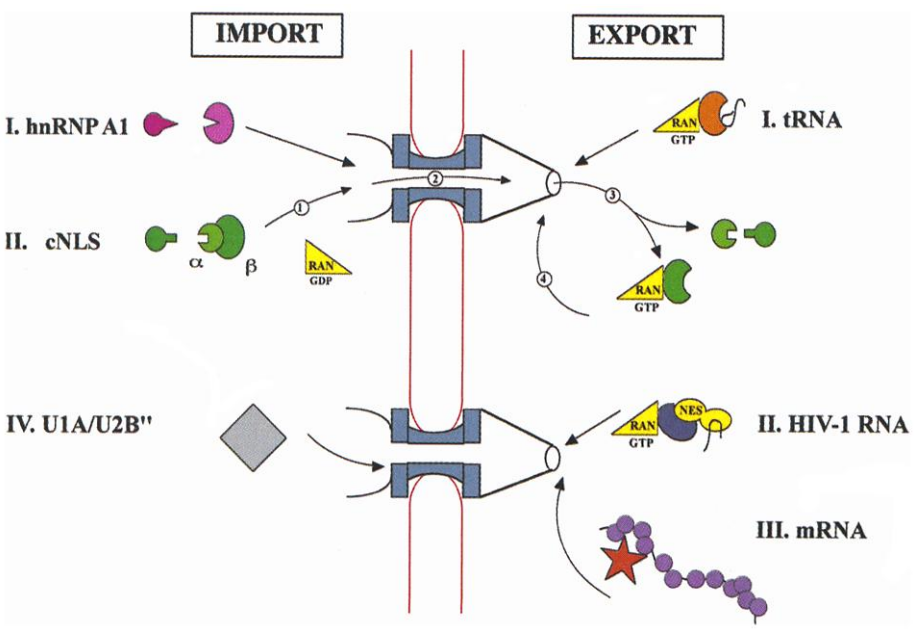

Fig. 2. Pathways for movement through the NPC. Examples of transport mechanisms for nuclear import (left) and export (right) are shown. In each case, a direct physical interaction with a Nup is presumably required. In mechanism I, cargo directly interacts with a karyopherin family member. This is typified by the import of hnRNP A1 by transportin (dark pink) (*68*) and the export of transfer RNA by exportin t (orange) (*69*). In mechanism II, cargo binds an adapter that interacts with a karyopherin. The pathway for importing cNLS substrates is shown (α and β in green) [reviewed in (*38, 39*)] as well as the export of human immunodeficiency virus RNA by Rev and Crm1/exportin (purple) (*70*). As shown in mechanism III, cargo can also bind directly to a non-karyopherin–shuttling transport factor. The export of mRNA by Mex67/Tap is possibly in this class (red star) [(*43, 44*); reviewed in (*24*)]. In mechanism IV, cargo may also be able to mediate its own transport by direct interaction with the NPC, as recently suggested for the import of U1A/U2B" spliceosome proteins (gray) (*42*). A general mechanism for nuclear import is illustrated with the cNLS pathway (mechanism II). The first committed step in karyopherin-based transport across the NPC requires receptor recognition of the particular signal. In a sequential manner, (i) the receptor-cargo complex docks at the NPC, (ii) translocates through the pore to the opposing face, (iii) the cargo is released, and (iv) the shuttling transport factors are recycled. For import, RanGTP in the nucleus triggers cargo release (yellow triangles). For export, RanGTP stabilizes the karyopherin-cargo interaction, and conversion to RanGDP in the cytoplasm results in the disassociation of receptor and cargo [reviewed in (*24*)].

Entering the Next NPC Century

Research efforts in the past decade have focused on identifying the factors required for nucleocytoplasmic exchange. The rapid progress has been in no small part due to the coupling of basic discoveries with information from both the yeast and human genome projects. The genomic information is currently primed for allowing further functional analysis on a grand scale, including pairwise protein-protein interaction analysis to identify transport substrates and Nup-Nup nearest neighbors (*60, 61*). The challenge now is to determine the precise mechanism for movement through the portal. In addition, we are only beginning to unravel the strategies for regulating and controlling transport (*62–65*). Continued fundamental work on the molecules that compose the transport machinery and examination of the dynamic interface between transport factors and Nups will undoubtedly provide key insight into the NPC gatekeepers.

References and Notes

1. N. G. Anderson, *Science* **117**, 517 (1953).
2. H. G. Callan and S. G. Tomlin, *Proc. R. Soc. London Ser. B* **137**, 367 (1950).
3. J. G. Gall, *Exp. Cell Res.* **7**, 197 (1954).
4. B. A. Afzelius, *Exp. Cell Res.* **8**, 147 (1955).
5. J. G. Gall, *J. Cell Biol.* **32**, 391 (1967).
6. M. L. Watson, *J. Biophys. Biochem. Cytol.* **6**, 147 (1959).
7. M. P. Rout and S. R. Wente, *Trends Cell Biol.* **4**, 357 (1994).
8. V. Doye and E. Hurt, *Curr. Opin. Cell Biol.* **9**, 401 (1997).
9. D. Stoffler, B. Fahrenkrog, U. Aebi, *Curr. Opin. Cell Biol.* **11**, 391 (1999).
10. M. P. Rout *et al.*, *J. Cell Biol.* **148**, 635 (2000).
11. B. Fahrenkrog, E. C. Hurt, U. Aebi, N. Panté, *J. Cell Biol.* **143**, 577 (1998).
12. Q. Yang, M. P. Rout, C. W. Akey, *Mol. Cell* **1**, 223 (1998).
13. B. M. Fontoura, G. Blobel, M. J. Matunis, *J. Cell Biol.* **144**, 1097 (1999).
14. C. M. Snow, A. Senior, L. Gerace, *J. Cell Biol.* **104**, 1143 (1987).
15. J. Sukegawa and G. Blobel, *Cell* **72**, 29 (1993).
16. D. Kraemer, R. W. Wozniak, G. Blobel, A. Radu, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 1519 (1994).
17. S. R. Wente and G. Blobel, *J. Cell Biol.* **125**, 955 (1994).
18. C. Dingwall, S. Sharnick, R. Laskey, *Cell* **30**, 449 (1982).
19. D. Kalderon, B. L. Roberts, W. D. Richardson, A. E. Smith, *Cell* **39**, 499 (1984).
20. D. Goldfarb, J. Gariepy, G. Schoolnik, R. Kornberg, *Nature* **322**, 641 (1986).
21. R. Lanford, P. Kanda, R. Kennedy, *Cell* **37**, 575 (1986).
22. D. Goldfarb and N. Michaud, *Trends Cell Biol.* **1**, 20 (1991).
23. E. Izaurralde and I. W. Mattaj, *Cell* **81**, 153 (1995).
24. S. Nakielny and G. Dreyfuss, *Cell* **99**, 677 (1999).
25. S. A. Adam, R. S. Marr, L. Gerace, *J. Cell Biol.* **111**, 807 (1990).
26. M. S. Moore and G. Blobel, *Nature* **365**, 661 (1993).
27. F. Melchior, B. Paschal, J. Evans, L. Gerace, *J. Cell Biol.* **123**, 1649 (1993).

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).

Swiss Institute for Experimental Cancer Research, Chemin des Boveresses 155, CH-1066 Epalinges, Switzerland. E-mail: sgasser@eliot.unil.ch