Commentary

How do tumors make ends meet?

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When Barbara McClintock irradiated strains of Indian corn in the early 30s, she identified ring chromosomes, which she soon realized were a special case of chromosomes broken by radiation; the broken ends sometimes fused to one another and formed a ring (1, 2). This discovery led McClintock to hypothesize the existence of a special structure at the chromosome tip that would maintain chromosome stability. In 1941 she described the breakage-fusion-bridge cycle, a model for a repeating pattern of chromosome behavior that is triggered by an initial breakage (3). Normally, each chromatid strand has one centromere, and the chromosome ends remain capped by the telomeres that protect the ends from sticking to one another. But sometimes, harmful substances or radiation damage a chromatid and cause it to break. Without telomere caps, the new ends stick to each other, and the resulting fused chromosome has two centromeres as well as a duplication of some of the genes from that chromosome. When cell division occurs, the two centromeres of this unusual chromosome may be pulled to the opposite spindle poles of the cell, forming an irregular, long chromosome bridge between the two newly forming daughter cells (Fig. 1 *A* and *B*). Eventually, the abnormal chromatid breaks in two or may be left behind during cell division. If the chromosome ends are broken, they are likely to rejoin again, reforming a chromosome bridge at the next division.

In this issue of PNAS, Gisselsson *et al.* (4) provide some experimental evidence that telomere dysfunction triggers extensive chromosome fragmentation through persistent bridge-breakage events in solid tumors that consequentially leads to a continuous reorganization of the tumor genome. Their findings shed some light on a possible connection between telomere function and the origin of chromosomal instability (CIN) in human cancers.

Over the last decades, the cytogenetic analyses of thousands of tumor karyotypes have lead to the conclusion that aneuploidy was rampant in cancers (5–7), so much so that some investigators questioned whether any truly diploid cancers could exist. When single clones derived from such aneuploid tumors are grown *in vitro* and analyzed cytogenetically, they virtually always display high rates of chromosome losses and gains, resulting in dramatic karyotypic variability from cell to cell (8). This form of genetic instability, termed CIN, has been observed in many solid tumors including those arising from the colon, breast, prostate, oropharynx, and lung (9–12). The important biological conclusion emerging from these studies was that the aneuploid state typical of most solid cancers does not simply reflect clonal expansion of a rare aberrant cell but that in fact the underlying rate of chromosome losses/gains is elevated in cells evolving toward cancer (13). These findings are consistent with the hypothesis that in order for a cancer cell to accumulate the multiple genetic alterations required for tumorigenesis during a single human lifetime, it must become genetically unstable, i.e., it must have a sustained increase in its mutation rate relative to normal, nonmalignant cell types (14).

It is also widely appreciated that the vast majority of cancers frequently exhibit gross structural alterations (i.e., translocations, deletions, and amplifications; ref. 6). New chromosome painting techniques have dramatically facilitated the analysis of such complex chromosomal changes (15, 16). Chromosome translocations are detectable cytogenetically as a fusion between different chromosomes or between normally noncontiguous segments of a single chromosome. At the molecular level, such translocations can give rise to fusions between two different genes, endowing the fused transcript with tumorigenic properties. Specific translocations can render a selective advantage to the cell and allow the outgrowth of cancer cell clones carrying such an aberration.

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In leukemias and lymphomas, translocations are characteristic of specific disease entities and seem critical for the development of these neoplasms (17, 18). The detailed molecular analysis of such breakpoint regions led to the identification of important oncogenes and in at least two cases helped the development of rationales for cancer drug implementation and efficacy, for example, acute promyelocytic leukemias (APL; refs. 19 and 20) and chronic myelogenous leukemias (CML; refs. 17, 21, and 22). Recent studies have generated optimism for the future impact of genetics on cancer drug target identification (23, 24).

Similar structural aberrations have been detected in some sarcomas, e.g., the 11;22 translocation in Ewing sarcoma (25). For most human carcinomas, however, links between a specific chromosomal breakpoint and consequential gene abnormalities that are involved frequently in a certain solid tumor type have not been described. The occurrence, frequency, and significance of translocations in solid tumors have remained a mystery. Are most translocations in solid tumors distributed randomly over the genome or much like in leukemias indicative of regions carrying specific cancer gene fusions? The majority of solid tumors exhibit complex patterns of chromosomal abnormalities, rarely showing any direct association with specific morphological or prognostic subgroups. And translocations, especially those occurring in common epithelial tumors, were reported to occur nearly random with respect to the chromosomal positions involved (26). The molecular characterization of each and every one of these numerous chromosomal translocations will reveal whether any (specific) genes or their regulatory sequences are disrupted frequently by such breakpoints.

Are translocations in solid tumors solely side effects of cells under stress? Is their frequency perhaps coupled to an abnormal chromosomal segregation at cell division indicative of CIN cancers? Or, do translocations represent an independent form of genetic instability? Most interestingly, what causes translocations in solid tumors? The existence of genetic alterations in a tumor, even when frequent, does not mean that the tumor is genetically unstable. Instability is by definition a matter of rate, and the mere existence of a translocation provides no information about the rate of its occurrence.

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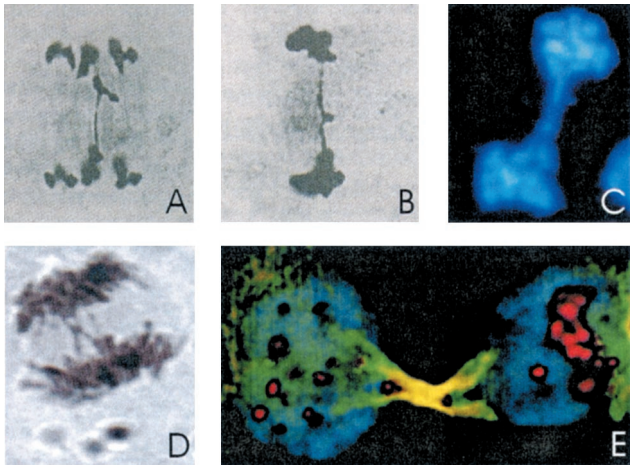

Fig. 1. Anaphase bridges. (*A* and *B*) Images from the late 1930s show Indian corn chromosomes that have duplicated and separated. A "chromatin bridge" connects one chromosome from the upper set to one from the lower set (ref. 53; reproduced courtesy of B. McClintock and the American Philosophical Society Library). (*C*) Chromosome spread prepared from pistils of a late-generation telomerase-deficient mustard weed mutant. End-to-end chromosome fusions are visible in anaphase. Chromosomes are stained with 4′,6′-diamidino-2-phenylindole (ref. 40; courtesy of K. Riha and D. E. Shippen, Texas A&M University, College Station, TX). [Reproduced with permission from ref. 40 (Copyright 2001, AAAS).] (*D*) Anaphase bridges in the moderately differentiated human pancreatic carcinoma LPC6 (ref. 4; courtesy of D. Gisselsson, University Hospital, Lund, Sweden). (*E*) Anaphase bridges in the oral carcinoma UPCI: SCC131. Centromeres (red) are trapped in the forming midbody as this late telophase cell divides. Immunolabeling with tubulin antibodies (yellow), chromosomes stained with 4′,6′-diamidino-2-phenylindole (blue; courtesy of W. S. Saunders and S. M. Gollin, University of Pittsburgh and the University of Pittsburgh Cancer Institute, Pittsburgh, PA). [Reproduced with permission from ref. 9 (Copyright 2000, National Academy of Sciences).]

Studies showing that the total number of such chromosomal aberrations is roughly proportional to the risk of metastases (27) could argue potentially for the existence of such an instability. However, although translocations in solid tumors may foster tumor progression, there has been no experimental evidence that translocations are the result of a specific genetic instability that allows them to occur at higher frequency than in normal cells. In contrast, it has been shown that some of these translocations represent an aberrancy of the normal recombination processes leading to Ig or T cell receptor gene rearrangements and that similar translocations occur in normal cells of lymphoid origin (28). Further progress in defining translocation instabilities are limited currently by the lack of simple assays to define the rate at which such endogenous events occur in either normal or neoplastic mammalian cells. Studies are complicated further by the fact that cells that have undergone DNA breakage are normally prevented from further proliferation by a series of checkpoints (29). A continuous accumulation of chromosomal changes in a cell population may thus arise through an elevated mutation frequency, a decreased efficiency to self-eliminate damaged cells, or both.

Gisselsson *et al.* present in their paper the construction of chromosome breakpoint profiles of ≈100 pancreatic carcinomas and 140 osteosarcomas (4). They report that tumors with few chromosomal alterations showed a preferential clustering of breakpoints to the terminal bands, whereas tumors with many changes showed primarily interstitial and centromeric breakpoints. This is an interesting finding. In addition, the authors evaluated mitotic figures of the tumors and could find anaphase bridges in all cases analyzed (Fig. 1*D*). Such anaphase abnormalities were shown before to occur in solid tumors with complex chromosome abnormalities including head and neck, ovarian, and pancreatic carcinomas (refs. 9 and 30; Fig. 1*E*); and breakage-fusion-bridge events have been shown previously to cause complex alterations in chromosome structure (9, 30).

Barbara McClintock suggested that nondisjunction of chromosomes suspended in anaphase bridges could lead to gains and losses of genetic material (3). Concurrent breaks in two different chromosomes may either give rise to translocations or dicentric chromosomes. Whereas translocation derivatives are stably transmitted through cell division, dicentric chromosomes may form anaphase bridges. These bridges may subsequently break, and the chromosomes are transmitted to the daughter cells with broken ends that may recombine further during the subsequent interphase. Thus, chromosomal damage may not only result in static aberrations such as translocations, inversions, deletions, and duplications, but it may result also in mitotically unstable chromosomes (3, 31) and contribute to CIN (13). Indeed, the data of Gisselsson *et al.* (4) as well as previous cytogenetic analyses indicate that the number of whole-chromosome gains and losses increases with the total number of structural aberrations in approximately equal proportions.

Surprisingly, the authors observed little impairment of cellular survival in the studied cells. Less than 2% of cells showed evidence of necrosis or apoptosis. An only slight impact on cellular survival was also observed in human cells with high levels of CIN (32, 33), indicating an impairment or abrogation of the systems normally causing arrest or apoptosis. The inactivation of certain tumor suppressor and checkpoint genes such as p53 or APC (34, 35) facilitate a rather unproblematic proliferation of these cells even though these genes do not cause genetic instability *per se* (F. Bunz, C. Fauth, M. R. Speicher, A. Dutriaux, J. M. Sedivy, K. W. Kinzler, B. Vogelstein, and C.L., unpublished data). Also, the common association with abnormal centrosome function (36–38) might indicate that highly malignant cells have acquired some tolerance to massive genomic imbalances.

Lastly, in the Gisselsson *et al.* paper, the terminal breakpoint frequency was correlated inversely to telomeric TTAGGG repeat length. Fluorescence *in situ* hybridization with telomeric TTAGGG probes consistently indicated shortened telomeres, and several chromosome ends were lacking telomeric signals completely. In all but one chromosome arm of the osteosarcomas analyzed, terminal breaks occurred at lower levels of cytogenetic complexity than interstitial breaks, suggesting that the terminal band broke first. Telomeric association occurred in tumors with few aberrations, followed by rings and dicentrics, gains, and finally losses of chromosome arms (4). These findings make perfect sense conceptually. Telomere shortening, seen as the absence of detectable TTAGGG repeats, may compromise—very much similar to double strand breaks induced by irradiation—the integrity of chromosome ends and lead to the formation of rings and dicentrics. These abnormal structures may form bridges at anaphase that either break and initiate a series of breakage-fusion-bridge events or induce cytokinetic failure leading to the formation of binucleated cells with supernumerary centrosomes. Cells with an abnormal centromeric number may form multipolar mitoses at the next cell division. Thus, telomeric dysfunction may result in both structural and numerical CIN (2, 30, 39). Practically, however, things are as usual more complicated, and the hypotheses mentioned above are far beyond proven. The Gisselsson *et al.* paper does not solve the many open questions about telomere dysfunction and its relationship to genomic instability but further strengthens our assumptions about the protective power of telomeres in chromosomal integrity and

their guardian role in preventing human cancers.

It is well established that disruption of telomere maintenance is associated with end-to-end chromosome fusion in many organisms. Plants display a more plastic pattern of development than animals and seem to better tolerate extensive chromosomal rearrangements and ploidy changes. The mustard weed *Arabidopsis thaliana* can survive up to 10 generations without telomerase, but the last five generations of these telomerase-deficient plants endure severe and increasing levels of cytogenetic anomalies (Fig. 1*C*), which are correlated with developmental anomalies in both vegetative and reproductive organs (40). In the fission yeast *Saccharomyces pombe*, deletion of the telomerase reverse transcriptase (trt+) gene or both ATM homologs (tel1+ and rad3+) causes telomere shortening and eventual loss of growth. The few cells that escape growth arrest have circularized all three of their chromosomes (41, 42). In *Saccharomyces cerevisiae*, telomere dysfunction is associated with an increased frequency of gross chromosomal rearrangements involving terminal deletions. End-to-end chromosome fusion junctions have been isolated recently from yeast with short telomeres, indicating that breakage-fusion bridge is one mechanism for the creation of terminal deletions (43).

The increased cancer incidence in late-generation mTR−/− telomerase null mice implicates telomere dysfunction as a factor that can contribute to tumorigenesis (44, 45). The frequency of tumor formation in mTR−/− mice is enhanced by p53 deficiency, possibly because the lack of a response to DNA damage allows increased genetic instability. All these data suggest that telomere dysfunction may initiate CIN. It seems that the decline in telomere length rather than the absence of telomerase activity *per se* is the most important parameter dictating chromosomal integrity, because early generation mTR−/− mice, which still possess long telomeres, are cytogenetically and biologically normal. In late-generation mTR−/− p53−/− tumors, the frequencies of chromosome fusions, anaphase bridges, and nonreciprocal translocations increase significantly (46, 47). Also, the majority of tumors analyzed by Gisselsson *et al.* and by other research groups in earlier studies seem to express normal levels of the telomerase catalytic subunit TERT (4, 48).

Nonreciprocal translocations (NRTs) represent a specific type of chromosomal abnormality that, along with aneuploidy, is characteristic of human carcinomas (49). Balanced translocations, on the other hand, are common in human hematopoietic tumors but remain rare in carcinomas. Unlike balanced translocations that harbor activated oncogenes at their breakpoints, NRTs lead to loss of heterozygosity and change in ploidy of multiple genes—two key features of CIN. A loss of telomere capping function is likely to promote carcinogenesis by promoting NRTs and aneuploidy, resulting in global and radical changes in gene dosage. The Gisselsson *et al.* paper, the telomerase-deficient mouse model (47), and studies on the evolution of intestinal carcinoma in humans (50) are consistent with a model (51) in which telomere dysfunction promotes CIN that drives early carcinogenesis (52), whereas telomerase activation restores genomic stability to a level permissive for tumor progression. The problem of how tumors make ends meet remains "fantastically complex" (53).

1. McClintock, B. (1932) *Proc. Natl. Acad. Sci. USA* **18,** 677–681.
2. McClintock, B. (1938) *Genetics* **23,** 215–376.
3. McClintock, B. (1941) *Genetics* **26,** 234–282.
4. Gisselsson, D., Jonson, T., Petersén, Å., Strömbeck, B., Dal Cin, P., Höglund, M., Mitelman, F., Mertens, F. & Mandahl, N. (2001) *Proc. Natl. Acad. Sci. USA*, **98,** 12683–12688. (First Published October 2, 2001; 10.1073/pnas.211357798)
5. Nowell, P. C. (1976) *Science* **194,** 23–28.
6. Mitelman, F., Johansson, B. & Mertens, F. (1994) *Catalog of Chromosome Aberrations in Cancer* (Wiley–Liss, New York).
7. Li, R., Yerganian, G., Duesberg, P., Kraemer, A., Willer, A., Rausch, C. & Hehlmann, R. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 14506–14511.
8. Lengauer, C., Kinzler, K. W. & Vogelstein, B. (1997) *Nature (London)* **386,** 623–627.
9. Saunders, W. S., Shuster, M., Huang, X., Gharaibeh, B., Enyenihi, A. H., Petersen, I. & Gollin, S. M. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 303–308.
10. Miyoshi, Y., Iwao, K., Takahashi, Y., Egawa, C. & Noguchi, S. (2000) *Cancer Lett.* **159,** 211–216.
11. Beheshti, B., Park, P. C., Sweet, J. M., Trachtenberg, J., Jewett, M. A. & Squire, J. A. (2001) *Neoplasia (Bratisl.)* **3,** 62–69.
12. Takahashi, T., Haruki, N., Nomoto, S., Masuda, A., Saji, S., Osada, H. & Takahashi, T. (1999) *Oncogene* **18,** 4295–4300.
13. Lengauer, C., Kinzler, K. W. & Vogelstein, B. (1998) *Nature (London)* **396,** 643–649.
14. Loeb, L. A. (1991) *Cancer Res.* **51,** 3075–3079.
15. Schrock, E., du Manoir, S., Veldman, T., Schoell, B., Wienberg, J., Ferguson-Smith, M. A., Ning, Y., Ledbetter, D. H., Bar-Am, I., Soenksen, D., Garini, Y. & Ried, T. (1996) *Science* **273,** 494–497.
16. Speicher, M. R., Gwyn Ballard, S. & Ward, D. C. (1996) *Nat. Genet.* **12,** 368–375.
17. Rowley, J. D. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 152–156.
18. Le Beau, M. M. & Rowley, J. D. (1986) *Adv. Hum. Genet.* **15,** 1–54.
19. Kakizuka, A., Miller, W. H., Umesono, K., Warrell, R. P., Frankel, S. R., Murty, V. V., Dmitrovsky, E. & Evans, R. M. (1991) *Cell* **66,** 663–674.
20. Lim, L. C., Vellupillai, M. & Ghafar, A. A. (2000) *Med. Oncol.* **17,** 301–306.
21. Nowell, P. & Hungerford, D. (1960) *J. Natl. Cancer Inst.* **25,** 85.
22. Ben-Neriah, Y., Daley, G. Q., Mes-Masson, A. M., Witte, O. N. & Baltimore, D. (1986) *Science* **233,** 212–214.
23. Druker, B. J., Talpaz, M., Resta, D. J., Peng, B., Buchdunger, E., Ford, J. M., Lydon, N. B., Kantarjian, H., Capdeville, R., Ohno-Jones, S. & Sawyers, C. L. (2001) *N. Engl. J. Med.* **344,** 1031–1037.
24. Druker, B. J., Sawyers, C. L., Kantarjian, H., Resta, D. J., Reese, S. F., Ford, J. M., Capdeville, R. & Talpaz, M. (2001) *N. Engl. J. Med.* **344,** 1038–1042.
25. Delattre, O., Olschwang, S., Law, D. J., Melot, T., Remvikos, Y., Salmon, R. J., Sastre, X., Validire, P., Feinberg, A. P. & Thomas, G. (1989) *Lancet* **2,** 353–356.
26. Johansson, B., Mertens, F. & Mitelman, F. (1996) *Genes Chromosomes Cancer* **16,** 155–163.
27. Mitelman, F., Johansson, B., Mandahl, N. & Mertens, F. (1997) *Cancer Genet. Cytogenet.* **95,** 1–8.
28. Tycko, B., Palmer, J. D. & Sklar, J. (1989) *Science* **245,** 1242–1246.
29. Nigg, E. A. (2001) *Nat. Rev. Mol. Cell. Biol.* **2,** 21–32.
30. Gisselsson, D., Pettersson, L., Hoglund, M., Heidenblad, M., Gorunova, L., Wiegant, J., Mertens, F., Dal Cin, P., Mitelman, F. & Mandahl, N. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 5357–5362. (First Published April 18, 2000; 10.1073/pnas.090013497)
31. Haber, J. E., Thorburn, P. C. & Rogers, D. (1984) *Genetics* **106,** 185–205.
32. Jallepalli, P. V., Waizenegger, I. C., Bunz, F., Langer, S., Speicher, M. R., Peters, J., Kinzler, K. W., Vogelstein, B. & Lengauer, C. (2001) *Cell* **105,** 445–457.
33. Cahill, D. P., Lengauer, C., Yu, J., Riggins, G. J., Willson, J. K., Markowitz, S. D., Kinzler, K. W. & Vogelstein, B. (1998) *Nature (London)* **392,** 300–303.
34. Hollstein, M., Sidransky, D., Vogelstein, B. & Harris, C. C. (1991) *Science* **253,** 49–53.
35. Vogelstein, B. & Kinzler, K. W. (1998) *The Genetic Basis of Human Cancer* (McGraw–Hill, New York).
36. Doxsey, S. (1998) *Nat. Genet.* **20,** 104–106.
37. Sato, N., Mizumoto, K., Nakamura, M., Maehara, N., Minamishima, Y. A., Nishio, S., Nagai, E. & Tanaka, M. (2001) *Cancer Genet. Cytogenet.* **126,** 13–19.
38. Ghadimi, B. M., Sackett, D. L., Difilippantonio, M. J., Schrock, E., Neumann, T., Jauho, A., Auer, G. & Ried, T. (2000) *Genes Chromosomes Cancer* **27,** 183–190.
39. Gisselsson, D., Bjork, J., Hoglund, M., Mertens, F., Dal Cin, P., Akerman, M. & Mandahl, N. (2001) *Am. J. Pathol.* **158,** 199–206.
40. Riha, K., McKnight, T. D., Griffing, L. R. & Shippen, D. E. (2001) *Science* **291,** 1797–1800.
41. Naito, T., Matsuura, A. & Ishikawa, F. (1998) *Nat. Genet.* **20,** 203–206.
42. Nakamura, T. M., Cooper, J. P. & Cech, T. R. (1998) *Science* **282,** 493–496.
43. Hackett, J. A., Feldser, D. M. & Greider, C. W. (2001) *Cell* **106,** 275–286.
44. Blasco, M. A., Lee, H.-W., Hande, P. M., Samper, E., Lansdorp, P. M., DePinho, R. A. & Greider, C. W. (1997) *Cell* **91,** 25–34.
45. Rudolph, K. L., Chang, S., Lee, H.-W., Blasco, M., Gottlieb, G., Greider, C. W. & DePinho, R. A. (1999) *Cell* **96,** 701–712.
46. Chin, L., Artandi, S., Shen, Q., Tam, S., Lee, S.-L., Gottlieb, G., Greider, C. W. & DePinho, R. A. (1999) *Cell* **97,** 527–538.
47. Artandi, S. E., Chang, S., Lee, S. L., Alson, S., Gottlieb, G. J., Chin, L. & DePinho, R. A. (2000) *Nature (London)* **406,** 641–645.
48. Ulaner, G. A., Hu, J. F., Vu, T. H., Giudice, L. C. & Hoffman, A. R. (1998) *Cancer Res.* **58,** 4168–4172.
49. Atkin, N. B. (1986) *Cancer Genet. Cytogenet.* **21,** 275–278.
50. Rudolph, K. L., Millard, M., Bosenberg, M. W. & DePinho, R. A. (2001) *Nat. Genet.* 155–159.
51. Chang, S., Khoo, C. & DePinho, R. A. (2001) *Semin. Cancer Biol.* **11,** 227–238.
52. Shih, I. M., Zhou, W., Goodman, S. N., Lengauer, C., Kinzler, K. W. & Vogelstein, B. (2001) *Cancer Res.* **61,** 818–822.
53. Comfort, N. C. (2001) *The Tangled Field: Barbara McClintock's Search for the Patterns of Genetic Control* (Harvard Univ. Press, Cambridge, MA).