A checkpoint on the road to cancer

Terry L. Orr-Weaver and Robert A. Weinberg

Mutations that disrupt a cell-division checkpoint, thereby causing alterations in chromosome number, have been identified in cancer cells. The accompanying increase in mutability helps to explain how tumours acquire large numbers of mutant genes during their development.

For the past two decades, cancer researchers have grappled with a paradox. The appearance of a human tumour is the culmination of a complex, multi-step process. The rate-limiting steps seem to be mutations in a half-dozen or more cellular genes that, directly or indirectly, affect tumour cell proliferation. However, from calculations using the known mutation rate in non-germline cells (~10^{-7} per gene per cell generation), one can predict that so many mutant genes would never accumulate in the genome of cell lineage during a human lifetime[1,2]. Hence, tumour formation is mathematically impossible. In the immortal words of the Maine farmer, answering a visitor's query for directions, "Ye cahn't get theah from heah". But experiments described by Cahill *et al.*[3] on page 300 of this issue provide a road map to solving this paradox. They show that control mechanisms that ensure the proper separation of chromosomes during cell division can be defective in colorectal cancer cells.

Lawrence Loeb, whose calculations indicated that the completion of human tumour progression is highly improbable, revisited his numbers and concluded that one critical parameter assumed in his initial calculations — the mutation rate — had been underestimated[1,2]. Tumorigenesis can occur, he concluded, only if the genomes of pre-malignant cells are far more mutable than those of their normal counterparts. Such mutability would hasten each rate-limiting mutational step in tumour progression, thereby accelerating completion of the process as whole. Still, calculations like these are at best well-informed surmises, because too many critical parameters cannot yet be measured and must, therefore, be assumed.

More compelling have been direct demonstrations of increased tumour cell mutability. Many colon cancer cells, for example, cannot effectively repair mismatched DNA bases. As a result, they sustain various types of mutation at a high rate , the most apparent of these being changes in their microsatellite DNA blocs — short stretches of DNA of very simple, repeating base sequence[4]. But these tumours account for only a portion of those surveyed.

Is acquired mutability typical of most tumours as they progress? Studies of hereditary non-polyposis colon cancer have provided a clue. Tumours of this type tend to have normal or quasi-normal complements of chromosomes (euploidy)[5,6], but show microsatellite instability[7–9]. Most colorectal tumours, however, show no microsatellite instability, but have abnormal chromosome number (aneuploidy) and loss of heterozygosity at many genetic loci. This suggests that generation of aneuploidy is an alternative mutagenic mechanism to defective mismatch repair for driving tumour progression.

Aneuploidy can result from improper allocation of the two chromatids of a chromosome to the two daughter cells during cell division (mitosis). Normally, a 'checkpoint' control monitors the proper assembly of the mitotic spindle — the cellular apparatus that will pull the chromatids apart. If chromosomes are not attached stably to the microtubules that form the spindle, this

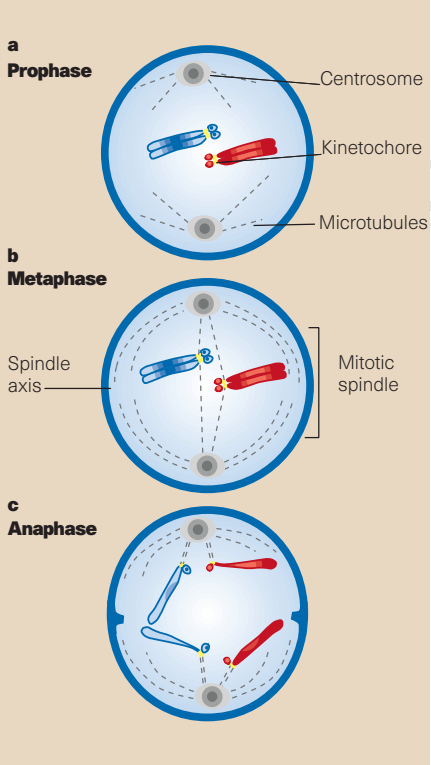

Figure 1 **Normal separation of chromosomes during cell division (mitosis).** a, **Prophase. The spindle begins to form, and is visible as a series of microtubules (polymers of the protein tubulin), which are organized by the centrosomes found at either end of the cell.** b, **Metaphase. The spindle takes shape, and the chromosomes align themselves along the spindle axis, attached to the microtubules at a specialized region called the kinetochore.** c, **Anaphase. The chromosomes move along the shortening microtubules, kinetochores first, to opposite poles of the cell.**

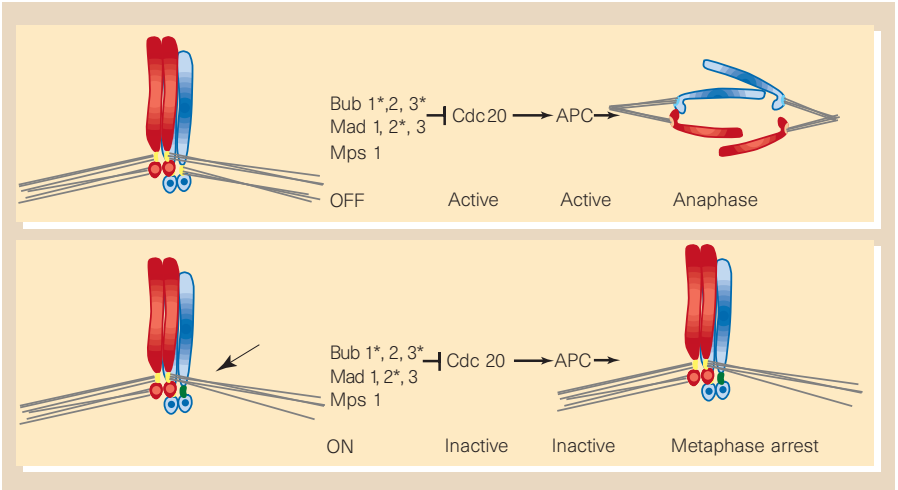

Figure 2 **How assembly of the mitotic spindle is normally monitored to ensure chromosomal stability. In yeast, the Bub, Mad and Mps1 proteins respond to improper assembly of the spindle by blocking anaphase, apparently through inhibition of Cdc20, which would normally activate the cyclin destruction machinery (the anaphase-promoting complex, APC). The mammalian BUB1, BUB3 and MAD2 proteins bind to the kinetochore region, and it is possible that all of the proteins form a complex there. Normally, stable, bipolar attachment of the kinetochores leads to dissociation of BUB1 and MAD2 proteins from the kinetochore. But if the kinetochore is not attached to the microtubules, the BUB and MAD proteins remain attached, and arrest the cell in metaphase by delaying activation of the cyclin destruction machinery. Cahill *et al.*[3] have found that this system is defective in tumour cells, leading to chromosomal instability (aneuploidy).**

checkpoint control blocks the onset of anaphase (a stage of mitosis; Fig. 1). This block is lifted only when all of the chromosomes are stably attached (at a specialized chromosomal region known as the kinetochore) to the microtubules. Indeed, unattached kinetochores seem to emit a signal that prevents the onset of anaphase[10]. So failure of the spindle assembly checkpoint machinery leads rapidly to aneuploidy.

Some of the proteins that mediate this inhibitory signal in yeast cells have been identified. Mutations in their respective genes result in increased sensitivity to drugs that cause the microtubules to depolymerize and fall apart. This sensitivity arises because the drug-treated cells do not undergo mitotic arrest, indicating a non-functional checkpoint. Six genes have been recovered using this strategy — three *BUB* genes (budding uninhibited by benomyl) and three *MAD* genes (mitotic arrest-deficient)[11,12]. A protein kinase (an enzyme that adds a phosphate group to its substrate), Mps1p, also seems to function in checkpoint signalling[13].

The proteins that make up the spindle assembly checkpoint machinery seem to act directly at the kinetochore, to sense microtubule attachment and to send an arrest signal in its absence (Fig. 2). In mammalian cells, the BUB1 and MAD2 proteins are present at the centromere region in prophase of mitosis, but not after stable microtubule attachments are made at metaphase[14,15]. MAD2 has been found to bind CDC20; this protein activates the cyclin destruction machinery[16,17]. This destruction machinery, also termed the anaphase-promoting complex (APC), degrades proteins that inhibit the separation of chromosomes, thereby permitting anaphase chromosome movement. So MAD2 — or a complex of checkpoint proteins —inhibits the APC after it has sensed that the spindle attachments are defective.

Many aneuploid colorectal tumour cell lines show continuous chromosomal instability when grown in culture[18]. Cahill *et al.*[3] now extend earlier experiments to reveal that this instability derives from defective control at the spindle assembly checkpoint. Moreover, they find mutant alleles of the human (*h*)*BUB1* gene in two of 19 colorectal tumour cell lines that show high rates of aneuploidy. By expressing the two mutant versions of the *hBUB1* gene in euploid cells, the authors can disrupt mitotic checkpoint control. Unexpectedly, however, only one of the two *hBUB1* gene copies present in the tumour-cell genomes described by Cahill *et al.* is mutant. Such heterozygosity might suggest that these mutant alleles act dominantly, as the authors propose, or it may indicate that a single, intact copy of the gene is inadequate for normal function.

Still, two swallows don't make a summer. The significance of these results will become apparent only when more mutant *hBUB1* alleles are found, and when mutant alleles of other mitotic checkpoint genes are documented and functionally characterized.

Much of contemporary cancer research is motivated by the tenet that if mutant alleles of a gene are repeatedly detected in tumour cell genomes, then these alleles probably confer selective advantage on evolving tumour cell clones[19]. In the present instance, the mutations affect a gene that controls the stability of a cell's chromosome complement, leading, in turn, to aneuploidy. Tumour-cell aneuploidy has long been speculated to be causally involved in tumorigenesis, but its importance has not been demonstrable. The work of Cahill *et al.*[3] represents the beginnings of a proof that acquired aneuploidy is a specific driving force in tumour progression, rather than a distracting epiphenomenon of this disease.

How does aneuploidy facilitate tumour progression? One attractive idea is that aneuploidy increases the rate at which tumour-suppressor genes are lost, through the loss of heterozygosity (which results in the conversion of genes having two dissimilar versions into identical versions). Elimination of these genes is known to cause deregulated cell proliferation. Perhaps most satisfying, however, is that Loeb's prediction — that mutability is a general characteristic of tumour-cell genomes — has, after so long, taken on new life and credibility. □

Terry L. Orr-Weaver and Robert A. Weinberg are at the Whitehead Institute for Biomedical Research and the Department of Biology, MIT, Cambridge, Massachusetts 02142-1479, USA.

1. Loeb, L. A. *Cancer Res.* **51,** 3075–3079 (1991).
2. Loeb, L. A., Springgate, C. F. & Battula, N. *Cancer Res.* **34,** 2311–2321 (1974).
3. Cahill, D. P. *et al. Nature* **392,** 300–303 (1998).
4. Eshleman, J. R. & Markowitz, S. D. *Curr. Opin. Oncol.* **7,** 83–89 (1995).
5. Kouri, M. *et al. Cancer* **65,** 1825–1829 (1990).
6. Frei, J. V. *Cancer* **69,** 1108–1111 (1992).
7. Ionov, Y., Peinado, M. A., Malkhosyan, S., Shibata, D. & Perucho, M. *Nature* **363,** 558–561 (1993).
8. Thibodeau, S. N., Bren, G. & Schaid, D. *Science* **260,** 816–819 (1993).
9. Aaltonen, L. A. *et al. Science* **260,** 812–816 (1993).
10. Rieder, C. L., Cole, R. W., Khodjakov, A. & Sluder, G. *J. Cell Biol.* **130,** 941–948 (1995).
11. Hoyt, M. A., Totis, L. & Roberts, B. T. *Cell* **66,** 507–517 (1991).
12. Li, R. & Murray, A. *Cell* **66,** 519–531 (1991).
13. Hardwick, K. G., Weiss, E., Luca, F. C., Winey, M. & Murray, A. W. *Science* **273,** 953–956 (1996).
14. Taylor, S. S. & McKeon, F. *Cell* **89,** 727–735 (1997).
15. Li, Y. & Benezra, R. *Science* **274,** 246–248 (1996).
16. Hwang, L. H. *et al. Science* **279,** 1041–1044 (1998).
17. Kim, S. H., Lin, D. P., Matsumoto, S., Kitazono, A. & Matsumoto, T. *Science* **279,** 1045–1047 (1998).
18. Lengauer, C., Kinzler, K. W. & Vogelstein, B. *Nature* **386,** 623–626 (1997).
19. Nowell, P. C. *Science* **194,** 23–28 (1976).

Geophysics

Finding chaos in abyssal hills

John A. Goff

Abyssal hills cover most of the Earth's sea floor. They are, in fact, the most common morphological feature on the Earth's surface, yet they are among the least understood. These elongate hills are created at mid-ocean-ridge spreading centres by faults which offset the volcanically created sea floor, and can be seen as both simple and complex (Fig. 1).

The simple view is often taken by physical modellers, who look upon oceanic rifting as a test case in which the dynamic processes of an active Earth are barely complicated by factors such as erosion or sedimentation. Modellers tend to speak a deterministic language, specifying their predictions of faults, for example, in terms of regularly spaced fault intervals and uniform fault offsets[1,2]. Observationalists, by contrast, tend to point to the random, chaotic-looking behaviour of the abyssal-hill fabric, and naturally choose the mathematical language of statistics to quantify their observations[3,4].

So observations and predictions have not been directly comparable, and this is the problem addressed by Buck and Poliakov in their paper on page 272 of this issue[5]. They have developed a numerical physical model of mid-ocean-ridge faulting that produces a chaotic-looking morphology very like that of abyssal hills observed on the sea floor.

Statistical descriptions of natural phenomena have been around for some time, but such applications reached truly phenomenal proportions following publication of Benoit Mandelbrot's *The Fractal Geometry of Nature*[6] in 1983. Although the mathematics described by Mandelbrot were not new (Hausdorff had much earlier explored the concept of fractional dimensions[7]), his book coincided with the rapid emergence of computer graphics. The resulting images were extraordinary — truly realistic representations of landscapes, seascapes, trees and other natural phenomena, as well as unreal, psychedelic drawings created from simple mathematical abstractions. Such graphics had an immediate impact on popular culture (the producers of the film *Star Trek: The Search for Spock* went so far as to create an entire world with fractal imaging techniques), as well as on education, with students scrambling to learn more about the equations that governed such 'cool' pictures.

Scientists also jumped on the bandwagon, wanting to understand how this 'new', fractal way of thinking about nature might bear on their particular speciality. Such enquiries, however, eventually lost a bit of