PERSPECTIVES: CELL CYCLE

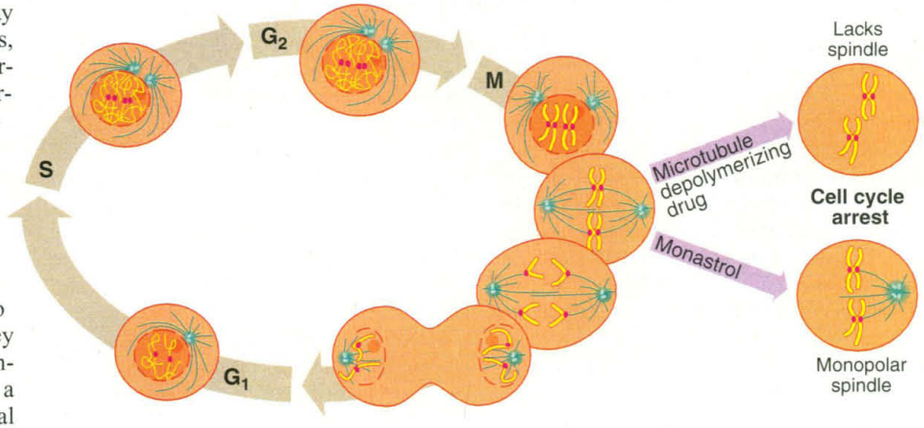
New Tools for the Antimitotic Toolbox

Duane A. Compton

About 560,000 people are estimated to die from some type of cancer in the United States each year (*1*), making cancer second only to heart disease as the most common cause of death. Although cancer is a generic term encompassing many different diseases, a unifying feature of many tumors is the uncontrolled proliferation of their cells. As they proliferate, cancer cells disrupt the normal function of surrounding tissues (or distant tissues in the case of metastases), leading to eventual organ failure and death. Based on the notion that some tumor cells may proliferate more rapidly than normal cells, a common strategy for cancer chemotherapy has been to develop drugs that interrupt the cell cycle. A particularly attractive stage of the cell cycle for intervention is mitosis, during which the mitotic spindle (a bipolar apparatus constructed of microtubules) separates the replicated chromosomes (see the figure). On page 971 of this issue, Mayer *et al.* (*2*) report that, by combining two phenotype-based screening assays, they have successfully identified a new compound that blocks mitosis by binding to a kinesin, a motor protein that is essential for production of a bipolar spindle.

The spindle is a complex microtubule-based superstructure that assembles during mitosis to separate replicated DNA. Chromosome attachment to and movement on the spindle is intimately tied to the dynamics of microtubule polymerization and depolymerization. The sister chromatid pairs must maintain a stable attachment to spindle microtubules as the microtubules interconvert between growing and shrinking states. Drugs that perturb microtubule lengthening (polymerization) or shortening (depolymerization) cause arrest of the cell cycle in mitosis because they perturb the normal microtubule dynamics necessary for chromosome movement (see the figure). A variety of these drugs (paclitaxel, docetaxel, etoposide, vinblastine, vincristine, and vinorelbine) are currently used in cancer chemotherapy (*3*). Unfortunately, this group is limited by the fact that they all share a common mechanism of action: They bind to tubulin, the molecule of which microtubules are composed, and arrest cells in mitosis by inhibiting spindle assembly.

To circumvent this limitation in their quest for new antimitotic agents, Mayer and colleagues performed an ambitious screen to identify small, cell-permeable compounds that inhibit mitosis by blocking the function of essential spindle proteins other than tubulin. Their screening strategy took advantage of a unique antibody that reacts with a mitosis-specific phosphorylation site on the nucleolar protein nucleolin (*4*). They reasoned that a small molecule that entered cells and blocked them in mitosis should cause mitotic cells to accumulate. Cells that are about to enter mitosis show an increase in the phosphorylated form of nucleolin, which the investigators could easily detect in immunoblots of whole-cell extracts using their anti-nucleolin antibody. The authors ran immunoblots in a high-density array format that permitted them to rapidly screen large numbers of small molecules (*5*). Their initial screen netted 139 (out of 16,320) cell-permeable compounds that caused cells to arrest in mitosis. As expected, many of the compounds identified interacted with tubulin, but these were readily discarded using an in vitro microtubule polymerization assay. The remaining compounds were analyzed in a visual screen aimed at identifying those that perturbed the organization of the mitotic spindle in cultured cells. This secondary screen divided the compounds into four groups: those that showed pleiotropic effects; those that perturbed microtubule organization in both mitotic and nonmitotic cells; those with no discernible effect on microtubule organization; and those that specifically perturbed the organization of the mitotic spindle without altering microtubule organization in nonmitotic cells. Five compounds were identified in this final category, and they represent the "winners" from the two-pronged screening strategy because they blocked cells in mitosis, disrupted microtubule organization specifically during mitosis, and did not interact directly with tubulin.



Spindle specters. DNA is replicated during the S phase of the cell cycle and condenses into discrete chromosomes during mitosis (M phase). Chromosomes are segregated equally into daughter cells by the microtubule-based structure called the spindle. Drugs that prevent assembly of tubulin into microtubules cause cell cycle arrest because the spindle does not form. Monastrol causes cell cycle arrest by disrupting another target, a kinesin-related motor protein that is necessary for formation of a bipolar spindle.

Although the protein targets of four of these compounds remain unknown, the manner in which one compound perturbed spindle organization immediately suggested a plausible target. This compound, called Monastrol, caused spindles to assemble as monopolar structures rather than the bipolar structures found in normal cells (see the figure). Monopolar spindles are commonly observed in cells in which a kinesin-related protein of the bimC family is disrupted (*6*). The authors used in vitro biochemical tests to verify that Monastrol specifically blocked the function of this class of kinesin-related proteins during spindle assembly. A variety of published data indicate that the

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The author is in the Department of Biochemistry, Dartmouth Medical School, 7200 Vail Building, Room 413, Hanover, NH 03755–3844, USA. E-mail: duane.a.compton@dartmouth.edu

bimC motor proteins play an essential role in organizing microtubules within the spindle during mitosis (*6*). These data support the Mayer findings and corroborate the accuracy of their screening strategy, which successfully identified an antimitotic compound targeting a nontubulin spindle protein. Although the clinical benefits of Monastrol require further evaluation, this compound will certainly be a useful molecular tool for studying the involvement of the bimC class of kinesin-related proteins in spindle assembly.

Mayer and co-workers have added several new, and potentially very important, molecular tools to the antimitotic toolbox. Clearly, the targets of the four remaining antimitotic compounds identified in this screen need to be characterized. Regardless of whether the four targets turn out to be proteins that are already known or new protein components of the spindle, their identification will shed more light on the steps of spindle assembly. Also, the group of compounds that blocked cells in mitosis without altering microtubule organization in any discernible way should not be ignored. Finally, the success of this screening strategy should stimulate similar screening approaches aimed at identifying small molecules that perturb other stages of the cell cycle. It is easy to envision how a suitable phosphorylation-specific antibody against the Rb tumor suppressor protein or one of the many proteins involved in DNA replication could be used in such a screen to identify new compounds that perturb other aspects of the cell cycle.

References and Notes

1. Statistics provided by the American Cancer Society.
2. T. U. Mayer *et al.*, *Science* **286**, 971 (1999).
3. E. Hamel, *Med. Res. Rev.* **16**, 207 (1996); A. Jordan, J. A. Hadfield, N. J. Lawrence, A. T. McGown, *Med. Res. Rev.* **18**, 259 (1998).
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5. B. R. Stockwell, S. J. Haggarty, S. L. Schrieber, *Chem. Biol.* **6**, 71 (1999)
6. C. E. Walczak and T. J. Mitchison, *Cell* **85**, 943 (1996).

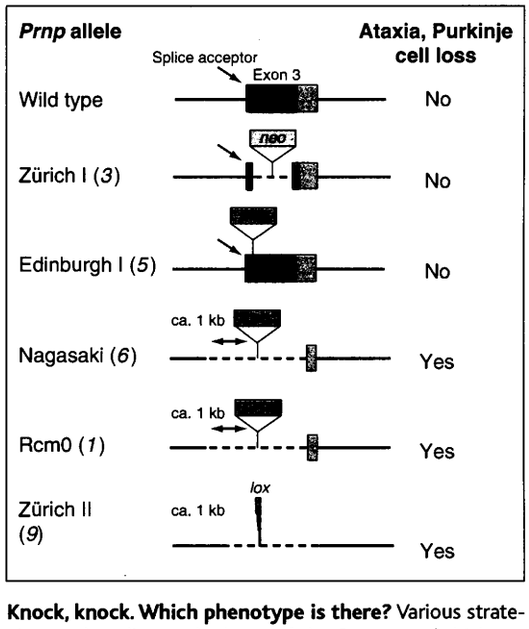
PERSPECTIVES: NEUROBIOLOGY

PrP's Double Causes Trouble

Charles Weissmann and Adriano Aguzzi

Disrupting ("knocking out") a mouse gene by homologous recombination is a widely used strategy for elucidating the function of that particular gene. However, the phenotype (or lack of it) displayed by the knockout mouse is not always conclusive. Although knockout experiments are conceptually simple—disrupt a gene and see what happens—the interpretation of their results is beset with pitfalls. For example, insertion of an interrupting sequence into an exon could result in the modified exon being spliced out after transcription; the truncated protein that is expressed could still be active or could be capable of interfering with the biochemical pathway in which the wild-type protein participates (dominant negative). If the promoter of a gene rather than the gene itself is deleted, transcription from an unrecognized upstream promoter could compensate for this loss. Deletion of the entire gene could result in ablation of an unrecognized open reading frame (the sequence that encodes the protein; ORF) or of important regulatory sequences, entailing loss of a function that is unrelated to the targeted ORF. Restoration of a gene's function in a knockout mouse by introduction of a transgene expressing the targeted protein is good evidence for linkage of the function to the gene in question. However, even then, the conclusion that a phenotype is due to the disruption of a particular gene can be erroneous, as demonstrated by a fascinating paper from Moore *et al.* published in a recent issue of the *Journal of Molecular Biology* (*1*). These investigators identify a second *Prnp*-like gene located 16 kb downstream of the murine *Prnp* gene, which encodes the prion protein (PrP). Prion proteins have been implicated in the propagation of the neurodegenerative prion diseases. Moore and colleagues call the new gene *Prnd* and the protein it encodes Dpl (Doppel, German for double). They go on to show that Dpl is overexpressed in certain strains of PrP knockout mice, possibly causing a neurological disease that some researchers had previously attributed to ablation of PrP.

Prion proteins play a central role in transmission of the prion diseases (transmissible spongiform encephalopathies), a group that includes sheep scrapie, mad cow disease, and Creutzfeldt-Jakob disease in humans. A conformational variant of the normal cellular protein PrP^C is either an essential component of the transmissible agent (the prion), or the prion itself (*2*). A crucial role for PrP was revealed by demonstrating that mice lacking PrP (*3*) were resistant to prion disease, failing to develop the illness when mouse scrapie prions were inoculated into their brains (*4*). These so-called Zürich I $Prnp^{0/0}$ mice, as well as a later PrP knockout line designated Edinburgh $Prnp^{-/-}$ (*5*), were clinically healthy, although they showed discrete neurophysiological changes and demyelination of peripheral nerves as they aged. However, mice of a third PrP knockout strain, Nagasaki $Prnp^{-/-}$ (*6*), developed ataxia and loss of cerebellar Purkinje cells at 6 to 12 months of age. Because the disease was prevented by introduction of a cosmid encoding PrP, this phenotype was attributed to PrP ablation (*7*). A comparison of the knockout strategies (see figure, this page) reveals that in the Zürich I mice, two-thirds of the ORF of *Prnp* was replaced by a cassette containing the neomycin resistance gene. In the Edinburgh mice a similar cassette was inserted into the ORF. However, in the Nagasaki mice not only the ORF, but also 0.9 kb of intron 2, the 5′ noncoding region, and 0.45 kb of the



Knock, knock. Which phenotype is there? Various strategies used to disrupt the locus of the *Prnp* gene (which encodes PrP) in the creation of different strains of *Prnp* knockout mice. The dotted line indicates the segment of *Prnp* DNA that has been deleted, and the green box indicates a sequence inserted into the gene. Dark red, ORF; pink, noncoding region; *neo*, neomycin phosphotransferase; HPRT, hypoxanthine phosphoribosyltransferase; *lox*, a 34-bp recombination site from phage P1.

C. Weissmann is at the MRC Prion Unit/Neurogenetics, Imperial College School of Medicine at St. Mary's, London W2 1PG, UK. A. Aguzzi is at the Institut für Neuropathologie, Universitätsspital Zürich, 8091 Zürich, Switzerland. E-mail: c.weissmann@ic.ac.uk, adriano@pathol.unizh.ch

References and Notes

[2] **Small Molecule Inhibitor of Mitotic Spindle Bipolarity Identified in a Phenotype-Based Screen**

Thomas U. Mayer; Tarun M. Kapoor; Stephen J. Haggarty; Randall W. King; Stuart L. Schreiber; Timothy J. Mitchison

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