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CARCINOGENESIS

Transient Expression of a Mutator Phenotype in Cancer Cells

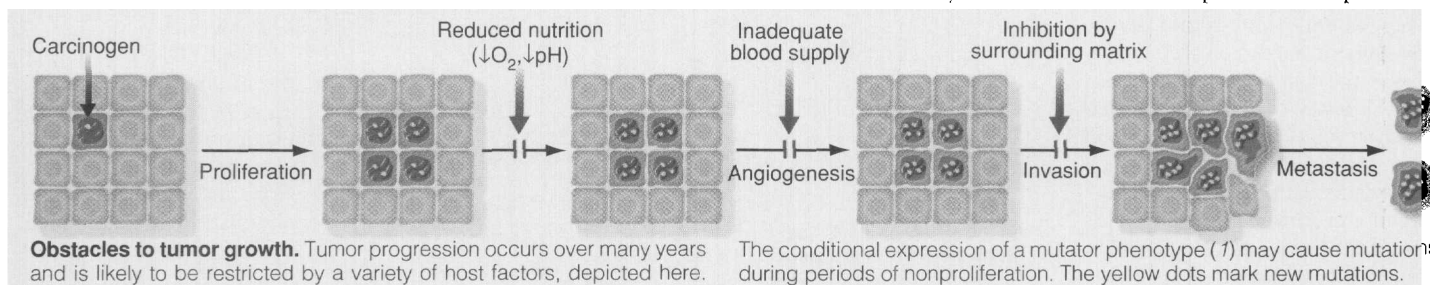
Lawrence A. Loeb

Characteristically, the chromosomes of cancer cells are rearranged in multiple ways, and their DNA contains insertions, deletions, duplications, and base substitutions. Each year more and more mutations are identified in human tumors. Yet, the mutation rates in cancer cells in culture are not always greater than those in nonmalignant cells. How and when do these mutations arise? The report by Richards *et al.* (*1*) on page 1523 of this issue provides an important clue for addressing this question.

The concept that cancer cells exhibit a mutator phenotype (mutate at a high rate) arose between 1974 and 1976 from studies on the infidelity of the DNA synthesis (*2*) and of clonal selection during tumor progression (*3*). Simply stated, the large number of mutations in tumor cells cannot be accounted for by the low mutation rates of normal somatic cells, but instead must be a manifestation of a mutator phenotype (*4*). The expansion and contraction of tandem repetitive nucleotide sequences (microsatellites) in many human tumors but not in nonmalignant cells (*5*) provided the first strong experimental support for a mutator phenotype in cancer. This alteration in microsatellite length is believed to result from slippage by DNA polymerases during DNA replication (*6*). Tumors exhibiting microsatellite instability frequently contain mutations in mismatch repair genes (*7*), which render the cells defective in correcting polymerase errors. Given the enormous numbers of microsatellites throughout the human genome, Perucho and colleagues (*8*) calculated that tumors exhibiting microsatellite instability could carry more than 100,000 mutations in these sequences. In addition, changes in repetitive sequences in tumors can occur within genes that are associated with specific types of cancers (*9*).

Meuth's laboratory initially observed that cell lines derived from colon cancers with a high degree of microsatellite instability also exhibit a several hundred-fold increase in mutations that cause resistance to 6-thioguanine and ouabain, providing evidence that microsatellite instability is a sentinel for a more general mutator phenotype (*10*). Now Richards *et al.* (*1*) report that two human tumor cell lines deficient in the key mismatch repair protein hMSH2 are not mutators in culture during periods of rapid growth. However, mutations accumulate when the cells are maintained at high density with greatly diminished proliferation. In one of the cell lines, mutation frequency was 7900 times as great at the HPRT locus and more than 67 times as great at the oubain locus as those in replica cultures kept under optimal growth conditions. These results support the suggestion of Strauss that mutations can accumulate in tumors in nondividing cells (*11*). This concept of mutation accumulation in the absence of DNA replication is derived from bacterial studies that show the accumulation of special mutations under restrictive conditions (*12*, *13*). Interestingly, many of the bacterial mutations are localized to runs of identical nucleotides that are similar to microsatellites.

Many years elapse between the initiation of a cancer and its detection. During this time malignant cells acquire the ability to grow under restrictive conditions as they negotiate a series of crisis points that could arrest the progressive growth of a tumor. Among the many obstacles that tumor cells must overcome are reduced nutritional requirements, inadequate blood supply, and impenetrable barriers generated by normal cellular matrices that limit expansion (see the figure). Thus, tumor cells must garner the ability to grow under adverse circumstances, to recruit new blood vessels, and to invade adjacent normal cells. The new studies from Meuth's laboratory provide a mechanism by which cells could overcome successive bottlenecks that limit expansion: the conditional generation of mutations during periods of arrested growth. Increasing the mutation rate during nonproliferation results in an increase in the likelihood that an advantageous mutation will arise in the tumor population X—one that can overcome the restriction and form a resistant clone. There are mechanisms for selection of mutators in bacteria also. Mao *et al.* (*14*) have shown that sequential rounds of selection for mutants in bacteria under restrictive conditions result in the selection of bacteria with an inherently high mutation rate. In these experiments, as few as two rounds of selection result in the simultaneous selection for mutators. Successive periods of nonproliferation could result in an increased number of mutations in a tumor cell population and facilitate the emergence of new mutant clones that would include mutators. Thus, the enhanced mutation frequency detected by Meuth's laboratory in density-dependent reduced growth may be only partially reversible when cells are replated in a serum-rich environment.



Obstacles to tumor growth. Tumor progression occurs over many years and is likely to be restricted by a variety of host factors, depicted here. The conditional expression of a mutator phenotype (*1*) may cause mutations during periods of nonproliferation. The yellow dots mark new mutations.

The new studies (*1*) are limited to two tumor cell lines that are deficient in mismatch correction. These studies on mutagenesis during periods of nonproliferation need to be extended to other tumor cell lines defective in mismatch repair. Mismatch repair deficiencies in hereditary nonpolyposis human colon cancer have been a paradigm for the mutator phenotype in cancers (*15*). However, many tumors exhibit microsatellite instability in the absence of mutations in the mismatch repair system. Do these tumors contain mutations in other genes that function to guard the genome (*16*)? Do these tumors exhibit increased mutagenesis under stress? If we assume that the generation of mutations is rate limiting for tumor progression, then the conditional generation of a mutator phenotype

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may provide a new mechanism for mutation accumulation during tumor progression. We need to understand this mechanism since tumors accumulate mutations over decades. Even a modest decrease in the rate of mutation accumulation may effectively prevent these cancers by simply delaying their onset sufficiently.

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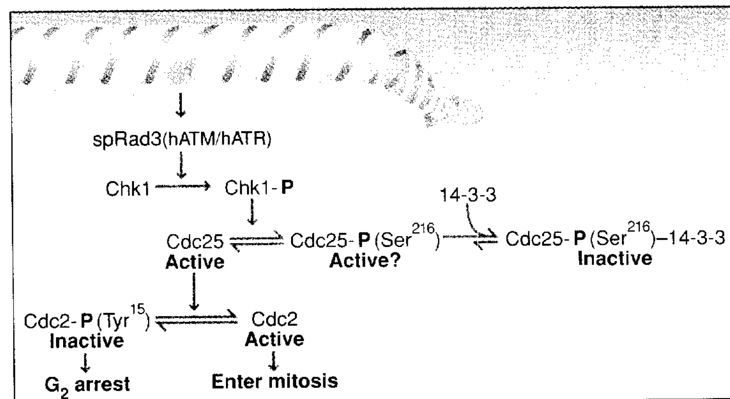
CELL CYCLE

A DNA Damage Checkpoint Meets the Cell Cycle Engine

Ted Weinert

Cell cycle regulators govern key transitions in the life of a cell—when to begin DNA replication and when to enter mitosis and divide. Preeminent among cell cycle regulators is the family of cyclin-dependent kinases ($p34^{CDK}$) and their partner cyclins, which together form heterodimer protein kinases (*1*). Reflecting that preeminence, Cdk-cyclin was crowned the cell cycle's "engine" (*2*): How goes Cdk-cyclin, so goes the cell cycle.

Changes in cell physiology, particularly damage to DNA, stop the cell cycle either before DNA replication in G_1 (termed the G_1 checkpoint) or before mitosis in G_2 [the G_2-M checkpoint (*3*, *4*)]. In many cell types DNA damage response pathways cause arrest by regulation of Cdk-cyclin through checkpoint proteins, which sense damage and transduce an inhibitory signal (*3*). Until recently it was unclear whether Cdc2, a prominent member of this Cdk family and a major mitotic activator in yeast, even plays a role in arrest in G_2 after damage. Nor was it clear how the checkpoint proteins transmit a signal to cause arrest. In the last year, the functions of both Cdc2 and checkpoint proteins have become clearer, and an ever more detailed hypothesis for a checkpoint pathway has emerged, culminating in three reports (pages 1495, 1497, and 1501) in this week's issue from fission yeast, human, and mouse (*5*–*7*).



A DNA break (a single-strand gap highlighted in yellow) activates the protein kinase Rad3 in fission yeast (and probably Rad3-like proteins ATM and ATR in human cells) (*3*). Activation of Rad3 probably occurs through association with other checkpoint proteins not shown (*3*). Active Rad3 then somehow activates the protein kinase Chk1 (Rad3 is required for phosphorylation of Chk1, but the exact mechanism of activation is unknown) (*13*). Activated Chk1 phosphorylates the phosphatase Cdc25 on Ser^{216} that then binds to and is sequestered by 14-3-3 protein. Sequestered Cdc25 is prevented from activating Cdc2. Cells arrest in G_2 when inhibitory phosphorylation of Cdc2 is intact. The dotted line highlights the aspects of this pathway discussed in three reports in this issue (*5*–*7*).

Evidence from several cell types has indicated that the G_2 arrest is caused by regulation of Cdc2; DNA damage results in the phosphorylation of inhibitory sites on the Cdc2 catalytic subunit in the filamentous fungi *Aspergillus*, in human cells, and in fission yeast (*8*–*10*). This inhibitory phosphorylation is required for arrest at the G_2-M checkpoint: in all three organisms nonphosphorylatable mutants of Cdc2 fail to fully arrest. How is this inhibitory phosphorylation of Cdc2 achieved? This question is addressed in three reports in this issue, which forge an attractive model explaining how upstream checkpoint proteins mediate the inactivation of Cdc25, a key activator of Cdc2.

The elegant model (see the figure) derived from these three reports seems destined for textbooks—regardless of whether it ultimately proves correct in detail. As yet, the single proposed pathway has not been demonstrated in its entirety in any one cell type but is instead synthesized from experiments in evolutionarily distant organisms—human, mouse, and yeast. Nevertheless, the mosaic nature of this model seems justified because the pathways in different cell types thus far have been found to be conserved.

After DNA damage, the inhibitory phosphorylation of Cdc2 that causes G_2 arrest occurs on Tyr^{15}. Genetic studies in fission yeast imply that this phosphorylation is maintained by the activity of protein kinases spWee1 [and the redundant spMik1 (here the prefix sp refers to *Schizosaccharomyces pombe*] and the simultaneous inactivity of phosphatase spCdc25 (*10*). In one of the new reports, human Cdc25 (here designated hCdc25) is shown to become phosphorylated in vivo on Ser^{216} after DNA damage in mammalian cells (*7*), and all three reports suggest that this is how Cdc25 (the *S. pombe* or human protein) is kept inactivated (*5*–*7*, *10*). This phosphorylation is clearly functionally important because the nonphosphorylatable allele hCdc25(S216A) (in which Ser^{216} is mutated to Ala) is defective for G_2-M arrest.

Phosphorylation of spCdc25 and hCdc25 is achieved by Chk1 protein kinase (*5*–*7*), a protein kinase required for arrest after DNA damage at least in fission yeast (*11*–*13*). From elegant genetic studies in fission yeast, Furnari *et al.* argue that Chk1 acts primarily through Cdc25 and not through Wee1 and Mik1 protein kinases, although they are required to maintain arrest (*6*, *10*). [The rel-

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