The Mutation Rate and Cancer

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ABSTRACT

The stability of the human genome requires that mutations in the germ line be exceptionally rare events. While most mutations are neutral or have deleterious effects, a limited number of mutations are required for adaptation to environmental changes. Drake has provided evidence that DNA-based microbes have evolved a mechanism to yield a common spontaneous mutation rate of ~0.003 mutations per genome per replication (Drake 1991). In contrast, mutation rates of RNA viruses are much larger (Holland *et al.* 1982) and can approach the maximum tolerable deleterious mutation rate of one per genome (Eigen and Schuster 1977; Eigen 1993). Drake calculates that lytic RNA viruses display spontaneous mutation rates of approximately one per genome while most have mutation rates that are approximately 0.1 per genome (Drake 1993). This constancy of germline mutation rates among microbial species need not necessarily mean constancy of the somatic mutation rates. Furthermore, there need not be a constant rate for somatic mutations during development. In this review, we consider mutations in cancer, a pathology in which there appears to be an increase in the rate of somatic mutations throughout the genome. Moreover, within the eukaryotic genome, as in microbes, there are "hot-spots" that exhibit unusually high mutation frequencies. It seems conceivable to us that many tumors contain thousands of changes in DNA sequence. The major question is: how do these mutations arise, and how many are rate-limiting for tumor progression?

CANCER is a multistage process that evolves over many years and is characterized by a series of chromosomal changes (Vogelstein *et al.* 1988). When detected, some cancers have accumulated as many as 50 chromosomal rearrangements and most likely an even greater number of changes in the nucleotide sequence of the tumor DNA. We have argued that the multiple mutations seen in human tumors cannot be accounted for by the spontaneous somatic mutation rate, and suggest that an early step in tumor progression involves creating a mutator phenotype (Loeb 1991). In this article, we will first consider the evidence that cancer cells manifest a mutator phenotype, and subsequently discuss the possibility that a mutator phenotype can arise transiently within a population of cells.

Cancer cells exhibit a mutator phenotype: Human cancers frequently exhibit many chromosomal abnormalities. Kallioniemi *et al.* (1994) demonstrated by comparative genomic hybridization that nearly all breast cancers, both primary cancers and cell lines, show alterations in DNA sequence copy number at one or more loci, with some tumors displaying more than 15 abnormalities. Iwabuchi *et al.* (1995) subsequently demonstrated by comparative genomic hybridization that low grade ovarian tumors contain as many as 20 abnormalities in DNA sequence copy number and high grade ovarian tumors contain as many as 30 such abnormalities, while benign tumors contain fewer than 2 copy number abnormalities. Among the cancers that have been documented to exhibit multiple and characteristic chromosomal aberrations are the following: malignant melanoma (Balaban *et al.* 1986), small-cell lung cancer (Naylor *et al.* 1987), and adenocarcinoma of the colon (Fearon and Vogelstein 1990). The sequential chromosomal changes in adenocarcinomas of the colon (Fearon and Vogelstein 1990) and melanoma (Balaban *et al.* 1986) have been correlated with the stage of the tumor. In addition to these large chromosomal changes, smaller deletions and rearrangements are found in many tumor suppresser loci, and single base pair substitutions are found in clones of cancer cells containing mutations in oncogenes such as *p53*, *ras*, and the *retinoblastoma* gene (Weinberg 1995). Current methods for the detection of mutations in cancer cells are for the most part restricted to situations in which all or most of the cells within each tumor exhibit the same mutation. Random changes in DNA sequence in tumors would not be detected by current methods unless they result in clonal proliferation. Thus, it seems conceivable to us that there are thousands and perhaps hundreds of thousands of random mutations in cancer cells (Loeb 1991).

We have asked what is the contribution of spontaneous mutations to the multiple mutations that are found in human cancers. Estimates of the spontaneous mutation rates in human cells have been mainly obtained from studies on cells grown in culture. The spontaneous mutation of a human diploid lymphoblast cell line, TK6, heterozygous for thymidine kinase and containing one copy of hypoxanthine-guanine phosphoribosyltransfer-

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TABLE 1

Calculation of the number of stem cells which could contain a certain number of mutations occurring either randomly throughout the genome, or restricted to cancer-associated genes, based upon the spontaneous mutation rate

	Stem cells containing "x" mutations	
"x" mutations	Random (genome-wide)	Cancer-associated genes
1	1.47×10^9	7.98×10^6
2	7.35×10^8	7.98×10^3
3	2.45×10^8	5
6	2.04×10^6	
9	4.05×10^3	
12	3	

For these calculations, we have made the following assumptions: The spontaneous mutation rate for a gene is 2×10^{-7} per gene per division; stem cells divide 100 times in lifetime; the number of stem cells in the body is 4×10^9; a cell contains 5×10^4 genes, but 100 cancer-associated genes. Probabilities were calculated by Poisson probability distribution. Populations were determined by multiplication of the probability by the number of stem cells.

ase has been measured extensively. The mutation rates for both genes are approximately 2.0×10^{-7} mutations/gene/division (Oller *et al.* 1989). Similar values have been obtained from measurements using primary diploid fibroblasts (DeMars and Held 1972). It does not appear that this estimate is biased toward target genes that mutate at abnormally high rates since a similar value has been reported for electrophoretically detected protein variants at unselected loci in human cells (Chu *et al.* 1988). Also, this value does not differ greatly from estimates on human germline mutation rates based on the frequency of hemoglobin variants in human populations (Neel *et al.* 1986).

In the absence of selection or clonal proliferation, one can estimate the number of spontaneous mutations that could accumulate in stem cells with the potential to proliferate into a tumor. Based on studies in human bone marrow, it has been estimated that only 0.002% of the cells can form colonies with high proliferative potential (McNieve *et al.* 1989) and thus can operationally be considered as stem cells. Assuming that a similar fraction of cells in other tissues is stem cells, and that the average stem cell undergoes 100 divisions during a human lifespan, then the cellular target size for mutagenesis is approximately (2×10^{14} cells per adult) (0.002%) or 4×10^9 cells. Thus, there is present in individuals more than enough stem cells that contain a sufficient number of mutations to account for the two-hit mechanism proposed by Knudsen, in which both alleles of a cancer-causing gene must be inactivated. Based on a Poisson probability distribution in which mutations are independent events, the average stem cell would accumulate 1 to 2 mutations. As many as 2×10^6 cells could contain as many as 6 mutations, and a few cells (3) could contain as many as 12 mutations (Table 1). If each of the cells with multiple mutations were able to proliferate continuously and form a tumor, then spontaneous mutation rates could account for the 6 to 10 events (mutations) that are predicted to be rate-limiting for tumor formation, based on the increase of cancer incidence with age (Armitage and Doll 1954). In this analysis, we assume that any mutation in any gene is on the pathway toward malignancy, that any stem cell has the potential to become a tumor, and that each of the mutations are rate-limiting for the development of a malignancy. If we restrict the analysis to mutations in cancer-associated genes, then the spontaneous mutation rate can account for as many as 8×10^3 stem cells containing as many as 2 mutations, and only a few cells (5) containing as many as 3 mutations.

Cancer cells contain much larger numbers of mutations, many of which may not be rate-limiting for the development of a tumor. Even at the cytologic level, many cancers have multiple mutations, and a greater number of changes may be present at the level of DNA sequence. We have argued that these multiple mutations could not be the result of spontaneous mutation rates, but instead must be a manifestation of a mutator phenotype. A mutator phenotype could result from the inactivation or dysregulation of any of a number of putative genomic stability genes. Among these are genes involved in DNA repair, DNA replication, chromosomal segregation, and cell cycle checkpoints. The elimination or decreased efficiency of these genomic stability functions could produce an increased overall mutation rate in the affected cell. As a consequence, the cell would be predisposed to the accumulation of further mutations which could contribute to cancer progression.

Clonal expansion and somatic selection contribute to the increased mutation rate: Repetitive rounds of clonal expansion of cells within a tumor can be an important factor in contributing to the increased number of mutations (Nowell 1976). A mutation conferring a proliferative advantage would result in an expansion of cells

containing that mutation. Each successive round of clonal expansion would yield a large number of cells harboring *N* mutations, thereby increasing the probability of incurring the $(N + 1)^{th}$ mutation. We have estimated that in the absence of clonal expansion, the spontaneous mutation rate in normal human cells can account for only two or three mutations. If we assume that each mutation confers a proliferative advantage that leads to a clonal expansion to 10^5 cells prior to the next mutation, then the chance of a given cell incurring two mutated genes would be increased from (10^{-7}) (10^{-7}) to (10^{-7}) $(10^{-7} \times 10^5)$. Thus, without an increase in the mutation rate, successive rounds of clonal selection could account for as many as five or six mutagenic events. In the absence of a mutator phenotype, clonal selection could account for the increased incidence of some but not all cancers (Armitage and Doll 1954).

Despite the above theoretical argument, clonal expansion in the absence of enhanced mutagenesis is unlikely to account for the large number of mutations observed in many cancers, because each mutation would have to exhibit a proliferative advantage. Even a mutation in one of the two alleles of a tumor suppresser gene would have to confer a proliferative advantage to cells harboring that mutation. Given that most mutations identified in cancer cells are recessive for tumorigenesis (Harris 1988), it is unlikely that mutations in only one of the two alleles would provide the proliferative advantage required by clonal expansion to produce multiple mutations. In addition, the mutant clones must be able to rapidly populate the tumor, which would require that the mutant have an exceptionally high selective advantage or prevent the proliferation of other cells within the tumor. Although a large number of mutations have been identified in tumor cells, it has not been determined whether this extensive accumulation of mutations is actually required for tumor development. It is conceivable that the accumulation of mutations in cancers may not be rate limiting for growth or progression, but may instead arise as a parallel event.

Microsatellite instability provides evidence for a mutator phenotype: The first strong evidence for the hypothesis that cancer cells harbor mutations in genome stability genes and manifest a mutator phenotype was provided by the demonstration that cells from patients with HNPCC (hereditary non-polyposis colon cancer) harbor mutations in mismatch DNA repair genes (Aaltonen *et al.* 1993; Leach *et al.* 1993; Peltomaki *et al.* 1993; Bronner *et al.* 1994; Papadopoulos *et al.* 1994). These DNA mismatch repair mutations transform these cells into mutators with mutator strengths of approximately 100-fold. Cell lines established from hereditary colon cancers exhibit variations in the lengths of repetitive microsatellite sequences (Peinado *et al.* 1992; Ionov *et al.* 1993; Umar *et al.* 1994), and exhibit increased mutation rates in endogenous genes such as *hprt* (Aaltonen *et al.* 1993; Bhattacharyya *et al.* 1994; Glaab and Tindall 1997). Microsatellite sequences, short repetitive sequences of 1–6 nucleotides, occur predominantly in non-coding regions of DNA, and are usually maintained with a stable number of repeat units in normal cells with functional mismatch repair mechanisms. However, in HNPCC cells deficient in mismatch repair mechanisms, as well as in many spontaneous colon tumor cells (Aaltonen *et al.* 1993; Ionov *et al.* 1993; Thibodeau *et al.* 1993; Eshleman and Markowitz 1995; Umar and Kunkel 1996), microsatellite sequences contain insertions and deletions of repeat units resulting from unrepaired polymerase slippage events during replication (Umar and Kunkel 1996, and references therein). Based on the large number of microsatellites in the human genome, Perucho and colleagues have calculated that each tumor could carry more than 100,000 mutations in these sequences alone. Correction of the mismatch repair deficiency in tumor cell lines by complementation with a chromosome harboring a functional mismatch repair gene, corrects both the microsatellite instability and the high mutation rate at endogenous genes (Koi *et al.* 1994; Li and Modrich 1995).

This demonstration of a linkage between microsatellite instability and widespread genomic instability in HNPCC has led to multiple studies on the occurrence of this form of genomic instability in sporadic cancers. Different types of sporadic cancers that have been demonstrated to display microsatellite instability include: colon cancer (Ionov *et al.* 1993), endometrial cancer (Risinger *et al.* 1993), breast cancer (Patel *et al.* 1994), small cell lung cancer (Chen *et al.* 1996), non-small cell lung cancer (Ryberg *et al.* 1995), stomach cancer (Dos Santos *et al.* 1996), ovarian cancer (Boyer *et al.* 1995), prostate cancer (Egawa *et al.* 1994), esophageal squamous cell carcinoma (Mironov *et al.* 1995; Ogasawara *et al.* 1995), bladder cancer (Mao *et al.* 1994; Mao *et al.* 1996), gliomas and glioblastomas (Dams *et al.* 1995), cervical carcinoma (Larson *et al.* 1996), pancreatic adenocarcinoma (Brentnall *et al.* 1995), and to a high degree in patients with multiple independent cancers (Shinmura *et al.* 1995). Although the extent of instability associated with microsatellite sequences in sporadic cancers is not as extensive as that observed for HNPCC, these observations by multiple independent laboratories demonstrate that microsatellite instability is a common feature of many sporadic cancers. Eshleman and Markowitz (1995) provide a detailed compilation of the percentage of different sporadic tumors exhibiting microsatellite alteration. It is interesting to note that defects in known mismatch repair genes have been detected in approximately half of the sporadic colorectal tumors that exhibit microsatellite instability (Papadopoulos *et al.* 1995; Huang *et al.* 1996). This suggests that other alterations or mutations in the mismatch correction system are responsible for the observed microsatellite instability in many of these tumors.

TABLE 2

Microsatellite instability within coding sequences correlates with altered protein function and the cancer phenotype

Gene	Sequence altered	Mutation	Resulting phenotype	Reference
TGF-β RII	$(A)_{10}$	−AA	Truncated receptor results in loss of growth inhibition of epithelial tissues in response to TGF-β	Markowitz *et al.* (1995) Togo *et al.* (1996) Parsons *et al.* (1995)
	$(GT)_3$	+GT		
APC	$(G)_8$	+G, −G	Loss of function of tumor suppressor gene implicated in the initiation of colorectal cancer	Huang *et al.* (1996)
BAX	poly(A)	−A, +A, −AA	Inactivation of Bax results in loss of the normal apoptotic pathway in response to DNA damage, despite wild-type p53	Rampino *et al.* (1997)
	poly(T)	−T, −TT		
	$(AG)_4$	−AG		

Instability of repetitive sequences within transcribed genes: In addition to serving as an indicator mutation for genomic instability, instability of repetitive DNA sequences may contribute directly to the cancer phenotype. Recently, a number of genes implicated in the development or progression of cancer have been found to contain repetitive sequences within their coding regions (Table 2). Changes in the lengths of these repetitive sequences frequently results in gene inactivation, usually by truncation of these gene products, thereby enhancing the progression of the cancer phenotype. The TGF-β receptor II inhibits growth of epithelial tissues in response to the growth suppresser TGF-β (Wrana *et al.* 1994). Frameshift mutations at two repetitive sequences, a GTGTGT repeat at nucleotides 1931–1936 and a poly(A) repeat at nucleotides 709–718, occur in colon and gastric tumors exhibiting microsatellite instability, and produce a truncated TGF-β receptor that is resistant to the growth suppresser TGF-β (Markowitz *et al.* 1995; Parsons *et al.* 1995; Togo *et al.* 1996). TGF-β RII inactivation would confer upon these genetically unstable tumors a growth advantage and is likely to be an important step in the progression of these tumors.

The *APC* (adenomatous polyposis coli) gene is mutated in familial polyposis coli and is believed to be an early mutation during tumor progression in sporadic colon cancer (Vogelstein *et al.* 1988). The APC protein is truncated or absent in the majority of colorectal tumors, even in very small adenomas (Powell *et al.* 1992; Ichii *et al.* 1993; Levy *et al.* 1994; Luongo *et al.* 1994) as well as in dysplastic aberrant crypt foci that appear amongst the earliest histological manifestation of neoplasia (Jen *et al.* 1994; Smith *et al.* 1994a). Although mutations in *APC* occur in both microsatellite-unstable and microsatellite-stable tumors, the *APC* mutational spectrum is significantly different in these two tumor types (Huang *et al.* 1996). The vast majority of mutations in microsatellite-unstable tumors are frameshift mutations, and these frameshifts occur in mononucleotide and dinucleotide repeat sequences; while in microsatellite-stable tumors, the majority of mutations are base substitutions, and frameshifts occur predominantly in non-repeat sequences.

BAX is a member of the Bcl2 family of apoptosis genes transactivated by p53 in response to DNA damage (Oltvai *et al.* 1993; Cory 1995; Korsmeyer 1995; White 1996). Recently, it was demonstrated that colon cancers that exhibit microsatellite instability also exhibit instability at a poly$(G)_8$ repetitive sequence within the coding region of *BAX* (Rampino *et al.* 1997). Changes in the length of this repetitive sequence are observed in both cell lines and primary tumors but not in microsatellite-stable colorectal carcinomas. It has been proposed that during tumorigenesis, there may exist a selection for the inactivating frameshift mutations observed in the poly(G) microsatellite repeat in *BAX* in microsatellite-unstable colon tumors (Rampino *et al.* 1997). Since the only known function of BAX is to directly promote apoptosis (Oltvai *et al.* 1993; Cory 1995; Korsmeyer 1995; White 1996), inactivation of BAX by frameshift at the $(G)_8$ hotspot may result in a diminished capacity to trigger apoptosis in response to DNA damage, despite the presence of functional p53 protein. As a result, cells with extensive DNA damage may persist and have an increased probability to generate mutations during subsequent DNA replication cycles.

These findings of inactivating frameshift mutations at microsatellite sequences within the coding regions of genes involved in tumorigenesis bear directly on the hypothesis that microsatellite instability serves as an indicator mutation for genomic instability (Loeb 1991). The demonstration that instability of microsatellite sequences is associated with many different tumor types may make it possible to utilize these sequences as markers for the detection of cancer (Mao *et al.* 1994).

Microsatellite instability is an early step in carcinogenesis: In order for a mutator phenotype to be a driving force in tumor progression, it would have to occur as an early event. An initial mutation in a key stability pathway such as DNA replication, DNA repair, or cell cycle checkpoints, could decrease the fidelity of these processes. As a result, each subsequent round of DNA replication

could result in an increase in mutations throughout the genome. Among these mutations would be mutations in other genes involved in maintaining genomic stability, thereby further increasing the accumulation of mutations and advancing the cancer phenotype. Thus, a mutator phenotype provides a positive feedback mechanism for the exponential accumulation of mutations during tumor progression.

The hypothesis that the mutator phenotype is an early event in tumorigenesis (Loeb *et al.* 1974; Nowell 1976; Loeb 1994; Shibata *et al.* 1996) is supported by several lines of evidence. The demonstration that cancers from individuals with multiple independent tumors exhibit a markedly increased frequency of microsatellite alterations, relative to that observed in solitary tumors (Shinmura *et al.* 1995), argues that microsatellite instability is an early event. Furthermore, it was demonstrated by Shibata *et al.* (1994) that mutations in microsatellite sequences are significantly associated with distinctively early characteristics of colon carcinomas, including low incidence of *ras* and *p53* mutations, a poorly differentiated phenotype, and a low incidence of metastases at diagnosis. Clonal mutations in microsatellite sequences were observed in all neoplastic areas of tumors from the same patient, including adenomas, documenting that they are very early events in tumor development. In this same study, it was demonstrated that microsatellite instability was associated with diploid tumors and cells lines, while all colon cancer cell lines which were negative for microsatellite instability were aneuploid. Shibata *et al.* subsequently microdissected different segments of colon cancers and chronographed clonal expansion based on the emergence of different microsatellite mutations (Shibata *et al.* 1996). Diversity of microsatellite sequences was found to be higher in adenomas adjacent to invasive carcinomas, consistent with the concepts that the adenomas preceded the carcinomas, and that microsatellite instability is associated with this earlier stage of tumor progression.

Additional support for microsatellite instability as an early event is its presence in aberrant crypt foci, microscopic lesions of the colon thought to be the earliest identifiable precursors of colon cancer (Heinen *et al.* 1996). Furthermore, inactivating frameshift mutations have been demonstrated to occur in *APC*, a gene central to both familial and sporadic colon cancer, as has already been discussed. Microsatellite instability appears to precede *APC* inactivation in some (Heinen *et al.* 1996; Huang *et al.* 1996) but not in all studies (Smith *et al.* 1994b). The demonstration of inactivating frameshift mutations in *APC* provides evidence that microsatellite instability may precede and be responsible for mutation in a gene believed to initiate colon cancer.

Also consistent with a mutator phenotype as an early event in tumor progression are the observations that microsatellite instability can be detected in chronic inflammatory disease associated with a high incidence of cancer. For instance, patients with chronic pancreatitis are at an increased risk for the development of pancreatic cancer. It has been reported that cells in pancreatic juice from patients with pancreatitis or pancreatic cancer demonstrate microsatellite instability (Brentnall *et al.* 1995). Furthermore, microsatellite instability has been found to be associated with approximately 50% of ulcerative colitis patients who did not exhibit early changes associated with dysplasia (Brentnall *et al.* 1996). Therefore, a mutator phenotype indicated by microsatellite instability appears to occur as a very early event in tumorigenesis, and may serve as a signpost to monitor the course of tumor progression.

Mutators can arise in non-dividing cells: The concept of a mutator phenotype is based upon the assumption that mutations arise in dividing cells and that the mutation rate in these cells is insufficient to produce the large numbers of mutations found in human cancers. An alternative hypothesis has been proposed by Strauss (1992), in which mutations accumulate in non-dividing cells. The implication is that cancers arise in non-dividing cells which, over time, have accumulated large numbers of mutations that increase the ability of these cells to overcome the normally restrictive blocks to cell division. In this scenario, the mutation rate is not proportional to the number of cell generations but instead to time.

MacPhee (1995) has offered an interesting hypothesis to explain the origin of these mutations in non-dividing cells. Polymerase errors arising during repair synthesis in non-dividing cells would be repaired by mismatch repair, but in the absence of strand discrimination. There is some evidence that in mammalian cells the signal for strand discrimination, a DNA nick (Holmes *et al.* 1990), is generated during DNA replication in dividing cells. In the absence of this signal to direct the mismatch repair machinery to the appropriate strand, 50% of the mismatches would be repaired correctly, and 50% would be converted to mutations. The resulting mutations, accumulating over time, could enable the cell to escape the barrier to cell division, and facilitate further progression toward tumor development. The concept that mutations are generated by functional mismatch repair operating in the wrong direction in non-dividing cells is not directly related to the observations that mutations in mismatch repair genes are frequently observed in human cancers, because the presence of the signal for strand-discrimination in dividing cells reduces or eliminates error-generation.

The coupling of clonal selection with the induction of mutators: Both genetic instability and clonal selection are likely to contribute to the emergence of a mutator phenotype in cancer. Moreover, genetic instability and clonal selection may be tightly linked and interdependent. Most environmental carcinogens are mutagens. These agents induce mutations throughout the genome. Among the mutations would be those that gener-

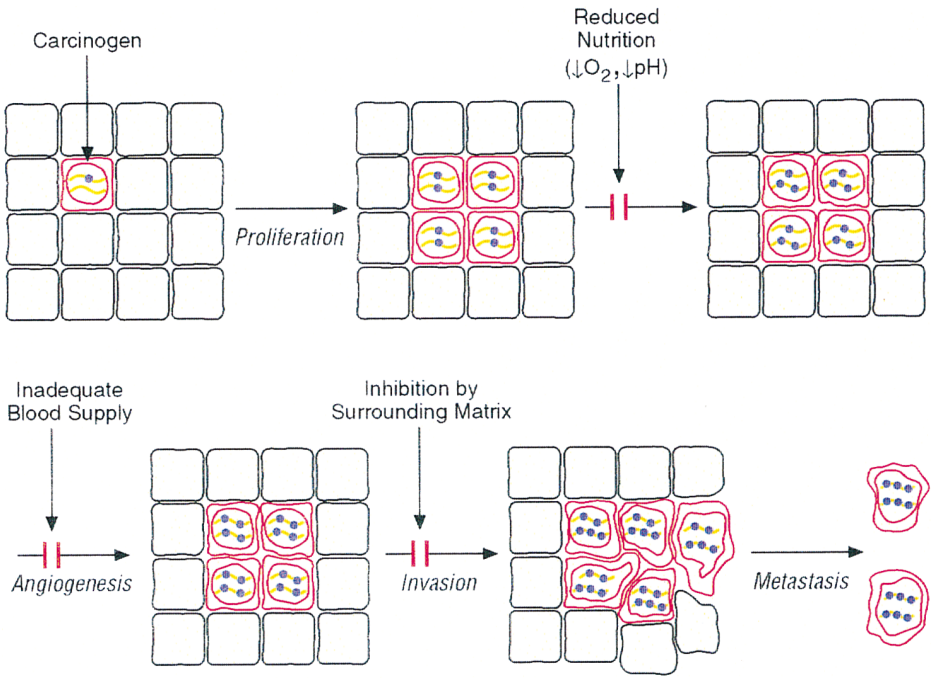

Figure 1.—Overcoming restrictive blocks to tumor progression. During the course of tumor development, progression through the multi-step process is likely to be hindered by restrictive blocks. During these periods of non-proliferation, selective pressure could result in the increased frequency of mutations, some of which could enable the tumor cell to overcome the restriction.

ate genetic instability. Thus, selection for mutations in a population would also result in the selection of mutators. By this logic, clonal selection for mutants would result in an enrichment of mutators within the population. During the course of subsequent divisions, further mutations could be generated, among which could be more mutators. Selection for a mutant phenotype in a cell population can greatly increase the proportion of mutators in the selected population. Even the very low level of mutators that occur spontaneously can be dramatically increased during selection.

This has been demonstrated several times, most recently by Mao *et al.* (1997), during selection for spontaneous Lac^+ revertants from a particular Lac^- bacterial population. This selection increases the percentage of mutators from 1/100,000 to 1/200. When subjected to a second-step selection, such as resistance to rifampicin, as many as 20% of the resulting two-step doubly-resistant mutants contain mutators. Therefore, clonal selection alone can produce a large number of mutators within a population. However, exposure to a mutagen also significantly increases the proportion of mutators in a population. Mutagenesis followed by as few as one or two selections can result in 100% of the population consisting of mutators.

Let us consider these experiments in the context of tumor progression. Following an initial mutagenic event, the frequency of mutators in a population of cells undergoing sequential rounds of selection (for uncontrolled growth, escape from tumor progression, etc.) could rapidly increase. Selection can occur in the form of the normally restrictive blocks to cell division and tumor progression which function in normal cells. These blocks include limitation for nutrients, decreased oxygen levels, decreased pH, and contact inhibition (Figure 1). The recent results by Richards *et al.* (1997) provide a mechanism for evading these successive blocks. It was demonstrated that two human tumor cell lines deficient in a key mismatch repair enzyme are not mutators in culture during periods of rapid growth. The maintenance of cells at high density in the absence of proliferation results in an increase in mutagenesis after cell division. Increasing the mutation rate during non-proliferation increases the likelihood that an advantageous mutation will arise in the tumor population which will escape the restriction and form a resistant clone. Thus, successive periods of non-proliferation caused by restriction on tumor growth could increase the number of mutations in a tumor cell population as well as increase the frequency of mutators.

Mutators may be required by tumors to adapt to changing environments. Taddei *et al.* (1997) utilized computer simulation to model the role of mutators in adaptive evolution. In the absence of selection for adaptive mutations, an equilibrium is reached whereby the spontaneous generation of mutators in a population is balanced by the increased production of deleterious and lethal mutations by the mutator. Therefore, mutator mutations are selected against in the absence of strong selection. However, adaptation can be very different when the ability of the population to adapt to a new environment is limited by mutation availability. Such a situation could exist in the developing tumor, in which the spontaneous mutation rate of the cells limits the ability of the population to overcome the restrictive blocks to tumor progression. Because these blocks provide strong selection against further tumor development, tumor cells must have an increased mutation rate

to overcome these blocks. This model is in accord with the hypothesis that cancers exhibit a mutator phenotype (Loeb 1991). A mutator phenotype is the result of a mutation in a gene whose product functions to maintain genomic stability. We have hypothesized that such a mutation is an early event in carcinogenesis and may be required for all the subsequent alterations necessary for a cell to progress from a pre-cancerous state to a metastatic tumor (Loeb 1991). Thus, the mutator phenotype resulting from selection for mutators can increase the probability that cells will gain the ability to adapt to the changing environment of the tumor, thereby enabling the tumor cell population to progress along the path toward metastasis. It is important to note that the mutation rate need not be elevated late in tumor progression; in highly developed tumors, there might be selection against cells exhibiting a mutator phenotype (Loeb 1998). However, the manifestations of a mutator phenotype, the multiple mutations in each cancer cell, should be detectable even in late-stage tumors.

Conclusion: DNA replication is a very accurate process in normal human cells. This consistency of DNA replicative processes is reinforced by the studies of Drake that establish in DNA-based microbes and RNA viruses a specific rate of mutations per nucleotide per generation. In contrast, there is increasing evidence to suggest that this accuracy is diminished during the development of cancers. As a result, cancers are likely to express a mutator phenotype early in their evolution. Quantitation of the number of mutations that a tumor accumulates may be important for both diagnosis and prognosis. To do this, will require new methodologies for the measurement of random mutations in DNA.

LITERATURE CITED

Aaltonen, L. A., P. Peltomaki, F. S. Leach, P. Sistonen, L. Pylkkanen *et al.*, 1993 Clues to the pathogenesis of familial colorectal cancer. Science **260:** 812–816.

Armitage, P., and R. Doll, 1954 The age distribution of cancer and a multi-stage theory of carcinogenesis. Br. J. Cancer **8:** 1–12.

Balaban, G. B., M. Herlyn, W. H. Clark, Jr. and P. C. Nowell, 1986 Karyotypic evolution in human malignant melanoma. Cancer Genet. Cytogenet. **19:** 113–122.

Bhattacharyya, N. P., A. Skandalis, A. Ganesh, J. Groden and M. Meuth, 1994 Mutator phenotypes in human colorectal carcinoma cell lines. Proc. Natl. Acad. Sci. USA **91:** 6319–6323.

Boyer, J. C., A. Umar, J. I. Risinger, J. R. Lipford, M. Kane *et al.*, 1995 Microsatellite instability, mismatch repair deficiency, and genetic defects in human cancer cell lines. Cancer Res. **55:** 6063–6070.

Brentnall, T. A., R. Chen, J. G. Lee, M. B. Kimmey, M. P. Bronner *et al.*, 1995 Microsatellite instability and K-*ras* mutations associated with pancreatic adenocarcinoma and pancreatitis. Cancer Res. **55:** 4264–4267.

Brentnall, T. A., D. A. Crispin, M. P. Bronner, S. P. Cherian, M. Hueffed *et al.*, 1996 Microsatellite instability in nonneoplastic mucosa from patients with chronic ulcerative colitis. Cancer Res. **56:** 1237–1240.

Bronner, C. E., S. M. Baker, P. T. Morrison, G. Warren, L. G. Smith *et al.*, 1994 Mutation in the DNA mismatch repair homologue *hMLH1* is associated with hereditary non-polyposis colon cancer. Nature **368:** 258–261.

Chen, X. Q., M. Stroun, J. L. Magnenat, L. P. Nicod, A. M. Kurt *et al.*, 1996 Microsatellite alterations in plasma DNA of small cell lung cancer patients. Nat. Med. **2:** 972–974.

Chu, E. H. Y., M. Boehnke, S. M. Hanash, R. D. Kuick, B. J. Lamb *et al.*, 1988 Estimation of mutation rates based on the analysis of polypeptide constituents of cultured human lymphblastoid cells. Genetics **119:** 693–703.

Cory, S., 1995 Regulation of lymphocyte survival by the *bcl-2* gene family. Annu. Rev. Immunol. **13:** 513–543.

Dams, E., E. J. Z. Van de Kelft, J.-J. Martin, J. Verlooy and P. J. Willems, 1995 Instability of microsatellites in human gliomas. Cancer Res. **55:** 1547–1549.

DeMars, R., and K. R. Held, 1972 The spontaneous azaguanine-resistant mutants of diploid human fibroblasts. Humangenetik **16:** 87–110.

Dos Santos, N. R., R. Seruca, M. Costancia, M. Seixas and M. Sobrinho-Simoes, 1996 Microsatellite instability at multiple loci in gastric carcinoma: clinicopathologic implications and prognosis. Gastroenterology **110:** 38–44.

Drake, J. W., 1991 A constant rate of mutation in DNA-based microbes. Proc. Natl. Acad. Sci. USA **88:** 7160–7164.

Drake, J. W., 1993 Rates of spontaneous mutation among RNA viruses. Proc. Natl. Acad. Sci. USA **90:** 4171–4175.

Egawa, S., T. Uchida, K. Suyama, C. Wang, M. Ohori *et al.*, 1994 Genomic instability of microsatellite repeats in prostate cancer: relationship to clinicopathological variables. Cancer Res. **55:** 2418–2421.

Eigen, M., 1993 Viral quasispecies. Sci. Amer. **269:** 42–49.

Eigen, M., and P. Schuster, 1977 The hypercycle. A principle of natural self-organization. Part A: Emergence of the hypercycle. Naturwissenschaften **64:** 541–565.

Eshleman, J. R., and S. D. Markowitz, 1995 Microsatellite instability in inherited and sporadic neoplasms. Curr. Opin. Oncol. **7:** 83–89.

Fearon, E. R., and B. Vogelstein, 1990 A genetic model for colorectal tumorigenesis. Cell **61:** 759–767.

Glaab, W. E., and K. R. Tindall, 1997 Mutation rate at the *hprt* locus in human cancer cell lines with specific mismatch repair-gene defects. Carcinogenesis **18:** 1–8.

Harris, H., 1988 The analysis of malignancy by cell fusion: the position in 1988. Cancer Res. **48:** 3302–3306.

Heinen, C. D., N. Shivapurkar, Z. Tang, J. Groden and O. Alabaster, 1996 Microsatellite instability in aberrant crypt foci from human colons. Cancer Res. **56:** 5339–5341.

Holland, J., K. Spindler, F. Horodyski, E. Grabau, S. Nichol *et al.*, 1982 Rapid evolution of RNA genomes. Science **215:** 1577–1585.

Holmes, J., S. J. Clark and P. Modrich, 1990 Strand-specific mismatch correction in nuclear extracts of human and *Drosophila melanogaster* cell lines. Proc. Natl. Acad. Sci. USA **87:** 5837–5841.

Huang, J., N. Papadopoulos, A. J. McKinley, S. M. Farrington, L. J. Curtis *et al.*, 1996 APC mutations in colorectal tumors with mismatch repair deficiency. Proc. Natl. Acad. Sci. USA **93:** 9049–9054.

Ichii, S., S. Takeda, A. Horii, S. Nakatsuru, Y. Miyoshi *et al.*, 1993 Detailed analysis of genetic alterations in colorectal tumors from patients without familial adenomatous polyposis (FAP). Oncogene **8:** 2399–2405.

Ionov, Y., M. A. Peinado, S. Malkhosyan, D. Shibata and M. Perucho, 1993 Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature **363:** 558–561.

Iwabuchi, H., M. Sakamoto, H. Sakunaga, Y-Y. Ma, M. L. Carcangiu *et al.*, 1995 Genetic analysis of benign, low grade, and high grade ovarian tumors. Cancer Res. **55:** 6172–6180.

Jen, J., S. M. Powell, N. Papadopoulos, K. J. Smith, S. R. Hamilton *et al.*, 1994 Molecular determinants of dysplasia in colorectal lesions. Cancer Res. **54:** 5523–5526.

Kallioniemi, A., O.-P. Kallioniemi, J. Piper, M. Tanner, T. Stoke *et al.*, 1994 Detection and mapping of amplified DNA sequences in breast cancer by comparative genomic hybridization. Proc. Natl. Acad. Sci. **91:** 2156–2160.

Koi, M., A. Umar, D. Chauhan, S. Cherian, J. M. Carethers *et al.*, 1994 Human chromosome 3 corrects mismatch repair deficiency and microsatellite instability and reduces *N*-methyl-*N*′-nitro-N-

nitroguanidine tolerance in colon tumor cells with homozygous *hMLH1* mutation. Cancer Res. **54:** 4308–4312.

Korsmeyer, S. J., 1995 Regulators of cell death. Trends Genet. **11:** 101–105.

Larson, A. A., S. Kern, R. L. Sommers, J. Yokota, W. K. Cavenee *et al.*, 1996 Analysis of replication error (RER+) phenotypes in cervical carcinoma. Cancer Res. **56:** 1426–1431.

Leach, F. S., N. C. Nicolaides, N. Papadopoulos, B. Liu, J. Jen *et al.*, 1993 Mutations of a *mutS* homolog in hereditary nonpolyposis colorectal cancer. Cell **75:** 1215–1225.

Levy, D. B., K. J. Smith, Y. Beazer-Barclay, S. R. Hamilton, B. Vogelstein *et al.*, 1994 Inactivation of both *APC* alleles in human and mouse tumors. Cancer Res. **54:** 5953–5958.

Li, G.-M., and P. Modrich, 1995 Restoration of mismatch repair to nuclear extracts of H6 colorectal tumor cells by a heterodimer of human MutL homologs. Proc. Natl. Acad. Sci. USA **92:** 1950–1954.

Loeb, L. A., 1991 Mutator phenotype may be required for multistage carcinogenesis. Cancer Res **51:** 3075–3079.

Loeb, L. A., 1994 Microsatellite instability: marker of a mutator phenotype in cancer. Cancer Res. **54:** 5059–5063.

Loeb, L. A., 1998 Cancer cells manifest a mutator phenotype. Adv. Cancer Res. (in press).

Loeb, L. A., C. F. Springgate and N. Battula, 1974 Errors in DNA replication as a basis of malignant change. Cancer Res. **34:** 2311–2321.

Luongo, C., A. R. Moser, S. Gledhill and W. F. Dove, 1994 Loss of Apc+ in intestinal adenomas from Min mice. Cancer Res. **54:** 5947–5952.

MacPhee, D. G., 1995 Mismatch repair, somatic mutations, and the origins of cancer. Cancer Res. **55:** 5489–5492.

Mao, E. F., L. Lane, J. Lee and J. H. Miller, 1997 Proliferation of mutators in a cell population. J. Bacteriol. **179:** 417–422.

Mao, L., D. J. Lee, M. S. Tockman, Y. S. Erozan, F. Askin *et al.*, 1994 Microsatellite alterations as clonal markers for the detection of human cancer. Proc. Natl. Acad. Sci. USA **91:** 9871–9875.

Mao, L., M. P. Schoenberg, M. Scicchitano, Y. S. Erozan, A. Merlo *et al.*, 1996 Molecular detection of primary bladder cancer by microsatellite analysis. Science **271:** 659–662.

Markowitz, S. J., J. Wang, L. Myeroff, R. Parsons, L. Sun *et al.*, 1995 Inactivation of the type II *TGF*-β receptor in colon cancer cells with microsatellite instability. Science **268:** 1336–1338.

McNieve, I. K., F. M. Stewart, D. M. Deacon, D. S. Temeles, K. M. Zsebo *et al.*, 1989 Detection of a human CFC with a high proliferative potential. Blood **74:** 609–612.

Mironov, N. M., A.-M. Aguelon, E. Hollams, J.-C. Lozano and H. Yamasaki, 1995 Microsatellite alterations in human and rat esophageal tumors at selective loci. Mol. Carcinog. **13:** 1–5.

Naylor, S. L., B. E. Johnson, J. D. Minna and A. Y. Sakaguchi, 1987 Loss of heterozygosity of chromsome 3p markers in small-cell lung cancer. Nature **329:** 451–454.

Neel, J. V., C. Satoh, K. Goriki, M. Fujita, N. Takahashi *et al.*, 1986 The rate with which spontaneous mutation alters the electrophoretic mobility of polypeptides. Proc. Natl. Acad. Sci. USA **83:** 389–393.

Ninio, J., 1991 Transient mutators: a semiquantitative analysis of the influence of translation and transcription errors on mutation rates. Genetics **129:** 957–962.

Nowell, P. C., 1976 The clonal evolution of tumor cell populations. Science **194:** 23–28.

Ogasawara, S., C. Maesawa, G. Tamura and R. Satodate, 1995 Frequent microsatellite alterations on chromosome 3p in esophageal squamous cell carcinoma. Cancer Res. **55:** 891–894.

Oller, A. R., P. Rastogi, S. Morgenthaler and W. G. Thilly, 1989 A statistical model to estimate variance in long term low dose mutation assays: testing of the model in a human lymphoblastoid mutation assay. Mutat. Res. **216:** 149–161.

Oltvai, Z. N., C. L. Milliman and S. J. Korsmeyer, 1993 Bcl-2 heterodimerizes *in vivo* with a conserved homolog, Bax, that accelerates programmed cell death. Cell **74:** 609–619.

Papadopoulos, N., N. C. Nicolaides, Y.-F. Wei, S. M. Ruben, K. C. Carter *et al.*, 1994 Mutation of a *mut*L homolog in hereditary colon cancer. Science **263:** 1625–1629.

Papadopoulos, N., N. C. Nicolaides, B. Liu, R. E. Parsons, C. Lengauer *et al.*, 1995 Mutations of *GTBP* in genetically unstable cells. Science **268:** 1915–1917.

Parsons, R., L. L. Myeroff, B. Liu, J. K. V. Willson, S. D. Markowitz *et al.*, 1995 Microsatellite instability and mutations of the transforming growth factor β type II receptor gene in colorectal cancer. Cancer Res. **55:** 5548–5550.

Patel, U., S. Grimdfest-Broniatowski, M. Gupta and S. Banerjee, 1994 Microsatellite instabilities at five chromosomes in primary breast tumors. Oncogene **9:** 3695–3700.

Peinado, M. A., S. Malkhosyan, A. Velazquez and M. Perucho, 1992 Isolation and characterization of allelic loss and gains in colorectal tumors by arbitrarily primed polymerase chain reaction. Proc. Natl. Acad. Sci. USA **89:** 10065–10069.

Peltomaki, P., L. A. Aaltonen, P. Sistonen, L. Pylkkanen, J.-P. Mecklin *et al.*, 1993 Genetic mapping of a locus predisposing to human colorectal cancer. Science **260:** 810–812.

Powell, S. M., N. Zilz, Y. Beazer-Barclay, T. M. Bryan, S. R. Hamilton *et al.*, 1992 APC mutations occur early during colorectal tumorigenesis. Nature **359:** 235–237.

Rampino, N., H. Yamamoto, Y. Ionov, Y. Li, H. Sawai *et al.*, 1997 Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. Science **275:** 967–969.

Richards, B., H. Zhang, G. Phear and M. Meuth, 1997 Conditional mutator phenotypes in hMSH2 deficient tumor cell lines. Science (in press).

Risinger, J. L., A. Berchuck, M. F. Kohler, P. Watson, H. T. Lynch *et al.*, 1993 Genetic instability of microsatellites in endometrial carcinoma. Cancer Res. **53:** 5100–5103.

Ryberg, D., B. A. Lindstedt, S. Zienolddiny and A. Haugen, 1995 A hereditary genetic marker closely associated with microsatellite instability in lung cancer. Cancer Res. **55:** 3996–3999.

Shibata, D., W. Navidi, R. Salovaara, Z.-H. Li and L. A. Aaltonen, 1996 Somatic microsatellite mutations as molecular tumor clocks. Nature Med. **2:** 676–681.

Shibata, D., M. A. Peinado, Y. Ionov, S. Malkhosyan and M. Perucho, 1994 Genomic instability in repeated sequences is an early somatic event in colorectal tumorigenesis that persists after transformation. Nat. Genet. **6:** 273–281.

Shinmura, K., H. Sugimura, Y. Naito, P. G. Shields and I. Kino, 1995 Frequent co-occurrence of mutator phenotype in synchronous, independent multiple cancers of the stomach. Carcinogenesis **16:** 2989–2993.

Smith, A. J., H. S. Stern, M. Penner, M. Hay, A. Mitri *et al.*, 1994a Somatic *APC* and K-*ras* codon 12 mutations in aberrant crypt foci from human colons. Cancer Res. **54:** 5527–5530.

Smith, R. J., K. Pack, S. Hodgson, S. K. Tay, R. Phillips *et al.*, 1994b *APC* mutation associated with late onset of familial adenomatous polyposis. J. Med. Genet. **31:** 888–890.

Strauss, B. S., 1992 The origin of point mutations in human tumor cells. Cancer Res. **52:** 249–253.

Taddei, F., M. Radman, J. Maynard-Smith, B. Toupance, P. H. Gouyon *et al.*, 1997 Role of mutator alleles in adaptive evolution. Nature **387:** 700–702.

Thibodeau, S. N., G. Bren and D. Schaid, 1993 Microsatellite instability in cancer of the proximal colon. Science **260:** 816–819.

Togo, G., N. Toda, F. Kanai, N. Kato, Y. Shiratori *et al.*, 1996 A transforming growth factor β type II receptor gene mutation common in sporadic cecum cancer with microsatellite instability. Cancer Res. **56:** 5620–5623.

Umar, A., J. C. Boyer, D. C. Thomas, D. C. Nguyen, J. I. Risinger *et al.*, 1994 Defective mismatch repair in extracts of colorectal and endometrial cancer cell lines exhibiting microsatellite instability. J. Biol. Chem. **269:** 14367–14370.

Umar, A., and T. A. Kunkel, 1996 DNA-replication fidelity, mismatch repair and genomic instability in cancer cells. Eur. J. Biochem. **238:** 297–307.

Vogelstein, B., E. R. Fearon, S. E. Kern, S. R. Hamilton, A. C. Preisinger *et al.*, 1988 Genetic alterations during colorectal-tumor development. N. Engl. J. Med. **319:** 525–532.

Weinberg, R. A., 1995 The molecular basis of oncogenes and tumor suppresser genes. Ann. NY Acad. Sci. **758:** 331–338.

White, E., 1996 Life, death, and the pursuit of apoptosis. Genes Dev. **10:** 1–15.

Wrana, J. L., L. Attisano, R. Weiser, F. Ventura and J. Massagué, 1994 Mechanism of activation of the TGF-β receptor. Nature **370:** 341–347.