Hypermutability in Carcinogenesis

Bernard S. Strauss

Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, Illinois 60637

ABSTRACT

The presence of numerous chromosomal changes and point mutations in tumors is well established. At least some of these changes play a role in the development of the tumors. It has been suggested that the number of these genetic changes requires that tumorigenesis involves an increase in mutation rate. However, the presence of numerous changes can also be accounted for by efficient selection. What is required to settle the issue is some measure of nonselected mutations in tumors. In order to determine whether the tumor suppressor TP53 (coding for the protein p53) is hypermutable at some stage of carcinogenesis, the frequency of silent and multiple mutations in this gene has been examined. Silent mutations make up ∼3% of the total recorded but constitute 9.5% of the mutations found in tumors with multiple mutations. Multiple closely linked mutations are also observed. Such multiple mutations suggest the operation of an error-prone replication process in a subclass of cells. The published data indicate that TP53 is hypermutable at some stage of tumor development. It is not yet clear whether TP53 is unique or whether other genes display a similar pattern of silent and multiple mutations.

Are tumors hypermutable? Tumors accumulate genetic alterations as they progress, and it seems very likely that at least a portion of these genetic alterations play a role in the etiology of the disease (Vogelstein *et al.* 1988; Goyette *et al.* 1992). This accumulation of changes is so striking that many investigators suppose that a condition of genetic instability is a necessary concomitant of tumorigenesis (Loeb *et al.* 1974; Loeb 1991; Cheng and Loeb 1993). The genetic changes observed are of different kinds and include both point mutations and qualitative and quantitative chromosome alterations. Chromosome instability might occur without affecting the fidelity of the replication process, that is, without changing the rate of gene mutation. The question to be addressed here is whether a general increase in the point mutation rate occurs at some stage in carcinogenesis.

It has been argued that the normal mutation rate is insufficient to generate the changes required in a single cell and that there must, therefore, be a special process at work in cells destined to engender tumors (Loeb 1991). Some years ago it would have been supposed that exogenous environmental agents acting as mutagens were in large part responsible for this increase (Ames 1973), but such arguments are no longer as compelling (Ames *et al.* 1995). Many of the changes observed in tumors are associated with G:C→A:T transitions characteristic of spontaneous change rather than the G:C→T:A transversions characteristic of chemical mutagenesis

(Hollstein *et al.* 1991). Current hypotheses suggest that the developing tumor cells carry "mutator" genes that diminish normal cellular surveillance mechanisms and increase the spontaneous mutation rate (Loeb 1994). Such surveillance mechanisms do exist (Modrich 1995), and certain types of tumors occur more frequently in individuals with defects in genes responsible for the coding of the necessary surveillance proteins. The most spectacular recent finding is the association of certain colon carcinomas with defects in the mismatch repair genes (Fishel *et al.* 1993; Modrich 1995), but the earlier association of a defect in excision repair with skin carcinomas is equally striking (Cleaver 1968). These correlations are persuasive arguments for the tumor hypermutability hypothesis.

The mutation frequency for point mutations is very significantly increased in the cells of individuals deficient in mismatch repair (Bhattacharyya *et al.* 1995; Malkhosyan *et al.* 1996). However, Parsons *et al.* (1995) found a subset of patients whose somatic cells were devoid of mismatch repair activity and were hypermutable, but such individuals did not have the number of early tumors that might be expected. In addition, the tumors that do occur in patients with hereditary nonpoyposis colorectal cancer represent a limited set, that is, certain kinds of tumors occur but not others. It may be that the importance of mismatch repair deficiency is not primarily its effect on the generalized mutation rate but rather on the mutation rate of genes containing repeated nucleotide sequences within the coding region of critical genes, for example, the observed frameshift in a run of 10 (A's) in the TGF-βII receptor gene (Lu *et al.* 1995; Markowitz *et al.* 1995; Akiyama *et al.* 1996; Rampino *et al.* 1997; Souza *et al.* 1996). Protection against single base changes that occur at the growing point during DNA synthesis is provided by both the

Address for correspondence: Department of Molecular Genetics and Cell Biology, The University of Chicago, 920 E. 58th St., Chicago, IL 60637. E-mail: bs19@midway.uchicago.edu

This paper is dedicated to Dr. John Drake on the occasion of his retirement as Editor of Genetics, in the hope that he may find it amusing.

mismatch repair and proofreading systems (Friedberg *et al.* 1995). Frameshifts in long-repeated sequences are to some extent ignored by the proofreading system but are efficiently recognized by the mismatch repair system (Strauss *et al.* 1997; Tran *et al.* 1997). Mismatch repair deficiency may therefore affect genes with such sequences to a disproportionate extent. If this explanation is correct, mismatch repair deficiency makes certain critical changes more likely, but the general increase in mutation need not result in generalized tumorigenesis. Demonstration that hypermutability is a general property of cells undergoing neoplastic transformation requires additional kinds of evidence.

The example of xeroderma pigmentosum has long been used as an argument for the role of DNA repair in removing tumorigenic lesions (Cleaver 1968) and, indirectly, for generalized mutagenesis as a causative factor in cancer induction. One of the signs of xeroderma pigmentosum is an increased frequency of sunlight-induced skin carcinoma. Such tumors are associated with dinucleotide "signature" mutations (Brash *et al.* 1991; Dumaz *et al.* 1993; Matsumura *et al.* 1996), and the lack of repair activity ensures the persistence of sunlight-induced lesions long enough to result in replication errors. These characteristics of xeroderma patients link mutation and cancer. Some years ago, Cairns (1981) proposed that the xeroderma defect resulted only in skin tumors, and his paper provoked the collection of data showing that internal tumors are also increased in xeroderma patients (Kraemer *et al.* 1984; Kraemer *et al.* 1994). "Knockout" xeroderma mice (XPA deficient) do develop spontaneous liver tumors and an increased frequency of benzpyrene-induced lymphomas as compared to normal mice (de Vries *et al.* 1997). Deficiency in repair synthesis, however, is not necessarily the cause of the tumorigenesis because individuals with trichothiodystropy and some cases of Cockaynes' syndrome have excision repair activities as low as some xeroderma patients but do not have an increased frequency of skin tumors, notwithstanding their sensitivity to light. Despite several attempts, this paradox has not been explained (Chu and Mayne 1996; Marionnet *et al.* 1996; Stary and Sarasin 1996; Takayama *et al.* 1996; Weeda *et al.* 1997). The genes involved are complex, with both repair and transcription functions. These examples suggest that heightened mutability is not necessarily accompanied by a general increase in tumorigenesis and also that increased mutability can be associated with only specific sorts of tumors.

Arguments against the hypothesis that tumors are necessarily hypermutable have a long history (Armitage and Doll 1957; Fisher 1958). Two recent discussions have been published by Tomlinson *et al.* (1996) and by Simpson (1997). The development of each tumor is an evolutionary event and, as in macroevolution, mutations are subject to selection, and some mutations are neutral and subject to genetic drift. The presence of any number of mutations in a tumor is the result of these different forces. Both very early and very recent calculations indicate that, given the power of selection and the biology of tumor development, selection is capable of yielding clones with multiple mutations without an increase in the normal mutation rate. Such calculations do not settle the issue, but they certainly indicate that the presence of multiple mutations in tumors is not sufficient to establish the case for general tumor hypermutability.

Some of the evidence that tumor cells are not necessarily hypermutable has already been summarized. As an example, Harwood *et al.* (1991) reported that two lines derived from a human colorectal carcinoma had mutation rates of 5×10^{-8} and 3×10^{-8} per cell per generation for the *apr*t gene, rates "as low or lower" than for diploid human cell lines. Wittenkeller *et al.* (1997) compared the mutation rates of hypoxanthine-guanine phosphoribosyltransferase in an immortalized human bronchial epithelial cell line and its tumorigenic derivative and found that the tumor line had slightly lower rates. Of course, the control "line" had been immortalized so that even though not a tumor, it was hardly "normal." Buettner *et al.* (1996) found an increased mutation frequency (for a transgenic *lac* gene) in only one of four tumors arising in p53 knockout mice, and even in that tumor the increase was only 2.3 fold. The p53 knockout mutation rate in normal tissue was not different from the normal (Nishino *et al.* 1995). The rate of point mutation is not permanently higher than the normal somatic mutation rate for some genes in (some) tumor cells.

***TP53* mutation:** The *TP53* tumor suppressor gene, whose product, the p53 protein, plays an important role in regulation of the cell cycle (Levine 1997), is much studied: numerous mutations have been reported, and there are several data bases available that summarize the information (DeVries *et al.* 1996; Carriello *et al.* 1997; Hainut *et al.* 1997). The analysis below utilizes the data base first prepared by Hollstein *et al.* (1996) which is available as an Excel spreadsheet (Hainut *et al.* 1997). This data base includes all reported mutations in tumors and attempts to exclude mutations at known polymorphic sites. Up to the end of 1996 (Hainut *et al.* 1997), there were 6005 total p53 mutations recorded. It seemed possible that an analysis of the mutational data base, and in particular the analysis of the frequency with which silent mutations occur, would permit an estimate of the nonselected mutation frequency for the *TP53* gene. Although knowledge of the mutation frequency does not permit estimation of the mutation rate, a comparison of the (nonselected) mutation frequencies of genes in normal and tumor tissue might give some indication of whether tumors are hypermutable.

Silent mutation: Silent mutations are defined as nucleotide substitutions that do not result in amino acid changes, owing to the redundancy of the genetic code.

There were 202 silent mutations in the tumors (Hainut *et al.* 1997). However, nine of the silent mutations recorded occur at codons reported as polymorphic. Consequently, there are $(193/6005) \times 100 = 3.2\%$ silent mutations in the sample. Their exact positions and the nature of the substitutions are readily retrieved from the data base (Hainut *et al.* 1997). The percentage of silent mutations is about the same in tumors and in cell lines (Strauss 1997).

A certain proportion of the recorded mutations are probably due to PCR errors, a problem that has been discussed previously (Strauss 1997). Although errors probably do occur, many of the papers reporting mutations do amplify several samples independently and take other precautions to reduce the possibility of error. The percentage of silent mutations reported in the 1996 update $(42/915)$ is slightly higher than the overall value, making it more likely that ~3% represents an accurate estimate of the frequency of silent mutations among all *TP53* mutations. The methodology for the detection of mutations is necessarily becoming more reliable as the utility of mutational analysis as a diagnostic tool becomes evident. The question of whether the changes represent preexisting polymorphisms is harder to address because many of the investigations utilize paraffin-embedded archival samples as their source of tissue and do not have normal tissue controls.

If mutation were random and neutral, 23.5% of all mutations in *TP53* would be silent. This figure is based on the actual usage of codons in p53 and the probability of a mutation in that codon being silent. The percentage of observed silent mutations is much lower. However, inactivation of p53 function plays a role in the etiology of tumors and so frameshifts, missense, and nonsense mutations would be expected to have a selective advantage. If we suppose that a first mutation in p53 inactivates its function and contributes to tumorigenesis, then it is possible that additional mutations can accumulate in a nonselective way. A tabulation of the occurrence of silent mutations in tumors with multiple p53 mutations might therefore make possible a nonbiased estimate of their frequency. In the most recent data, there are 305 tumors with multiple mutations, and these tumors had a total of 693 mutations. After removal of four possibly polymorphic silent changes, 66 mutations, or 9.5%, were silent. This figure is still an underestimate of the total percentage of silent mutations because, as indicated above, a nonsilent mutation is (presumably) required for entry into the data set. If one assumes that among the tumors with multiple p53 mutations, silent mutations are only found in tumors with a p53-inactivating missense, nonsense, or frameshift mutation, then a corrected silent mutation frequency can be obtained by dividing the total number of silent mutations (66) among the multiple mutations (693) by the total number of mutations in that sample minus the total number of tumors with multiple mutations (305) as a correction

for the ascertainment bias. The corrected frequency is 17% $([66/693\text{-}305] \times 100)$. Because not all silent mutations need be nonselective (see below), the figure for silent mutations is probably somewhere between 9.5% and 17%. What is critical is that considerable numbers of silent mutations occur. An essentially similar argument, in which the second mutations in doublets (including the silent mutations) are considered as "hitchhikers," has been made by Rodin *et al.* (1998).

Individual investigations from the published data base in which substantial numbers of p53 mutations have been recorded do give results nearer to the expected value for silent mutations. In one of the cases in which silent mutations were found and in which the surrounding tissue was also sequenced, 11 out of 29 single nucleotide changes were silent, and of these, only two were found in the surrounding tissue at a known polymorphic site (Hongyo *et al.* 1995). In another investigation, a total of 64 bladder tumors yielded 45 p53 mutations of which 11 (24%) were silent (Taylor *et al.* 1996). Two of the tumors had two silent mutations each, and for these polymorphism was eliminated by sequencing blood DNA. None of the other silent mutations were at the sites of known polymorphisms (Weston and Godbold 1997).

Silent mutations need not be selectively neutral and, in fact, are not in many organisms (Sharp *et al.* 1995). Codon usage differs, and the availability of tRNAs is different for the different codons. In Drosophila, in yeast, and in *E. coli* it has been shown that codons are selected. This does not seem to be true for humans in which codon usage for the same amino acid in different genes expressed at the same time may differ widely (Sharp *et al.* 1993; see Table 6 in Strauss 1997). It has also been pointed out that silent mutation might produce alternative splice sites (Hongyo *et al.* 1995). The production of a 5′ GT, for example, could result in a new splice site and an altered p53 protein, but no such altered protein has been isolated. Notwithstanding these caveats, the hypothesis that most of the silent mutations are selectively neutral for tumor formation in humans remains a reasonable one.

If silent mutations are effectively neutral, then their frequency in tumors, as compared to the frequency of newly occurring (*i.e.*, nonpolymorphic) mutations in normal tissue, should provide a clue to the question of the mutation rate in tumors. There is only the most limited data that would permit an estimate of what the expected frequency of a newly arising mutation in a tumor might be, considering that a sufficient number of generations would have had to occur between the mutation and the assay to result in a clone of cells, most of which contained the mutation, so that it would be detected by current PCR methodology. Aguilar *et al.* (1994) estimated the frequency for mutation at one nucleotide of the frequently mutated p53 codon 249 in nonmalignant samples of liver from hepatocellular carci-

noma patients. The detection methodology depends on the resistance of mutated sites to restriction-enzyme digestion. Values of <2 to 13×10^{-7} per base were obtained for mutation at the third position of codon 249 in three nonmalignant liver samples. Assuming that all positions in the *TP53* gene have the same mutability, but that only one-fourth of the changes would give silent mutations, the highest frequency of silent mutations would be $1/4 \times 1179$ coding bases $\times 1.3 \times 10^{-6} = 3.8 \times 10^{-4}$, or 0.04%. This is about 1/100 of the overall frequency of silent mutations in the p53 set and 1/400 of the corrected frequency in multiple mutants. Another estimate can be obtained from values for transgenic *lacI* mutations in the mouse, where Nishino *et al.* (1995) report a frequency of 3.7×10^{-5} mutations per gene. The effective mutational size of the *lacI* gene is ~210 bp, or ~one-fifth the p53 size. If this frequency applied to humans, one might expect $3.7 \times 10^{-5} \times 5 \times 1/4$ (to correct for silent mutations), or $4.6 \times 10^{-5} = 0.005$%. Once again, the measured p53 silent mutation frequency in human tumors is much higher.

Values for mutation frequencies in blood cells of normal young adults are available for four genes (Albertini *et al.* 1993): hemoglobin (4×10^{-8}; red blood cells), *hprt* (5×10^{-6}; T cells), glycophorin A (10×10^{-6}; red blood cells), and *HLA* (3×10^{-5}; T cells). The *hprt* frequency has been described in human kidney epithelium as age-dependent, ranging from 5×10^{-5} (children) to 2.5×10^{-4} (>80 yr) (Martin *et al.* 1996). Measurement of the somatic mutation frequency of a gene involved in O-acetylation of sialic acid in normal colonic mucosa from patients with either Crohn's disease, hereditary nonpolyposis colorectal cancer, or sporadic colorectal cancer gave values of 4.2×10^{-4}, 4.3×10^{-4}, and 6.6×10^{-4}, respectively, with no significant differences between the sets, although there was a significant age dependence seen (Williams *et al.* 1995). These frequencies are about two orders of magnitude lower than the frequency of silent mutations (even the uncorrected frequency) observed in *TP53*. The data on silent mutations in *TP53* show that this gene is hypermutable in tumors.

Multiple *TP53* mutations: Are there any other indications of a special nature for p53 mutations in tumors? To answer this question, it is useful to look at the distribution of mutations in tumors with multiple p53 mutations (Table 1). A problem with the tabulated data (Hollstein *et al.* 1996; Hainut *et al.* 1997) is that it is not easy to find the number of tumors scored with no mutations, but it can be estimated (M. Hollstein, personal communication) as ~24,000 or 48,000 *TP53* genes. There were 6005 mutations recorded. This gives a $P(0)$ of 0.87 and a value of 0.13 mutations per p53 gene based on the Poisson expectation, $P(0) = e^{-m}$.

There is a deficiency of double mutations but an excess of single and multiple (>2) mutations (Table 1). There are several interpretations possible for the devia-

TABLE 1

Reported mutations in the *TP53* gene

p53 mutations	Data to 1995	1996 update	Total	Expected
1	5090	787	5877	5377
2	213	49	262	343
3	46	6	52	15
4	8	3	11	0.48
5	1	0	1	0.01

Data from Hollstein *et al.* (1996) and Hainut *et al.* (1997). The expected numbers are calculated from the Poisson distribution as described in the text.

tions of the observed from expected value. A trivial explanation is that because only ~16% of the publications report sequencing all exons, many second mutations were just not observed. A second possibility (suggested by J. Cairns in a personal communication) is that there is a fraction of hypermutable tumors. Yet a third, not necessarily exclusive, possibility is suggested by an examination of the position of the mutations, some of which are closely linked. A tabulation of tumors with two closely linked mutations, and including six tumors with three mutations located no more than 10 codons from each other, has recently been presented (Strauss 1997). The current data set provides additional cases. A particularly interesting example is provided by Shipman *et al.* (1996) who carefully examined *TP53* mutations in 24 paired samples of normal and non-small-cell lung carcinomas from the same individuals. Three of these tumors had multiple *TP53* mutations, including one with four mutations, shown by cloning to be the result of three mutations in one allele and one in the other. Shipman *et al.* (1996) eliminated germinal polymorphism as an explanation for the findings. Counting the site of the first change, a deletion at position (1), a second deletion occurred at base 36 followed by a base substitution at position 46. The authors suggest that it is the first change that creates a stop codon 23 bp downstream, and the following mutations are presumably of no physiological importance. Taylor *et al.* (1996) studied 64 bladder tumors. Thirty-two had p53 mutations, and of these, nine had multiple p53 mutations. One of these tumors had five mutations at four codons (nos. 89, 101, 132 and 214). Although this tumor came from an individual exposed to arylamines, multiple mutations occurred about equally in exposed and control groups, and there were no differences between exposed and control groups in the codons mutated or in the pattern of changes—most were transitions. There is no evidence that such multiple changes occur as part of a single (replicative) event, but the proximity of such changes and their frequency certainly suggests that hypothesis. This possibility was first raised by Seidman *et al.* (1987) and by Harwood *et al.* (1991).

Seidman *et al.* (1987) found multiple point mutations in a shuttle vector introduced into repair-proficient lymphoblastoid cells, especially after introduction of a nick. The tumor cell lines studied by Harwood *et al.* (1991) were peculiar in that although the spontaneous mutation rate was low, those cells that did have mutations tended to have multiple mutations.

Mutations in other genes: The frequency of silent mutations and the observation of closely linked multiple mutations are arguments for the hypothesis that at least at some stage of tumorigenesis, the *TP53* gene is hypermutable and/or mutates by a special mechanism. The question is then whether genes other than *TP53* are hypermutable at some stage of tumorigenesis. Useful data are available for only a few genes. These include the *APC* gene (Smith *et al.* 1994; Beroud and Soussi 1996, 1997; DeVries *et al.* 1996), the *BRCA1* (Futreal *et al.* 1994; Merajver *et al.* 1995) and *BRCA2* cancer susceptibility genes (Lancaster *et al.* 1996; Miki *et al.* 1996; Teng *et al.* 1996), *CDKN2* (cyclin-dependent kinase inhibitor, p16) (Pollock *et al.* 1996; Smith-Sorensen and Hovig 1996), *WAF1* (Shiohara *et al.* 1994; Koopman *et al.* 1995), and *HPRT* (Cariello *et al.* 1997). Although numerous germinal mutations have been reported in *BRCA1* and *BRCA2*, there is a paucity of somatic point mutations, probably because of the presence of numerous deletions that occur at a high-enough frequency to mask the point mutations (Smith *et al.* 1996). Pollock *et al.* (1996) have compiled a list of 124 somatic mutations in the *CDKN2* (p16) tumor suppressor. Of these, four were silent but one silent mutation occurred at the site of a dipyrimidine change and a second at the site of a known polymorphism. This small sample therefore gives a frequency of 2/124, or ~1.6%. Data bases are available for the *APC* gene in tumors (Beroud and Soussi 1996, 1997) and for *HPRT* in cell cultures. The *APC* data base includes 339 germ line mutations and 486 from tumors. Most of the mutations are either frameshift or stop mutations. Only 6 of 339 germ line mutations and 20 of 486 mutations in tumors were missense. (The difference is barely significant; $P < 0.05$.) No silent mutations have been recorded. Double or triple mutations were reported in 18 of the tumors. In one report (Oda *et al.* 1996), eight of 13 sporadic hepatoblastomas were reported to have *APC* point mutations, and of these, two cases had double mutations. One of these had a CCT to ACT change at codon 1319 and a AGC to AAC change at codon 1321! The other double had mutations in codon 1395 and 1534. Tests for loss of heterozygosity were inconclusive.

WAF1 mutations were not found in a study of 315 samples from 14 different malignancies, although two polymorphisms were detected, validating the sensitivity of the methodology (Shiohara *et al.* 1994). A second study of *WAF1* in a different set of tumors also found several polymorphisms in 158 brain tumors but no mutations, although p53 mutations were found in 22 of the

tumors (Koopman *et al.* 1995). If hypermutability was widespread, and mutation to silent alleles was at least as frequent as in *TP53*, ~13 silent mutations might have been expected.

The data are inconclusive, but it certainly remains possible that mutations in *TP53* are particularly easy to detect or that the gene is uniquely mutable and/or that hypermutation of p53 is a likely (but not necessary) part of tumorigenesis. The particular structure of a functional p53 protein might make dominant negative effects common so that the first mutation inactivates function and that many second mutations would have no effect (this suggestion is from M. Hollstein). As has been suggested by several authors, inactivation of p53 might be important in inactivating the cell death mechanisms, thereby permitting the accumulation of deleterious changes. However, in contrast to the *APC* gene (Powell *et al.* 1992), mutation in p53 is often a late event in carcinogenesis (Baker *et al.* 1990; Neri *et al.* 1993; Ohgaki *et al.* 1993; Berchuck and Boyd 1995; Craanen *et al.* 1995; Ilyas *et al.* 1996; Kahlenberg *et al.* 1996). For that reason, it is unlikely that inhibition of cell death due to p53 mutation permits p53 mutations to accumulate. It is also unlikely that *TP53* mutation is the initiating event in carcinogenesis.

Other examples of hypermutability: The data indicate that the mutation process in (some) tumor cells and in (some) genes is different from mutation in normal cells. What occurs in tumorigenesis is reminiscent of the events involved in the generation of antibodies in which a limited portion of the genome becomes hypermutable for a limited time (Neuberger and Milstein 1995). Antibody production is an example of microevolution in much the same way as tumor formation is because a large number of random mutations are generated, and those mutations that produce a more efficient antibody molecule are selected. The mutations occur at random. Silent mutations are found in 17% of nonproductively rearranged V_H chains and in 29% of productively rearranged chains (Dorner *et al.* 1997), close to the expected value. Antibody molecules may contain multiple changes, some possibly occurring in the same replication cycle (Storb 1996). This special mutational process occurs exclusively in a sequence defined by precise boundaries (Lebecque and Gearhart 1990) and is limited to a particular time in development. The nature of the error-generating mechanism in that process remains a mystery, although it appears to be linked to transcription (Peters and Storb 1996).

The hypermutability that occurs in *TP53* during tumorigenesis is reminiscent of the hypermutability of starving bacterial cells (Strauss 1992), a comparison that has not eluded the attention of workers in that field (Sniegowski *et al.* 1997; Taddei *et al.* 1997; Torkelson *et al.* 1997). A subpopulation of *E. coli* cells kept under starvation conditions become hypermutable. Although hypermutability is attained by modulation of

the mismatch repair system, a special mode of DNA replication may also be required, possibly set off by double-strand breaks in the DNA (Torkelson *et al.* 1997). The mutational process affects many genes, and selection gives the appearance of adaptation (Foster 1997). The factors that result in a hypermutable state remain to be determined.

Both experimental and modeling studies suggest that clonal populations may increase their rate of evolution by transient changes in their mutation rates (Sniegowski *et al.* 1997; Taddei *et al.* 1997). In bacterial populations, it would appear that selection is not the unique determinant of adaptation, as has been generally accepted (Drake 1991, 1992). The demonstration of a surprisingly high frequency of mutator strains among bacterial pathogens appears to illustrate this paradigm (LeClerc *et al.* 1996), an example of the advantage of high mutability in populations themselves subject to selection (Mao *et al.* 1997). Recent experiments with certain of the mismatch repair-deficient tumor cells indicate that mutations accumulate only when the cells are maintained at high density, rather than under conditions of rapid growth (Richards *et al.* 1997). The data suggest that in tumors, as in starved bacteria, mutation rates are transiently increased under conditions of stress and that mutation rates in general may be influenced by the conditions of growth (Boesen *et al.* 1994). It may be that the initial events in carcinogenesis result in a condition of transient hypermutability, perhaps as a result of a peculiar form of DNA synthesis related to the functional mismatch repair deficiency (Richards *et al.* 1997). The processivity factor for DNA synthesis, proliferating cell nuclear antigen has been reported to form a physical complex with the mismatch repair proteins, and mutations in proliferating cell nuclear antigen have a mutator effect (Johnson *et al.* 1996; Umar *et al.* 1996). Some of the genes mutated as a result of this hypermutable state may then play a role in the rapid progression/evolution of the tumor.

Noted added in proof: A 1997 update of the databases is available (Hainut, P., T. Hernandez, A. Robinson, P. Rodriguez-Tome, T. Flores, M. Hollstein, C. C. Harris, and R. Montesano, 1988 IARC Database of p53 gene mutations in human tumors and cell lines: updated compilation, revised formats and new visualisation tools. Nucleic Acids Res. **26:** 207–215.) The overall set of over 8100 mutations now includes 3.4% silent mutations. Ponten *et al.* (1997) report a study of basal cell cancer in which tumor progression could be followed. Subclones with two or three mutations were identified indicating a progressive (rather than simultaneous) accumulation of mutations. Surprisingly, this set of 78 samples with 109 mutations included no silent mutations. (Ponten, F., C. Berg, A. Ahmadian, R. Zhi-Ping, M. Nister, J. Lundeberg, M. Uhlen and J. Ponten, 1997 Molecular pathology in basal cell cancer with p53 as a genetic marker. Oncogene **15:** 1059–1067.)

LITERATURE CITED

Aguilar, F., C. C. Harris, T. Sun, M. Hollstein and P. Cerutti, 1994 Geographic variation of p53 mutational profile in nonmalignant human liver. Science **264:** 1317–1319.

Akiyama, Y., R. Iwanaga, T. Ishikawa, K. Sakamoto, N. Nishi *et al.*, 1996 Mutations of the transforming growth factor-beta type II receptor gene are strongly related to sporadic proximal colon carcinomas with microsatellite instability. Cancer **78:** 2478–2484.

Albertini, R. J., J. A. Nicklas, J. C. Fuscoe, T. R. Skopek, R. F. Branda *et al.*, 1993 In vivo mutations in human blood cells: biomarkers for molecular epidemiology. Env. Health Perspectives **99:** 135–141.

Ames, B. N., W. Durston, E. Yamasaki and F. Lee, 1973 Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. Proc. Natl. Acad. Sci. USA **70:** 2281–2285.

Ames, B. N., L. S. Gold and W. C. Willett, 1995 The causes and prevention of cancer. Proc. Natl. Acad. Sci. USA **92:** 5258–5265.

Armitage, P., and R. Doll, 1957 A two-stage theory of carcinogenesis in relation to the age distribution of human cancer. Brit. J. Cancer **11:** 161–169.

Baker, S. J., A. C. Preisinger, J. M. Jessup, C. Paraskeva, S. Markowitz *et al.*, 1990 p53 gene mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. Cancer Res. **50:** 7717–7722.

Berchuck, A., and J. Boyd, 1995 Molecular basis of endometrial cancer. Cancer **76** (10 Suppl.): 2034–2040.

Beroud, C., and T. Soussi, 1996 APC gene: database of germline and somatic mutations in human tumors and cell lines. Nucleic Acid Res. **24:** 121–124.

Beroud, C., and T. Soussi, 1997 p53 and APC gene mutations: software and databases. Nucleic Acids Res. **25:** 138.

Bhattacharyya, N. P., A. Ganesh, R. G. Phea, B. Richards, A. Skandalis *et al.*, 1995 Molecular analysis of mutations in mutator colorectal carcinoma cell lines. Human Molecular Genetics **4:** 2057–2064.

Boesen, J. J., M. J. Niericker, N. Dieteren and J. W. Simons, 1994 How variable is a spontaneous mutation rate in cultured mammalian cells? Mutat. Res. **307:** 121–129.

Brash, D. E., J. A. Rudolph, J. A. Simon, A. Lin, G. J. McKenna *et al.*, 1991 A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. Proc. Natl. Acad. Sci. USA **88:** 10124–10128.

Buettner, V. L., K. A. Hill, H. Nishino, D. J. Schaid, C. S. Frisk *et al.*, 1996 Increased mutation frequency and altered spectrum in one of four thymic lymphomas derived from tumor prone p53/Big Blue double transgenic mice. Oncogene **13:** 2407–2413.

Cairns, J., 1981 The origin of human cancers. Nature **289:** 353–357.

Cariello, N. F., G. R. Douglas, M. J. Dycaico, G. S. Provost and T. Soussi, 1997 Databases and software for the analysis of mutations in the human p53gene, the human hprt gene and both the lacI and lacZ gene in transgenic rodents. Nucleic Acids Res. **25:** 136–137.

Cheng, K. C., and L. A. Loeb, 1993 Genomic instability and tumor progression: mechanistic considerations. Advances in Cancer Research **60:** 121–56.

Chu, G., and L. Mayne, 1996 Xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy: do the genes explain the diseases? Trends in Genetics **12:** 187–192.

Cleaver, J. E., 1968 Defective repair replication of DNA in xeroderma pigmentosum. Nature **216:** 652–656.

Craanen, M. E., P. Blok, W. Dekker, G. J. Offerhaus and G. N. Tytgat, 1995 Chronology of p53 protein accumulation in gastric carcinogenesis. Gut **36:** 848–852.

de Vries, A., C. T. van Oostrom, P. M. Dortant, R. B. Beems,

C. F. van Kreijl *et al.*, 1997 Spontaneous liver tumors and benzo[a]pyrene-induced lymphomas in XPA-deficient mice. Mol. Carcinog. **19**: 46–53.

DeVries, E. M., D. O. Ricke, T. N. DeVries, A. Hartmann, H. Blaszyk *et al.*, 1996 Database of mutations in the p53 and APC tumor suppressor genes designed to facilitate molecular epidemiological analyses. Human Mutation **7**: 202–213.

Dorner, T., H. P. Brezinschek, R. I. Brezinschek, S. J. Foster, R. Domiati-Saad *et al.*, 1997 Analysis of the frequency and pattern of somatic mutations within nonproductively rearranged human variable heavy chain genes. J. Immunol. **158**: 2779–2789.

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

Drake, J. W., 1992 Mutation rates. BioEssays **14**: 137–140.

Dumaz, N., C. Drougard, A. Sarasin and L. Daya-Grosjean, 1993 Specific UV-induced mutation spectrum in the p53 gene of skin tumors from DNA-repair-deficient xeroderma pigmentosum patients. Proc. Natl. Acad. Sci. USA **90**: 10529–10533.

Fishel, R., M. Lescoe, M. Rao, N. Copeland, N. Jenkins *et al.*, 1993 The human mutator gene homolog *MSH2* and its association with hereditary nonpolyposis colon cancer. Cell **75**: 1027–1038.

Fisher, J. C., 1958 Multiple-mutation theory of carcinogenesis. Nature **181**: 651–652.

Foster, P. L., 1997 Nonadaptive mutations occur on the F′ episome during adaptive mutation conditions in *Escherichia coli*. J. Bacteriol. **179**: 1550–1554.

Friedberg, E. C., G. C. Walker and W. Siede, 1995 *DNA Repair and Mutagenesis*. ASM Press, Washington, DC.

Futreal, P. A., Q. Liu, D. Shattuck-Eidens, C. Cochran, K. Harshman *et al.*, 1994 *BRCA1* mutations in primary breast and ovarian carcinomas. Science **266**: 120–122.

Goyette, M. C., K. Cho, C. L. Fasching, D. B. Levy, K. W. Kinzler *et al.*, 1992 Progression of colorectal cancer is associated with multiple tumor suppressor gene defects but inhibition of tumorigenicity is accomplished by correction of any single defect via chromosome transfer. Mol. Cell. Biol. **12**: 1387–1395.

Hainut, P., T. Soussi, B. Shomer, M. Hollstein, M. Greenblatt *et al.*, 1997 Database of p53 gene somatic mutations in human tumors and cell lines: updated compilation and future prospects. Nucleic Acids Res. **25**: 151–157.

Harwood, J., A. Tachibana and M. Meuth, 1991 Multiple dispersed spontaneous mutations: a novel pathway of mutation in a malignant human cell line. Mol. Cell. Biol. **11**: 3163–3170.

Hollstein, M., D. Sidransky, B. Vogelstein and C. C. Harris, 1991 p53 mutations in human cancers. Science **253**: 49–53.

Hollstein, M., B. Shomer, M. Greenblatt, T. Soussi, E. Hovig *et al.*, 1996 Somatic point mutations in the p53 gene of human tumors and cell lines: updated compilation. Nucleic Acids Res. **24**: 141–146.

Hongyo, T., G. S. Buzard, D. Palli, C. M. Weghorst, A. Amorosi *et al.*, 1995 Mutations of the K-ras and p53 genes in gastric adenocarcinomas from a high-incidence region around Florence, Italy. Cancer Res. **55**: 2665–2672.

Ilyas, M., M. Kendall, H. Jalal, C. Linton and N. Rooney, 1996 Changes in Bcl-2 and p53 expression in recurrent B-cell lymphomas. J. Pathol. **180**: 249–253.

Johnson, R. E., G. K. Kovvali, S. N. Guzder, N. S. Amin, C. Holm *et al.*, 1996 Evidence for involvement of yeast proliferating cell nuclear antigen in DNA mismatch repair. J. Biol. Chem. **271**: 27987–27990.

Kahlenberg, M. S., D. L. Stoler, M. Basik, N. J. Petrelli, M. Rodriguez-Bigas *et al.*, 1996 p53 tumor suppressor gene status and the degree of genomic instability in sporadic colorectal cancers. J. Natl. Cancer Inst. **88**: 1665–1670.

Koopman, J., D. Maintz, S. Schild, J. Schramm, D. N. Louis *et al.*, 1995 Multiple polymorphisms, but no mutations, in the WAF1/CIP1 gene in human brain tumours. Brit. J. Cancer **72**: 1230–1233.

Kraemer, K. H., M. M. Lee and J. Scotto, 1984 DNA repair protects against cutaneous and internal neoplasia: evidence from xeroderma pigmentosum. Carcinogenesis **5**: 511–514.

Kraemer, K. H., D. D. Levy, C. N. Parris, E. M. Gozukara, S. Moriwaki *et al.*, 1994 Xeroderma pigmentosum and related disorders: examining the linkage between defective DNA repair and cancer. Journal of Investigative Dermatology **103** (5 Suppl.): 96S–101S.

Lancaster, J. M., R. Wooster, J. Mangion, C. M. Phelan, C. Cochran *et al.*, 1996 BRCA2 mutations in primary breast and ovarian cancers. Nature Genetics **13**: 238–240.

Lebecque, S. G., and P. J. Gearhart, 1990 Boundaries of somatic mutation in rearranged immunoglobulin genes: 5′ boundary is near the promoter and 3′ boundary is ∼1 kb from V(D)J gene. J. Exp. Med. **172**: 1717–1727.

LeClerc, J. E., B. Li, W. L. Payne and T. A. Cebula, 1996 High mutation frequencies among *Escherichia coli* and *Salmonella* pathogens. Science **274**: 1208–1211.

Levine, A. J., 1997 p53, the cellular gatekeeper for growth and division. Cell **88**: 323–331.

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

Lu, S. L., Y. Akiyama, H. Nagasaki, K. Saitoh and Y. Yuasa, 1995 Mutations of the transforming growth factor-beta type II receptor genes and genomic instability in hereditary nonpolyposis colorectal cancer. Biochem. Biophys. Res. Commun. **216**: 452–457.

Malkhosyan, S., A. McCarty, H. Sawai and M. Perucho, 1996 Differences in the spectrum of spontaneous mutations in the hprt gene between tumor cells of the microsatellite mutator phenotype. Mutat. Res. **316**: 249–259.

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

Marionnet, C., X. Quilliet, A. Benoit, J. Armier, A. Sarasin *et al.*, 1996 Recovery of normal DNA repair and mutagenesis in trichothiodystrophy cells after transduction of the XPD human gene. Cancer Res. **56**: 5450–5456.

Markowitz, S., 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.

Martin, G. M., C. E. Ogburn, L. M. Lolgin, A. M. Gown, S. D. Edland *et al.*, 1996 Somatic mutations are frequent and increase with age in human kidney epithelial cells. Hum. Mol. Genet. **5**: 215–221.

Matsumura, Y., C. Nishigori, T. Yagi, S. Imamura and H. Takebe, 1996 Characterization of p53 gene mutations in basal-cell carcinomas: comparison between sun-exposed and less-exposed skin areas. Int. J. Cancer **65**: 778–780.

Merajver, S. D., T. M. Pham, R. F. Caduff, M. Chen, E. L. Poy *et al.*, 1995 Somatic mutations in the BRCA1 gene in sporadic ovarian tumours. Nature Genetics **9**: 439–443.

Miki, Y., T. Katagiri, F. Kasumi, T. Yoshimoto and Y. Nakamura, 1996 Mutation analysis in the BRCA2 gene in primary breast cancers. Nature Genetics **13**: 245–247.

Modrich, P., 1995 Mismatch repair, genetic stability and tumour avoidance. Phil. Trans. R. Soc. Lond. B **347**: 89–95.

Neri, A., L. Baldini, D. Trecca, L. Cro, E. Polli *et al.*, 1993 p53 gene mutations in multiple myeloma are associated with advanced forms of malignancy. Blood **81**: 128–135.

Neuberger, M., and C. Milstein, 1995 Somatic hypermutation. Curr. Opin. Immunol. **7**: 248–254.

Nishino, H., A. Knoll, V. L. Buettner, C. S. Frisk, Y. Maruta *et al.*, 1995 p53 wild-type and p53 nullizygous Big Blue transgenic mice have similar frequencies and patterns of observed mutation in liver, spleen and brain. Oncogene **11**: 263–270.

Oda, H., Y. Imai, Y. Nakatsuru, J. Hata and T. Ishikawa, 1996 Somatic mutations of the APC gene in sporadic hepatoblastomas. Cancer Res. **56**: 3320–3323.

Ohgaki, H., P. Kleihues and P. U. Heitz, 1993 p53 mutations in sporadic adrenocortical tumors. International Journal of Cancer **54**: 408–410.

Parsons, R., G. M. Li, M. Longley, P. Modrich, B. Liu *et al.*, 1995 Mismatch repair deficiency in phenotypically normal human cells. Science **268**: 738–740.

Peters, A., and U. Storb, 1996 Somatic hypermutation of immunoglobulin genes is linked to transcription initiation. Immunity **4**: 57–65.

Pollock, P. M., J. V. Pearson and N. K. Hayward, 1996 Compilation of somatic mutations of the CDKN2 gene in human cancers: non-random distribution of base substitutions. Genes, Chromosomes & Cancer **15**: 77–88.

Powell, S. M., N. Zilz, Y. Beazer-Barclay, T. Bryan, S. 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 **277**: 1523–1526.

Rodin, S. N., G. P. Holmquist and A. S. Rodin, 1998 CpG transition strand asymmetry and hitch-hiking mutations as measures of tumorigenic selection in shaping the p53 mutation spectrum. Int. J. Molec. Med. (in press).

Seidman, M. M., A. Bredberg, S. Seetharam and K. H. Jraemer, 1987 Multiple point mutations in a shuttle vector propagated in human cells: evidence for an error-prone DNA polymerase activity. Proc. Natl. Acad. Sci. USA **84**: 4944–4948.

Sharp, P. M., M. Averof, A. T. Lloyd, M. G. and J. F. Peden, 1995 DNA sequence evolution: the sounds of silence. Phil. Trans. Roy. Soc. Lond. B **349**: 241–247.

Sharp, P. M., M. Stenico, J. F. Peden and A. T. Lloyd, 1993 Codon usage: mutational bias, translational selection, or both? Biochemical Society Transactions **21**: 835–841.

Shiohara, M., W. S. el-Deiry, M. Wada, T. Nakamaki, S. Takeuchi *et al.*, 1994 Absence of WAF1 mutations in a variety of human malignancies. Blood **84**: 3781–3784.

Shipman, R., P. Schraml, M. Colombi, G. Raefle, P. Dalquen *et al.*, 1996 Frequent *TP53* gene alterations (mutation, allelic loss, nuclear accumulation) in primary non-small cell lung cancer. Europ. J. Cancer **32A**: 335–341.

Simpson, A. J. G., 1997 The natural somatic mutation frequency and human carcinogenesis. Adv. Cancer Res. **71**: 209–240.

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

Smith, T. M., M. K. Lee, C. I. Szabo, N. Jerome, M. McEuen *et al.*, 1996 Complete genomic sequence and analysis of 117 kb of human DNA containing the gene *BRCA1*. Genome Research **6**: 1029–1049.

Smith-Sorensen, B., and E. Hovig, 1996 *CDKN2A* (p16[INK4A]) somatic and germline mutations. Human Mutation **7**: 294–303.

Sniegowski, P. D., P. J. Gerrish and R. E. Lenski, 1997 Evolution of high mutation rates in experimental populations of *E. coli*. Nature **387**: 703–705.

Souza, R. F., R. Appel, J. Yin, S. Wang, K. N. Smolinski *et al.*, 1996 Microsatellite instability in the insulin-like growth factor II receptor gene in gastrointestinal tumours. Nature Genetics **14**: 255–257.

Stary, A., and A. Sarasin, 1996 The genetic basis of xeroderma pigmentosum and trichothiodystrophy syndromes. Cancer Surveys **26**: 155–171.

Storb, U., 1996 The molecular basis of somatic hypermutation of immunoglobulin genes. Curr. Opin. Immunol. **8**: 206–214.

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

Strauss, B. S., 1997 Silent and multiple mutations in p53 and the question of the hypermutability of tumors. Carcinogenesis **18**: 1445–1452.

Strauss, B. S., D. Sagher and S. Acharya, 1997 Role of proofreading and mismatch repair in maintaining the stability of nucleotide repeats in DNA. Nucleic Acids Res. **25**: 806–813.

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.

Takayama, K., E. P. Salazar, B. C. Broughton, A. R. Lehmann, A. Sarasin *et al.*, 1996 Defects in the DNA repair and transcription gene ERCC2(XPD) in trichothiodystrophy. Am. J. Hum. Genet. **58**: 263–270.

Taylor, J. A., Y. Li, M. He, T. Mason, C. Mettlin *et al.*, 1996 p53 mutations in bladder tumors from arylamine-exposed workers. Cancer Res. **55**: 294–298.

Teng, D. H., R. Bogden, J. Mitchell, M. Baumgard, R. Bell *et al.*, 1996 Low incidence of BRCA2 mutations in breast carcinoma and other cancers. Nature Genetics **13**: 241–244.

Tomlinson, I. P. M., M. R. Novelli and W. F. Bodmer, 1996 The mutation rate and cancer. Proc. Natl. Acad. Sci. USA **93**: 14800–14803.

Torkelson, J., R. S. Harris, M. J. Lombardo, J. Nagendran, C. Thulin *et al.*, 1997 Genome-wide hypermutation in a subpopulation of stationary-phase cells underlies a recombination-dependent adaptive mutation. EMBO J. **16**: 3303–3311.

Tran, H. T., J. D. Keen, M. Kricker, M. A. Resnick and D. A. Gordenin, 1997 Hypermutability of homonucleotide runs in mismatch repair and DNA polymerase proofreading yeast mutants. Mol. Cell. Biol. **17**: 2859–2865.

Umar, A., A. B. Buermeyer, J. A. Simon, D. C. Thomas, A. B. Clark *et al.*, 1996 Requirement for PCNA in DNA mismatch repair at a step preceding DNA resynthesis. Cell **87**: 65–73.

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

Weeda, G., E. Eveno, I. Donker, W. Vermeulen, O. Chevallier-Lagente *et al.*, 1997 A mutation in the XPB/ERCC3 DNA repair transcription gene, associated with trichothiodystrophy. Am. J. Hum. Genet. **60**: 320–329.

Weston, A., and J. H. Godbold, 1997 Polymorphisms of H-ras-1 and p53 in breast cancer and lung cancer: a meta-analysis. Env. Health Perspect. **105** (Suppl. 4): 919–926.

Williams, G. T., J. M. Geraghty, F. Campbell, M. A. C. Appleton and E. D. Williams, 1995 Normal colonic mucosa in hereditary non-polyposis colorectal cancer shows no generalized increase in somatic mutation. Brit. J. Cancer **71**: 1077–1080.

Wittenkeller, J. L., B. Storer, G. Bittner and J. H. Schiller, 1997 Comparison of spontaneous and induced mutation rates in an immortalized human bronchial epithelial cell line and its tumorigenic derivative. Oncology **54**: 335–341.