

Mutation Studies With Human Fibroblasts

by Robert DeMars*

My attitude toward studying mutation in human fibroblasts at this time is related to that expressed by Dylan Thomas in a note to his "Collected Poems" (1): "I read somewhere of a shepherd who, when asked why he made, from within fairy rings, ritual observances to the moon to protect his flocks, replied: 'I'd be a damn fool if I didn't!' These poems, with all their crudities, doubts, and confusions, are written for the love of man and in praise of God, and I'd be a damn fool if they weren't."

The experimental study of mutation in diploid human cells is in its infancy and is still prey to crudities, doubts and confusion in comparison to some submammalian mutation detection systems that are technically neat and genetically characterized. It is generally assumed that, ultimately, we will want to use human cells for mutagenesis detection, but that this will not be possible for some time to come because of their technical limitations. I will submit two propositions for your consideration: (1) technical limitations in using diploid human cells are far from decisive and several opportunities for detailed mutation studies are now available; (2) presented with opportunities for making these studies, "I'd be a damn fool if I didn't!"

My plan in this presentation is to summarize with little documentation some ongoing studies of hereditary variation in cultured human fibroblasts. Many of the details are available in papers that are published (2, 3) or submitted for publication (4). I

will try to use only the details needed to define the present status of the studies and the main questions that must be answered if they are to become useful for mutagenesis detection in human cells.

Human Cells for Mutation Studies

Cultures of skin fibroblasts that are usable for mutation studies can be obtained from virtually any human. Comparisons of cultures from different humans will allow us to derive information about cell genetics that is representative of humanity. A corollary advantage of this approach is the opportunity to relate the genetic behavior of cells *in vitro* to the inheritance of genetic factors in families: in favorable cases, pedigree analysis will provide part of the evidence that cells with altered phenotypes are genetic variants. These advantages might appear to be overridden by two often-cited technical limitations of diploid fibroblasts: low cloning efficiency and limited life span.

Cloning efficiencies from laboratories in different nations often range from 0.2 to 0.8. Subcloning efficiencies sometimes fall in this range, too, and are large enough for practical work in many cases. Low cloning efficiencies are often limitations of investigators or laboratory conditions, not fibroblasts.

Life span is still an ineluctable limitation, which just hasn't prevented us and others (5,6) from making progress in the study of mutation. The number of doublings allotted to fibroblasts *in vitro* is usually estimated at 50-60. Only about 20 doublings are used when a single mutant cell generates

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tatively studying mutation in human cells. Here are some observations.

Properties of AG^r Mutant Fibroblasts

Two broadly defined categories of spontaneous mutants have been observed. Type I mutants have very low HG-PRT activity and are unable to grow in H[AS]. These mutants resemble cells cultured from boys having X-chromosomal mutant alleles that cause the Lesch-Nyhan syndrome and have the properties we expected to find in AG^r mutants. However, only three Type I mutants have been found among 20 independent, spontaneous mutants tested. About 85% of the mutants are Type II. The range of apparent HG-PRT activities in these mutants extends from Type I amounts to those found in normal cells. Almost all of them have distinct reductions in apparent HG-PRT activity but all of them can grow in H[AS]. Type II mutants resemble cells from some humans who have X-chromosomal mutant alleles that change the properties and reduce the function of HG-PRT but do not cause the Lesch-Nyhan syndrome (2).

Is AG Resistance a Stable Hereditary Change?

Several independent mutants have been subcloned in the absence of AG and shown to retain their characteristic AG resistance, HG-PRT activity, and ability or inability to grow in H[AS], depending on whether they were Type I or Type II. We think that, at least in most cases, AG resistance associated with reduced HG-PRT activity is not an adaptation induced in the presence of AG and readily reversible in its absence. This notion is supported by the results of several fluctuation tests, which provided evidence that most AG-resistant colonies resulted from changes in cell heredity that occurred before the cells were subjected to selection with AG. The averaged upper and lower estimates of the rate of spontaneous origin of AG-resistance are 0.34 to 1.34×10^{-6} per cell generation (2).

Does AG Resistance Result from Genetic Changes, i.e., from Changes in Genes or Chromosomes?

There are various ways of trying to answer this question; head-on enzymology is one of them. We have shown that the residual HG-PRT activity of a Type I mutant had abnormal thermolability and a distinctly increased affinity for one of its substrates, PRPP (2). One Type II mutant had a distinctly abnormal pH optimum (unpublished data). This sort of characterization of mutants will finally permit estimation of the fraction of independent mutations that cause abnormalities in HG-PRT other than simple reductions in activity. Electrophoretic alterations have not been observed yet but methods with greater resolving power will be tried. Finally, Dr. Karsten Held has prepared antibodies against partially purified HG-PRT so that, using a sensitive serum-blocking test, we can determine if enzyme-deficient mutants have an amount of antigenically reactive protein in excess of that corresponding to the apparent HG-PRT activity. Such excesses would be evidence that the mutations had altered the properties of the enzyme protein and not merely reduced the amount of enzyme produced by the cells.

It would be surprising if a variety of regulatory mutations affecting HG-PRT did not occur. In a general sense, some of them could correspond to *cis*-dominant operator and promoter mutations or *trans*-dominant regulator mutations in bacteria. Mutations affecting post-transcriptional processes influencing the amount of HG-PRT activity might also occur e.g. messenger RNA stability, stabilization of the enzyme protein or processing of HG-PRT zymogen, if such occurs. Excepting the analogs of operator and promoter mutations associated with the structural gene on the X, mutations reducing HG-PRT activity could occur on any chromosome. Therefore, we have set out to map AG-resistance mutations to a chromosome by means of somatic cell hybridization. The principle is now familiar. Most

AG^r mutants are Type II and able to grow in H[AS]. By use of Sendai virus, Type II mutants are fused with a nonreverting mutant of mouse cell clone ID that is AG^r, HG-PRT-deficient and unable to grow in H[AS]. In this medium, mouse parental cells are quickly and totally eliminated. Hybrid cells are readily identified by their morphology and ability to grow as discrete colonies upon a background of Type II mutant, human cells. They are then isolated and propagated as pure cultures in H[AS] medium. Human chromosomes are gradually eliminated from the hybrid cells until only those remain that bear the genes necessary for growth in the selective medium. Therefore, we expect to find the human X-chromosome because it bears structural genes for HG-PRT. If AG-resistance in Type II mutants results directly from a change in an X-chromosomal gene, the H[AS]-selected hybrids should still be AG-resistant and have HG-PRT activity that corresponds qualitatively and quantitatively to that present in the human cell parent. AG-resistance might be phenotypically and genetically more complicated in some cases. For instance, full resistance to AG in Type II mutants might require two conditions: (1) a mutation in an X-chromosomal gene that reduced HG-PRT activity but not enough to, by itself, reduce the production of AG-nucleotide to sub-toxic amounts; (2) the overproduction of purines by the *de novo* pathway, which occurs in cells from HG-PRT-deficient humans. The excess normal nucleotides might minimize the effects of AG-nucleotide. If genes necessary for the overproduction are located on autosomes two relevant types of clones may segregate out of H[AS]-selected hybrids: clones retaining only the human X, which are either sensitive or slightly resistant to AG but whose HG-PRT may, nevertheless, be demonstrably abnormal; AG-resistant clones retaining the human X and at least one other, specific, human chromosome.

If AG resistance resulted from the trans-dominant effect of an autosomal mutation on a normal X-chromosomal HG-PRT locus

then, again, two types of clones might segregate from H[AS]-selected hybrids: AG-sensitive clones retaining only the human X and producing qualitatively and quantitatively normal human HG-PRT; AG-resistant clones that retain the human X and at least one other, specific, human chromosome. HG-PRT activity would be reduced but qualitatively normal.

Other genetic bases for AG resistance are conceivable but will not be discussed. My object is simply to emphasize that it will be possible to attempt a genetic analysis of the commonest type of AG^r mutants of human fibroblasts. Three crosses with independent Type II mutants are in progress. One was performed with cells from the human prototype of Type II mutants, which I mentioned above. In this case, we know that the HG-PRT is qualitatively altered as the result of a mutation in an X-chromosomal gene (2). Therefore, the properties of hybrids obtained with this mutant will provide a standard for evaluating the results obtained with *in vitro* mutants. Furthermore, the AG resistance and HG-PRT activity of these hybrids can be compared with those of H[AS]-selected hybrids produced from the same strain of mouse cells and normal, AG-sensitive human cells. Subclones of the first hybrid to be analyzed so far are still AG-resistant. The other two crosses utilized Type II mutants that originated *in vitro*. H[AS]-selected hybrids have been obtained. These studies are obviously incomplete but should finally tell us whether or not the commonest type of AG^r mutants result from changes that can be assigned to specific human chromosomes.

If an adequately firm genetic foundation for AG-resistant mutations in human cells can be laid down, additional information will be needed to make them useful for mutagenesis testing. First, we should determine that the mutation-detection method is responsive to known mutagens. Some information is available but more is needed. There is published evidence that x-rays increase the rate of mutation to AG resistance (3) and evidence, to be published, that

MNNG has spectacular mutagenic effects (?). Second, more reliable and representative estimates of the rate of spontaneous mutation are needed, since this is the most useful standard for quantitatively evaluating mutagenic effects, especially small ones. These estimates are best derived from fluctuation tests, some repeated, with cultures from a number of humans of different sexes and races.

Resistance to 2,6-Diaminopurine

The preceding discussion makes possible a concise description of a second selection system, which Dr. Harriet Rappaport and I have recently been studying with human cells (4). 2,6-Diaminopurine (DAP) is an adenine analog that becomes toxic to cells when converted to a ribonucleotide by the enzyme A-PRT (Fig. 1). A-PRT is dispensable under ordinary conditions of growth, and enzyme-deficient mutants should be selected for when DAP is present. Furthermore, if the *de novo* pathway is blocked with [AS], normal cells should be able to grow in a medium containing adenine as the sole exogenous purine (i.e., A[AS] medium), but A-PRT-deficient cells should be unable to do so.

DAP-resistant (DAP^r) fibroblast clones have been recovered from 13 people belonging to 11 unrelated families. Two general categories of mutants occur, corresponding to the two categories of AG^r mutants. Type I mutants have very low A-PRT activity and are unable to grow in A[AS]. One Type I mutant has been found so far among at least four independent mutants that we have studied.

Type II mutants have had apparent A-PRT activities ranging between about 0.2 of normal and normal but all can grow well in A[AS]. One mutant with about 0.02 of normal activity had only a slight ability to grow in A[AS]. Individual subclones of Type II mutants that initiate growth in DAP can continue growth when the medium is replaced with A[AS]. Finally, since all of the mutants increased manyfold in the absence of DAP before they were char-

acterized we believe that DAP resistance is a stable hereditary alteration of normal cells.

From the outset we expected the genetics of DAP resistance to be more complicated than that of AG resistance and that it would be difficult to obtain mutants. There was evidence that A-PRT was determined by autosomal genes. Dr. Brenda Kahan and I have since determined that hybrids of normal human fibroblasts and A-PRT-deficient mouse cells isolated by selection for ability to utilize adenine characteristically retain human autosome No. 16 (to be published). We expected DAP resistance to be recessive in heterozygous cells and, therefore, our attempts to obtain DAP^r mutants began with a search for heterozygous people: only in heterozygous cell populations would homozygous mutants resulting from single genetic changes be frequent enough to permit practical experimentation. The rationale used in identifying heterozygous cultures was simple and successful. It is described here as an idea because it should be applicable in developing new selection methods for human cells.

There was only one report of hereditary, partial A-PRT deficiency, which was non-pathological. If homozygosity for deficiency alleles was lethal before birth, a heterozygote incidence of 1/200 to 1/25 was fairly likely, to judge from the frequency of many deleterious, autosomal recessive genes. Alternatively, A-PRT variation could be innocuous and might have escaped notice because the commonest variant alleles neither altered the electrophoretic behavior nor the apparent activity of the enzyme. Nonpathological allelic variations of some other enzymes occur with frequencies of 0.1–0.5. In either case, then, there was a reasonable chance of identifying a heterozygote if we examined as few as 200 people using the right kind of detection method.

A discontinuous variable was used to distinguish between normal and heterozygous cell cultures. The rate of spontaneous mutation to AG-resistance is, roughly, 10⁻⁶ per cell generation. Assuming this is a typical

rate for single mutations, homozygous DAP^r mutants should be undetectable in samples of 10⁶ homozygous normal cells but should often be detected in similar samples of 10⁶ heterozygous cultures. We simply cultured foreskin fibroblasts from normal newborns and subjected samples of about 10⁶ cells from each boy to selection with DAP after a mutagenic treatment with MNNG. The treatment was known to increase the rate of mutation to AG-resistance 10 to 100 times and raised the probability of detecting mutants in heterozygous cultures without permitting their detection in homozygous cultures. These small-scale mutagenesis experiments worked. DAP^r mutants having the phenotypes described above were detected in eleven unrelated, foreskin-derived cultures among 83 that were grown.

Our estimate of the incidence of apparent heterozygosity is in the range 0.1 to 0.5. Such estimates are useful in planning further studies but do not prove the hypothesis on which they are founded. They will not mean much with regard to mutagenesis testing until the actual genetic basis for DAP resistance is worked out.

Genetic Analysis of DAP Resistance

According to the hypothesis that DAP^r mutants originate in cultures of cells that are heterozygous for A-PRT deficiency alleles, boys whose cultures yield mutants should have one parent, sometimes two parents, who should be heterozygous and whose cultures should yield mutants. This will less frequently be true for boys whose cultures fail to yield DAP^r mutants and quantities can be assigned to these expectations as our estimates of the allele frequencies improve. In two families studied so far, mutants were recovered from just one parent of boys yielding mutants. We hope these family studies will show that the mutations we are studying *in vitro* correspond to hereditary factors in humans. Furthermore, if we can define the inheritance of distinctive A-PRT alleles in families the information will be of

great value in distinguishing between different possible genetic origins of DAP^r mutants, which are discussed below.

The approaches to biochemical and genetic analysis of AG resistance that were described above are also being used in characterizing DAP^r mutants. For instance, Type II DAP^r mutants, the commonest type, can be fused with A-PRT-deficient mouse cells and hybrids isolated in A[AS] selective medium. The genetically simplest outcome will be hybrids that are DAP-resistant and retain human autosome No. 16. More complicated outcomes, analogous to those mentioned above with reference to AG-resistance, will not be discussed further here. My main point is that a formal genetic analysis of the commonest type of DAP^r mutant can be attempted.

These approaches will have to be complemented by special studies of the mutant, human cells, themselves. Selectable DAP^r variants could result from more than one kind of genetic event involving autosome No. 16 in cells that are already heterozygous: (1) gene mutation; (2) terminal deletion of the normal allele; (3) monosomy for No. 16 resulting from simple loss or non-disjunction of the homolog with the normal allele; (4) somatic crossing over. There are practical ways of distinguishing between all of these possibilities, even between gene mutation and somatic crossing over. I will not discuss them here but want to emphasize that these genetic processes have to be evaluated in every mutation detection system utilizing diploid cells, regardless of the species from which they are derived.

The chance that phenotypic mutants will originate from heterozygous cells through processes other than gene mutation is strongly influenced by two factors: distance between the locus and the centromere and deleterious effects of partial or complete monosomy for the chromosome bearing the locus. This suggests that cells with mutant phenotypes will most often result from gene mutations of some loci, from chromosome mutations involving other loci and, perhaps, from somatic crossing in some cases. A vig-

orous effort to develop additional selective systems for human cells and to analyze them thoroughly may provide us with sensitive ways of screening for chromosome breaks that are propagated (i.e., the kind that cause trouble for people) and, in addition, a possible cause of somatic disease that receives little serious consideration: somatic crossing over.

Some Perspectives in Mutagenesis Testing with Human Cells

We are interested in two related applications of scoring mutations in human cells, one concerned with mutagenesis detection and the other with mutagen identification. Somatic mutations are important as possible indicators of germinal mutations that can affect future generations and as indicators of genetic damages that can harm the individuals in which they occur. I think of the first application as "direct" testing: the incidences of mutants already present among somatic or germinal cells are determined immediately upon their removal from the bodies of a properly chosen sample population, before the cells have a chance to proliferate outside the body. The main objective of direct testing is the prompt detection of mutagenic influences on cells *in vivo*. Such discovery must be followed up by detective work needed to identify the mutagen.

One problem associated with direct testing is the cumulative incidence of mutants that results from their proliferation after they originate in a population of dividing cells. This is the background above which mutagenic effects must be detected *in vivo*. Its magnitude can be roughly evaluated by using information that is available now and the assumption that mutation rates are similar in somatic and germinal cells. This assumption is not proven, but comparative information has begun to accumulate. The incidences of gametes having a mutation that causes one specific human disease or another is about 10^{-5} to 10^{-4} for many human diseases. This range represents the accumulation of mutants with regard to a single locus in a

population of cells resulting from about 50 prior doublings of the gonads in the case of males. This number of doublings may pertain to some populations of somatic cells, too. Therefore, an influence that doubled the mutation rate per cell generation wouldn't be detectable as a doubled incidence of mutants until it had acted for about 50 doublings of a cell population. This means that direct testing will promptly discover mutagenic effects only when they are very large and that less powerful influences may cause significant damage before they are detected. I know of no way to eliminate or reduce the high background of *in vivo* mutants that I have referred to. It may be lower than I have estimated and may be a smaller problem than I have indicated. Little information is available about the *in vivo* incidences of somatic cells with genetically defined mutations. The accumulation of such information should be an explicit objective of the immediate future if direct testing is to become practical. I hope this will be achieved because experience indicates that despite our efforts at prospective diagnostic testing we are likely to discover some mutagens only after people have been exposed to them. Direct testing may provide a relatively sensitive method of discovering their effects before they are manifested as increased incidences of cancer, congenital deformity, or disease.

The need for information about the incidences of mutant somatic cells *in vivo* introduces the second difficulty with direct testing: deciding if a cell with a specific abnormal phenotype is a genetic variant or a phenocopy. Mostly histochemical, serological, or other methods that kill individual cells in the process of defining their phenotypes will be used in direct testing. We will need background genetic information, which will give us confidence that a cell with a specific abnormal phenotype is probably a mutant and not a phenocopy. One approach to solving this problem is to choose mutant phenotypes that almost certainly will not occur unless an individual has a specific genotype. The studies of diaminopurine resistance that I described exemplify this approach but one

would want to apply it using phenotypes scorable in single cells for direct testing.

I think of the second application of scoring mutations in human cells as "diagnostic" testing. Its principal purpose is the identification of mutagens before they have acted on people. Diagnostic testing could be executed, in part, with cells proliferating *in vitro*, where the problems of high background and genetic analysis are at least partly overcome. Single abnormal cells can be amplified into clonal populations, permitting one to determine if an abnormal phenotype is hereditary and to analyze it chemically and in other ways. Genetic analysis by means of karyotype study, complementation, and somatic cell hybridization is also possible. Information obtained in this way with cultured cells could be applied to the development of methods for direct testing.

Here is an example of what I mean. While Dr. Richard Albertini was studying in my laboratory we tried to detect AG-resistant circulating lymphocytes in normal individuals. Our initial feasibility study involved quantitating the incorporation of tritiated thymidine into lymphocytes stimulated by phytohemagglutinin in the presence of AG. Comparisons of HG-PRT-deficient lymphocytes from boys who had the Lesch-Nyhan syndrome with normal lymphocytes and artificial mixtures of the two cell types showed that the method was roughly quantitative for the detection of AG^r lymphocytes down to about the one percent level. We detected in normal lymphocyte populations an AG-resistant incorporation that was about 1/1000 that observed in the absence of AG (8). The next step is to determine if this results mainly from slight incorporation into all the cells or from very active incorporation into rare AG^r cells. An obvious method of tackling this question is to use autoradiographic procedures that have been thoroughly worked out with fibroblasts. An additional method, derived from experience in bacteriophage genetics will also be tried. The lymphocytes would be infected with vesicular stomatitis virus and the infected cells would be introduced into a film of nutrient

agar atop a monolayer of AG-resistant mouse cells, on which the virus could form plaques. In the presence of AG, plaques would be initiated only by AG^r lymphocytes in which the virus could multiply. The quantitative sensitivity and accuracy of the method can be ascertained with mixtures of Lesch-Nyhan and normal lymphocytes. AG resistance is surely the way to try out such methods now because we know quite a lot about it. But the results will still be subject to the doubt: Are mutants or phenocopies being counted? Family studies will not be much help here, since AG-resistance mutations occur in everyone. I imagine that if the initial studies with AG resistance, which Albertini is continuing at the University of Vermont, give neat results, it might be interesting to apply the same principle in comparing lymphocytes from people who have yielded DAP-resistant fibroblasts (presumed heterozygotes) with lymphocytes from people presumed to be normally homozygous.

In general then, we should plan on using the genetic and phenotypic analysis that is possible with cells proliferating *in vitro* to devise tests that are faster, less expensive and more compact than the selection methods being used currently.

These improved tests could include automated procedures for scanning large populations of cells for rare, abnormal histochemical phenotypes. For instance, it might be worthwhile to try and find an inducible enzyme that has a cheap fluorogenic substrate. The enzyme chosen should not be inducible in AG-sensitive cells but should be in AG-resistant cells when AG is present. One might detect and count the mutants after brief selection with AG in the presence of the inducer by adding the substrate and passing the suspension of counted cells through a device that senses and counts the fluorescent cells. Methods of this general sort would be useful for both direct and diagnostic testing.

A major difficulty with strictly *in vitro* methods is a partial loss of realism: the cells are removed from the metabolic influences of the intact human, which sometimes deter-

mine if a chemical will be mutagenic or not. There are severe difficulties in solving this problem with human cells. One approach to a solution is a host-mediated assay that uses human cells and nonhuman hosts. Another approach is to subject human cells *in vitro* to conditions that are supposed to simulate *in vivo* influences relevant to mutagenesis.

Two questions apply to any mutation detection method: (1) is it genetically sound? (2) Is it technically practical and sufficiently economical? I have emphasized the basic genetic questions that must be answered if maximum use is to be made of AG^r and DAP^r mutants in mutagenesis detection with human cells. The question about their genetic soundness is not yet answered but is accessible to experimental analysis. The same question must be answered for all current mammalian cell methods that I know of. Both selection systems have a favorable genetic aspect that I hope can pertain to selection systems that will be adopted for discovering mutagenic effects: they detect forward mutations in dispensable genes. Deficiencies of A-PRT and HG-PRT correspond to auxotrophic mutations but the methods for detecting them are more nearly quantitative than other methods for detecting auxotrophic mutations. The entire variety of mutational changes that can affect a gene is sampled. The only equivalent to this whole-spectrum sensitivity is the study of reverse mutation using a panel of mutants that resulted from different specified kinds of genetic events spanning the entire spectrum of mutagenic effects. Original site reversion is usually a less frequent event than forward mutation and can be simulated by extragenic suppressor mutations. Therefore, reversion studies would substitute a set of tests that must be individually large enough to detect a specific kind of mutation for a single, all-purpose test.

The serious technical limitation called "metabolic cooperation" (9) afflicts the AG-resistance selection system and, probably, the DAP-resistance selection system as used with human fibroblasts and Chinese hamster cells: AG^r mutants become AG-sensitive pheno-

copies as a result of contact with normal cells in the presence of AG. Maximum recovery of mutants for purposes of quantitation requires that selection be applied to populations less dense than about 60 cells/mm² of surface. This requirement causes experiments to be larger and more expensive than is desirable. To me, the cost-benefit ratio will still be small if application of these methods prevents the dissemination of just one weak mutagen. Nevertheless, elimination of the complication created by metabolic cooperation from these selection systems or others should be an important objective of future investigation.

Metabolic cooperation may be insignificant in cultures of human lymphoblastoid cells and mouse lymphoma cells. Some cultures of lymphoblastoid cells have high cloning efficiencies and can be propagated without limit *in vitro*. The mouse lymphoma cells have the added advantage of being unhampered by major histocompatibility barriers in host-mediated applications. Both types of cells may have much to offer in mutagenicity testing. At this time, lymphoblastoid lines exhibit great variability in cloning efficiencies and in sensitivities to the lethal and mutagenic effects of chemical mutagens (10). Their indefinitely prolonged *in vitro* propagability may depend on the presence of a viral (Epstein-Barr) genome, which might conceivably affect the mutational behavior of the cells. Nevertheless, human lymphoblastoid cells, mouse lymphoma cells, Chinese hamster cells and, perhaps, others, are potential substitutes for human fibroblasts. It would be important in some applications to show that their mutation mechanisms and mutagenic responsiveness are representative of normal human cells. They should not be markedly hyper- or hyporesponsive in comparison to normal cells, but the latter is the more dangerous difference. The only way this can be determined is by a detailed comparison of fibroblast strains and other cell lines with regard to mutagenic responsiveness at several loci. A comparison of DNA repair mechanisms should be a part of such studies. Finally, it will be of considerable in-

terest to determine if lymphoblasts and fibroblasts from the same person exhibit similar mutational behavior.

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