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DNA Sequence Analysis of Spontaneous Mutations at the *aprt* Locus of Hamster Cells

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To determine the nature of spontaneous mutational events in cellular genes in hamster cells, mutant adenine phosphoribosyltransferase (*aprt*) genes were cloned and the regions to which we mapped alterations were sequenced. A variety of nucleotide changes were found to occur in the 12 mutant genes analyzed. Most mutations were simple base-pair substitutions-transitions (both G · C → A · T and A · T → G · C) and transversions. The only multiple mutation was a simple transition next to a single-base-pair insertion. Of the 12 mutations, 4 were more complex, involving small deletions or duplications. Two of these were similar to previously described deletions in that they occurred between short direct sequence repeats. No hot spots were detected. Three independent mutations were characterized at one restriction endonuclease site, although no other mutations were detected in the nucleotides surrounding this site in other mutant strains. At a functional level, sequence changes were either in exons (resulting in missense and, in one instance, nonsense mutations) or at splicing sites.

Mutational alterations or deficiencies in the products of selectable genetic loci in cultured somatic cells have been demonstrated in numerous laboratories (for reviews, see references 7 and 27). However, very little is known of the structural alterations in genes giving rise to variations in phenotype. To examine these events in Chinese hamster ovary (CHO) cells, we collected a large number of strains with spontaneous mutations of the autosomal locus coding for the nonessential salvage enzyme adenine phosphoribosyltransferase (APRT) and analyzed the strains at the molecular level for alterations in *aprt* gene structure (19).

aprt is a particularly attractive locus for analyzing mutational events. The gene is autosomal, but a strain hemizygous for *aprt* has been identified (4, 19), making it possible to collect single-step spontaneous mutants. Furthermore, *aprt* is a small gene (14; Fig. 1): a 3.8-kilobase *Bam*HI fragment contains all the sequences necessary to transform $APRT^-$ cells to $APRT^+$. This allows, in many instances, localization of mutational events in vivo to restriction endonuclease sites and rough mapping of deletion or insertion termini (19). Also crucial for the consideration of *aprt* as a model for the study of gene variation in mammalian cells is that it is a true endogenous gene with the low mutational rate inherent to such loci, unlike many exogenously introduced genes (28) or shuttle vectors (6) which have unusually high mutational rates or high frequencies of rearrangements.

In our previous investigations we found that only about 10% of APRT mutants resulted from deletions or insertions of more than 30 base pairs (bp). The great majority had no detectable alterations of gene structure, as judged by fine-structure restriction endonuclease mapping. A number of these mutations were, however, localized to restriction endonuclease recognition sites in the *aprt* gene, since the nucleotide alterations produced by the mutations led to the loss or gain of the site. We cloned these mutant *aprt* genes and sequenced the base-pair changes causing the alterations of the sites. Here we report the properties of these mutational events and discuss mechanisms for their production.

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MATERIALS AND METHODS

Mutant collection. Cell culture techniques and mutant selections have been described previously (19). Spontaneous APRT mutants were selected from CHO strain D422, which is hemizygous for *aprt* (4, 19). Replica cultures were grown from an initial 100-cell inoculum to 2×10^6 cells in α medium (GIBCO Laboratories), 4 μM thymidine, and 2.5% dialyzed fetal bovine serum-2.5% horse serum. All the cells from each replica were plated in 8-azaadenine-containing selective medium. To ensure that all the mutants obtained were truly independent, only one drug-resistant colony was picked from each positive replica culture. With this protocol, the rate of occurrence of APRT mutants was 4×10^{-8} per cell per generation. In this manner, a collection of 120 mutant strains was obtained.

Southern blot analysis. The protocols used for extraction of high-molecular-weight DNA, restriction endonuclease digestion, and Southern blot analysis have been described in detail (19). All probes were labeled to high specific activity (2×10^8 dpm/min per μg) by nick translation (23).

Hybridization with oligonucleotides. The oligonucleotide 5′-CCTAACAGGCCTAGACTC-3′ was synthesized by an automated solid-phase phosphite triester method (Applied Biosystems 380B). Oligonucleotide probes (150 ng) were endlabeled by using T4 polynucleotide kinase in the presence of 50 mM Tris hydrochloride (pH 7.6)–10 mM $MgCl_2$–5 mM dithiothreitol–0.1 mM spermidine–0.1 mM EDTA–250 μCi of [γ-^{32}P]ATP (>3,000 Ci/mmol) for 60 min at 37°C. The labeled molecules were purified by electrophoresis on a 20% denaturing polyacrylamide gel, followed by elution into 500 μl of 1 mM EDTA. This yielded probes with a specific activity of 5×10^8 to 1×10^9 dpm/μg.

Restriction endonuclease digests of genomic DNAs were electrophoresed on a 1% agarose gel and treated as described previously (29), and the gel was dried under vacuum. The gel was wetted, and hybridization was performed for 14 to 18 h at 50°C in 6× SSC (0.9 M sodium chloride, 0.09 M sodium citrate) containing 10× Dendhardt solution, 50 μg of sheared herring sperm DNA per ml, 10 μg of poly(A) per ml,

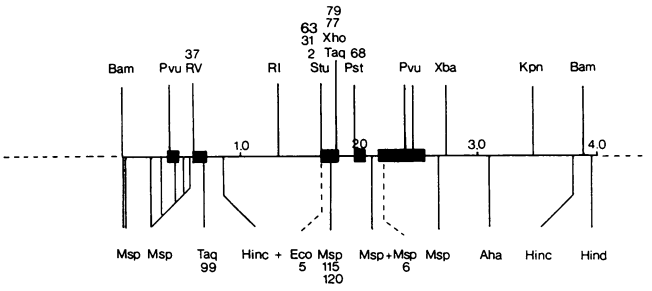

FIG. 1. Restriction map of *aprt* locus of CHO cells. The positions of spontaneous mutations localized by lost or gained restriction endonuclease sites are indicated by the mutant strain numbers above or below the sites affected. The restriction sites of the 4.0-kilobase fragment were assigned on the basis of our DNA sequence for this fragment (21). ■, Exons determined by homology (87%) with the mouse cDNA sequence (9).

0.1% sodium dodecyl sulfate, 10% dextran sulfate, and labeled probe (6×10^6 cpm/ml). The gel was rinsed quickly three times in 6× SSC–0.1% sodium dodecyl sulfate at 50°C. The most stringent wash was performed in 6× SSC–0.1% sodium dodecyl sulfate at 54°C, 2° the melting temperature for this oligonucleotide, for 4 min with constant agitation.

Cloning and sequencing of mutant *aprt* genes. Genomic DNA which had been digested with *Eco*RI and *Hin*dIII was size fractionated on agarose gels and cloned into *Eco*RI-*Hin*dIII-digested bacteriophage λNM1149. Ligation, in vitro packaging, and screening of recombinants were performed by using previously described protocols (20). Fragments of interest were subcloned directly from λ minipreps (15) into M13 vectors mp18 or mp19 (22). Sequencing was performed by the dideoxy chain termination method (25) using [α-^{35}S]dATP (3). The polymerization reaction was primed either by the 17-mer universal primer (22) or by appropriate internal primers (19-mer). Sequencing reaction mixtures were doubly loaded onto 5% polyacrylamide denaturing gels (thickness, 0.4 mm; length, 65 cm), allowing analysis of the sequence up to 500 bp from the priming site.

RESULTS

Mutant *aprt* alleles with altered restriction endonuclease sites. Restriction endonuclease digests of mutant DNAs were analyzed for alterations of *aprt* gene structure after fractionation on agarose gels and transfer to nitrocellulose filters. In a number of strains (12 of the 120 analyzed; Fig. 1), losses or gains of restriction endonuclease sites were detected, thus allowing localization of mutational events to these modified sites in the mutant genes. The sites which were affected were fairly evenly distributed over the *aprt* locus. Although two or three independent mutants may have lost the same site, no single hot spot predominated. One small region, however, appeared to be the target of most of the mutations, the third exon between the *Stu*I site (which includes the splice acceptor) and the *Xho*I site.

To analyze the mutations of the *aprt* locus which were localized to these restriction endonuclease recognition sequences, mutant genes were cloned from genomic libraries produced in the λ vector NM1149. The region of the *aprt* gene bearing the mutation was then sequenced after subcloning into the appropriate M13 vector. The results of these analyses are presented below according to the type of modification which occurred.

Single-base-pair substitutions. The type of event most frequently observed (5 of 12) was a simple transition mutation (Fig. 2). Both A · T → G · C and G · C → A · T transitions were found, with three of the five being A · T → G · C. Only one of the G · C → A · T transitions (S120) occurred at a potentially methylatable CpG site, although this dinucleotide was present in many of the affected restriction endonuclease recognition sequences.

Two of the mutations analyzed were transversions (S31 and S77; Fig. 2). Mutant S115 is slightly more complex, having two base-pair alterations of the *Msp*I site (nucleotide position 1756). There was both a G · C → A · T transition and an adjacent insertion of an A · T base pair (Fig. 2). The net result was a transition and frameshift mutation. No

strain	map site	wild type sequence	mutant sequence	alteration
transitions:				
S5	gained Eco RI	CAGGG G ATTCT / GTCCC C TAAGA (1700)	CAGGG A ATTCT / GTCCC T TAAGA (Eco RI)	G:C → A:T
S6	gained Msp I	GCTGC T GGGCC / CGACG A CCCGG (2190)	GCTGC C GGGCC / CGACG G CCCGG (Msp I)	A:T → G:C
S68	lost Pst I	TCTGC A GGCTG / AGACG T CCGAC (1970)	TCTGC G GGCTG / AGACG C CCGAC (Pst I)	A:T → G:C
S99	lost Taq I	GATCG A CTACA / CTAGC T GATGT (700)	GATCG G CTACA / CTAGC C GATGT (Taq I)	A:T → G:C
S120	lost Msp I	CATCC G GAAGC / GTAGG C CTTCG (1760)	CATCC A GAAGC / GTAGG T CTTCG (Msp I)	G:C → A:T
transversions:				
S31	lost Stu I	AGGCC T AGACT / TCCGG A TCTGA (1690)	AGGCC A AGACT / TCCGG T TCTGA (Stu I)	T:A → A:T
S77	lost Xho I	CTCTC G AGTAT / GAGAG C TCATA (1810)	CTCTC T AGTAT / GAGAG A TCATA (Xho I)	G:C → T:A
multiple changes:				
S115	lost Msp I	CTCAT C CGGAA / GAGTA G GCCTT (1760)	CTCAT TT CGGAA / GAGTA AA GCCTT (Msp I)	C:G → TT:AA

FIG. 2. Spontaneously occurring base-pair substitutions at the *aprt* locus. The nucleotide substitutions resulting in the loss or gain of restriction endonuclease sites are indicated for each strain in which alterations were detected. The numbering of nucleotides represents the positions of these nucleotides in the 4.0-kilobase fragment.

further alterations were detected in about 1,000 bp of adjacent DNA sequenced in S115 and the other mutants.

The mutations also generated a variety of alterations of the *aprt* gene product. All occurred in coding regions or splice junctions even though these sequences represent only about 15% of the target locus. S68 contains a modification of a splice acceptor sequence, as determined by the alignment of the genomic hamster sequence with the mouse cDNA (9) and the presence of a consensus splice acceptor sequence at this site (Fig. 2; 18). Most of the other base-pair substitutions resulted in missense mutations, whereas the alteration in mutant S77 produced an in-frame nonsense codon.

Complex mutations. Four of the mutant genes had either small deletions or insertions which were not detectable by altered mobility of *aprt* restriction fragments on Southern blots. S2 contains a 9-bp deletion including the *Stu*I site (Fig. 3). It occurred between two short (4-nucleotide) direct repeats, one copy of which is retained in the mutant gene. S63 contains another small deletion at the same *Stu*I site (Fig. 3). The nucleotides deleted in this mutant are different, being adjacent to those affected in S2. This deletion is also flanked by short nucleotide repeats (CC), with one copy retained in the mutant allele.

Mutant S37 lost an *Eco*RV site at nucleotide position 604 in the second exon of the *aprt* gene as a result of a 24-bp deletion (Fig. 3). Again, short direct repeats (CTCC) surrounded the deleted nucleotides, but unlike the events described above, both copies are largely retained in the mutant gene and three new nucleotides, or perhaps rearranged nucleotides from the deleted fragment, were introduced. Analysis of the surrounding and deleted nucleotides revealed some short inverted repeats, although none of these was predicted to form potentially stable secondary structures (5).

Mutant S79 like S77 lost the *Xho*I site at nucleotide position 1805. However, the sequence of that region of S79 demonstrates that a very different event took place (Fig. 3). The loss of the *Xho*I site was the result of a duplication of 7 bp 5′ to the *Xho*I site, which were then inserted into the middle of the site, with the addition of two more nucleotides. The heptanucleotide duplicated is flanked by a CTC trinucleotide, and a CCTCC pentanucleotide is found 5′ to the duplicated fragment.

Is the region of *aprt* which includes the *Stu*I site a mutational hot spot? The observation that the three mutations at the *Stu*I site all altered different nucleotides, as well as the occurrence of another mutation (S5) nearby, raised the possibility that this region of the *aprt* gene might be prone to mutation to produce the APRT$^-$ phenotype. To test this, we probed genomic DNA digests of our collection of spontaneous APRT mutants with a labeled oligonucleotide spanning the *Stu*I site and six nucleotides on either side of it (a total of 18 nucleotides). Under conditions in which a single-base-pair mismatch would prohibit hybridization, digests of DNA from mutants known to have alterations in this region, including S31, which has only a single-base-pair substitution, did not hybridize with the end-labeled probe (Fig. 4). In contrast, a clear signal was obtained after hybridization with all the other mutant DNAs. Thus, no other alterations were detected in the nucleotides immediately surrounding the *Stu*I site in 120 independent mutants.

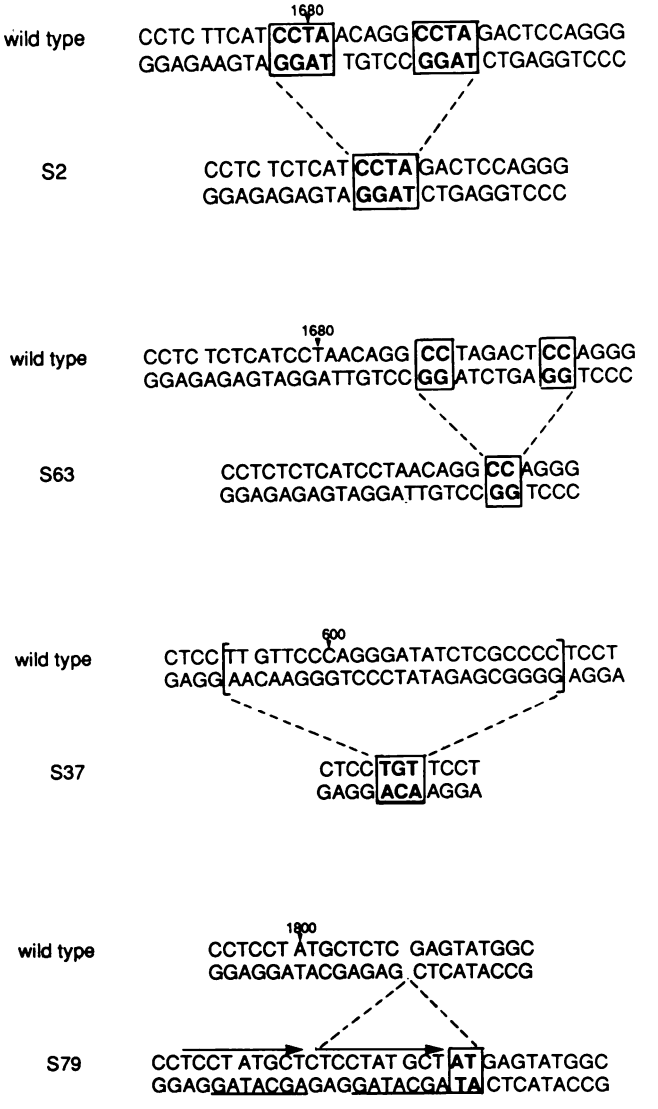

FIG. 3. Nucleotide sequence of complex alterations at the *aprt* locus eliminating restriction endonuclease sites. The boxed regions represent short sequence homologies at the two deletion termini in S2 and S31 and newly inserted nucleotides in the mutant alleles in S31 and S79. Short direct repeats formed by the duplication in S79 are indicated by arrows. The numbering represents the positions of the nucleotides on the map in Fig. 1.

DISCUSSION

Our findings indicate that a variety of events produce spontaneous mutations at the *aprt* locus. In the mutants examined, no one type of alteration or target sequence predominated. Independent mutations occurred at some sites (e.g., three *aprt* mutants lost the *Stu*I site), but in each instance different nucleotides were the targets and different changes occurred. However, the analyses reported here were limited to mutants which lost restriction endonuclease sites, since these provide in vivo confirmation of the sequences determined in vitro. We cannot eliminate the possibility that a hot spot outside these regions might exist and upset our spectrum.

Several mechanisms have been proposed to account for simple base-pair substitutions. Transitional hot spots in the *lacI* gene of *Escherichia coli* have been identified at 5-methylcytosine residues (8) at which deamination produces G · C → A · T substitutions. A similar mechanism has been suggested for 5-methylcytosine at methylated CpG sites in

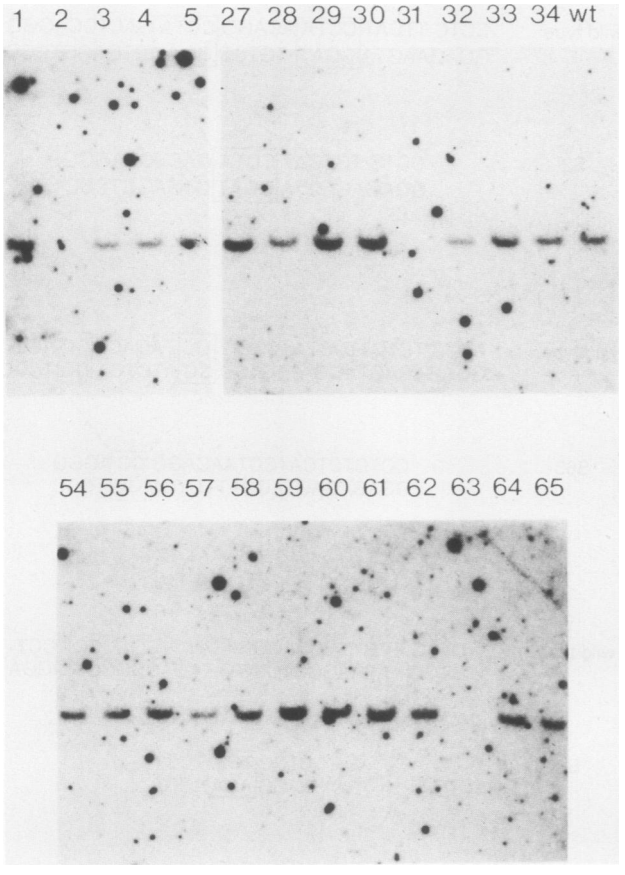

FIG. 4. Probe of digests of genomic DNAs from spontaneous APRT mutants, using an end-labeled oligonucleotide. Mutant DNAs (indicated by the number above each lane) digested with *Pvu*II were fractionated by electrophoresis on agarose gels. The gels were then dried and hybridized with a labeled 18-mer oligonucleotide containing the *Stu*I site (position 1684).

the human genome (2). Of the seven single-base-pair substitutions characterized in this study, only two occurred at potential methylcytosine sites, and only one of these was the type of transition predicted by the deamination pathway. Thus, it appears that this mechanism cannot explain the events at the *aprt* locus. This is not particularly surprising since active genes are typically undermethylated (30) and analyses of *Msp*I-*Hpa*II sites have indicated that these are unmethylated in the *aprt* structural gene (M. Meuth, unpublished observations). The presence of the two transversions among the simple base-pair substitutions is notable since it has been proposed that this type of mutation is the consequence of a replicational bypass of spontaneously occurring apurinic sites in DNA by an error-prone DNA polymerase (13, 16, 26). But even though mammalian polymerases behave as though error prone in vitro, (13), such events do not appear to be the dominant cause of spontaneous mutation in vivo.

Four of the mutations were the result of small (less than 25 bp) rearrangements of nucleotides at the *aprt* locus. Again, all four involved different nucleotides and events. The small deletions in mutants S2 and S63 resembled previously reported larger (40 to 2,000 bp) deletions in that they occurred between two short direct repeat sequences, only one of which is retained in the mutant gene (20). In S2 an inverted repeat is also present in the nucleotides which would be looped out by slipped mispairing of the two nearby direct repeats, potentially stabilizing this structure (Fig. 5). Thus, an attractive rationalization of the event in S2 is that the nucleotides looped out were the ones deleted (Fig. 4). But this is not the situation for S63 since the deletion occurred at the base of the proposed stem loop or else partially eliminated the structure on the opposite strand. In the larger deletions reported previously, deletion termini occurred in regions rich in inverted repeats capable of forming stable secondary structures, although these were not necessarily eliminated by the mutation (20). Structures similar to the one in which the mutation in S2 occurred have been identified as hot spots for mutation in the RII region of phage T4 (24) and the *lacI* gene of *E. coli* (10). It clearly cannot be called hot in *aprt* since it represented only about 1% of spontaneous mutations and, apart from the two affecting the *Stu*I site, no other mutations in our entire collection mapped to the nucleotides immediately surrounding this site. On the other hand, this region of the *aprt* locus appears to be a target for gene rearrangements. In addition to the two small deletions reported here, one of the termini of the 421-bp deletion in S118 falls about 30 bp away (20) and a γ radiation-induced deletion-insertion mutation occurred at the same site as those in S2 and S63 at nucleotide 1690 (which is the nucleotide next to the one altered in S31) (4a).

Mutants S37 and S79 have small rearrangements quite different in nature from those described previously. S37 contains a small deletion, and S79 contains a duplication occurring some distance away in the genome. There are similarities between the two in that the nucleotides altered in both mutant alleles are bounded by the tetranucleotide CTCC and both copies of this short direct repeat are largely retained in the mutant genes. This is in contrast to deletion events we have characterized in which only one copy of the repeat is retained (20). Numerous mechanisms could be proposed to explain the generation of these mutations. Alterations similar to that in S37 have been generated in vitro during replication of DNA templates by DNA polymerase I in the presence of terminal transferase (12), and therefore this mutant gene could be a result of a replication error. One could also imagine the duplication in S79 being formed by slipped mispairing of the CTCC in the nascent DNA chain during replication. Insertion and excision of procaryotic transposable elements in vivo can also generate small deletions or duplications (11).

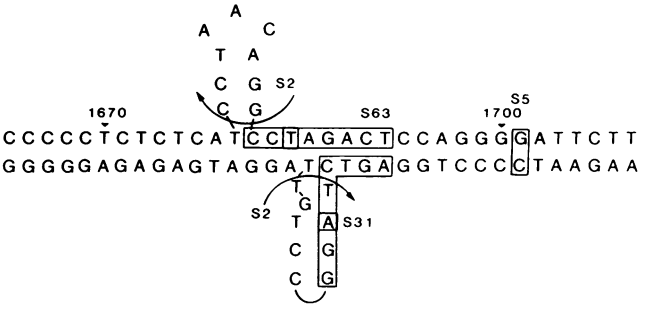

FIG. 5. Possible structure produced by slipped mispairing of short direct repeats flanking the deletion in mutant S2. The positions of other mutations mapped to this region of the *aprt* gene are indicated by arrows (S2) or boxed nucleotides. A splice site consensus sequence occurs at nucleotide 1685. Nucleotide numbering is in accordance with the map in Fig. 1.

At a functional level all of the mutations occurred within coding regions of *aprt* or at splice junctions. Over half of the mutants which lost sites did so within a short 200-bp region of *aprt* (*Stu*I to *Xho*I), although it could be argued that there are simply more vulnerable restriction endonuclease sites within this region. Most of the mutations were simple missense mutations, but splice acceptors were also a significant target. Two of the small deletions, those in S2 and S37 (but not that in S63, which is immediately adjacent to that in S2) included splice acceptor sites. One of the single-base-pair alterations (S68) modified a consensus splice acceptor; in this instance ApG was modified to GpG. A similar ApG → GpG alteration in the human β-globin gene totally inactivates the acceptor site (1). In a similar study of a small collection of spontaneous mutations at the dihydrofolate reductase locus of CHO cells, modifications of splice sites were predominant (17). We also detected an in-frame amber mutation in S77 which could be of use in analyzing suppression of cellular genes in mammalian cells.

These analyses, together with our earlier work (20), show that a variety of events are responsible for spontaneous gene variation at cellular loci in hamster cells. They also provide a data base for analyzing the effects of chemical mutagens, carcinogens, and cellular mutator genes in modifying this spectrum.

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LITERATURE CITED

1. **Antonarakis, S. E., S. H. Orkin, T.-C. Cheng, A. F. Scott, J. P. Sexton, S. P. Trusko, S. Charache, and H. H. Kazazian, Jr.** 1984. β-Thalassemia in American blacks: novel mutations in the "TATA" box and an acceptor splice site. Proc. Natl. Acad. Sci. USA **81**:1154–1158.
2. **Barker, D., M. Schafer, and R. White.** 1984. Restriction sites containing CpG show a higher frequency of polymorphism in human DNA. Cell **36**:131–138.
3. **Biggin, M. D., T. J. Gibson, and G. F. Hong.** 1983. Buffer gradient gels and ^{35}S label as an aid to rapid DNA sequence determination. Proc. Natl. Acad. Sci. USA **80**:3963–3965.
4. **Bradley, W. E. C., and D. Letovanec.** 1982. High frequency nonrandom mutation event at the adenine phosphoribosyl transferase (*aprt*) locus of sib-selected CHO variants heterozygous for *aprt*. Somatic Cell Genet. **8**:51–66.

4a. **Breimer, L., J. Nalbantoglu, and M. Meuth.** 1986. The structure and sequence of mutations induced by ionizing radiation at selectable loci in Chinese hamster ovary cells. J. Mol. Biol. **192**:669–674.

5. **Brutlag, D. L., J. Clayton, P. Friedland, and L. H. Kedes.** 1982. SEQ: a nucleotide sequence analysis and recombination system. Nucleic Acids Res. **10**:279–294.
6. **Calos, M. P., J. S. Lebkowski, and M. R. Botchan.** 1983. High mutation frequency in DNA transfected into mammalian cells. Proc. Natl. Acad. Sci. USA **80**:3015–3019.
7. **Caskey, C. T., and G. D. Kruh.** 1979. The HPRT locus. Cell **16**:1–9.
8. **Coulondre, C., J. H. Miller, P. J. Farabaugh, and W. Gilbert.** 1978. Molecular basis of base substitution hotspots in *Escherichia coli*. Nature (London) **274**:775–780.
9. **Dush, M. K., J. M. Sikola, S. A. Kahn, J. A. Tischfield, and P. J. Stambrook.** 1985. Nucleotide sequence and organization of the mouse adenine phosphoribosyl transferase gene: presence of a coding region common to animal and bacterial phosphoribosyltransferases that has a variable intron/exon arrangement. Proc. Natl. Acad. Sci. USA **82**:2731–2735.
10. **Glickman, B. W., and L. S. Ripley.** 1984. Structural intermediates of deletion mutagenesis: a role for palindromic DNA. Proc. Natl. Acad. Sci. USA **81**:512–516.
11. **Kleckner, N.** 1981. Transposable elements in prokaryotes. Annu. Rev. Genet. **15**:344–404.
12. **Kunkel, T. A., K. P. Gopinathan, D. K. Duke, E. T. Snow, and L. A. Loeb.** 1986. Rearrangements of DNA mediated by terminal transferase. Proc. Natl. Acad. Sci. USA **83**:1867–1871.
13. **Loeb, L. A.** 1985. Apurinic sites as mutagenic intermediates. Cell **40**:483–484.
14. **Lowy, I., A. Pellicer, J. F. Jackson, G.-K. Sim, S. Silverstein, and R. Axel.** 1980. Isolation of transforming DNA: cloning the hamster aprt gene. Cell **22**:817–823.
15. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Miller, J. H., and K. B. Low.** 1984. Specificity of mutagenesis resulting from the induction of the SOS system in the absence of mutagenic treatment. Cell **37**:675–682.
17. **Mitchell, P. J., G. Urlaub, and L. Chasin.** 1986. Spontaneous splicing mutations at the dihydrofolate reductase locus in Chinese hamster ovary cells. Mol. Cell. Biol. **6**:1926–1935.
18. **Mount, S. M.** 1982. A catalogue of splice junction sequences. Nucleic Acids Res. **10**:459–472.
19. **Nalbantoglu, J., O. Goncalves, and M. Meuth.** 1983. Structure of mutant alleles at the *aprt* locus of Chinese hamster ovary cells. J. Mol. Biol. **167**:575–594.
20. **Nalbantoglu, J., D. Hartley, G. Phear, G. Tear, and M. Meuth.** 1986. Spontaneous deletion formation at the *aprt* locus of hamster cells: the presence of short sequence homologies and dyad symmetries at deletion termini. EMBO J. **5**:1199–1204.
21. **Nalbantoglu, J., G. A. Phear, and M. Meuth.** 1986. Nucleotide sequence of hamster adenine phosphoribosyl transferase (aprt) gene. Nucleic Acids Res. **14**:1914.
22. **Norrander, J., T. Kempe, and J. Messing.** 1983. Construction of improved M13 vectors using oligodeoxynucleotide-directed mutagenesis. Gene **26**:101–106.
23. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
24. **Ripley, L. S.** 1982. Model for the participation of quasi-palindromic DNA sequences in frameshift mutation. Proc. Natl. Acad. Sci. USA **79**:4128–4132.
25. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
26. **Schaaper, R. M., T. A. Kunkel, and L. A. Loeb.** 1983. Infidelity of DNA synthesis associated with bypass of apurinic sites. Proc. Natl. Acad. Sci. USA **80**:487–491.
27. **Siminovitch, L.** 1976. On the nature of hereditable variation in cultured somatic cells. Cell **7**:1–11.
28. **Tindall, K. R., L. F. Stankowski, Jr., R. Machanoff, and A. W. Hsie.** 1984. Detection of deletion mutations in pSV2*gpt*-transformed cells. Mol. Cell. Biol. **4**:1411–1415.
29. **Tsao, S. G. S., C. F. Brunk, and R. E. Pearlman.** 1983. Hybridization of nucleic acids directly in agarose gels. Anal. Biochem. **131**:365–372.
30. **van der Ploeg, L. H. T., and R. A. Flavell.** 1980. DNA methylation in the human γδβ-globin locus in erythroid and nonerythroid tissues. Cell **19**:947–958.