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Genetic Instability at the Adenine Phosphoribosyltransferase Locus in Mouse L Cells

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Resistance to adenine analogs such as 2,6-diaminopurine occurs at a rate of $\sim 10^{-3}$ per cell per generation in mouse L cells. This resistance is associated with a loss of detectable adenine phosphoribosyltransferase activity. Other genetic loci in L cells have the expected mutation frequency ($\sim 10^{-6}$). Transformation of L cell mutants with Chinese hamster ovary cell DNA results in transformants with adenine phosphoribosyltransferase activity characteristic of Chinese hamster ovary cells. No activation of the mouse gene occurs on hybridization with human fibroblasts. That this high frequency event is the result of mutation rather than an epigenetic event is supported by antigenic and reversion studies of the 2,6-diaminopurine-resistant clones. These results are consistent with either a mutational hot-spot, a locus specific mutator gene, or a site of integration of an insertion sequence.

Examples of unusually high mutation frequencies have been reported for *Escherichia coli* (27), corn (20), and *Drosophila melanogaster* (9). Such instances are believed to derive from three distinct etiologies: (i) highly mutable genes—genes which show mutational instability; (ii) mutator genes—altered functions capable of inducing gene mutation, e.g., the Treffers mutator gene (*mu T*) in *E. coli* (6, 38); and (iii) extrachromosomal factors or agents such as viruses, plasmids, or transposons (3, 27).

There has been much discussion as to whether or not variants of mammalian cells are true mutants, that is, the result of some base sequence perturbation at the DNA level, leading to a stable alteration in phenotype, or due to "epigenetic" events (8, 29). Epigenetic events activate or suppress certain types of information within the cell independent of mutagenic pressure and independent of base sequence alterations in DNA. In the past, epigenetic hypotheses have been invoked to explain some unusually high mutation rates observed for mammalian cells in culture (21). However, that many variants (e.g., thioguanine-resistant cells) are true mutants has been firmly established (29).

Examples of possible events other than single base changes or small deletions capable of modifying gene function are chromosomal rearrangements (position effects), transposable elements or insertion sequences, heterochromatization, site-specific inversions, or site-specific modifications (e.g., methylation). All of these, as well as mutation by known mutagens, will result in permanent or semipermanent (depending on reversion frequency) phenotypic alterations. Analysis of the altered gene product has the potential to discriminate among some of these different classes of events.

In this paper, we report an example of gene instability in a mammalian cell line. Mouse L cells exhibit an abnormally high spontaneous mutation frequency to 2,6-diaminopurine resistance (DAP^r) (approximately 10^{-3}), whereas the frequencies of resistance to other drugs (thioguanine, azaserine, ouabain) are within the expected range (approximately 10^{-6}). The DAP^r mutants are stable with an accompanying defect in the enzyme adenine phosphoribosyltransferase (APRT), indicating that these "variants" result from true mutational events.

MATERIALS AND METHODS

Cell culture and mutagenesis. L cells were usually grown in Eagle minimal essential medium supplemented with 5 to 10% fetal calf serum, penicillin (100 U/ml), and streptomycin (100 μg/ml). Later experiments utilized 10% calf serum. Cells were routinely tested for the presence of mycoplasma by a modification of the method of Hayflick (11) using both aerobic and anaerobic incubation in broth and agar. Also, no bands of mycoplasma-specific APRT activity were observed on electropherograms of cell lysates (31, 35). L cells were from three sources: those carried in Bloomington, Ind., (M.W.T.) for the past 10 years and supplied to J.A.T., and cultures from the American Type Culture Collection, Rockville, Md., and the Institute for Medical Research, Camden, N.J.

To eliminate preexisting mutants in cultures before selection with adenine analogs, cells were grown for

two passages in medium containing 50 μM azaserine, 4 μg of alanosine per ml, and 50 μM adenine as the sole purine source. Under such conditions, only cells with APRT activity can survive (16, 36). The frequency of drug resistance was measured by plating 10^4 to 10^5 cells per 100-mm petri plate in Eagle minimal essential medium containing 40 to 75 μg of DAP per ml, 6 μg of thioguanine per ml, 50 μM azaserine, 20 μg of 8-azaadenine (AZA) per ml, 4 μg of 2-fluoroadenine (FA) per ml, or 1 μg of oubain per ml. Selective media were changed at 3-day intervals, and surviving colonies were stained after 10 to 14 days. To measure induced mutation, cells were treated for 16 h with 1 μg of ICR-170 or ICR-191 per ml or 400 μg of ethyl methane sulfonate (EMS) per ml and selected after a 6-day period of growth in drug-free medium. Variations of this protocol are noted in the text. There was 30 to 40% cell survival under these conditions. Clones isolated without mutagen treatment were designated "LS" (spontaneous), whereas those clones isolated after ICR treatment were designated "LI" (induced). For reversion studies, 10^7 cells (3×10^5 cells per 100-mm dish) or more (10^9 for LS-24b) of 10 independent mutants were plated into selective medium (alanosine, azaserine, adenine). Cells were also treated with either EMS or ICR-191, as indicated above, before selection for revertants. Spontaneous revertants were designated "RS", whereas EMS-induced and ICR-induced revertants were designated "RE" and "RI", respectively.

APRT assays, adenine incorporation, and immunological analyses. Incorporation of labeled adenine into trichloroacetic acid-insoluble and -soluble material was done by the method of Sharp et al. (28) or Stambrook and Sisken (30). Cell extracts were assayed for APRT activity by previously published methods (12, 31).

Determination of immunologically cross-reacting material (CRM⁺). Anti-rat liver APRT was produced in rabbits. This antiserum has been shown to cross-react with mouse and other mammalian APRTs but not with yeast, sea urchin, or *E. coli* APRT (33). The immunoprecipitation reaction between L cell APRT and rabbit anti-APRT serum was performed by mixing 5 μl of bovine serum albumin (10 mg/ml), 10 μl (0.057 mg of protein) of wild-type cell extract, 20 μl of rabbit anti-APRT serum (previously titered, and used at 1:100 dilution), and a serial dilution of mutant cell extract (100 μl). This mixture was incubated at 4°C for 2 h, at which time 5 μl of fixed *Staphylococcus aureus* (Pansorbin, Calbiochem, La Jolla, Calif.) was added and the mixture was retained at 4°C for an additional 20 min. The antibody-bound CRM was precipitated by centrifugation for 1 min in a microcentrifuge. APRT activity in the supernatant was measured, and the fraction of APRT activity precipitated by the antiserum was determined. Controls using normal rabbit serum showed no precipitation of APRT activity (33).

Fluctuation analysis. To determine whether or not DAP^r colonies randomly arise independently of the selective agent, a Luria and Delbruck (19) type fluctuation analysis (shown in Table 2) was performed using the protocol described by DeMars and Held (7). In set I, the incidence of DAP^r colonies was determined by seeding 10^4 cells into dishes containing medium plus DAP. At the same time the plating efficiency in drug-free medium was determined for use in future calculations. Set II consisted of a series of independent cultures initiated from either 10 or 100 cells. These cultures were grown for 10 days in drug-free medium at which time there were an average of 3.13×10^4 and 2.50×10^4 cells per culture in experiments 1 and 2, respectively. On day 10 the cells from each set II culture were removed with Viokase (GIBCO Laboratories, Grand Island, N.Y.) and split into three dishes containing medium plus DAP. DAP^r colonies were enumerated 13 days later. The numbers shown are the sum of the colonies in all three dishes.

Cell hybridization and DNA-mediated gene transfer. Cell hybrids were made between one APRT-deficient (APRT⁻) mutant (LS-24b) and two different strains of primary human fibroblasts (14). Fusion was mediated by polyethylene glycol and dimethy sulfoxide (22), and selection was in medium containing alanosine and adenine (36). The species origin of the APRT expressed by the hybrids was determined by gel electrophoresis (35), and the hybrids were tested for 28 different human enzymes which define 18 different linkage groups, each assigned to a specific human chromosome. These data, including electropherograms of the hybrids, are presented elsewhere (14).

High-molecular-weight Chinese hamster ovary cell DNA was used to transform cells from APRT⁻ clones LS-24b and LI-10 (37). The species origin of the APRT expressed by transformants was determined by gel electrophoresis (35). Transformation was accomplished via a modification of the method of Wigler et al. (40). Cells were exposed for 20 h to one part calcium phosphate-precipitated DNA mixed with eight parts whole medium. Each flask of 4×10^6 cells contained 100 μg of DNA at a concentration of 2 μg/ml. The cells were washed to remove the DNA and seeded at a density of 2×10^5 cells per 100-mm dish containing Eagle minimal essential medium, and after 24 h medium containing alanosine, azaserine, and adenine was substituted. Colonies were ready for picking after 14 to 28 days.

RESULTS

Mutation frequencies. Table 1 lists the frequencies of DAP^r, FA^r, and AZA^r colonies with and without pretreatment with mutagens. These experiments were done independently in two different laboratories, originally with the same strain of L cells, but later with L cells purchased from the American Type Culture Collection and The Institute for Medical Research. The average incidence of adenine analog resistance for all experiments was 1.3×10^{-3}. Pretreatment with mutagen appears to yield little or no enhancement.

Fluctuation analysis. The observation that the variance in the colony counts was less than the mean number of colonies in set I and that it was much greater than the mean (by a factor of at least 500) in set II (Table 2) indicates that DAP^r cells arise independently of the selecting drug (DAP) by a spontaneous and random process. The data are, thus, consistent with a mutational origin for DAP^r. The rate at which DAP^r colonies arise was calculated to be 7.91×10^{-4} to

TABLE 1. Frequency of adenine analog-resistant colonies[a]

Expt[a]	Treatment	Frequency	Adenine analog(s)
1	None	1.4×10^{-3}	DAP
2	None	2.7×10^{-3}	DAP
3a	None	1.0×10^{-3}	AZA
3b	None	1.9×10^{-3}	DAP + AZA
4a	None	3.0×10^{-3}	DAP
4b	ICR-170H	2.3×10^{-3}	DAP
5a (A)[b]	None	8.3×10^{-4}	DAP
5b[b]	EMS	7.8×10^{-4}	DAP
5c[b]	None	6.1×10^{-4}	FA
5d[b]	EMS	6.1×10^{-4}	FA
6a (A)[b]	None	2.8×10^{-4}	DAP
6b[b]	EMS	6.7×10^{-4}	DAP
6c[b]	None	2.1×10^{-4}	FA
6d[b]	EMS	3.0×10^{-4}	FA
7 (A)	None	1.1×10^{-3}	DAP
8 (A)	None	3.1×10^{-3}	DAP

[a] (A) denotes experiments done in Augusta, Ga.; all others were done independently in Bloomington, Ind.

[b] These experiments had a different protocol resulting in artificially low frequencies. Dishes were seeded with cells, and after 5 days the selective analog(s) was added. Calculations are based on the number of cells at the time of analog addition; thus, mosaic colonies arising from more than one resistant cell were scored as single colonies. In all other experiments, cells were seeded directly into medium containing one or more adenine analogs, and calculations are based on the number of viable cells seeded.

10.37×10^{-4} per cell per generation using two different statistical methods (4, 17) (Table 2).

Characterization of DAP[r] clones. (i) Adenine incorporation and APRT activity. Twenty-one independent DAP[r] clones were studied. The incorporation of [^{14}C]adenine (or [^{3}H]adenine) into trichloroacetic acid-precipitable and -soluble material was measured after a 24-h labeling period. Previous experiments had shown that, under the conditions utilized, wild-type cells demonstrate linear incorporation of label with time (J. A. Tischfield and M. W. Taylor, unpublished data). None of the clones showed adenine incorporation significantly above background, whereas the wild-type L cells incorporated large amounts of adenine (400× background). Additionally, none of the DAP[r] clones had detectable APRT enzyme activity (less than 0.1% of wild-type levels) (Table 3).

(ii) Growth in selective media. The average population doubling time for wild-type L cells in drug-free medium was 19.1 h. The average doubling times for mutant LS-24b in drug-free medium and medium containing 100 μg of DAP per ml were 17.5 and 17.7 h, respectively. Similarly, the average doubling times for clone LS-25b were 17.9 and 19.5 h. Clearly these mutants are very resistant to DAP. Wild-type L cells show complete growth inhibition at DAP concentrations above 30 μg/ml.

When (with the exception of LS-9) mutants were seeded into either azaserine-adenine (200 cells per dish) or alanosine-adenine medium (1,000 cells per dish), no colonies were observed. This observation is consistent with their completely APRT-deficient (APRT$^-$) phenotype.

(iii) Selection for other markers. To investigate whether or not a high frequency of mutation is a general property of L cell genetic loci, the frequencies of resistance to thioguanine, oubain, azaserine, AZA, and FA were measured. The spontaneous incidence of resistance to all the drugs, except AZA and FA, was within the expected range (approximately 10^{-6} to 10^{-7}). AZA[r] and FA[r] both had an incidence of approximately 10^{-3} (Table 1). Both of these latter resistant phenotypes, like DAP resistance, result from APRT deficiency.

(iv) Reversion studies. Reversion studies are important to distinguish between complete loss of a locus and alterations that have the potential to revert, such as point, frameshift, and insertion mutations. When 10 independent mutants were plated into selective medium, no spontaneous APRT$^+$ revertant colonies were observed, with one exception (Table 3). Clone LS-9 reverted to an apparently wild-type phenotype (as defined by APRT activity level and growth in selective media) at a frequency of 10^{-3}. This clone exhibits a high rate of transition between the APRT$^+$ and APRT$^-$ states and will be described in a future publication. However, when the other mutants were treated with EMS or ICR-191, revertants could be detected in some instances (Table 4). These revertants appeared at low frequencies, between 1×10^{-7} and 9×10^{-7}, and exhibited partially restored APRT activity (Table 4). This observation eliminates the possibility that these mutants arose from the physical loss of a relatively large segment of DNA.

(v) Immunological assay. Cross-reacting material (CRM) in APRT$^-$ mutant cell lysates was measured based upon the inhibition of immunoprecipitation of the wild-type enzyme (Table 4). Figure 1 shows typical results of such competition assays using cell extracts from LS-9, LS-10 (CRM$^+$), LI-8, and LI-10 (CRM$^-$). It should be noted that this type of competition assay would not detect very low levels of APRT protein.

(vi) Cell hybridization and gene transfer. Mutant LS-24b was fused with two different human adult primary fibroblast strains. These experiments were done to determine whether or not a human genetic element(s) is able to activate the expression of mouse APRT in such cell hybrids.

TABLE 2. L cell fluctuation analysis

Determination	Expt 1	Expt 2
Set I		
No. dishes	25	20
No. cells/dish	10^4	10^4
Medium	DVME[a] + 75 μg of DAP per ml	MEM[b] + 75 μg of DAP per ml
Results (set I)		
Colonies/dish	27, 30, 28, 31, 35, 28, 30, 28, 27, 34, 29, 29, 33, 31, 34, 34, 38, 30, 29, 31, 36, 31, 31, 31, 33, 32	8, 3, 8, 13, 11, 9, 8, 12, 12, 6, 9, 13, 12, 13, 14, 14, 13, 14, 12, 10
Total no. cells plated	2.5×10^5	2×10^5
Total no. colonies	769	214
Mean no. colonies/dish	30.8 (variation, 6.34; SD, 2.61)	10.7 (variation, 8.5; SD, 2.9)
Frequency DAP^r	3.08×10^{-3}	1.07×10^{-3}
Plating efficiency		
No. dishes	7	5
No. cells/dish	50	10
Medium	DVME	MEM
Colonies/dish	36, 31, 33, 34, 37, 35	8, 8, 9, 7, 7
Plating efficiency	76.4%	78.0%
Set II		
No. dishes	30	25
No. cells/dish	100	10
Medium (initially)	DVME	MEM
Results (set II)		
Colonies/dish	151, 150, 139, 152, 137, 136, 82, 51, 194, 238, 221, 197, 287, 217, 153, 192, 205, 214, 143, 185, 175, 161, 131, 163, 124, 148, 158, 172, 135, 119	48, 232, 109, 20, 122, 172, 82, 51, 91, 113, 89, 59, 56, 152, 97, 139, 162, 160, 135, 141, 104, 134, 116, 351, 128
Total no. cells plated	9.475×10^5	6.25×10^5
Total no. colonies	4,930	3,063
Mean no. colonies/dish	164.3 (variation, 2,093; SD, 45.81)	122.5 (variation, 4,244; SD, 66.5)
Rate of DAP^r (per cell per generation)[c]		
(1)	7.95×10^{-4}	7.87×10^{-4}
(2)	10.67×10^{-4}	10.10×10^{-4}

[a] In this experiment, Dulbecco-Vogt-modified Eagle (DVME) medium was used.
[b] MEM, Eagle minimal essential medium.
[c] (1) Calculated from equation 8 of Luria and Delbruck (19) using tables in Capizzi and Jameson (4). (2) Calculated from Lea and Coulson median method, Table 3 (17).

Alternatively, it is theoretically possible that a mouse element could suppress the expression of the human APRT gene. Previous fusions of human primary cells with L cell-derived APRT$^-$ cells (line A9) suggested that neither possibility would apply (36). A total of 14 hybrid clones were isolated. Each hybrid clone expressed only human APRT and a variable assortment of other human enzyme markers (14). The average APRT activity of the hybrids was 5.8 nmol of AMP/min per mg of protein, approximately 56% of the activity expressed by the human parents. As expected for mouse × human hybrids, DAP^r APRT$^-$ segregants could be selected at a frequency of 4.7×10^{-2}. Previous experiments done with similar hybrids demonstrated that these high-frequency, APRT$^-$ segregants arise mainly as a consequence of the loss of human chromosome 16 which bears the human APRT gene (36).

Mutants LS-24b and LI-10 were used as recipients in gene transfer experiments using Chinese hamster ovary cell (CHO) DNA. Thirty independent transformants expressed only hamster APRT at levels ranging from 0.5 to 6 times that of wild-type L cells. To date, no clones express-

TABLE 3. Presence of cross-reacting material and reversion

Clone	CRM	Spontaneous reversion[a]	Induced reversion[a]	
			EMS	ICR-191
LI-1	−	0	0	0
LI-3	−	0	0	0
LI-4	NT[b]	0	NT	6×10^{-7}
LI-8	−	0	9×10^{-7}	8×10^{-7}
LI-10	−	0	1×10^{-7}	4×10^{-7}
LS-1	+	0	NT	0
LS-9	+	$\sim 10^{-3}$	Spontaneous	Too high
LS-2a	+	0	5×10^{-7}	2×10^{-7}
LS-24b	+	0	0	0
LS-25b	+	0	1×10^{-7}	0
LS-10	+	0	NT	0

[a] See text for number of cells tested.
[b] NT, Not tested.

ing mouse APRT have been observed (37). Figure 2 shows the electrophoretic characterization of APRT from seven independent gene transfer clones. It was previously shown that mouse L cells produce an APRT with electrophoretic mobility identical to that exhibited by 40 different mouse strains (35, 36). It is clear that the transformants express only hamster (donor DNA) and not mouse APRT.

The above data indicate that APRT deficiency in clones LS-24b and LI-10 behaves recessively and that there is no evidence for any mouse cell activity that affects the expression of either the human or hamster APRT genes or enzyme activity. Furthermore, it demonstrates no difference between CRM$^+$ (LS-24b) and CRM$^-$ (LI-10) mutants in their ability to support the expression of a hamster APRT gene. This suggests that there is no trans-operating APRT gene "suppression" in either instance.

DISCUSSION

In this report, we describe a very high frequency of adenine analog resistance concomitant with loss of APRT activity in mouse L cells. Resistance to thioguanine, oubain, and azaserine occurs at much lower frequencies (e.g., by a factor of 10^{-3} to 10^{-4}). Our initial prejudice was to ascribe the high-frequency events to some sort of regulatory (epigenetic) phenomenon involving "turn off" of the APRT gene or to gene loss. If loss of APRT activity were due to chromosomal rearrangement, a position effect, or an event usually associated with cell differentiation, one would expect that none of the variants would retain CRM. Furthermore, in the case of the loss of a single active APRT gene, one would not expect reversion. Thus, our data argue against such hypotheses and indicate that this phenotype is due to an event(s) which sometimes permits retention of CRM and that this event(s) can sometimes be reverted after treatment with known mutagens, even in CRM$^-$ clones, albeit at a low frequency. The demonstration of revertants with less than wild-type levels of APRT activity suggests the possibility of amino acid substitutions or second-site reversion. The fluctuation analysis, cell hybridization, and gene transfer data, and the stability of the DAP^r phenotype taken with the above-mentioned data strongly suggest that these variants are true mutants. (Clone LS-9 is the exception and will be dealt with separately in a future publication.) The possibility exists, however, that different clones may be the result of different classes of mutational events, each having unique phenotypic consequences.

TABLE 4. APRT activity of revertant subclones

Clone	APRT activity (% of wild type)
LS-25bRE-4	38
LS-2aRE-13	31
LI-10RI-4	43
LI-10RI-5	58
LI-10-RI-6	60
LS-9RS-21	100
LS-9RS-39	100

This high rate of mutation to the APRT$^-$ phenotype may be unique to mouse L cells. Other permanent mouse cell lines, such as 3T6 (16), MMT (mammary tumor), TA3 (mammary tumor) (J. A. Tischfield, Ph.D. thesis, Yale University, New Haven, Conn., 1973), and teratoma (25), do not share this property. Also, the frequency of adenine analog-resistant clones is much lower in primary human cells (24) and CHO cells (5, 13, 32, 33; J. A. Tischfield, In Vitro **13**:152a, 1977; J. A. Tischfield, Proc. XIV Int. Cong. Genet., 1979, p. 415). In the latter case, analysis of mutant APRTs has demonstrated that many, if not all, adenine analog-resistant

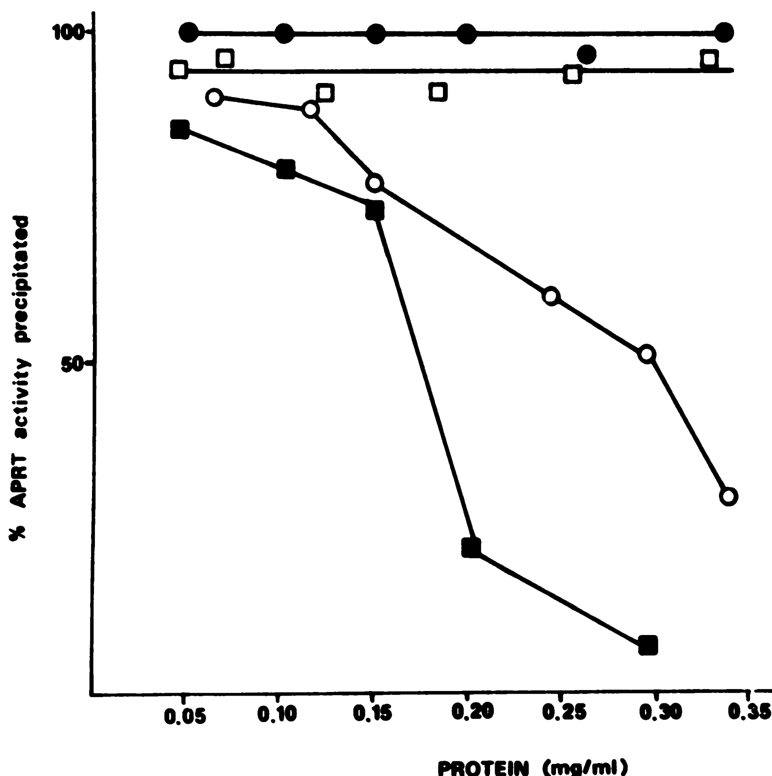

FIG. 1. Antibody competition (rabbit anti-rat liver APRT serum) curves. Symbols: LI-8, □; LI-10, ●; LS-9, ○; LS-10, ■. Various amounts of mutant cell extract (0.5 to 0.05 mg of protein) were added to 0.057 mg of L cell wild-type protein in the presence of antibody (1:100 dilution) sufficient to precipitate 80% wild-type activity. The immunoprecipitation reaction was as described in the text.

variants are true mutants resulting from point mutations or frameshifts in the APRT gene. It is significant that the data obtained with the original strain of L cells can be essentially reproduced with L929 cells from two independent sources. One outstanding difference between L cells and most other mouse cell lines is that L cells are extremely heteroploid, exhibiting a subtetraploid modal chromosome number with many biarmed chromosomes (36).

The question remains as to why there is such a high rate of mutation to the APRT$^-$ phenotype in L cells. Since the APRT locus is autosomal in the mouse (15), there could theoretically be as many as four copies of this locus in L cells. However, our data suggest that only one copy of the APRT gene is actually functioning in L cells. This is consistent with proposals that a substantial region of the genome of cultured somatic cells may be functionally hemizygous (29) or "silent." The latter term refers to the inactivation of linked genes such that the stability of their silence approaches that usually associated with mutation (2). Given that there is only one functioning copy of the APRT gene in L cells, several high-frequency mechanisms for mutation may be proposed. Perhaps the particular APRT allele functioning in L cells contains a base sequence which is highly mutable, similar, perhaps, to "hot-spots" in procaryotes. Also, the presence of an allele or locus specific "mutator gene" or an insertion sequence that transposes near the APRT locus could result in extremely high mutation frequencies and mutants of variable phenotype. In *D. melanogaster*, presumptive insertion mutants fall into two general classes: stable mutants which revert at a frequency of 10^{-5} to 10^{-6} per gamete, and unstable mutants which revert at a frequency of 10^{-3} to 10^{-4} per gene (9). This appears analogous to our findings at the mouse APRT locus.

It may be significant that L cells and other mouse cell lines commonly express retroviruses. The striking similarity between retrovirus proviruses, bacterial transposons, and movable genetic elements in yeast (TY1) and *Drosophila* (*copia*) has been noted (3, 34). Evidence suggests that retroviruses have no apparent specificity in the nucleotide sequence of their cellular DNA integration site (34). Likewise, the inser-

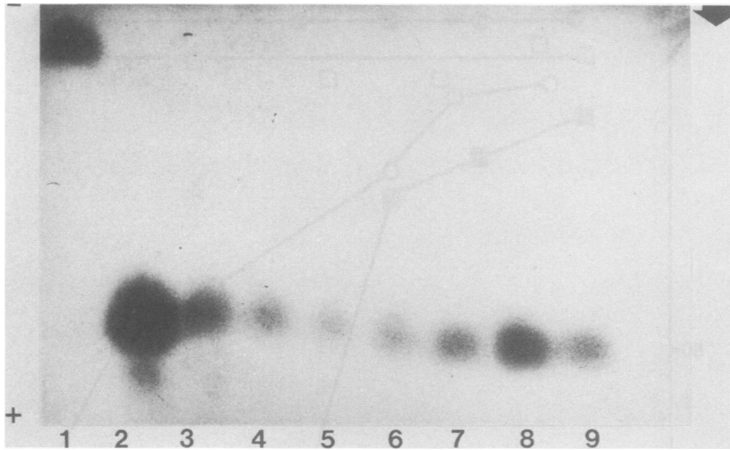

FIG. 2. Separation of mouse and CHO APRT as shown by autoradiograph of APRT activity in cell extracts after electrophoretic separation on a starch gel (35). Channel 1, Mouse L cell control; 2, CHO cell control; 3 to 9, transformants of APRT$^-$ mouse clone LS-24b obtained after treatment with CHO cell DNA (37). The arrow indicates the direction of migration. Only hamster APRT activity was detected in the transformants.

tion of transforming DNA into the mammalian genome may not be restricted to a unique chromosome or chromosomal region (26). In the process of insertion, transforming DNA may also generate mutations secondary to chromosomal rearrangements (25). It was recently shown that retroviruses can act as insertion mutagens, causing the inactivation of cellular genes by insertion of viral DNA, in the manner exhibited by the mutator bacteriophage, Mu-1, or by other transposable elements in yeast, *Drosophila*, and maize (39). In the above cases, insertion can be into a large number of sites in the host genomes (3, 39). Thus, there are features in common between DNA-mediated transformation and insertion of retroviruses and transposable elements.

It is worthwhile to note that DNA-mediated transfer of the thymidine kinase and dihydrofolate reductase genes is much higher with L cell recipients as compared to CHO recipients (18). L cells were also better recipients for the APRT gene than were human or hamster cells (35). It is difficult to evaluate the above data, but they suggest that L cells may be particularly susceptible to insertion of DNA sequences, and, thus, insertional mutagenesis. A high rate of insertional mutagenesis could help explain the highly rearranged karyotype of L cells. This could also account for the high rate of mutation at the APRT locus, but we do not know if loci, other than those tested, can be affected in a similar manner.

Atkins and Gartler (1) isolated DAP^r clones from L cells of unknown origin. Of eight DAP^r clones, seven incorporated very little adenine and showed negligible APRT activity. An eighth clone showed 8% of wild-type adenine incorporation and 7.1% of wild-type APRT activity (our calculations). We have not observed any clones similar to this latter type, although a clone with a high spontaneous reversion frequency such as LS-9 could assay in this manner if maintained in nonselective medium. They observed DAP^r clones at frequencies between 1.4×10^{-4} and 7.0×10^{-4}, with an average frequency of 2.6×10^{-4} (our calculations). This is somewhat lower than our average frequency but still unusually high for a putative autosomal recessive mutation. The differences could be due to variables in culture conditions or to fundamental differences between cell lines. Atkins and Gartler (1) found only one putative revertant in one cell line in 3×10^6 cells. No spontaneous revertants were observed for seven other cell lines after testing 2.2×10^7 cells. These latter data are consistent with our data.

High spontaneous frequencies have also been reported for loss of adenosine kinase activity in CHO cells (10, 23). As in the case reported herein, these variants do not revert spontaneously. The extent to which the CHO adenosine kinase variants and L cell APRT mutants are homologous remains to be determined.

Thus, our data are consistent with either a "hot-spot(s)," some type of locus-specific "mutator" gene, or an insertion sequence that specifically interacts with DNA at or near the APRT locus. Analysis of mutant APRT proteins and direct examination of mutant DNA should illuminate the situation.

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