Structural Alterations of the *aprt* Locus Induced by Deoxyribonucleoside Triphosphate Pool Imbalances in Chinese Hamster Ovary Cells

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Mutants induced at the adenine phosphoribosyl transferase (*aprt*) locus by dTTP or dCTP pool imbalances were examined for alterations in genomic DNA sequences. No observable changes were detected by Southern blot analysis of most mutant DNAs, suggesting induction of base pair alterations or other events below our level of detection (~30 base pairs). However, in a few strains (11 from a total collection of 125 mutant cell strains), we were able to localize these events to restriction endonuclease recognition sequences when the mutations resulted in the loss or gain of a particular site. The distribution of lost or gained sites in aprt-deficient mutants induced by the two types of pool imbalances clearly varied, with those occurring in a mutator strain with increased dCTP clustering at one end of the *aprt* gene. Mutants induced by dTTP also revealed novel events: multiple restriction site modifications in a small region of the *aprt* gene in one mutant and a small (~50 base pairs) insertion or duplication of DNA sequences. As in previous studies, very few deletion or insertion mutants were detected at the *aprt* locus. The significance of these findings in terms of the known biochemical and genetic consequences of these pool imbalances is discussed.

Imbalances of the pools of DNA synthetic precursors, the deoxyribonucleoside triphosphates, have profound genetic effects on cells (12, 17). Thymidylate starvation induces a *recA*-dependent, error-prone repair system in *Escherichia coli* (7) and recombination in yeast cells (13). Excess thymidylate induces mutations in phage (3) and yeast cells (2). In cultured mammalian cells, both thymidylate deprivation and excess produce gross genetic abnormalities—chromosome breaks, deletions, and sister chromatid exchanges (1, 12, 22, 24). Effects of pool imbalances on single-gene loci have also been reported in the form of increased mutational frequencies (5) or rates (16, 20, 29) and altered sensitivities to DNA-damaging agents (15, 23). Despite the wide range of events induced by deoxyribonucleoside triphosphate pool imbalances, the molecular mechanisms by which the events arise are unknown. In vitro DNA-synthesizing systems with either procaryotic or eucaryotic enzymes indicate that pool balance plays an important role in accurate base pairing (11) and, in procaryotes, "editing" activities (9). However, the contribution of these factors to the final effects observed in vivo (mutations and gross chromosomal abnormalities) is not clear.

In the experiments reported here, we made use of a cloned probe for a "selectable locus" to determine the molecular basis of mutations induced by imbalances of both dTTP (produced with excess exogenous thymidine) and dCTP (with somatic cell mutations; Table 1). The selectable locus investigated was that coding for adenine phosphoribosyl transferase (*aprt*). This locus is attractive for these analyses for a number of reasons. (i) Selection systems exist for both forward and reverse mutations (10). (ii) The gene is nonessential, so a wide range of mutations can be analyzed (deletions and insertions as well as point mutations [21]). (iii) Although the locus is autosomal, strains hemizygous for the locus have been identified (6, 21). (iv) The gene is small (a 3.8-kilobase [kb] fragment is sufficient to transform aprt⁻

cells to aprt⁺ in transfection experiments [14; I. Lowy, personal communication]), facilitating localization and analysis of mutational events.

The data presented here suggest that most mutants induced at the *aprt* locus by both types of pool imbalance are either point mutations or deletions or insertions below our level of resolution (about 30 base pairs), though the data indicate some difference in the distribution of the mutations over the *aprt* gene. Furthermore, two alterations detected among the mutants induced by high thymidine suggest novel mechanisms of mutation.

MATERIALS AND METHODS

The strains used in these experiments are described in Table 1. The Chinese hamster ovary wild-type strain, pro⁻, was obtained from Lou Siminovitch; D422, isolated from the same pro⁻ strain, was obtained from Ted Bradley (6). Cell strains were routinely maintained in suspension culture in α minimal essential medium (GIBCO; without ribonucleosides or deoxyribonucleosides) supplemented with 10 μM thymidine and 5% sera (2.5% [vol/vol] fetal bovine serum–2.5% [vol/vol] horse serum [GIBCO]). Two new mutant strains are reported here, D422 thy1-1 and 2-1. These strains were isolated from cultures of D422 mutagenized with 1.2 mM ethyl methanesulfonate and then (after allowing 5 days of expression time) plated in medium selective for the thy mutator phenotype (19). This medium contains 1 μM arabinosyl cytosine, 10 μM 5-fluorouracil, and 1 μM thymidine supplemented with 5% dialyzed sera. Colonies forming after 2 to 3 weeks were picked, transferred to nonselective medium, and screened for their growth dependence upon thymidine, resistance to arabinosyl cytosine and 5-fluorouracil, and mutational rates at independent genetic loci.

Mutant selections. Adenine phosphoribosyl transferase-deficient mutants were obtained by selecting for resistance to 8-azaadenine (aa) (10). Only 500,000 cells were plated per 100-mm dish in aa medium containing aa (0.4 mM), thymidine (10 μM), and 5% dialyzed sera. In some experiments,

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TABLE 1. Properties of Chinese hamster ovary strains used

Cell strain	Parental strain	Phenotype	References
pro⁻		Wild type	
D422	pro⁻	Hemizygous for aprt (+/0)	6, 21
thy⁻49	pro⁻	Arabinosyl cytosine, 5-fluorouracil, thymidine resistant; dCTP pool increased 5- to 10-fold; dependent upon thymidine (auxotrophic) for growth as a result of a mutation of CTP synthetase; mutator strain, 5- to 300-fold increased rate of mutation at certain loci	16, 19, 20, 28
D422 thy⁻2-1	D422	Selected for resistance to arabinosyl cytosine, 5-fluorouracil as strain above	

mutational frequencies or rates were also determined at other genetic loci. 6-Thioguanine-resistant mutants (6-tg^r, hypoxanthine guanine phosphoribosyl transferase [hprt] deficient) were selected in 10 μM 6-thioguanine, 10 μM thymidine, and 5% dialyzed sera. The frequency of ouabain-resistant mutants (Oua^R) was determined by plating in α medium containing 2 mM ouabain, 10 μM thymidine, and 5% dialyzed sera. Finally, the presence of emetine-resistant (emt^r) mutants was assayed by plating cell populations in 0.2 μM emetine, 10 μM thymidine, and 5% dialyzed sera.

Thymidine-induced mutants were produced by treating cultures with 10 mM thymidine in α medium and 5% dialyzed sera for 24 h. At that time, the medium was removed, the cultures were washed, and medium containing 10 μM thymidine with dialyzed fetal bovine-horse sera was added back. Cell survival after treatment by this protocol was ca. 30%. Cultures were maintained in nonselective medium for 5 to 6 days to allow expression of the mutant aprt phenotype before being plated on selective media to allow formation of mutant colonies. Only one aa^r mutant was picked from each independently treated culture for further screening, and sufficiently small inocula were used to preclude preexisting mutants.

TABLE 2. Induction of mutation at various loci by excess thymidine

Treatment	Relative survival	Frequency of mutation (10⁶)^a			
		6-tg^r	Oua^R	emt^r	aa^r
pro⁻					
None	1.0	3.6	0.86	0.17	<0.1
10 mM dT	0.30	105	12	2.1	1.0
dT + dC	1.0	2.5	0.2	0.2	
D422					
None	1.0	1.1			0.7
10 mM dT	0.3	63			22

^{a} The frequency of mutation is defined as follows: number of colonies growing in selective agent/(number of cells plated × the fraction-forming colonies in nonselective medium).

The effect of deoxycytidine on the frequency of thymidine-induced aprt mutants was also examined. The addition of 10 mM thymidine to cultures causes a large increase in the dTTP pool and, because dTTP is a negative effector of dCTP synthesis (8), a decrease in the dCTP pool (4). DNA synthetic rates decrease in parallel with the decline of the dCTP pool (4), ultimately resulting in cell death. The addition of deoxycytidine restores the dCTP pool to near-normal levels, allowing resumption of DNA synthesis and recovery of cell viability. To determine the effect of deoxycytidine on the frequency of mutations in thymidine-treated cultures, 0.1 mM deoxycytidine was added with the 10 mM thymidine for 24 h.

To determine mutational rates in thy⁻ strains as well as to isolate spontaneous aprt mutants, replica cultures inoculated with 100 cells each were grown to high density in α medium containing 4 μM thymidine and 5% dialyzed sera. Each replica (usually 1 × 10⁶ to 2 × 10⁶ cells) was then plated in the desired selective medium (aa, 6-thioguanine, or ouabain) as described above. After growth for 7 to 10 days, plates were fixed or stained, and mutational rates were calculated on the basis of the fraction of replica populations lacking mutant colonies (a Po calculation; 16, 20). For the collection of aprt mutants from thy⁻ strains, only one aa^r colony was picked from each positive culture.

The aa^r mutants isolated were maintained in nonselective medium for several weeks before being screened in aa (0.8 mM) and also in AAT media (100 μM adenine, 1 μM aminopterin, 10 μM thymidine) to check for revertants (10).

Extraction, restriction enzyme digestion, and Southern blot analysis of wild-type and mutant DNAs. High-molecular-weight DNA was prepared from cell pellets of each of the mutant strains as described in detail previously (21). DNAs were digested with the following restriction endonucleases or combinations: *Msp*I, *Taq*I, *Pst*I, *Pvu*II, *Xba*I-*Bam*HI, *Kpn*I-*Eco*RI, and *Eco*RV-*Hind*III under conditions described by the supplier (Bethesda Research Laboratories). Digested DNAs were usually fractionated on 1% (wt/vol) agarose gels and transferred to nitrocellulose filters (Schleicher and Schuell; BA85) as described by Southern (27). The hybridization of the nitrocellulose filter-bound DNA with labeled pHaprt probe DNA was performed as described earlier (18, 21). The probe used was the 3.8-kb *Bam*HI fragment.

Sequences coding for *aprt* were then visualized on hybridized and washed filters by autoradiography in X-ray cassettes at −70°C with Dupont Cronex Lightning-Plus screens and Kodak X-Omat AR film.

RESULTS

Isolation and nature of thymidine-induced mutants. Treatment of CHO cells with 10 mM thymidine significantly

TABLE 3. Spontaneous mutational rates in wild-type and thy⁻ strains

Cell strain	Rate of mutation (10⁸) to:^{a}		
	Oua^r	6-tg^r	aa^r
pro⁻	1.0	6.6	<1.0
thy⁻49	27	89	<1.0
D422	1.8	4.3	7.3
D422 thy⁻1-1		86	47
D422 thy⁻2-1	140	170	53

^{a} Mutational rates were determined by fluctuation tests and a Po calculation as described in the text.
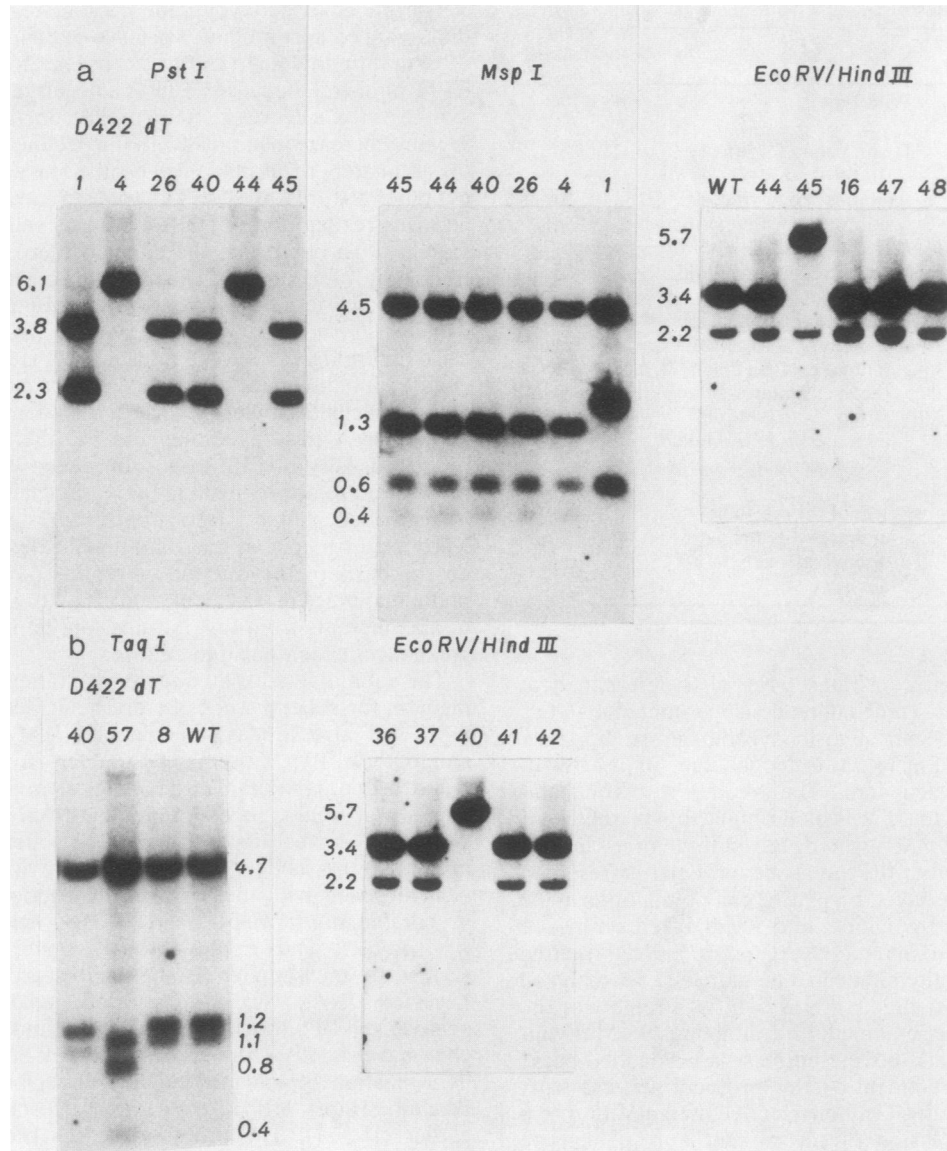
FIG. 1. Southern blot analysis of mutant *aprt* alleles in DNAs from wild-type (WT), D422, and thymidine-induced mutants of D422. (a) Mutants losing *Pst*I, *Msp*I, or *Hind*III sites; (b) two thymidine-induced mutants gaining sites and one losing the *Eco*RV site. Thymidine-induced aprt mutants are identified by the number above each lane. DNA (12 μg) from each cell strain was digested with the indicated restriction endonucleases and fractionated by electrophoresis on 1% agarose gels. DNA was denatured, transferred to nitrocellulose, and hybridized with a ^{32}P-labeled *Bam*HI fragment as the probe (see Fig. 2). Fragment sizes, indicated on the side of the blot (both in this figure and the following ones), were determined by comparison with *Hind*III-*Eco*RI digests of λ DNA run on the same gel.

increased the frequency of mutations at several genetic loci (Table 2). As previously reported by Bradley and Sharkey (5), this mutagenicity was relieved by the addition of deoxycytidine to the medium. Even though there are significant increases in mutational frequencies for all markers tested, thymidine is clearly not as potent a mutagen as equally cytotoxic doses of the DNA-alkylating agent ethyl methanesulfonate (15). To examine mutations induced by thymidine at the *aprt* locus, the frequency of these mutants in the diploid wild-type strain and the hemizygous D422 derivative was first determined. Surprisingly, aprt mutants could be detected in the diploid wild-type strain as well as D422, though the frequency was 20 to 30 times higher in the hemizygous strain (Table 2).

A collection of ca. 75 independent aprt mutants from both

wild-type and D422 strains was then made. All of the mutants were screened both in aa, to be sure the mutants were stable, and in AAT medium, to examine reversion frequencies (10). All mutants were stably resistant to aa, but the ability of the mutants to form colonies on AAT medium varied considerably. Roughly one-third of the strains derived from D422 or pro⁻ plated with high frequencies on AAT. Such mutants presumably have an enzyme which lacks affinity for aa but retains some affinity for adenine. AAT⁺ colonies were not detected in most of the remaining thymidine-induced aprt⁻ strains, except in four which produced revertant colonies at measurable frequencies (~10⁻⁶).

Isolation and properties of aprt mutants isolated from thy⁻ strains. Even though we have been unsuccessful in obtaining spontaneous aprt mutants from the wild-type pro⁻ strain,
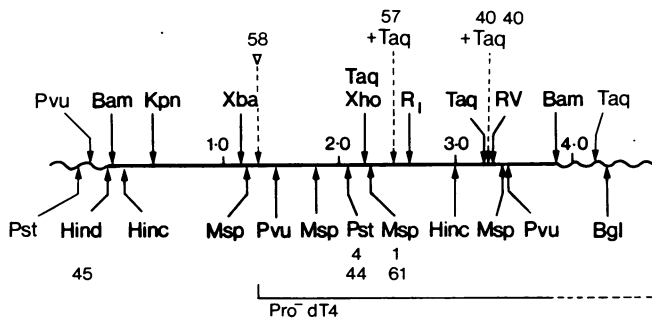
FIG. 2. Map of thymidine-induced mutations of the hamster genomic *aprt* locus localized by Southern blot analysis. Mutants losing a restriction endonuclease recognition site are indicated by the number of the mutant strain above the site. Mutants gaining sites are identified by broken arrows. The region of the gene deleted in pro⁻dT4 where it could be measured (i.e., within the region covered by our probe) is indicated by the line. The position of sequences inserted into the *aprt* gene of dT58 is indicated by a broken line. All mutant strains specified here were isolated from D422 unless otherwise indicated. Most restriction sites (filled arrows) are from Lowy et al. (14) and our own studies (18, 21). The straight line represents the fragment used as the probe in these experiments; the wavy line represents flanking genomic sequences. Restriction endonuclease sites are as follows: Hind, *Hind*III; Bam, *Bam*HI; Kpn, *Kpn*I; Hinc, *Hinc*II; Xba, *Xba*I; Msp, *Msp*I; Pvu, *Pvu*I; Pst, *Pst*I; Xho, *Xho*I; Taq, *Taq*I; RI, *Eco*RI; RV, *Eco*RV; and Bgl, *Bgl*II.

which is diploid for *aprt* (21), we attempted to isolate aprt mutants from pro⁻ thy⁻ mutator strains. Table 3 shows that these attempts were equally unsuccessful.

To overcome this limitation, we isolated thy⁻ derivatives of a strain hemizygous for *aprt*, D422. This was accomplished with the arabinosyl cytosine–5-fluorouracil selection, described previously (19) on ethyl methanesulfonate-mutagenized populations of D422. The frequency of the mutants was somewhat lower in D422 than in other pro⁻ strains. However, the properties of the strains were identical: stable low-level resistance to arabinosyl cytosine and 5-fluorouracil, thymidine auxotrophy with a high reversion frequency (10^{-4}) to thymidine prototrophy, and increased mutational rates at independent genetic loci (Table 3).

We then examined the effect of the mutator gene on the rate of mutation of the *aprt* locus in D422 thy⁻ strains. Rates were significantly increased, though the increases were not as great as those for the *hprt* or *oua* loci (Table 3). Several other D422 thy⁻ strains were isolated. None of these showed any further increase in mutational rates to aprt deficiency (J. Nalbantoglu, unpublished data).

A collection of ca. 50 independent aprt⁻ strains was produced with D422 thy⁻2-1 under conditions optimizing mutator activity (low concentrations of exogenous thymidine which gives the maximum increase in dCTP). Several of the mutants isolated were screened in aa and checked for their ability to form colonies in AAT in the same manner as for thymidine-induced mutants. All mutants remained stably resistant to aa, though some (5 of the 15 tested) were able to form colonies in AAT. AAT⁺ colonies were not detected in the rest of the mutants (frequency, $<10^{-6}$).

Southern blot analysis of thymidine-induced aprt⁻ strains. To examine structural alterations of *aprt* sequences occurring in these mutants, DNA was purified from each and digested with 10 restriction endonucleases either singly or in combination. After fractionation by agarose gel electrophoresis, *aprt*-containing fragments were visualized by Southern blotting and hybridization to a nonrepetitive portion of the

aprt gene (18). Most thymidine-induced aprt⁻ strains had no observable alterations of *aprt*-containing DNA fragments (Fig. 1). However, in a few of the digests of mutant DNAs—D422 dT1, 4, 44, 45, and 61—wild-type bands disappeared and new fragments of higher molecular weight appeared. The size of these new fragments was roughly equal to the sum of the fragments normally produced by cleavage with the restriction enzyme. Excess enzyme failed to restore the normal pattern, and other restriction endonuclease sites were unaffected, even though some of these sites map within 50 bp of the one affected. These observations indicated that the mutant *aprt* allele in question had lost that particular restriction enzyme site, presumably as a result of a mutation in the recognition sequence of the enzyme. Mutants D422 dT4 and 44 appear to have lost the *Pst*I site at map position 2.1, whereas D422 dT1 and 61 appear to have lost an *Msp*I site at map position 2.3. (Fig. 1a and 2; data for dT61 not shown). D422 dT45 lost a *Hind*III site at the very beginning of our map (position 0.0).

In another thymidine-induced mutant, the pattern of *aprt*-containing DNA fragments observed suggested the gain of a new restriction endonuclease site within the mutant allele (Fig. 1b). Mutant dT57 loses the 1.2-kb *Taq*I fragment, and new fragments of 0.8 and 0.4 kb appear. Evidence indicates that the new *Taq*I site maps approximately at position 2.5 (Fig. 2).

DNA fragments of mutant dT40 containing *aprt* were altered in a more complex manner. The *Eco*RV site at map position 3.5 was apparently lost, leading to the generation of a fragment ca. 5.7 kb in size from the 3.4 and 2.2-kb fragments usually produced in a *Hind*III-*Eco*RV double digest (Fig. 1b, 2, and 3). All other digestions of DNA from this mutant showed the normal pattern of fragments, with the exception of the *Taq*I digest which revealed an increase in mobility of the 1.1-kb fragment (Fig. 1b and 3). *Pvu*II-*Bgl*II and *Pvu*II-*Eco*RI double digests failed to show any similar alterations in the pattern of *aprt* fragments (Fig. 2 and 3). However, a *Taq*I-*Bam*HI double digest was altered, indicating that the *aprt* gene of dT40 had gained a new *Taq*I site ca. 50 bp from the original at position 3.3, very close to the lost *Eco*RV site. As these map sites are only approximate, the possibility existed that some small rearrangement of sequences of the *Eco*RV site (recognition sequence, GATATC) had given rise to the new *Taq*I site (TCGA). In that instance, one would predict that a *Taq*I-*Eco*RV double digest of wild-type DNA would produce a pattern similar to that generated by *Taq*I digestion of dT40 DNA. Southern blot analysis revealed that this was not the case, as a *Taq*I-*Eco*RV double digest of wild-type DNA produced a fragment 40 to 50 bp shorter than the mutant fragments and ca. 100 bp shorter than the wild-type 1.1-kb *Taq*I fragment (Fig. 3). This observation indicates that the lost *Eco*RV site is near, though distinct from, the gained *Taq*I site.

Evidence for rearrangements of *aprt* sequences in thymidine-induced mutants. Deletion of *aprt* sequences and insertions of sequences into the gene were rarely found in our collection of mutants. One arpt mutant (pro⁻dT4) obtained from the diploid wild-type strain appeared to have lost *aprt* sequences in both alleles. From the mobility of the fragments obtained upon digestion of the remaining sequences homologous to our probe, we were able to map one of the deleted alleles as beginning approximately at map position 1.3 and eliminating all other restriction sites covered by our probe to the right of this point (Fig. 2 and 4).

A small rearrangement of *aprt*-containing sequences was detected in *Pvu*II digests of DNA from mutant D422 dT58
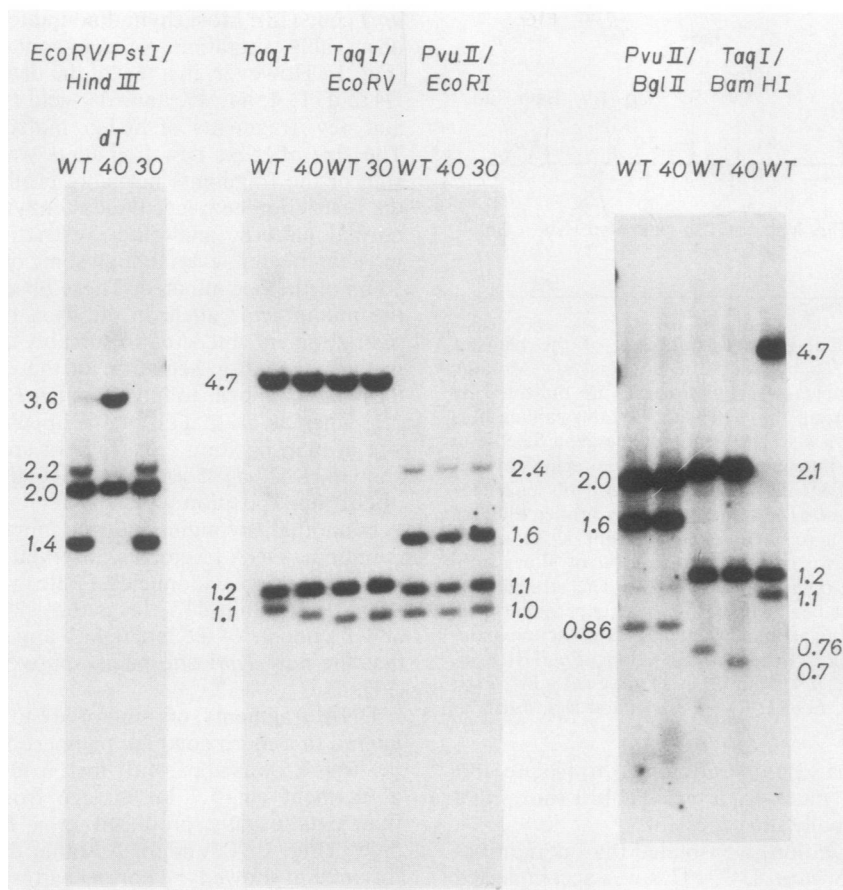
FIG. 3. Detailed Southern blot analysis of aprt mutant D422 dT40. DNAs from wild-type, dT40, and another thymidine-induced mutant were digested with the indicated restriction endonucleases or combinations. Fragment sizes were determined as described previously. The lane on the far right-hand side represents a *Taq*I digest (not indicated) of wild-type DNA for comparison with the *Taq*I-*Bam*HI double digest.

(Fig. 4). The 1.6-kb fragment obtained by *Pvu*II digestion increased slightly in size (ca. 40 to 50 bp). This increase was mapped to the *Xba*I-*Pvu*II fragment of the *aprt* locus (Fig. 2 and 4). No further changes were observed in other regions of the *aprt* gene of this mutant.

Southern blot analysis of mutants induced by the thy⁻ mutator gene (excess dCTP). DNAs purified from aprt mutants induced in thy⁻ strains were analyzed by restriction enzyme digestion and Southern blotting as described above. Most mutants showed no change in the pattern of *aprt*-containing sequences. However, one (thy 9) gave patterns suggesting the loss of a *Pvu*II site at position 3.5, and three others (thy 14, 22, and 53) had patterns of *aprt*-containing sequences which indicated the loss of the *Eco*RV site (position 3.4) (Fig. 5a, b, and 6). All other restriction enzyme digests of these four mutant DNAs gave normal patterns of *aprt*-containing fragments. In another mutant, thy 32, an increase in mobility of the 1.3-kb *Msp*I fragment of *aprt* was detected, indicating a reduction in size of ca. 50 to 100 bp (Fig. 5b). Digestions with other enzymes (e.g., *Taq*I) did not reveal similar alterations, suggesting the gain of a new *Msp*I site ca. 100 bp from one of the existing sites rather than a small deletion of *aprt*-containing sequences. Double digestion with *Msp*I-*Taq*I restored the wild-type pattern of fragments to thy 32, indicating that the new site maps between the *Taq*I and *Msp*I sites at position 3.3 (Fig. 5b and 6).

No deletions, insertions, or other rearrangements of *aprt*-containing sequences were detected among the 45 mutants isolated from D422 thy⁻ strains.

DISCUSSION

Our data suggest that the predominant type of mutation induced by dTTP or dCTP pool imbalances is either point mutation or rearrangement below our level of resolution (ca. 30 bp). In several mutants, such alterations were localized to restriction endonuclease recognition sequences, causing either loss of existing sites or generation of the new sites (Fig. 2 and 6), though the nucleotide changes occurring have not yet been defined. Among alterations which were localized in this manner, we noted a marked similarity between those lost in our collection of thymidine-induced aprt mutants (Fig. 2) and those lost in spontaneous or ethyl methanesulfonate-induced mutants (21). However, the distribution of mutants localized in the thy⁻ background (i.e., dCTP induced) appeared to be different (Fig. 6). These mutations clustered at one end of the *aprt* gene, and one of the sites lost (the *Pvu*II site at 3.5) was not lost in any other mutant. These observations could suggest a difference in the spectrum of mutations being induced by thy⁻ or variations in the mutability of specific regions of DNA by the mutator gene. If our latter interpretation is correct, it is possible that the locus-specific
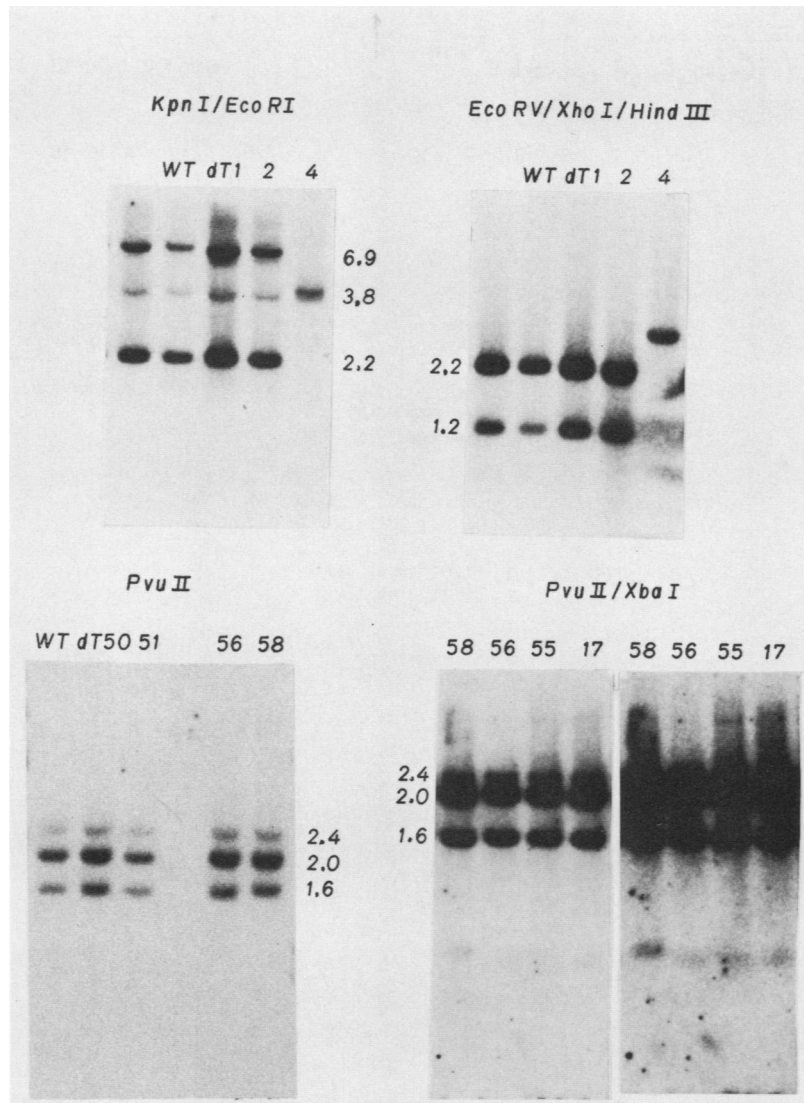
FIG. 4. Blot hybridization of DNAs from thymidine-induced mutants showing rearrangements of *aprt*-containing sequences. Mutant strains are indicated by the number above the lane. The digests in the upper two blots are from pro⁻ strains, and those in the lower two are from D422. In the lower right panel, two exposures of the same blot are presented; the one furthest to the right is a prolonged exposure to facilitate visualization of the small *Xba*I-*Pvu*II fragment (Fig. 2).

effects of thy⁻ (16, 20; Table 3) might relate to the presence of such "hot spots" in target regions of these loci.

The properties of one thymidine-induced mutant, dT40, suggest a novel mutational event as evidenced by two distinguishable alterations of restriction enzyme sites (one *Eco*RV site lost, one *Taq*I site gained) within a very small region of the *aprt* gene. Double digestions indicated that these changes are not due to the "conversion" of one site into another since the new *Taq*I site is ca. 40 to 50 bp from the lost *Eco*RV site. Our evidence argues against a deletion eliminating the *Eco*RV site and creating a *Taq*I site since several double digestions (producing small fragments in the affected region) failed to reveal any fragment with an alteration similar to that in the *Taq*I digest. An inversion event in the region is also unlikely, as we did not observe an increase in size of the 1.2-kb *Taq*I fragment corresponding to the decrease found in the 1.1-kb fragment. Thus, the most likely explanation of our data is that both changes were the result of separate nucleotide alterations. As nucleotide changes

resulting in gains or losses of restriction enzyme sites would be expected to be uncommon, there may well be numerous other alterations within this region of the *aprt* gene of dT40, although the neighboring *Taq*I, *Msp*I, and *Pvu*II sites are apparently unaffected. The occurrence of these two neighboring events is significant in view of our knowledge of the biochemical effects of high thymidine—that is, retardation but not complete arrest of the DNA replication fork due to deprivation of dCTP (4, 26). It is possible that the replication of DNA under such restrictive conditions is highly inaccurate, at least for some regions of the genome, leading to intense localized clusters of mutations. Certainly the other thymidine-induced mutations we have localized will be useful to determine the ubiquity of this effect.

Also deserving comment is mutant dT45, which lost the *Hind*III site at position 0.0 of our map. This position is supposed to be downstream from the *aprt* structural gene, as the 3.8-kb *Bam*HI fragment (beginning at 0.1 [14]) codes for sufficient information to transform aprt⁻ cells to the aprt⁺
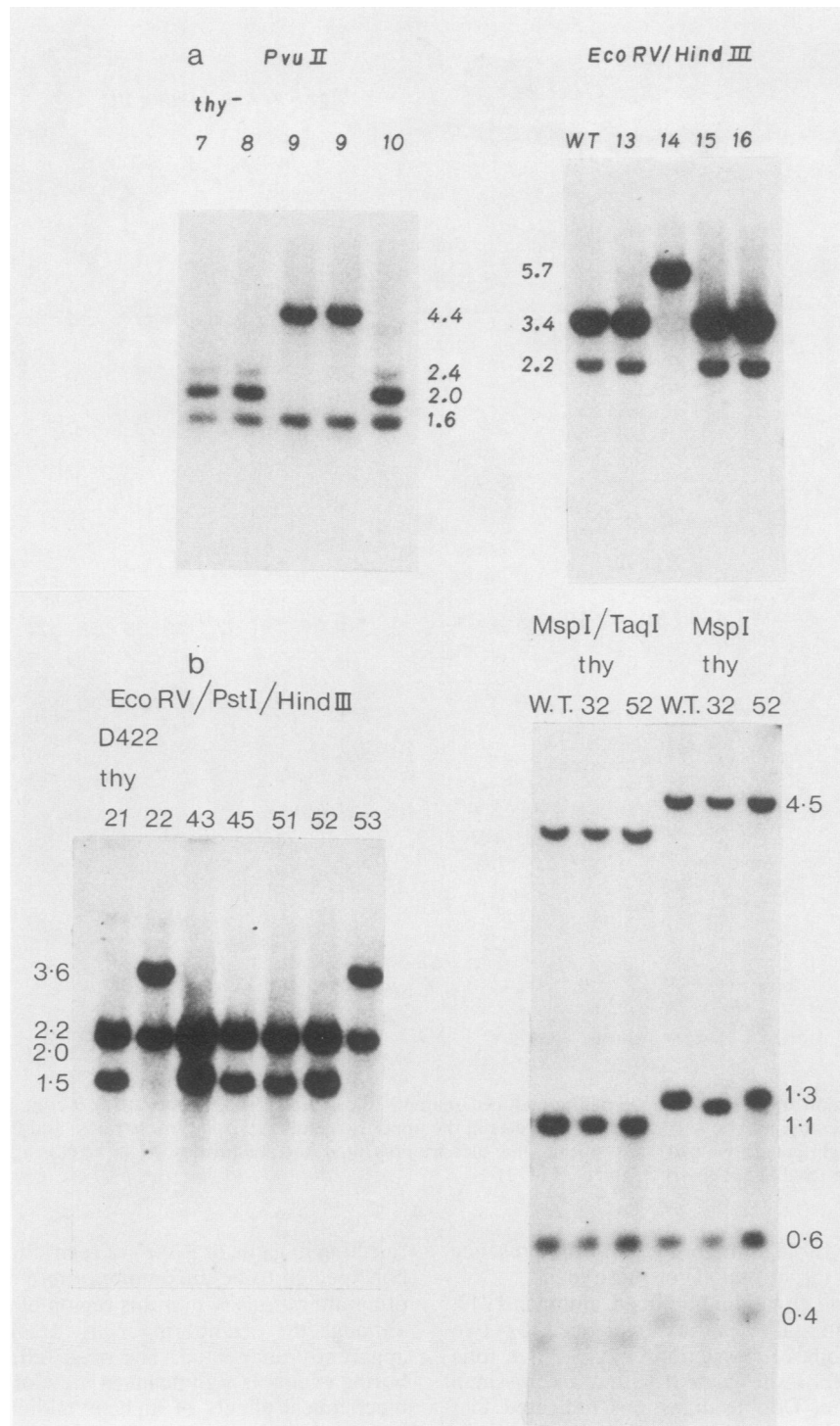
FIG. 5. Southern blot analysis of DNAs from aprt mutants induced in thy⁻ strains. aprt⁻ strains from D422 thy2-1 are identified by the number above each lane. In 5a, mutant thy 9 was digested with both the usual amount (1.5 U/μg DNA) and excess PvuII (3.0 U/μg).

state (I. Lowy, personal communication). It is possible that the alteration observed is not responsible for the mutant phenotype but is just one of many other alterations within the aprt region as discussed above. On the other hand, downstream sequences have been shown to influence gene expression (25). No other alterations of restriction endonu-

clease sites outside the aprt structural gene have been observed in our studies (18, 21).

Rearrangements of aprt-containing sequences were uncommon in our collection of mutants induced by pool imbalances. Only 2 were detected among 120 mutants screened. This frequency is significantly lower than that of
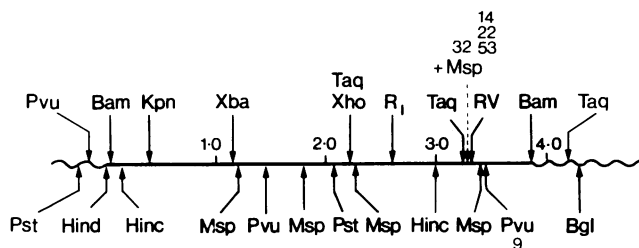
FIG. 6. Map of aprt mutants of D422 thy⁻2-1 localized by loss or gain of restriction endonuclease sites. Mutant strains with altered sites are indicated by the number of that strain above the site. (See the legend to Fig. 2 for further details concerning the map of the restriction endonuclease sites in the genomic DNA fragment coding for hamster aprt.)

our collection of spontaneous mutants (9 of 120). It seems quite likely, then, that the chromosome breakage and deletions observed among metaphase chromosomes of thymidine-treated cells (1, 12, 24) may contribute to cell lethality though not significantly to mutagenesis. Interestingly, one mutant was found which gained sequences in the region covered by our probe. Mutant dT58 gained ca. 50 bp in the region thought to include the 3' end of the *aprt* gene. It cannot yet be determined whether these represent novel sequences or a duplicated region of the *aprt* gene. The cloning and sequencing of this mutant *aprt* gene, as well as others in which mutations have been localized by lost or gained restriction sites, will elucidate the precise nucleotide base-pair changes occurring. Once these alterations have been defined, significant information concerning the cellular control of the accuracy of DNA replication, and the mechanisms by which dNTP pools alter these controls, will be gained.

ACKNOWLEDGMENTS

LITERATURE CITED

1. Anderson, D., C. R. Richardson, and P. J. Davies. 1981. The genotoxic potential of bases and nucleosides. Mutat. Res. 91:265–272.
2. Barclay, B. J., and J. G. Little. 1981. Mutation induction in yeast by deoxythymidine monophosphate: a model. Mol. Gen. Genet. 181:279–281.
3. Bernstein, C., H. Bernstein, S. Mufti, and B. Strom. 1972. Stimulation of mutation in phage T4 by lesions in gene 32 and by thymidine imbalance. Mutat. Res. 16:113–119.
4. Bjursell, G., and P. Reichard. 1973. Effects of thymidine on deoxyribonucleoside triphosphate pools and deoxyribonucleic acid synthesis in Chinese hamster ovary cells. J. Biol. Chem. 248:3904–3909.
5. Bradley, M. O., and N. A. Sharkey. 1978. Mutagenicity of thymidine to cultured Chinese hamster cells. Nature (London) 274:607–608.
6. Bradley, W. E. C., and D. Letovanec. 1982. High frequency non-random mutational event at the adenine phosphoribosyl transferase (aprt) locus of sib-selected CHO variants heterozygous for arpt. Somat. Cell Genet. 8:51–56.
7. Bridges, B. A., J. Law, and R. J. Munson. 1968. Mutagenesis in *Escherichia coli*. II. Evidence for a common pathway for mutagenesis by ultraviolet light, ionizing radiation and thymine deprivation. Mol. Gen. Genet. 103:266–273.
8. Ericksson, S., L. Thelander, and M. Akerman. 1979. Allosteric regulation of calf thymus ribonucleoside diphosphate reductase. Biochemistry 18:2948–2952.
9. Fersht, A. R. 1979. Fidelity of replication of phage φX174 DNA by DNA polymerase III holoenzyme: spontaneous mutation by misincorporation. Proc. Natl. Acad. Sci. U.S.A. 76:4946–4950.
10. Jones, G. E., and P. A. Sargent. 1974. Mutants of cultured Chinese hamster cells deficient in adenine phosphoribosyl transferase. Cell 2:43–54.
11. Kunkel, T. A., and L. A. Loeb. 1979. On the fidelity of DNA replication: effect of divalent metal ion activators and deoxyribonucleoside triphosphate pools on *in vitro* mutagenesis. J. Biol. Chem. 254:5718–5725.
12. Kunz, B. A. 1982. Genetic effects of doxyribonucleotide pool imbalances. Environ. Mutagene. 4:695–725.
13. Kunz, B. A., B. J. Barclay, J. C. Game, J. G. Little, and R. H. Haynes. 1980. Induction of mitotic recombination in yeast by starvation for thymine nucleotides. Proc. Natl. Acad. Sci. U.S.A. 77:6057–6061.
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. Meuth, M. 1981. Role of deoxynucleoside triphosphate pools in the cytotoxic and mutagenic effects of DNA alkylating agents. Somat. Cell Genet. 7:89–102.
16. Meuth, M. 1981. Sensitivity of a mutator gene in Chinese hamster ovary cells to deoxynucleoside triphosphate pool alterations. Mol. Cell. Biol. 1:652–660.
17. Meuth, M. 1984. The genetic consequences of nucleotide precursor pool imbalance in mammalian cells. Mutat. Res. 126:107–112.
18. Meuth, M., and J. E. Arrand. 1982. Alterations of gene structure in ethyl methane sulfonate-induced mutants of mammalian cells. Mol. Cell. Biol. 2:1459–1462.
19. Meuth, M., O. Goncalves, and P. Thom. 1982. A selection system specific for the thy⁻ mutator phenotype. Somat. Cell Genet. 8:423–430.
20. Meuth, M., N. L'Hereux-Huard, and M. Trudel. 1979. Characterization of a mutator gene in Chinese hamster ovary cells. Proc. Natl. Acad. Sci. U.S.A. 76:6505–6509.
21. 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.
22. Perry, P. E. 1983. Induction of sister chromatid exchanges (SCES) by thymidine and the potentiation of mutagen induced SCEs in Chinese hamster ovary cells. Mutat. Res. 109:219–230.
23. Peterson, A. R., J. R. Landoph, H. Peterson, and C. Heidelberger. 1978. Mutagenesis of Chinese hamster cells is facilitated by thymidine and deoxycytidine. Nature (London) 276:508–510.
24. Potter, C. G. 1971. Induction of polyploidy by concentrated thymidine. Exp. Cell Res. 68:442–448.
25. Queen, C., and D. Baltimore. 1983. Immunoglobulin gene transcription is activated by downstream sequence elements. Cell 33:741–478.
26. Reichard, P. 1978. From deoxynucleotides to DNA synthesis. Fed. Proc. 37:9–14.
27. Southern, E. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503–517.
28. Trudel, M., T. Van Genechten, and M. Meuth. 1984. Biochemical characterization of the thy mutator gene and its revertants. J. Biol. Chem. 259:2355–2359.
29. Weinberg, G., B. Ullman, and D. W. Martin, Jr. 1981. Mutator phenotypes in mammalian cell mutants with distinct biochemical defects and abnormal deoxyribonucleoside triphosphate pools. Proc. Natl. Acad. Sci. U.S.A. 78:2447–2451.