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Molecular Characterization of 15 Rearrangements among 90 Human In Vivo Somatic Mutants Shows that Deletions Predominate

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Ninety hypoxanthine phosphoribosyltransferase-deficient mutants were isolated from lymphocytes of 31 individuals drawn from both control populations and populations exposed to low doses of ionizing radiation. Southern analysis of the DNA revealed altered hybridization patterns in 15 mutants. Of these, 14 changes consisted of deletions of 2 to 40 kilobases or more.

Recent advances in T-lymphocyte cloning procedures have led to the development of methods to estimate in vivo human mutation frequencies (1, 12). These techniques have been used to measure the effect of ionizing radiation on mutation frequency in groups of individuals exposed to high (10) and low (11) doses of low linear energy transfer radiation, and an effect was observed at both levels. This led to an estimate for the rate of radiation-induced mutation, a value which previously could only be guessed at by a variety of indirect measures of genetic damage. The lymphocyte cloning procedure can be further exploited since many of the mutant colonies isolated have sufficient growth potential to yield mass cultures suitable for biochemical or molecular analysis (2, 17). Therefore, the possibility exists of establishing in vivo mutational spectra for different genotoxic agents (4–6, 13, 18).

In the course of our previous work, many 6-thioguanine (6-TG)-resistant mutant lymphocytes were isolated from subjects differing with respect to radiation exposure, as well as age, sex, and health status. We have begun a systematic analysis of the hypoxanthine phosphoribosyltransferase (*hprt*) gene structure of these mutants and present here results comparing 90 mutants from 31 individuals. These individuals included seven nuclear medicine patients (Messing, Bradley, and Seifert, Mutat. Res., in press) and 17 female technicians (11) from the nuclear medicine and radiotherapy departments of Notre-Dame Hospital. The control population consisted of one female and six male unexposed hospital workers, with an average age of 30 years. From these subjects more than 600 mutants were cloned, and of these, 250 were transferred into wells of increasing capacity in order to generate mass cultures. Of these, 90 mutants yielded sufficient DNA for analysis. Southern blots were prepared according to standard procedures (9, 16) and probed with insertions from plasmids pHPT5 (8) and pHPT31 (3), kindly provided by C. T. Caskey.

The analysis of the Southern blot data depends on the work of Caskey and colleagues (14, 15, 19), who have mapped the exons of human *hprt* and assigned them to individual *Pst*I fragments. A map is shown in Fig. 1, which also shows the conclusions from the analyses of all of the mutants included in this study. Recent mapping data (14) have also assigned the exons to the various fragments generated by digestion with *Eco*RI, *Hin*dIII, and *Bam*HI.

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Most of the control group were male, and the hemizygosity of the X-linked *hprt* gene facilitated analysis of these mutants. The wild-type *Pst*I pattern (19) was observed in most of the 30 mutants from controls (designated mutants 61 through 90). Six mutants, all from males, carried detectable alterations (Fig. 2 and Table 1). Mutant 62, isolated from individual C1, and mutant 81 (data not shown), isolated from individual C3, had virtually identical patterns without any of the exon bands but with a new band visible at about 6.0 kilobases (kb). The simplest interpretation is that a deletion occurred in each mutant including exons 2 through 6, but leaving all or part of either extremity of the gene (exon 1 or the exon 7-8-9 region), and probably extending past the gene in one direction. Analogous reasoning led us to conclude that mutants 72, 79, and 83 had deletions of exons 1 through 3, 5 through 9, and 6, respectively. Mutant 85 was identical to another clone from the same individual, so probably did not represent an independent event.

DNAs from mutants isolated from the technicians were digested with *Eco*RI, *Hin*dIII, and *Bam*HI, as well as with *Pst*I. As with the controls, the established wild-type pattern was seen in most mutants. A striking variation in intensity ratio between bands corresponding to *hprt* and pseudogene sequences was evident (this was most apparent when we compared *Hin*dIII bands at 7.0 and 7.8 kb [Fig. 3]) and correlated with the expected X-chromosome dosage difference between males and females. New bands or lost bands or both occurred in 4 of 36 mutants from technicians and in 5 of 24 mutants from patients (Table 1). Mutant 1, from female radiotherapy technician RTT1, yielded a new *Hin*dIII band at 4.4 kb (Fig. 3); this most likely resulted from a change in the band at 7.0 kb since it was the same intensity as the pseudogene at 7.8 kb. The *Pst*I digestion mixture (data not shown) showed about one-half the relative intensity in the band corresponding to exon 2. The simplest interpretation is that a 2.6-kb deletion incorporating exon 2 occurred. The *Eco*RI-generated pattern is in complete agreement with this conclusion.

By similar reasoning, we were able to conclude that mutant 11 had a deletion which was larger than 3 kb and included exons 2 and 3. Mutant 39 also had suffered a deletion event, about 5 kb long, which eliminated exon 2 and sequences upstream, generating new *Eco*RI and *Hin*dIII fragments that were the sizes shown in Fig. 3. Mutant 54 (data not shown) was altered such that exon 4 was deleted.

Mutants 14 and 32 (Fig. 3) each produced new *Hin*dIII bands at about 15 to 16 kb, which were generated from the

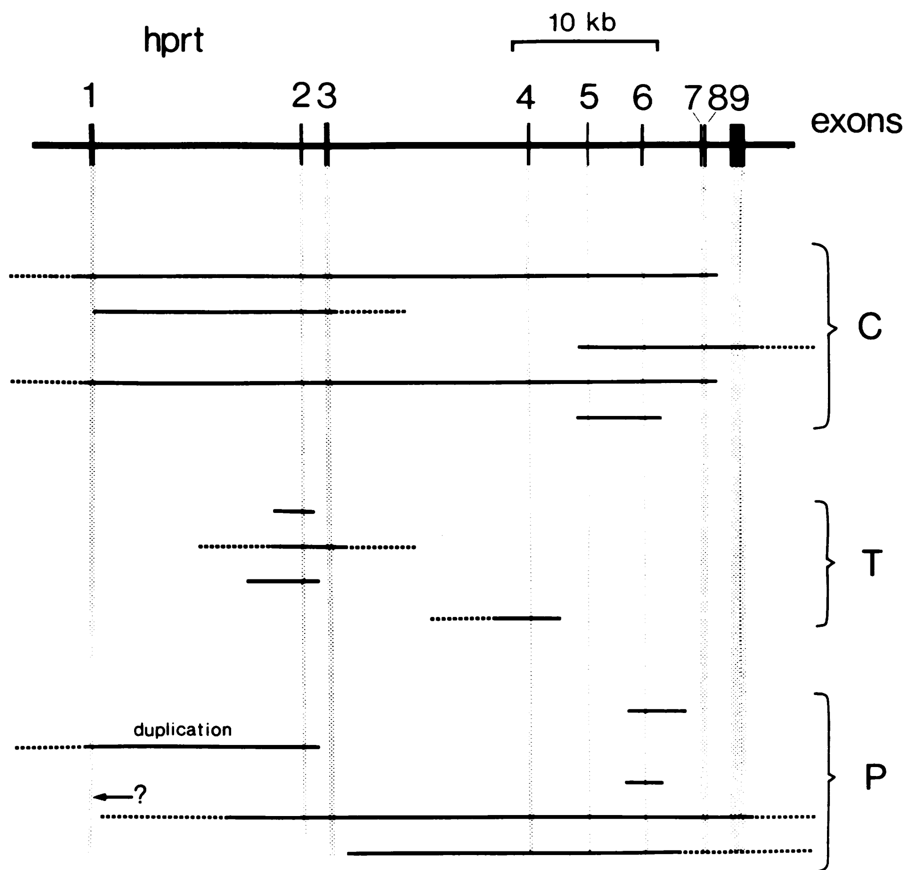

FIG. 1. Summary of detectable *hprt* alterations in 14 independent 6-TG-resistant mutant lymphocytes from the control group (C), technicians (T), and patients (P). The gene structure showing the nine exons (top) is taken from reference 14. The order of the mutants, from the top, is 62, 72, 79, 81, 83; 1, 11, 39, 54; and 32, 49 (unexposed), 14, 43, and 48 (exposed). Solid horizontal lines represent the approximate extents of deleted sequences, with the exception of mutant 49, which is a duplication. The hybridizing ends of mutants 62, 72, and 81 may be at the opposite ends from what is depicted. Deletions 79 and 83 are each portrayed as including exon 5, although this is not certain. Uncertainty of more than ±2 kb in the endpoint of the deletion is indicated by dotted lines. The arrow and question mark indicate that the exon 1-specific probe was not used, so no information is available concerning whether this deletion includes the 5′ end of the gene.

17.5-kb normal band (in the case of mutant 14, from a female, a relative intensity decrease occurred at 17.5 kb). For mutant 14 the *Eco*RI blot pattern showed reduced intensity in the 8.0- and 10.5-kb bands and appearance of a new band at 16 kb, so the deletion had removed the *Eco*RI site close to exon 6 which defined the 10.5- and 8.0-kb fragments. *Pst*I digestion (data not shown) confirmed this conclusion and also indicated that the small deletion in mutant 32 also involved exon 6.

Patient NMP2, from whom mutant 14 was obtained, generated three other rearranged mutants (data not shown). The mutant 43 had undergone a complete deletion of all detectable coding sequences, since no new bands were visible, but all of the pseudogene bands had twice the relative hybridization intensities as in the standard female pattern. The mutation occurring in clone 48 probably was a deletion starting downstream from exon 3 and including all coding sequences to the right, but this conclusion is not in complete accordance with all of the mapping data. Thus, some complex rearrangement may have occurred. Mutant 49 was unusual in that the only changes were increased hybridization intensity to *Pst*I fragments carrying exons 1 and 2. Thus, a duplication of sequences carrying these exons probably occurred.

The 14 independent rearrangements which we identified among 90 6-TG-resistant mutants are shown in Fig. 1. All but one are deletions, and for all but three (mutants 32, 48, and 49) the simplest possible interpretation is entirely consistent with all of our data and also with known mapping data. In all groups studied the incidence of detectable rearrangements was less than one-half of the mutants isolated, and the overall incidence was less than 20%. This compares with frequencies of about 30 to 50% found by other workers (2, 17), although the ages and health status of donors in these smaller surveys were not completely specified. An additional discrepancy which we cannot explain is the frequency of amplification of individual exons which in one study (17) appeared to be the predominant event.

TABLE 1. Summary of origin and characteristics of mutants

Donor individual[a]	No. of mutants	Rearranged mutants	Nature of rearrangement
C1	3	62	Deletion, >38 kb
C3	16	72	Deletion, >15 kb
		81	Deletion, >38 kb
		83, 85	Deletion, <11 kb
C5	3	79	Deletion, >14 kb
Four other controls	8	None	
RTT1	3	1	Deletion, 2.6 kb
RTT2	1	54	Deletion, <12 kb
Nine other RRTs	15	None	
NMT1	6	11	Deletion, ≥3 kb
NMT2	2	39	Deletion, 5 kb
Four other NMTs	9	None	
NMP1 (b)	1	32	Deletion, ca. 5-10 kb (complex)[b]
NMP2 (b)	5	49	Duplication (complex)[b]
Five other NMP (b)	9	None	
NMP1 (a)	1	None	
NMP2 (a)	4	14	Deletion, 2.5 kb
		43	Deletion, >27 kb
		48	Deletion, >25 kb (complex)[b]
Five other NMP (a)	4	None	

[a] C, Control; RTT, radiotherapy technician; NMT, nuclear medicine technician; NMP, nuclear medicine patient; (b), before radiation exposure; (a), after exposure.

[b] Complex means that all of the blot data were not consistent.

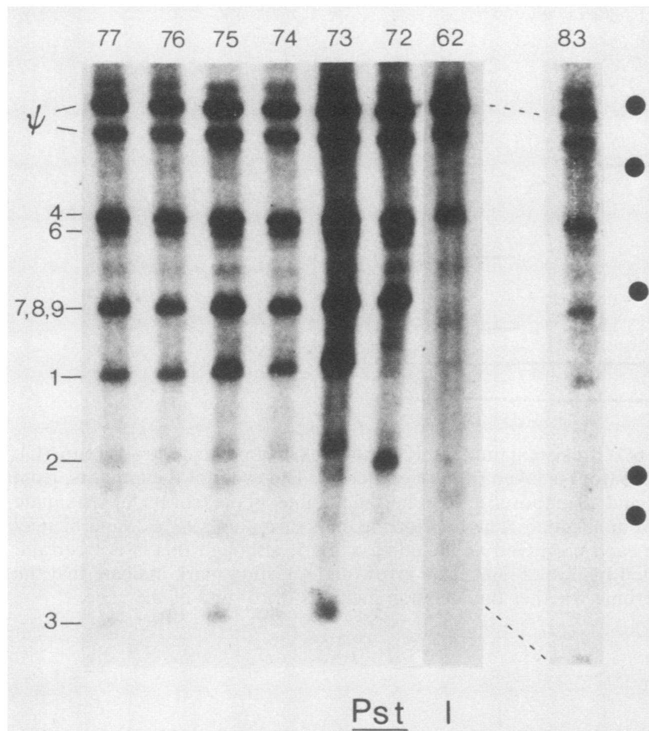

FIG. 2. Southern blot analyses of representative mutants among the 6-TG-resistant clones isolated from the control male individuals. Lymphocytes were isolated as described previously (10) and grown to 10^7 cells, and DNA was extracted. Samples were digested with *Pst*I, size fractionated in a 0.8% agarose gel, transferred to nitrocellulose, and probed under stringent conditions with the *Pst*I insertion of pHPT31. Dots on the right and in Fig. 3 indicate λ *Hin*dIII-cleaved markers at 23.1 (not included in this figure), 9.4, 6.6, 4.4, 2.3, and 2.0 kb (from the top). Bands corresponding to pseudogene sequences are identified (ψ). Numbers on the left identify the exons of *hprt* which are carried by the *Pst*I fragments (19).

Molecular analysis of in vivo mutants can be useful for a variety of purposes. At a fundamental level, the demonstration that some 6-TG-resistant lymphocytes have major rearrangements in their *hprt* gene suggests a genetic basis for the drug resistance and gives confidence that the selected lymphocyte clones are, indeed, mutants. In addition, our results and those of other workers (2, 17) show that in vivo mutation does indeed occur in nongerm cells at measurable frequencies. This is perhaps not surprising, but nevertheless had not been directly demonstrated before the development of the T-cell cloning procedure. Also of fundamental interest are the relative frequencies of the different categories of events, such as amplifications, deletions, or point mutations, since this information may contribute to an understanding of the nature of in vivo mutation and repair.

At a more applied level, analysis of many mutants can lead to establishing mutational spectra which may be of value in comparing groups of individuals or classes of toxic environmental agents. This approach has been exploited with bacterial (7) and mammalian cells (13, 18) in vitro, and our intention is to establish an analogous in vivo system. This initial survey was designed to test the feasibility of such a

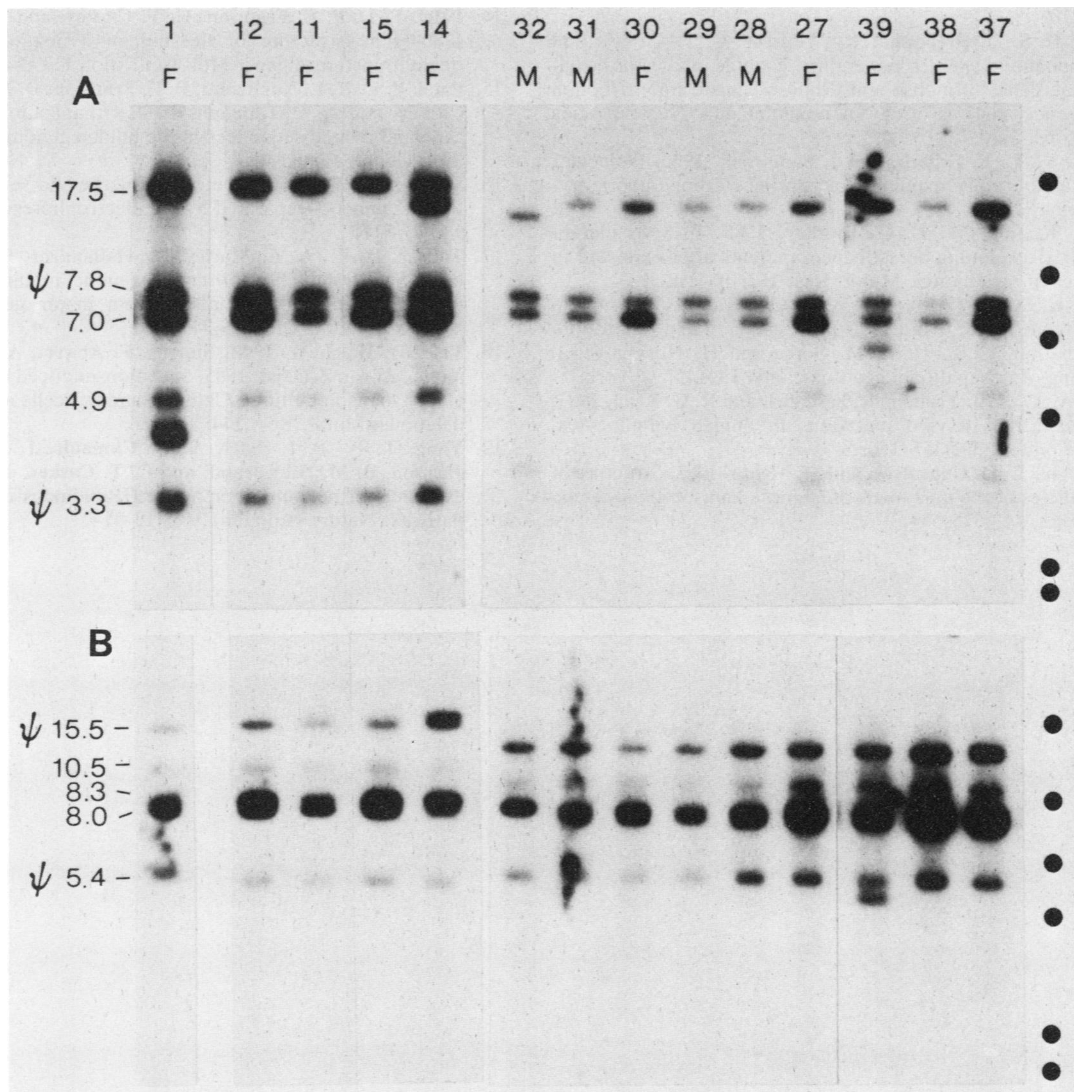

FIG. 3. Southern analyses of 14 mutants (F, female donors; M, male donors) after digestion with either *Hin*dIII (A) or *Eco*RI (B). The conditions used were the same as those described in the legend to Fig. 1, except that the blots were probed with the insertion from pHPT5, (8), which does not hybridize with exon 1.

system. Our choice of subjects was influenced to a large degree by factors such as availability and technical ease of analysis, since we had no prior knowledge of the overall frequency of rearrangements in different groups of individuals. Therefore, although statistically valid comparisons among the groups are not possible with our present results, sufficient information was obtained to suggest future experimental design.

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