Ala-Cys-Gly sequence. The homologous region becomes a heptapeptide by inclusion of the amino acid residues in the box in Fig. 1. In every case the amino acid replacements in the heptapeptides are possible by a single base change in a codon and are those found most frequently in families of homologous proteins (*22*). We were struck to discover such related peptides at the outset of our sequencing, covering only 56 residues in the two enzymes. No homology of similar extent exists among any of the other cysteine-containing peptides in Fig. 1, showing all those so far published for any aminoacyl-tRNA synthetase (*23, 24*). Since all of the cysteine-containing tryptic peptides of *E. coli* isoleucyl-tRNA synthetase are known, the lack of homology with any cysteine sequence in that enzyme is certain.

The homologous region may be more extensive. If the proline residue next to the COOH-terminus in peptide IA91 has been deleted during evolution of peptide TC2, then the COOH-terminal lysine residues of peptides IA91 and TC2 correspond, and the homologous region may extend from these lysine residues. Moreover, the homologous region may extend from the NH_2-terminals, since the isoleucine residue next to the NH_2-terminus of peptide TC2 may represent a replacement allowable by a single base change in a codon of lysine, which may be the COOH-terminal residue of the tryptic peptide preceding IA91. The ultimate extent and role of the homologous region and whether other cognate series of aminoacyl-tRNA synthetases have homologies remain to be determined.

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21. The abbreviations for the amino acid sequences are as follows: Ala, alanine; Arg, arginine; Asp, aspartic acid; Asn, asparagine; Asx, aspartic acid or asparagine; Cys, cysteine; Glu, glutamic acid; Gln, glutamine; Glx, glutamic acid or glutamine; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Trp, tryptophan; Tyr, tyrosine.
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Chromosome-Wide Event Accompanies the Expression of Recessive Mutations in Tetraploid Cells

Abstract. *Mutants resistant to 6-thioguanine were induced in pseudotetraploid hybrid Chinese hamster cells homozygous wild-type at the locus for hypoxanthine phophoribosyl transferase but heterozygous for the linked marker glucose-6-phosphate dehydrogenase. About half of these mutants had concomitantly lost the wild-type allele for glucose-6-phosphate dehydrogenase, as expected if mutation plus chromosome segregation had occurred.*

The interpretation that variant clones of cultured animal cells arise from mutations is complicated by the fact that the cells of higher organisms can also give rise to somatically heritable phenotypic changes by epigenetic processes. The latter are presumed to underlie cellular differentiation. If mutation in cultured cells is to be studied as a phenomenon per se, and if it is to be used as a tool to illuminate cell function, it is important to be able to distinguish mutation from epigenetic processes in any particular system.

There are now several systems in which the demonstration of an altered gene product in variant cells provides strong evidence for a mutational event (*1*). In most of these systems, a constitutive and ubiquitous enzyme has been studied, which makes it less likely that variants would arise epigenetically. However, in several of the same or similar systems, predictions of variant frequencies as a function of gene dosage, made on the assumption that purely mutational events were occurring, have not always been borne out. Thus Harris (*2*) found no difference in the spontaneous mutation rate to 8-azaguanine resistance in diploid, tetraploid, and octaploid Chinese hamster cells, and Mezger-Freed (*3*) found no difference between haploid and diploid frog cells in the frequency of 5-bromodeoxyuridine–resistant variants. Resistance to each of these drugs is usually associated with a recessive loss in enzyme activity (*4–6*), although this point was not tested in the experiments cited. In the case of recessive mutations, it should be necessary to mutate each copy of the gene in question before a mutant phenotype can be expressed and recognized. If each mutation is an independent event, the mutant frequency should be an exponential function of the gene dosage; for example, the frequency with two genes should be approximately the square of the frequency in the single gene case. The failure of this prediction has led to the idea that mutation is not operating in these systems (*2, 3*).

We have previously examined this question, comparing mutation frequencies induced by ethyl methanesulfonate (EMS) in (pseudo) diploid and tetraploid Chinese hamster ovary (CHO) cells (*7*). Mutants resistant to 6-thioguanine (TG) were induced at a frequency of 2.5×10^{-4} in diploid cells, while in tetraploids the figure was 0.9×10^{-5}. Drug resistance was shown to be recessive and associated with the loss of hypoxanthine phosphoribosyltransferase (HPRT) activity. Since the gene specifying this enzyme is X-linked in humans (*8*) and probably also in Chinese hamster cells (*9, 10*) there is presumably only one active gene in diploid cells and two active genes in tetraploids (*11*). While the 25-fold difference was highly

Table 1. Distribution of G6PD phenotypes among TG-resistant mutant colonies derived from a tetraploid hybrid clone. The hybrid clone Y143 was induced to mutate with EMS and TG-resistant cells selected as previously described (*7*). Colonies that grew in TG were stained directly for G6PD activity (*10*).

Experiment	Frequency of TG-resistant colonies	Colonies (No.)	
		$G6PD^+$	$G6PD^-$
1	5×10^{-6}	40	59
2	1.1×10^{-6}	19	20
3	4.2×10^{-6}	53	71

significant, the mutant frequency in the tetraploids was still about 50 times higher than that expected on the basis of two independently occurring gene mutations. Rather than invoking an epigenetic explanation, we proposed a mechanism combining the spontaneous loss of one chromosome bearing an HPRT gene with mutation of the single remaining gene (*7*). The numerical results were consistent with such an interpretation based on segregation rates of the $HPRT^+$ (TG-sensitive) phenotype from heterozygous tetraploid hybrid cells (*12*).

This explanation leads to at least three predictions: (i) TG-resistant mutants derived from tetraploid cells should all have lost one particular chromosome; (ii) since spontaneous chromosome loss is constantly occurring, segregants should accumulate with time, and the induced mutation rate should increase with clonal age; and (iii) markers linked to the HPRT locus should also have been segregated in mutants derived from tetraploids. The first prediction has been difficult to test, since most of these mutants have lost one to five chromosomes, and even within a clone the distribution of chromosome numbers in tetraploids is not as narrow as that found in diploid CHO cells (*7*).

The second point was examined by cloning a tetraploid subline of CHO cells that had been produced by treatment of the diploid with low doses of Colcemid (*7, 13*). The clonal population was grown in nonselective medium, and the EMS-induced mutation rate was measured at intervals over a period of 3 months. As can be seen in Fig. 1, the general trend (with the exception of one point) indicates increased mutability with clonal age. Although this result is in agreement with the segregation plus mutation hypothesis it does not represent strong evidence in its favor, as almost any proposal involving a spontaneous change in a cell as a prerequisite for mutability would predict such a time-dependent increase.

The third prediction is a much more stringent one, and can be tested by exploiting a recently isolated CHO mutant lacking glucose-6-phosphate dehydrogenase (G6PD) activity (*10*). The wild-type alleles of G6PD and HPRT are linked in CHO cells, since they cosegregate from heterozygous hybrid cells more than 92 percent of the time (*10*). If the segregation of one chromosome bearing the HPRT gene is almost always involved in the generation of TG-resistant ($HPRT^-$) mutants from tetraploid cells, then segregation of the linked G6PD locus should also occur. This prediction can be tested by using as a tetraploid cell a hybrid heterozygous for G6PD and homozygous wild type for HPRT. Such a hybrid was therefore constructed, the parental lines being two mutant subclones of CHO cells, strain 43-64 ($glyB^-$) and strain Y113 ($glyA^-$ $G6PD^-$) (*10*). Hybrids were selected on the basis of the complementing glycine auxotrophies carried by the two parental lines (*14*). One hybrid clone (strain Y143) was recloned in selective (glycine-free) medium and used for subsequent experiments. Its genetic constitution can be represented as

$$\frac{glyA^+\ glyB^-\ HPRT^+\ G6PD^+}{glyA^-\ glyB^+\ HPRT^+\ G6PD^-}$$

The hybrid has a modal chromosome number of 40, close to the sum of those of the parents (42 chromosomes). Its phenotype is $G6PD^+$, the $G6PD^-$ phenotype being a recessive trait (*10*). The G6PD phenotype of cells and colonies can be clearly demonstrated by using a histochemical stain for enzyme activity (*10*).

If random segregation of either of the chromosomes bearing the HPRT and G6PD loci were occurring before mutation, then 50 percent of the $HPRT^-$ mutants derived from this hybrid should be $G6PD^+$ and 50 percent $G6PD^-$. The results of three separate experiments are shown in Table 1. There is good agreement with the predicted 50-50 distribution of $G6PD^+$ and $G6PD^-$ colonies among the mutants. It can also be seen that the average induced mutation frequency in these near-tetraploid hybrid cells is about 1/70 of that usually found in diploid cells (2.5×10^{-4}) (*7*) and is similar to the frequency found in tetraploid cells of similar clonal age that had been formed by low-Colcemid treatment (Fig. 1).

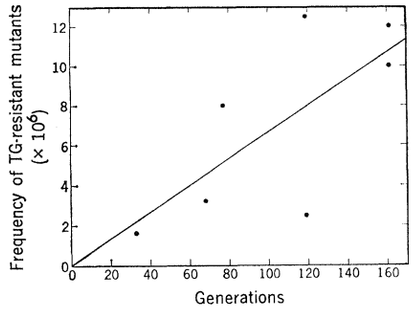

Fig. 1. Frequency of EMS-induced mutation as a function of clonal age. The pseudotetraploid line 5T11 of CHO cells was recloned and grown in monolayer culture in F-12 medium with 10 percent fetal calf serum. Cells were passaged when they reached confluence: at least 2×10^6 cells were transferred at each passage. Subpopulations were periodically induced to mutate and selected for TG-resistant cells as described previously (*7*). The total number of mutant colonies scored was 114, with a range of 5 to 25 in individual experiments.

Although these results do implicate one chromosome-wide event in the generation of TG-resistant mutants from tetraploid cells, they do not clearly discriminate between chromosome loss, homozygosis through mitotic recombination, and X chromosome inactivation as a mechanism. A careful study of the karyotypes of these mutants should be able to demonstrate the first possibility. The results are not consistent with the occurrence of two point mutations as the basis for the TG-resistant phenotype, and they argue against a mechanism that specifically turns off HPRT activity in these cells by an epigenetic process.

There is little doubt that stable, heritable phenotypic changes that represent epigenetic alterations in regulatory programs can be demonstrated in cultured animal cells. The most convincing examples are the extinction (*15*) or induction (*16*) of differentiated cell products in hybrids formed between different types of specialized cells. It is equally clear that mutational changes can also be induced in cultured animal cells. If mutation is to be useful as a means to study gene regulation in culture, it will be necessary to distinguish these two types of change within one system.

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Prostaglandins in Human Seminal Fluid: Two Novel Compounds

Abstract. *Human seminal fluid frozen immediately after ejaculation contains two novel prostaglandins. These are present in larger quantities than the previously reported prostaglandins. They are characterized by gas chromatography and mass spectrometry as 19-hydroxyprostaglandins E_1 and E_2. Most of the previously identified prostaglandins may be artifacts.*

The prostaglandin content of human seminal fluid that had been frozen immediately after ejaculation was found to differ significantly from that which had not been so treated (*1*). The initial studies of prostaglandins were performed on pooled samples of human seminal fluid obtained from fertility investigation laboratories (*2*). Thirteen prostaglandins were identified: A_1, A_2, B_1, B_2, E_1, E_2, E_3, $F_{1\alpha}$, $F_{2\alpha}$, and 19-hydroxy A_1, A_2, B_1, and B_2. The 19-hydroxy compounds were the most abundant, their concentrations being approximately four times those of prostaglandins of the E series.

Human seminal fluid was obtained from a healthy fertile donor on two separate occasions and cooled to −10°C within 5 minutes. An extract in a mixture of chloroform and methanol (2 : 1) was evaporated under nitrogen and remaining traces of water were removed by dissolving the residue in benzene and evaporating to dryness (*3*). On silicic acid chromatography, "neutral lipid," "prostaglandin," and "polar lipid" fractions were eluted, respectively, by chloroform, a mixture of chloroform and methanol (8 : 1), and methanol. The "prostaglandin" fraction was examined by gas chromatography–mass spectrometry (GC-MS) without further purification (*4*).

The following derivatives of the prostaglandin fraction were prepared for GC-MS analyses (*5*): the *O*-ethyloxime, trimethylsilyl ester, trimethylsilyl ether (EO-TMS); the *O*-methyloxime (MO)-TMS; and the MO-[9-^{2}H]TMS derivatives. Selective ion monitoring (*6*) at a mass-to-charge ratio (*m/e*) of 611 during GC-MS of EO-TMS derivatives revealed the presence of prostaglandins E_1 and E_2 as well as more abundant components of longer retention time. The GC data (Table 1) indicated that the additional components (compounds **1** and **2**) were 19-hydroxyprostaglandins E_1 and E_2 (*7*). Comparison of the mass spectral data (Table 2) with those of the primary prostaglandin derivatives (*8*) confirmed that the additional hydroxyl groups were at the 19-position. Ions in the spectra of derivatives of prostaglandins E_1 and E_2 which do not contain the C_{16} to C_{20} chain are present in spectra of derivatives of **2** and **1**, respectively. Ions characteristic of the unsubstituted C_{16} to C_{20} chain are absent. Instead, corresponding ions containing the trimethylsilyloxy group appear at 88 mass units higher. Additional ions are formed by elimination of trimethylsilanol from these ions. The powerful fragmentation-directing properties of the 19-trimethylsilyloxy groups give rise to characteristic ions of *m/e* 129 and 143 comprising C_{17} to C_{19} and C_{17} to C_{20}, respectively.

Thus, all the data are consistent with compounds **1** and **2** being 19-hydroxy prostaglandins E_2 and E_1, respectively. Since meticulous precautions were taken to minimize the possibility of modifications of the samples during storage, isolation, and characterization, it is reasonable to assume that 19-hydroxy prostaglandins E_1 and E_2 are indeed present in human seminal fluid immediately after ejaculation.

Prostaglandins A_1, A_2, B_1, B_2, and their 19-hydroxy analogs were not found in our study. Since others have found them in seminal fluids, that had not been frozen immediately after collection, the possibility exists that they are metabolites produced after ejaculation. However, since prostaglandins of the E series are readily dehydrated to those of the A and B series by heat, light, and extremes of *p*H (*9*), it seems more likely that some, if not all, of the prostaglandins of the A and B series (and their 19-hydroxy analogs) previously found in human seminal fluid are artifacts.

19-Hydroxy prostaglandins E_1 and E_2 were found in approximately five times the concentration of prostaglan-

Table 1. Gas chromatographic data for derivatives of compounds **1** and **2**. Expected values are computed by adding the appropriate retention increments (+230 for 19-hydroxy, +310 for 20-hydroxy) to retention indices of prostaglandins E_2 and E_1. Each compound affords both a *syn* and an *anti* isomer, which are separated by gas chromatography. Abbreviations: 19-hydroxy, 19-OH; 20-hydroxy, 20-OH.

Derivatives	Compound **1**	Compound **2**
	Observed	
MO-TMS	3040, 3090	3070, 3120
EO-TMS	3095, 3130	3105, 3145
	Expected	
19-OH MO-TMS	3060, 3110	3090, 3140
19-OH EO-TMS	3090, 3145	3120, 3175
20-OH MO-TMS	3140, 3190	3170, 3220
20-OH EO-TMS	3170, 3225	3200, 3255

Table 2. Mass spectral data for isomers of the MO-TMS derivative of compound **1**. Parentheses indicate relative abundances.

Peak	M$^+$	Base peak	Major ions					
Peak 1	685 (2)	75	143 (55)	117 (45)	73 (45)	217 (44)	133 (43)	129 (38)
Peak 2	685 (3)	143	75 (87)	133 (78)	223 (73)	353 (64)	129 (63)	73 (43)