Somatic Cell Hybrids from Frog Lymphocytes and Mouse Myeloma Cells

Abstract. *Stable somatic cell hybrids were obtained by fusing* Xenopus *lymphocytes with mouse myeloma cells. These hybrids contained one to four* Xenopus *chromosomes and expressed* Xenopus *gene products, one of which was a lymphocyte membrane protein of 85,000 daltons precipitated by a monoclonal antibody.*

Somatic cell fusion between phylogenetically distant species (*1–3*) has important applications. It is a powerful tool in gene mapping, and it can be used to produce homogeneous cell products encoded by the genome of one of the partners. Moreover, the simple existence of stable hybrids between two partners belonging to very different zoological groups gives information about both the flexibility and stability of regulation in cell division and gene expression during evolution. We have obtained stable somatic cell hybrids between *Xenopus laevis* lymphocytes and murine myeloma cells, hybrids that express *Xenopus* membrane antigens.

We centrifuged 5×10^7 pooled frog splenocytes (from *Xenopus* immunized with 2,4-dinitrophenyl and keyhole-limpet hemocyanin in Freund's adjuvant) with 1×10^7 to 5×10^7 BALB/c myeloma cells and fused them by using polyethylene glycol (molecular weight 1500 or 4000) (*4*). Two types of myeloma cells were used, P3-x63Ag8 (*5*) and Fo (*6*), a nonsecreting line. Both cell lines are azaguanine-resistant. The cells were cultured in hypoxanthine, aminopterin, and thymidine (HAT) (*7*) with 10 percent fetal calf serum in microtiter plates, each 0.2-ml well containing approximately 10^5 cells (lymphocytes and myeloma cells) and 0.5×10^4 to 1×10^4 BALB/c peritoneal exudate cells (*8*). Experience has shown that viable hybrids appear only under optimal tissue culture conditions for mammalian cells (mammalian osmolarity and 37°C). The cultures were fed twice a week and observed for 5 weeks.

From a total of seven experiments, nine frog-mouse hybrid clones were obtained. The clones appeared not earlier than day 17 and not later than day 35. Four of them grew poorly, and died after a few weeks. The remaining five hybrids were finally cloned (M_{x1} to M_{x5}) and subjected to further analysis. Hybrid M_{x1} was the product of a fusion with P3-x63Ag8 cells, and hybrids M_{x2} to M_{x5} were obtained with Fo cells. Chromosome analysis of the hybrids was facilitated by the distinguishability of the chromosomes of the two species. The *Xenopus* chromosomes were all submetacentric and telocentric. The mouse chromosomes, which were all acrocentric except for one metacentric chromosome, could be stained by the C banding technique (*9*). The centromeres of *Xenopus* chromosomes do not stain.

Figure 1 shows the chromosome content of an M_{x1} hybrid cell. The hybrid cells derived from a single fusion event exhibited (after 5 weeks) 61 to 63 mouse chromosomes and two to four *Xenopus* chromosomes, indicating that the original clone was not stable. In January 1979 a very stable line was obtained and has since been maintained. It contains two *Xenopus* chromosomes, one telocentric (chromosome 13-18) and one belonging to the small metacentrics (chromosome 10 or 11 or 5-7) (*10*). Hybrids M_{x2} and M_{x4} did not contain any visible *Xenopus* chromosome, so expression of *Xenopus* hypoxanthine-guanine phosphoribosyl transferase (HGPRT) and any *Xenopus* antigens may be accounted for by translation or by the presence of a small chromosome fragment. Hybrids M_{x3} and M_{x5} had one (as yet uncharacterized) *Xenopus* chromosome.

The hybrids were obtained in HAT selective medium, which requires the expression of the *Xenopus* HGPRT. Under our conditions of selection, the growth of any cell in HAT medium would imply the expression of *Xenopus* HGPRT or a reversion of the HAT-sensitive mouse myeloma line. Since no revertant was observed in control cultures of 2×10^8 Fo or P3-x63Ag8 cells, we assume that the HGPRT is of *Xenopus* origin. In this context it was interesting to check whether the linkage between HGPRT and glucose-6-phosphate dehydrogenase (G6PD) observed on the mammalian X chromosome (*2*) also existed in *Xenopus*. The electrophoretic mobility of *Xenopus* G6PD is much lower than that of mouse G6PD, and it is easy to detect the *Xenopus* enzyme on cellulose acetate gel (*11*). None of the M_x lines expressed the G6PD of *Xenopus*, meaning that the enzyme cannot be expressed in a mouse environment or that it is not closely linked to HGPRT.

To test whether these somatic cell hybrids secreted any *Xenopus* products, we cultured the hybrid cell line in leucine-free medium supplemented with [^{14}C]leucine. The culture supernatant was analyzed by gel electrophoresis. No frog immunoglobulin or any other frog-

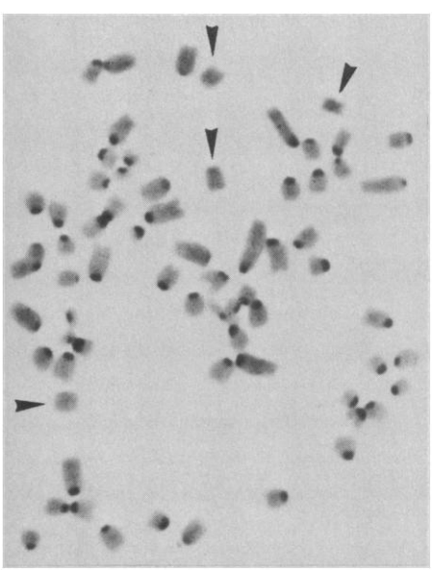

Fig. 1. Hybrid M_{x1} chromosomes. The original P3-x63Ag8 myeloma cell contains 64 acrocentric chromosomes and one metacentric chromosome, and the frog lymphocytes contain 36 chromosomes. This hybrid cell has 63 mouse chromosomes and four *Xenopus* chromosomes (arrows) after C banding.

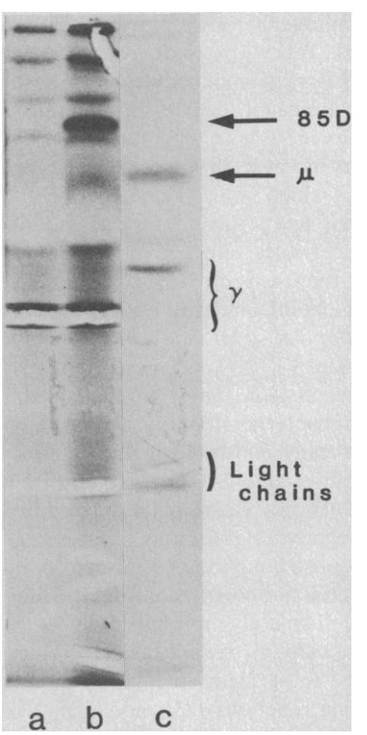

Fig. 2. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of [^{35}S]methionine labeled, solubilized *Xenopus* lymphocytes after precipitation by supernatants of P3-x63Ag8 myeloma cells (a) and Xen 10 hybridoma (b). (c) The immunoglobulin light-chain markers and μ and γ heavy chains.

0036-8075/81/0529-1034$00.50/0

specific molecule was detected by electrophoresis and immunoprecipitation. The cell lines produced either the myeloma immunoglobulin (in the case of M_{x1}) or nothing (all the other hybrids were obtained with a nonsecreting line).

The hybrid cells were injected repeatedly into BALB/c mice to raise antibodies against frog antigens that could be expressed at the surface of the hybrid cell. In no case did the injection of 2.5×10^6 to 5×10^6 hybrid cells (all lines) cause death; an injection of myeloma cells is fatal within 3 weeks. All the hybrids except for M_{x2} induced the production of antibodies. M_{x2}, after multiple injections over 2 months, finally provoked a slow-growing tumor that killed the mice 1 month later. It is possible that during growth the tumor retained only the *Xenopus* HGPRT and lost all genes expressing *Xenopus* surface antigens.

Antibodies to hybrid M_{x1} have been characterized in some detail. Indirect immunofluorescence showed that, at a final dilution of 1:60, serum samples from mice injected with M_{x1} reacted with antigens on *Xenopus* red cells and leukocytes. These serums did not, at a final dilution of 1:2, react with any antigens on mouse tissue, nor did the supernatant of P3-x63Ag8 myeloma cells. Absorption with red cells eliminated the activity of all but one of the antiserums, which retained some antileukocyte activity. The mouse that produced this leukocyte-specific serum was killed for fusion to produce monoclonal antibodies. From this fusion 11 hybridomas were selected, some of which reacted with both *Xenopus* frog red cells and leukocytes, others only with leukocytes, suggesting that at least two *Xenopus* antigens were expressed against M_{x1}. Xen 10, one of the hybridomas that reacted only with leukocytes, was used to precipitate a frog antigen from the membranes of M_{x1} and *Xenopus* spleen cells. The precipitate, analyzed by electrophoresis, revealed a single band in the region of 85,000 daltons (Fig. 2). *Xenopus* cells yielded a large amount of this material, whereas the M_{x1} hybrid cells yielded only a very narrow band in the corresponding molecular weight region. This may indicate differential expression of the membrane product under natural and hybrid conditions. The red cell extract showed no band, and no material beyond background amounts was precipitated from labeled myeloma cells.

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Purine Resistant Mutants of *Drosophila* Are Adenine Phosphoribosyltransferase Deficient

Abstract. *A deficiency for adenine phosphoribosyltransferase activity is the primary biochemical defect in mutants of* Drosophila *selected for resistance to purine-induced lethality.*

Although many techniques have been used for the direct selection of specific gene mutations in prokaryotes and eukaryotic cells in culture (*1*), only rarely has this been possible in intact higher animals. The absence of selective systems for the recovery of specific gene alterations in whole, developmentally complex organisms has been an obstacle to the study of gene organization and the associated biochemical mechanisms that give rise to the differentiated state. The single exception is the alcohol dehydrogenase (*Adh*) locus of *Drosophila*, where mutations resulting in the absence of alcohol dehydrogenase (ADH) activity rescue flies from an otherwise lethal exposure to certain secondary alcohols (*2*). A large number of point mutations and small deletions in the *Adh* genetic element were rapidly isolated through this specific selection procedure (*3*). In an effort to reveal other genetic loci in whole complex organisms at which mu-

Table 1. Adenine phosphoribosyltransferase (APRT) activity in *Drosophila melanogaster*. Heterozygotes were constructed reciprocally between the purine-resistant mutant, *aprt*^{1}, and the wild type (+) *Ore-R*. Since there was no significant difference in APRT activity between the adult progeny of reciprocal matings, the data for the heterozygote were combined. APRT activity is expressed as the mean number of disintegrations per minute of [8-^{14}C]AMP per minute per microgram of protein. APRT activity was measured at 25°C (*p*H 7.5) by a modification of the procedure of Merril (*13*). Assay mixtures contained: 60 m*M* tris-HCl, 0.1 m*M* Na-EDTA, 26.0 m*M* $MgCl_2 \cdot 7H_2O$, 2.0 m*M* dithiothreitol, 6.25 m*M* Na-PRPP, 7.6×10^{-5} *M* [8-^{14}C]adenine (62 mCi/mmole), and 10 μl of *Drosophila* homogenate in a total volume of 35 μl. All chemicals were purchased from Sigma except [8-^{14}C]adenine, which was obtained from Amersham. Homogenates of *Drosophila* were prepared by extraction of ten newly eclosed adult flies (5♀ and 5♂) in 100 μl of deionized H_2O in a Kontes Duall all-glass tissue grinder at 4°C, or single flies were homogenized in 20 μl of deionized water and assayed as described above. Separate 5 μl portions of reaction mixture were removed at 5, 10, and 15 minutes after initiation of the reaction and placed on thin-layer chromatography (PEI-TLC) plates (20 by 20 cm; Brinkmann) at points previously overlaid with 5 μl of unlabeled 5′ AMP and adenosine (2 mg/ml). The AMP and adenosine markers were located using a shortwave ultraviolet mineral light after development of the TLC plate in 0.1*M* LiCl. The TLC chips corresponding to AMP and adenosine were dried; the radioactivity was determined by liquid scintillation counting. Radioactivity associated with the adenosine marker represented less than 1 percent of that in AMP, and therefore AMP nucleotidase (E.C. 3.1.3.5) activity was ignored in the determination of APRT activity. Protein was measured by the method of Lowry (*14*). The synthesis of [8-^{14}C]AMP from [8-^{14}C]adenine and PRPP by extracts of *Drosophila* is a linear function of time and protein concentration. S.D., standard deviation.

Genotype	APRT activity	S.D.	*N*	Wild-type APRT activity (%)
+/+	97.3	7.0	8	100
aprt^1/*aprt*^{1}	1.8	0.2	7	2
aprt^2/*InP18 kar ry*^{41} *Ubx e*^{4}	13.4	1.4	3	14
aprt^1/ +	60.2	4.9	5	62
aprt^1/*aprt*^{2}	5.5	1.3	4	6
aprt^1 *ry*^{2}/*aprt*^{1} *ry*^{2}	1.8	0.6	7	2
ry^2/*ry*^{2}	40.5	3.7	7	42

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