(*10*) also have not accounted for the degree of demineralization reported. Carbonic anhydrase, on the other hand, has been shown to be intimately involved with hydrogen ion secretion in other organ systems (*1*), and if it is present in bone, as the data presented above suggest, then it may function as a mechanism for hydrogen ion secretion in areas undergoing bone resorption. In each of the secretory systems which involve CA the fundamental process of "OH-separation" is linked with another specific process involving the transport of a cation or another anion (*14*). It will be worthwhile to examine the relationship of CA activity to the influx and efflux of calcium in bone cells in view of recent suggestions regarding the role of ionic calcium in hormonal regulatory mechanisms mediated by cyclic adenosine monophosphate (*15*).

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Reversion of Murine Sarcoma Virus Transformed Mouse Cells: Variants without a Rescuable Sarcoma Virus

Abstract. *Murine sarcoma virus transformed mouse 3T3 cells, which are negative for murine leukemia virus and which yield sarcoma virus after superinfection with murine leukemia virus, spontaneously give rise to flat variants from which murine sarcoma virus can no longer be rescued. The revertants support leukemia virus growth and show an enhanced sensitivity to murine sarcoma superinfection and, like normal cells, do not release RNA-dependent DNA polymerase activity. Because revertants could be obtained with high frequency from progeny of single transformed cells, each cell that contains the sarcoma virus genome seems to have the capacity to suppress or eliminate an RNA tumor virus native to its species of origin.*

Murine sarcoma virus (MSV) can both transform and replicate in mouse cells in the presence of murine leukemia virus (MuLV), but can only transform them in its absence (*1–3*). Mouse cells that have been infected and morphologically altered by MSV in the absence of MuLV have been isolated and are readily detected by both their altered morphology and the presence of a rescuable MSV genome (*2, 3*). Some of these MSV-transformed cell lines originating from soft agar colonies have been named sarcoma-positive leukemia-negative (S+L−) cells (*2*). These S+L− cells contain no infectious MSV or MuLV but release small amounts of "C"-type particles and some but not all MuLV group-specific antigens (*4*). Superinfection of S+L− cells with MuLV leads to a rescue of MSV and replication of MuLV and serves as a simple, rapid assay for MuLV (*5*). During extended cultivation of single colonies of S+L− cells in liquid medium, we observed the appearance of flat variant cells which, when isolated, had many properties of untransformed 3T3 cells and from which MSV could no longer be rescued by MuLV. We now describe the morphology, frequency of occurrence, and some of the special features of these revertant cells. This seems to be the first observation that cells transformed by an oncornavirus native to the species can revert in their morphology and function to resemble normal cells.

Table 1. Frequency of occurrence of flat, low density, S+L− cell-derived clones which were negative for rescue of MSV. The designation of the cell lines refers to clonally derived cells from single agar colonies. The derived cells were grown in liquid medium and the subline numbers refer to clonal isolates tested in different laboratories over an 18-month test period. Rescue of the MSV genome was tested by passing the clone to obtain several dishes with 10^5 cells per 20-cm² dish. These were infected with MuLV (1 to 3 FIU per cell) and followed for characteristic changes. Supernatants of infected cultures were verified by the focus assay for the presence of free MSV in 3T3 cells with optimum helper MuLV. The cell density was determined at or beyond confluence, but the medium was not changed. Not every S+L− or revertant subline was tested. The values represent an average of at least two representative determinations; NT, not tested.

Cloned subline	Clones tested	Clones positive for MSV rescue	Maximal density (No./cm²)	
			S+L− cells	Sarcoma-negative cells
		EC-10 cell line		
FG-10	40	11/40	1.9×10^5	3.2×10^4
FG-10T	11	5/11	2.8×10^5	3.5×10^4
FG-10M	4	4/4	$0.7–2.4 \times 10^5$	
FG-10-0	19	15/19	2.2×10^5	4.9×10^4
Flat variant of FG-10T	7	0/7		4.2×10^4
		3-19 cell line		
3-19-7	25	25/25	2.4×10^5	
3-19-2T	10	10/10	2.7×10^5	
3-19-6T	21	20/21	2.7×10^5	4.4×10^4
2-43-3H	14	14/14	NT	NT
3-197-3	239	238/239	2.4×10^5	1.5×10^4
Flat variant "R9" of 3-19-6T	9	0/9		4.3×10^4
Flat variant "D" of 3-197-3	27	0/27		3.0×10^4

Propagation and transformation of Swiss 3T3 cells under both liquid medium and soft agar have been described (*2*). Both defective and competent MSV stocks have been analyzed for their content of focus-forming units (FFU), and their endogenous replicating MuLV has been determined by end-point dilution methods, or as focus-inducing units (FIU) in S+L− cells, or as leukemia virus helper units (*6*). MuLV stocks having $\sim 10^6$ FIU per milliliter were obtained from 3T3 cells infected with Moloney leukemia virus, which were subsequently cloned and passaged in Swiss mice (*7*). Cloning of cells was performed in microtiter wells (Falcon Plastics, Microtest II), with the multiplicity of cells per well adjusted so that the Poissonian probability times the efficiency of plating gave an actual distribution of $\leqq 0.3$ cell per well. Each well was examined visually after plating and several times thereafter, and the occurrence of negative, single, and multiple clones closely followed the theoretical expectation (*8*). We ascertained that single clones contained a rescuable MSV genome as follows. The contents of each well were cultured in dishes or flasks, then plated at 10^5 cells on 20-cm^2 plastic dishes, and superinfected with a multiplicity of MuLV of 3 FIU per cell. The cells were followed for morphological changes associated with MuLV growth and MSV rescue; the supernatants were tested for rescued infectious MSV in normal 3T3 cells in the presence and absence of adequate MuLV helper virus (*6*). RNA-dependent DNA polymerase activity in cell culture supernatants was assayed with the use of the synthetic RNA-DNA template polyriboadenylic • oligodeoxythymidilic acid [poly(rA) • oligo-$(dT)_{12-18}$] (*9*).

Two dissimilar cultures, EC-10 and 3-19, each cloned twice, initially in soft agar and once in liquid medium, were compared by repeated cloning over a period of 18 months. The derived sublines were tested for MSV rescue and cell density (Table 1).

Culture EC-10 consisted of a monolayer of hyperrefractile fusiform cells and flat epithelioid cells, while culture 3-19 was composed solely of very dark, loosely attached cells, which did not form a compact layer. Cloning efficiency of both lines was greater than 50 percent, and their cell density in confluent cultures was well in excess of 2×10^5 cell/cm^2. The MSV was readily recovered in high titer from both lines only after MuLV superinfection. To ascertain that MSV genetic information was vertically transmitted, additional cycles of cloning were carried out on both cell derivatives for more than 1 year. From the EC-10 line, a hyperrefractile transformed colony (FG-10) was found positive for MSV rescue and was passaged for several weeks in liquid medium. The culture again became composed of both flat and hyperrefractile cells, and, when cloned in turn, a typical single S+L− colony gave rise to transformed, flat, and mixed colonies after several passages. The flat variant cloned cells of the EC-10 line were examined for rescuable MSV. The addition of MuLV resulted in no morphological changes. No MSV was rescued, but MuLV replication occurred, as determined by helper activity for MSV and by FIU's in S+L− cell assays. The clones derived from the 3-19 line were predominantly parental type, with occasional flatter cuboidal variants. Essentially all colonies isolated from line 3-19 were S+L−; of several hundred individual clones examined, only two clones were very flat and negative for MSV rescue. The frequency of isolation of MSV negative variants among the progeny of the two cell lines is shown in Table 1. From the FG-10 line, the frequency of revertant clone isolation became reduced after 1 year in culture. In contrast, the 3-19 S+L− derivatives almost invariably gave rise to transformed cells, although some S+L− sublines now adhere to a plastic surface more efficiently. Repeated cloning of sarcoma-negative flat variants of either line gave rise only to new flat colonies which, when repeatedly tested for MSV, did not behave as back-revertants with a rescuable MSV genome. Isolated revertants formed colonies in liquid medium with essentially the same or slightly lower (~40 percent) efficiency as compared to S+L− cells. Thus the isolation procedure did not discriminate against the growth of flat variants. The morphological criterion of cell density became less clear as numbers of clones were examined; a morphological gradation was observed so that occasionally rather flat colonies from the 3-19 line were positive for MSV rescue, and progeny from MSV negative variants gave rise to more dense colonies but which were still negative for a rescuable MSV genome. All revertant colonies readily replicated MuLV without transformation or rescue of infectious MSV. When tested for superinfection with MSV, the revertants could be readily transformed and subsequently produced both MSV and MuLV. Quantitative titrations of MSV revealed that all revertants tested were at least several times more susceptible to MSV focus formation than were normal 3T3 cells (Table 2). The titration patterns of MSV in the revertants were analogous to those in 3T3 cells; competent MSV gave linear responses,

Table 2. Susceptibility of revertants to superinfection by MSV. Cells were first treated for 0.5 hour at 37°C with 25 μg of DEAE-D in 1 ml of medium. This procedure gave optimum enhancement of focus formation (*10*). Virus inoculums were either a 70 percent competent stock 130 or a defective stock 164 (*6*). Optimum helper virus was added to the 164 stock to express all defective MSV as foci. The enhancement factor (EF) for the MSV foci is the ratio of FFU in revertants or in DEAE-D–treated cells to FFU in normal 3T3 cells. The titers of MSV 130 and 164 assayed in untreated 3T3 cells with optimal MuLV helper were 1.0×10^6 and 4.2×10^6 FFU per milliliter, respectively.

Revertant	Treatment of cells	EF
	EC-10 cell line	
R8	MSV 130	4.2
R11	MSV 130	5.4
R4	MSV 130	8.8
	3-19 cell line	
R9	MSV 130	3.0
D	MSV 164	3.1
D	DEAE-D, then MSV 164	6.1
	3T3 cell line	
Control	MSV 164	1.0
Control	DEAE-D, then MSV 164	2.2

Table 3. Virus production measured by RNA-dependent DNA polymerase activity. The supernatants from 10^8 cells were concentrated 100-fold after the cells had been in culture for 120 hours. The RNA-dependent DNA polymerase activity was measured by the incorporation of tritiated thymidine (7400 count^{-1} min^{-1} pmole^{-1}) into acid-precipitable material, which was placed on Millipore filters and counted in a liquid scintillation counter. Each reaction mixture (total volume, 0.125 ml) was incubated for 60 minutes at 37°C and contained 0.025 ml of the potential virus preparation, 50 m*M* tris-HCl (*p*H 7.8), 20 m*M* KCl, 1 m*M* dithiothreitol, 0.5 m*M* manganese acetate, 0.003 m*M* [methyl-^{3}H]thymidine triphosphate, 0.005 absorbancy units at 260 nm of poly(rA) • $oligo(dT)_{12-18}$, and 0.04 percent (by volume) of Nonidet P-40.

Supernate source	Polymerase activity (picomoles per milliliter of supernatant)
MuLV-infected 3T3 cells	135.00
S+L− cells (3-19)	3.15
Revertant "D" (3-19 derived)	<0.03
Uninfected 3T3 cells	<0.03

and defective MSV's still needed MuLV for maximum early focus detection (*6*). Because surface alterations could have been responsible for the increased MSV focus formation, an MSV infection was also carried out in the presence and absence of diethylaminoethyldextran (DEAE-D, Pharmacia) on normal 3T3 cells and revertants (*10*). The DEAE-D enhanced the number of MSV foci on both revertants and 3T3 cells; the DEAE-D–treated revertant cells displayed the greatest sensitivity to MSV. The enhanced MSV detection is thus an intrinsic quality of revertants superposed on the surface effects mediated by DEAE-D treatment.

The detection of RNA tumor viruses has been accomplished by an assay of RNA-dependent DNA polymerase activity (which suggests RNA tumor virus presence) and which detected virus in minute amounts (*11*). Table 3 shows comparative activities found in the supernatant fluids of normal 3T3 cells, S+L− cultures, revertants, and in 3T3 cells infected with MuLV. Whereas S+L− cell supernatants had a low but definite level of reverse transcriptase activity (*12*), the revertants and normal 3T3 cells are comparably negative. Infection with MuLV, however, greatly increases the enzyme activity. Initial data also indicate that revertants, unlike S+L− cells, have no detectable "C"-type particles and do not form colonies in soft agar (*13*).

Reversion of transformed cells has been reported for both the papova and the Rous sarcoma systems but in either instance the transforming viral genome was foreign to the altered cell (*14*). Either a loss of viral genome or adequate suppression can result in nontransformed variants. The chromosomal state seems to be important in polyoma-transformed hamster cells which contain the virus genome so that specific regrouping of chromosomes tends to promote or inhibit the transformation regardless of the total chromosome number (*14*). The present revertants, negative for sarcoma rescue, contained either more or fewer chromosomes than the modal number of the S+L− cells. In our revertants, the integration of the viral genome is uncertain, but their increased susceptibility to MSV infection may reflect a residual repressed MSV genome. A loss of a potentially integrated viral genome is difficult to show, yet in the lambda prophage system high rates of cure are associated with lambda plasmid association rather than chromosomal covalent linkage (*15*). Viral antigen and virus induction by bromo- or iododeoxyuridine (*13*, *16*) can be used to test for the presence of MSV.

It is of interest that a transforming RNA tumor virus native to the species can be either lost or phenotypically repressed because procedures that would efficiently accelerate such natural trends could have obvious practical therapeutic value.

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Sex Differences in Electric Signaling in an Electric Fish

Abstract. *The electric discharge of* Sternopygus macrurus *is distinctly different from the discharges of ten sympatric species of electric fish in Guyana, South America.* Sternopygus *is the first known example of a fish with sexually different electric discharges. Males and females differ in the steady-state frequency of their discharges, and males produce variations in their discharge during courtship. Playback experiments demonstrate that species and sex differences in electric discharges have communicative significance.*

The gymnotid fish of Central and South America have specialized electric organs and electroreceptors for the production and reception of electric currents (*1*). These structures function together in an active sensory system for the location of conducting and nonconducting objects in the environment (*2*). The same structures function in intraspecific communication (*3–5*). Black-Cleworth (*4*) has described the extensive use of electric signals and their role in agonistic behavior in *Gymnotus carapo*. Little is known of the breeding behavior of the gymnotids since they have proved difficult to breed in aquariums. This study was undertaken to explore the role of electric signals in the reproductive behavior of gymnotids.

The research reported here was carried out from April to July 1971 in the Rupununi District of Guyana, South America. *Sternopygus macrurus* (Bloch and Schneider) is a common fish in mountain streams in fairly rapidly moving water. I made most of my observations in Moco-moco Creek, one of the few in the district that continues to flow during the dry season. The electric signals were detected with wire electrodes, amplified with a portable audio amplifier, and tape-recorded for later analysis with a sound spectrograph. Since the fish are nocturnal, most of the fieldwork was done at night.

Sternopygus macrurus produces an electric discharge that is easily distinguishable from those of all other species of gymnotids found in the Rupununi. Of the 11 species of gymnotids from Moco-moco Creek, seven produce pulse discharges, in which the discharge is brief with respect to the period between discharges, and in which the frequency is variable. The remaining four species, including *Sternopygus*, produce tone discharges, in which the discharge is nearly as long as the period between discharges and in which the frequency is highly stable (*6*). The individual pulses of the electric organ