a given kill, than ultraviolet did ($b = -0.66$), in enhancing the trp^+ reversion. The chloramine sensitivity of 12 trp^+ revertants was also studied, and they were as sensitive to chloramine as the *trp*C cells.

The effect of chloramine on the yield of *trp*C to trp^+ of other different strains carrying various mutation pertinent to DNA repair was also studied on CH medium. The preliminary experiments showed that the trp^+ yields of *pol*A5 (SB1060), *rec*3 (BD193), *rec*A (BD194), and *uvr* (SB879) were not significantly different from the yields of $polA5^+$ rec^+ control strains (168, BD170) when treated with chloramine. Strain *rec*B (BD191), however, had a comparatively lower reversion frequency whether the cells were chlorinated or not. No increase of absolute number of revertants in chloramine-treated cells was seen. Similar phenomena have been reported also in studying the mutability of recombination-deficient and ultraviolet-sensitive strains of *E. coli* toward ultraviolet light (*9*).

The trp^+ revertants of 168 have been studied for the transforming activity of the linkage group ($aroB^+$ $trpC^+$ $hisB^+$). Of 75 chloramine-induced revertants, 24 percent showed reduced trp^+ transforming activity compared to wild type trp^+. Spontaneous reversions showed about 8 percent such transforming-defective types. These may represent more complex genetic changes than base substitution.

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Increased Sarcoma Virus RNA in Cells Transformed by Leukemia Viruses: Model for Leukemogenesis

Abstract. *A morphologically flat revertant of mink cells nonproductively infected with Moloney sarcoma virus exhibited contact inhibition and lacked detectable sarcoma virus RNA. Superinfection by usually nontransforming type C mammalian leukemia-causing viruses induced transformation and increased sarcoma virus RNA. The results suggest a model for leukemogenesis in animals by increasing, during replication of usually nontransforming leukemia viruses, the levels of RNA from potentially oncogenic cell or integrated virus transforming genes.*

Mammalian type C RNA tumor viruses exist in two functional categories.

1) There are viruses that, when injected into animals, cause sarcoma or leukemia within a few weeks (*1*). These viruses are able to enter and transform fibroblasts and other cells in cell culture. The viruses that can transform fibroblasts have been well characterized and are defective in their ability to replicate progeny virions in the absence of replicating nontransforming helper viruses (*2*). There is evidence that these replication-defective but transforming viruses directly code for a function required for the maintenance of transformation (*3*).

2) There are helper-type viruses that replicate progeny without causing transformation in cell cultures and are capable of helping transforming viruses in replication of their progeny (*2, 4*). While some of these viruses (for example, the feline-primate viruses RD-114, CCC, and M-7) are not associated with disease, others are known to cause leukemias (*5*). These leukemias, as exemplified by the thymic leukemia of AKR mice, are unlike the leukemias caused by the transforming RNA tumor viruses and occur only after a long latent period (*6*). Since replicating viruses cause no transformation in vitro, the manner by which they induce transformation of hematopoietic cells in vivo, that is, leukemia and lymphoma, is obscure.

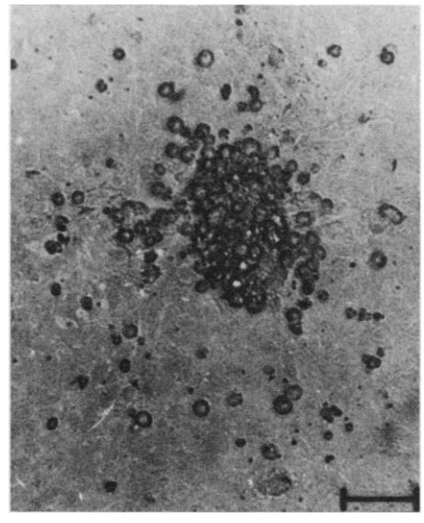

Fig. 1. A focus induced on S+L− mink cells by a single RD-114 replicating helper virus (*8*). The RD-114 superinfected S+L− mink cells continue to divide after confluency and form the focus of transformed cells seen above. The surrounding nonsuperinfected S+L− mink cells maintain contact inhibition and do not divide after the cell monolayer reaches 100 percent confluency. The bar represents 100 μm.

We have recently isolated mink lung cells which, although infected by replication-defective Moloney murine sarcoma virus (S+L−) strain MSV, were not like other murine and nonmurine cells infected by MSV and had phenotypically reverted to a flat morphology exhibiting contact inhibition similar to uninfected mink lung cells (*7*). Superinfection of these MSV infected cells (termed S+L− mink cells) by a variety of replicating but usually nontransforming type C viruses resulted in small foci (Fig. 1) of transformed cells on the contact inhibited monolayer; and this focus induction was useful as a quantitative assay for infectious replicating type C viruses (*8*).

Since this system involved transformation by usually nontransforming type C viruses, we investigated further the mechanism by which S+L− mink cells were caused to undergo transformation by the replicating mammalian type C viruses. We recently were able to prepare a complementary DNA (cDNA) probe that represents the sarcoma specific sequences of Moloney sarcoma virus (*7, 9*). The endogenous reverse transcriptase reaction from MSV obtained by rescue from S+L− dog cells (*7*), superinfected with RD-114 helper virus, was used to synthesize [^{3}H]deoxycytidine-labeled DNA. The DNA transcripts were recycled against RD-114 RNA and Moloney MuLV RNA to leave only MSV specific cDNA. Using this probe, we examined the levels of sarcoma virus specific RNA in S+L− mink cells before and after superinfection with various mammalian type C viruses. In addition, we examined mouse cells derived from 3T3 mouse cells and infected by the same MSV (*10*).

These S+L− mouse cells have also been used as a quantitative assay for replicating MuLV because of similar morphologic changes after superinfection with murine leukemia viruses (*10*).

Total cell RNA was extracted (*9*) from S+L− mouse and mink cells and helper virus superinfected S+L− cells (Fig. 2). Each of these RNA's was hybridized to the MSV cDNA probe, and the reaction was analyzed by examining the RNA·[^{3}H]cDNA reassociation kinetics. The results are shown in Fig. 2.

Before superinfection with MuLV, S+L− mouse cells have detectable MSV specific RNA with a half C_rt value of approximately 2×10^2 mole sec liter^{-1} (Fig. 2A). After superinfection with MuLV the number of copies of sarcoma specific RNA increases fourfold. This increase in sarcoma specific information can be correlated not only with the previously reported foci induced in S+L− mouse cells by MuLV (*10*), but also with our cell density studies indicating increased transformation with further loss of contact inhibition (Table 1). S+L− mouse cells and MuLV infected normal mouse cells grow after confluency to approximately the same cell density (86 and 89 percent, respectively) of normal uninfected mouse cells. S+L− mouse cells superinfected with MuLV grow to a higher cell density (146 percent of normal uninfected cells) after reaching confluency (Table 1).

Table 1. Contact inhibition control of cell division as measured by cell density after confluency of uninfected and helper virus infected normal and S+L− mink and mouse cells. Cell lines, viruses, and culture methods have been described (*7*). The maximal cell density is expressed as the average number of cells $\times$ 10^5 per square centimeter present 3 weeks after plating 5×10^5 cells into triplicate 250-cm^3 (75 cm^2) tissue culture flasks (Falcon). Helper virus infected cell lines were infected 5 weeks before density studies were begun. Ranges are shown in parentheses.

Cell line	Helper virus infection	Maximal cell density
Mink		
Normal		2.5 (1.8–3.6)
Normal	RD-114	2.2 (1.9–2.5)
S+L−		2.7 (2.3–3.3)
S+L−	RD-114	6.9 (5.1–8.3)
Mouse		
Normal		3.4 (3.1–3.6)
Normal	MuLV	2.9 (2.9–2.9)
S+L−		3.0 (2.6–3.2)
S+L−	MuLV	4.9 (4.8–5.0)

Because S+L− mouse cells were like their parental uninfected mouse cells and were not as flat and contact inhibited as normal and S+L− mink cells, we examined the levels of sarcoma specific RNA in nonsuperinfected S+L− mink cells and S+L− mink cells superinfected with various mammalian helper viruses, including feline leukemia virus strain C (FeLV) and feline-primate viruses RD-114 and M-7 (*5, 11*). The results of these hybridization experiments are presented in Fig. 2B. S+L− mink cells have twice been derived from single cell clones to ensure the presence of the sarcoma genome in every cell; yet the levels of sarcoma specific RNA were essentially undetectable up to C_rt values of $> 10^3$ mole sec liter^{-1}. However, in each instance where S+L− mink cells were superinfected with the different helper viruses, the levels of sarcoma specific RNA rose significantly. When even more S+L− mink cells are infected, as in the cells with the higher multiplicity of FeLV superinfection, the levels of sarcoma virus RNA increased even more.

This dramatic increase in the sarcoma specific RNA in S+L− mink cells is concomitant with transformation of the cells as shown by cell density studies (Table 1). Before superinfection with helper viruses S+L− mink cells are similar to uninfected normal and helper virus infected normal mink cells and are quite contact inhibited. Helper virus superinfection, however, causes a marked loss in contact inhibition control of cell division, and cell cultures reach very high cell densities (almost three times that of normal uninfected cells).

In the experiment shown in Fig. 2C, a cDNA probe from FeLV was used to compare the amount of FeLV RNA present in the same S+L− mink cells of Fig. 2B infected with FeLV at a high MOI (*12*) to the RNA in S+L− mink cells infected at the lower MOI. There is, as expected, an increased amount of FeLV RNA with higher MOI; and with more S+L− mink cells infected with FeLV, there is more sarcoma virus RNA (Fig. 2B).

These results indicate that, in both mouse and mink cells infected by Moloney sarcoma virus, superinfection of phenotypic revertants of such cells by replicating, but usually nontransforming, mammalian helper viruses leads to increased levels of sarcoma specific RNA in such cells (Fig. 2), concomitant induction of foci (Fig. 1), and an increase in

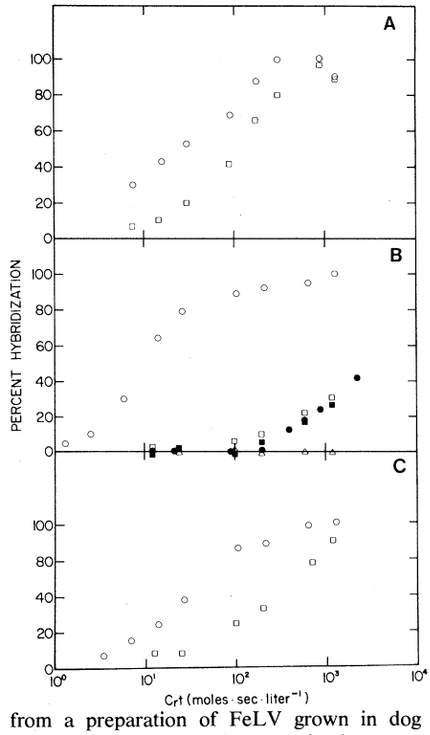

Fig. 2. Levels of Moloney sarcoma virus specific RNA in flat revertants of Moloney mouse and mink cells infected with sarcoma virus before and after superinfection with replicating helper viruses. Each hybridization reaction (0.05 ml) was incubated at 66°C for varying periods of time with 0.02*M* tris-HCl, *p*H 7.2, 0.60*M* sodium chloride, 5×10^{-5}*M* EDTA, 0.05 percent sodium dodecyl sulfate, and the RNA's and [^{3}H]cDNA's, as indicated below. Hybridization assays were analyzed with the use of S1 nuclease (*13*). All superinfected S+L− cells were harvested for RNA extraction after 10 days in culture. (A) Hybridization of the Moloney sarcoma virus specific cDNA (1000 count/min; 2×10^7 count/min per microgram) to either uninfected (□) or Moloney murine leukemia virus superinfected (○) S+L− mouse cell RNA. (B) Hybridization of the same Moloney sarcoma virus specific tritiated cDNA to uninfected S+L− mink cells (△); S+L− mink cells infected with FeLV strain C at a multiplicity of infection (MOI) of 0.5 (○); S+L− mink cells infected with FeLV at an MOI of 0.03 (□); S+L− mink cells infected with RD-114 virus at an MOI of 0.03 (●); or S+L− mink cells infected with M7 baboon virus at an MOI of 0.03 (■). (C) Hybridization of FeLV cDNA to RNA of S+L− mink cells of (B) superinfected with FeLV at an MOI of 0.5 (○) or 0.03 (□). The FeLV cDNA was prepared from a preparation of FeLV grown in dog thymus cells. The acid-precipitable fraction of each hybridization reaction contained approximately 4000 count/min. All hybridization values have been normalized to 100 percent of the actual final levels of hybridization in the S1 nuclease assay ranging from 50 to 70 percent of the input of [^{3}H]cDNA. The 100 percent value hybridization for the Moloney sarcoma virus specific probe was achieved with the RNA from a Moloney sarcoma virus transformed nonproducer rat cell (*9*); and the 100 percent value of hybridization with the FeLV probe was achieved with the dog thymus cell producing FeLV. C_rt values [initial concentration of total RNA (mole sec liter^{-1}) $\times$ time (seconds)] were calculated by the methods of Birnsteil and corrected to 0.18*M* concentration of monovalent cation (*14*).

transformation (Table 1). This effect can be achieved by either homologous mouse type C replicating viruses or heterologous viruses capable of replicating in such cells (*8*).

The increased levels of Moloney sarcoma specific RNA might be achieved by increased rates of transcription of the MSV genome or decreased rates of degradation of the sarcoma specific RNA. In addition, as a secondary effect, increases of sarcoma RNA may occur by gene amplification of the MSV genome. Within an S+L− mink cell infected with helper virus the presence of helper virus reverse transcriptase may increase the copy number of integrated MSV or the pseudotype MSV rescued by helper virus may be superinfecting surrounding S+L− mink cells and thereby increase the copy number.

Whatever the molecular explanation, the results observed in cells exogenously infected with Moloney sarcoma virus and superinfected with the replicating type C viruses suggests a hypothesis concerning a similar possible phenomenon in cells that might be carrying latent oncogenic information not acquired by exogenous infection. Thus, it is possible that a usually nontransforming type C virus might act in vivo in a similar manner by leading to an increase in RNA from potentially oncogenic normal cellular or viral genomes during the replication of the nontransforming type C virus. Such a model would predict that some forms of leukemia or lymphoma caused by type C viruses (particularly those characterized by a long latent period and involving replicating but nontransforming viruses) might be due to effects on the levels of RNA of cellular genes by the replicating type C viruses rather than to a direct effect of a transforming gene of the viruses themselves on the growth potential of the cell.

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On Competitive Innervation of Goldfish Eye Muscles

The report by Scott (*1*), appears to contradict the conclusions we drew from similar experiments published in 1970 and 1972 (*2–4*). If her interpretation is correct, Scott's result would also throw doubt on other experiments on competitive reinnervation done by us (*5*), as well as on similar experiments she cites, done by others.

The question is whether the function of regenerated foreign nerve terminals in a multiply innervated striated muscle can be repressed when the muscle accepts further regenerating synapses from its embryologically appropriate nerve. When Scott repeated our procedure on goldfish eye muscles she observed behavioral repression of the function of foreign nerves supplying the superior oblique muscle in about the same proportion of cases we did, about one third of the animals. The behavioral test involves measurement of static ocular counterrotation in response to tilting the whole animal head up and head down. In Scott's experiment, electrical stimulation of both appropriate and inappropriate nerve trunks resulted in strong contraction of the superior oblique muscle, in apparent refutation of our conclusion that the foreign innervation had become ineffective in causing muscle contraction.

Regeneration of the antagonistic inferior oblique muscle is a problem in these experiments, as Scott points out. The behavioral method we used, however, can detect defects in coordination due to simultaneous contraction of antagonists (*3*, p. 139), and this cannot explain our results. We also checked all fish by dissection for regenerated muscles and found them in only two cases (*2*, p. 47; *4*, p. 153).

Why then should tests of innervation by the use of natural reflex activation of the oculomotor system give results that are incompatible with those from tests by electrical stimulation of motor nerves? We think it likely that the discrepancy arises because static vestibulo-ocular reflexes and direct electrical stimulation of muscle nerves in fact test the function of two separate classes of muscle fibers.

In a recent paper (*6*), we have described two patterns of innervation corresponding to the two kinds of muscle fibers found in these muscles. The larger fibers, comprising the bulk of the oblique muscles (*7*), have elongated nerve endings that come from a small number of parent nerve fibers and spiral round the fiber almost from one end to the other. These muscle fibers would contract almost synchronously along their whole length when an action potential was propagated along the extended nerve ending, and they appear to be designed to produce strong and rapid contractions. The smaller fibers, whose ultrastructure suggests that they are involved in tonic contraction, are very densely multiply innervated by nerve fibers that run transversely across the muscle fibers at intervals of about 50 μm and supply a nerve terminal to each one they cross.

It is now known that the static ocular reflexes are a function of the smaller tonic muscle fibers (*8*). In a test of innervation by electrical stimulation of the nerve trunk, however, the bulk of the muscle tension would come from the larger muscle fibers. Large fibers will also be the main contributors to any sample of junctional activity gathered by an intracellular microelectrode because they are easier to penetrate and hold. The majority of small muscle fibers are 5 μm or less in diameter, and it must be very hard to record from inside them.

If the above is true, Scott's results may mean that there is selective competitive innervation of the population of small, multiply innervated muscle fibers, which leads to repression of previously functional foreign synapses, but that