the presence of the three bands in the *E. coli* extract, but physical, chemical, and DNA sequence analysis should help us select the correct one.

The results of Ouchterlony double diffusion experiments (Fig. 2) support the conclusion that the extract of *E. coli* carrying pJRS42 contains a molecule with antigenic determinants identical to those of streptococcal M6 protein.

Thus the M6 protein synthesized in *E. coli* has the same type-specific determinants as the M protein extracted from type 6 streptococci, although the *E. coli* product has a higher apparent molecular weight. The expression of the M protein gene in *E. coli* will allow us to dissect the molecular structure of the M molecule, which may lead to an understanding of how M proteins function to prevent phagocytosis as well as the mechanism by which streptococcal M types change during evolution.

Note added in proof: As determined by bactericidal assay, rabbits immunized with purified M6 proteins produced by the *E. coli* develop antibodies which allowed phagocytosis of type 6 streptococci.

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X Chromosome–Linked Transmission and Expression of Retroviral Genomes Microinjected into Mouse Zygotes

Abstract. *A genomic clone consisting of the Moloney leukemia proviral genome with moderately repetitive mouse sequences was microinjected into the pronucleus of a mouse zygote. An animal was derived that carried multiple copies of proviral DNA in a tandem array. No evidence for homologous recombination was obtained. The viral genome was expressed in this animal and was transmitted as a single unit to its offspring. Subsequent breeding studies revealed that the proviral DNA had integrated on an X chromosome.*

The introduction of viral (*1–7*) and cellular (*8–14*) genes into mouse embryos has been used as a means to study gene regulation during mammalian development. In our laboratory, the Moloney strain of leukemia virus (M-MuLV) was inserted into the germ line of mice and used as a model gene, because the activation of an integrated retroviral genome can be easily monitored in animals. A number of different mouse substrains, each carrying a single M-MuLV provirus at a different chromosomal locus, have been derived by exposing embryos to the infectious virus (*6*). In contrast, nonviral genes microinjected into the pronucleus were frequently carried as multiple copies integrated in tandem in the genomes of the resulting animals (*8–14*). We compared exposure of embryos to infectious virus with microinjection of cloned proviral DNA into the pronucleus as alternative means of introducing genes into the germ line. We also investigated the effect of repetitive sequences flanking the provirus on integration.

The DNA we used consisted of the linearized pMov-3 Eco RI fragment (Fig. 1A) lacking the pBR322 vector sequences. This genomic clone was obtained from the Mov-3 substrain of mice (*15*), in which infectious virus is regularly activated, with development of viremia and leukemia (*5*). The pMov-3 clone, which consists of the entire M-MuLV genome and 8 kilobases (kb) of moderately repetitive adjacent mouse sequences, is highly infectious when transfected to 3T3 cells (*15*).

Fertilized eggs from C57BL/6 females mated with C57BL/6 males were collected on the morning of the mating. One pronucleus was injected with cloned DNA by means of a glass micropipette. Of 150 eggs injected with approximately 1 picoliter of DNA solution at a concentration of 60 μg/ml, 92 survived the injection procedure and were transferred to the oviducts of pseudopregnant foster mothers and allowed to develop to term. Twelve individuals were born and were screened at age 6 to 8 weeks for virus expression by radioimmunoassay of the serum (*16*). One mouse (No. 69) was viremic. Partial hepatectomies were performed. The DNA was extracted, cleaved with Eco RI, and subjected to blot hybridization analysis. The injected sequences were detected by hybridization with the Hpa II–Hpa II fragment (Fig. 1A) derived from the 5′ flanking cellular sequences of the pMov-3 clone; this fragment cross-hybridizes to multiple bands of normal mouse DNA (Fig. 1B) (*15*). This probe detects the M-MuLV provirus carried in Mov-3 mice as a 16.8-kb fragment (lane b in Fig. 1B) (*15*). An intensely hybridizing fragment of the same size was detected in the DNA of female No. 69 (lane c), indicating that the injected DNA had been integrated, with the Eco RI sites of the flanking sequences preserved. The other nonviremic animals did not show these sequences. By comparing the intensity of this band to the single copy of proviral DNA at the Mov-3 locus (lane b in Fig. 1B), we estimate that mouse No. 69 contained approximately 10 to 20 integrated copies of proviral DNA [see also (*17*)]. When the filter in Fig. 1B was hybridized to a M-MuLV–specific probe, the results obtained with the flanking probe were confirmed (data not shown).

The arrangement of the integrated DNA was analyzed with Hind III digestion. Figure 1C shows a predominant 9.4-kb band, which was to be expected if most of the injected DNA molecules had integrated in a tandem head-to-tail arrangement (compare Fig. 1A). In addition, a weak 14.6-kb band was seen, indicating that some of the molecules had integrated in a head-to-head arrangement at the same integration site.

The 8-kb band corresponding to the site where M-MuLV was inserted in Mov-3 mice was present in the DNA of animal No. 69 (lane c in Fig. 1B) with the same intensity as is found in normal mouse DNA (lane a in Fig. 1B); this indicates that the injected pMov-3 sequences had not integrated at the Mov-3 locus [compare the DNA from a heterozygous Mov-3 animal (lane b in Fig. 1B), which shows half the intensity of the 8-kb fragment from mouse No. 69, and, in

addition, the 16.8-kb fragment carrying the integrated M-MuLV genome]. Similarly, the intensity of none of the other bands was changed in the DNA of animal No. 69, suggesting that these cross-hybridizing sequences were not involved in the integration of the injected DNA. This would have been expected if homologous recombination between the adjacent sequences of the clone and the respective cellular sequences had taken place. Additional experiments are needed to evaluate the role, if any, of the repetitive sequences in the process of integration.

Genetic transmission of the integrated virus was monitored by two experimental approaches. Since development of viremia is a dominant marker for the presence of a functional M-MuLV genome, numerous offspring of viremic males were screened by radioimmunoassay for the presence of virus in their serum (*6, 16*). Because viremic females transmit virus to all of their offspring by congenital infection (*4*), maternal transmission of viremia does not indicate genetic transmission of the proviral genome. Therefore, genetic transmission from the mother was monitored by blot hybridization analysis (Fig. 1) of DNA obtained from liver biopsies. Mouse No. 69 was mated with a normal male and gave birth to five offspring (BC-1 generation), two of which, one a male, the other a female, contained the integrated pMov-3 sequences (lanes d to k in Fig. 1B, and Table 1). The male was mated with normal females to derive BC-2 mice, and the offspring were analyzed for viremia and for the presence of pMov-3 sequences. Table 1 shows that all of the 20 daughters of the BC-1 male, but none of the sons, became viremic. Analysis of liver DNA from five of the daughters and four of the sons revealed that only the daughters contained the injected pMov-3 sequences. When the daughters were mated with normal males to derive BC-3 animals, the pMov-3 sequences were transmitted to about 50 percent of daughters and sons. BC-3 males transmitted the integrated viral sequences only to their daughters. Our results therefore indicate that the proviral genomes were stably transmitted at a single locus on the X chromosome. The substrain with this new virus locus was named Mov-14. The previously derived mouse substrains Mov-1 to Mov-13 carry M-MuLV at different sites on autosomal chromosomes (*6*).

Our results show that, in contrast to exposure of embryos to infectious virus, injection of proviral DNA into the pronucleus can lead to integration in tandem of multiple copies of the proviral DNA into the germ line. This is in agreement with experiments performed by others (*8–14*), in which nonviral DNA was microinjected into the pronucleus. So far, only transient expression of genes injected into mouse zygotes has been observed (*7, 18*), but no evidence exists for embryonic expression of the genes after genomic integration. An efficient de novo methylation activity, which is characteristic for early embryonic cells, is involved in inactivation of retroviral genomes upon genomic integration (*17, 19*). Before integration, the DNA remains unmethylated (*19*) and can be expressed. This is supported by earlier experiments in which the clone used for derivation of Mov-14 was microinjected into the cytoplasm of a zygote rather than into the pronucleus (*7*). In those earlier experiments the injected DNA was expressed, the flanking Mov-3 sequences were lost, and a copy of the

Table 1. Sex-linked transmission of microinjected pMov-3 sequences. Female No. 69 was derived as described in the text. Maternal transmission of the microinjected pMov-3 sequences was monitored by blot hybridization (see Fig. 1) of DNA obtained by liver biopsies. Paternal transmission was monitored by DNA hybridization and by radioimmunoassay to test the serum for the presence of viral p30 (*17*); N.T., not tested.

Back-cross generation	Parents	Ratio of virus-positive offspring to total tested with			
		Viremia		pMov-3 DNA sequences	
		♂	♀	♂	♀
	♀ No. 69 (Microinjected into zygote) × ♂ (normal)	N.T.		1/2	1/3
BC-1	♀ (Normal) × ♂ (Mov-14)	0/17	20/20	0/4	5/5
BC-2	♀ (Mov-14) × ♂ (Normal)	N.T.		11/24	6/16
BC-3	♀ (Normal) × ♂ (Mov-14)	0/35	43/43	N.T.	

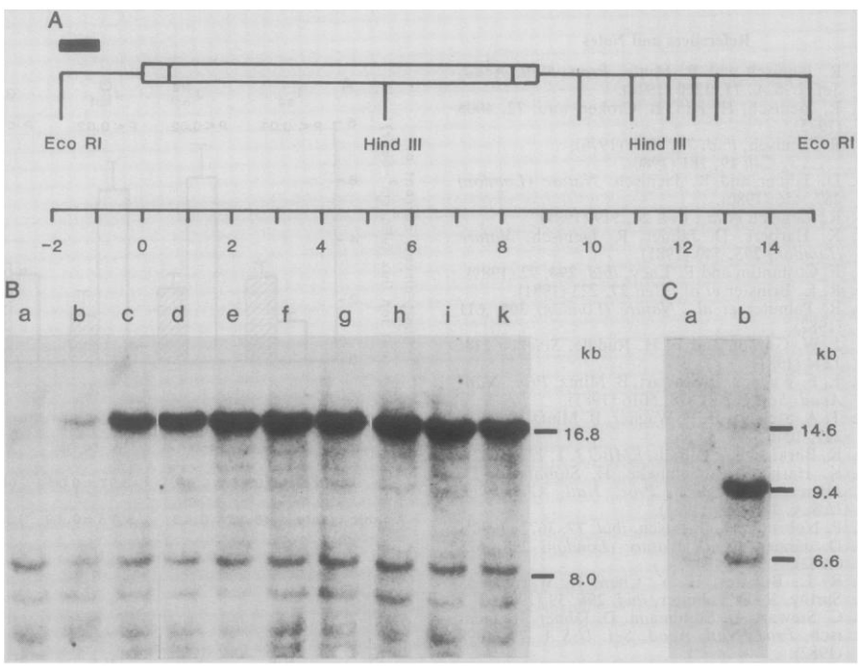

Fig. 1. Analysis of DNA's from mouse No. 69 and its offspring. (A) Restriction enzyme map of the integrated M-MuLV provirus of Mov-3 mice. The molecular cloning of the Eco RI fragment and derivation of the restriction map have been described (*16*). Only restriction sites relevant to the present study are indicated. The black bar indicates a 0.8-kb Hpa II–Hpa II fragment which was used as a probe for the experiments described below. (B) DNA (15 μg) from mouse No. 69 and offspring was cleaved with Eco RI, subjected to electrophoresis on a 0.5 percent agarose gel, transferred to nitrocellulose, and hybridized with the nick-translated Hpa II probe (A). The filters were washed with 0.1 strength standard saline citrate at 68°C. (Lanes a to d) Liver DNA from (a) C57BL, (b) Mov-3, (c) mouse No. 69, and (d) the son of mouse No. 69. (Lanes e to k) DNA's from (e) liver, (f) spleen, (g) kidney, (h) brain, (i) testis, and (k) heart of the daughter of mouse No. 69 (see Table 1). (C) DNA from the liver of mouse No. 69 was cleaved with Hind III and analyzed as described in (B) except that a 0.6 percent gel was used. (Lane a) C57BL liver DNA and (lane b) mouse No. 69 liver DNA.

proviral DNA was inserted at a new chromosomal site (*7*).

So far, all functional proviral copies carried in the different Mov substrains have been activated during later development (*20*). This activation may depend on the chromosomal position of the proviral DNA (*5, 6*). Thus, retroviral genomes are favorable tools to study mechanisms of gene regulation in embryonic development. The retroviral genomes carried in the Mov-14 substrain are invariably expressed during the lifetime of the animals. The presence of these proviral genomes on the X chromosome should provide a useful marker for studying the molecular events associated with X chromosome inactivation (*21*), since monitoring virus expression does not require the use of the selective methods needed for the study of other X-linked genes (*22, 23*).

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Entamoeba histolytica Causes Intestinal Secretion: Role of Serotonin

Abstract. *Lysates of the protozoan parasite* Entamoeba histolytica *altered active electrolyte transport when present on the serosal surface of rabbit ileum and rat colon. The lysate-induced effects on electrolyte transport were similar to those caused by serotonin, and were blocked by bufotenine, an analog known to inhibit the action of serotonin. The transport effects were partially inhibited by antibody to serotonin. The amebic lysates were shown to contain serotonin by radioimmunoassay, high-performance liquid chromatography, and thin-layer chromatography. These results suggest that the serotonin present in* Entamoeba histolytica *may be important in the diarrhea seen in amebiasis.*

Entamoeba histolytica is a common cause of diarrhea in humans, but the pathophysiology of amebiasis is poorly understood. Lysates of axenically cultivated *E. histolytica* have both cytoxic and enterotoxic activities which may contribute to the pathogenesis of the diarrhea (*1–3*). In the present study, cell-free extracts of *E. histolytica* affected active electrolyte transport when added to the serosal side of rabbit ileum or rat colon. The changes produced were similar to those caused by several neurohumoral substances, including serotonin, which was identified in amebic lysates. During the course of infection, amebas are located in the lamina propria at the bases of intestinal epithelial cells where released neurohumoral substances could contribute to the alteration in intestinal transport to produce diarrhea.

Strains of *E. histolytica* of varying virulence based on animal model criteria were cultured axenically in Diamond's medium (*4, 5*). Trophozoites were harvested in the late log phase of growth, washed three times in phosphate-buffered saline, and resuspended to a final concentration of 10^5 trophozoites per milliliter. A cell-free extract, termed the crude lysate, was obtained by freeze-thawing and sonication. The crude lysate was centrifuged ($10^5 g$ for 1 hour) and a 30 to 70 percent ammonium sulfate precipitate prepared. Previous studies indi-

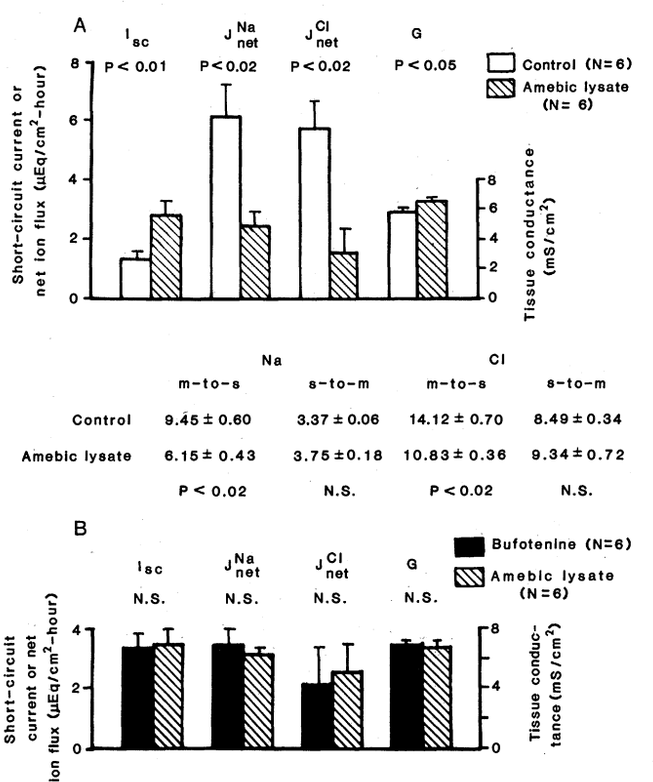

Fig. 1. The effect of *E. histolytica* lysate on rat colonic electrolyte transport. (A) Untreated control tissue. (B) Tissue first exposed to bufotenine. In (A), fluxes were determined in untreated control tissue 20 to 60 minutes after the addition of isotope; amebic lysate was then added and 70 to 110 minutes after isotope addition the effect of the lysate was measured. In (B), bufotenine was added at time 0 and its effect was determined between 20 and 60 minutes after isotope addition; then amebic lysate was added and the fluxes were measured between 70 and 110 minutes. Units of short-circuit current (I_{sc}) and fluxes are microequivalents per square centimeter per hour; conductance (G), millisiemens per square centimeter. Unidirectional (mucosal-to-serosal or serosal-to-mucosal) Na^+ and Cl^- fluxes are shown below (A) to show the effect of lysate. The P values represent comparisons of transport in (A) between untreated control and lysate addition to the same tissues and in (B) between bufotenine and lysate addition to the same tissues (paired t-test). Bufotenine treatment prevented the effect of lysate.