zation of genes on mouse chromosomes prepared for high-resolution analysis should more precisely delineate regions of genetic homology between man and mouse and should facilitate the use of the mouse as a model for human genetic disease.

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13. Supported in part by EPA Assistance Agreement CR-809737.

13 December 1985; accepted 15 April 1986

Viral Shedding and Transmission Between Hosts Determined by Reovirus L2 Gene

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Two reovirus isolates (type 1 Lang and type 3 Dearing) differ in their transmissibility between littermates of newborn mice. They also differ in the amounts of virus excreted by the gastrointestinal tract. With the use of reassortant viruses, these properties were mapped to the L2 gene. Thus environmental spread of reovirus is a genetic property.

MANY PATHOGENIC MICROORGANISMS have an obligatory stage in a eukaryotic host. Such a "parasitic" microorganism, whether virus, bacterium, or parasite, must develop a mechanism for efficient transmission from the infected host to another susceptible host. Transmission between animal hosts often takes place by defined routes such as respiratory, fecal-oral, or inoculation. The route of transmission is often related to the localization of the infecting microorganism. Thus, influenza, which localizes in the lung, is spread by respiratory secretions, whereas poliovirus, an enteric virus, is spread in stool.

Much has been learned in recent years about the mechanisms by which viruses localize in certain tissues, and hence about why viruses might select different strategies for transmission. There is also considerable information about the stability of viruses on release into the environment and the way in which properties such as droplet size, heat, and moisture might affect spread. Little is known, however, about how the genetic properties of viruses affect the capacity of viruses to be transmitted between hosts and to survive in the environment. Schulman (*1*) attempted to define differences in transmissibility between two strains of influenza that differed in virulence. He was able to show clearly that virulence and transmissibility were genetically determined, but the specific genetic basis for transmission was difficult to define (*2*). In fact, there is no example in which a specific gene (or genes) has been shown to play a clear role in transmission.

We used mammalian reoviruses to define the genetic basis of viral-host interactions. The reoviruses are widely distributed in nature, infecting humans, cows, mice, and many other species. By age 15, 50 to 80 percent of humans have antibody to reovirus (*3*). Although not important pathogens, they produce multiple pathologic changes in mice after experimental inoculation (*4*). The reovirus genome consists of ten segments of double-stranded (ds) RNA; there are three serotypes. Coinfection of L-cell cultures with different strains of virus results in reassortant virions (*5*). The relative ease of generating reassortants has enabled us and others to identify the functions of a number of genes [reviewed in (*4*)], including the gene responsible for receptor binding and serotype-specific immunity (S1), growth in differentiated tissue and protease sensitivity (M2), and initiation of persistent infection in cell culture (S4).

We used reovirus infection of newborn mice to define the gene responsible for transmission between mice and for enhanced secretion from the gastrointestinal tract. The gene that confers these properties is L2.

Initially we asked whether reovirus isolates could be identified that differed in their capacity to be transmitted between littermates. The standard laboratory strains, type 1 Lang and type 3 Dearing, showed marked differences in transmission under standard conditions (see Fig. 1). When a dose of 10^5 plaque-forming units (PFU) of type 1 Lang was given to two members of a litter (five litters inoculated), 57 ± 10 percent of the littermates were infected. Only one instance of transmission (five litters inoculated) was observed when type 3 Dearing was given at the same dosage (a transmission frequency of 3.7 ± 6 percent). The murine reovirus

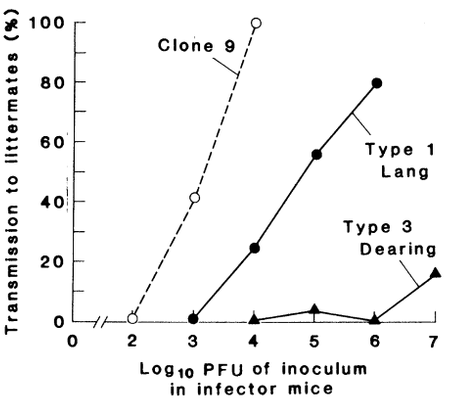

Fig. 1. Frequency of reovirus transmission among members of a litter as a function of inoculum given to infector mice. In standard transmission experiments (five replications), one litter of eight to ten suckling mice of the NIH/Swiss strain was placed in each cage. At age 1 to 2 days, two members of the litter were infected by orogastric intubation with a dose of virus in 30 μl of gelatin saline, as previously described (*6*). These "infector mice" were designated by clipping the tail and were replaced in the litter. At 10 days the littermates were killed and their intestines were removed (the site of primary replication for reovirus) (*14*). Intestines were placed in 1 ml of gelatin saline, frozen and thawed three times, and sonicated. The sonicate was diluted from 10^{-1} to 10^{-5}, and titers of virus were determined in the plaque assay previously described (*15*). Because of the toxicity of the intestinal contents to the L cells used in the assay, the 10^{-1} dilution wells were difficult to interpret. However, the assay detected the presence of virus at ≥1000 PFU per intestine. Presence of virus in the intestines of the littermates was scored as (+) transmission and its absence as (−) transmission.

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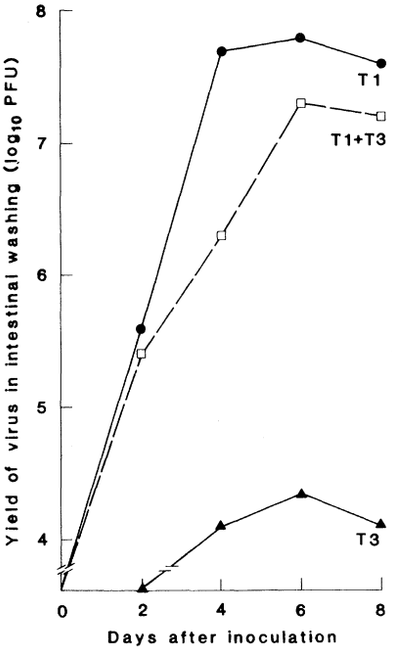
Fig. 2. Shedding from the gastrointestinal tract of mice infected with reovirus type 1 (T1), type 3 (T3), or type 1 and type 3 together (T1 + T3). All members of a litter of 1- to 2-day-old NIH/Swiss mice were inoculated by orogastric intubation with 10^5 PFU of virus in 30 μl of gelatin saline. Pairs of mice were killed at 2, 4, 6, and 8 days after infection. The abdomen was opened in sterile fashion, and the intestine was dissected from duodenum to anus. The duodenum was ligated over a tuberculin syringe, and 1 ml of gelatin saline was gently flushed through the intestine. Titers of virus were determined for the intestinal washings by methods described previously (*15*). Mice that died shortly after inoculation or that showed signs of peritonitis due to the intubation were not used. Each point represents the mean of four animals.



type 3 isolate, clone 9, showed 100 percent transmission at 10^4 PFU. This indicated that the differences in transmission were properties of individual isolates and were not serotype-specific. For all isolates examined, infection in littermates appeared first in the small intestine; this is consistent with the known transmission of reovirus by the fecal-oral route (*3*). The electropherotype of the virus recovered from four of the infected littermates was identical to that used to inoculate the infector mice for both type 1 and clone 9. The first cases of transmission of type 1 virus were observed on day 6 after infection, and the frequency rose to a plateau by day 8. Smaller litters (four to six pups) had a lower frequency of transmission for a given inoculum. These studies indicate that different viruses show reproducible differences in their capacity to transmit between littermates.

In order to determine the genetic basis for differences in transmission, we inoculated mice with reassortants composed of a mixture of genome segments derived from the high transmission strain (type 1 Lang) and the low transmission strain (type 3 Dearing) (Table 1). The reassortants were separable into two categories, those that were capable of being transmitted between littermates and those that were not. In each case of positive transmission (upper section of Table 1), the L2 dsRNA segment of the reassortant virus was derived from the type 1 parent. Every other dsRNA segment was derived from the type 3 parent in at least one of the reassortant viruses demonstrating transmission. Thus, the L2 dsRNA segment of type 1 was sufficient to confer the phenotype of high transmission. Cases of negative transmission (absence of virus in the intestine) are listed in the lower part of Table 1. Each reassortant that failed to be transmitted contained the L2 dsRNA segment of type 3. Furthermore, the presence of any other type 1 dsRNA did not confer transmissibility when the L2 was derived from type 3. This suggests that the L2 segment of type 1 was necessary as well as sufficient for transmission.

A wide variation in the rate of transmission was observed among reassortants bearing the L2 gene of type 1 (30 to 78 percent), suggesting that other genes may modulate the effect of L2. However, the litter-to-litter variation in transmission of the type 1 strain

Table 1. Transmission in reovirus reassortants. All stocks and reassortants were plaque-purified three times and used as second-passage stocks. Generation of reassortants by coinfection of mouse L cells and their analysis by polyacrylamide gel electrophoresis have been described previously (*5, 16*). Standard transmission experiments were conducted with one litter per cage of eight to ten suckling mice of the NIH/Swiss strain. 10^5 PFU of virus in 30 μl of gelatin saline was given to two members of the litter at 1 to 2 days after birth (the infectors). All mice were killed at 7 days (eight experiments) or 10 days (45 experiments). The day of death did not affect the result. Titers of virus were determined for the intestine, and transmission was scored as positive or negative, as described for Fig. 1.

Virus	dsRNA segment encoding										Transmission (littermates infected/total)
	Capsid polypeptides			Core polypeptides					Nonstructural polypeptides		
	S_1	M_2	S_4	L_1	L_2	L_3	M_1	S_2	M_3	S_3	
Parental											
T1	1	1	1	1	1	1	1	1	1	1	17/30 (57%)
T3	3	3	3	3	3	3	3	3	3	3	1/27 (3.7%)
Reassortants											+ *Transmission*
85	1	1	1	1	1	1	1	3	3	1	11/14 (78%)
143	3	1	1	3	1	1	1	1	1	1	10/22 (45%)
144	1	3	1	1	1	1	1	1	3	3	7/19 (37%)
146	1	1	3	1	1	1	3	1	1	1	6/14 (43%)
H14	1	1	1	1	1	3	1	1	1	3	5/14 (36%)
H24	1	1	3	1	1	1	1	1	1	1	6/20 (30%)
											− *Transmission*
1	1	3	1	1	3	1	1	1	1	3	0/13
13	3	3	1	3	3	3	3	3	3	3	0/6
18	1	3	1	3	3	1	3	1	3	3	0/6
28	3	3	3	3	3	1	3	1	3	3	0/13
39	3	3	3	1	3	3	1	3	3	3	0/14
68	1	3	3	1	3	1	1	1	1	3	0/7
88	3	1	3	3	3	3	3	3	3	3	0/6
126	3	1	3	3	3	3	3	3	3	3	0/18
136	3	3	3	3	3	3	1	3	1	3	0/6
145	1	3	3	3	3	3	3	3	3	3	0/6
H9	3	1	3	3	3	1	3	3	1	3	0/7
G2	3	1	1	1	3	1	1	1	1	1	0/13

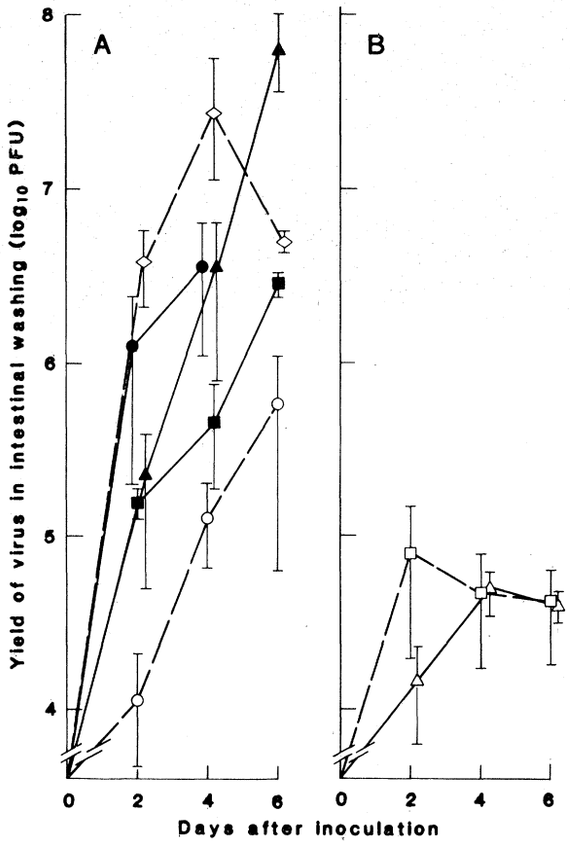
itself (57 ± 10 percent) made it impossible to discern the effect of additional genes from these data.

Although these studies showed that the L2 dsRNA segment is responsible for differences in transmission, they did not reveal how the L2 segment might mediate these differences in transmission. Since reoviruses multiply in the gastrointestinal tract and are shed into the environment like other enteric viruses, we considered the possibilities that the L2 genes influence (i) the growth of virus in the small intestine, (ii) the release of virus from the large intestine into the environment, or (iii) the stability of the virus in the environment outside the host. Earlier studies suggested that the M2 gene determines growth in the small intestine (*6*). A study of the capacity of various reassortant reoviruses to grow in intestinal tissue will determine whether the L2 gene has a role in this growth. Resistance to environmental stresses such as heat, drying, and high ionic strength might determine transmission if persistence outside the host for long periods is necessary. Drayna and Fields (*7*) examined the relation of intertypic differences in stability to a number of such stresses and found that the capsid genes (S1, M2, or S4) were responsible for resistance to these stresses; L2 did not have a central role.

We tested the hypothesis that the rate of shedding affects transmission, and we were able to demonstrate marked differences in the release of type 1 Lang and type 3 Dearing from the gastrointestinal tract after oral inoculation of the viruses. After an inoculum of 10^5 PFU, type 1 was shed in high titers ($>10^7$ PFU) within 4 days after infection, whereas type 3 was shed at titers below 10^5 PFU (Fig. 2). In order to determine whether this property of shedding is related to the differences in transmission, we used the reassortant clones to determine whether the L2 dsRNA segment is responsible for allowing type 1 virus to be shed in the stool in high titer (Fig. 3). The analysis of reassortants showed that the high shedding phenotype correlated with the presence of the L2 gene segment of type 1 (Fig. 3). All clones showing transmission in the earlier experiments were shed at titers greater than 10^5 PFU, whereas clones that did not show transmission were shed in titers below 10^5 PFU (see Table 1 for parental origin of dsRNA segments).

When the L2 dsRNA segment of type 3 was present in a reassortant, no other type 1 dsRNA segment conferred the high shedding phenotype of the type 1 parent (clones G2 and 126). All reassortants bearing the L2 dsRNA segment of type 1 were shed in titers greater than 10^5 PFU. A type 3 dsRNA segment occurring in any other locus did not abolish the effect of L2. However, one reassortant (clone 143) did not shed in titers above 10^6 despite the presence of the L2 gene of type 1. This suggests that some type 3 dsRNA segments may attenuate the effect of the type 1 L2 segment. Thus these experiments indicated that the L2 dsRNA segment of type 1 reovirus is responsible for allowing reovirus type 1 both to transmit efficiently between littermates and to be shed from the gastrointestinal tract at high titer. Since both shedding and transmission are under the control of the same dsRNA segment, they may be occurring through the same mechanism; that is, the capacity to be shed from the gastrointestinal tract at high levels allows the virus to be transmitted to other hosts.

Fig. 3. Shedding from the gastrointestinal tract of mice infected with reassortant reoviruses. The protocol is discussed in the legend to Fig. 2. The electropherotypes of the clones studied is given in Table 1. (A) Yield of virus for those viral clones that shed virus in the intestinal washings at high titers. (B) Viral clones that shed virus at low titer. Each point represents the mean of four animals ± SD. (◇) Clone 85, (○) clone 143, (●) clone 144, (■) clone 146, (▲) clone H14, (□) clone G2, and (△) clone 126.



The L2 dsRNA segment encodes the λ2 spike protein of reovirus (*8*). The spike is composed of pentamers of λ2 that form hollow tubes stretching from the viral core to the vertices of the icosahedral outer capsid (*9*). Removal of the λ2 protein eliminates the RNA transcriptase activity of isolated cores, and ultrastructural studies suggest that messenger RNA is actively extruded through the spikes during RNA transcription (*10, 11*). The λ2 protein also serves a major structural role in the virus. Temperature-sensitive mutants of L2 show defective assembly at the nonpermissive temperature (*12*). The L2 gene also governs the generation of defective virus in high-passage stocks in vitro. Reassortants bearing the L2 dsRNA segment of type 3 generate deletions at high frequency in high-passage cell cultures, whereas those bearing the L2 genes of type 1 do not (*13*).

The role of the L2 segment in determining the generation of deletion mutants suggested to us the possibility that the low shedding of type 3 Dearing might be related to high levels of interfering virus in the large intestine of mice infected with type 3 Dearing. The possibility of defective virus interference was investigated by coinfection of suckling mice with both parental strains (see Fig. 2). Both parental strains were inoculated into infector mice in experiments similar to the initial transmission experiments. Transmission occurred in 50 percent of littermates, a rate equivalent to that seen with type 1 alone (see Fig. 1). Thus the presence of the parental type 3 had little effect on the high shedding phenotype of type 1. These results suggest that high levels of interference from defective type 3 virus did not appear to cause the low yield. Similarly, maternal immunity does not account for the differences in transmission, since the NIH/Swiss mice used for the experiments were tested for the presence of antibody to reovirus type 1 and type 3 and found to be negative.

In summary, we studied transmission of

reoviruses under controlled conditions of host and environment and found that high transmission is a genetically determined property controlled by the L2 dsRNA segment. Release of high levels of virus (shedding) from the gastrointestinal tract is also a property controlled by the L2 segment, suggesting that the differences in transmission are mediated by the level of shedding. These findings indicate that a single reovirus gene can influence the capacity of the virus to be transmitted in the environment. A further understanding of the L2 gene and how other genes affect its properties should provide new insights into the behavior of viruses in the natural environment.

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17. We gratefully acknowledge the expert technical assistance of E. Freimont and A. Agulnick and the help of C. King in preparing the manuscript. This work was supported by grant 5R01 AI13178 of the National Institute of Allergy and Infectious Disease. Reassortant viruses were kindly provided by Max Nibert from the stocks of E. Brown and D. Drayna.

8 October 1985; accepted 15 April 1986

Suppression of Neurite Elongation and Growth Cone Motility by Electrical Activity

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Electrical activity may regulate a number of neuronal functions in addition to its role in transmitting signals along nerve cells. The hypothesis that electrical activity affects neurite elongation in sprouting neurons was tested by stimulating individual snail neurons isolated in cell culture. The findings demonstrated that growth cone advance, and thus neurite elongation, is reversibly stopped during periods when action potentials are experimentally evoked. A decrease in filopodial number and growth cone area was also observed. Thus, action potentials can mediate the cessation of neurite outgrowth and thereby may influence structure and connectivity within the nervous system.

ELECTRICAL ACTIVITY PLAYS A PROMinent role in communication within the nervous system. It has long been suggested that electrical activity might also play a role in shaping the morphology and connectivity of the nervous system both during development and in adult neuroplastic events (*1*). One plausible mechanism for such regulation is through the control of neurite outgrowth. While, in recent years, chemical factors have been shown to influence neurite outgrowth (*2*), a similar role for electrical activity has not been unequivocally demonstrated. However, electrical activity has been shown to regulate many other cellular processes including: the synthesis of acetylcholine receptors (*3, 4*), the synthesis of neurotransmitters and enzymes used in the production of neurotransmitters and hormones (*5*), the formation and pattern of synaptic connections (*6*), and sprouting at the neuromuscular junction (*7*). Electrical fields have been implicated in influencing the direction of neurite outgrowth (*8*), and shifts in the membrane potential induced by changes in ion concentrations or the addition of ionophores and growth factors can cause changes in the state of differentiation and the amount of neurite outgrowth in culture (*9*). By stimulating specific neurons growing in cell culture, we directly tested the hypothesis that action potentials can affect neurite outgrowth. Our findings show that the generation of action potentials is a sufficient signal to halt neurite outgrowth abruptly and reversibly.

We used cultured neurons from the snail *Helisoma*, as they afford a high level of resolution in the analysis of neurite outgrowth. Single identified neurons were removed from buccal ganglia and plated in cell culture (*10*). Within 3 days of plating, these neurons extended neurites via large growth cones that typically measured 20 μm in width. A direct test of the hypothesis required that three experimental conditions be met. First, growth cone advance should be quantitatively measured during stimulation of the neuron. Second, action potentials must be evoked at will and their occurrence monitored. Third, the techniques used to stimulate the neurons and record the responses must not interfere with the motile properties of the growth cones. We have now been able to satisfy all of these conditions in a single set of experiments with a total of 143 growth cones from 21 neurons. Quantitative measurements of the advance of each growth cone were readily obtained from photographs taken at constant time intervals (*11*). Direct stimulation was accomplished by extracellularly stimulating the somata of individual neurons during the period of neurite outgrowth. We initially tested two stimulation techniques. Penetrating the cell body with an intracellular microelectrode frequently stopped neurite elongation; in contrast, extracellular patch pipettes (*12*) attached to the soma membrane by a gigohm seal did not alter elongation (see below). Consequently, we used extracellular patch pipettes in these experiments. Because the patch pipette could not reveal the magnitude of membrane potential change during stimulation, we performed control experiments to evaluate the effect of current pulses delivered through the patch pipette by simultaneously recording from the soma with an intracellular microelectrode (Fig. 1).

Neurons were stimulated under current-clamp conditions with 20-msec pulses (amplitudes of 1 to 6 nA, depending on the seal resistance). Current pulses delivered through the patch pipette produced small depolarizations (approximately 10 mV in amplitude), which evoked action potentials (Fig. 1) as measured intracellularly (*13*). Furthermore, we could monitor these action potentials with the patch pipette as a result of capacitive coupling between the pipette and the cell. Thus, individual neurons could be stimulated extracellularly and the evoked action potentials could be recorded through the same noninvasive pipette.

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