

# Appearance of Defective Virions in Clones of Reovirus

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Virus obtained from five plaques of reovirus was serially passaged in L cells. Defective virions arose in each clone by the seventh passage. Such defective virions lacked the largest of the 10 segments of the double-stranded ribonucleic acid genome.

Recently, we have shown that some preparations of type 3 reovirus contain defective virions which multiply only in the presence of infectious virions (1). In the one case analyzed, the defective virions were shown to be lacking the largest of the 10 double-stranded ribonucleic acid (dsRNA) segments of the genome. Most clones of virus prepared by plaque isolation from a mixed population of complete and defective virions contained no detectable defective virions. In the present paper, it is shown that defective virions again appear during serial passage of such clones and that in each of five clones tested the defective virions lacked the largest dsRNA segment of the genome.

There is only a very slight difference in buoyant density in CsCl between complete and defective virions. However, the two types of virion can be distinguished by the following method which, both in the present and previous work, has provided a criterion to determine whether a viral population contains defective virions. A culture of L cells is infected with virus and labeled with <sup>3</sup>H-uridine in the presence of actinomycin D. The resulting progeny are purified and then digested with chymotrypsin to remove quantitatively the capsomeres from virions and release the viral core particles (1-3). This chymotrypsin digest is subjected to isopycnic centrifugation in a preformed gradient of CsCl, and the presence of cores is detected by assaying the fractions for <sup>3</sup>H. Heavy (H) cores band at a density of 1.43 g/ml and are derived from complete, infectious (H) virions. Light (L) cores have a density of 1.415 g/ml and are obtained from defective (L) virions. H cores are infectious for L cells, whereas L cores almost certainly are not infectious. All of

the procedures involved in this work have been described in detail elsewhere (1).

To obtain a virus population free from pre-existing L virions, a single plaque formed by H cores was picked and passaged twice in monolayers of L cells, and the second lysate was plated on monolayers of L cells to give three to four plaques per plate. Five of these plaques were picked and used for the rest of the study. Virus from each plaque was used to infect a monolayer

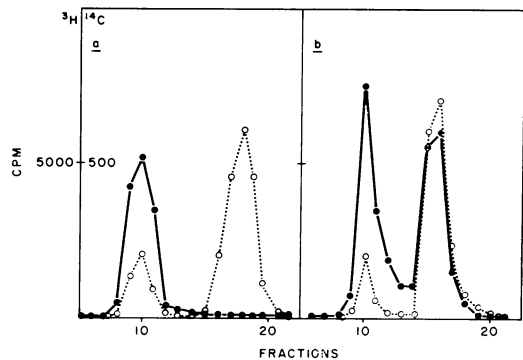


FIG. 1. Separation of heavy (H) and light (L) cores by isopycnic centrifugation in a gradient of CsCl. Purified virus was digested with chymotrypsin (100  $\mu$ g/ml for 30 min at 37 C), and the products were layered over a preformed gradient of CsCl ( $\rho = 1.35 - 1.45$ ) and centrifuged at 41,000 rev/min for 3 hr in an SW-50 rotor. The <sup>3</sup>H-virus was a purified population obtained after serial passage of a single plaque. <sup>14</sup>C-virus was a purified preparation known already to contain H and L virions and was used as a density marker for H and L cores. <sup>3</sup>H- and <sup>14</sup>C-labeled viruses were mixed before chymotrypsin digestion. (a) Cores obtained from third-passage progeny virus. (b) Cores from eighth-passage progeny. Symbols: ●, <sup>3</sup>H-labeled cores; ○, <sup>14</sup>C-labeled marker cores. Direction of sedimentation is from right to left. H cores band at a density of 1.43 g/ml; L cores band at 1.415 g/ml.

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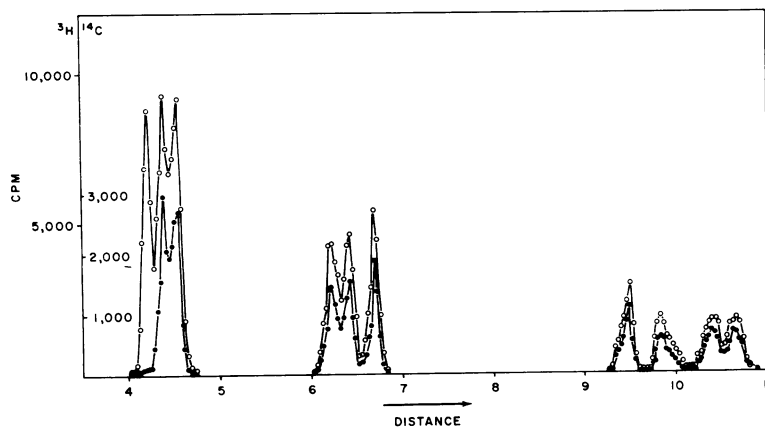


FIG. 2. Electrophoretic analysis on polyacrylamide gel of dsRNA from defective cores.  $^3\text{H}$ -labeled L cores were obtained from a gradient as in Fig. 1b and mixed with  $^{14}\text{C}$ -labeled, purified virus that contained only H cores (passage 3 of a single plaque). dsRNA was obtained from the mixture by phenol extraction and analyzed by electrophoresis for 48 hr at 8 ma/tube on 5% acrylamide gel (1). The gel was stained with methylene blue and cut into 0.3-mm slices with a freezing microtome; the slices were assayed for  $^3\text{H}$  and  $^{14}\text{C}$ . Symbols: ●,  $^3\text{H}$ ; ○,  $^{14}\text{C}$ . Direction of migration is from left to right.

of L cells in a petri dish (passage 1), and then monolayers of L cells in 1-liter blake bottles were infected with the resulting progeny (passage 2). With these lysates, suspension cultures of L cells were infected at a multiplicity of 30 plaque-forming units (PFU)/cell (passage 3). Portions of each infected culture were labeled with  $^3\text{H}$ -uridine in the presence of actinomycin D between 7 and 20 hr after infection, and the resulting progeny were tested for the presence of H and L cores. As shown in Fig. 1a, only H cores were detectable.

Virus from the passage 3 lysates was serially passaged in suspension cultures of L cells at multiplicities of infection of 30 PFU/cell. By passage 8, all five clones contained defective virions as shown by the presence of L cores in the CsCl gradient of Fig. 1b. In fact, passage 7 progeny already contained H and L cores in the proportion of 10 to 1, although this was the first passage at which L cores could be detected. Ninth-passage progeny virus had a ratio of H to L cores of approximately 1 to 4, and this ratio was unchanged by further serial passage. Large changes in multiplicity of infection, 5 to 100 PFU/cell, did not change the ratio further.

Since the five clones independently accumulated defective (L) virions, it was of interest to determine whether all had the same defect. To this end,  $^3\text{H}$ -labeled L cores were obtained from each clone, and the dsRNA was extracted and subjected to electrophoretic analysis on polyacrylamide gel as described in the legend to Fig. 2. As shown for one clone in Fig. 2, the L cores lacked the largest (slowest moving) dsRNA segment. L cores from the other four clones gave

the same result as did the L cores in the previous work (1).

Thus, defective virions arise frequently in clonal populations of type 3 reovirus and it is interesting to speculate on the reason. Recent results have indicated that the fragmentation of the reovirus genome is not merely an artefact of the extraction procedure but that the dsRNA segments exist as such in situ (S. Millward and M. Nonoyama, Cold Spring Harbor Symp. Quant. Biol., *in press*). If this is so, an occasional mistake in replication might be expected to occur, resulting in a virion with a deleted segment. One might also expect to find a spectrum of defective virions with any one of the 10 genomic segments missing, but, thus far, all six defective populations examined have lacked the same segment. Perhaps there is some selective pressure against other types of defective virion developing during viral multiplication. In any event, it is worth noting that if one could obtain defective virion populations, each with a different segment deleted, they would be of great assistance in studying the genetics of reovirus.

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#### LITERATURE CITED

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