# CONSERVATION AND TRANSFER OF THE RNA OF ANIMAL VIRUSES

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It has been shown that all the components of viral DNA are fully conserved during the replication of phage T2 in E.coli and are transferred to progeny particles (Cocito and Hershey, 1960). Such a conclusion is valid in spite of the fragmentation of viral chromosome which has been reported to occur during the multiplication of T-phages, as an event linked to genetic recombination (Kozinski, 1961). Since the patterns of nucleic acid metabolism in cells infected with DNA- and with RNA-viruses are different (Doi and Spiegelman, 1963; Cocito, 1963 a,b), it would be interesting to know whether the components of viral RNA are conserved and transferred as are the components of viral DNA.

Moreover, the biophysical and biological properties of the chromosomes of enteroviruses and myxoviruses, two groups of particles which contain RNA, are quite different. RNA molecules that have molecular weights of about two million and are fully infectious have been isolated from enteroviruses. Conversely, myxoviruses have been reported to carry RNA molecules of the order of 30 million, and isolation of infectious nucleic acids from these particles has failed in most cases (cf. the reviews of Schramm, 1959; Schuster, 1960; Colter and Ellem, 1961; Cocito, 1963b). One could, then, speculate that the RNAs of these two groups of viruses might follow two different replication patterns and have different fates.

The fate of RNA of enteroviruses and myxoviruses multiplied in different host cells, in vitro and in vivo, has been the object of our

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investigation. Transfer of parental nucleic acid to progeny particles was judged on the basis of the conservation of a characteristic ratio of labeled ribonucleotides in the RNA of viruses multiplied for several generations in unlabeled cells. A microanalytical procedure recently described (Cocito et al., 1963; Cocito and Laduron, 1963) for electrophoretic analysis of nucleotides proved to be a suitable method for this study.

In vitro experiments were performed as follows : Monolayers of HeLa cells infected with about 50 particles of Coxsackie virus strain B5 per cell were incubated at 37° C with medium containing $P^{32}$-orthophosphate. To the supernatant collected 24 hr later, 500 µg of yeast RNA per ml was added as carrier, and labeled virus was precipitated by lowering the pH to 3.5 with HCl at 0° C. The sediment was dissolved in saline buffered at pH 7.4, and the suspension of labeled particles of first generation was then divided into two portions. One aliquot was used for nucleotide analysis of viral RNA. For this purpose labeled virus was reversibly adsorbed on HeLa cells at 0° C (Fenwick and Cooper, 1962), extracted with phenol and duponol at 0° C ( Cocito and De Somer, 1961), and then fractionated by chromatography on columns of methylated albumin (Lerman, 1955; Mandell and Hershey, 1960). This method of fractionation proved able to separate viral RNA from cellular nucleic acids (Cocito et al., 1961; Kubinski et al., 1962; Cocito, 1963 a,b). Viral RNA was then precipitated with ethanol, dialyzed at 0° C, concentrated, and hydrolyzed with alkali (0.5 M KOH, 37° C, 18 hr), and the resulting 2'3'-$P^{32}$-ribonucleotides were separated by electrophoresis on strips of cellulose acetate, eluted and counted. The other aliquot of the labeled virus preparation was used to infect monolayers of unlabeled HeLa cells. From the 24 hr lysate the virus of second generation was precipitated and purified as described. Again one part of the virus suspension was employed for nucleotide analysis of viral RNA, and the remaining part for a third replication cycle in unlabeled cells. The efficiency of transfer of parental label to progeny can be evaluated from the following data. Of the radioactivity of purified labeled virus of second generation, 4.5 % was present in the low-speed sediment of cells and debris, and 95 % in the supernatant of the 24 hr lysate. Of the latter fraction 36.4 % was recovered by precipitation at pH 3.5, or by adsorption on HeLa cells at 0° C, and 63.6 % was left in the supernatant fluid. It

was concluded, therefore, that parental label was conserved through subsequent generations with an efficiency of transfer of about 30 %. Data summarized in Table 1 show that the ratio of $P^{32}$-nucleotides in the RNAs isolated from viral particles of first, second, and third generation was practically the same. Results obtained with another enterovirus, poliomyelitis Mahoney strain, were quite similar.

In vivo experiments were carried out with influenza B virus, strain Lee, replicated in the chorioallantoic membrane. Into the allantoic cavity of 9-day-old embryonated eggs were injected 0.1 mC of $P^{32}$-orthophosphate and, 24 hr later, the suspension of viral particles. From the allantoic fluid collected after incubation for 48 hr at 39° C the virus was purified by reversible adsorption on 10 % suspension of chick erythrocytes at 0° C. The cells were centrifuged and repeatedly washed with ice-cold buffered saline, and the virus was finally eluted by incubation for 2 hr at 37° C with 0.1 M NaCl buffered at pH 7.4 with 0.1 M Na phosphate. One aliquot of labeled virus of first generation was extracted at 60° C with water-saturated phenol and 2 % Na dodecyl sulfate and the protein-free viral RNA was purified by column chromatography and then hydrolyzed for nucleotide analysis (Table 1). The remaining part was injected into the allantoic cavity of 9-day-old unlabeled eggs for a second replication cycle. After incubation for 8 hr at 39° C, the chorioallantoic membranes were removed, repeatedly rinsed in buffered saline, and further incubated in vitro with Eagle-Earle medium supplemented with 10 % decomplemented calf serum. About 18 % of the radioactivity of labeled virus was recovered in the membranes isolated at this stage : 26 % of this amount was found intracellularly, and 74 % extracellularly, at the 48th hour of the replication cycle in vitro. Of the latter fraction about 31 % was adsorbed reversibly at 0° C on suspension of chick erythrocytes, and was considered as associated with purified particles of second generation. Thus, the efficiency of transfer of parental label to progeny was about 4 %. Data reported in Table 1 indicate that the RNA of viral progeny bears a nucleotide composition similar to that of the original injected virus. Similar results were obtained with two other myxoviruses, influenza A, strain PR8, and fowl plague virus.

A possible objection to our results is that the RNA of the infected virus might be degraded and the resulting building blocks reincorporated with high efficiency into the newly formed viral particles. This pos-

Table 1

NUCLEOTIDE COMPOSITION OF RNA FROM VIRUSES OF SEVERAL GENERATIONS

| Viruses species | gener. | Cells | RNA | Nucleotide composition (%) of the RNAs CMP | AMP | UMP | GMP | G + C |
|---|---|---|---|---|---|---|---|---|
| CB5 | 1 | | vRNA[a] | 20.6 | 27.5 | 28.9 | 23.0 | 43.6 |
| | 2 | | vRNA | 21.1 | 27.0 | 29.4 | 22.5 | 43.6 |
| | 3[b] | | vRNA | 20.8 | 29.6 | 25.5 | 24.1 | 44.9 |
| | 3[c] | | vRNA | 19.7 | 30.0 | 27.3 | 23.0 | 42.7 |
| | | HeLa | R-RNA[a] | 29.2 | 19.9 | 20.2 | 30.7 | 59.9 |
| | | | S-RNA[a] | 29.8 | 18.8 | 22.1 | 29.3 | 59.1 |
| IBL | 1 | | vRNA[a] | 25.7 | 22.1 | 32.3 | 19.9 | 45.6 |
| | 1 | | vRNA | 26.0 | 21.7 | 32.1 | 20.2 | 46.2 |
| | 2 | | vRNA | 26.4 | 26.1 | 29.7 | 17.8 | 44.2 |
| | | CAC | R-RNA[a] | 27.5 | 17.1 | 20.8 | 34.7 | 62.2 |
| | | | S-RNA[a] | 27.9 | 18.3 | 25.3 | 28.5 | 56.4 |

Nucleotide composition of viral ribonucleic acid (vRNA) from purified particles of Coxsackie B5 (CB5) and influenza B-Lee (IBL) of first, second, and third generation is compared with the nucleotide ratios of ribosome (R-RNA) and transfer (S-RNA) ribonucleic acids of the corresponding host cells (HeLa and chorioallantoic cells or CAC).
[a] RNA purified by chromatography on methylated albumin column ; [b] extracellular virus ; [c] intracellular virus.

sibility, however, can be ruled out when the size of the precursor pool, which is enormous in mammalian cells, is compared with the relatively small number of particles released by one infected cell (200 to 300). In other words, though labeled nucleotides could originate by hypothetical degradation of the injected virus, the specific activity of the nucleotide pool would be so low that viral progeny would not incorporate appreciable amounts of parental radioactivity. Finally, the different efficiency of transfer of parental label displayed by the two groups of viruses can be explained by their different content of nucleic acid (1 % for myxoviruses and 27 % for enteroviruses) and phospholipids (about one third of the weight of a myxovirus is accounted for by lipids).

We may, then, conclude that conservation and transfer of viral RNA is a common feature of all the animal viruses that we have so far examined, with the possible exception of Rous sarcoma virus

(work in progress). In the latter case we have observed that a transfer, if any, might occur only with very low efficiency : defectiveness and interference make it difficult to decide with certainty whether the RNA of this tumor virus is conserved. It is tempting to extrapolate the present and previous results, and to suppose that conservation and transfer of viral genome occurs during the replication process of both DNA- and RNA-viruses. It can be mentioned that minute phages carrying single-stranded DNA represent so far the only case in which transfer of viral nucleic acid definitely does not occur (Sinsheimer et al, 1962).

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