A Possible Subunit Structure of Rous Sarcoma Virus RNA

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Rous sarcoma virus (RSV) RNA from both the BRYAN and SCHMIDT-RUPPIN strains has been extracted as a single piece with a sedimentation constant of 65 to 70S (Robinson, Pitkanen & Rubin, 1965; Harel et al. 1965). According to the relationship between sedimentation constant and molecular weight established for smaller single-stranded RNAs (Spirin, 1963), this value would correspond to a molecular size of about $10^7$ daltons, and, therefore, to the entire RNA content of the mature virion (Crawford & Crawford, 1960). We have since obtained evidence that, unlike some other RNAs of cellular and viral origin, RSV-RNA can be dissociated irreversibly into smaller pieces of approximately equal size, by using dimethylsulphoxide, a solvent known for its ability to disrupt the secondary structure of RNAs (Katz & Penman, 1966; Kelly & Sinsheimer, 1967). A similar independent finding has been recently reported for the BRYAN strain of RSV-RNA by Duesberg (1968).

In control experiments, we first found that the relative sedimentation rates of the two ribosomal RNA components and of encephalomyocarditis virus RNA were unchanged in 99% dimethylsulphoxide-sucrose gradients according to the technique of Kelly & Sinsheimer (1967), and, after this treatment, in aqueous gradients, provided that special care was taken against nuclease contamination throughout all steps of the preparation; i.e. phenol extractions were carried out in the presence of bentonite and versene (Montagnier & Sanders, 1963; Montagnier & Bellamy, 1964). Moreover, the dimethylsulphoxide treatment did not affect the infectivity of encephalomyocarditis virus RNA, which always sedimented at the same position as the labelled material (L. Montagnier, unpublished).

Tritium-labelled RSV-RNA was then prepared from the BRYAN high-titre strain or from a high-titre variant of the SCHMIDT-RUPPIN strain (Goldé & Latarjet, 1966) and sedimented in sucrose gradient as previously described (Harel et al. 1965). Fractions of the 65 to 70S peak were pooled and 0.1 ml aliquots were resedimented under various conditions, with 14C-labelled or unlabelled ribosomal RNA and 32P-labelled encephalomyocarditis virus RNA used as velocity markers (Fig. 1).

The purity of the 65 to 70S RSV-RNA is shown in the control sedimentation in aqueous sucrose gradient (Fig. 1a). When sedimented in a 99% dimethylsulphoxide-sucrose gradient, however, the relative sedimentation rate of RSV-RNA changed markedly. Under these conditions, the RSV-RNA sedimented just behind the encephalomyocarditis virus RNA and in front of the fast sedimenting component of ribosomal RNA (Figs. 1b, d). The effect of dimethylsulphoxide treatment was irreversible, for when the RSV-RNA peak was taken from the dimethylsulphoxide gradient and then sedimented in aqueous medium, its position between the two marker RNAs remained unchanged (Fig. 1c).

A trail of slower sedimenting polydisperse material was always observed in RSV-RNA, and varied in amount from one preparation to another: it was minimal with RNA from fresh virus preparations (Fig. 1d), and increased considerably with 65S
RNA extracted from frozen virus in the presence of pronase (Harel et al. 1965). No discrete and constant peak could be recognized in this trial; thus it is likely that it represents fragments originating from breaks located randomly in the RNA molecule.

In contrast, the main peak was constantly found in all preparations at the same sedimentation velocity. It can be concluded, therefore, that the major part, if not the totality of RSV-RNA, can be dissociated into units of identical or similar size, since the technique used was not adequate to detect small variations in size.

The fact that the BRYAN strain RSV, which is associated with an excess of RAV particles (Hanafusa, 1964) and the SCHMIDT-RUPPIN strain RSV, which does not appear to be accompanied by a helper lymphomatosis virus, both yielded the same RNA subunits further suggests that their production in the presence of dimethylsulphoxide is a feature common to all viruses of the avian leukemia–sarcoma group.
Size and number of the units. The S value of encephalomyocarditis virus RNA is 35 to 37S (Burness, Vizoso & Clothier, 1963; Montagnier & Sanders, 1963). From the position of their peak in the gradients compared to this marker and to the ribosomal marker, an S value of 32 to 34S can be assigned for the RSV–RNA pieces obtained in dimethylsulphoxide. Using different markers, a value of 36S was obtained by Duesberg (1968). Assuming that the configuration of the units in moderate ionic strength is similar to that of other single-stranded RNAs, a molecular weight of 2.5 x 10^6 daltons can be derived for a 34S value from the Spirin’s formula (1963). There would thus be room for four such subunits in a whole RSV–RNA molecule.

The calculated size of the RSV–RNA subunits further agrees with data obtained with the electron microscope. Indeed, the modal length of the marker encephalomyocarditis–RNA measured on micrographs was recently found to be 2.4 μm. (N. Granboulan & L. Montagnier, in preparation), whereas that of avian myeloblastosis virus–RNA, which appears to have the same size as that of RSV–RNA (Robinson, Robinson & Duesberg, 1967), was shown to be 8.7 μm. (Granboulan, Huppert & Lacour, 1966): one quarter of this length would be 2.15 μm., slightly less than that of encephalomyocarditis virus RNA, as expected. It should be further noted that fragments of about 2.1 μm. were actually seen in large numbers by Granboulan et al. in their avian myeloblastosis virus–RNA preparations in which 8 M urea was used for spreading the RNA. Thus it is likely that some viral RNA molecules were dissociated by this treatment in the same way as by dimethylsulphoxide.

The significance of the subunit structures obtained by Duesberg and ourselves is unknown. They might result from fracture of the large viral RNA molecule at equidistant ‘weak points’ in the chain, but they could also correspond to replication units reassembled before virus maturation. These units could further be genetic units coding for different viral components and functions. Two results are consistent with this hypothesis. First, the curve for inactivation of the transforming capacity of RSV by X-rays (Latarjet & Goldé, 1962) is similar to that for inactivation of the infectivity of...
tobacco mosaic virus–RNA (Ginoza & Norman, 1957), suggesting similar target sizes (Latarjet & Chamaillard, 1962). Therefore, the transforming function of RSV could be coded by an RNA molecule having the size of one of the postulated subunits. Secondly, irradiation of the sR–RSV by γ-rays can give rise to deficient mutants falling in two classes, some having lost the infective capacity while remaining oncogenic (Goldé & Latarjet, 1966), the others having lost the oncogenic function while being capable of giving progeny (Goldé, in preparation). Since the radiation doses required to produce either type of mutant were similar, the infective capacity may also be carried by a single subunit.

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REFERENCES


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