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Electron Microscopy Study of Reovirus Reaction Cores

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A combined staining and shadowing method has been used to enhance the contrast of both reovirus cores and the RNA product that they have synthesized. After limited synthesis when all RNA strands associated with the core are nascent, many cores are observed that have more than 10 strands associated with each particle. The lengths of individual nascent mRNA molecules are not identical: many strands are extruded in the form of loops. These observations are consistent with the notion that synthesis of individual mRNA molecules occurs simultaneously at different rates at a number of different sites. The sites of extrusion are probably the 12 hollow projections located on the surface of the core.

Reovirus contains an RNA transcriptase that is an integral part of the virus particle. Treatment of purified virions with α-chymotrypsin (14) or heat (5) removes the outer viral capsomeres and results in the activation of the transcriptase. In vitro, the transcriptase synthesizes copies of all ten segments of viral RNA and the resulting 10 single-stranded mRNA molecules correspond in size and sequence to those synthesized in vivo (8). Cores in the process of synthesizing RNA in vitro have been termed reaction cores (16).

Since the in vitro product synthesized by the transcriptase consists of intact mRNA strands, it has been possible to study the initiation of RNA synthesis and the time taken for the three size-classes of mRNA (*l*, *m*, and *s*) to be synthesized. These studies have demonstrated that the time taken for the synthesis of intact molecules at 37 C is approximately 1 to 2 min for *s*-sized mRNA, 4 to 5 min for *m*-sized molecules, and 8 to 10 minutes for *l*-sized molecules (2, 16).

Biochemical studies of the reovirus RNA transcriptase have been carried out by following the incorporation of radioactive triphosphate into an acid-insoluble product. However, there is no way in which the average product synthesized by a complete reaction mixture, containing 10^{13} to 10^{14} particles, can be related to the products synthesized by individual reovirus cores.

In attempts to circumvent this restriction, Gillies, Bullivant, and Bellamy (6) utilized the Kleinschmidt spreading technique to examine individual reaction cores by electron microscopy. These studies demonstrated that not all cores were able to synthesize mRNA in vitro as evidenced by the fact that most of the particles lacked attached RNA strands. Those reaction cores that were observed frequently exhibited more than one mRNA molecule, suggesting that each core was capable of simultaneously synthesizing a number of strands from a number of discrete enzymic sites.

A limitation of this preliminary study was that the detail of the reaction cores was usually obscured by the heavy deposits of platinum-palladium shadow used to enhance the contrast of the single-stranded RNA. Therefore, we have used a combination of staining and shadowing to permit the structural detail of both cores and RNA to be viewed simultaneously. Using this technique we have studied reaction cores at very short incubation times when all the RNA attached to cores consists of nascent mRNA molecules.

MATERIALS AND METHODS

Cells and virus. Reovirus type III (Dearing strain) was propagated at 34 C in mouse L cells by using established methods (3). Virus was purified from the infected cells by the method of Smith et al. (17) and stored in 1× SSC at 4 C prior to use.

Preparation of viral cores. Viral cores were prepared by digesting purified virus (1 mg/ml in 1× SSC) with 50 μg of α-chymotrypsin per ml for 90 min at 37 C (14). The resulting cores were separated from the digest mixture by isopycnic sedimentation on preformed CsCl gradients (ρ = 1.4 to 1.5; SW65 rotor, Spinco L2-65B, 2 h, 45,000 rpm). The band of cores that formed at ρ = 1.43 was collected with a Pasteur pipette and diluted with 1× SSC, and the cores were

concentrated by centrifugation (50,000 rpm, SW 65 rotor, 25 min). Cores were resuspended in 1× SSC prior to use.

RNA transcriptase reaction mixture. The complete reaction mixture contained the following components in a volume of 1.5 ml: Tris-hydrochloride buffer, pH 8.5, 30 μmol; $MgCl_2$, 5.0 μmol; ATP, GTP, CTP, and UTP, 0.5 μmol each; [^{3}H]GTP, 10 to 200 μCi; purified reovirus cores, approximately 1 mg. After incubation at 37 C, 0.4-ml samples were removed from the reaction mixture with a chilled pipette and synthesis was terminated by rapidly chilling the sample to 0 C. RNA synthesis would not continue under these conditions since it is known that the transcriptase shows little synthetic activity below 30 C (11). Reaction cores were further purified by isopycnic sedimentation on CsCl gradients (see Results).

Kleinschmidt-surface spreading. Reaction cores taken from CsCl gradients were diluted with the components of a cytochrome-*c* spreading mixture to give a final concentration of 100 μg of cytochrome-*c* per ml and 1% formaldehyde. The resulting mixture containing the reaction cores was spread by a modification of the drop method of Inman and Schnös (10). A 1.2-ml drop of distilled water was pipetted onto a square of Parafilm (American Can Co.). The end of a Pasteur pipette which had been sealed in a gas flame was placed in the drop at an angle of approximately 45°. A 20-μliter amount of the spreading solution which contained the reaction cores was expelled from a 20-μliter disposable Micropet (Clay Adams) onto the exterior of the 45° glass surface and allowed to drain onto the surface of the drop of water. A 0.1-ml amount of water was removed from within the drop with a hypodermic syringe in order to compress the film. The film was then transferred to carbon-coated grids.

Electron microscopy. Copper grids were coated with collodion by standard water/surface film techniques. Carbon was evaporated onto the surface of the collodion-coated 400 mesh grids by using heated carbon rods in an A.E.I. vacuum evaporator. The collodion was removed from the grids by treatment with amyl acetate immediately before use. Cytochrome-*c* films containing reaction cores were transferred to grids by touching grids to the surface of the drop. Grids were rinsed in distilled water for 1 s, 0.08% uranyl acetate in 95% ethanol for 20 s, 100% ethanol for 1 s (13). They were then rotary shadowed with platinum-palladium at an angle of 2 to 3° in an A.E.I. evaporator. A Philips EM 200 was used for examination and photography of the grids. Contour lengths of RNA molecules were determined by projecting the negatives onto paper, tracing the profiles, and measuring strand lengths with a map measurer. Magnifications were determined by measurements carried out on photographs of a calibration grid.

Preparation of reovirus mRNA. Purified reovirus (10 mg in 10 ml of 1× SSC) was digested with 50 μg of α-chymotrypsin per ml for 90 min at 37 C. The resulting cores formed a floccular aggregate and were collected by low-speed centrifugation (2,000 rpm International PR2 centrifuge) and resuspended in 1× SSC. The concentrated cores were added to a standard (1.0 ml) transcriptase reaction mixture and incubated at 37 C for 10 min. At the end of this incubation the cores were concentrated by low-speed centrifugation, the supernatant was replaced with a freshly prepared reaction mixture, and the cores were reincubated for a further 30 min. After four successive incubations the supernatants that contained the bulk of the newly synthesized mRNA were pooled and extracted with water-saturated phenol. The RNA was precipitated from the phenol supernatant by addition of two volumes of ethanol, and the resulting mixture was held at −70 C overnight. The precipitated RNA was collected by centrifugation, redissolved in 1× SSC, and further purified by an additional precipitation step from 2 M LiCl (1). The resulting single-stranded mRNA was collected by centrifugation and held under 70% ethanol prior to use.

Zone sedimentation on sucrose gradients. Single-stranded RNA synthesized by reovirus cores was analysed by zone sedimentation on 15 to 30% (wt/wt) sucrose gradients containing 0.1 M LiCl, 0.01 M Tris, pH 7.0, and 0.5% *n*-lauroyl sarcosine. Fractions were collected and analyzed as described previously (4).

RESULTS

Effect of staining and shadowing techniques on the contrast achieved for single-stranded reovirus mRNA. To establish the optimum conditions for spreading both single-stranded RNA and reovirus cores a series of preliminary experiments were carried out that involved spreading reovirus cores and messenger RNA both singly and in combination. Satisfactory contrast was achieved for purified reovirus mRNA after staining with 0.08% uranyl acetate in anhydrous ethanol (Fig. 1d), a procedure devised by Robberson (13).

However, this staining procedure positively stained reovirus cores and no surface detail of the core was evident (Fig. 1b). In contrast to the lack of surface detail obtained for stained cores, cores first stained with uranyl acetate and then shadowed showed good surface projections with the platinum-palladium (Fig. 1a). As well as enhancing the contrast obtained for cores, shadowing of the grid also resulted in somewhat improved contrast of the single-stranded RNA (Fig. 1c). Although excellent contrast was obtained by using this combined staining and shadowing procedure, there was often significant variation in contrast achieved on different areas of the same grid, presumably as a result of surface irregularities which results in a variable amount of shadow falling on the grid.

Analysis of the transcriptase reaction products on isopycnic CsCl gradients: the problem of aggregation. It has been reported that the presence of a nuclease-inhibitor such as macaloid is necessary for synthesis of intact mRNA (16). We have found that this material causes reaction cores to aggregate and pellet on

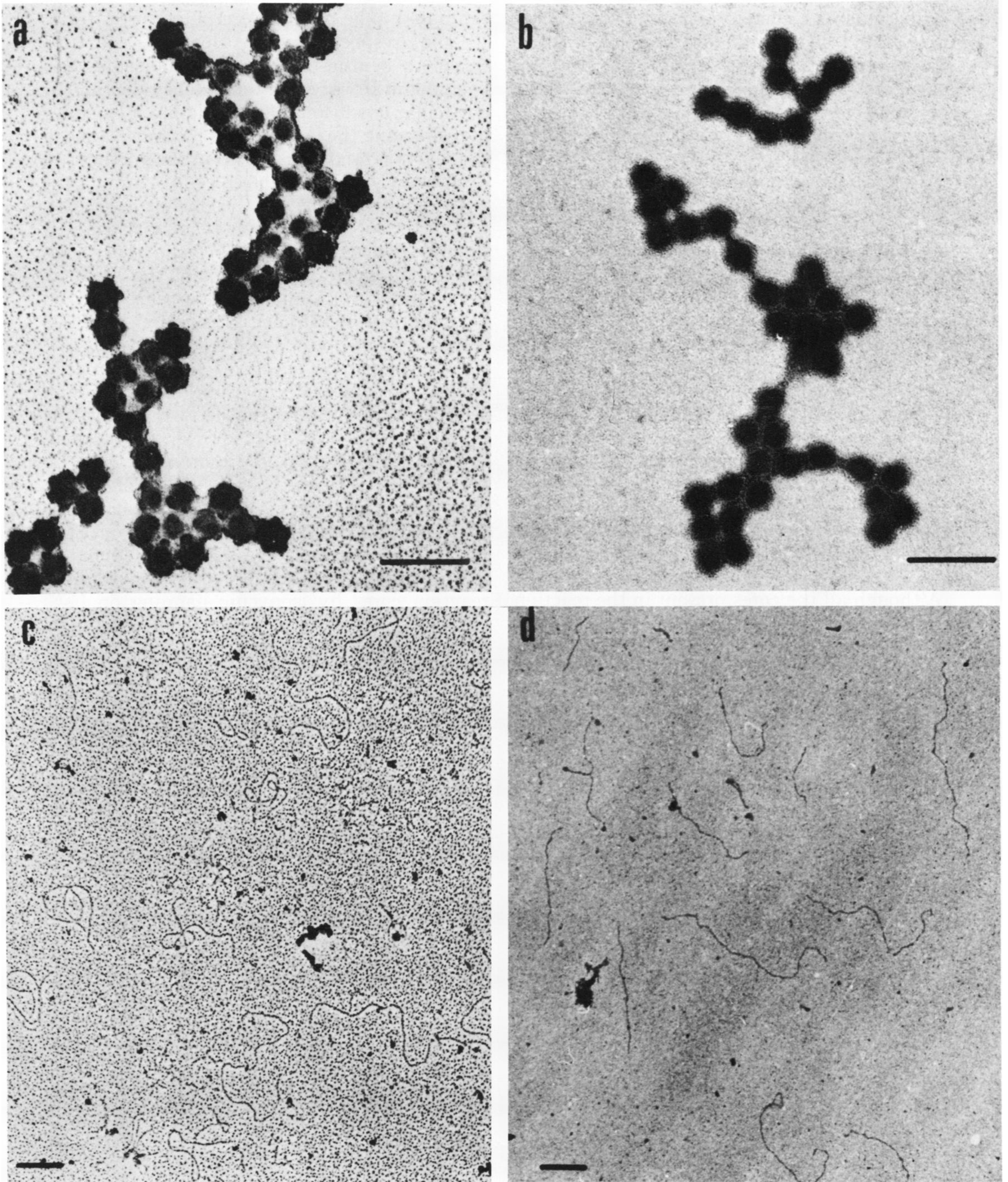

FIG. 1. *Effect of staining and shadowing on the contrast achieved for reovirus single-stranded RNA and reovirus cores. Reovirus single-stranded RNA was synthesized in vitro by the RNA transcriptase and purified (see text). Reovirus cores were prepared by α-chymotrypsin digestion and purified by isopycnic banding on CsCl gradients. Both materials were surface-spread by the drop method of Inman and Schnös (10). (a) Reovirus cores stained with uranyl acetate and then lightly shadowed with platinum-palladium; (b) reovirus cores stained with uranyl acetate; (c) reovirus single-stranded RNA stained with uranyl acetate and then lightly shadowed with platinum-palladium; (d) reovirus single-stranded RNA stained with uranyl acetate. The bar represents 0.2 μm.*

CsCl gradients. Therefore, macaloid was not incorporated in the standard reaction mixture used in this study and its absence did not appear to affect the integrity of the RNA provided that the cores used for RNA synthesis were previously purified by isopycnic banding on CsCl gradients.

Aggregation was also found to be a function of the time for which RNA synthesis was permitted to proceed. The results of a typical experiment are shown in Fig. 2. The reaction mixture held at 0 C or lacking ATP (nil-synthesis sample) yielded a single opalescent band of cores which formed at a buoyant density of 1.43. As synthesis proceeded, an increasing proportion of the opalescent core band shifted to a buoyant density greater than 1.43, due to the synthesis of mRNA that remains attached to the core particles. After 15 min of synthesis, aggregation became evident; the total core material present became floccular and shifted to a buoyant density of 1.47 to 1.48. This floccular material presumably corresponded to the band of reaction cores described by other workers (16).

Although all the cores present in the reaction mixture shifted to a density of 1.48 it is unlikely that all of the cores became reaction cores since electron microscope examination of the floccular aggregate revealed that most of the cores that were present were unreactive. Since it was difficult to individually identify or examine reaction cores once flocculation had taken place, incubation times used in this study were less than 8 min.

Nature of RNA strands associated with reaction cores. The time taken for the reovirus RNA transcriptase to synthesize complete *l*, *m*, and *s* mRNA molecules at 37 C has been estimated to be 2, 4, and 8 min (16) or 1, 5, and 10 min, respectively (2). However, the time taken to synthesize complete molecules has been found to vary markedly depending on the particular conditions of the reaction. For example, changing the concentration of viral cores in the reaction mixture resulted in the synthesis of complete *l*, *m*, and *s* molecules in 1 min (2).

To determine whether the strands associated with reaction cores after short incubations represented nascent strands or completed molecules that had failed to detach, the products synthesized in a 5-min reaction were analyzed by zone sedimentation on sucrose gradients (Fig. 3).

As anticipated significant amounts of full-length molecules of the three size classes of reovirus mRNA were synthesized during the 5-min reaction (Fig. 3a). However, only a small fraction of the total radioactivity (15%) remained associated with the reaction cores when they were subjected to isopycnic banding on the CsCl gradient; this material did not consist of intact *l*, *m*, or *s* molecules (Fig. 3b). Therefore, most completed molecules are removed from reaction cores by isopycnic banding on CsCl gradients and those molecules that remain attached comprise partially synthesized (nascent) strands. Thus, although we may anticipate that under some conditions intact strands will be synthesized during very short incubations, most of these strands will be removed during the subsequent purification of the reaction cores.

Appearance of reaction cores under the electron microscope. When cores were permitted to synthesize RNA for 1 min and the resulting "reaction cores" were examined by electron microscopy, many particles were observed that had attached nascent RNA strands

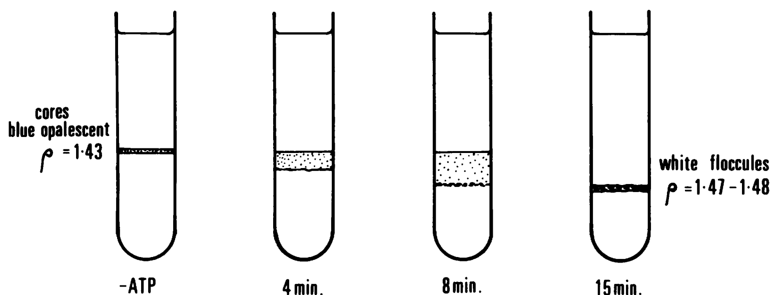

FIG. 2. *Effect of RNA synthesis on the banding properties of reovirus cores in CsCl gradients. Purified reovirus cores were incubated at 37 C in a 1.5-ml standard RNA transcriptase incubation mixture (see text). A reaction mixture lacking ATP was incubated as a control. After 4, 8, and 15 min of incubation, samples were chilled to 0 C and transferred to a 4.4-ml preformed CsCl gradient (ρ = 1.4 to 1.5). Centrifugation was carried out for 2 h at 45,000 rpm in the SW65 rotor of a Spinco L2-65B. Densities were determined by refractometry using the relationship ($\rho^{25} = 10.8601_n 25 - 13.4974$) (9).*

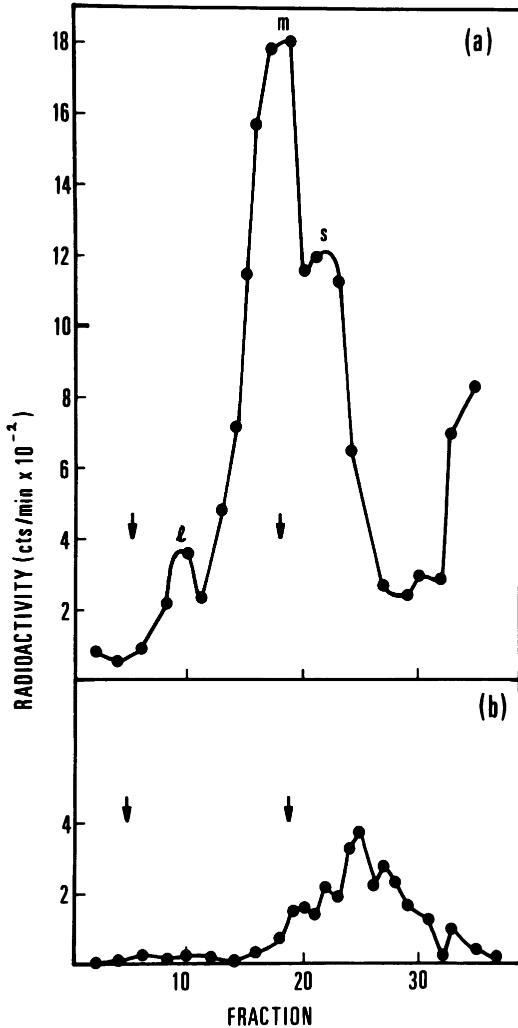

FIG. 3. *Demonstration that strands associated with reaction cores are nascent. Purified reovirus cores (2 mg) were incubated at 37 C in a 1.2-ml standard reaction mixture containing 80 µCi of [³H]GTP (see text). After 5 min of synthesis the sample was chilled and divided into two portions. n-Lauroyl sarcosine was added to one portion to a concentration of 0.5% and the sample was incubated at 37 C for 10 min. Reaction cores were purified from the other portion by isopycnic banding on a CsCl gradient (see text). The sample was then brought to 0.5% n-lauroyl sarcosine and incubated at 37 C for 10 min. Both RNA samples were then applied to 15 to 30% sucrose gradients and centrifuged for 20 h at 20 C and 24,000 rpm in the SW27 rotor of a Spinco L2-65B centrifuge. Arrows indicate the position of 18 and 28S, ¹⁴C-labeled L cell rRNA marker. (a) RNA extracted from the total unfractionated reaction mixture; (b) RNA associated with reaction cores; (l, m, s) three size classes of reovirus mRNA.*

of variable length (Fig. 4). Thus it is likely that synthesis proceeds at a different rate at different enzymic sites; or that synthesis is not initiated in a synchronous fashion. The rate of synthesis at different sites may be calculated by measuring the contour length of the individual nascent strands and by assuming that synthesis begins synchronously at the time of addition of triphosphates. The average internucleotide distance for reovirus single-stranded RNA may be estimated to be 0.310 nm (4, 18) which agrees with the value of 0.317 nm determined for bacteriophage R17 RNA by Granboulan and Franklin (7). The contour length of the individual molecules attached to the reaction cores shown in Fig. 4 were measured and the apparent rate of RNA synthesis calculated. The maximum apparent rate of RNA synthesis was 50 nucleotides per s and the minimum rate was 2 nucleotides per s; the average rate was 18 nucleotides per s. As anticipated, the maximum strand length recorded from Fig. 4 (0.93 µm) was less than the 1.1-µm length predicted for completed *l*-sized molecules (18), confirming that the longer strands observed associated with reaction cores are nascent rather than completed molecules.

It is of interest to determine the number of nascent strands extruded simultaneously from individual core particles since these values should provide an estimate of the number of enzymic sites active at any one time. Figures 5 and 6 present such an analysis of the number of strands associated with individual reaction cores after synthesis of RNA for 30 and 60 s, respectively. After synthesis for 30 s there is a pronounced maximum in the region of 10 to 12 strands per reaction core and a minimum in the region of five strands. A significant number of reaction cores show more than 12 strands per core, particularly if loops are scored as strands (Fig. 5). After 1 min of RNA synthesis the distribution is similar (Fig. 6) but the number of reaction cores that have one to five strands per core has increased, suggesting that these reaction cores have released completed strands and have subsequently initiated a second round of RNA synthesis.

Origin of unattached mRNA strands. In some fields containing reaction cores, particularly those that had been permitted to synthesize RNA for greater than 2 min, a number of strands were observed that were not attached to cores. Since all preparations examined were taken from CsCl gradients, these strands presumably represented either completed strands that had sedimented to the same position in the gradient as the reaction core band, or incom-

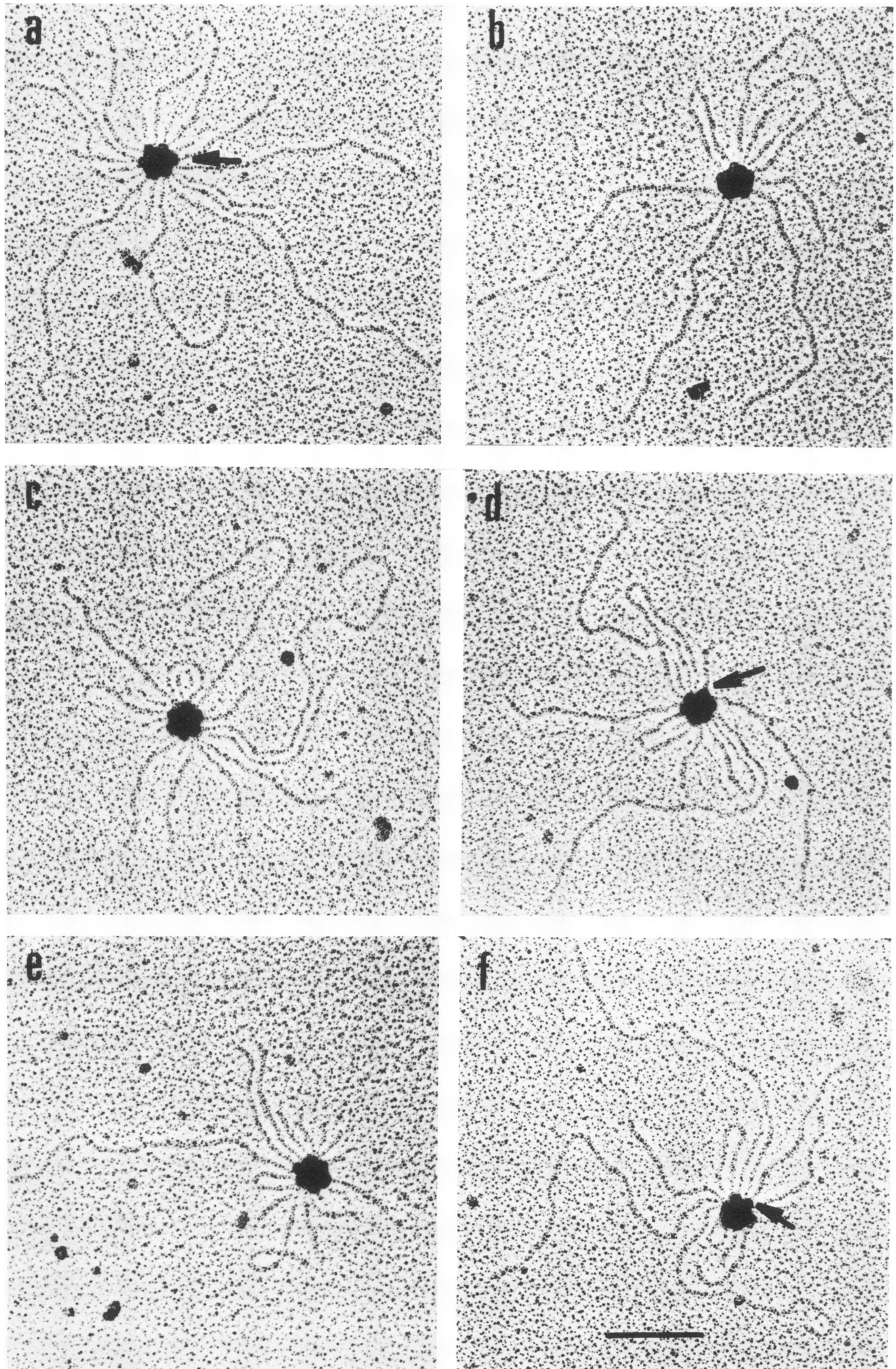

FIG. 4. *Reovirus reaction cores: electron micrographs of stained and shadowed preparations. Reaction cores harvested from CsCl gradients were surface-spread by the drop method of Inman and Schnös (10). Preparations were stained with uranyl acetate and shadowed with platinum-palladium. RNA synthesis was for 1 min at 37 C (see text). Arrows indicate where strands appear to emerge from spikes. The bar represents 0.2 μm.*

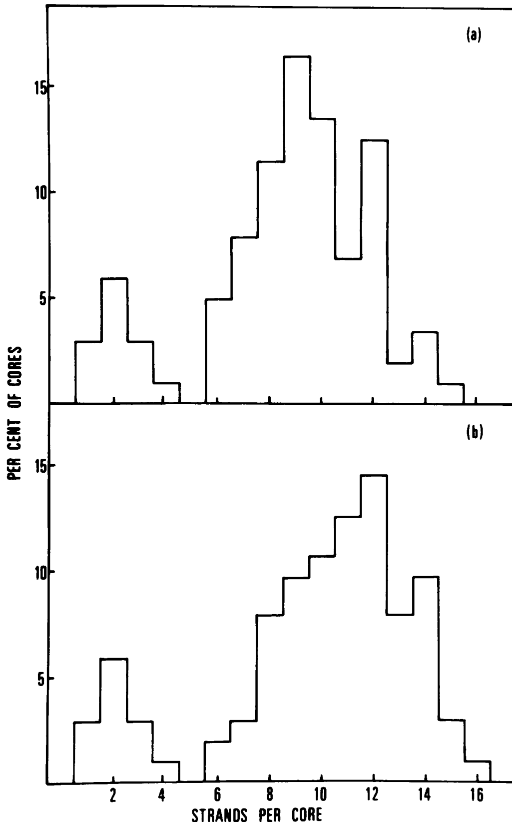

FIG. 5. *Analysis of the number of nascent strands associated with individual reaction cores, after incubation for 30 s at 37 C. Randomly selected reaction cores were examined under the electron microscope and the number of strands and loops associated with each core were counted. The ordinates are the percentage of reaction cores in the sample and the abscissae are the number of strands associated with the reaction core. Ninety-seven reaction cores were counted. (a) Strands counted but loops ignored; (b) strands and loops both counted.*

plete (nascent) strands that had been removed from the core by either nuclease action or shearing forces.

To distinguish between these possibilities the contour length distribution of these strands was measured to determine whether it exhibited the trimodal size distribution expected for intact *l*-, *m*-, and *s*-sized reovirus mRNA (Fig. 7). It is clear that the unattached strands exhibit maxima at 0.36, 0.61, and 1.11 μm (Fig. 7b). This size distribution agrees closely with that of denatured reovirus RNA which has three size classes of 0.38, 0.64, and 1.10 μm (18). In contrast, nascent RNA strands attached to cores are of shorter length and exhibit a maxima at 0.3 μm (Fig. 7a). Thus we may conclude that the free strands observed on grids are not nascent strands released from reaction cores by nuclease action but are probably completed strands that are released on the monolayer.

Site of extrusion of mRNA and the origin of loops. Reovirus cores bear 12 hollow projections on the surface of the core particle (12). It has been suggested by some workers that these spikes may be the sites from which RNA is extruded (12, S. C. Gillies, M. Sc. thesis, University of Auckland, 1972). Although the combined staining and shadowing procedure provides sufficient contrast for both the spikes and the strands to be observed simultaneously, we have encountered technical difficulties in attempting to determine whether the strands indeed emerge from the spikes. There are 12 spikes per core, and if a core displays threefold

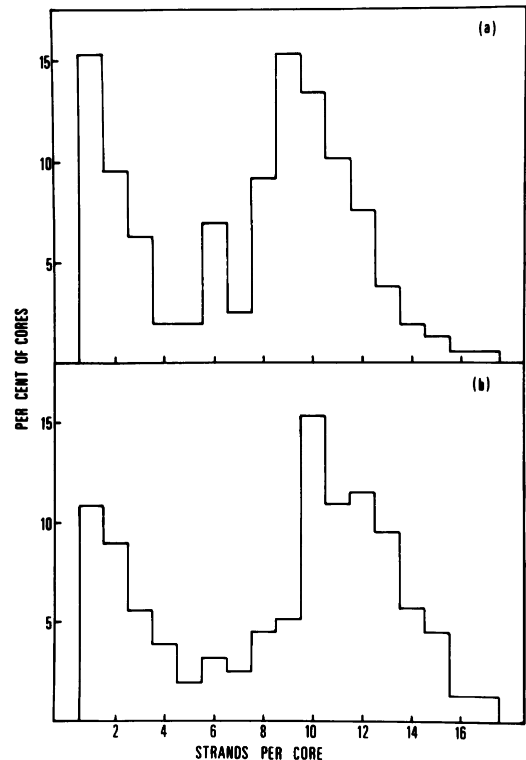

FIG. 6. *Analysis of the number of nascent strands associated with individual reaction cores after incubation for 1 min at 37 C. Randomly selected reaction cores were examined under the electron microscope and the number of strands and loops associated with each core were counted. The ordinates are the percentage of reaction cores in the sample, and the abscissae are the number of strands associated with the reaction core. One hundred fifty-seven reaction cores were counted. (a) Strands counted but loops ignored; (b) strands and loops both counted.*

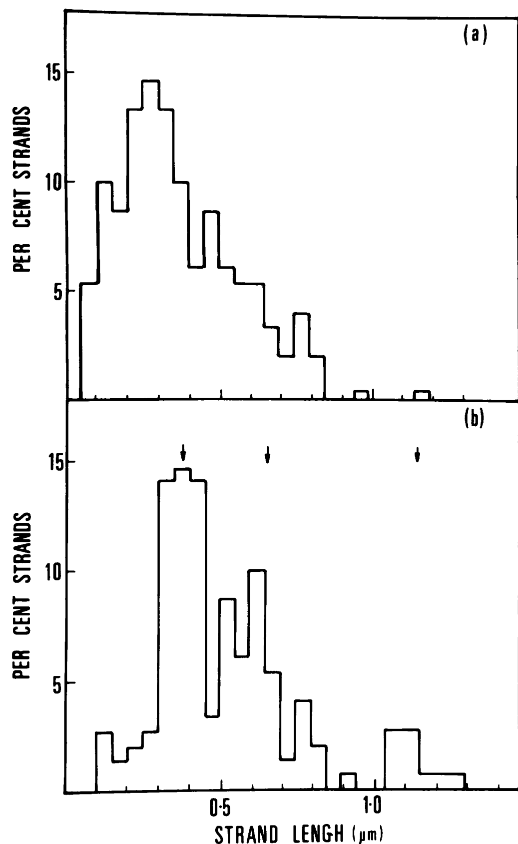

FIG. 7. *The contour length of RNA strands observed in reaction core preparations. Random fields of grids containing reaction cores were photographed and the length of RNA filaments associated with reaction cores and those free in the monolayer were measured (see text). The ordinates are the percent number of strands per 0.77 μm and the abscissae are the lengths of the RNA strands. (a) RNA strands associated with reaction cores; 200 filaments were measured; (b) RNA strands free in the monolayer; 150 filaments were measured. Note that those strands free in the monolayer show length maxima close to those expected for complete molecules. Those associated with cores show a length distribution that indicates that they are incomplete (nascent) strands. Arrows mark the length of complete l, m, and s messenger molecules.*

symmetry three of these spikes are above and three are below the core and six are peripheral. In some reaction cores, strands appear to originate from all six of the peripheral spikes but it is difficult to determine the origin of strands that do not coincide with peripheral spikes because the strands are not visible where they lie across the surface of the core.

Figure 8 shows individual reaction core particles viewed down a threefold and fivefold axis of symmetry together with a model of the core that has been aligned in the same manner. Relatively good contrast of both the spikes and RNA strands has been achieved. Spikes appear to be associated with the nascent strands but we are unable to exclude the possibility that extrusion occurs at a point near the spike rather than through the hollow tube itself. Examination of any single electron micrograph does not provide conclusive evidence that the spike is the site from which mRNA is extruded. However, examination of numbers of reaction cores that show favourable contrast for both spikes and strands (indicated by arrows in Fig. 4 and 8) indicates that many instances occur in which strands appear to emerge from spikes.

Fields that contain many reaction cores show evidence for a preferential alignment of the reaction core in the cytochrome-*c* monolayer (Fig. 9). Most reaction cores exhibit six peripheral spikes and are therefore aligned with a threefold axis of symmetry perpendicular to the monolayer.

A striking feature of reaction cores was the extrusion of many RNA strands in the form of loops (Fig. 10). Many reaction cores show more than one loop, but there does not appear to be any marked correlation between the frequency of loops and other factors such as the time of synthesis or the length of the particular RNA strand involved.

DISCUSSION

Banerjee and Shatkin (2) have reported that during the early periods of in vitro transcription of RNA by reovirus cores the rate of synthesis of *s*, *m*, and *l* mRNA molecules is different and in the order $s > m > l$. These workers used sucrose density gradient centrifugation to determine the time taken for synthesis of completed mRNA molecules. Values were derived for the rates of RNA synthesis that ranged from 6 to 16 nucleotides per s.

It is not known whether synthesis of all 10 reovirus mRNA molecules is initiated synchronously or sequentially. Estimates of the rate of chain elongation that are based on the time taken to synthesize a molecule of given length, therefore are subject to varying interpretations. However, if it is assumed that synthesis is initiated synchronously, the electron microscope evidence presented in this communication suggests that the synthesis of individual reovirus mRNA molecules does indeed proceed at different rates. Values for the rate of polymerisation calculated from the contour length measurements of individual nascent strands yielded a range in apparent rates of synthesis from 2 nucleotides per s to 50 nucleotides

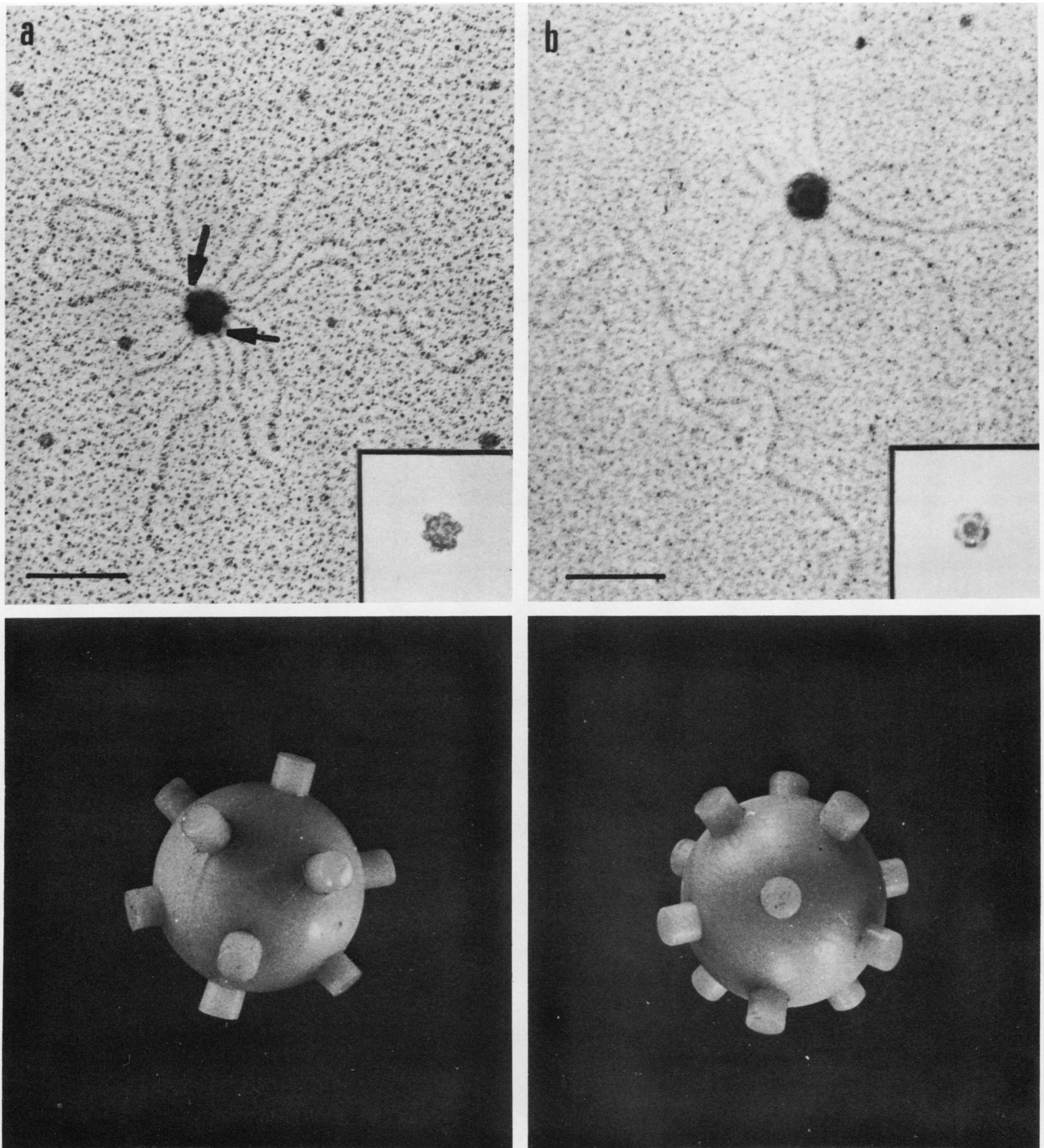

FIG. 8. *Reovirus reaction cores: alignment with respect to threefold and fivefold axis of symmetry. (a) The upper micrograph is of a reovirus reaction core aligned on a threefold axis of symmetry. The lower photograph shows a model aligned in the same manner. The insert has been under-developed to provide better contrast for the surface spikes. Note that six peripheral spikes are visible. (b) Similar to (a) except that both reaction core and model are aligned on a fivefold axis of symmetry. Note that five peripheral spikes are visible but a second series of peripheral spikes obscures detail. The model was constructed according to the dimensions determined by Luftig et al. (12). Arrows indicate where strands appear to emerge from spikes. The bar represents 0.2 μm.*

per s: the average value was 18 nucleotides per s. These values are in reasonable agreement with the range of 6 to 16 nucleotides per s estimated by Banerjee and Shatkin (2).

The site of extrusion of RNA strands has not been conclusively identified by this study, but many reaction cores were observed in which strands did appear to emerge from spikes. The

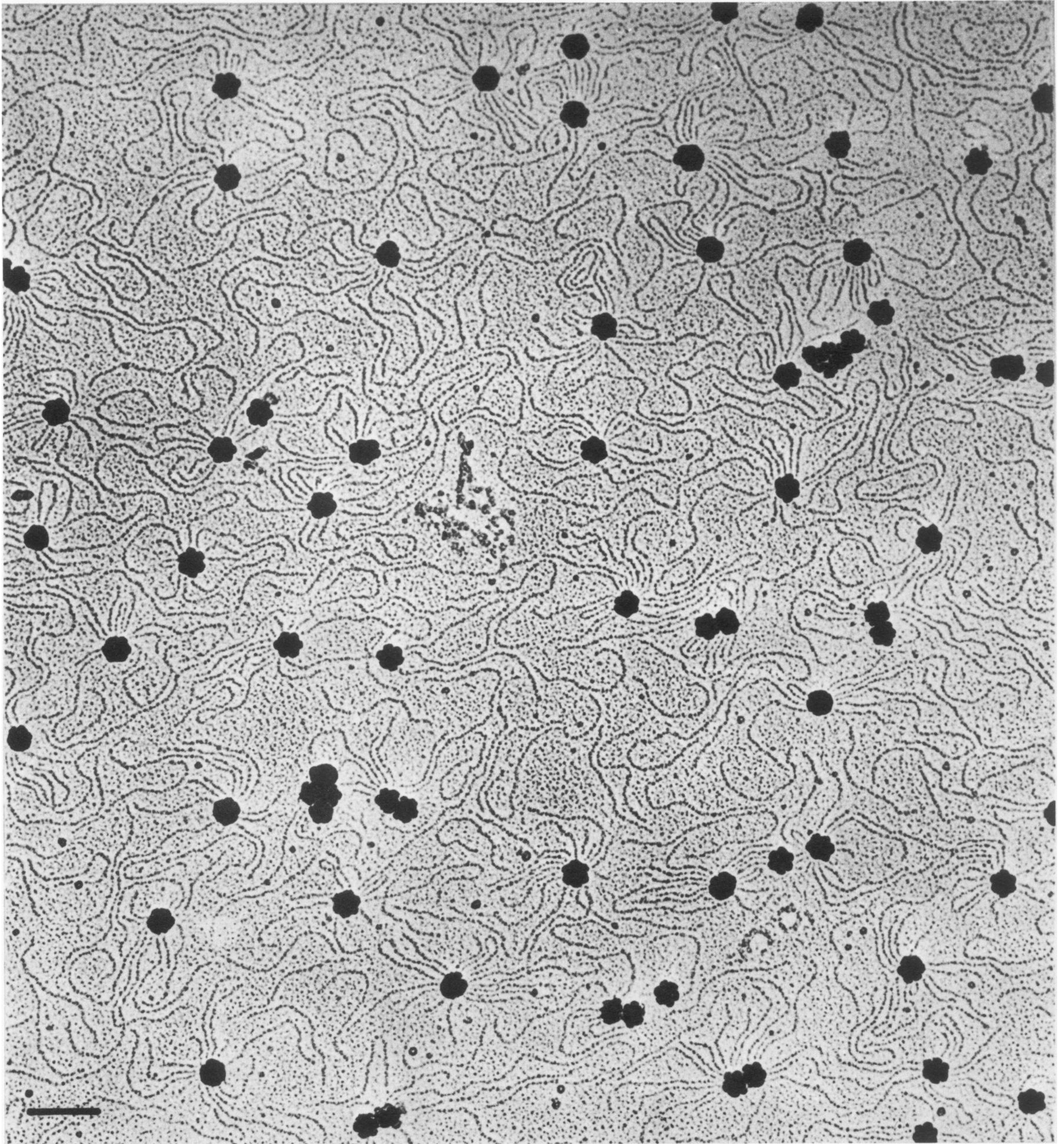

FIG. 9. *Reovirus reaction cores: preferential alignment on the threefold axis of symmetry. Note that most cores exhibit the six peripheral spikes provided by the threefold alignment. RNA synthesis was for 8 min at 37 C. The bar represents 0.2 μm.*

fact that the 12 spikes are hollow (12; S. C. Gillies, M. Sc. thesis, University of Auckland, 1972) supports this view. The spike might be the site of both synthesis and extrusion since such an arrangement would obviate the requirement for a mechanism to transport nucleoside triphosphates to an internal polymerization site. An enzymic site located close to the extrusion point would also have the advantage of permitting rapid and efficient extrusion of RNA strands as nucleic acid synthesis proceeds.

There are 12 spikes on the surface of the core but a total of only 10 unique mRNA molecules are transcribed in vitro (2, 16). Electron micros-

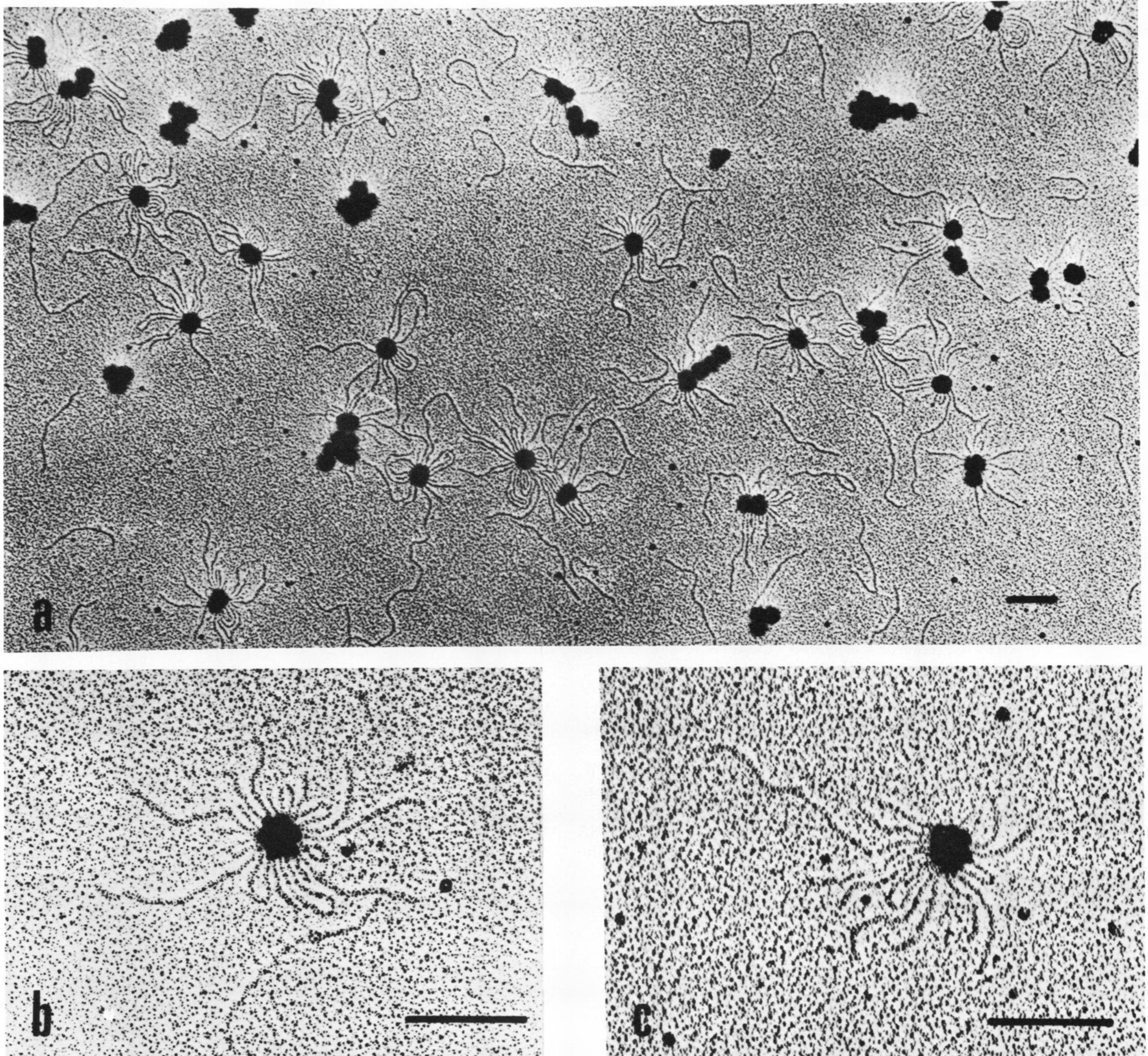

Fig. 10. *Reovirus reaction cores: apparent formation of loops during extrusion of the product. (a) A number of reaction cores that exhibit loop formation. RNA synthesis was for 1 min at 37 C. (b and c) Two reaction cores that exhibit loops, viewed at higher magnification. The bar reprsents 0.2 μm.*

copy reveals that up to 16 strands may be associated with an individual particle. Since some free strands are present on grids (Fig. 10a) it is possible that reaction cores sometimes fortuitously become associated with one or more free strands and it would not be possible to distinguish these strands from those actually attached to the particle. Alternative explanations for core particles having up to 16 strands might be that multiple copies of some individual genome segments are synthesized simultaneously, or reaction cores may fail to complete synthesis of some mRNA molecules before initiating a second round of synthesis. It is unlikely that reaction cores having more than 10 strands represent those that bear significant numbers of completed strands that have failed to detach from the core; sedimentation analysis of the RNA associated with reaction cores indicated that this material did not contain completed molecules (Fig. 3).

Finally, mRNA often appeared to be extruded in the form of a loop. It is unlikely that loops are solely an artifact of the preparative procedure since they were frequently observed attached to cores that also bore linear filaments.

Thus, loops may have some functional significance: for example the 5′-triphosphate end of the nascent mRNA may not always be set free immediately synthesis is initiated. In such instances the 5′-triphosphate end of the nascent mRNA may remain associated with a site in the internal region of the spike and it is possible that in such cases the first end to be set free outside the particle might be the 3′-OH end of the molecule rather than the expected 5′-triphosphate end.

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LITERATURE CITED

1. Baltimore, D. 1966. Purification and properties of poliovirus double-stranded ribonucleic acid. J. Mol. Biol. **18**:421–428.
2. Banerjee, A. K., and A. J. Shatkin. 1970. Transcription in vitro by reovirus-associated ribonucleic acid-dependent polymerase. J. Virol. **6**:1–11.
3. Bellamy, A. R., J. L. Nichols, and W. K. Joklik. 1972. Nucleotide sequences of reovirus oligonucleotides: evidence for abortive RNA synthesis during virus maturation. Nature N. Biol. **238**:49–51.
4. Bellamy, A. R., L. Shapiro, J. T. August, and W. K. Joklik. 1967. Studies on reovirus RNA. I. Characterisation of reovirus genome RNA. J. Mol. Biol. **29**:1–17.
5. Borsa, J., and A. F. Graham. 1968. Reovirus: RNA polymerase activity in purified virions. Biochem. Biophys. Res. Commun. **33**:895–901.
6. Gillies, S., S. Bullivant, and A. R. Bellamy. 1971. Viral RNA polymerases: electron microscopy of reovirus reaction cores. Science **174**:694–696.
7. Granboulan, N., and R. M. Franklin. 1966. Electron microscopy of viral RNA, replicative form and replicative intermediate of the bacteriophage R17. J. Mol. Biol. **22**:173–177.
8. Hay, A. J., and W. K. Joklik. 1971. Demonstration that the same strand of reovirus genome RNA is transcribed in vitro and in vivo. Virology **44**:450–453.
9. Ifft, J. B., D. H. Voet, and J. Vinograd. 1961. The determination of density distributions and density gradients in binary solutions at equilibrium in the ultracentrifuge. J. Phys. Chem. **65**:1138–1145.
10. Inman, R. B., and M. Schnös. 1970. Partial denaturation of thymine- and 5-bromouracil-containing λ DNA in alkali. J. Mol. Biol. **49**:93–98.
11. Kapuler, A. M. 1970. An extraordinary temperature dependence of the reovirus transcriptase. Biochemistry **9**:4453–4457.
12. Luftig, R. B., S. S. Kilham, A. J. Hay, H. J. Zweerink, and W. K. Joklik. 1972. An ultrastructural study of virions and cores of reovirus type 3. Virology **48**:170–181.
13. Robberson, D., Y. Aloni, and G. Attardi. 1971. Expression of the mitochondrial genomé in HeLa cells. VI. Size determination of mitochondrial ribosomal RNA by electron microscopy. J. Mol. Biol. **60**:473–484.
14. Shatkin, A. J., and J. D. Sipe. 1968. RNA polymerase activity in purified reoviruses. Proc. Nat. Acad. Sci. U.S.A. **61**:1462–1469.
15. Shatkin, A. J., J. D. Sipe, and P. Loh. 1968. Separation of ten reovirus genome segments by polyacrylamide gel electrophoresis. J. Virol **2**:986–991.
16. Skehel, J. J., and W. K. Joklik. 1969. Studies on the in vitro transcription of reovirus RNA catalysed by reovirus cores. Virology **39**:822–831.
17. Smith, R. E., H. J. Zweerink, and W. K. Joklik. 1969. Polypeptide components of virions, top component and cores of reovirus type 3. Virology **39**:791–810.
18. Vasquez, C., and A. K. Kleinschmidt. 1969. Electron microscopy of RNA strands released from individual reovirus particles. J. Mol. Biol. **34**:137–147.