Poliovirus subviral particles associated with progeny RNA in the replication complex

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The poliovirus replication complex (RC), the site of genomic 36S RNA synthesis, was previously shown to contain subviral particles of 5S protomer and 14S pentamer antigenicity. The present investigation demonstrates that 5S/14S antigenic subviral particles can be cross-linked to viral RNA by UV irradiation of a subcellular fraction containing the poliovirus RC. Each capsid protein of the subviral particles, i.e. VP0, VP1 and VP3, was cross-linked to viral RNA. SDS-PAGE analysis of the cross-linked capsid proteins revealed a bandshift for VP1, whereas VP0 migrated in several bands, which were interpreted to be multimers of VP0 linked by short stretches of RNA. It was found that 36S RNA rather than replicative intermediate RNA was cross-linked to capsid proteins. Our results indicate that encapsidation of poliovirus RNA starts in the RC and is initiated by 14S pentamers.

Introduction

Poliovirus has a single-stranded RNA genome of positive polarity and a non-enveloped capsid. The icosahedral capsid is composed of 60 subunits, called protomers, each consisting of four capsid proteins: VP1 to VP4. Five protomers make up a pentamer and are arranged around the twelve five-fold symmetry axes within the icosahedron (Hogle et al., 1985). From extracts of infected cells, several subviral particles have been isolated and described: 5S protomers, 14S pentamers, 65S and 74S empty capsids (EC) and 125S provirions, in addition to 150S infectious virus particles (for a recent review see Hellen & Wimmer, 1992). The biological significance of EC is not clear. These particles are not present in native (i.e. not detergent-treated) subcellular fractions and it was consequently proposed that EC are formed artificially by a detergent-dependent assembly pathway (Pfister et al., 1992). The type of subviral particle that associates with progeny RNA and proceeds to RNA encapsidation has not yet been identified (see also Ansardi & Morrow, 1993), although some investigators have proposed that 14S pentamers, rather than EC, may be involved in encapsidation of progeny plus-strand RNA (Ghendon et al., 1972; Marongiu et al., 1981; Rombaut et al., 1990).

Progeny plus-strand RNA is asymmetrically synthesized from a minus-strand RNA template (Girard, 1969). Plus-strand synthesis takes place in a virus-induced structure called the replication complex (RC) (Caliguiri & Tamm, 1970; Bienz et al., 1980). Electron microscopic studies of isolated RC showed that the RC is anchored to vesicles which are arranged in a rosette-like fashion (Bienz et al., 1990). The vesicular membrane, in concert with virus-encoded (Andino et al., 1993; Bienz et al., 1987; Flanagan & Baltimore, 1979; Li & Baltimore, 1988; Molla et al., 1993) and cellular proteins (Andino et al., 1990, 1993; Takeda et al., 1986), provide the structural requisites needed for initiation of viral RNA synthesis and for elongation and release of progeny plus-strand RNA (Bienz et al., 1992).

In the infected cell, progeny RNA synthesis and RNA encapsidation have been proposed to be coupled processes, since (i) progeny RNA becomes encapsidated immediately after its synthesis (Baltimore et al., 1966) and (ii) progeny virus (Caliguiri & Compans, 1973) as well as subviral particles (Caliguiri & Mosser, 1971; Pfister et al., 1992; Yin, 1977) could be detected in subcellular RC-containing fractions.

In an earlier report we demonstrated the presence of 5S protomers and/or 14S pentamers within the RC (Pfister et al., 1992). The present investigation was done to elucidate whether subviral particles located in the RC are involved in RNA encapsidation. We report that subviral particles of 5S/14S antigenicity associate with genomic 36S RNA within the RC and that progeny RNA starts to be encapsidated by 14S subviral particles.

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Table 1. Monoclonal antibodies

<table>
<thead>
<tr>
<th>Systematic designation of monoclonal antibody</th>
<th>Recognition of protein on Western blots†</th>
<th>Recognition of subviral particles by immunoprecipitation</th>
<th>In the present study used and designated as</th>
</tr>
</thead>
<tbody>
<tr>
<td>B3/H.1* and B3/H.2†</td>
<td>VP1</td>
<td>5S protomers, 14S pentamers, 74S EC</td>
<td>Anti-14S</td>
</tr>
<tr>
<td>14/2.A3.1*</td>
<td>VP0 and VP2</td>
<td>74S EC</td>
<td>Anti-74S</td>
</tr>
<tr>
<td>14/1.A1.1* and 14/1.D1.1‡</td>
<td>VP0 and VP2</td>
<td>None</td>
<td>Anti-VP0/2</td>
</tr>
<tr>
<td>12/A.A3.1* and 12/B.D4.1§</td>
<td>VP3</td>
<td>None</td>
<td>Anti-VP3</td>
</tr>
<tr>
<td>39/1‖</td>
<td>None</td>
<td>None</td>
<td>Determination of background</td>
</tr>
</tbody>
</table>

* Pfister et al. (1992).
† Subclone of B3/H.1.
‡ Same characteristics as 14/1.A1.1.
§ Same characteristics as 12/A.A3.1.
‖ Bienz et al. (1987).
¶ All precursor peptides are also recognized.

Methods

Cells, virus and in vivo radiolabelling of viral proteins and RNA. HEp-2 cells and poliovirus type 1 (Mahoney) were grown in suspension cultures with a cell density of 10^6 cells/ml with an m.o.i. of 30 p.f.u. per cell.

Radiolabelling was done in suspension cultures with a cell density of 10^6 cells per ml. Viral proteins were metabolically labelled with [3H]methionine (Amersham International) from 2:25 h to 4:25 h post-infection (p.i.) in methionine-free Eagle's MEM (Gibco) and in the presence of 120 mM excess NaCl to inhibit residual host protein synthesis (Nuss et al., 1975). Viral RNA was metabolically labelled with [3H]uridine (Amersham International) from 3.0 h to 4:25 h p.i. in the presence of actinomycin D to block any cellular RNA synthesis.

Isolation of the replication complex. The poliovirus RC was isolated from infected cells at 4:25 h p.i. by discontinuous sucrose gradient centrifugation of a cytoplasmic extract as described (Bienz et al., 1990), except that a 20% sucrose solution was layered on top of the 30% 45% sucrose cushions. The vesicular fractions banding on top of each of the sucrose layers were harvested and examined by (immuno-) electron microscopy (IEM) (Bienz et al., 1990; Pfister et al., 1992).

Cross-linking of proteins and RNA by UV irradiation. A Stratagene 2400 (Stratagene) containing UV bulbs with maximum emission at 254 nm was used to irradiate the vesicular fractions. Aliquots (20 µl) of the fractions were put on glass slides and irradiated on ice with a distance of 10 cm to the bulbs and energies ranging from 0:1 to 3:2 J/cm².

Filter-binding assay for RNA cross-linked to proteins. The filter-binding assay was done essentially as described earlier (Bienz et al., 1990). [3H]uridine-labelled 30% vesicular fractions were treated with 4% SDS (final concentration), diluted in TE buffer (10 mM-Tris–HCl, pH 7.5, 1 mM-EDTA) to a concentration of SDS of 0.024% and filtered through nitrocellulose filters (0.45 µm; BA 85; Schleicher & Schuell) (Kuno & Kihara, 1967; Oberste & Flanagan; 1988; Zimmern & Butler, 1977). RNA retained on the filters was quantified by liquid scintillation counting (LSC).

Monoclonal antibodies and immunoprecipitation. Monoclonal antibodies (MAb) recognizing different configurations of capsid proteins have been characterized earlier (Pfister et al., 1992) and are listed in Table 1. For immunoprecipitation, sheep anti-mouse antibody-coated magnetic beads (M 280; Dynal) were used to immobilize MAb and antigen. Beads (0.5 mg) were washed three times with Tris–HCl buffer pH 7.0 containing 0.1% BSA. The beads were incubated with MAb-containing hybridoma culture supernatant adjusted to 20 mM-Tris–HCl pH 7.2, 5 mM-EDTA and 0.005% NP40 for 4 h at 4°C on a rotating tray. The beads were washed as before and incubated with the antigen in a Tris–HCl buffer (pH 7.0) containing 0.1% BSA and 0.005% NP40 for 1 h at 4°C before being washed in the same buffer. If RNA cross-linked to an antigen was to be quantified, immunoprecipitation was performed with DTT (1 mM) and RNase inhibitor from human placenta (1 unit/µl) (Boehringer-Mannheim) added to the antigen-containing buffer. After the final washing, RNA was digested by pancreatic RNase A (100 µg/ml, 4°C, 30 min) (Boehringer) and the radioactivity in the supernatant was determined by LSC. To analyse immunoprecipitated proteins, 20 µl SDS–PAGE sample buffer was added to the beads. After boiling for two minutes and centrifugation, the supernatant was either subjected to SDS–PAGE or radioactivity was measured by LSC. To estimate the background of the immunoprecipitation, MAb 39/1, which does not recognize any viral or cellular protein (Bienz et al., 1987), was used (Table 1).

SDS–PAGE and Western blotting. The 30% vesicular fractions were first solubilized with 1% NP40 and RNase A-treated before being processed for SDS–PAGE on 15% polyacrylamide gels and electroblotting onto nitrocellulose filters according to Laemmli (1970) and Towbin et al. (1979). The blotted proteins were immunostained by the method of Mertens et al. (1983). To detect capsid proteins, the MAb anti-VP1, anti-VP0/2 and anti-VP3 (Table 1) were used individually or in an anti-VP cocktail. For the detection of non-structural viral proteins (NCVP), an anti-NCVP cocktail was used containing MAb against 2C (Pasamontes et al., 1986), 2B and 3D (not published).

Isolation of RNA by sucrose gradient centrifugation. The 30% vesicular fractions with radiolabelled RNA were denatured by incubation at 80°C for 2 min in the presence of 2% SDS and 6 mM-mercaptoethanol (Wetz & Habermehl, 1982), in order to disrupt non-covalent RNA–protein interactions. Subsequently, the denatured material was centrifuged through linear gradients of 15–30% sucrose in TE buffer. Centrifugation was done in a SW 55 rotor (Beckmann) at 5°C at 50000 r.p.m. for 5:7 h. The gradients were harvested from the bottom in 0.25 ml aliquots. The radioactivity of each fraction was quantified by LSC after TCA precipitation.

Isolation of RNA and RNA-bound proteins using CsCl gradients. The 30% vesicular fractions containing either radiolabelled RNA or
proteins were denatured by 4 M-guanidinium thiocyanate (MacDonald et al., 1987) and centrifuged through a two-step CsCl cushion with densities of 1.76 g/ml and 1.37 g/ml (Bienz et al., 1990). The supernatant fraction and the two CsCl cushions were harvested from the top of the gradient. The pellet was dissolved in 200 µl TE buffer and harvested. Each fraction was TCA precipitated and radioactivity was quantified by LSC.

**Results**

Poliovirus RC were isolated by centrifugation of a cytoplasmic extract through a discontinuous sucrose gradient of 20%, 30% and 45% sucrose. The 30% sucrose fraction consists of membrane-bound RC which have been actively synthesizing plus-strand RNA in vivo (Bienz et al., 1992) and contain large amounts of 5S and 14S subviral particles (Pfister et al., 1992). This fraction, called 30% vesicular fraction, was used throughout this study. To keep any soluble 5S/14S subviral particles separated from the 30% sucrose, a 20% sucrose layer was put on top of the 30% sucrose. Neither the ultrastructure nor immunocytochemical pattern of the RC were changed as compared to RC obtained with the previous isolation procedure (Bienz et al., 1990, 1992), which lacked the 20% sucrose layer (not shown).

**UV cross-linking of viral RNA and protein in vesicular fractions**

To test for an association of capsid proteins with viral RNA, i.e. the first step in virus formation, cross-linking of RNA and protein by UV irradiation of 30% vesicular fractions was used (Greenberg, 1980). To optimize the UV dose for cross-linking RNA to proteins, a filter-binding assay was performed. Viral RNA was metabolically labelled with \[^{3}H\]uridine in the presence of actinomycin D, the 30% vesicular fraction was isolated, UV-irradiated with increasing energy from 0 to 3.2 J/cm², denatured with SDS and filtered through a nitrocellulose filter. Retained RNA was quantified by LSC (Fig. 1). For further experiments, a UV dose of 3.2 J/cm² was used unless otherwise stated.

Immunoprecipitation with anti-14S MAb was performed to test whether the 5S/14S subviral particles of the 30% vesicular fraction can be cross-linked to viral RNA. The MAb used recognizes 5S protomers and 14S pentamers as well as 74S EC (Table 1). The presence of 74S EC within the RC, however, has been excluded by IEM with an anti-74S MAb (Pfister et al., 1992). The 30% vesicular fractions containing radiolabelled viral RNA were UV-irradiated or not irradiated, solubilized with 1% NP40 in the presence of RNase inhibitor and immunoprecipitated with anti-14S or 39/1 MAb, the latter recognizing neither cellular nor viral protein (Table 1). In the UV-irradiated 30% vesicular fraction, the precipitate of anti-14S MAb contained 10-fold more viral RNA as compared to the precipitates from a non-irradiated fraction (Table 2). The amount of non-specifically precipitated RNA (background) was determined with MAb 39/1. This indicates that, in the 30% vesicular fraction, viral RNA is so tightly associated with 5S/14S subviral particles that it can be cross-linked by UV irradiation.

To characterize the precipitated viral proteins further, the anti-14S precipitate of the UV-irradiated vesicular fraction was RNase-treated, separated by SDS–PAGE and analysed on Western blots using anti-VP MAb cocktail (Fig. 2). Expectedly, VP0, VP1 and VP3 were found. The VP1 band was shifted and additional protein bands with apparent Mr of ~70000 (70K) and higher were observed.

Table 2. **Immunoprecipitation of 14S antigenic subviral particles from UV irradiated and not irradiated 30% vesicular fractions containing radiolabelled viral RNA**

<table>
<thead>
<tr>
<th>Monoclonal antibody</th>
<th>Viral RNA recovered in the immunoprecipitate (% of total viral RNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without UV</td>
</tr>
<tr>
<td>Anti-14S</td>
<td>0.46</td>
</tr>
<tr>
<td>39/1</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Fig. 1. Filter-binding assay for RNA cross-linked to protein. The 30% vesicular fractions with radiolabelled viral RNA were UV-irradiated as indicated, SDS-treated and filtered through nitrocellulose. Radioactivity retained on the filter was determined by LSC. The background of 1.8 %, obtained with non-UV-irradiated fractions, was subtracted.
viral proteins in the cross-linking of viral RNA to 5S/14S subviral particles, the immunoprecipitates of anti-14S and 39/1 MAb were analysed on a Western blot with an anti-NCVP MAb cocktail (Fig. 3c). Since no specific immune reaction could be found, the proteins 2B, 2C, 3D and their precursors seem not to participate in the association of viral RNA and 5S/14S subviral particles. However, participation of viral proteins not recognized by the MAb used or of cellular proteins can not be excluded.

**Capsid protein and viral RNA species involved in UV-induced RNP formation**

To determine the extent of UV-induced protein–protein cross-links, the buoyant density of viral protein from irradiated and non-irradiated 30% vesicular fraction was compared. Proteins which are covalently bound to RNA show a higher buoyant density in CsCl gradients than proteins and protein aggregates not cross-linked to RNA (Wagenmakers et al., 1980). From a vesicular fraction, containing radiolabelled viral protein, one aliquot was not UV-irradiated, a second was UV-irradiated and a third was irradiated and RNase-treated. All samples were denatured with 4M-guanidinium thiocyanate and centrifuged through discontinuous CsCl gradients. The amount of labelled proteins in the supernatants, CsCl fractions and pellets was determined by LSC (Fig. 4). UV irradiation increased the amount of proteins with higher buoyant density. RNase treatment of irradiated vesicular fraction reduced the amount of proteins sedimenting with higher densities and increased the amount of proteins in the supernatant again, almost to the value found with non-irradiated fractions. This indicates that UV irradiation produces covalently bound ribonucleoprotein (RNP), although the formation of some protein–protein cross-links can not completely be ruled out.

In order to identify the viral RNA species which was UV cross-linked to capsid proteins and to identify the capsid protein(s) directly involved in RNP formation, RNA and protein of 30% vesicular fractions were analysed by rate-zonal and isopycnic centrifugation. The 30% vesicular fractions containing [3H]labelled viral RNA were irradiated or not irradiated, SDS-denatured and centrifuged into continuous 15–30% sucrose gradients. Viral RNA from a non-UV-irradiated 30% vesicular fraction sedimented in two peaks (Fig. 5), representing 36S RNA and replicative intermediate (RI) RNA (Bienz et al., 1992). After UV irradiation, the sedimentation rate of RI RNA was virtually not affected. The amount of RNA sedimenting with 36S, however, was reduced, whereas more viral RNA sedimented faster than 36S.

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**Fig. 2.** Western blot of 30% vesicular fractions, immunostained with an anti-VP MAb cocktail. The vesicular fractions were UV-irradiated (lane 2) or not irradiated (lane 1) before being immunoprecipitated with anti-14S MAb and processed for SDS-PAGE. In the UV-irradiated vesicular fraction, the VP1 is shifted and several additional protein bands of approximately 70 kDa and above 200 kDa appear. HC and LC indicate the heavy and light chains of the murine MAb used for immunoprecipitation. Symbols: *, additional protein bands in UV irradiated vesicular fraction; M, not immunoprecipitated 30% vesicular fraction used as marker.
Fig. 3. (a) Western blot of 30% vesicular fractions, immunostained with an anti-VP MAb cocktail. The vesicular fractions were UV-irradiated (lane 1) or not irradiated (lane 2) before being processed for SDS–PAGE. The UV-irradiated vesicular fraction exhibits a bandshift of VP1 as well as additional protein bands with higher Mr, comparable to the findings with immunoprecipitated vesicular fractions (Fig. 2). (b) Western blot of a UV-irradiated 30% vesicular fraction. Lanes 1 to 3 were immunostained individually with the indicated MAb. The UV-induced additional protein bands react only with VP0 MAb. (c) Western blot of 30% vesicular fractions, immunostained with an anti-NCVP MAb cocktail. The vesicular fractions were UV-irradiated (lanes 2 and 4) or not irradiated (lanes 1 and 3) before being immunoprecipitated with anti-14S MAb (lanes 1 and 2) or 39/1 (lanes 3 and 4) and processed for SDS–PAGE. 2B, 2C, 3D and their precursors were not detected, with the exception of trace amounts of 3CD which is non-specifically precipitated also from non-irradiated vesicular fractions. Lane M1 contains cytoplasmic extract from polio-infected cells and lane M2 contains non-immunoprecipitated 30% vesicular fraction as marker.
Fig. 4. Buoyant density profile of radiolabelled viral proteins of 30% vesicular fractions in a discontinuous CsCl gradient. The vesicular fractions were not irradiated ( ■ ), UV-irradiated ( □ ) or UV-irradiated and RNase-treated ( □ ) before being denatured in guanidinium thiocyanate and subjected to isopycnic centrifugation. UV irradiation of the vesicular fraction caused an increase in apparent buoyant density of the radiolabelled proteins which was reversed by RNase digestion.

Fig. 5. Sedimentation profile of radiolabelled viral RNA from 30% vesicular fractions. The vesicular fractions were UV-irradiated ( — — — ) or not irradiated ( — — — ) before being SDS-treated and centrifuged into 15–30% sucrose gradients. The gradients were fractionated and TCA-precipitable radioactivity was determined in each fraction. Viral RNA of not UV-irradiated vesicular fractions sediments in two peaks. UV irradiation reduced the amount of RNA sedimenting with 36S, whereas more viral RNA was found sedimenting with higher S-values than 36.

To determine which capsid protein(s) were cross-linked to viral RNA, the pellet fraction and fractions 1–13 of the sucrose gradients were pooled and immunoprecipitated with anti-VP1, anti-VP0/2 and anti-VP3 MAb (Table 3). UV-irradiated vesicular fraction yielded VP1-, VP0/2- and VP3-immunoprecipitates containing, by LSC, between 5- and 11-fold more labelled and thus

Table 3. Immunoprecipitation of capsid proteins crosslinked to radiolabelled viral RNA

<table>
<thead>
<tr>
<th>Monoclonal antibody</th>
<th>Ratio with UV/without UV*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-VP1</td>
<td>10.9</td>
</tr>
<tr>
<td>Anti-VP0/2</td>
<td>5.3</td>
</tr>
<tr>
<td>Anti-VP3</td>
<td>11.1</td>
</tr>
</tbody>
</table>

* Prior to ratio calculation, the radioactivity found in the immunoprecipitates was corrected for background obtained with MAb 39/1.

Fig. 6. Buoyant density profile of radiolabelled viral RNA of 30% vesicular fractions. The vesicular fractions were not irradiated ( ■ ), UV-irradiated ( □ ) or UV-irradiated and RNase-treated ( □ ) before being denatured in guanidinium thiocyanate and subjected to isopycnic centrifugation in a discontinuous CsCl gradient. (a) UV irradiation of the 30% vesicular fraction caused viral RNA to shift from the pellet into the CsCl cushions and the supernatant. (b) RNase treatment reduced not only the amount of free RNA in the pellet but also in the two CsCl fractions where RNA is present covalently linked to protein.
viral RNA, as compared to corresponding precipitates from non-irradiated vesicular fractions. This result shows that each of capsid proteins VP0, VP1 and VP3 was covalently cross-linked to viral RNA during UV irradiation of the vesicular fraction.

The change in sedimentation rate of viral RNA after UV irradiation of a 30% vesicular fraction (Fig. 5) suggests that mainly 36S RNA is cross-linked to capsid proteins. To test this hypothesis, first the buoyant density of labelled viral RNA from UV-irradiated and non-irradiated vesicular fractions were compared. UV irradiation reduced the amount of RNA in the pellet fraction (buoyant density greater than 1.76 g/ml) concomitantly increasing the amount with lower density (Fig. 6a). This indicates that a substantial amount of RNA was present as RNP. To identify the RNA species involved, a UV-irradiated, native vesicular fraction as above was RNase-treated. Under these conditions, only 36S RNA is digested and RI RNA is left intact (Bienz et al., 1992). The vesicular fractions were then guanidinium-dissociated and subjected to CsCl gradient centrifugation. The RNase treatment had reduced the amount of viral RNA not only in the pellet, containing non-cross-linked RNA, but also in all other fractions of the CsCl gradient, which contain cross-linked RNP (Fig. 6b). This indicates that during UV-induced formation of RNP, mainly RNase-accessible RNA, i.e. 36S RNA but not RI RNA, was cross-linked to proteins.

Discussion
Several authors (Bienz et al., 1990; Etchison & Ehrenfeld, 1981; Takeda et al., 1986) have reported the isolation of functional poliovirus RC from infected cells by centrifugation of a cytoplasmic extract through a discontinuous sucrose gradient. The subcellular fraction banding in 30% sucrose contains RC which have been actively synthesizing plus-strand RNA in the infected cell. They continue to initiate and elongate genome-length 36S plus-strand RNA in vitro (Bienz et al., 1992; Takeda et al., 1986). Such RC have previously been shown to contain 5S protomers and/or 14S pentamers but not EC and virions (Pfister et al., 1992). The present investigation demonstrates, by UV cross-linking experiments, that 5S/14S subviral particles associate with 36S RNA in the RC, probably as an initial event in virus encapsidation.

In the virion, all capsid proteins are associated with RNA (Wetz & Habermehl, 1982). We also found this to be true for 5S/14S particles, where VP0, VP1 and VP3 could be UV cross-linked to viral RNA. In Western blot analysis, VP1 showed a bandshift, whereas VP0 was found in additional protein bands with apparent Mr of 70K and more than 200K. These bands are thought to represent multimers of VP0. Their mode of origin can be explained in the following way: in the complete virion, the cleavage products of VP0, i.e. VP2 and VP4, are associated with genomic RNA (Wetz & Habermehl, 1982) and we report here that already during virus formation, VP0 associates with progeny RNA. If a 14S pentamer associates with RNA by virtue of its VP4 moieties, this would lead, upon UV cross-linking, to multimers of VP0, held together by short stretches of RNA. The largest multimers would be pentamers, because, in analogy to virions, the VP4 moieties of VP0 are thought to be arranged around the fivefold symmetry axis of the 14S pentamer (Hogle et al., 1985). Thus, the appearance of VP0 multimers in our experiments argues for 14S pentamers, rather than 5S protomers, associating with RNA. UV-cross-linking-induced formation of protein multimers, connected by RNase-resistant RNA, has also been reported for the rotavirus non-structural protein NS35 (Kattoura et al., 1992). However, the formation of small amounts of VP0 multimers by protein–protein cross-linking can not be ruled out by our experiments (see Fig. 4).

In two structurally related RNA viruses, flock house virus (Fisher & Johnson, 1993) and satellite tobacco mosaic virus (Larson et al., 1993), a double-stranded RNA segment was found lying across the twofold symmetry axis connecting two neighbouring pentamers. In the poliovirus capsid, trp-38 and tyr-41 of VP2, both located near the twofold symmetry axis, are putative RNA-binding sites (Filman et al., 1989). During poliovirus maturation, VP2 (in addition to VP4) moieties of VP0 in the 14S pentamers may associate with RNA. This would connect individual pentamers with each other. UV irradiation would cross-link two VP0 molecules of adjacent 14S pentamers to one RNA molecule, leading to a dimer of VP0. The UV-induced 70K protein in Fig. 2 and Fig. 3(a and b) might represent such dimers. Thus, dimerization of VP0 would indicate the beginning of capsid assembly. That capsid assembly starts with 14S pentamers was also suggested by Ghendon et al. (1972), Marongiu et al. (1981) and Rombaut et al. (1990).

Two observations point to 36S RNA, rather than RI RNA, as the RNA species associating with subviral particles. First, after UV irradiation of the 30% vesicular fraction, the sedimentation rate of 36S RNA is increased, whereas that of the RI is not affected. Second, after RNase treatment of a native UV-irradiated 30% vesicular fraction, we found viral RNA cross-linked to proteins to be digested (Fig. 6b). Since it has been shown that in a native 30% vesicular fraction, 36S but not RI RNA, is accessible to RNase (Bienz et al., 1992), we conclude that preferentially 36S RNA and not RI RNA associates with subviral particles.

By combining some of our earlier results with the
present findings, the following sequence of events during RNA encapsidation seem plausible. RNase digestion experiments with isolated RNA-synthesizing RC have shown, that upon completion, virtually all 36S RNA (in contrast to RI RNA) becomes RNase accessible (Bienz et al., 1992), presumably after changing its location in the RC. The altered RNase accessibility seems to be coupled to or closely following the release of 36S RNA from the RI, since no RNase-protected 36S RNA can be found in an actively RNA-synthesizing RC (Bienz et al., 1992). Concomitantly or following the completion of 36S RNA, its association with 14S pentamers occurs. Our results indicate that the process of RNA–protein association takes place within the RC of the 30% vesicular fraction, whereas it is not yet clear where the subsequent events in RNA encapsidation (condensation of RNA and capsid formation) occur. The final maturation step of VP0 cleavage, however, has to take place in RNP complexes detached from the RC since no complete virus can be detected in 30% vesicular fractions as judged by a lack of VP2 in SDS-PAGE (Fig. 3a) and of virions by EM (Pfister et al., 1992).

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References


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