Association of Ribosomes with Intracellular Vesicular Stomatitis Virus Particles

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Ribosomes are observed intimately associated with nucleocapsids of vesicular, stomatitis virus, especially those that line structures that are either cytoplasmic vesicles or invaginations of the plasma membrane.

Vesicular stomatitis virus (VSV) is a bacillus-shaped, enveloped virus with a single-stranded RNA genome (J. Orenstein, L. Johnson, E. Shelton, and R. A. Lazzarini, manuscript in preparation; 6, 7, 14). Both in the mature virus and in the cytoplasm of infected cells the genomic RNA exists as a ribonucleoprotein complex with approximately 1,100 subunits of the viral nucleocapsid protein, the N protein (3, 11, 13, 16). These nucleocapsid structures are easily identified by electron microscopy of thin sections of infected cells. The most frequently encountered forms are: (i) the tightly wound rod-like structures found in budding and mature viruses, (ii) the extended forms seen in the filamentous cytoplasmic inclusions, and (iii)

the oval structures which may be cross sections of the rod-like forms (12, 13, 17).

Biochemical data indicate that the RNA that directs viral protein synthesis is not the virion (genomic) RNA but its complement (9, 10). This mRNA is synthesized by viral transcriptase using the viral RNP as a template (1, 2, 8, 11). Consequently, it would appear that the viral RNP found in the cytoplasm of infected cells functions in two ways: one as a precursor of the nucleocapsid of progeny virus and two as a source of mRNA for viral protein synthesis. In an earlier report Murphy and Lazzarini (11) speculated that a single RNP complex might function in both capacities.

We have recently observed that ribosomes are

FIG. 1. VSV budding perpendicularly from the marginal plasma membrane. One of the three perpendicularly-oriented, budding VSV particles (left) has several ribosomes associated with the planar end of its bullet-shaped nucleocapsid, whereas another particle has only one ribosome in the vicinity (right). The ribosomes in the lower part of the field may be associated with the membrane of rough endoplasmic reticulum cut tangentially. Note the two virus particles cut in cross section in the upper left of the field. $\times 130,000$.

frequently found to be closely associated with various types of viral RNP structures in thin sections of VSV-infected BHK-21 cells. Virtually all cells which were harvested in h 9 of infection, fixed, stained, and sectioned (Oren-

stein et al., manuscript in preparation) have virus budding from their plasma membranes. The most common orientation of the bullet-shaped nucleocapsids in these maturing particles is perpendicular to the surface of the

FIG. 2. Transversely oriented VSV nucleocapsids. (a) The bullet-shaped, striated nucleocapsid material, oriented parallel to the plasma membrane, has at least four ribosomes intimately associated with its cytoplasmic side and spikes covering part of its membranous side. $\times 130,000$. (b) A cross sectioned virus particle transversely oriented to the plasma membrane (arrow) has two ribosomes in contact with its cytoplasmic side. $\times 130,000$. (c) Bullet-shaped nucleocapsid material with several intimately associated ribosomes apparently lies free in the cytoplasm. These ribosomes are indistinguishable from those on the rough endoplasmic reticulum in the lower part of the field. $\times 130,000$.

FIG. 3. Cytoplasmic vesicles rimmed with VSV nucleocapsids. (a) Part of a low-power electron micrograph of a VSV-infected cell showing three vesicles lined with nucleocapsids associated with ribosomes. These ribosomes are indistinguishable from those elsewhere in the field. In this particular field, cross sections of the nucleocapsid material are notable, but transverse and oblique sections are also present. Cells containing these vesicles frequently have abundant cytoplasmic filaments, seen in this field cut both longitudinally and in cross section. $\times 72,000$. (b) The intimate association of the ribosomes and the nucleocapsid material is shown clearly. Higher magnification of the middle vesicle shown in (a). $\times 130,000$. (c) Another vesicle lined by nucleocapsid material having ribosomes intimately associated with their cytoplasmic sides. The two particles cut in cross section in the lower right have spikes on their luminal sides (arrowheads). The tangentially cut nucleocapsid (arrow) could be misinterpreted as being free in the cytoplasm if the rest of the vesicle was not visible. $\times 130,000$.

membrane. Occasionally ribosomes can be seen in the immediate vicinity of these structures (Fig. 1). Nucleocapsids whose orientation is not perpendicular but parallel to the surface of the plasma membrane also are present. Although these transversely oriented particles are observed less often than perpendicularly oriented ones when they are encountered, they very frequently have ribosomes associated with them. Examples of this type of particle are shown in Fig. 2a and b. There, the longitudinally and cross-sectionally displayed particles both have ribosomes associated with the cytoplasmic side of the nucleocapsids. Spikes similar to the glycoprotein spikes of the mature virus frequently decorate the extracellular side of the membrane in the vicinity of the nucleocapsid. Highly structured RNP with clearly defined striations can be found which appear to be free in the cytoplasm of the infected cells (Fig. 2c). Examination of serial sections of several such particles indicates that they are special examples of the transverse orientation in which the RNP is transversely associated with a membrane in the sections immediately above or below the particle.

Ribosomes associated with viral RNP are encountered most frequently at the surfaces of what appear to be intracytoplasmic vesicles or invaginations of the plasma membrane (for convenience, called vesicles). Five to twenty percent of the cells examined at h 9 of VSV infection have vesicles which are lined with oval or bullet-shaped nucleocapsid material. The low magnification image in Fig. 3 shows three such viral-rimmed vesicles. The ribosomes on the rough reticula in the same field are indistinguishable from those associated with the viral structures. From the high magnification images of Fig. 3b and c it can be seen that ribosomes are virtually in contact with all aspects of the VSV nucleocapsid material. The perpendicularly oriented particles that are observed budding from the plasma membrane are conspicuously absent from the membranes of the vesicles.

The functional significance of the ribosome-RNP association can only be guessed at. Two sets of biochemical observations seem relevant, however. First, studies by Wagner et al. (15) indicate that the synthesis of the VSV envelope proteins M and G in infected cells is specifically associated with the plasma membrane and endoplasmic reticulum. More recently these findings have been corroborated by the demonstration that the G protein is specifically synthesized by the membrane-bound polysomes (4, 5). The ribosomes associated with the RNP may

be those synthesizing the G protein. The proximity of the ribosomes to the RNP may result from simple coincidence of the sites of G protein synthesis and viral assembly without a physical link connecting them. Second, the RNP transcription complex which synthesizes mRNA in VSV-infected cells may also function as precursor to the nucleocapsids of the progeny virus (11). Viewed within this framework the particles we have described here may be RNP which is still being actively transcribed and whose product mRNA is actively translated. In this case the close association of the ribosomes with the particles might result from the physical constraints of coupled transcription and translation.

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