Local Expression and Exocytosis of Viral Glycoproteins in Multinucleated Muscle Cells
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Abstract. We have analyzed the distribution of enveloped viral infections in multinucleated L6 muscle cells. A temperature-sensitive vesicular stomatitis virus (mutant VSV ts045) was utilized at the nonpermissive temperature (39°C). As expected, the glycoprotein (G protein) of this mutant was restricted to the ER when the multinucleated cells were maintained at 39°C. We demonstrate that this G protein remained localized when the infection was performed at low dose. By 4 h after infection the G protein patches spanned an average of 220 μm. The localization was independent of nuclear positions, showing that the ER was a peripheric structure. Thus, the infection did not recognize nuclear domains characteristic of nuclearly encoded proteins. After release of the 39°C block, transport through a perinuclear compartment into a restricted surface domain lying above the internal G protein patch occurred. Accordingly, the transport pathway was locally restricted. After a 16-h infection the G protein spanned 420 μm, while the matrix protein occupied 700–800 μm of the myotube length. Double infection of multinucleated L6 muscle cells with Semliki Forest virus and VSV at high multiplicities showed that the glycoprotein of each virus occupied intracellular domains which were devoid of the other respective glycoprotein. Taken together, these findings indicate that the viral glycoproteins did not range far from their site of synthesis within the ER or other intracellular membrane compartments in these large cells. This result also suggests that relocation of viral RNA synthesis occurred slowly.

Materials and Methods

Cells and Viruses

Rat L6 myoblastic cells (Yaffe, 1968) were grown in DME (Gibco Laboratories, Grand Island, NY) containing 10% FCS (Gibco Laboratories). Sub
confluent monolayers of L6 myoblasts were induced to fuse by growing the cells in DMEM containing 1% horse serum and 0.4 U/ml insulin. 4 d after the induction of fusion, the cells appeared as long, branched structures containing hundreds of nuclei which were gathered into groups. Wild-type vesicular stomatitis virus (VSV) (Indiana serotype) was grown in BHK cells (Matsin et al., 1983). The mutant ts045 was a clone ts045-6 (Griffiths et al., 1985). Semliki Forest virus (SFV) was propagated in BHK cells as described (Kääriäinen et al., 1969). Virus titers were determined on 2-d-old L6 myoblast monolayers. In brief, infected cells were identified by immunofluorescence staining for the viral glycoprotein, while the number of cells was determined by staining the nuclei with Hoechst dye 33258 (Sigma Chemical Co., St. Louis, MO). Multiplicities so obtained were regarded equivalent to plaque forming unit (pfu). The multinucleated cells were usually infected at 0.01 pfu/cell with VSV ts045 for 1 h at 32°C and then grown at 39°C for indicated periods. Infections with wild-type VSV and SFV were done at 37°C.

Metabolic Labeling and Analysis of the Labeled Virions

The multinucleated muscle cells were infected with VSV at 2 pfu and grown for 4 h at 37°C. A 10-min pulse with 50 μCi/ml of [35S]methionine (Amersham Corp., Bucks, England) was then given, followed by a 60-min chase. The chase medium was centrifuged (5000 g, 10 min) to remove cellular debris, and virions were then pelleted (30000 g, 60 min). The pellet was analyzed by SDS-PAGE (Laemmli, 1970).

Electron Microscopy

For thin section EM, myotubes were cultured on microscope object glasses. Cells were fixed with 3% glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.2, for 4 h. The specimens were stored in the same buffer supplemented with 7.5% sucrose. Postfixation was for 1 h in 4% OsO4. Specimens were dehydrated in ethanol and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate and examined with a Philips 410 electron microscope (Philips Electronic Instruments Co., Mahwah, NJ).

Antibodies

Polyclonal antibodies against VSV glycoprotein (G protein) were prepared by immunizing a rabbit with G protein liposomes. The liposomes were prepared by the octylglucoside dialysis method (Eidelberg et al., 1984). A viral lipid mixture was used for the reconstitution as described (Mettsikkö et al., 1982). About 10 μg protein were injected intradermally three times at 2-wk intervals. The antisera was collected 3 wk after the last booster and absorbed with a monolayer of multinucleated L6 muscle cells that were fixed and permeabilized. The preabsorbed antiserum specifically recognized the viral G protein when a cell lysate from BHK cells, infected with VSV, was analyzed by Western blotting at 1:200 antisera dilution.

mAbs against the VSV matrix (M) protein were prepared with some modifications as described (Gallpré and Mülio, 1981). VSV nucleocapsids containing the M protein (skeletons) (Newcomb et al., 1982) were prepared as described by Petri and Wagner (1979), and injected with Freund's adjuvant intraperitoneally into BALB/c mice (100 μg protein). During fusions, the ratio of spleen cells to P3-NS1-Ag4-1 myeloma cells was 1:5. Screening of the hybridomas was performed by Western blotting. In brief, polypeptides in cell lysates from BHK cells infected with VSV were separated by SDS-PAGE and transferred onto nitrocellulose (Risau et al., 1981). The culture medium of clone 2F9 specifically recognized VSV M protein and it was used for immunofluorescence studies.

A mAb against the VSV G protein was prepared by Henrik Garoff and Brian Burke at the European Molecular Biology Laboratory (EMBL). Ascites fluid was used at 1:400 dilution for immunofluorescence studies. Polyclonal antibodies against SFV p62/E2 glycoprotein have been described (Metsikkö and Garoff, 1990). They were directed against a peptide of 32 amino acids, corresponding to the cytoplasmic tail of p62 glycoprotein. Polyclonal antibodies against rat RER were prepared by Louvard et al. (1982). A mannosidase II specific mAb (53FC3) (Burke et al., 1982; Baron and Garoff, 1990) was used as a Golgi marker.

Immunofluorescence Studies

Cells were fixed with 3% paraformaldehyde in PBS and permeabilized with Triton X-100 as described (Lowyrd, 1980). Polyclonal anti-G protein serum was used at 1:200 dilution, followed by TRITC-conjugated anti-rabbit IgG (DAKO PATTS, Glostrup, Denmark; 1:400). Culture medium from clone 2F9 containing monoclonal anti-M protein antibody was diluted 1:5. For double immunofluorescence staining, the polyclonal and monoclonal antibodies were mixed at appropriate dilutions, applied to fixed cells, and incubated. TRITC-conjugated swine anti-rabbit IgG (DAKO PATTS; 1:200) and FITC-conjugated goat anti-mouse IgG (DAKO PATTS; 1:200) were mixed and used as secondary antibodies. Nuclei were visualized with Hoechst dye 33258. Simultaneous visualization of the internal and cell surface G proteins was performed in two stages. First, the fixed cells were incubated with polyclonal antibodies, followed by the TRITC-conjugated IgG. The cells were then permeabilized with Triton X-100 and internal G protein was visualized with a monoclonal anti-G protein antibody and FITC-conjugated anti-mouse IgG. Specimens were mounted with Mowiol (Hoechst, Frankfurt, Germany) in PBS containing 2.5% 1,4-diazobicyclo[2.2.2]octane (Sigma Chemical Co.) and viewed under the Leitz Aristoplan fluorescence microscope (E. Leitz Inc., Rockleigh, NJ), using appropriate filters.

Results

Infection of Multinucleated L6 Cells

The L6 myoblasts were induced to fuse by growing them in a medium containing horse serum and insulin. Under these conditions, branched myotubes were generated which contained several groups of nuclei separated by stretches of cytoplasm. To examine whether the L6 myotubes were successfully infected with VSV or the temperature-sensitive mutant of VSV ts045, we performed immunofluorescence staining for the VSV G glycoprotein. We found that the myotubes were infected throughout their length at viral doses corresponding to 0.5 pfu/cell in the parental mononucleated L6 myoblasts. Furthermore, by using [35S]methionine pulse-labeling of a myotube culture where 90% of the nuclei were counted in myotubes, we observed that the label was transferred into the medium in the form of virions (Fig. 1A). The viral titer of a VSV-infected myotube culture medium was 1.7 x 105 pfu/ml when determined after a 6-h infection period. Thus, viral particle formation occurred in the multinucleated muscle cells. Examination of the infected cells by EM indicated that budding occurred at the cell surface (Fig. 1B). Rounding and detachment of the myotubes was not observed after a 24-h infection period with the wild-type VSV. While the VSV M protein probably disrupts the cytoskeleton and causes a cytopathic effect in mononucleated cells (Blondel et al., 1990), it appears that myotubes are more resistant to this effect.

Infection at Low Dose Remains Local

We used the mutant VSV ts045 at 39°C to analyze whether the infection generated by a single viral particle remained local in the large multinucleated cells. At this temperature the mutant G protein should remain restricted to the ER and no infective particles should be formed (Schnitzer et al., 1979; Zilberstein et al., 1980; Metsikkö and Simons, 1986). We determined that the conditioned medium from multinucleated muscle cells infected with <2 pfu/cell of VSV ts045 was unable to infect a fresh culture of muscle cells, indicating that no infective progeny was generated to propagate the infection. At pfu <0.01 the ts045 infection was local as evaluated by indirect immunofluorescence for the G protein in

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1. Abbreviations used in this paper: pfu, plaque forming unit; SFV, Semliki Forest virus; VSV, vesicular stomatitis virus.
with a group of nuclei, fluorescent dots were often observed to surround vicinar nuclear groups.

To analyze whether the dispersion of the G protein was because of the exchange of ER membranes between nuclear groups, we stopped protein synthesis with cycloheximide (0.4 mM). Cycloheximide was added at 4 h after infection and the cells were fixed at 5 h after infection. Visualization of the G protein showed that the intense central staining disappeared (Fig. 3), suggesting that the G protein was part of a diffusible vesicle population. However, we could not demonstrate an increase in the size of the fluorescent G protein patches during the 1-h cycloheximide treatment, indicating that the G protein did not migrate far from the site of synthesis.

At 16 h after infection G protein localization spanned 420 ± 76 μm (SD) when measured on 10 myotubes. In the same myotubes, the viral matrix protein showed smooth staining spanning 700–800 μm on the average (Fig. 4). Phase contrast microscopy revealed usually that the M protein staining did not cover the entire length of the myotubes. These measurements show that the G protein dispersed at a rate of ~26 μm/h in multinucleated muscle cells while the corresponding rate for M protein was 40–50 μm/h. At this speed the viral infection should fill the cytoplasm of a 20 μm diameter mononuclear cell within 45 min.

**Exocytic Transport Is Vertical in Multinucleated Muscle Cells**

Incubation of mononucleated cells at 20°C blocks exocytic membrane traffic into a compartment called the trans-Golgi network (Matlin and Simons, 1983). We applied this block on myotubes infected with the VSV ts045. Accordingly, after a 3-h infection period at 39°C the locally infected myotubes were transferred to 20°C for 2 h. A prominent discontinuous perinuclear staining with diffuse dots in the cytoplasm was seen (Fig. 5A and C). The infection remained local but in the majority of cases vicinar groups of nuclei shared the perinuclear G protein. A Golgi-specific marker partially colocalized with the G protein in the infected area after the 2-h incubation at 20°C (Fig. 5B and D). Some G protein was probably left at the ER, explaining why the colocalization was not complete. It seems plausible that the perinuclear and dotty cytoplasmic staining represented the myotube trans-Golgi network. The presence of Nocodazole during the 20°C block did not scatter the observed circumnuclear ring structures (data not shown). Thus, depolymerization of microtubuli had little effect on the 20°C compartment. Furthermore, microtubuli were not essential for the transport from the ER into the 20°C compartment. This situation is thus comparable to that in the Cos cells where Nocodazole does not inhibit exocytic transport of the G protein (Featherstone et al., 1985).

Incubation for 30 min at 32°C after the 20°C block resulted in the appearance of the G protein on the cell surface. The surface staining was located over the sites where the internal G protein was seen, as shown by double immunofluorescence staining for the G protein before and after permeabilizing the cells (Fig. 6). Thus the G protein was transported from the presumed trans-Golgi network to the cell surface along the shortest route. After 2 h at 32°C, the surface G protein dispersed to a larger area on the myotubes, suggesting that at least a portion of the total G protein frac-
Figure 2. Distribution of VSV ts045 G protein in locally infected L6 multinucleated muscle cells at the nonpermissive temperature. In A, a local infection patch is shown. Polyclonal anti-G protein antibodies and TRITC-conjugated anti-rabbit IgG were used for the visualization. Nuclei in the same microscope field were visualized by Hoechst 33258 dye and are shown in B, together with the cell borders. The total length of the cell shown was 1.8 mm. C and D show double immunofluorescence staining with a mAb against the G protein and polyclonal anti-ER antibodies, followed by the appropriate FITC- and TRITC-coupled secondary antibodies. Note that the infection (C) remains local. Bars, 50 μm.

Expression was mobile. In mononucleated cells 75% of the surface G protein has been shown to be mobile (Scullion et al., 1987).

Double Infection in the Multinucleated Cells

Lytic viruses such as SFV or VSV cut off cellular protein synthesis and generally will not grow in the same cell (Metsikkö and Garoff, 1989). To establish whether multinucleated cells could be simultaneously infected by two lytic viruses, we infected myotubes with VSV and SFV and followed the progress of this double infection. We found that double infection in a multinucleated muscle cell was possible but that the two viral glycoproteins generally did not intermingle. As shown in Fig. 7, intracellular domains of VSV G protein were separated from those containing SFV p62 glycoprotein.

Extent of the mutual exclusion of VSV and SFV infections in multinucleated L6 muscle cells (as shown below). Infected areas for the VSV and SFV glycoproteins (see Fig. 7) were counted in five microscope fields. Percentages of the total number of the infected areas are given. The means and SD are shown:
Discussion

Our data indicate that the viral G protein was locally synthesized and inserted into the ER. This finding implies that the mRNA for the glycoprotein remained locally restricted, suggesting that the intracytoplasmic translocation of viral replication occurred slowly. Because the VSV ts045 G protein was blocked at the ER, we could follow the time course of its subsequent distribution within the ER. Since the VSV G protein remained localized for hours, we conclude that exchange of ER material within these large cells is slower than that of G protein degradation. Our results with cycloheximide suggest, however, that the protein in the ER could migrate short distances (Fig. 3). The average span of the G protein was 220 μm at 4 h after infection and 420 μm at 16 h after infection. For the CD8 membrane protein a 68 μm intracellular range was found (Ralston and Hall, 1989a). The reason for the discrepancy between these two results is probably that the viral infective RNA is more mobile than that of the nuclei. Moreover, CD8 was not blocked at the ER and therefore did not have time to disperse within the ER. We conclude that the longitudinal exchange of ER material occurred slowly. Thus, the ER in these large cells appears to be a relatively static compartment.

The infection patches were constantly found at areas without nuclei. This supports the idea that rough ER structures in myotubes do not belong to individual nuclei. Thus, the localization of the ER in multinucleated muscle cells is independent of nuclear positions. It appears that after myoblast fusion, their respective ER membranes completely interm-
Figure 4. Double immunofluorescence staining of VSV G and M proteins at 16 h after infection in locally infected multinucleated cells. The cells were infected with VSV ts045 at 0.003 pfu for 1 h, then incubated at 39°C for 15 h, and fixed. A shows G protein distribution. Two local infections are seen. B shows the M protein distribution. Bar, 300 μm.
Figure 6. Examples of the surface appearance of the G protein in locally infected multinucleated muscle cells. Muscle cells were infected at 0.01 pfu and then grown for 3 h at 39°C, followed by a 2-h growth period at 20°C. To facilitate transport to the cell surface, the temperature was shifted to 32°C for 30 min. The left panels show internal staining while the corresponding surface stainings are shown on the right. Bar, 100 μm.

Figure 5. Distribution of the VSV ts045 G protein in multinucleated L6 muscle cells during a 20°C transport block. The G protein was visualized using polyclonal anti–G protein antibodies and a TRITC-conjugated anti-rabbit IgG. Perinuclear G protein staining is shown in A and C. The corresponding double immunofluorescence stainings with a monoclonal anti-Golgi antibody and a FITC-conjugated anti-mouse IgG are shown in B and D. The infections (A and C) are local. Bar, 50 μm.
Figure 7. Double infection of multinucleated muscle cells with VSV and SFV. Cells were infected at 2 pfu of both VSV and SFV simultaneously. At 5 h after infection the cells were fixed and subjected to double immunofluorescence staining for the VSV G protein (A) or the SFV p62 glycoprotein (B). Bar, 100 μm.

in BHK or CHO cells (Froshauer et al., 1988). It seems that the replication of the SFV RNA is restricted to the sites of viral entry. However, Perännen and Kääriäinen (1991) found that one incoming virion induced the formation of numerous cytopathic vacuoles, suggesting that viral RNA can move from membrane to membrane. The situation with the VSV polymerase remains to be elucidated. We think that the dispersion of the G protein was not exclusively due to the movement of ER membranes. Rather, viral replication moved short distances. The finding that the M protein dispersed faster than the G protein could be because of the cytoplasmic nature of the M protein. It is known that a fraction of the M
protein is soluble in infected cells (Knipe et al., 1977). Furthermore, cytoplasmic proteins have been shown to diffuse freely in myotubes (Minz and Baker, 1967; Ralston and Hall, 1989b).

We show here that SFV and VSV can be propagated in a single multinucleated cell but that both viruses occupy their own domains of glycoprotein expression (Fig. 7). It is likely that the mRNA synthesis of both viruses was also restricted to such domains. It is reasonable to think that neither the glycoprotein nor the replicative viral RNA ranged more than ~200 μm in the doubly infected cells. We think that the first virus to be uncoated by endocytosis initiated local mRNA synthesis and occupied the protein synthesis machinery in a domain spanning ~200 μm.

In summary, in multinucleated L6 myoblast-derived muscle cell viral protein synthesis did not recognize nuclear domains at the ER level but still remained localized for a considerable period of time. This behavior reflects a static characteristic of the ER. Thus, there was little exchange of material between the ER membranes at different longitudinal parts of myotubes. At later stages of exocytosis, the membrane compartments were at least to some extent associated with individual nuclei. Thus there is strong supporting evidence for the functionality of nuclear domains. First, there is restricted longitudinal transport and second, organelles such as the Golgi network or the 20°C compartment are preferentially associated with individual nuclei. This situation provides the basis for positional information in multinucleated cells.

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References
Baron, M. D., and H. Garoff. 1990. Mannoosidase II and the 135 kDa Golgi-specific antigen recognized monoclonal antibody 53FC3 are the same di- meric protein. J. Biol. Chem. 265:19928–19931.
Blondel, D., G. G. Harmison, and M. Schubert. 1990. Role of matrix protein in cytopathogenesis of vesicular stomatitis virus. J. Virol. 64:1716–1725.
Burke, B., G. Griffiths, H. Reggio, D. Louvard, and G. Warren. 1982. A monoclonal antibody against a 135-K Golgi membrane protein. EMBO (Eur. Mol. Biol. Organ.) J. 1:1621–1628.
Eide1man, O., R. Schiegel, T. S. Traika, and R. Blumenthal. 1984. pH-dependent fusion induced by vesicular stomatitis virus glycoprotein reconstituted into phospholipid vesicles. J. Biol. Chem. 259:4622–4628.
Featherstone, C., C. Griffiths, and G. Warren. 1985. Newly synthesized G protein of vesicular stomatitis virus is transported to the Golgi complex in mitotic cells. J. Cell Biol. 101:2036–2046.
Fontaine, B., and J.-P. Changeux. 1989. Localization of nicotinic acetylcholine receptor alpha-subunit transcripts during myogenesis and motor endplate development in the chick. J. Cell Biol. 108:1025–1037.
Frosheuer, S., J. Kartenbeck, and A. Helenius. 1988. Alphavirus RNA replicase is located on the cytoplasmic surface of endosomes and lysosomes. J. Cell Biol. 107:2075–2086.
Galfré, G., and C. Millstein. 1981. Preparation of monoclonal antibodies: strategies and procedures. In Methods in Enzymology vol. 73B. J. J. Langone and H. van Vunakis, editors. Academic Press, New York. 3–46.
Griffiths, G., and K. Simons. 1986. The trans-Golgi network: sorting at the exit site of the Golgi complex. Science (Wash. DC). 234:438–443.
Griffiths, G., S. Pfeiffer, K. Simons, and K. Matlin. 1985. Exit of newly synthesized membrane proteins from the trans-cisterna of the Golgi complex to the plasma membrane. J. Cell Biol. 101:949–964.
Gu, Y., E. Ralston, C. Murphy-Erdosh, R. A. Black, and Z. W. Hall. 1989. Acetylcholine receptor in a C2 muscle cell variant is retained in the endoplasmic reticulum. J. Cell Biol. 109:729–738.
Hall, Z. W., and E. Ralston. 1989. Nuclear domains in muscle cells. Cell. 59:771–772.
Käräriinen, L., K. Simons, and C. H. von Bonnordoff. 1969. Studies of Semliki Forest virus subviral components. Ann. Med. Exp. Biol. Fenn. 47:255–268.
Knipe, D. M., D. Baltimore, and H. F. Lodish. 1977. Separate pathways of maturation of the major structural proteins of vesicular stomatitis virus. J. Virol. 21:1128–1139.
Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of bacteriophage T4. Nature (Lond.). 227:680–685.
Louvard, D. 1980. Apical membrane aminopeptidase appears at sites of cell-cell contact in cultured epithelial cells. Proc. Natl. Acad. Sci. USA. 77:4132–4136.
Louvard, D., H. Reggio, and G. Warren. 1982. Antibodies to the Golgi complex and the rough endoplasmic reticulum. J. Cell Biol. 92:92–107.
Matlin, K. S., and K. Simons. 1985. Reduced temperature prevents transfer of a membrane glycoprotein to the cell surface but does not prevent terminal glycosylation. Cell. 34:233–242.
Matlin, K., D. F. Bainton, M. Pesonen, D. Louvard, N. Genty, and K. Simons. 1983. Transmembrane transport of a viral membrane glycoprotein implanted into the apical plasma membrane of MDCK cells. I. Morphological evidence. J. Cell Biol. 97:627–637.
Merlie, J. P., and J. R. Sanes. 1985. Concentration of acetylcholine receptor mRNA in synaptic regions of adult muscle fibres. Nature (Lond.). 317:66–68.
Mettsikkö, K., K. Garoff. 1989. Role of heterologous and homologous glycoproteins in phenotypic mixing between Sendai virus and vesicular stomatitis virus. J. Virol. 63:511–524.
Mettsikkö, K., and H. Garoff. 1990. Oligomers of the cytoplasmic domain of the p62/E2 membrane protein of Semliki Forest virus bind to the nucleocapsid in vitro. J. Virol. 64:4678–4682.
Mettsikkö, K., and K. Simons. 1986. The budding mechanism of spikeless vesicular stomatitis virus particles. EMBO (Eur. Mol. Biol. Organ.) J. 5:1913–1920.
Mettsikkö, K., G. van Meer, and K. Simons. 1986. Reconstitution of the fusogenic activity of the vesicular stomatitis virus. EMBO (Eur. Mol. Biol. Organ.) J. 5:3429–3435.
Mintz, B., and W. W. Baker. 1967. Normal mammalian muscle differentiation and gene control of aspartate dehydrogenase synthesis. Proc. Natl. Acad. Sci. USA. 58:592–598.
Newcomb, W. G., J. J. Tobin, J. J. McGowan, and J. C. Brown. 1982. In vitro reassembly of vesicular stomatitis virus skeletons. J. Virol. 41:1055–1062.
Pavlath, G. P., K. Rich, S. G. Webster, and H. M. Blau. 1989. Localization of muscle gene products in nuclear domains. Nature (Lond.). 337:570–573.
Peränen, J., and L. Käräriinen. 1991. Biogenesis of type I cytopathic vacuoles in Semliki Forest virus-infected BHK cells. J. Virol. 65:1623–1627.
Petri, W. A., Jr., and R. R. Wagner. 1979. Reconstitution into liposomes of the glycoproteins of vesicular stomatitis virus by detergent dialysis. J. Biol. Chem. 254:4313–4316.
Ralston, E., and Z. W. Hall. 1989a. Intracellular and surface distribution of a membrane protein (CD8) derived from a single nucleus in multinucleated myoblasts. J. Cell Biol. 109:2345–2352.
Ralston, E., and Z. W. Hall. 1989b. Transfer of a protein encoded by a single nucleus to nearby nuclei in multinucleated myoblasts. Science (Wash. DC). 242:1066–1069.
Risa, W., H. Saumweber, and P. Symmons. 1981. Monoclonal antibodies against a nuclear membrane protein of Drosophila. Exp. Cell Res. 137:43–54.
Rotundo, R. L., and A. M. Gomez. 1990. Nucleus-specific translation and assembly of acetylcholinesterase in multinucleated muscle cells. J. Cell Biol. 110:715–719.
Schnitzer, T. J., C. Dickson, and R. A. Weiss. 1979. Morphological and biochemical characterization of viral particles produced by the ts45 mutant of vesicular stomatitis virus at restrictive temperature. J. Virol. 29:185–195.
Scullion, B. F., Y. Hou, L. Puddington, J. K. Rose, and K. Jacobson. 1987. Effects of mutations in three domains of the vesicular stomatitis virus glycoprotein on its lateral diffusion in the plasma membrane. J. Cell Biol. 105:69–75.
Tassin, A. M., B. Maro, and M. Bornens. 1985a. Fate of microtubule-organizing centers during myogenesis in vitro. J. Cell Biol. 100:35–46.
Tassin, A. M., M. Paintrand, E. G. Berger, and M. Bornens. 1983b. The Golgi apparatus remains associated with microtubule organizing centers during myogenesis. J. Cell Biol. 101:630–638.
Yaffe, D. 1968. Retention of differentiation potentials during prolonged cultivation of myogenic cells. Proc. Natl. Acad. Sci. USA. 61:477–483.
Zilberstein, A., M. D. Snider, M. Porter, and H. F. Lodish. 1980. Mutants of vesicular stomatitis virus blocked at different stages in maturation of the viral glycoprotein. Cell. 21:417–427.