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Infection with paramyxoviruses stimulates synthesis of cellular polypeptides that are also stimulated in cells transformed by Rous sarcoma virus or deprived of glucose

(simian virus 5/Sendai virus/membrane proteins/glucose uptake/glycosylation)

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ABSTRACT **Infection of several types of cultured cells with the paramyxoviruses simian virus 5 and Sendai virus stimulates synthesis of four polypeptides (I–IV) with molecular weights of approximately 99,000, 97,000, 86,000, and 78,000, respectively. That these are host polypeptides encoded in cellular mRNAs has been shown by the inhibition of their synthesis by actinomycin D and by the similarity of the peptide maps of them and of polypeptides with the same electrophoretic mobility from uninfected cells. Peptide mapping as well as identical migration in polyacrylamide gels has also indicated that polypeptides I, II, and IV are the same as plasma membrane polypeptides whose synthesis is enhanced in cells transformed by Rous sarcoma virus and in normal cells by glucose deprivation or treatment with 2-deoxyglucose. Polypeptides I and II appear to be the same polypeptides, with the observed differences in migration reflecting the glycosylation of polypeptide I, a relationship previously shown to exist between polypeptides in glucose-deprived and glucose-fed cells. Infection with paramyxoviruses does not significantly increase the transport of glucose by cells, and the maintenance of a high concentration of glucose in the medium does not prevent the enhanced synthesis of these polypeptides. This is in contrast to the situation in transformed cells in which stimulation of synthesis of these polypeptides is secondary to depletion of glucose in the medium due to increased glucose uptake by the cells. Thus, although paramyxovirus infection and transformation by Rous sarcoma virus result in stimulation of the synthesis of the same membrane polypeptides, the mechanism of stimulation differs.**

We have reported (1) that, in addition to the viral structural polypeptides and a nonstructural polypeptide, four polypeptides were synthesized in increased amounts in cells infected with the paramyxovirus simian virus 5 (SV5). These polypeptides, designated I–IV, had estimated molecular weights of 99,000, 97,000, 86,000, and 78,000, respectively. It was suggested that these were cellular polypeptides whose synthesis was enhanced by infection because: (*i*) polypeptides with identical electrophoretic mobilities were detected in uninfected cells; (*ii*) not all four polypeptides were enhanced in every cell type; and (*iii*) the synthesis of polypeptides with identical mobilities was enhanced in cells infected with Sendai virus, another paramyxovirus.

In this communication we present further evidence, including peptide maps, that these are cellular polypeptides and that polypeptides I, II, and IV are the same membrane polypeptides whose synthesis is stimulated in cells transformed by avian sarcoma viruses (2–4) and in cells deprived of glucose or under conditions in which glycosylation is inhibited (4–6). Maintenance of a high concentration of glucose in the medium decreases the synthesis of these polypeptides in transformed cells, and thus their enhancement in transformed cells appears to be secondary to the increased transport of glucose in such cells (4, 7). On the basis of these results, these polypeptides have been referred to as "glucose-regulated proteins" (4). As described here, paramyxovirus infection, unlike transformation, does not cause a significant increase in glucose transport, and maintenance of a high glucose concentration does not prevent the enhanced synthesis of these polypeptides in paramyxovirus-infected cells. Thus, paramyxovirus infection apparently produces a perturbation in the cell membrane, different from transformation, that alters the rate of synthesis of these polypeptides.

MATERIALS AND METHODS

Cells. CV-1 cells (TC7 clone) were grown in Eagle's medium with 10% fetal calf serum, and HeLa cells were grown in reinforced Eagle's medium with 5% fetal calf serum as described (1) (Eagle's medium contains 5.5 mM glucose and the reinforced medium contains 22 mM glucose). Primary chicken embryo fibroblasts (CEFs) and chorioallantoic membrane cells were prepared as described (8, 9) and grown on plastic surfaces in medium 199 with 7.5% calf serum, 1.5% heat-inactivated chicken serum, and 10% tryptose phosphate broth.

Virus. Stock SV5 virus was grown in MDBK cells or CV-1 cells; Sendai virus was grown in embryonated chicken eggs as described (1, 10).

Infection and Transformation of Cells. Cells were infected with SV5 or Sendai virus at a multiplicity of ≈50 plaque-forming units per cell as described (1). For transformation of CEFs, secondary cultures were inoculated with the Schmidt–Ruppin strain B of Rous sarcoma virus at a multiplicity of ≈0.5 and monitored for transformation by morphological changes as described by Goldberg (11). By 3 days, essentially all cells had been transformed and were used in experiments.

Labeling, Polyacrylamide Gel Electrophoresis, and Autoradiography. These were carried out as described (1).

Peptide Mapping. The limited proteolysis technique of Cleveland *et al.* (12) with minor modifications (13) was used; 5 μg of chymotrypsin or 0.25 μg of *Staphylococcus aureus* protease V8 was used per gel slot.

Hexose Transport. Cells were assayed for uptake of 2-[^{3}H]deoxyglucose by the technique of Hatanaka and Hanafusa (7). Cells were washed five times with warm phosphate-buffered saline (P_i/NaCl), incubated for 10 min at 37°C in P_i/NaCl containing 0.2 μCi of the isotope per ml, and rapidly washed five times with P_i/NaCl. Monolayers were scraped into 1 ml of water and sonicated, and samples were removed for protein determination (14) and scintillation counting. Results were expressed as cpm/mg of protein. Triplicate samples were used for each determination.

Abbreviations: SV5, simian virus 5; CEFs, chicken embryo fibroblasts; P_i/NaCl, phosphate-buffered saline.

RESULTS

Polypeptides Synthesized in Paramyxovirus-Infected Cells. As reported (1), all the known viral structural proteins (L, HN, NP, $F_{1,2}$, P, and M), the uncleaved precursor to $F_{1,2}$ (F_0), and a nonstructural viral protein (V) could be identified in SV5-infected cells. In addition, polypeptides I–IV with molecular weights of ≈99,000, 97,000, 86,000, and 76,000 were also seen, beginning 12–14 hr after infection (Fig. 1). The enhanced synthesis of polypeptides I–IV can be seen in both the autoradiograph of the polyacrylamide gel and the densitometer tracing. The synthesis of these polypeptides is also enhanced in cells infected with Sendai virus (1), and this can be detected as early as 8 hr after infection.

Inhibition of Synthesis of Polypeptides I–IV by Actinomycin D. Because polypeptides with similar electrophoretic mobilities are present in uninfected cells as well as in cells infected with two different paramyxoviruses, it was concluded that these were cellular polypeptides whose synthesis was enhanced on infection. To obtain additional evidence that these polypeptides are translated from host cell mRNAs whose synthesis is enhanced in infection, the ability of actinomycin D to inhibit the synthesis of these polypeptides was investigated. CEFs were infected with SV5 or Sendai virus and treated with actinomycin D (1 μg/ml) for 6 hr, beginning at 10.5 hr after infection, and then were pulse-labeled for 30 min with [^{35}S]-methionine in the presence of the drug. The synthesis of polypeptides I–IV was increased in the infected cells, and actinomycin D inhibited this enhancement (Fig. 2). As expected with paramyxoviruses (16, 17), the synthesis of viral polypeptides was not inhibited by the drug.

Synthesis of Polypeptides I–IV in Uninfected Cells. It has been reported that cells deprived of glucose or grown in the presence of 2-deoxyglucose or high concentrations of glucosamine synthesized increased quantities of plasma membrane polypeptides with molecular weights of ≈95,000 and 78,000 (or 90,000 and 75,000, depending on the gel system used) (4, 6) and that a 97,000 (or 92,000) polypeptide present in glucose-fed cells was the glycosylated form of the 95,000 polypeptide. Furthermore, the 95,000 and 78,000 polypeptides found under conditions of glucose deprivation corresponded to the 90,000 and 75,000 polypeptides described in transformed cells (2, 3). With allowances for minor differences in estimated molecular weights due to the gel systems used, it appeared likely that polypeptides I, II, and IV which we found (1) in paramyxovirus-infected cells correspond to the 97,000, 95,000, and 78,000 polypeptides seen in transformed and glucose-deprived cells. To investigate this, the polypeptides synthesized in SV5- and Sendai virus-infected cells were compared with those synthesized in glucose-free medium or in the presence of 2-deoxyglucose. Polypeptides II and IV in the infected cells comigrated with polypeptides induced in glucose-free medium or with 2-deoxyglucose (Fig. 3). Although both I and II were found in infected cells, a polypeptide corresponding to I was not increased in cells maintained in glucose-free medium or 2-deoxyglucose, as would be expected if polypeptide I were the glycosylated form of II as is the case with the glucose-regulated polypeptides with molecular weights of ≈97,000 and 95,000. Additional evidence for the identity of these polypeptides is presented below in peptide maps.

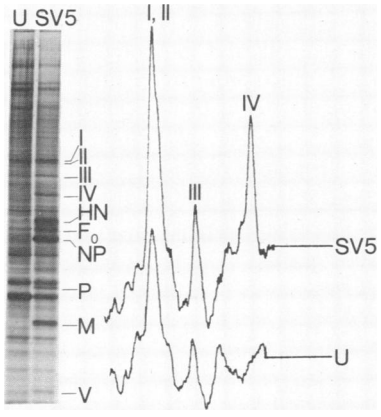

FIG. 1. Effect of infection with SV5 on the synthesis of polypeptides in primary CEF cells. (*Left*) Cells were labeled with [^{35}S]-methionine (10 μCi/ml) for 60 min 20 hr after infection and then processed for electrophoresis and autoradiography. U, uninfected cells; SV5, SV5-infected cells. (*Right*) Densitometer tracing of the upper portions of the gels containing polypeptides I–IV.

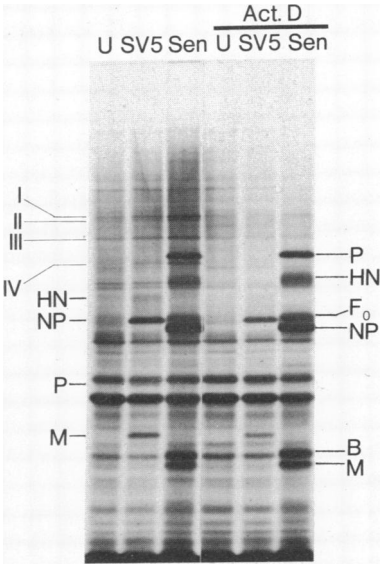

FIG. 2. Effect of actinomycin D (Act. D) on synthesis of polypeptides I–IV in paramyxovirus-infected cells. CEFs were infected with SV5 or Sendai virus (Sen), and 10.5 hr later the medium was removed and replaced with fresh medium, with or without actinomycin D (1 μg/ml). After 6 hr, the medium was removed and replaced with methionine-free medium containing [^{35}S]methionine (10 μCi/ml), with or without actinomycin D. After 30 min the cells were prepared for electrophoresis. All media contained 22 mM glucose. B, phosphorylated form of polypeptide M (15). U, uninfected.

Comparison of Polypeptides Synthesized in Paramyxovirus-Infected and Transformed Cells. The stimulation of synthesis of membrane polypeptides with molecular weights of ≈95,000 and 78,000 in cells transformed by avian sarcoma viruses has been shown to be suppressed by maintenance of high concentrations of glucose in the medium, and these experiments led to the conclusion (4) that the synthesis of these polypeptides is not a direct effect of transformation but is secondary to depletion of glucose in the medium as a result of an increased rate of glucose transport in transformed cells (7). To determine if glucose deprivation played a role in the enhanced synthesis of polypeptides I–IV in paramyxovirus-infected cells and to compare these polypeptides with those synthesized in transformed cells, the synthesis of polypeptides in Sendai virus-infected CEFs and cells transformed by Rous sarcoma virus was examined in various concentrations of glucose. Regardless of whether the cells were maintained in relatively low (5.5 mM) or high (22 or 110 mM) levels of glucose, or if fresh medium containing a high concentration of glucose was added at 12 hr after infection, the synthesis of polypeptides I–IV was enhanced (Fig. 4). In contrast to these results with Sendai virus, the en-

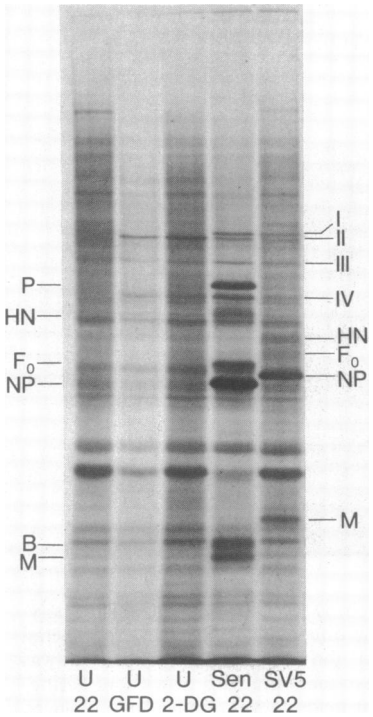

FIG. 3. Comparison of polypeptides synthesized in cells deprived of glucose, treated with 2-deoxyglucose, or infected with paramyxoviruses. CEFs were grown in glucose-free medium or medium containing 5 mM 2-deoxyglucose for 24 hr or were infected with SV5 or Sendai virus for 16 hr prior to labeling. All cells were pulsed with [^{35}S]methionine (10 μCi/ml) for 30 min and prepared for electrophoresis. U, uninfected cells; Sen, Sendai virus-infected; SV5, SV5-infected; 22, mM glucose; GFD, cells in glucose-free medium; 2-DG, cells treated with 2-deoxyglucose.

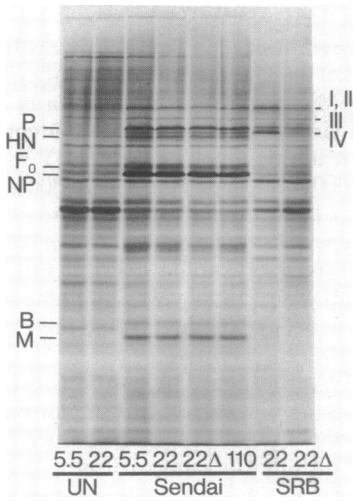

FIG. 4. Comparison of polypeptides synthesized in cells infected with Sendai virus or transformed by Rous sarcoma virus, with different concentrations of glucose in the medium. Secondary cultures of normal CEFs or cells transformed with the Schmidt–Ruppin B strain of Rous sarcoma virus (SRB) were used. All cells were changed to fresh medium containing 5.5 or 22 mM glucose and the untransformed cells were infected with Sendai virus. After 12 hr, medium was removed from some cells and replaced with fresh medium containing 22 mM glucose; other cells were left unchanged. After 4 hr, all cells were labeled with [^{35}S]methionine (10 μCi/ml) for 60 min and then prepared for electrophoresis. The numbers refer to glucose concentration (mM); Δ indicates the cells whose medium was changed at 12 hr.; UN, uninfected.

hanced synthesis of polypeptides I, II, and IV in transformed cells in which the glucose level was not maintained by changing the medium (lane SRB 22) was largely abolished by addition of fresh glucose-containing medium (lane SRB 22Δ). Thus, although maintenance of a high glucose concentration prevents the enhancement of synthesis of polypeptides in transformed cells (4), a high glucose concentration does not affect the enhanced synthesis of these proteins in paramyxovirus-infected cells. In this case the synthesis of these polypeptides is not turned on by depletion of glucose, and therefore increased glucose transport in infected cells is apparently not the cause of their enhancement.

Glucose Transport in Paramyxovirus-Infected Cells. To investigate further the possible effects of paramyxovirus infection on hexose transport, the uptake of 2-[^{3}H]deoxyglucose was investigated in four cell types infected with SV5 or Sendai virus. With the possible exception of a slight stimulation in Sendai virus-infected CEFs, there was no detectable effect on sugar uptake in infected as compared to normal cells (Table 1). In contrast, as reported earlier by Hatanaka and Hanafusa (7), there was marked stimulation of uptake in transformed cells. These results and the above data on the polypeptides indicate that different mechanisms are responsible for the enhanced synthesis of these polypeptides in paramyxovirus-infected and in transformed cells.

Peptide Mapping of Polypeptides. On the basis of identical migration in polyacrylamide gels and their behavior with respect to glucose deprivation, polypeptides I, II, and IV apparently correspond to the glucose-regulated polypeptides with molecular weights of ≈97,000, 95,000, and 78,000 described by Pouysségur *et al.* (6) and Shiu *et al.* (4), which in turn appear to be the same polypeptides stimulated in transformed cells (2, 3). Results of experiments with glucose deprivation or inhibition of glycosylation suggested that the 97,000 polypeptide is the glycosylated form of the 95,000 polypeptide, and antiserum against the former also reacted with the latter (4, 6, 18). Furthermore, an antiserum prepared against the 78,000 polypeptide from transformed cells reacted with the polypeptide of similar mobility from glucose-deprived cells (4). To investigate further the identity of polypeptides I–IV and the comparable polypeptides in glucose-deprived, glucose-fed, and transformed cells, these polypeptides were compared by peptide mapping.

Polypeptide I from uninfected glucose-fed cells, polypeptide II from uninfected glucose-deprived cells, polypeptides I and II from SV5-infected cells, and polypeptides I and II from Sendai virus-infected cells contained similar peptides (Fig. 5). (The two polypeptides from infected cells were mapped together because they are both present in significant amounts, and it is not possible to excise them separately from gels.) These results confirm that the polypeptides from normal and infected cells are the same, and that I and II are different forms of the same polypeptide, with I being glycosylated as shown in other systems (4, 6) as well as above. Polypeptide IV from SV5- or Sendai virus-infected cells and the polypeptides of the same mobility in normal, glucose-deprived, and transformed cells gave similar peptide maps (Fig. 6). The polypeptides designated

Table 1. Effect of paramyxovirus infection and transformation by Rous sarcoma virus on uptake of 2-deoxyglucose by cells

	Sugar uptake in infected or transformed cells*		
Cell type	Sendai virus	SV5	Transformed cells
CEF	1.8	1.0	14.0
CAM†	1.1	0.8	
CV-1	1.2	0.9	
HeLa	1.0	1.0	

* At 12–18 hr after infection with SV5 or Sendai virus or at 3 days after infection with Rous sarcoma virus (when essentially all cells appeared to be transformed), the uptake of 2-[^{3}H]deoxyglucose (0.2 μCi/ml) was determined. Data are shown as relative to value in uninfected cells, means of two to five experiments.

† Chorioallantoic membrane cells.

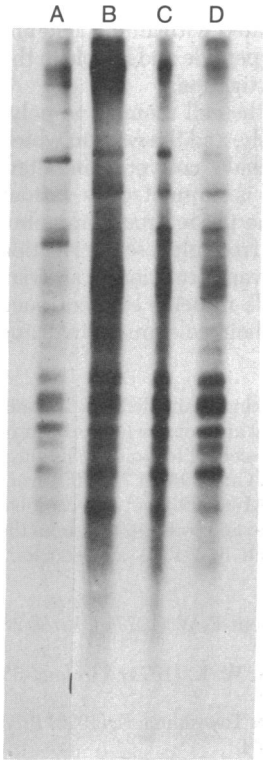

FIG. 5. Peptide mapping by limited proteolysis of polypeptides I and II from glucose-deprived or paramyxovirus-infected cells. CV-1 cells were infected with SV5 or Sendai virus or were deprived of glucose for 24 hr. At 18 hr after infection, cells were labeled with [^{35}S]methionine (150 μCi per culture) in methionine-free medium for 4 hr, and cell lysates were prepared for electrophoresis. The appropriate bands were excised from wet gels and digested with chymotrypsin (5 μg), and the peptides were separated by electrophoresis. Infected cells were maintained in 22 mM glucose; Lanes: A, polypeptide II from uninfected cells deprived of glucose for 24 hr; B, polypeptide I from uninfected cells maintained in 22 mM glucose; C, polypeptides I and II from SV5-infected cells; D, polypeptides I and II from Sendai virus-infected cells.

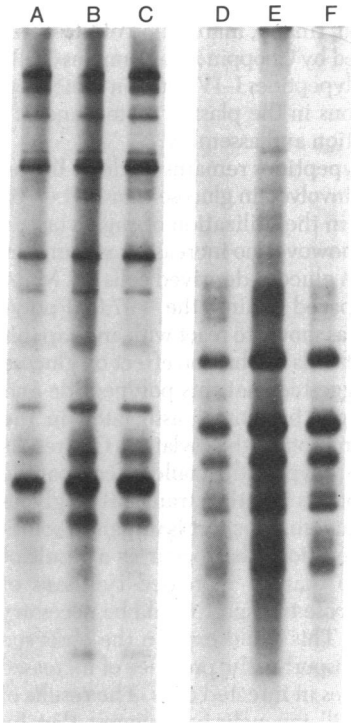

FIG. 7. Peptide mapping of polypeptide III from uninfected and paramyxovirus-infected cells. CEFs were infected with SV5 or Sendai virus and labeled with [^{35}S]methionine, the polypeptides were isolated and digested, and the peptides were separated as in Fig. 5. Lanes A–C are after digestion with chymotrypsin (5 μg); lanes D–F are after digestion with *S. aureus* protease (0.25 μg). Lanes: A and D, polypeptide III from uninfected cells; B and E, polypeptide III from SV-5 infected cells; C and F, polypeptide III from Sendai virus-infected cells.

III from uninfected, SV5-infected cells, and Sendai virus-infected cells gave similar peptide maps (Fig. 7). These results provide additional evidence that the polypeptides whose synthesis is enhanced under the various conditions are the same and that they exist in normal cells, confirming their cellular rather than viral origin.

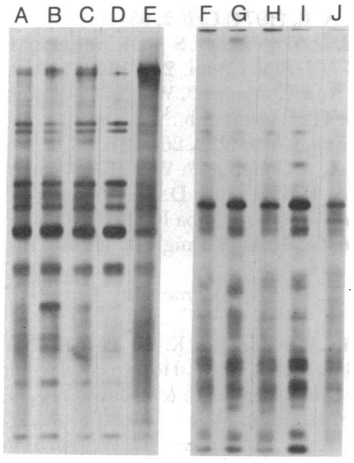

FIG. 6. Peptide mapping of polypeptide IV from paramyxovirus-infected, transformed, and glucose-deprived cells. CEFs infected with SV5 or Sendai virus or transformed with Rous sarcoma virus were labeled with [^{35}S]methionine, the polypeptides were isolated and digested, and the peptides were separated by electrophoresis as in Fig. 5. Transformed cells were grown in medium containing 5.5 mM glucose for 48 hr prior to labeling to allow maximal synthesis of polypeptide IV. Lanes A–E are after digestion with chymotrypsin (5 μg); lanes F–J are after digestion with *S. aureus* V8 protease (0.25 μg). Lanes: A and F, polypeptide IV from SV5-infected cells; B and G, polypeptide IV from Sendai virus-infected cells; C and H, polypeptide IV from uninfected cells deprived of glucose for 24 hr; D and I, polypeptide IV from transformed cells; E and J, polypeptide IV from uninfected cells.

DISCUSSION

On the basis of electrophoretic mobility, behavior in the presence of various concentrations of glucose and 2-deoxyglucose, and peptide maps, polypeptides I, II, and IV, which we previously reported (1) to be synthesized at enhanced rates in cells infected with paramyxoviruses, are shown to be the same polypeptides that are induced in normal cells under conditions of glucose deprivation or inhibition of glycosylation (4–7) and in cells transformed by avian sarcoma virus (2–4, 6). Further evidence that these are cellular rather than viral polypeptides has been provided by peptide maps and the inhibition of their synthesis by actinomycin D. The latter is in agreement with the finding (19) of increased translation, in a cell-free system using mRNAs from transformed cells or from glucose-deprived cells, of polypeptides with molecular weights of ≈95,000 and 78,000, thus suggesting that control of the synthesis of these polypeptides is at the level of transcription. The present results also indicate that polypeptide I in paramyxovirus-infected cells is the glycosylated form of polypeptide II, as was shown with the 97,000 and 95,000 polypeptides in glucose-fed and glucose-deprived cells, respectively (4, 6, 18).

Although both paramyxovirus infection and transformation enhance the synthesis of similar peptides, the precise mechanisms for their stimulation appear to be different. In transformed cells there is a greatly increased rate of glucose transport (7), and the increased synthesis of these polypeptides is secondary to this and inhibited by maintenance of a high glucose concentration in the medium (4, 6). In contrast, in paramyxovirus-infected cells we found little or no increase in glucose transport, and maintenance of high glucose concentrations did not prevent enhanced polypeptide synthesis. Thus, paramyxovirus infection apparently causes membrane alterations that involve the enhanced synthesis of these membrane polypeptides, which is not the result of increased glucose transport. In this regard, it is pertinent that paramyxovirus replication involves the synthesis of two viral glycoproteins, their incorporation into the plasma membrane, the association of a nongly-

cosylated viral membrane polypeptide with the inner surface of the plasma membrane, and, finally, maturation of the virus by a budding process [reviewed by Choppin and Compans (20)]. The enhanced synthesis of polypeptides I–IV could in some way be related to the perturbations in the plasma membrane occurring during virus replication and assembly.

The function of these polypeptides remains unclear. It was suggested that they could be involved in glucose transport (4–6) or as enzymes participating in the utilization of sugars or energy-yielding reactions (4); however no increase was found in a number of such enzymes in glucose-deprived cells (4). More recently, an antiserum prepared against the ≈97,000 polypeptide from 3T3 cells (18) was shown to react with and cap this polypeptide on the surface of cells but had no effect on glucose transport. These authors suggested that this polypeptide and the 78,000 polypeptide might be closely associated in the membrane and be involved in protein glycosylation. Our results on the enhancement of these polypeptides could be compatible with a role of these polypeptides in either transport or glycosylation. It is possible that glucose transport is impaired somewhat by the membrane alterations that occur as a result of paramyxovirus infection, so that an increased synthesis of polypeptides involved in glucose transport would be necessary to maintain normal uptake. This could explain the observed essentially normal rate of transport in the presence of increased amounts of these polypeptides in infected cells. The results of preliminary experiments, still inconclusive, suggest that infection of transformed cells with paramyxoviruses diminishes the increased glucose transport in transformed cells. The fact that polypeptide II, the unglycosylated form of polypeptide I, is also stimulated in paramyxovirus-infected cells might also be construed as a reflection of decreased availability of glucose, because this is the form stimulated in glucose-deprived cells. On the other hand, it is also plausible that, if these polypeptides were involved in glycosylation of proteins, their synthesis would be stimulated by paramyxovirus infection, in which the synthesis of two new virus-specific viral membrane glycoproteins occurs and host cell protein synthesis is not inhibited by infection with SV5 or Sendai virus within the time period of these experiments (1, 10, 21). An understanding of the precise role of these polypeptides, which appear to be involved in some aspect of glucose transport or utilization or glycosylation, must await further study.

Lee and Lipmann (22) have isolated a glucose-binding factor from CEFs that is increased in cells transformed by Rous sarcoma virus. The addition of this factor to the medium increased the uptake of glucose by cultured cells. To investigate the possibility that this factor represented one or more of polypeptides I–IV, a sample, kindly supplied by Lee, was subjected to coelectrophoresis with the polypeptides from paramyxovirus-infected and glucose-deprived cells: the glucose-binding factor migrated significantly faster than any of the other polypeptides, with an estimated molecular weight of ≈70,000. This does not definitely exclude a relationship among these polypeptides because it is conceivable that size or electrophoretic mobility may have been altered in the process of isolation or preparation for electrophoresis. Further comparisons of the polypeptides are necessary to explore the possible relatedness of these polypeptides.

Although polypeptides I, II, and IV correspond to polypeptides whose synthesis is enhanced in glucose-deprived or transformed cells, polypeptide III of paramyxovirus-infected CEFs has not been noted under these conditions; thus, the function of this polypeptide may be different from that of the others. Polypeptide III does appear to correspond to one of the polypeptides synthesized in cells treated with amino acid analogs (23, 24). The nature of this polypeptide and its role in the uninfected cell require further investigation.

Although the exact functions of the cell membrane polypeptides described here and previously, and the ways in which they may interact with each other, have not yet been determined, the fact that their synthesis is stimulated by various conditions, each of which affects the plasma membrane but vary significantly in nature ranging from glucose deprivation to infection with paramyxoviruses to transformation by an avian sarcoma virus, provides considerable impetus for continued study of these polypeptides and their role in normal and virus-modified cells.

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