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Phosphorylation of Ribosomal Proteins in Hamster Fibroblasts Infected with Pseudorabies Virus or Herpes Simplex Virus

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In BHK cells infected with pseudorabies virus, there was a substantial increase in the phosphorylation of ribosomal protein S6. This increase occurred between 2 and 4 h after infection and persisted at least until 9 h. We estimated that in mock-infected cells S6 contained, on an average, one phosphate group per protein chain, whereas in infected cells this rose to between four and five phosphate groups per protein chain. A second ribosomal protein, either S16 or S18, was also phosphorylated after infection. No increase in cyclic AMP was found at the time of phosphorylation. We also found an increased phosphorylation of S6 in herpes simplex virus-infected BHK cells.

In a wide variety of eucaryotic cells, only 2 of the 70 to 80 ribosomal proteins are phosphorylated in vivo (20). One of these is a small acidic protein of the 60S ribosomal subunit (22, 39), the extent of phosphorylation of which is altered only under very abnormal circumstances (24, 25). In contrast, the extent of phosphorylation of the other phosphoprotein, a basic protein of the 40S ribosomal subunit designated S6, can be increased by a variety of stimuli (see reference 20 for a recent review), including cyclic AMP (5, 10, 30), inhibitors of protein biosynthesis (9, 11), and conditions which stimulate cellular growth (18, 26, 35). The phosphorylated forms of ribosomal protein S6 can be readily identified by two-dimensional gel electrophoresis in the system of Kaltschmidt and Wittmann (14) because they migrate to the anodic side of the parent spot in the first dimension. When present, five discrete increasingly phosphorylated derivatives can be resolved under optimal conditions (8).

Despite the fact that ribosomal protein S6 is usually more highly phosphorylated on polyribosomes than on monoribosomes (11, 23, 28), it has not been possible to detect any quantitative functional difference among ribosomes in which the extent of phosphorylation of S6 is markedly different. One possibility that has not been adequately tested is that the phosphorylation of ribosomal protein S6 affects the relative affinity of ribosomes for different mRNA's. In this respect, it is interesting that increased labeling of ribosomal protein S6 by ^{32}P has been reported in cells infected with vaccinia virus (12, 13), adenovirus (2, 33), and mengovirus (31), suggesting a possible role for the phosphorylation in the selective translation of viral, rather than host cell, mRNA's. In none of the above reports, however, was there a clear difference in electrophoretic migration of S6 accompanying the increased radiolabeling. Thus, there is doubt as to whether the increased ^{32}P radioactivity of S6 reflected an increase in its phosphate content or whether it resulted from an increased specific radioactivity of the nucleotide precursor or an increase in the turnover of the phosphoryl groups. In contrast, the results presented here clearly show that infection of BHK cells (baby hamster kidney fibroblasts) with pseudorabies virus or herpes simplex virus causes extensive conversion of ribosomal protein S6 to more highly phosphorylated derivatives.

MATERIALS AND METHODS

Cells. BHK-21/C13 cells were maintained in monolayer cultures in modified Eagle medium containing 10% calf serum, as previously described (26), and experiments were performed with cells grown in roller bottles for 3 days, by which time they had just reached confluence.

Virus. Pseudorabies virus was originally derived from a stock preparation (15) and has been plaque purified several times. Virus stocks were prepared from infected BHK monolayer cultures as previously described (4). Herpes simplex virus type 1 was the F strain, and the original stock was obtained from Y. Becker, Hebrew University, Jerusalem.

Infection. The methods used for infection have been described previously (34) but, briefly, were as follows. The volume of medium in the roller bottles was decreased from 180 to 25 ml, and pseudorabies virus was added to a multiplicity of 20 PFU per cell or herpes simplex virus was added to a multiplicity of 10 PFU per cell. A similar volume of medium was added

for mock infection. The virus was allowed to adsorb for 1 h, and the medium was removed and replaced by 50 ml of the original medium. Infection was allowed to continue for various times, with the time of infection quoted being taken from the initial addition of the virus.

Labeling. In those experiments in which the cells were labeled with $^{32}P_i$, the medium was replaced by 50 ml of one in which the phosphate had been decreased to 1/10 of its normal concentration and from which tryptose phosphate broth had been excluded. After 30 min the medium was replaced by 50 ml of the same, 1 mCi of $^{32}P_i$ (Radiochemical Centre, Amersham, United Kingdom) was added per roller bottle, and incubation was continued for a further 3 h before the cells were harvested.

Cell fractionation and isolation of ribosomal proteins. Ribosomes were isolated from the cells (26) and dissociated into their subunits (29), and their proteins were extracted (26), all as previously described.

Polyacrylamide gel electrophoresis. Ribosomal protein (200 μg) was subjected to two-dimensional gel electrophoresis, using the Lastick and McConkey modification (17) of the general method of Kaltschmidt and Wittmann (14), except a miniaturized system (19) was used for which the conditions of electrophoresis were 3 mA per gel for 3 h in the first dimension (or as indicated by an external cytochrome *c* tracker dye [21]) and 6 mA per gel for 16 h in the second dimension. The gels were stained for 3 h with Coomassie brilliant blue R and destained with 1 M acetic acid, and the proteins were designated according to standard nomenclature (38). In some cases the gels were sliced longitudinally, dried, and subjected to autoradiography as described previously (29). In other cases the stained gel spots were cut out of the gel and treated with Protosol (New England Nuclear Corp., Boston, Mass.), and their radioactivity was determined by liquid scintillation spectrometry. For a more detailed analysis of the distribution of radioactivity in ribosomal protein S6, segments of rectangular cross section containing this protein and its derivatives were excised from the gels, scanned densitometrically at a wavelength of 586 nm, using the linear transport accessory to a Gilford 240 spectrophotometer, and then cut into slices with an assembly of razor blades, and the radioactivity in each slice was determined as described above.

In some experiments (data not shown) the protein was subjected to electrophoresis in the presence of sodium dodecyl sulfate, as previously described (22).

Estimation of stoichiometry of phosphorylation of ribosomal proteins. In each radioactive experiment the specific radioactivity of the cellular ATP was measured as previously described (29) and used to convert to moles of phosphate the ^{32}P radioactivity in the excised gel spots of S6. A correction had to be made to allow for the fact that all of the protein applied to the two-dimensional gels does not enter the gels. This was done by comparing the relative recovery in S6 of ^{32}P per microgram of applied protein with that obtained with gels containing sodium dodecyl sulfate, for which 100% recovery was assumed.

Protein was estimated by the dye-binding method of Bradford (3), using bovine serum albumin as a standard.

ATP and cyclic AMP. ATP (29) and cyclic AMP (26) were determined as described previously.

RESULTS

Pseudorabies virus. Infection of BHK cells for 5 h with pseudorabies virus had a marked effect on the phosphorylation of the ribosomal proteins of the 40S subunit (Fig. 1). Not only was the ^{32}P labeling of ribosomal protein S6 markedly increased compared with that in uninfected cells, but most of the stained S6 protein was displaced anodically from the position to which it migrated in uninfected cells at this stage of growth. In addition, a ribosomal protein that was not phosphorylated in uninfected cells, either S16 or S18 (S16/18), became labeled by $^{32}P_i$. The alteration in the position of electrophoretic migration of S6 did not occur in mock-infected cells, nor did mock infection cause any labeling of protein S16/18 (Fig. 2).

The relationship between the phosphorylation of ribosomal protein S6 and the position of its electrophoretic migration is better illustrated in Fig. 3 and 4, which show the 40S ribosomal proteins of cells infected for 9 h with pseudorabies virus. In this case, the overall extent of phosphorylation was somewhat less than at earlier times (Fig. 1 and 2), and an appreciable amount of stained material was in the original position of protein S6 or in anodic positions of less extreme displacement. The majority of the radioactivity coincided with the peak of the most anodic position of protein S6, with little being found at the peak of the most cathodic position (Fig. 4). Figure 3 also shows, more clearly than do Fig. 1 and 2, a definite appearance of stained protein at the anodic side of the position to which proteins S16 and S18 migrated in uninfected cells; this was coincident with the blackened area on the autoradiograph.

The changes in phosphorylation of protein S6 in BHK cells infected with pseudorabies virus were clearly of considerable magnitude and involved most of the molecules of S6 on the ribosome. However, the predominance of a single species in Fig. 1 and 2 and the incomplete resolution of the different species in Fig. 4 made it difficult to quantitate the phosphorylation exactly. A comparison with published work in which the various phosphorylated species of ribosomal protein S6 were all present and resolved (9, 36, 37) suggests that the major species of S6 from the virus-infected cells of Fig. 1 and 2 contains four or five phosphoryl groups per molecule of S6. We also estimated the extent of phosphorylation from measurements of the specific radioactivity of the ATP pool (see above),

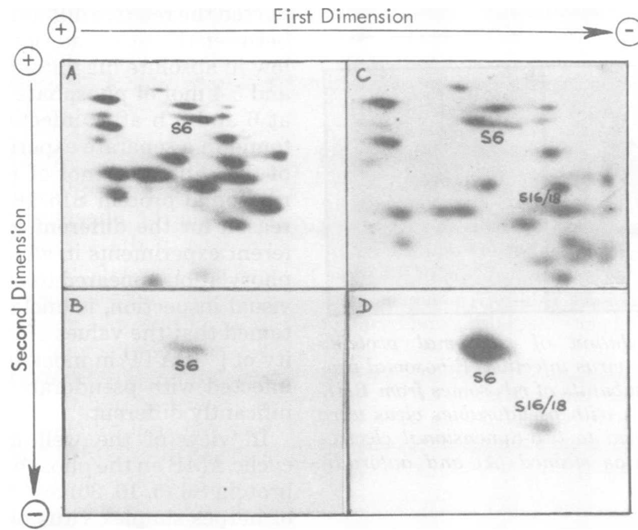

FIG. 1. *Phosphorylation of ribosomal proteins during pseudorabies virus infection. Ribosomal proteins from the 40S subunit of ribosomes from BHK cells were isolated and subjected to two-dimensional electrophoresis. The gels were stained with Coomassie brilliant blue (A and C) and autoradiographed (B and D). The patterns were obtained from uninfected cells (A and B) and from cells infected for 5 h with pseudorabies virus (C and D).*

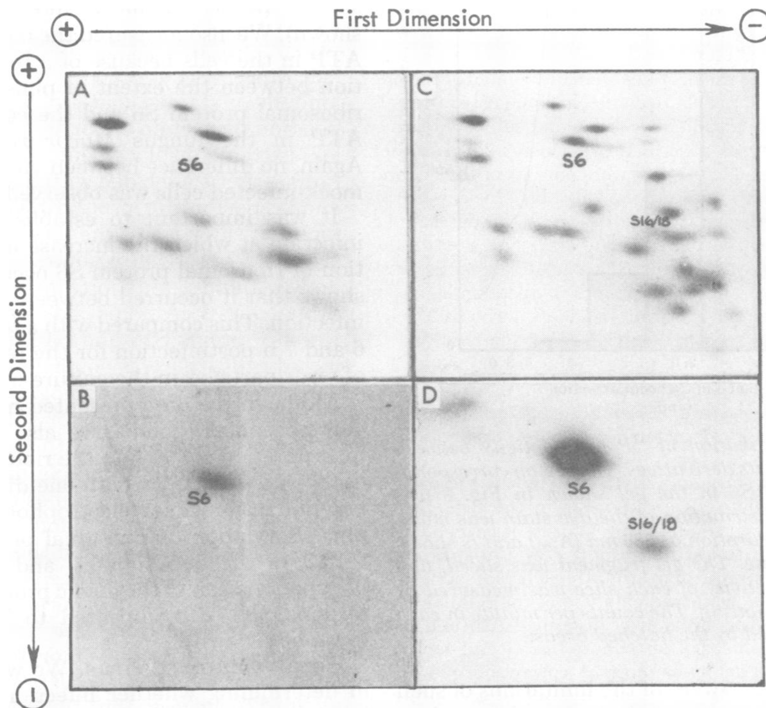

FIG. 2. *Phosphorylation of ribosomal proteins during pseudorabies virus infection. Ribosomal proteins from the 40S subunit of ribosomes from BHK cells were isolated and subjected to two-dimensional electrophoresis. The gels were stained with Coomassie brilliant blue (A and C) and autoradiographed (B and D). The patterns were obtained from mock-infected cells (A and B) and from cells infected for 6 h with pseudorabies virus (C and D).*

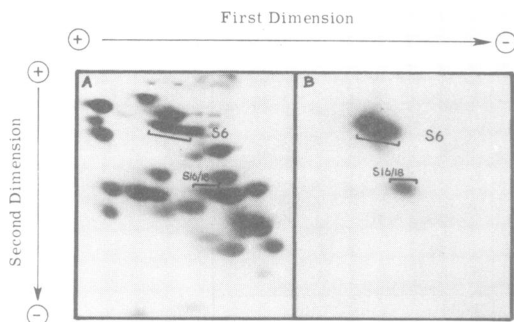

FIG. 3. *Phosphorylation of ribosomal proteins during pseudorabies virus infection. Ribosomal proteins from the 40S subunits of ribosomes from BHK cells infected for 9 h with pseudorabies virus were isolated and subjected to two-dimensional electrophoresis. The gel was stained (A) and autoradiographed (B).*

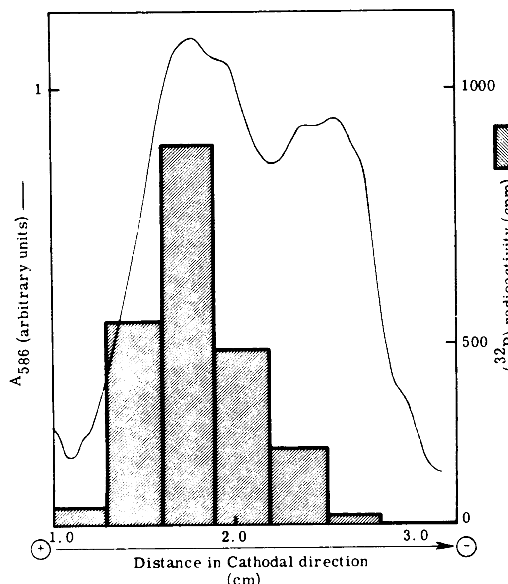

FIG. 4. *Distribution of ^{32}P radioactivity between protein S6 and its derivatives. The region corresponding to protein S6 in the gel shown in Fig. 3 was excised. The distribution of the dye stain was monitored by its absorption at 586 nm (A_{586}) and is shown by the solid line. The gel fragment was sliced, and the ^{32}P radioactivity of each slice was measured by scintillation counting. The counts per minute in each slice is indicated by the hatched areas.*

although we are aware of the limitations of such an approach (35). Indeed, the absolute values obtained were rather variable. In one experiment, in which infection was for 9 h, we estimated values of 1.0 and 0.2 mol of phosphate per mol of protein S6 in infected and mock-infected cells, respectively. These values may have reflected the relative difference in phosphorylation between the two conditions, but were clearly too low in absolute magnitude. Higher values of 2.9 and 5.4 mol of phosphate per mol of protein S6 at 6 and 9 h after infection, respectively, were found in a separate experiment, in which values of 0.35 and 1.06 mol of phosphate per mol of ribosomal protein S16/18 were estimated. The reason for the different values obtained in different experiments in which the extent of phosphorylation appeared to be similar, as judged by visual inspection, is unclear. It should be mentioned that the values of the specific radioactivity of [^{32}P]ATP in mock-infected cells and cells infected with pseudorabies virus were not significantly different.

In view of the well-documented effects of cyclic AMP on the phosphorylation of ribosomal protein S6 (5, 10, 30) and the report of an effect of herpes simplex virus on the cellular concentration of cyclic AMP in BHK cells (1), we felt that it was important to determine the concentration of this nucleotide in our own cells. No significant difference in concentration was found between cells after infection with pseudorabies virus for 6 h and cells which had undergone a similar period of mock infection (data not shown). We also measured the concentrations of ATP in the cells because of a reported correlation between the extent of phosphorylation of ribosomal protein S6 and the concentration of ATP in the fungus *Mucor racemosus* (16). Again, no difference between virus-infected and mock-infected cells was observed.

It was important to establish the stage of infection at which the increase in phosphorylation of ribosomal protein S6 occurred. Figure 5 shows that it occurred between 2 and 4 h after infection. This compared with a time of between 6 and 7 h postinfection for the first appearance of virus particles in the culture medium.

Although the data presented here are for the 40S ribosomal subunit, we also examined the incorporation of $^{32}P_i$ into the ribosomal proteins of the 60S subunit by both one-dimensional and two-dimensional gel electrophoreses. No new phosphorylation of ribosomal proteins was detected in the 60S subunit, and the extent of phosphorylation of the acidic protein (Lγ, in our terminology [22]) appeared to be unchanged (data not shown).

Herpes simplex virus. We were interested in determining whether infection with the related herpes simplex virus type 1 would cause effects similar to those found with pseudorabies virus. As far as ribosomal protein S6 was concerned, this was indeed the case at 13 h, but there was no labeling of S16/18 after infection with this virus (Fig. 6).

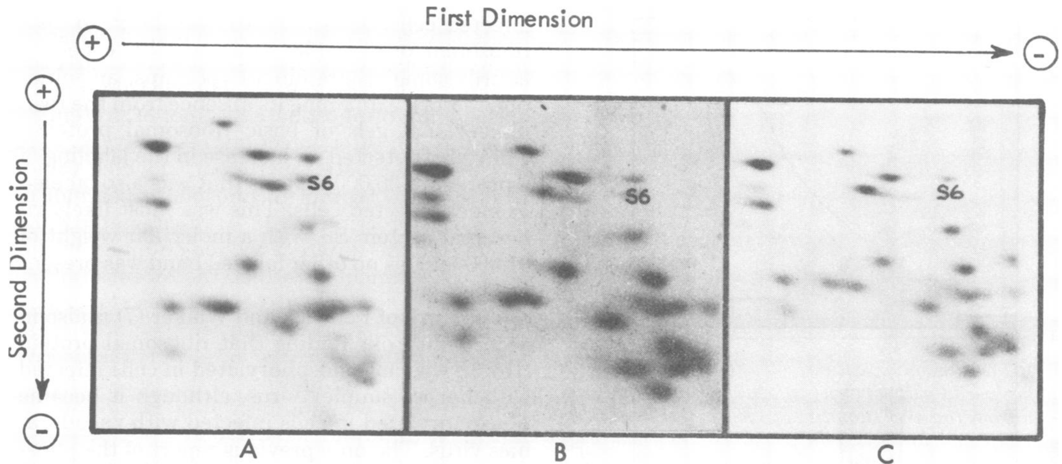

FIG. 5. *Time course of phosphorylation of ribosomal protein S6 during pseudorabies virus infection. Ribosomal proteins from the 40S subunits of ribosomes from BHK cells infected with pseudorabies virus were isolated and subjected to two-dimensional electrophoresis. The gels were stained with Coomassie brilliant blue. The patterns shown were obtained from cells infected for 2 h (A), 4 h (B), and 6 h (C).*

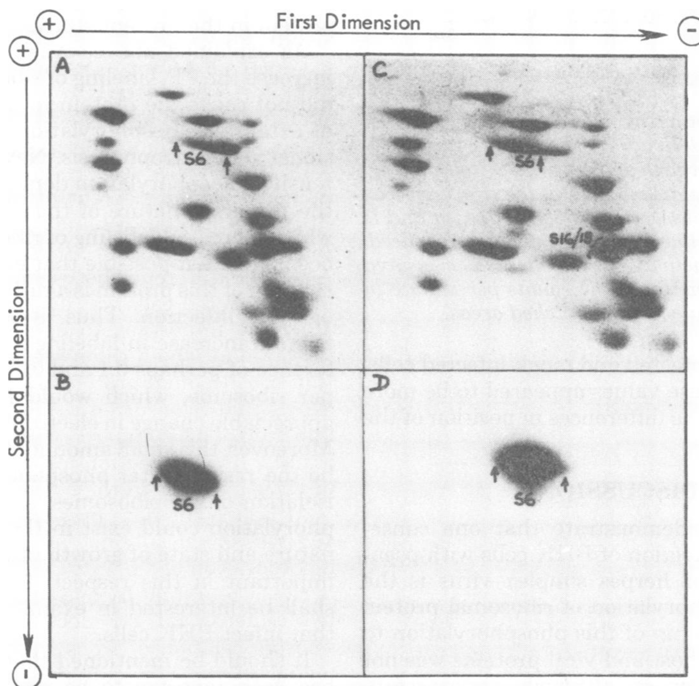

FIG. 6. *Phosphorylation of ribosomal proteins during herpes simplex virus infection. Ribosomal proteins from the 40S subunits of ribosomes from BHK cells were isolated and subjected to two-dimensional electrophoresis. The gels were stained with Coomassie brilliant blue (A and C) and autoradiographed (B and D). The patterns shown were obtained from mock-infected cells (A and B) and from cells infected for 13 h with herpes simplex virus (C and D).*

The results of a more quantitative analysis of the changes in ribosomal protein S6 are shown in Fig. 7. Although there was only a twofold increase in radioactivity in protein S6 from virus-infected cells compared with mock-infected cells, the specific radioactivity of the ATP was much higher in the mock-infected cells because of the use of dissimilar quantities of radioactivity and cells. In fact, we calculated that there were 4 and 0.64 mol of phosphate per mol of protein

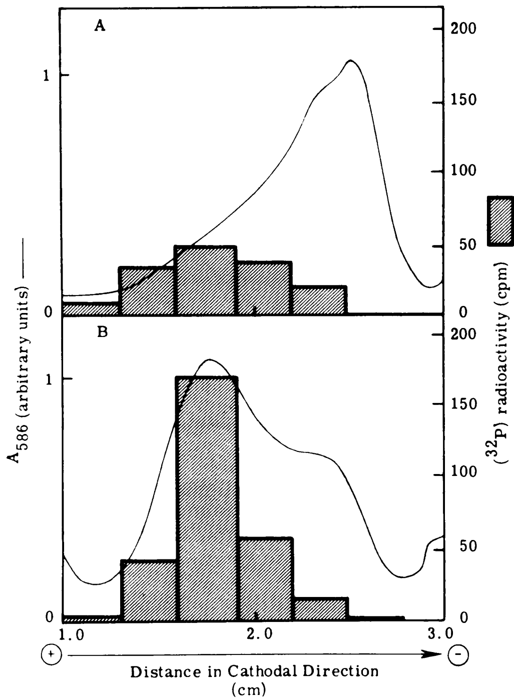

FIG. 7. *Distribution of ^{32}P radioactivity between protein S6 and its derivatives. The region corresponding to protein S6 in each of the two gels shown in Fig. 6 was excised. The distribution of the dye stain was monitored by its absorption at 586 nm (A_{586}) and is shown by the solid line. The gel fragments were sliced, and the ^{32}P radioactivity of each slice was measured by scintillation counting. The counts per minute in each slice is indicated by the hatched areas.*

S6 in the virus-infected and mock-infected cells, respectively. These values appeared to be more consistent with the differences in position of the stained proteins.

DISCUSSION

These results demonstrate that one consequence of the infection of BHK cells with pseudorabies virus or herpes simplex virus is the extensive phosphorylation of ribosomal protein S6. The relationship of this phosphorylation to the synthesis of host and viral proteins was not explored in this work, although it was established that phosphorylation precedes the formation of new virus particles. While this work was in progress, there was another report of ribosomal protein phosphorylation in cells infected with herpes simplex virus (7). In that study, only one-dimensional gel electrophoretic analysis was performed, and attention centered on the phosphorylation of a supposed ribosomal protein of the 40S subunit with a molecular weight of 48,000 that was unlabeled in mock-infected cells. This is clearly too large to be a ribosomal protein (38) and must have been a nonribosomal contaminant (perhaps, an initiation factor), explaining its absence from the two-dimensional gels of basic ribosomal proteins. They also detected an increase in the labeling of a protein of 36,000 daltons that was also labeled in mock-infected cells. This was most likely ribosomal protein S6, with a molecular weight of 31,000 (38), as no other labeled band was present on their gel.

The work of Fenwick and Walker (7) is also in accord with our finding that ribosomal protein S16/18 was not phosphorylated in cells infected with herpes simplex virus, although it became phosphorylated in cells infected with pseudorabies virus. The only previous report of the phosphorylation of ribosomal proteins in this region of the gel is for HeLa cells infected with vaccinia virus (12), in which the protein was identified as S16 (13). In cells infected with vaccinia virus, ribosomal protein S2 was also phosphorylated, but we did not detect phosphorylation of this protein in the present study.

As explained above, other viruses shown to increase the $^{32}P_i$ labeling of ribosomal protein S6 did not cause the protein to move to a position of extensive phosphorylation upon two-dimensional gel electrophoresis. Nevertheless, the extensive phosphorylation demonstrated here and the disparate nature of the different viruses in which increased labeling of ribosomal protein S6 occurs make it possible that extensive phosphorylation of this protein is a rather general feature of virus infection. Thus, it is possible that a fivefold increase in labeling might reflect a difference of perhaps 0.1 and 0.5 mol of phosphate per ribosome, which would not result in any appreciable change in electrophoretic migration. Moreover, this small amount of phosphate might be the residue after phosphatase action during isolation of the ribosomes, and extensive phosphorylation could exist in the intact cells. The nature and state of growth of the cells might be important in this respect; for this reason, we shall be interested in examining other viruses that infect BHK cells.

It should be mentioned that a protein kinase has been reported to be present in enveloped virions of herpes simplex virus (27, 32). The fact that several hours elapse before the phosphorylation of ribosomal protein S6 occurs tends to argue against such a kinase being solely responsible for this phosphorylation, but more definitive experiments are clearly required. The phosphorylation in pseudorabies virus-infected cells of protein S16/18, which has never been found to be phosphorylated in uninfected cells, suggests the possibility of a virus-specified protein

kinase. One possibility is that the viruses code for S6 protein kinases which in some cases are less specific than the host enzyme and fortuitously phosphorylate protein S16/18. It will be interesting to examine the specificity of protein kinases from pseudorabies virus-infected BHK cells.

In the introduction, we drew attention to a possible function for the phosphorylation of protein S6 in determining the relative affinity of ribosomes for different mRNA's. However, since protein synthesis is rapidly inhibited in cells infected with either herpes simplex virus or pseudorabies virus, the phosphorylation might be seen as a cellular response to the inhibition. We have already cited reports (9, 11) in which an increase in phosphorylation of protein S6 occurs in the presence of inhibitors of protein synthesis.

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LITERATURE CITED

1. **Bittlingmaier, K., D. Schneider, and D. Falke.** 1977. Influence of dibutyryl cyclic AMP on thymidine uptake by herpes simplex virus infected cells and the intracellular level of cyclic AMP. Biochim. Biophys. Acta **477**: 228–238.
2. **Blair, G. E., and I. Horak.** 1977. Phosphorylation of ribosomes in adenovirus infection. Biochem. Soc. Trans. **5**:660–661.
3. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding. Anal. Biochem. **72**:248–254.
4. **Chantler, J. K., and W. S. Stevely.** 1973. Virus-induced proteins in pseudorabies-infected cells. I. Acid-extractable proteins of the nucleus. J. Virol. **11**:815–822.
5. **Cawthorn, M. L., L. F. Bitte, A. Krystosek, and D. Kabat.** 1974. Effect of cyclic adenosine 3':5'-monophosphate on ribosomal protein phosphorylation in reticulocytes. J. Biol. Chem. **249**:275–278.
6. **Collatz, E., I. G. Wool, A. Lin, and G. Stöffler.** 1976. The purification and characterization of the 40S ribosomal subunit proteins S2, S3, S4, S5, S6, S7, S8, S9, S13, S23/S24, S27 and S28. J. Biol. Chem. **251**:4666–4672.
7. **Fenwick, M. L., and M. J. Walker.** 1979. Phosphorylation of a ribosomal protein and of virus-specific proteins in cells infected with herpes simplex virus. J. Gen. Virol. **45**:397–405.
8. **Gressner, A. M., and I. G. Wool.** 1974. The phosphorylation of liver ribosomal proteins *in vivo*: evidence that only a single small subunit protein (S6) is phosphorylated. J. Biol. Chem. **249**:6917–6925.
9. **Gressner, A. M., and I. G. Wool.** 1974. The stimulation of the phosphorylation of ribosomal protein S6 by cycloheximide and puromycin. Biochem. Biophys. Res. Commun. **60**:1482–1490.
10. **Gressner, A. M., and I. G. Wool.** 1976. Influence of Glucagon and cyclic adenosine 3':5'-monophosphate on the phosphorylation of rat liver ribosomal protein S6. J. Biol. Chem. **251**:1500–1504.
11. **Kabat, D.** 1970. Phosphorylation of ribosomal proteins in rabbit reticulocytes. Characterisation and regulatory aspects. Biochemistry **9**:4160–4170.
12. **Kaerlein, M., and I. Horak.** 1976. Phosphorylation of ribosomal proteins in HeLa cells infected with vaccinia virus. Nature (London) **259**:150–151.
13. **Kaerlein, M., and I. Horak.** 1978. Identification and characterisation of ribosomal proteins in vaccinia-virus-infected HeLa cells. Eur. J. Biochem. **90**:463–469.
14. **Kaltschmidt, E., and H. G. Wittmann.** 1970. Ribosomal proteins. VII. Two-dimensional polyacrylamide gel electrophoresis for fingerprinting of ribosomal proteins. Anal. Biochem. **36**:401–412.
15. **Kaplan, A. S., and A. E. Vatter.** 1959. A comparison of herpes simplex and pseudorabies viruses. Virology **7**: 394–407.
16. **Larsen, A., and P. Sypherd.** 1980. Physiological control of phosphorylation of ribosomal protein S6 in *Mucor racenosus*. J. Bacteriol. **141**:20–25.
17. **Lastick, S. M., and E. H. McConkey.** 1976. Exchange and stability of HeLa ribosomal proteins *in vivo*. J. Biol. Chem. **251**:2867–2875.
18. **Lastick, S. M., P. J. Nielsen, and E. H. McConkey.** 1977. Phosphorylation of ribosomal protein S6 in suspension cultured HeLa cells. Mol. Gen. Genet. **152**:223–230.
19. **Leader, D. P.** 1975. Enzymic iodination of eukaryotic ribosomal subunits. Characterization and analysis by two-dimensional gel electrophoresis. Biochem. J. **152**: 373–378.
20. **Leader, D. P.** 1980. The control of phosphorylation of ribosomal proteins, p. 203–233. *In* P. Cohen (ed.), Protein phosphorylation in regulation. Elsevier/North-Holland Publishing Co., Amsterdam.
21. **Leader, D. P.** 1980. A tracking marker for the first dimension of the two-dimensional gel electrophoresis. J. Biochem. Biophys. Methods **3**:247–248.
22. **Leader, D. P., and A. A. Coia.** 1977. The phosphorylation of an acidic protein of the large ribosomal subunit of Krebs II ascites cells. Biochem. J. **162**:199–206.
23. **Leader, D. P., and A. A. Coia.** 1978. The phosphorylation of ribosomal protein S6 on the monoribosomes and polyribosomes of baby hamster kidney fibroblasts. FEBS Lett. **90**:270–274.
24. **Leader, D. P., and A. A. Coia.** 1978. The phosphorylation of ribosomal proteins L14 and S3 in Krebs II ascites cells. Biochim. Biophys. Acta **519**:224–232.
25. **Leader, D. P., A. A. Coia, and L. H. Fahmy.** 1978. The extent of phosphorylation of the acidic 60S ribosomal phosphoprotein, Lγ, in Krebs II ascites cells and in the skeletal muscle of normal and diabetic rats. Biochem. Biophys. Res. Commun. **83**:50–58.
26. **Leader, D. P., A. D. Rankine, and A. A. Coia.** 1976. The phosphorylation of ribosomal protein S6 in baby hamster kidney fibroblasts. Biochem. Biophys. Res. Commun. **71**:966–974.
27. **Lemaster, S., and B. Roizman.** 1980. Herpes simplex virus phosphoproteins. II. Characterization of the virion protein kinase and of the polypeptides phosphorylated in the virion. J. Virol. **35**:798–811.
28. **Martin, O. H. W., and J. Kruppa.** 1979. Ribosomal phosphoproteins of mouse myeloma cells. Changes in the degree of phosphorylation induced by hypertonic initiation block. Eur. J. Biochem. **95**:349–358.
29. **Rankine, A. D., D. P. Leader, and A. A. Coia.** 1977. The phosphorylation of the ribosomal proteins of Krebs II ascites cells. Biochim. Biophys. Acta **474**:293–307.
30. **Roberts, S., and C. D. Ashby.** 1978. Ribosomal protein phosphorylation in rat cerebral cortex *in vitro*: influence of cyclic adenosine 3':5'-monophosphate. J. Biol. Chem. **253**:288–296.
31. **Rosnitschek, I., U. Traub, and P. Traub.** 1978. Enhanced phosphorylation of ribosomal protein S6 and

other cytoplasmic proteins after mengovirus infection of Ehrlich ascites tumour cells. Z. Physiol. Chem. **359:** 593-600.

32. **Rubenstein, A. S., M. Gravell, and R. Darlington.** 1972. Protein kinase in enveloped herpes simplex virions. Virology **50:**287-290.
33. **Russell, W. C., and G. E. Blair.** 1977. Polypeptide phosphorylation in adenovirus-infected cells. J. Gen. Virol. **34:**19-35.
34. **Stevely, W. S.** 1975. Virus-induced proteins in pseudorabies-infected cells. II. Proteins of the virion and nucleocapsid. J. Virol. **16:**944-950.
35. **Thomas, G., M. Siegmann, and J. Gordon.** 1979. Multiple phosphorylation of ribosomal protein S6 during transition of quiescent 3T3 cells into early G1, and cellular compartmentalization of the phosphate donor. Proc. Natl. Acad. Sci. U.S.A. **76:**3952-3956.
36. **Thomas, G., M. Siegmann, A. M. Kubler, J. Gordon, and L. Jimenez de Asua.** 1980. Regulation of 40S ribosomal protein phosphorylation in Swiss mouse 3T3 cells. Cell **19:**1015-1023.
37. **Treloar, M. A., M. E. Treloar, and R. Kisilevsky.** 1977. Ethionine and the phosphorylation of ribosomal protein S6. J. Biol. Chem. **252:**6217-6221.
38. **Wool, I. G.** 1979. The structure and function of eukaryotic ribosomes. Annu. Rev. Biochem. **48:**719-754.
39. **Zinker, S., and J. R. Warner.** 1976. The ribosomal proteins of *Saccharomyces cerevisiae*: phosphorylated and exchangeable proteins. J. Biol. Chem. **250:**1799-1807.