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Cell-Free Synthesis of Herpes Simplex Virus Proteins

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Polyribosomes isolated from herpes simplex virus type I (HSV-1)-infected cells have been used to program a eucaryotic cell-free translation system. At least 10 HSV-specific polypeptides, with apparent molecular weights of 25,000 to 160,000, are synthesized by wild-type HSV-infected polyribosomes. Polyribosomes prepared from thymidine kinase-negative mutants of HSV direct the synthesis of three putative nonsense termination polypeptides. HSV-specific polypeptides synthesized in vitro are precipitated with antiserum to HSV-infected cell proteins.

Late after infection of mammalian cells with herpes simplex virus type I (HSV-1), viral genes specify nearly all macromolecular synthesis. By 10 to 12 h, a large fraction of the polysomal mRNA is of viral origin, and synthesis of viral structural polypeptides accounts for most of the total proteins synthesized (12).

A number of nonstructural viral polypeptides are also synthesized, including viral thymidine kinase (viral TK). Mutants in viral TK have been selected and characterized genetically and biochemically (22). A number of the mutants neither direct the synthesis of the intact viral TK polypeptide nor induce any detectable TK enzymatic activity. However, four of these mutants induce the synthesis of new shorter polypeptides which have the properties expected of peptide chain termination mutants.

A number of cell-free translation systems have been utilized in the analysis of animal virus genomes. Isolated simian virus 40 mRNA has been used to program cell-free translation into viral capsid proteins (19) and the T antigen of simian virus 40 (5). A linked transcription-translation cell-free system primed with simian virus 40 DNA fragments generated by restriction endonucleases was used to biochemically map the viral capsid proteins of simian virus 40 (20). The genes for each of the major adenovirus 2 polypeptides, expressed early and late after infection, were located on the adenovirus 2 DNA by in vitro translation of the infected cell RNA selected by hybridization to specific endonuclease fragments of adenovirus 2 DNA (16, 17). Equine herpesvirus mRNA has been translated into nucleocapsid polypeptides in a reticulocyte cell-free system (1).

Here we report an investigation of the translation of HSV-1 proteins in a preinitiated cell-free system from HSV-infected mouse cells. At least 10 major virus-specific polypeptides, ranging in apparent molecular weight from 25,000 to 160,000 on electrophoresis in sodium dodecyl sulfate (SDS) polyacrylamide gels, were synthesized in vitro using infected cell polyribosomes. In addition, the putative nonsense polypeptide fragments from three different TK-negative mutants of HSV were synthesized in vitro. Immunological characterization of the in vitro protein products confirm their identity as viral proteins.

MATERIALS AND METHODS

Growth and preparation of virus. African green monkey cells (Vero) were infected with 0.1 PFU/cell of HSV-1 strain Cl 101 or TK^- mutants of this strain (22) and grown in 29-oz (ca. 0.856-liter) bottles at 37°C in medium 199 containing 2% fetal calf serum. At 40 to 48 h later, infected cultures were collected, resuspended in medium 199 plus 10% fetal calf serum, and sonically treated in a water-cooled Bronwell sonic oscillator for 3 min. Cellular debris was removed by centrifugation for 5 min at 1,200 × *g*, and the virus-containing supernatant was assayed by plaque formation and stored at −70°C.

Preparation of polyribosomes. Polyribosomes were prepared from mouse $LMTK^-$ cells (deficient in cellular TK) infected with wild-type or mutant strains of HSV-1 at multiplicities of 10 to 20 PFU/cell. Ten to 12 h after infection, 5×10^8 infected cells and medium were poured over frozen, crushed phosphate-buffered saline. The cells were collected by centrifugation at 1,200 × *g* for 5 min at 0°C and washed once with cold phosphate-buffered saline. The packed cells were resuspended in three volumes of ice-cold KMTE buffer (10 mM KCl, 2.5 mM magnesium acetate, 10 mM Tris-hydrochloride [pH 7.6], 0.5 mM EDTA, and 1 mM dithiothreitol) containing

150 μg of sodium heparin per ml (6). After 3 min at 0°C, the cells were disrupted in a Dounce homogenizer ("A" pestle). The cells and nuclei were observed by phase-contrast microscopy to monitor cell breakage.

Immediately, a 0.5 volume of a concentrated salt solution containing 1,200 mM KCl, 50 mM magnesium acetate, and 200 mM Tris-hydrochloride (pH 7.6; 18) was added, and the extract was centrifuged for 10 min at 12,000 × *g*. The postmitochondrial supernatant was layered over 5 ml of 1 M sucrose in buffer A (100 mM NH_4Cl, 5 mM magnesium acetate, 20 mM Tris-hydrochloride [pH 7.6], 1 mM dithiothreitol; 21) and centrifuged for 2.5 h at 48,000 rpm (230,000 × *g*) in a Spinco 50 Ti rotor at 4°C. The surface of the polyribosome pellet was rinsed with buffer A, and the pellet was resuspended in buffer A containing 0.25 M sucrose and stored at −70°C in 0.5-ml portions. The final concentration of polysomes was such that there were 250 units of absorbancy at 260 nm.

The "pH 5 fraction" was prepared from Krebs II ascites cells or rat liver by the method of Schreier and Staehelin (21). Crude initiation factors from rabbit reticulocytes were prepared from the high-salt wash of reticulocyte ribosomes (21). These factors were separated into two fractions: fraction A, the material precipitating between 30 and 40% saturated $(NH_4)_2SO_4$, and fraction B, the material precipitating between 40 and 60% saturated $(NH_4)_2SO_4$.

Assay for protein synthesis. Standard reaction mixtures of 25 μl contained polyribosomes having 0.75 to 1.5 units of absorbancy at 260 nm, 0 to 2 μl of the pH 5 fraction, 0.5 μl of initiation factor fraction A (16 μg of protein), and 1.5 μl of initiation factor fraction B (44 μg of protein). The other ingredients were 1.0 mM ATP, 0.4 mM GTP, 10 mM creatine phosphate, 20 μg of creatine phosphokinase per ml, 30 mM NH_4Cl, 82 mM KCl, 2.5 mM magnesium acetate, 8 mM putrescine, 1 mM dithiothreitol, 30 μM each of 19 amino acids (minus methionine), and 10 to 15 μCi (1.5 μM) of [^{35}S]methionine (specific activity, greater than 400 Ci/mmol). Reaction mixtures were incubated for 60 or 90 min at 30 or 36°C.

At the end of the synthesis, each reaction was treated with 30 mM EDTA and 30 μg of RNase for 15 min at 37°C. Incorporation of [^{35}S]methionine into polypeptides was determined by precipitating 2.5 μl of the RNase-treated reaction with 5% trichloroacetic acid at 0°C for 30 min and collecting the precipitate on Whatman GF/C glass fiber filters. Dried filters were counted in a liquid scintillation counter.

Polypeptides synthesized in vitro were prepared for analysis on polyacrylamide gels by precipitating 3 to 10 μl of the RNase-treated reaction with 0.5 ml of 80% acetone. After 30 min at 0°C, the samples were centrifuged for 10 min at 1,000 × *g*. The acetone supernatant was removed, and the precipitate was dried in vacuo. A 10- to 25-μl portion of SDS-sample buffer (see below) was added to each sample, and they were incubated for 5 min at 90°C.

Electrophoretic analysis of proteins. The proteins synthesized in vivo (22) or in vitro were analyzed by electrophoresis in 15-cm-long slab gels using the discontinuous buffer system with SDS as described by Laemmli (15). The best resolution was observed when the separating gel contained 7.5 M urea, 10% acrylamide, and 0.15% *N,N'*-methylene bisacrylamide (Eastman Organic Chemicals Div., Eastman Kodak Co., Rochester, N.Y.). Samples were prepared for electrophoresis by boiling for 3 min in SDS-sample buffer (0.0625 M Tris-hydrochloride, pH 6.8; 2% SDS [BDH Chemicals, Poole, England]; 10% glycerol; 0.001% bromophenol blue; 0.1 M dithiothreitol). After 4 to 5 h of electrophoresis (150 to 200 V, 25 to 35 mA), the gel was fixed in 7% acetic acid and 15% methanol, stained with 0.25% Coomassie brilliant blue, dried, and autoradiographed by contact with X-ray film (SB 5, Eastman Kodak).

Immunoprecipitation procedures. Portions of RNase-treated [^{35}S]methionine protein from cell-free synthesis reactions were adjusted to 35 μl in 0.15 M NaCl, 0.01 M EDTA, and 0.5% Triton X-100. The sample was centrifuged for 2 min at 10,000 × *g*, and the supernatant was recovered.

Infected cells labeled with [^{35}S]methionine were harvested into phosphate-buffered saline plus 10 mM EDTA and centrifuged for 3 min at 2,000 × *g*. The cell pellet was resuspended in 35 μl of 0.15 M NaCl, 0.01 M EDTA, and 0.5% Triton X-100 and subjected to sonic treatment for 1 min. The extract was centrifuged for 2 min at 10,000 × *g*. Portions (10 μl) of the supernatant solutions were used for each immunoprecipitation.

In vitro or in vivo labeled proteins were reacted with immunoglobulin G prepared from rabbit antiserum raised against HSV-infected cells protein (kindly provided by Alec Buchan). The optimum amount of immunoglobulin G was determined by titration experiments.

After 12 h at 4°C, formalin-fixed *Staphylococcus aureus* (ATCC 12598) was added to each sample. The A protein of the *S. aureus* was used as an immune absorbant as described by Kessler (13). After 10 min at 0°C, the samples were centrifuged for 2 min at 10,000 × *g*, and the supernatant removed. The immune absorbant and bound antigen-antibody complex were washed three times with 1 ml of 0.5 M NaCl, 0.01 M EDTA, and 0.4% Triton X-100. A 20-μl portion of SDS-sample buffer was added to each sample, after which they were incubated at 90°C for 5 min. This procedure eluted the antigens and dissociated the antigen-antibody complex. The insoluble immune abosrbant was removed by centrifugation for 2 min at 10,000 × *g*, and the supernatants were analyzed by gel electrophoresis.

RESULTS

Polyribosomes prepared from LMTK$^-$ cells infected with HSV-1 can be used to program the incorporation of radioactive amino acids into trichloroacetic acid-precipitable material. The incorporation of [^{35}S]methionine is linear for 20 to 30 min and continues for 60 to 90 min at 30°C. Under the conditions of these experiments, 15 to 20% of the isotope is incorporated (Fig. 1).

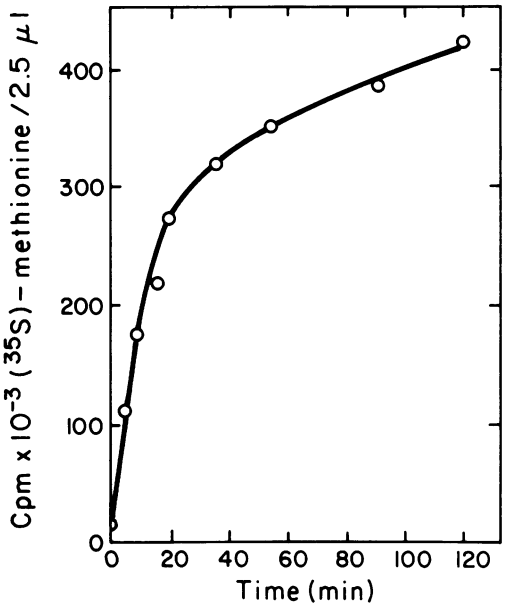

FIG. 1. *Kinetics of incorporation of [^{35}S]methionine into trichloroacetic acid-insoluble radioactivity. A 100-µl cell-free reaction mixture, consisting of polyribosomes from wild-type HSV-infected cells and the components specified in Materials and Methods, was incubated at 37°C. At the times indicated, 2.5-µl portions were removed and assayed for trichloroacetic acid-insoluble radioactivity.*

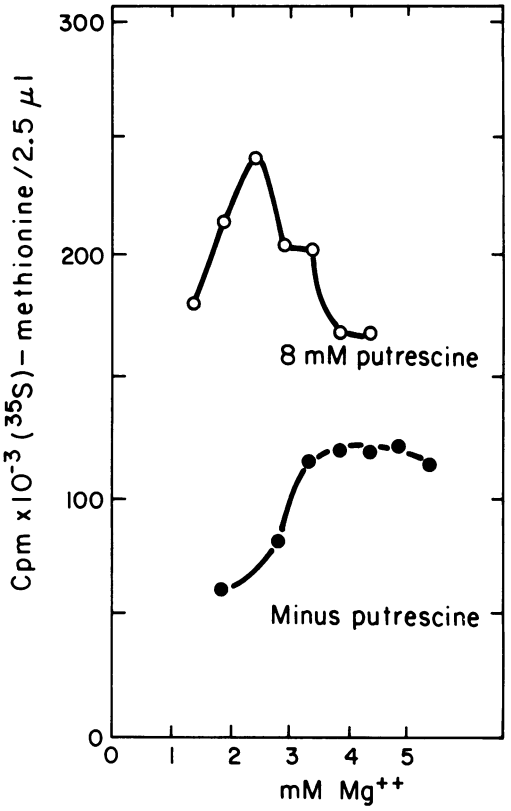

FIG. 2. *Effect of polyamine addition on cell-free protein synthesis at different Mg^{2+} concentrations. Trichloroacetic acid-insoluble radioactivity was assayed on a 2.5-µl portion of a 25-µl reaction mixture. Incubation was at 36°C for 90 min. The remainder of the samples were analyzed by SDS-polyacrylamide gel autoradiography. The intensities of the [^{35}S]methionine protein bands were proportional to the overall trichloroacetic acid insolubility.*

The magnesium ion dependence of this cell-free translation system, in the presence and absence of 8 mM putrescine, is shown in Fig. 2. In the absence of the polyamine, the magnesium optimum is quite broad, between 3 and 5 mM. However, in the presence of 8 mM putrescine, there is a sharp optimum at 2.6 mM magnesium. In addition, the incorporation is at least double that seen in the absence of putrescine. All further results presented here are from experiments in which putrescine was included in the cell-free incubation mixture.

To determine if there had been any initiation of polypeptide synthesis as well as elongation of nascent proteins, the compound 7-methylguanosine-5'-monophosphate was tested for its inhibitory effect in this system. Little or no effect of this compound was noted, even at quite high concentrations (Table 1).

Reaction mixtures programmed with polyribosomes isolated from cells infected with wild-type HSV or TK$^-$ mutant HSV were fractionated by electrophoresis on SDS-polyacrylamide slab gels. A comparison of the [^{35}S]methionine-labeled proteins synthesized in vivo and in vitro is seen in Fig. 3 and 4. It can be seen that at least 10 polypeptides synthesized in vitro have

TABLE 1. *Effect of 7-methylguanosine-5'-monophosphate on cell-free protein synthesis*[a]

Polyribosomes	Incubation	Addition	Incorporation (cpm)
LMTK$^-$	0°C		22,000
LMTK$^-$	30°C		542,000 (100)[b]
LMTK$^-$	30°C	1.7 mM m^{7}G^5'p[c]	334,000 (61.9)
21TK$^+$	0°C		17,000
21TK$^+$	30°C		337,000 (100)
21TK$^+$	30°C	1.7 mM m^{7}G^5'p	281,000 (78.3)

[a] Cell-free protein synthesis reactions were prepared as in the text with polyribosomes from uninfected LMTK$^-$ or wild-type (21TK$^+$) HSV-infected cells. Incubation was for 90 min. The counts per minute of [^{35}S]methionine incorporated are for a 2.5-µl portion of the reaction mixture.

[b] The numbers in parentheses represent the percentage of cell-free protein incorporated.

[c] m^{7}G^5'p, 7-methylguanosine-5'-monophosphate.

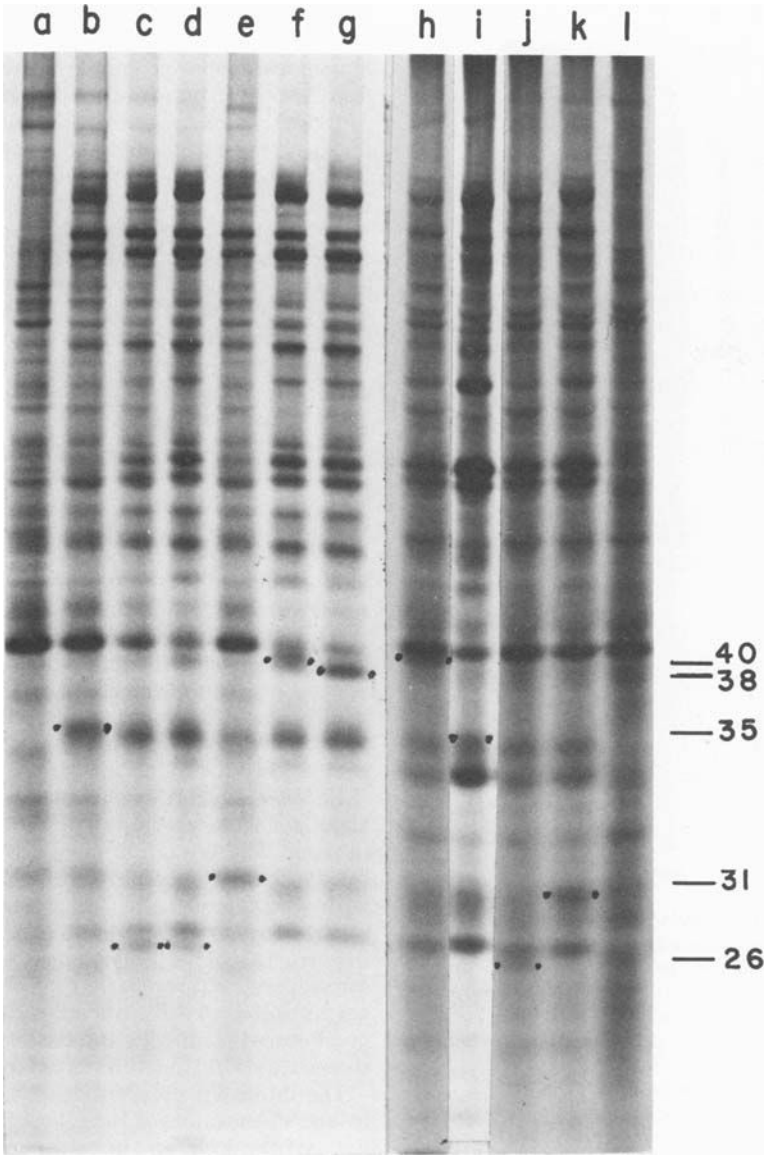

FIG. 3. *SDS-polyacrylamide gel autoradiogram of [^{35}S]methionine-labeled in vivo cell extracts and [^{35}S]methionine-labeled products of cell-free protein synthesis. The gel contained 10% acrylamide and 0.15% bisacrylamide without urea. Vero cells infected with wild type and TK$^-$ mutants of HSV were labeled from 4 to 5 h postinfection with 50 μCi of [^{35}S]methionine per ml (100 Ci/mmol). Samples (a-g) are Vero cells infected in vivo with the following virus: (a) uninfected, (b) 38TK$^-$, (c) 37TK$^-$, (d) 21TK$^-$, (e) 20TK$^-$, (f) 21TK$^+$, and (g) 20R1TK$^+$. Samples (h-l) are the cell-free translation products of reactions prepared from polyribosomes from LMTK$^-$ cells infected with the following virus: (h) 21TK$^+$, (i) 38TK$^-$, (j) 21TK$^-$, (k) 20TK$^-$, and (l) uninfected. The autoradiograms were exposed for: (a-g) 3 days and (h-l) 6 h. The dots indicate the intact TK polypeptide and the fragments. Their molecular weights in (×10^3) are indicated.*

electrophoretic mobilities indistinguishable from in vivo virus-specific proteins.

Polyribosomes from wild-type and TK mutant-infected cells direct the synthesis of an almost identical set of 10 herpesvirus-specific polypeptides, which completely differed from that observed when polyribosomes from uninfected cells are used. The only discernible difference between parent and TK mutant is the presence or absence of the intact TK polypeptide (molecular weight, about 40,000).

The molecular weights of the [^{35}S]methio-

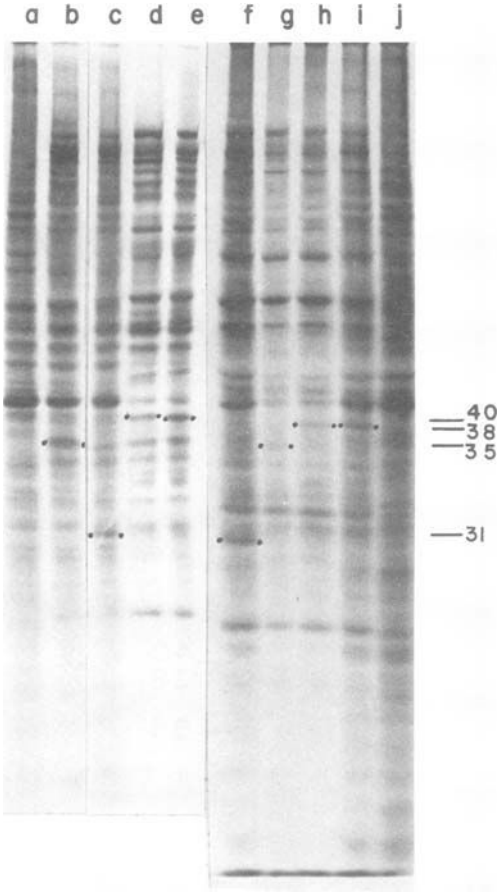

FIG. 4. *SDS-polyacrylamide gel autoradiogram of [^{35}S]methionine-labeled in vivo cell extract and [^{35}S]methionine-labeled products of cell-free protein synthesis. The samples and gel electrophoresis are identical to Fig. 3, except that 7.5 M urea was included in the acrylamide gel during polymerization. Samples (a–e) are Vero cells infected in vivo with the following virus: (a) uninfected, (b) 38TK$^-$, (c) 20TK$^-$, (d) 21TK$^+$, and (e) 20R1TK$^+$. Samples (f–j) are the cell-free translation products of reactions prepared from polyribosomes from LMTK$^-$ cells infected with the following virus: (f) 20TK$^-$, (g) 38TK$^-$, (h) 20R1TK$^+$, (i) 21TK$^+$, and (j) LMTK$^-$. The autoradiograms were exposed for: (a–e) 3 days and (f–j) 6 h. The dots indicate the intact TK polypeptide or the TK polypeptide fragments. Their molecular weights (×10^3) are shown.*

nine-labeled polypeptides were determined by coelectrophoresis with standard proteins of known molecular weight. All autoradiographs displayed in this paper were made from 10% polyacrylamide gels, although the molecular weights of the largest polypeptides were determined on gels containing 7.0 or 8.0% acrylamide.

Immunoprecipitation procedures were employed to further characterize the products of in vitro reaction mixtures programmed with polyribosomes prepared from infected and uninfected cells. To determine whether [^{35}S]methionine-labeled virus-specific proteins were synthesized in vitro, the product was analyzed immunologically with antiserum from rabbits immunized with total HSV-infected cell protein (HSV grown in rabbit kidney cells). An immune absorbant procedure described by Kessler (13), in which formalin-fixed *S. aureus* is used to precipitate the antigen-antibody complex, was utilized to circumvent overloading of the gels with immunoglobulin G, which occurred in standard indirect immunoprecipitation experiments.

Autoradiographs of slab gels (Fig. 5) indicate that proteins synthesized in vivo in infected cells and in vitro in the cell-free translation system are immunologically reactive with the anti-HSV antibody. Virus-specific polypeptides having molecular weights of 160,000, 130,000, 80,000, 61,000, 58,000, and 40,000 are efficiently precipitated with anti-HSV serum. The same polypeptides are precipitated from cell-free reactions programmed with polyribosomes from wild-type and TK mutant-infected cells but not from reactions programmed with uninfected control polyribosomes.

HSV-specific TK, with a molecular weight of about 40,000, is precipitated with anti-HSV antiserum. The protein is precipitated only from in vivo extracts prepared from cells infected with wild-type or 20R1 TK revertant virus (a revertant derived from mutant 20 TK$^-$) or from in vitro reactions programmed with polyribosomes prepared from wild-type or 20R1 TK revertant-infected cells. Such a polypeptide is not precipitated from cells infected with TK-negative mutants or from uninfected control cells.

The intact TK polypeptide is not synthesized in any of the mutant infections in vivo and is not synthesized in in vitro reactions programmed with polyribosomes from TK mutant-infected cells. However, these polyribosomes do direct the in vitro synthesis of polypeptides that are smaller than the 40,000-molecular-weight intact TK peptide and are not seen in reactions programmed with wild-type polysomes (Fig. 3 and 4). These short polypeptides have electrophoretic mobilities identical to those of the new shorter polypeptides synthesized in vivo by the mutants (22). Mutant 20R1 directs the synthesis of a polypeptide of slightly greater mobility than the wild-type TK polypeptide (Fig. 3–5). This variant polypeptide is observed neither in the wild-type nor TK$^-$ mutant in vitro reactions.

A large number of herpesvirus-specific poly-

peptides in addition to the TK polypeptide are synthesized in the cell-free translation system (Fig. 6). Polypeptides with molecular weights of about 160,000, 130,000, and 122,000 are synthesized in response to HSV-infected cell polyribosomes. These polypeptides are conspicuously absent from incubations programmed with uninfected control cell polyribosomes. In addition, the cell-free system directs the synthesis of two polypeptides with molecular weights of about 110,000 and 61,000. These polypeptides appear to have slightly greater electrophoretic mobilities than the in vivo polypeptides that are absent from the in vitro products.

It has been reported that several herpes simplex proteins undergo post-translational modification by addition of carbohydrate (7, 14), sulfate (8), or phosphate (10) groups. Such additions are known to alter the mobility of the protein in SDS-polyacrylamide gel electrophoresis (3). It is possible that some of the in vitro products that do not comigrate with any virus-specific protein represent such unmodified proteins. Likewise, the absence of an expected polypeptide from the in vitro pattern may result from the lack of in vitro modification. To identify those in vivo glycoproteins, [^{14}C]glucosamine-labeled proteins were subjected to electrophoresis in parallel with [^{35}S]methionine-labeled proteins made in vivo (Fig. 6). The in vivo

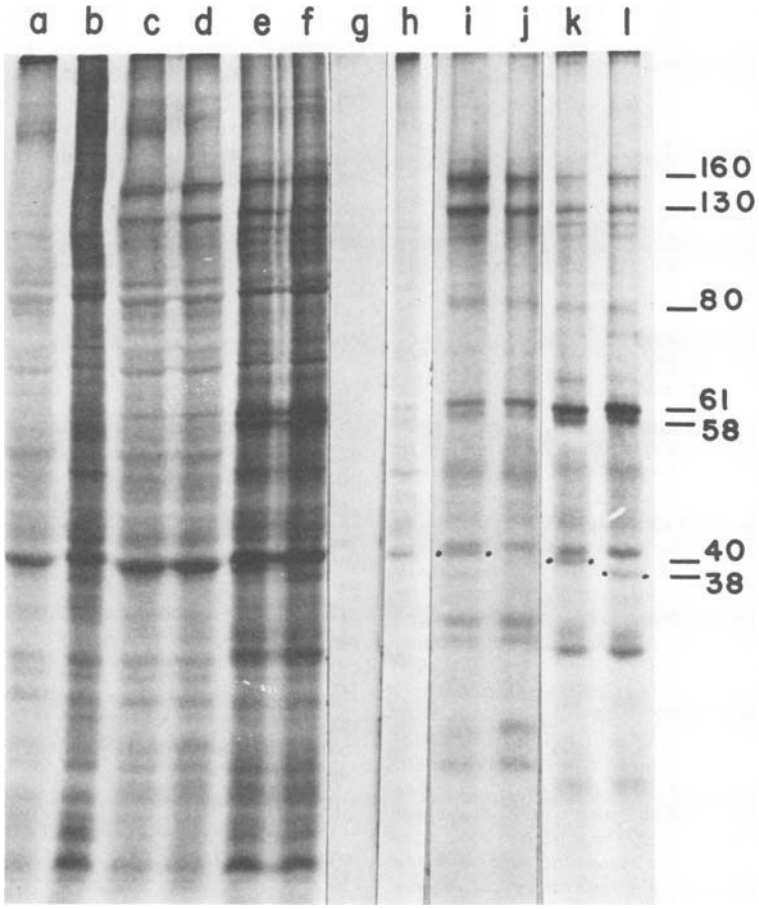

FIG. 5. *SDS-polyacrylamide gel autoradiogram of immunoprecipitates of [^{35}S]methionine-labeled in vivo extract and [^{35}S]methionine-labeled products of cell-free protein synthesis. Immunoprecipitates were prepared as in Materials and Methods. The gel contained 10% acrylamide and 0.15% bisacrylamide without urea. Vero cells infected with HSV were labeled from 4 to 6 h after infection. Samples (a–f) show total proteins prior to immunoprecipitation. Samples (g–l) are immunoprecipitates. Samples are as follows: (a, g) uninfected Vero cells, in vivo; (b, h) uninfected LMTK$^-$, in vitro; (c, i) Vero cells infected with 21TK$^+$, in vivo; (d, j) Vero cells infected with 20TK$^-$, in vivo; (e, k) LMTK$^-$ infected with 21TK$^+$, in vitro; (f, l) LMTK$^-$ infected with 20R1TK$^+$, in vitro. The autoradiograms were exposed for: (a–f) 4 days and (g–l) 14 days. The molecular weights (×10^{-3}) of the immunoprecipitated proteins are shown.*

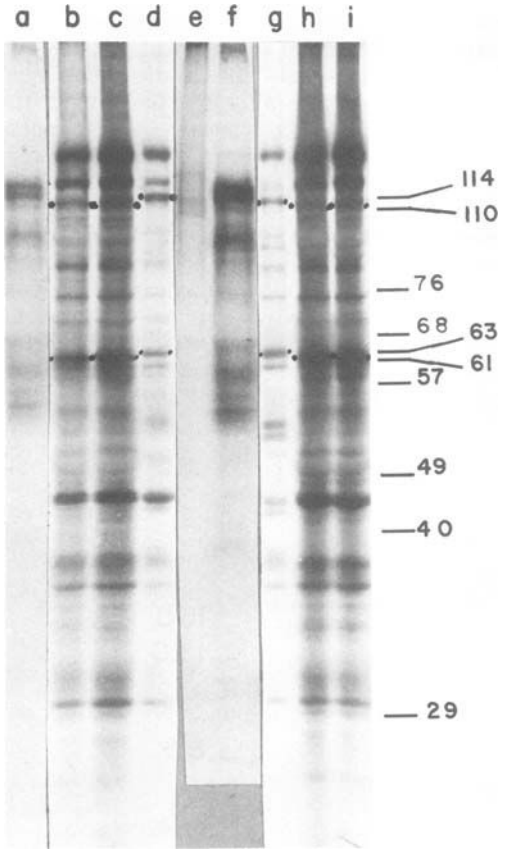

FIG. 6. *SDS-polyacrylamide gel autoradiogram of [^{35}S]methionine and [^{14}C]glucosamine-labeled in vivo extracts of infected cells and [^{35}S]methionine-labeled products of cell-free protein synthesis. The gel contained 10% acrylamide and 0.15% bisacrylamide without urea. Vero cells infected with HSV were labeled from 5 to 7 h after infection with [^{35}S]methionine (25 μCi/ml, samples d and g) or from 5 to 18 h after infection with [^{14}C]glucosamine (5 μCi/ml; 250 mCi/mmol). (Samples a and f show Vero cells infected with 21TK$^+$ virus and sample e shows uninfected Vero cells.) Cell-free reactions (b, c, h, and i) are from four different preparations of polyribosomes from LMTK$^-$ cells infected with wild-type 21TK$^+$ virus. The autoradiograms were exposed for 2 days (a–d, g–i) or for 5 days (e, f). The dots indicate the glyco- (114,000 and 63,000 molecular weight) and aglyco- (100,000 and 61,000 molecular weight) polypeptides. The molecular weights of the standard proteins used as molecular-weight makers are also shown.*

proteins having molecular weights of about 114,000 and 63,000, which are absent from the in vitro pattern, are indeed glycoproteins.

DISCUSSION

In this paper we describe a cell-free protein synthesis system which at least completes the synthesis of 10 or more HSV-specific polypeptides. The proteins synthesized in vitro are judged with the following criteria to be similar or identical to those proteins synthesized in vivo during HSV infection. (i) Proteins synthesized in vitro have electrophoretic mobilities in SDS-polyacrylamide gels identical or similar to proteins synthesized in vivo during HSV infection. We observed a slight diminution in the apparent molecular weight of two proteins synthesized in vitro, which may be ascribed to the lack of glucosylation in vitro. (ii) Proteins synthesized in vitro and in vivo are immunologically precipitated by antiserum specific for herpes virus-infected cell protein. These two sets of proteins have identical electrophoretic mobilities on SDS-polyacrylamide gels. (iii) Specific mutants in the virus result in specific changes in the in vitro products.

The system described here depends on the presence of the heparin in the buffer for polysome preparation so that each preparation is reproducibly active. This may reflect a high level of ribonucleases in the infected cells or, alternately, unresolved shortcomings in the polysome preparation. Another interesting feature of this system is its stimulation by polyamines, a phenomenon that has been noted in some other in vitro protein synthetic systems (2).

Although the total incorporation was often stimulated two- or threefold by the addition of the reticulocyte initiation factor fractions, the experiments with 7-methylguanosine-5′, monophosphate suggest that extensive initiation of polypeptide synthesis is not occurring in this system. Hickey and co-workers (11) have shown that 7-methylguanosine-5′-monophosphate acts as an analogue of the 5′ "cap" structure on mRNA and, hence, blocks initiation, which apparently requires a free cap-binding site on the ribosomes. The addition of purified RNAs from HSV-infected cells did not stimulate either the uninfected polysomes or the infected polysomes to make additional HSV-specific polypeptides (data not shown), thus confirming the lack of translation initiation in this system. Although this system does not allow study of the events involved in the initiation of translation, it has the advantages of incorporating a large amount of label into the very large virus-specific proteins, which is not the case in some more highly fractionated systems. Further, it will allow study of the nature of the mutations in the HSV TK gene, since it is expected that suppressor tRNA's can function in a dominant fashion upon addition to the system described here (4, 9). It is possible that the products of this system will provide useful substrates for the study of

post-translation modification reactions.

The in vitro synthesis of three specific TK polypeptide fragments with molecular weights identical to those synthesized in vivo suggest that our cell-free translation system is faithfully carrying out the biosynthetic processes which we observe in vivo. On the basis of genetic data, it was previously suggested that these fragments may be the result of chain-terminating mutations in the TK gene of the virus (22). An alternative hypothesis is that the fragments arise by some proteolytic or "processing" event. The fact that the TK polypeptide fragments have identical molecular weights in vitro and in vivo leads us to believe that these putative nonsense fragments are not the result of protein processing that might lead to the production of stable protein fragments. If, however, the TK polypeptide fragments are the result of post-translational cleavage, we are observing exactly the same process in vivo and in vitro.

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