JOURNAL OF VIROLOGY, Jan. 1979, p. 275-284
0022-538X/79/01-0275/10$02.00/0

Vol. 29, No. 1

Control of Herpes Simplex Virus Type 1 mRNA Synthesis in Cells Infected with Wild-Type Virus or the Temperature-Sensitive Mutant *tsK*

C. M. PRESTON

Medical Research Council Virology Unit, Glasgow G11 5JR, Scotland

Received for publication 2 August 1978

This paper deals with control of mRNA levels, assayed by in vitro translation, in cells infected with herpes simplex virus type 1 (HSV-1). A particularly useful marker has been pyrimidine deoxyribonucleoside kinase (dPyK) mRNA, for which the enzymatically active product can be assayed quantitatively. Cells infected with the HSV-1 temperature-sensitive mutant *tsK* at the nonpermissive temperature (38.5°C) or with wild-type HSV-1 in the continuous presence of cycloheximide contained no detectable dPyK mRNA. Upon temperature shift-down of *tsK*-infected cells to 31°C, dPyK mRNA was produced, and this event was inhibited by actinomycin D but not cycloheximide. This result demonstrated that the defective polypeptide in *tsK*-infected cells was involved in transcription of the dPyK gene and could regain activity at 31°C. Because *tsK*-infected cells synthesized mainly immediate early polypeptides at 38.5°C, the involvement of this polypeptide class in synthesis of dPyK mRNA was investigated. Analysis of the kinetics of induction of dPyK mRNA indicated that the temperature-sensitive lesion in *tsK* lies in an immediate early polypeptide which is directly responsible for activation of the dPyK gene at the transcriptional level.

One of the earliest events detectable after infection of mammalian cells with herpes simplex virus type 1 (HSV-1) is transcription of a limited portion of the viral genome to give products known as immediate early (IE) RNA (1, 8, 20, 24). This transcription does not need prior protein synthesis in infected cells and is mediated by cellular RNA polymerase II (2). IE RNA is defined here as virus-specific RNA synthesized in cells infected with HSV-1 in the continuous presence of the drug cycloheximide, and it is similar to the α-RNA class described by Roizman and co-workers (9, 11). The translated products of IE RNA are IE polypeptides, of which six have been identified in HSV-1-infected BHK cells (19a).

Synthesis of one or more polypeptides, probably virus specified, is necessary to overcome the initial restriction of transcription of the viral genome. Transition from the synthesis of IE polypeptides to later species occurs shortly after resumption of protein synthesis in "cycloheximide-blocked" cells (7). This finding, along with analysis of the rates of synthesis of virus-induced polypeptides during the infection cycle, has led to the "cascade regulation" model for control of viral protein synthesis (6, 7). According to this scheme, synthesis of α proteins (encoded by α RNA) is necessary before synthesis of β proteins can occur and, similarly, β proteins precede a late class, the γ proteins. Although kinetic experiments with different systems support this model (14, 17), analysis of polypeptides induced by temperature-sensitive (*ts*) mutants of HSV-1 shows that a large number of characteristically restricted polypeptide profiles can be obtained (3, 14). It is possible, therefore, that the α, β, and γ classes of polypeptides encompass a larger number of functionally distinct regulatory pathways which can only be detected by introducing mutations into specific gene products.

In the experiments reported here, we have investigated viral mRNA synthesis at early times during infection. One approach has been to analyze mRNA synthesis after removal of cycloheximide from drug-treated cells, and this has established conditions in which the functions of IE polypeptides can be studied. Another approach has been to use the *ts* mutant, *tsK*, which has suitable properties because it is arrested at an early stage in the replication cycle (14). At the nonpermissive temperature, the major *tsK*-induced polypeptides are of the IE class, this being the most restricted polypeptide profile of HSV-1 mutants so far described. It is important to note, however, that *tsK*-induced polypeptides differ from IE polypeptides in two respects: an IE polypeptide ($V_{mw}136$) is overproduced and a late polypeptide ($V_{mw}155$) is synthesized in small amounts in *tsK*-infected cells (D. MacDonald,

M. Suh, and H. Marsden, manuscript in preparation).

The system used to study mRNA synthesis, and hence protein synthesis, is cell-free translation of cytoplasmic RNA from infected cells (18), and of particular usefulness has been the demonstration that enzymatically active HSV-coded pyrimidine deoxyribonucleoside kinase (dPyK) can be synthesised in vitro (19). In vitro protein synthesis as used here has provided three major advantages over analysis of intracellular protein synthesis. First, the concentration of specific cytoplasmic mRNA's can be estimated accurately, because complications due to changes in the overall rate of protein synthesis or cellular amino acid pools are avoided. The ability to direct synthesis of an enzymatically active product permits precise estimates of dPyK mRNA levels to be made. Second, production of specific mRNA's in the absence of protein synthesis can be followed, thus allowing detailed analysis of the roles of early polypeptides. This approach has enabled a synchronized induction of mRNA's after resumption of protein synthesis to be investigated. Third, situations in which mRNA is present but untranslated in infected cells could be detected.

MATERIALS AND METHODS

Isotopes and chemicals. L-[^{35}S]methionine (300 to 800 Ci/mmol) and [5-^{3}H]thymidine (25 Ci/mmol) were obtained from the Radiochemical Centre, Amersham, England. Cycloheximide and actinomycin D were obtained from Sigma, London, England.

Cells and virus. BHK cells (13) were grown on 90-mm-diameter plastic petri dishes for subsequent RNA extraction or on 30-mm-diameter plastic dishes for labeling of intracellular proteins.

Cells were infected with 20 PFU per cell of wild-type (WT) HSV-1 strain 17 or the mutant *tsK* (isolated from HSV-1 strain 17). Cycloheximide, when present, was added at a final concentration of 200 μg/ml during absorption of virus and subsequent maintenance of cells. Cultures to be infected with *tsK*, along with the virus inoculum, were prewarmed at 38.5°C before commencement of the experiment.

Removal of cycloheximide from infected cells was achieved by washing monolayers three times with growth medium prewarmed to 38.5°C. This operation was performed in a warm room (37°C), and each wash represented addition of medium for 1.5 min with constant agitation of cultures.

Temperature shiftdown experiments involved replacing the growth medium of cells incubated at 38.5°C with medium equilibrated to 31°C and subsequent incubation at 31°C.

Actinomycin D, when present, was added at a final concentration of 2.5 μg/ml.

In vitro protein synthesis. Cytoplasmic RNA was extracted as described previously (18), and 5 μg per 25-μl reaction mixture was added to a fractionated reticulocyte cell-free protein-synthesizing system (18). Enzymatically active dPyK synthesized in vitro was assayed after a 3-h incubation of the cell-free system with cytoplasmic RNA (19). For the examination of [^{35}S]methionine-labeled polypeptides synthesized in vitro, the cell-free system was preincubated with micrococcal nuclease to reduce the background (15; C. M. Preston, *In Oncogenesis and Herpesviruses 3*, in press). Radiolabeled polypeptides were analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis as described by Marsden et al. (14).

The nomenclature of virus-induced polypeptides synthesized in vitro follows that described previously (18).

RESULTS

Quantitative assay of dPyK mRNA. An essential prerequisite for the experiments described here was a quantitative assay for dPyK mRNA. An experiment was carried out in which cytoplasmic RNA from HSV-1-infected cells was mixed in varying proportions with RNA from mock-infected cells or from cells infected with a dPyK-deficient mutant, $dPyK_1^{-1}$, before addition to the cell-free protein-synthesizing system (Fig. 1). RNA was extracted at 5 h postinfection at 31°C, a time at which maximum levels of dPyK mRNA exist (data not shown), and therefore this reconstruction experiment pertains to all situations except any in which dPyK mRNA is overproduced.

A linear relationship between enzymatically active dPyK synthesized in vitro and the amount of HSV-1-infected cell RNA was obtained (Fig. 1), and therefore addition of 5 μg or less of various cytoplasmic RNA preparations allows comparison of their contents of functional dPyK mRNA.

Induction of IE mRNA by HSV-1 and mutant *tsK*. Two "early" stages of infection are analyzed here. The first is infection of cells with WT HSV-1 strain 17 in the presence of 200 μg of cycloheximide per ml, in which, by definition, IE mRNA's are the only virus-induced species. The second is infection with the mutant *tsK* at the nonpermissive temperature (38.5°C), when IE polypeptides are overproduced. Cytoplasmic RNA extracted from cells infected under either of these conditions contained no detectable dPyK mRNA (Table 1). This is in agreement with the finding that the enzyme is not induced in cells infected with *tsK* at 38.5°C (I. K. Crombie, Ph.D thesis, University of Glasgow, Scotland, 1976) or after release from a cycloheximide block in the presence of actinomycin D (5; C. Preston, unpublished data).

Analysis of the polypeptides synthesized in vitro in response to IE mRNA shows production of species designated VI63, VI109, VI136, and VI173 (Fig. 2). Two of these comigrated with major IE polypeptides synthesized in infected cells (VI63 and VI136 with $V_{mw}63$ and $V_{mw}136$), whereas VI109 and VI173 migrated more rapidly

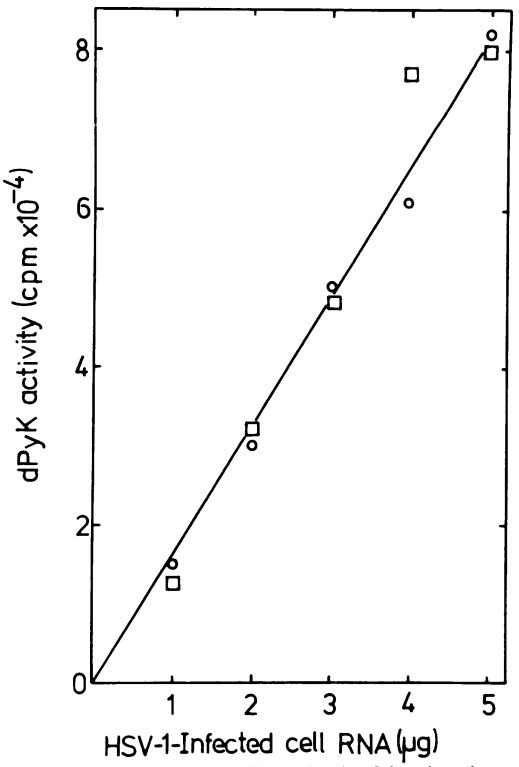

FIG. 1. *Levels of dPyK synthesized in vitro in response to HSV-1-infected cell RNA. Varying amounts of WT HSV-1-infected cell RNA were added to the cell-free protein-synthesizing system, and the total added RNA adjused to 5 µg by addition of cytoplasmic RNA from uninfected (□) or dPyK$_1^{-1}$-infected (○) BHK cells.*

TABLE 1. *Induction of dPyK mRNA in HSV-infected cells*

Source of cytoplasmic RNA[a]	Temp (°C)	dPyK mRNA level (cpm × 10^{-3})[b]
Uninfected cells	31	2.3
Uninfected cells	38.5	2.4
WT HSV-1-infected cells	31	140.3
WT HSV-1-infected cells	38.5	57.2
WT HSV-1-infected cells, cycloheximide present	31	2.5
WT HSV-1-infected cells, cycloheximide present	38.5	2.3
tsK-infected cells	31	180.0
tsK-infected cells	38.5	2.4
E. coli rRNA	—	2.3

[a] All RNA preparations were extracted at 5 h after infection.
[b] Assay of in vitro-synthesized dPyK was performed at 30°C.

than the other major IE polypeptides, V_{mw}110 and V_{mw}175 (compare tracks 9 and 10, also tracks 4 and 5 in Fig. 4). It is likely that VI173 represents the precursor of V_{mw}175 which has been described by Pereira et al. (16), whereas VI109 may be an unprocessed form of the phosphoprotein V_{mw}110.

In vitro translation of RNA from *tsK*-infected cells maintained at 38.5°C (Fig. 2, tracks 1 and 6) also revealed synthesis of VI63, VI109, VI136, and VI173, and this result is similar to the pattern seen in infected cells (Fig. 2, tracks 2 and 7). The enhanced amount of V_{mw}136 compared with the IE situation (compare Fig. 2, tracks 7 and 9) is reflected in the products of cell-free protein synthesis, (compare Fig. 2, tracks 6 and 10), but the minor species V_{mw}155 which is in small amounts in infected cells was not detected. Additional polypeptides V_{mw}26, V_{mw}90 and V_{mw}100 which are synthesized in *tsK*-infected cells during long labeling periods (D. MacDonald, M. Suh, and H. Marsden, manuscript in preparation) were not detected in the cell-free translation system, supporting the suggestion that these are not primary translation products (D. MacDonald, M. Suh, and H. Marsden, manuscript in preparation).

Comparison of RNA from mock-infected BHK cells after 5 h of incubation with cycloheximide with RNA from untreated cells shows an increase in mRNA coding for low-molecular-weight species (compare tracks 4 and 5, Fig. 2). These polypeptides are of corresponding molecular weights to histones, whose mRNA's are stabilized by inhibition of protein synthesis (21), and they are not considered to be virus induced.

Induction of dPyK mRNA after shiftdown of *tsK*-infected cells. All available data indicate that cells infected with *tsK* at 38.5°C are arrested at an early stage in the infection cycle. Shiftdown experiments, in which cultures were transferred to the permissive temperature (31°C), were performed to investigate further the nature of this lesion.

Cells were infected with *tsK* and incubated at 38.5°C for 5 h. At this time, cultures were either kept at 38.5°C, or shifted down in the presence, or absence, of the RNA synthesis inhibitor actinomycin D or the protein synthesis inhibitor cycloheximide. The dPyK mRNA content of cytoplasmic RNA was analyzed at various times (Fig. 3). Shiftdown of cultures in the absence of inhibitors resulted in production of dPyK mRNA, a feature which was not observed in cultures maintained at 38.5°C. Addition of actinomycin D during the shiftdown prevented induction of dPyK mRNA, indicating the necessity for new transcription. In the presence of cycloheximide, however, greater amounts of dPyK mRNA were produced than in its absence. This result shows that a polypeptide(s) synthesized in *tsK*-infected cells at 38.5°C is responsible for induction of dPyK mRNA and further demonstrates that the polypeptide(s), although inactive at the nonpermissive temperature, is relatively stable in cells and has a reversible action. Examination of the kinetics of induction of

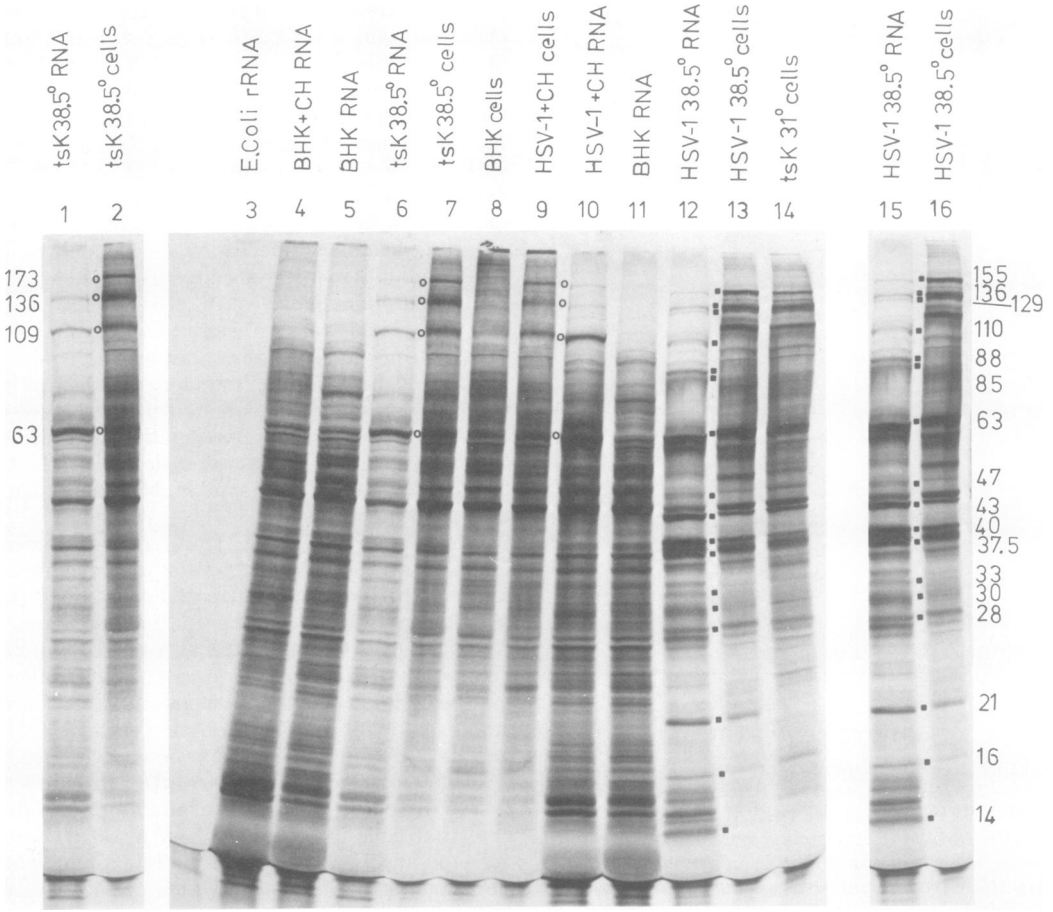

FIG. 2. *Polypeptides synthesized in vitro in response to various cytoplasmic RNA preparations extracted from cells incubated at 38.5°C. The RNA added to the cell-free system was from E. coli rRNA (track 3), uninfected cells (tracks 5 and 11), uninfected cells after incubation for 5 h with cycloheximide (track 4), tsK-infected cells (track 6), WT HSV-1-infected cells with cycloheximide present throughout infection (track 10), and WT HSV-1-infected cells without drug treatment (track 12). The standards included are [^{35}S]methionyl polypeptides labeled in a 1-h period from uninfected cells (track 8), tsK-infected cells maintained at 38.5°C for 5 h (track 7), tsK-infected cells maintained at 31°C for 5 h (track 14) and WT HSV-1-infected cells maintained at 38.5°C for 5 h (track 13). IE polypeptides were labeled by incubating WT HSV-1-infected cells at 38.5°C for 5 h in the presence of cycloheximide, washing out the inhibitor in the presence of actinomycin D, and labeling in the presence of actinomycin D (track 9). Tracks 1 and 2 are identical to tracks 6 and 7, and tracks 15 and 16 are identical to tracks 12 and 13. IE polypeptides synthesized in vitro* (○) *are classified to the left of track 1. Late polypeptides synthesized in vitro* (■) *are classified to the left of track 15 according to Preston (18).*

dPyK mRNA suggests that a polypeptide responsible for switch-off or destabilization of dPyK mRNA may be synthesized in the absence of cycloheximide.

The mRNA's present after shiftdown of *tsK*-infected cells were also studied by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the products of in vitro protein synthesis. Fig. 4 shows the characteristic production of VI63, VI109, VI136, and VI173 after incubation for 5 h at 38.5°C. This pattern did not change during a further incubation for 2.5 h at 38.5°C or during shiftdown in the presence of actinomycin D. Shiftdown in the presence or absence of cycloheximide, however, resulted in the production of mRNA for polypeptide VI43 and, to a lesser extent, VI37.5.

These results show that failure of the RNA preparations, analyzed in Fig. 3, that were kept at 38.5°C or shifted down in the presence of actinomycin D to direct synthesis of dPyK enzyme was not due to an overall loss of mRNA activity or to the presence of inhibitory materials. A further interesting point is that IE mRNA's appeared to be stable under the conditions of the experiment and were present in

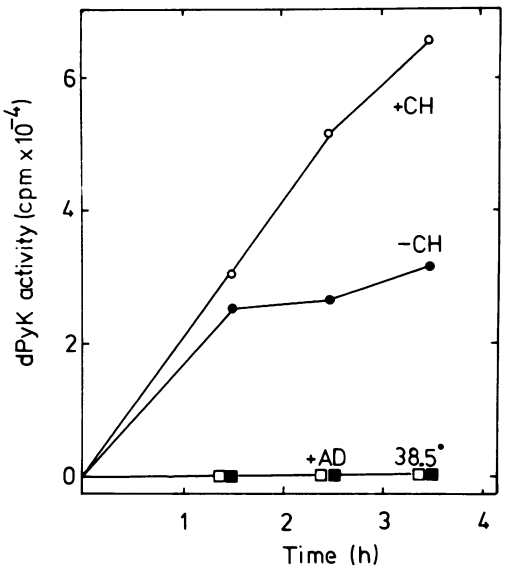

FIG. 3. *Synthesis of dPyK mRNA after shiftdown of tsK-infected cells. Cell cultures were infected with tsK and incubated at 38.5°C for 5 h. One set was maintained at 38.5°C (□), one set was shifted down in the presence of actinomycin D (■), one set was shifted down in the presence of cycloheximide (○), and the final set was shifted down without inhibitors (●). Cytoplasmic dPyK mRNA was assayed at various times.*

approximately equal amounts after 5 and 7.5 h at 38.5°C.

Involvement of IE polypeptides in induction of dPyK mRNA. Polypeptides induced in *tsK*-infected cells mainly represent the IE class, although some additional species have been detected (D. MacDonald, M. Suh, and H. Marsden, manuscript in preparation). Any of these could be the polypeptides which are defective in the ability to induce dPyK mRNA. To classify further this polypeptide(s), the involvement of IE polypeptides in the induction of dPyK in WT HSV-1-infected cells was investigated. The desired situation was one in which the only virus-induced polypeptides present in infected cells were the IE polypeptides, so that their activities could be examined. This was achieved by investigation of protein synthesis after release of infected cells from a "cycloheximide block."

BHK cell cultures were infected with HSV-1 in the presence of cycloheximide and maintained at 38.5°C for 5 h, after which the inhibitor was washed rapidly from the cells. Provided the temperature of the washing medium was kept high (38 to 40°C), protein synthesis resumed rapidly at rates varying from 70 to 110% of that in untreated, uninfected cells (data not shown). Under these conditions, dPyK mRNA was first detectable in cytoplasmic RNA at 40 min after removal of cycloheximide, after which the level rose for more than 140 min (Fig. 5). The presence of actinomycin D after removal of cycloheximide prevented appearance of dPyK mRNA.

The time of synthesis of the polypeptide responsible for induction of dPyK mRNA after removal of cycloheximide was then determined. Cells were infected in the presence of cycloheximide as described above, and the inhibitor was removed after 5 h. Cycloheximide was then replaced at various times, and incubation of all cultures continued until 3 h after washing of monolayers. Cytoplasmic RNA was then extracted, and dPyK mRNA activity was measured (Fig. 6). The results show that by 15 min after the start of washing of the cells some inducing activity was present, and this had reached saturating levels by 30 min, a time before dPyK mRNA was detectable in the cytoplasm (Fig. 5). Therefore, by allowing cellular protein synthesis to proceed for 20 to 30 min after removal of cycloheximide, followed by readdition of the inhibitor, a situation was established in which induction of dPyK mRNA could be studied.

The polypeptides produced in vitro by translation of the RNA samples used in Fig. 6 were also examined (Fig. 7). This shows that 0, 5, or 10 min of protein synthesis resulted in the production only of IE mRNA's, whereas after this time mRNA's for new virus-induced polypeptides were detected. The mRNA for VI43, the putative dPyK polypeptide (23), followed kinetics similar to those shown for dPyK mRNA (Fig. 6). other mRNA's (those for VI16, VI37.5, VI85, VI110, VI136, and VI155) were also detectable when 15 to 20 min of protein synthesis was permitted. Induction of these mRNA's coincided with inactivation of the mRNA's for the IE polypeptides VI109 and VI173.

The minimum period of protein synthesis after removal of cycloheximide necessary for synthesis of dPyK mRNA inducer was 15 min. Protein synthesis would not be expected to proceed at normal rates during the first 5 min of this period, because this represents the time during which the inhibitor was washed out, and therefore a reasonable interpretation of the data is that this period represents the time necessary for synthesis of IE polypeptides. To test this assumption, the polypeptides synthesized from 5 to 25 min after removal of cycloheximide were examined. Figure 8 shows that, regardless of the presence of actinomycin D, only IE polypeptides were labeled during this period. Later labeling times (60 to 80 min after removal of cycloheximide) showed no change in the polypeptide profile in the presence of actinomycin D but synthesis of additional polypeptides in its absence,

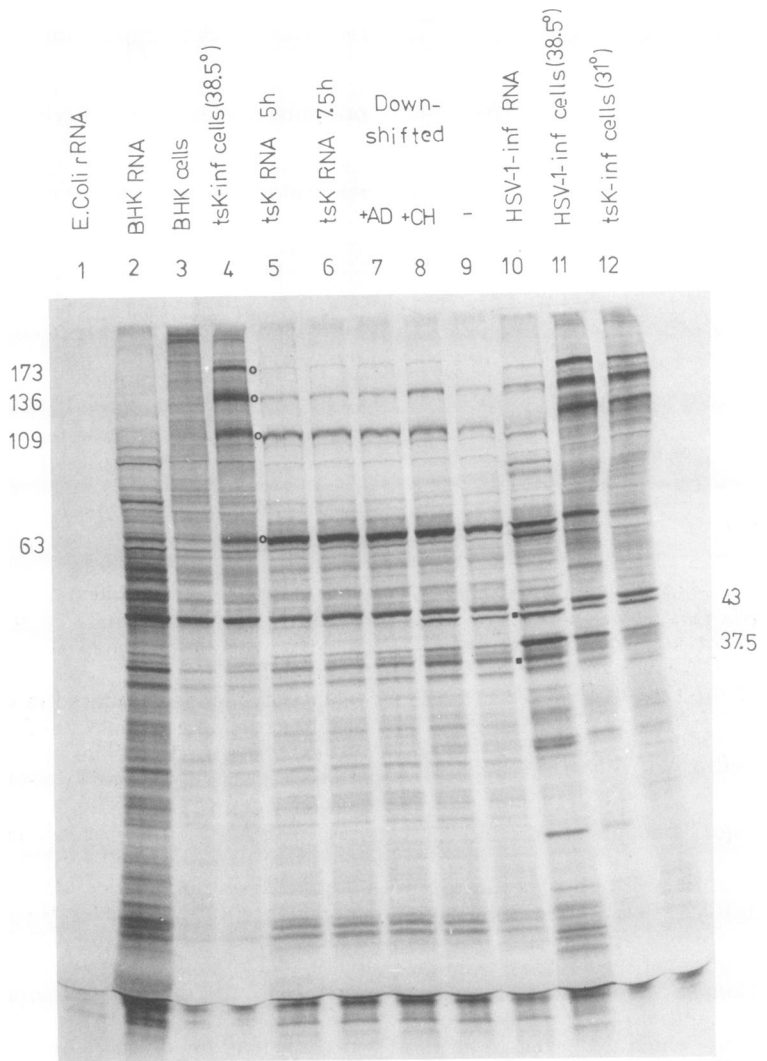

FIG. 4. *Viral mRNA synthesis after downshift of tsK-infected cells. Cells were treated as described in the legend to Fig. 3, and in vitro polypeptide synthesis directed by cytoplasmic RNA extracted 2.5 h after shiftdown was examined. The source of RNA was E. coli. RNA (track 1); tsK-infected cells 5 h postinfection at 38.5°C (track 5); tsK-infected cells 7.5 h postinfection at 38.5°C (track 6); tsK-infected cells after shiftdown in the presence of actinomycin D (track 7); tsK-infected cells after shiftdown in the presence of cycloheximide (track 8); tsK-infected cells after shiftdown without inhibitors (track 9); and WT HSV-1-infected cells after incubation for 5 h at 38.5°C (track 10). Standards included for comparison are [^{35}S]methionyl polypeptides synthesized in a 1-h labeling period by uninfected BHK cells (track 3); tsK-infected cells, 5 h postinfection at 38.5°C (track 4); WT HSV-1-infected cells at 5 h postinfection at 38.5°C (track 11); and tsK-infected cells at 5 h postinfection at 31°C (track 12). IE polypeptides synthesized in vitro are labeled between tracks 4 and 5 (○) and classified to the left of track 1. Polypeptides whose mRNA's are induced upon shiftdown are labeled between tracks 9 and 10 (■) and classified to the right of track 12.*

confirming that recovery from inhibition of protein synthesis had occurred.

Failure of IE polypeptides from *tsK*-infected cells to induce dPyK mRNA. The above results indicate that an IE polypeptide(s) is directly responsible for the induction of dPyK mRNA. This conclusion can be tested, because *tsK* is defective in this activity at 38.5°C. Cells were infected with WT HSV-1 or *tsK* and maintained at 38.5°C in the presence of cycloheximide for 5 h. Keeping all cultures at 38.5°C, cycloheximide was removed for 25 min to allow synthesis of IE polypeptides and then replaced. Cultures were then maintained at 38.5°C, or shifted down to 31°C, and cytoplasmic RNA was extracted after 2.5 h.

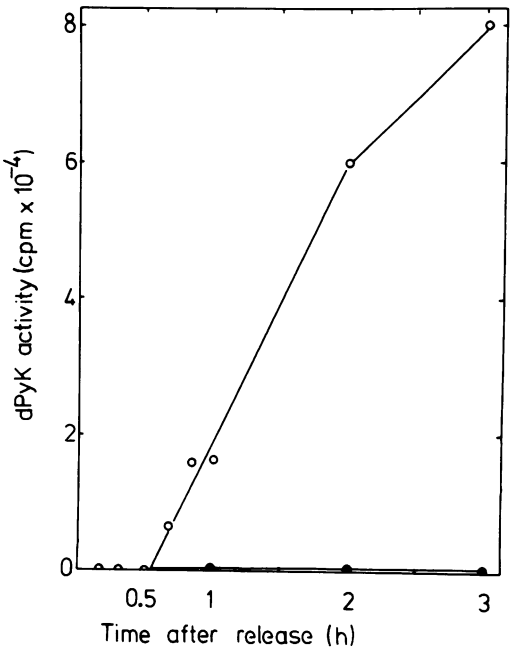

FIG. 5. *Induction of dPyK mRNA after release from a cycloheximide block. Cells were infected with HSV-1 in the presence of cycloheximide and maintained at 38.5°C for 5 h. Cells were then washed, and dPyK mRNA content of cytoplasmic RNA was measured. Symbols: ●, actinomycin D present after washing cells, ○, no inhibitors present after washing cells.*

Table 2 shows that in both HSV-1 and *tsK*-infected cells, dPyK mRNA was induced at 31°C, but in *tsK*-infected cells this did not occur at 38.5°C. This experiment emphasizes that *tsK*-infected cells fail to induce dPyK mRNA at 38.5°C due to the reversible *ts* activity of an IE polypeptide(s).

DISCUSSION

The experiments described here have used in vitro translation of mRNA for HSV-coded dPyK to investigate the control of this gene in infected cells. A major advantage of this approach is that the presence of the mRNA can be determined in the absence of cellular protein synthesis, thus allowing a greater synchronization and more detailed analysis of early events in infection than is possible by examination of protein synthesis in intact cells. We have established that expression of the dPyK gene is controlled by the direct action of one or more IE polypeptides and that the mutant *tsK* has a *ts* lesion affecting the activity of an IE polypeptide responsible for this event. Analysis of mRNA species present at early times in the infection cycle has also yielded information on the intracellular stability of IE mRNA.

Two results exclude the possibility that post-transcriptional or translational events are the major controls regulating the synthesis of dPyK. First, the presence of dPyK mRNA in the cytoplasm correlates with production of the enzyme in infected cells. Thus, cells infected with *tsK* at 38.5°C or with HSV-1 in the presence of cycloheximide have no cytoplasmic dPyK mRNA that can be detected by in vitro translation. Second, the appearance of dPyK mRNA after shiftdown of *tsK*-infected cells or upon removal of cycloheximide is inhibited totally by actinomycin D. The conclusion that this major control is at the level of transcription is in agreement with previous findings. Analysis of cytoplasmic HSV-specified RNA has demonstrated a restricted pattern of molecular species in the absence of protein synthesis (1, 9). Furthermore, *tsK*-infected cells have a similarly restricted content of viral RNA, and the synthesis of additional transcripts occurs upon shiftdown in the

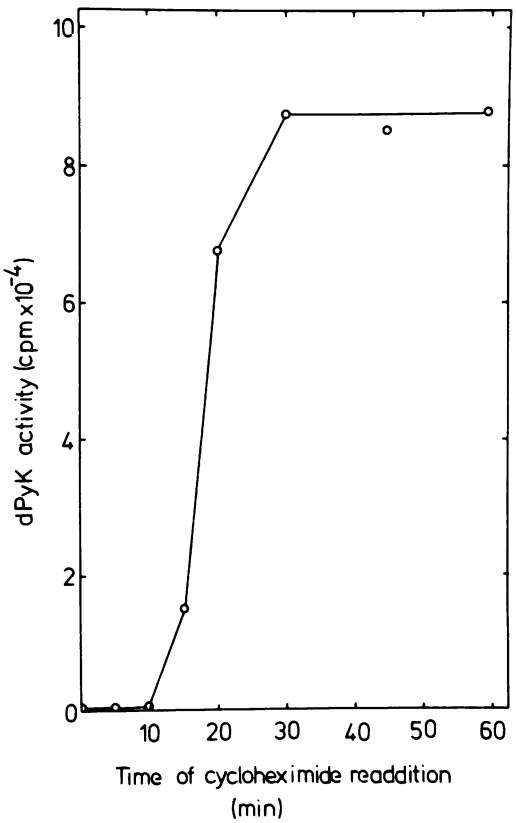

FIG. 6. *Time of synthesis of inducer polypeptide for dPyK mRNA. Cells were infected with WT HSV-1 in the presence of cycloheximide and incubated at 38.5°C for 5 h. Cells were then washed, and cycloheximide was added at various times. All cultures were harvested at 180 min after the initial removal of cycloheximide, and the dPyK mRNA content of cytoplasmic RNA was measured. The period 0 to 5 min represents the time during which cells were washed.*

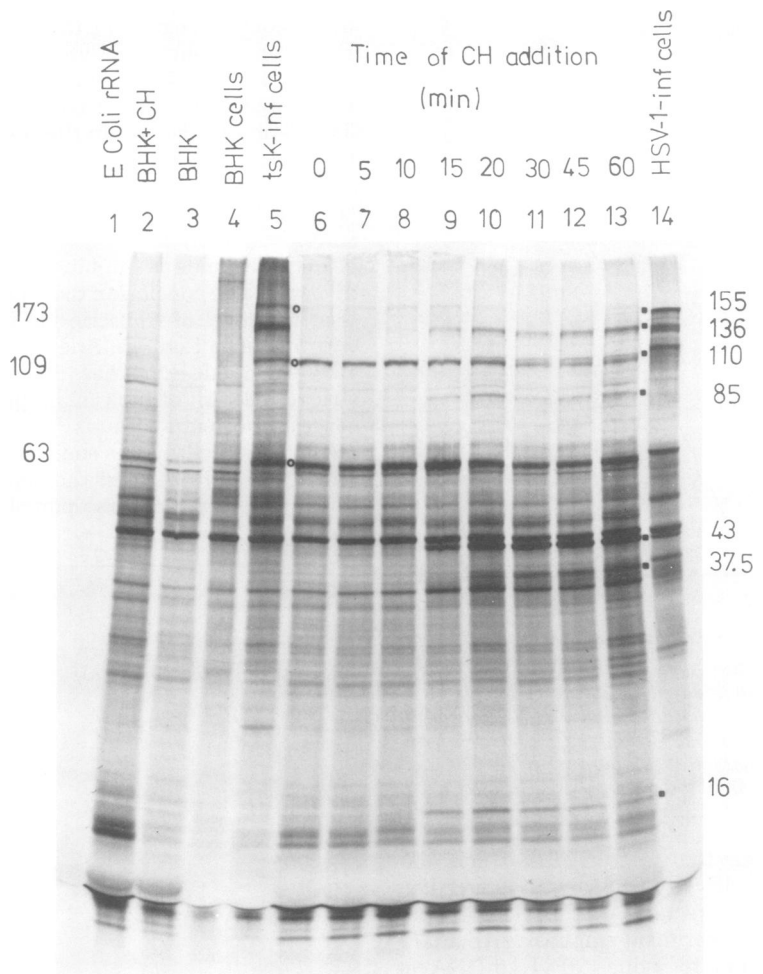

FIG. 7. *Time of synthesis of inducer polypeptides for viral mRNA's. Polypeptides specified by the RNA samples whose dPyK mRNA content is shown in Fig. 6 were analyzed. Readdition of cyclohexmide was at 0 min (track 6), 5 min (track 7), 10 min (track 8), 15 min (track 9), 20 min (track 10), 30 min (track 11), 45 min (track 12), or 60 min (track 13). Polypeptides specified by E. coli rRNA (track 1); uninfected cells after incubation for 5 h with cycloheximide (track 2); and uninfected, untreated cells (track 3) are shown. The standards are [^{35}S]methionyl polypeptides synthesized in a 1-h labeling period by uninfected cells (track 4), tsK-infected cells incubated for 5 h at 38.5°C (track 5), and HSV-1-infected cells incubated for 5 h at 38.5°C (track 14). IE polypeptides synthesized in vitro are labeled between tracks 5 and 6 (○) and classified to the left of track 1. Polypeptides whose mRNA's are induced after removal of cycloheximide are labeled between tracks 13 and 14 (■) and classified to the right of track 14.*

presence, or absence, of cycloheximide (R. J. Watson and J. B. Clements, Virology, in press). Hybridization studies, however, could not determine the mRNA species present in cytoplasmic RNA.

The cascade regulation model suggests that α polypeptides act at the transcriptional level (7), and more recent studies have demonstrated that IE polypeptides are translocated to the nucleus, the expected site of action of transcriptional control (16). It also seems likely that dPyK genes present in HSV-transformed cells can be activated in a similar way (10, 12).

The results presented here define a function of the *ts* lesion in the HSV mutant *tsK*. It seems likely that a single lesion is responsible for the temperature sensitivity of growth of *tsK*, because single-step revertants have been obtained (D. Dargan and J. H. Subak-Sharpe, personal communication). All revertants so far tested exhibit normal polypeptide production, and dPyK induction, at 38.5°C.

Physical mapping, by marker rescue, has shown that the *tsK* lesion is located in the repeats flanking the (S) region of HSV-1 DNA (22; N. M. Wilkie, N. D. Stow, H. S. Marsden, V.

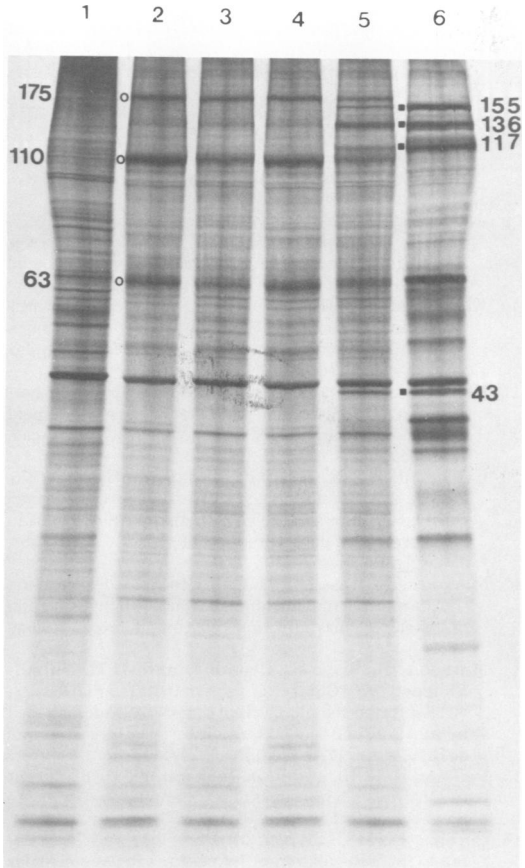

FIG. 8. *Polypeptides synthesized after removal of cycloheximide from infected cells. Cells were infected with HSV-1 in the presence of cycloheximide and incubated for 5 h at 38.5°C, after which they were washed. Polypeptides were labeled by incubation with [^{35}S]methionine from 5 to 25 min after washing in the presence (track 2) or absence (track 4) of actinomycin D, or from 60 to 80 min in the presence (track 3) or absence (track 5) of actinomycin D. Polypeptides synthesized in a 20-min labeling period by uninfected cells (track 1) and WT HSV-1-infected cells (track 6) are shown. IE polypeptides (○) are classified to the left of track 1. Polypeptides induced after removal of cycloheximide (■) are classified to the right of track 6.*

Preston, R. Cortini, M. C. Timbury, and J. H. Subak-Sharpe, *In Oncogenesis and Herpesviruses 3,* in press). This location is represented in IE transcripts (1, 9), and recent analysis of HSV-1/HSV-2 intertypic recombinants (19a), as well as previous studies (4), indicates that it encodes the IE polypeptide $V_{mw}175$. It is possible, therefore, tentatively to assign a function to this specific HSV-coded polypeptide.

Differences exist between polypeptides induced by *tsK* at 38.5°C and IE polypeptides (D. MacDonald, M. Suh, and H. Marsden, manuscript in preparation, and Fig. 2). It appears, therefore, that the polypeptides whose synthesis at 38.5°C is induced ($V_{mw}155$) or amplified ($V_{mw}136$) in *tsK*-infected cells compared with the IE species are themselves induced by IE functions distinct from the *tsK* lesion. Thus, two separate classes of early polypeptides, one dependent upon and the other unaffected by the *tsK* lesion, have been identified. A similar situation has been recognized by Periera et al. (16), who, on the basis of the effects of the arginine analog canvanine, defined the polypeptide classes β_1 and β_2. Induction of only one of these was inhibited by addition of canvanine to infected cells, but Pereira et al. (16) were unable to distinguish a sequential regulatory pathway ($\alpha \rightarrow \beta_1 \rightarrow \beta_2$) from two independent pathways ($\alpha_1 \rightarrow \beta_1$ and $\alpha_2 \rightarrow \beta_2$). The data presented here favor the latter scheme and raise the possibility that the defective polypeptide of *tsK* may be analogous to the component which is selectively inactivated by canvanine.

A noteworthy observation is that recovery of viral mRNA production after release from a cycloheximide block is more extensive than after shiftdown of *tsK*-infected cells. This may be due to a slower activation of genes at 31°C compared with 37°C. Alternatively, *tsK*-infected cells may regain the ability to induce only a subset of mRNA's (including dPyK mRNA), or the disruption of normal temporal events may have resulted in inactivation of some genes. These experiments, therefore, may define a further point for control of protein synthesis, and this possibility is currently under investigation.

Immediate early polypeptides are synthesized transiently during the very early stages of the HSV-1 infected cycle. One factor causing their limited production is probably an early cessation of IE RNA synthesis (1), but a further control may be exerted on the functional stability of IE mRNA. In the presence of cycloheximide, or in *tsK*-infected cells, IE mRNA (as identified by in vitro translation) is stable, and this probably

TABLE 2. *Induction of dPyK mRNA by IE polypeptides of WT HSV-1 or tsK*[a]

Virus	Temp (°C)	dPyK mRNA level[b] (cpm × 10^{-3})
WT HSV-1	31	37.3
WT HSV-1	38.5	54.0
tsK	31	24.6
tsK	38.5	0.2

[a] BHK cells were infected with WT HSV-1 or *tsK* in the presence of cycloheximide and incubated at 38.5°C for 5 h. Cycloheximide was then removed by washing, and incubation continued for 25 min at 38.5°C. After this time, cycloheximide was added, and cultures were incubated at 31 or 38.5°C for 3 h. Cytoplasmic RNA was then extracted and analyzed for dPyK mRNA content.

[b] A background of 1.9×10^3 cpm has been subtracted in all cases.

contributes to the overproduction of IE polypeptides in these situations. Once the infection cycle reaches a certain stage, IE mRNA is rapidly inactivated, thus implicating a virus-specified polypeptide in this process. It is not possible at present to determine whether mRNA inactivation is a general event or is specific to IE mRNA.

The studies presented here show that IE polypeptides control activation of the HSV genome, and, as with most examples of transcriptional controls, little is known of the molecular mechanisms by which this occurs. One possibility is that IE polypeptides modify cellular RNA polymerases to cause a change in template specificity; another is that IE polypeptides interact with viral DNA or a nucleoprotein complex to activate specific genes. Pertinent to this discussion is the finding that IE polypeptides bind strongly to immobilized DNA in vitro (R. Hay and J. Hay, personal communication).

The mutant *tsK* provides a basis for further examination of the action of IE polypeptides. These can be radiolabeled during incubation of *tsK*-infected cells at 38.5°C, and, after addition of cycloheximide, their intracellular properties in their active (at 31°C) or inactive (at 38.5°C) states can be followed in the absence of further protein synthesis. Alternatively, the action of IE polypeptides from *tsK* or WT HSV-1-infected cells can be examined if only a short period (less than 30 min) of protein synthesis is permitted after release from a cycloheximide block. It is anticipated that these will be useful systems in the study of the biochemical mechanisms of transcriptional control of the HSV genome.

ACKNOWLEDGMENTS

I am grateful to J. H. Subak-Sharpe for encouragement and critical appraisal of the manuscript and to M. Dunlop for skilled technical assistance.

LITERATURE CITED

1. **Clements, J. B., R. J. Watson, and N. M. Wilkie.** 1977. Temporal regulation of herpes simplex virus type 1 transcription: location of transcripts on the viral genome. Cell **12**:275-285.
2. **Constanzo, F., G. Campadelli-Fiume, L. Foa-Tomasi, and E. Cassai.** 1977. Evidence that herpes simplex virus DNA is transcribed by cellular polymerase B. J. Virol. **21**:996-1001.
3. **Courtney, R. J., P. A. Schaffer, and K. L. Powell.** 1976. Synthesis of virus-specific polypeptides by temperature-sensitive mutants of herpes simplex virus type 1. Virology **75**:306-318.
4. **Frenkel, N., R. J. Jacob, R. W. Honess, G. S. Hayward, H. Locker, and B. Roizman.** 1975. Anatomy of herpes simplex virus DNA. III. Characterization of defective DNA molecules and biological properties of virus populations containing them. J. Virol. **16**:153-167.
5. **Garfinkle, B., and B. R. McAuslan.** 1974. Regulation of herpes simplex virus-induced thymidine kinase. Biochem. Biophys. Res. Commun. **58**:822-829.
6. **Honess, R. W., and B. Roizman.** 1973. Proteins specified by herpes simplex virus. XI. Identification and relative molar rates of synthesis of structural and nonstructural herpes virus polypeptides in the infected cell. J. Virol. **12**:1347-1365.
7. **Honess, R. W., and B. Roizman.** 1974. Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. J. Virol. **14**:8-19.
8. **Jean, J.-H., T. Ben-Porat, and A. S. Kaplan.** 1974. Early functions of the genome of herpesvirus. III. Inhibition of the transcription of the viral genome in cells treated with cycloheximide early during the infective process. Virology **59**:516-523.
9. **Jones, P. C., G. S. Hayward, and B. Roizman.** 1977. Anatomy of herpes simplex virus DNA. VII. α RNA is homologous to noncontinguous sites in both the L and S components of viral DNA. J. Virol. **21**:268-276.
10. **Kit, S., and D. R. Dubbs.** 1977. Regulation of herpesvirus thymidine kinase activity in LM (TK^-) cells transformed by ultraviolet light-irradicated herpes simplex virus. Virology **76**:331-340.
11. **Kozak, M., and B. Roizman.** 1974. Regulation of herpesvirus macromolecular synthesis: nuclear retention of nontranslated viral RNA sequences. Proc. Natl. Acad. Sci. U.S.A. **71**:4322-4326.
12. **Leiden, J. M., R. Buttyan, and P. G. Spear.** 1976. Herpes simplex virus gene expression in transformed cells. I. Regulation of the viral thymidine kinase gene in transformed L cells by products of superinfecting virus. J. Virol. **20**:413-424.
13. **Macpherson, I. A., and M. G. P. Stoker.** 1962. Polyoma transformation of hamster cell clones—an investigation of genetic factors affecting cell competence. Virology **16**:147-151.
14. **Marsden, H. S., I. K. Crombie, and J. H. Subak-Sharpe.** 1976. Control of protein synthesis in herpesvirus-infected cells: analysis of the polypeptides induced by wild type and sixteen temperature-sensitive mutants of HSV strain 17. J. Gen. Virol. **31**:347-372.
15. **Pelham, H. R. B., and R. J. Jackson.** 1976. An efficient mRNA-dependent translation system from reticulocyte lysates. Eur. J. Biochem. **67**:247-256.
16. **Pereira, L., M. H. Wolff, M. Fenwick, and B. Roizman.** 1977. Regulation of herpesvirus macromolecular synthesis. V. Properties of α polypeptides made in HSV-1 and HSV-2 infected cells. Virology **77**:733-749.
17. **Powell, K. L., and R. J. Courtney.** 1975. Polypeptides synthesized in herpes simplex virus type 2-infected HEp-2 cells. Virology **66**:217-228.
18. **Preston, C. M.** 1977. The cell-free synthesis of herpesvirus-induced polypeptides. Virology **78**:349-353.
19. **Preston, C. M.** 1977. Cell-free synthesis of herpes simplex virus-coded pyrimidine deoxyribonucleoside kinase enzyme. J. Virol. **23**:455-460.
19a. **Preston, V. G., A. J. Davison, H. S. Marsden, M. C. Timbury, J. H. Subak-Sharpe, and N. M. Wilkie.** 1978. Recombinants between herpes simplex virus types 1 and 2: analyses of genome structures and expression of immediate early polypeptides. J. Virol. **28**:499-517.
20. **Rakusanova, T., T. Ben-Porat, M. Himeno, and A. S. Kaplan.** 1971. Early functions of the genome of herpesvirus 1. Characterization of the RNA synthesized in cycloheximide-treated, infected cells. Virology **46**: 877-889.
21. **Stahl, H., and D. Gallwitz.** 1977. Fate of histone messenger RNA in synchronized Hela cells in the absence of initiation of protein synthesis. Eur. J. Biochem. **72**: 385-392.
22. **Stow, N. D., J. H. Subak-Sharpe, and N. M. Wilkie.** 1978. Physical mapping of herpes simplex virus type 1 mutations by marker rescue. J. Virol. **28**:182-192.
23. **Summers, W. P., M. Wagner, and W. C. Summers.** 1975. Possible peptide chain termination mutants in thymidine kinase gene of a mammalian virus, herpes simplex virus. Proc. Natl. Acad. Sci. U.S.A. **72**:4081-4084.
24. **Swanstrom, R. C., and E. K. Wagner.** 1974. Regulation of synthesis of herpes simplex type 1 virus mRNA during productive infection. Virology **60**:522-533.