JOURNAL OF VIROLOGY, Apr. 1989, p. 1540–1548
0022-538X/89/041540-09$02.00/0

Vol. 63, No. 4

Relationship between RNA Polymerase II and Efficiency of Vaccinia Virus Replication

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Received 19 August 1988/Accepted 14 December 1988

It is clear from previous studies that host transcriptase or RNA polymerase II (pol II) has a role in poxvirus replication. To elucidate the participation of this enzyme further, in this study we examined several parameters related to pol II during the cycle of vaccinia virus infection in L-strain fibroblasts, HeLa cells, and L_6H_9 rat myoblasts. Nucleocytoplasmic transposition of pol II into virus factories and virions was assessed by immunofluorescence and immunoblotting by using anti-pol II immunoglobulin G. RNA polymerase activities were compared in nuclear extracts containing crude enzyme preparations. Rates of translation into cellular or viral polypeptides were ascertained by labeling with [^{35}S]methionine. In L and HeLa cells, which produced vaccinia virus more abundantly, the rates of RNA polymerase and translation in controls and following infection were higher than in myoblasts. The data on synthesis and virus formation could be correlated with observations on transmigration of pol II, which was more efficient and complete in L and HeLa cells. The stimulus for pol II to leave the nucleus required the expression of both early and late viral functions. On the basis of current and past information, we suggest that mobilization of pol II depends on the efficiency of vaccina virus replication and furthermore that control over vaccinia virus production by the host is related to the content or availability (or both) of pol II in different cell types.

It has been clearly established that host RNA polymerase II (pol II) is required for vaccinia virus replication (6, 23, 24). Evidence against transfer of inoculum genomes into the nucleus (2) or the presence of any intranuclear viral DNA during infection (12, 14) minimizes the likelihood that vaccinia virus replication depends on host transcription. On the other hand, transposition of pol II out of the nucleus into viroplasmic foci during poxvirus infection (13, 33) favors the view that pol II participates in virus-specific cytoplasmic transcription. In further attempts to elucidate the role of pol II in the replication strategy of these agents, in this study we investigated factors affecting nucleocytoplasmic transfer of pol II. The data obtained suggest, on the one hand, that the efficiency of vaccinia virus replication controls mobilization of pol II out of the nucleus and, on the other hand, that levels of pol II activity have a bearing on the viral replication process.

MATERIALS AND METHODS

Cells and viruses. Human HeLa (20) and rat myoblast L_6H_9 (25) cell lines were propagated as monolayers, and mouse L_2 cells (19) were propagated as suspension cultures in Eagle minimal essential medium supplemented with 10% fetal bovine serum. The IHD-W strain of vaccinia virus (7) was used throughout at various multiplicities of infection (MOI), as specified for individual experiments. In some cases, conditionally lethal, temperature-sensitive (*ts*) mutants of Dales et al. (4) were used. Infectivity assays conformed to previously published procedures (2).

Virus purification and dismemberment. Suspensions of purified vaccinia virus particles were produced as previously described (26). The envelopes were stripped away from cores by treatment with Nonidet P-40 and β-mercaptoethanol in buffer solution.

Preparation of cell fractions for enzymatic assays of RNA polymerase and immunoblotting. The following procedures were adapted from the methods described previously (15). Briefly, monolayers of HeLa and L_6H_9 cells were washed with phosphate-buffered saline and then treated with 0.05% trypsin until cell rounding was evident. Tryptic action was arrested, and the cells were freed by gentle shaking and collected into pellets by centrifugation at 2,000 × *g*. L cells from suspension were similarly washed and collected. To promote swelling, 7×10^7 to 7×10^8 pelleted cells were suspended on ice for 20 min in 1 ml of hypotonic buffer A containing 10 mM Tris hydrochloride, 5 mM $MgCl_2$, and 6 mM KCl (pH 7.9). After addition of Nonidet P-40 to a final concentration of 0.5%, the suspensions were aspirated through syringes fitted with needles of progressively diminishing 19 to 22-gauge bore sizes until rupture in at least 90% of the cells was evident by phase-contrast microscopy. The free nuclei were sedimented at 1,000 × *g* for 5 min, washed in phosphate-buffered saline, and suspended in buffer A (to a final volume equaling that of the cytoplasmic fraction supernatant remaining after removal of nuclei plus the nuclear-fraction wash). Nuclear fractions contained 3×10^7 to 5×10^7 nuclei per ml. The following additions, at the final concentrations indicated, were made to cytoplasmic and nuclear fractions: 12.5% glycerol, 1 mM dithiothreitol, and 0.3 M ammonium sulfate. The preparations, in 3.0-ml samples, were sonicated for 5-s intervals in a Fisher Sonic Dismembrator (microtip output, 0.3) to reduce or eliminate viscosity. Subsequently, buffer B, containing 50 mM Tris hydrochloride (pH 7.9), 12.5% (vol/vol) glycerol, 5 mM $MgCl_2$, 6 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, and 0.1 mM phenylmethylsulfonyl fluoride, was rapidly added at twice the original volume of each fraction. The particulate material was sedimented at 105,000 × *g* for 90 min, and 4.2 g of crystalline ammonium sulfate per ml was added slowly to the clarified supernatant. Protein precipitation was completed by keeping the mixture at 0°C for 18 h, and the precipitates were collected at 135,000 × *g* for 60 min,

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suspended in buffer B, and then dialyzed for 24 h against buffer B containing 0.05 M ammonium sulfate. Dialysates of nuclear and cytoplasmic fractions were sonicated for 3 s and stored in aliquots at −70°C for use in Western blots (immunoblots) and RNA polymerase assays. Protein concentrations were determined as described previously (11).

Assay for RNA polymerase activity. Enzyme aliquots prepared as described above, containing 200 μg of cellular protein, were assayed at 37°C for 30 min as described previously (15, 18, 34). The reaction mixtures contained 7.5 μmol of Tris hydrochloride (pH 7.9); 0.3 μmol of $MnCl_2$; 0.09 μmol each of ATP, GTP, and CTP; 0.015 μmol of UTP; 1.0 μCi of [^{3}H]UTP (specific activity, 17.7 Ci/mmol); and 22.5 μg of native calf thymus DNA in a final volume of 150 μl. When appropriate, actinomycin D was added at a final concentration of 4 μg/ml and α-amanitin was added at 3 μg/ml. The effect of the latter was tested by preincubation with the enzyme for 15 min at 37°C. The RNA product synthesized in the reaction was measured as 10% trichloroacetic acid-insoluble material. Trichloroacetic acid precipitates were collected and washed extensively with 5% trichloroacetic acid on Whatman GF/C filters, and radioactivity was determined in a Beckman scintillation counter at an efficiency of 58% for ^{3}H.

Rates of translation. Subconfluent (50 to 75% complete) monolayers of the three cell lines were inoculated with IHD-W vaccinia virus at an MOI of 7 or 10. At 4, 7, and 11 h postinoculation, [^{35}S]methionine (specific activity, 1,140 Ci/mmol; New England Nuclear Corp.) was added to the culture medium at a final concentration of 30 μCi/ml, and pulse-labeling was allowed to proceed for 60 min. In one set of experiments, preincubation in starvation medium preceded pulse-labeling with [^{35}S]methionine. In another set, starvation conditions were omitted to account for any possible differences in methionine pool size among the cell lines used.

Inhibition of translation. Reduction of protein synthesis by 95% was effected within a few minutes by addition of 10 μg of the translation inhibitor streptovitacin A (1, 5) per ml at various times postinoculation of HeLa cells with the wild-type virus. The drug was a gift from The Upjohn Co., Kalamazoo, Mich.

Localization of pol II during infection by immunofluorescence. Labeling by indirect immunofluorescence for light microscopy was performed by standard procedures (3). Two methods of fixation and permeation of cells were used. Cultures on glass cover slips were either fixed in acetone at −20°C or fixed for 5 min with 1% paraformaldehyde in phosphate-buffered saline at neutral pH and then permeabilized with cold acetone. The overall preservation after paraformaldehyde plus acetone was superior (see Fig. 1 and 2). The homogeneous distribution of the pol II antigen in nuclei after paraformaldehyde treatment should be compared with the spotty localization of pol II following acetone fixation (see Fig. 1A and 2A and D). Polyclonal goat antibodies to *Drosophila* pol II (anti-pol II immunoglobulin G [IgG]) (17, 31) were kindly provided by Arno Greenleaf of Duke University. The anti-pol II IgG was identified by rabbit anti-goat IgG coupled to rhodamine (Jackson Laboratories, Avondale, Pa.).

Immunoblotting. Antigen identification by Western blotting (28) conformed to established procedures used routinely in our laboratory (32, 33). Separation of polypeptides by polyacrylamide gel electrophoresis was done in 5 to 18% polyacrylamide gradient gels.

TABLE 1. Effects of inhibitors, irradiation, and inoculation with *ts* mutants on intracellular distribution of pol II

Inoculum[a]; treatment	Intracellular location of antigen		Matching images from immune labeling (Fig. 1)
	Nuclei	Cytoplasm[b]	
Uninfected controls	+	−	A
WT; 37°C, 18 h	−	+	B
WT; streptovitacin A added at 2 h, 37°C, 18 h	+	−	C
WT; streptovitacin A added at 5 h, 37°C, 18 h	+/−	+/−	D
WT; streptovitacin A added at 12 h, 37°C, 18 h	−	+	E
ts 6389; 39.5°C, 12 h	+	−	F
ts 6389; 33°C, 12 h	−	+	G
ts 6389 with hydroxyurea[c]; 33°C, 12 h	+	−	NS[d]
ts 6389 in UV-pretreated cells[e]; 33°C, 12 h	+	−	NS
ts 9179; 39.5°C, 12 h	+	−	H
ts 9179; 33°C, 12 h	−	+	I
WT in γ-preirradiated cells[f]; 37°C, 18 h	−	+	NS

[a] For inoculation with wild-type vaccinia virus (WT), an MOI of 6 to 10 PFU per cell was used, and with *ts* mutants an MOI of 50 PFU per cell was used. Infection was allowed to proceed for either 12 or 18 h, as indicated.

[b] In all of the cell types examined, a low level of specific fluorescence was observed in the cytoplasm (Fig. 1A).

[c] Hydroxyurea was present at 5×10^{-3} M.

[d] NS, Data not shown.

[e] Cells were irradiated for 40 s by a UV lamp delivering 1,900 ergs/cm^2 per s.

[f] Cells were irradiated with 50,000 rads as described by Silver et al. (24).

RESULTS

Relationship between infection and translocation of pol II out of the nucleus. Migration of pol II, or the largest subunit of this enzyme, from the cell nucleus into cytoplasmic foci occurs during infection with vaccinia virus and rabbit poxvirus (13, 33). Our previous studies with polyvalent *Drosophila* anti-pol II IgG (17) showed that pol II antigen was completely depleted from nuclei of L cells within 12 h after inoculation and became concentrated within cytoplasmic foci, concordant with viral factories (33). The known involvement of pol II in the vaccinia virus cycle suggested that pol II participates in cytoplasmic transcription of some viral function. In the current studies, the translation inhibitor streptovitacin A was applied at various times postinoculation to suppress vaccinia virus-specific synthesis (5). Addition of the drug at 2 h blocked transfer of pol II antigen into the cytoplasm, but addition at 12 h had little or no effect on pol II transfer (Table 1; corresponding data in Fig. 1A to D). Application of streptovitacin A at 5 h produced an intermediate effect, whereby depletion of antigen from some nuclei was extensive and the presence of pol II in small cytoplasmic foci was clearly recognizable. It was also found, when hydroxyurea was used to block vaccinia virus DNA synthesis at 33°C (16) and a DNA$^-$ *ts* 6389 mutant was used at the restrictive temperature of 39.5°C, that at 12 h after inoculation pol II remained inside nuclei (Table 1; corresponding data in Fig. 1). Following infection for 12 h at 39.5°C with a DNA$^+$ *ts* 9179 mutant expressing both early and late functions, albeit very slowly, the pol II antigen also remained within nuclei (combined data in Table 1 and Fig. 1). By contrast, during infection at 33°C with both *ts* mutants pol II was transferred into cytoplasmic foci in the same manner as with wild-type vaccinia virus (Table 1; Fig. 1). Therefore,

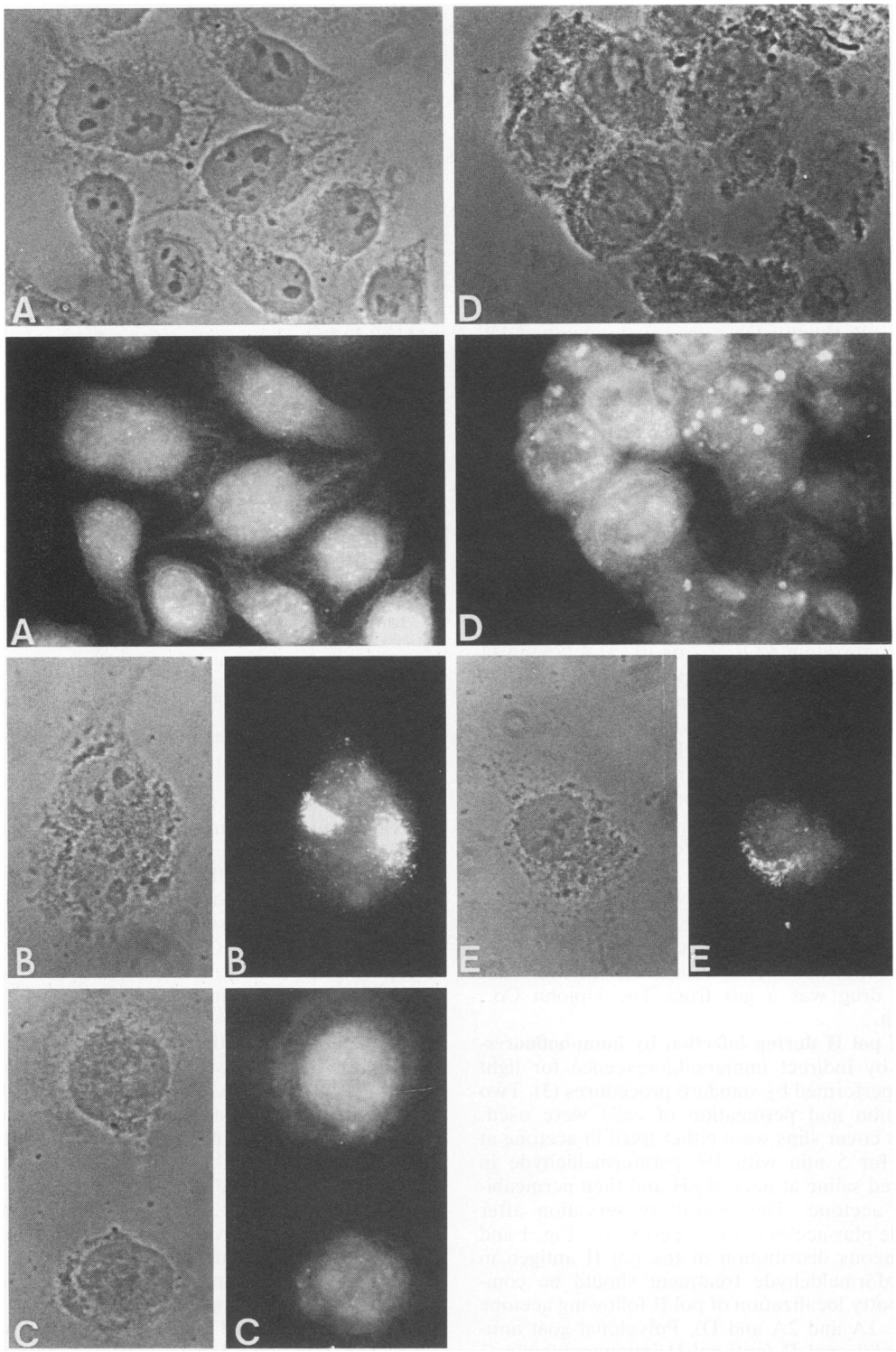

FIG. 1. Intranuclear localization by immunofluorescence of pol II antigen in HeLa cells following infection with *ts* mutants and treatment with streptovitacin A as described in Materials and Methods and Table 1. In panels A and D, the phase-contrast image is above and the companion images under UV optics are below. In all of the other examples, images under phase contrast are on the left and those under UV are on the right. For a description of the experimental material illustrated in panels A to I, see Table 1. Magnification, ×1,200.

there is an indication that transmigration of pol II is influenced by overall rates of vaccinia virus-specific synthesis and is not due per se to expression of an early viral function(s). In the above-described experiments with *ts* mutants, when pol II transfer into the cytoplasm did not occur, the antigen occasionally became concentrated inside nuclei at the periphery (13).

Previous studies demonstrated that vaccinia virus replica-

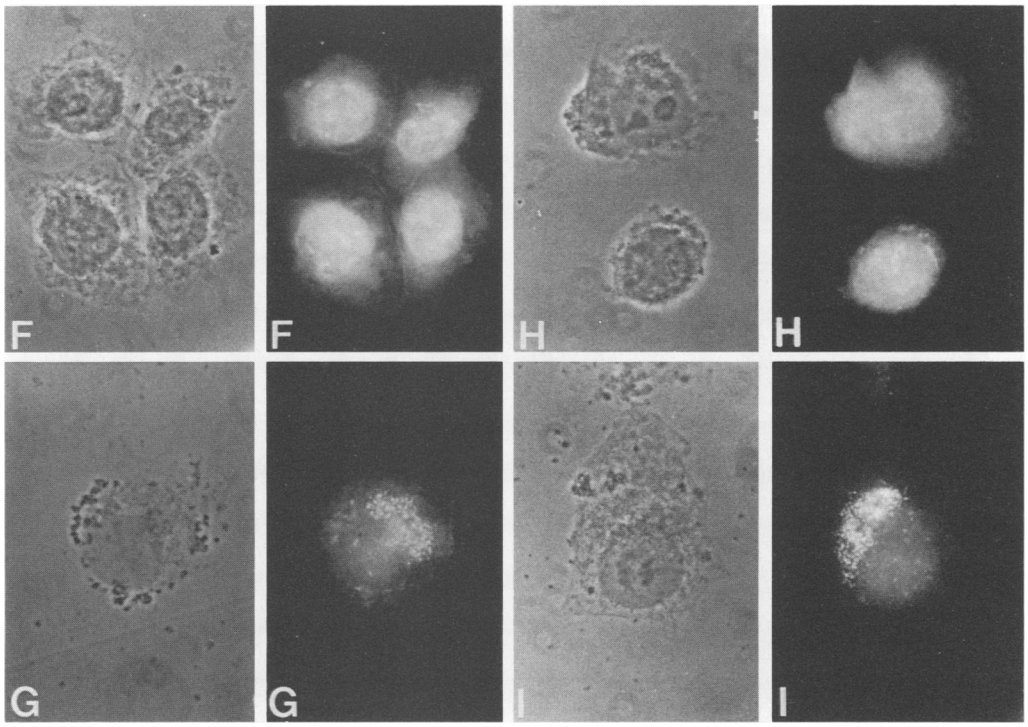

FIG. 1—*Continued.*

tion is suppressed when UV irradiation of host cells is performed before infection (6, 9). In this enquiry, we investigated the effect of UV and γ preirradiation on pol II antigen translocation. At 50,000 rads, γ irradiation does not inhibit vaccinia virus (24), and it did not prevent release of pol II into the cytoplasm (Table 1). The UV dose effective in suppressing virus production in L cells by 99% was 1,900 ergs/cm^2 per s. At this dose, UV pretreatment affected transfer of the pol II antigen out of the nucleus (Table 1). The evidence that UV irradiation can cause cross-linking between nucleic acids and proteins in nucleoprotein complexes (reviewed in the Discussion) makes it plausible that by cross-linking pol II to the host DNA UV prevented migration of the enzyme associated with normal infection.

To further test the notion that the stimulus for pol II translocation is related to rates of vaccinia virus protein synthesis, three host cells were examined for (i) the time course of virus production with respect to both the PFU and particles formed, (ii) time and MOI-related pol II transfer, and (iii) rates of translation. Subconfluent cultures during the exponential phase of growth were challenged with vaccinia virus to ensure as much as possible metabolic equivalence among the three cell lines.

Concerning virus production, the rates and maximum PFU titers produced by HeLa and L cells (4×10^7 PFU/ml) were about equal, whereas in L_6H_9 myoblasts infectious virus was initially produced at about the same rate as in the other lines (Fig. 2). However, between 8 and 12 h the rate of PFU formation reached a plateau at 7×10^5 PFU/ml. This result indicates that L_6H_9 cells supported replication of vaccinia virus at near the normal rate during the early part of the virus growth cycle, but thereafter virus production declined rapidly. Judging by the ratios of particles to PFU, there was no profound difference in the quality of vaccinia virus originating from high- and low-yield cell strains. This

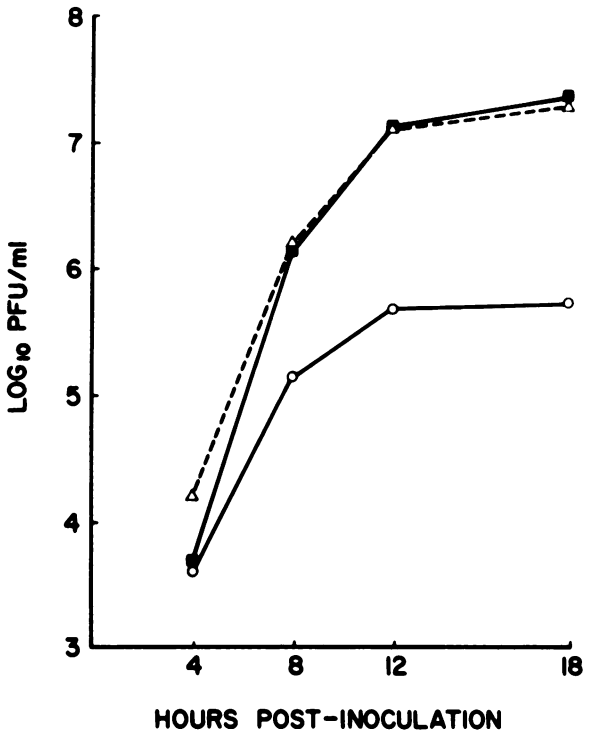

FIG. 2. Vaccinia virus replication in three host cell lines. Cultures were inoculated at an MOI of 10 PFU per cell with wild-type IHD-W and sampled at regular intervals. Symbols: ■, L cells; △, HeLa cells; ○, L_6H_9 cells.

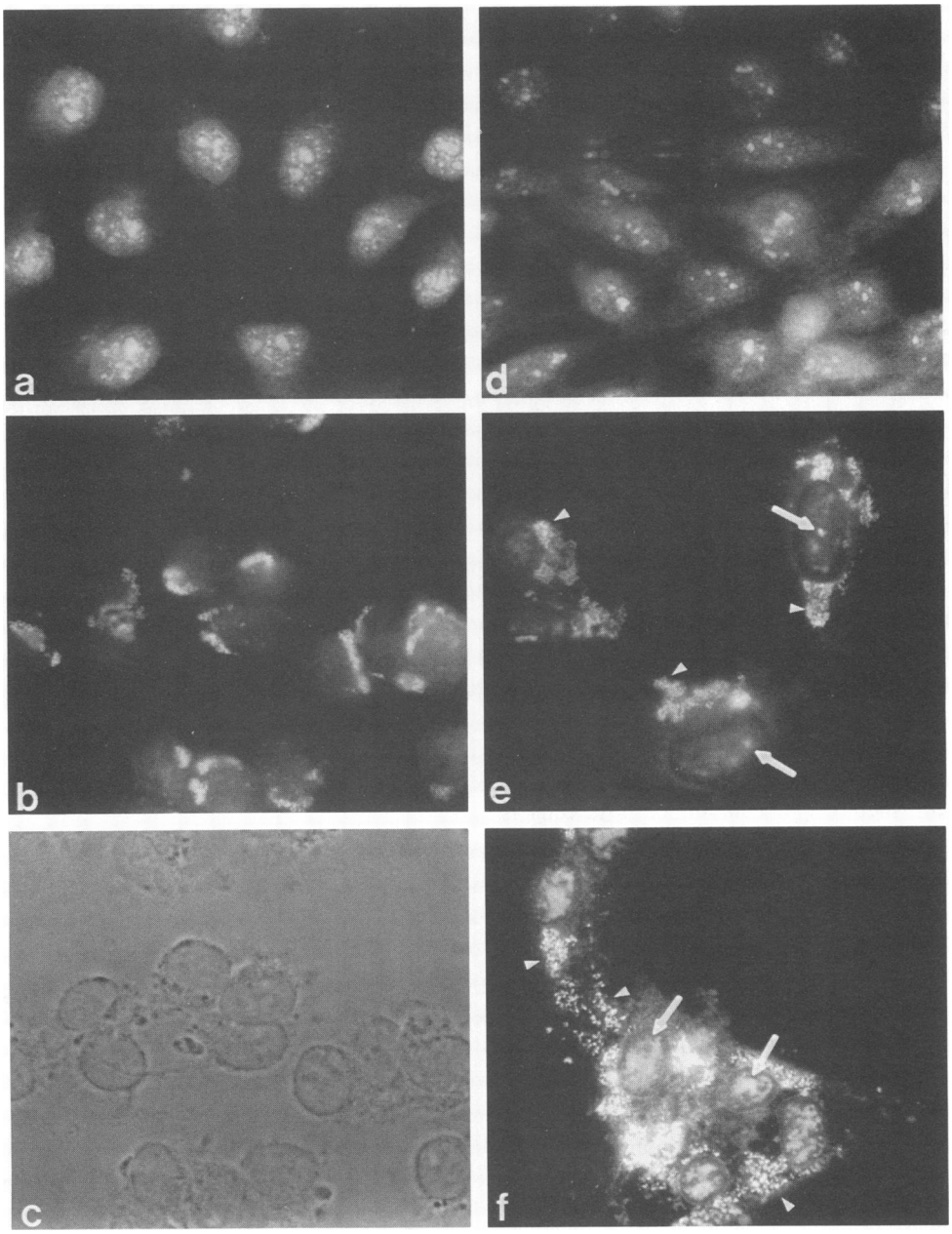

FIG. 3. Mobilization and transfer of pol II antigens from the nucleus into the cytoplasm in HeLa and L_6H_9 cells following infection with wild-type IHD-W at an MOI of 10 PFU per cell. Panels: a, uninfected HeLa cells; b, HeLa cells at 12 h postinoculation; c, phase-contrast image of panel b; d, uninfected L_6H_9 cells; e, L_6H_9 cells at 12 h postinoculation; f, L_6H_9 cells at 24 h postinoculation. In panels e and f, the arrows indicate antigens within nuclei and the arrowheads indicate cytoplasmic spots of fluorescence. Magnification, ×1,200.

ratio was established by relating PFU to the number of mature virions, enumerated as previously described (5), in thin sections of L_6H_9 and L cells or by comparing yields of purified virions from different host cells. Thus, 18 h after inoculation per 100 sectioned cell profiles L cells contained 2,400 mature vaccinia virus particles compared with 325 particles identified in L_6H_9 cells. It was also found that about 3×10^8 L cells yielded about 20 optical density units of purified vaccinia virus, but from the same number of L_6H_9 myoblasts only 3.5 optical density units were derived. These data show that L_6H_9 cells generated about 1/8 of the particles and 1/50 of the PFU made in L cells.

Regarding the effects of cell type, MOI, and duration of infection on pol II transposition, it was found that infection of HeLa cells with an MOI of 10 resulted, within 12 h, in transfer of pol II into the cytoplasm (Fig. 3A and B). In a parallel infection of L_6H_9 cells, both nuclear and cytoplasmic pol II antigens were evident (Fig. 3E). Even at 24 h postinoculation of these myoblasts, after extensive cell-cell fusion had occurred, the intranuclear antigen remained (Fig. 3F). It should be noted that in Fig. 3E and F pol II was distributed among clusters of minute cytoplasmic spots, implying that the antigen was present within individual virions.

Detection of pol II antigen in uninfected cells and vaccinia virions by immunoblotting. Evidence that differing rates of

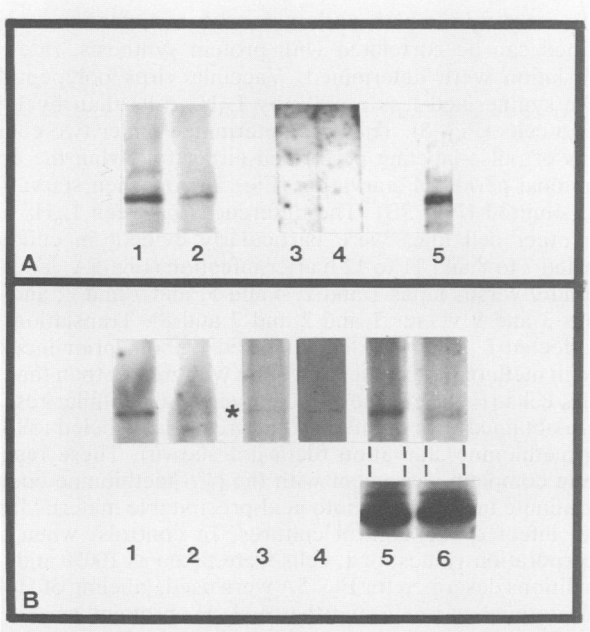

FIG. 4. Identification by immunoblotting of pol II antigens within nuclear or cytoplasmic fractions of uninfected cells and in purified virions. Unless otherwise specified, each lane received 60 to 70 μg of protein. Antigens were reacted with either anti-pol II immunoglobulins or nonspecific goat serum and then with rabbit anti-goat antibodies and marked by [^{125}I]protein A or peroxidase-conjugated anti-goat antibodies with 4-chloro-1-naphthol as the peroxidase substrate. (A) Uninfected cell material. Lanes: 1, L-cell nuclear fraction; 2, L-cell cytoplasmic fraction; 3, L_6H_9 cell nuclear fraction; 4, L_6H_9 cell cytoplasmic fraction; 5, concentrated (180 μg of protein) HeLa cell nuclear fraction. Lanes 1 to 5 were probed with anti-pol II IgG. (B) Purified IHD-W virion material. Lanes 1 to 5, vaccinia virus derived from L cells; 6, vaccinia virus from L_6H_9 cells; 1, reaction with immune serum; 2, reaction with control serum; 3, radiofluorogram demonstrating the presence of a [^{35}S]methionine-labeled ~200,000-M_r polypeptide (*); 4, material in lane 3 reacted with antiserum and labeled by the immunoperoxidase reaction; 5 and 6, virus from L and L_6H_9 cells, respectively, reacted with anti-pol II serum and MAb I30-1.2.

viral replication are dependent on the host strain and that replication is associated with nucleocytoplasmic migration of pol II suggested that differences in pol II exist among the cell type studied. The host enzyme was identified by means of Western blotting by using as starting material crude pol II preparations from L cells. The polyclonal *Drosophila* anti-pol II IgG used by us contained antibodies reactive to enzyme subunits, including the largest component(s) (17, 31). Two specific proteins were labeled in the nucleus, the more abundant one at about 200,000 M_r and a minor component at about 220,000 M_r (Fig. 4A, lane 1). Other bands (not shown) which were present in the blots were presumably unrelated to pol II because they also appeared in blots made with nonspecific goat antiserum. Equivalent enzyme preparations made from extracts of the cytoplasmic fraction from L cells contained reduced amounts of the antigen (Fig. 4A, lane 2), confirming the predominance of pol II within the nuclear compartment. When quantitatively comparable extracts from L_6H_9 and HeLa cells were subjected to Western blotting, pol II antigen bands were not evident (Fig. 4A, lanes 3 and 4). However, in three- to fivefold-concentrated HeLa nuclear extracts the 200,000-M_r antigen (and perhaps also the 220,000-M_r antigen) was demonstrable (Fig. 4A, lane 5). To ascertain whether a 200,000-M_r pol II antigen could be detected in the purified virus, vaccinia virus suspensions were subjected to immunoblotting as described above. Virions from L cells contained an about 200,000-M_r antigen (Fig. 4B; compare lanes 1 and 2). This antigen was unlikely to be a nonvirion contaminant which was carried over during virus purification, because it could be detected specifically in viral cores after stripping away of the envelopes (data not shown). Host derivation of the 200,000-M_r material in purified virions was implied by extensive prelabeling of L cells with [^{35}S]methionine. The presence in purified virions of a radioactive band matching the position of the 200,000-M_r band, demonstrable by immunoperoxidase labeling in Western blots on the same nitrocellulose strip (Fig. 4B, lanes 3 and 4), indicated that a host pol II antigen became an integral virion component.

TABLE 2. Conditions establishing RNA pol II enzyme activity

Condition	^{3}H-labeled nucleotide incorporation at 37°C (dpm/mg of nuclear protein)	% of maximum
Complete mixture	28,200	100
0°C	1,550	5
No enzyme	625	2
No DNA template	3,650	13
No ATP	2,850	10
With actinomycin D (4 μg/ml)	2,400	9
With α-amanitin (3 μg/ml)	8,875	31

When equal quantities of vaccinia virus polypeptides derived from L or L_6H_9 cells and separated by polyacrylamide gel electrophoresis were probed in immunoblots, differences in the amounts of the 200,000-M_r antigen were detected. Equivalence in the quantities of viral antigens in the blots was assessed in terms of optical density units or by marking an indicator 28,000-M_r viral polypeptide with monoclonal antibody (MAb) I30-1.2 (Fig. 4B, lanes 5 and 6). The content of the 200,000-M_r pol II antigen was much lower in virions derived from L_6H_9 cells than in those from L cells (Fig. 4B, lanes 5 and 6). These data not only confirmed previously shown packaging of a pol II subunit from rabbit kidney cells within rabbit pox virions (14) but also indicated that the quantity of the 200,000-M_r pol II polypeptide incorporated per virion is related to the host cell of origin.

Rates of transcription and translation in the three cell lines. Our evidence that nucleocytoplasmic transfer and virus-associated pol II antigen content are correlated with efficiency of virus replication in L, HeLa, and L_6H_9 cells suggests that differences in pol II exist among the cell types used. Activities were compared on crude enzyme preparations of extracts from nuclear and cytoplasmic fractions, as described in Materials and Methods. The standard assay was established with L-cell material. For maximum RNA synthesis, the presence of all four bases was required (Table 2). Actinomycin D inhibited transcription by over 90%. By contrast, α-amanitin, which at lower concentrations specifically inhibits pol II (8), reduced transcription by about 70%. As expected, comparison of specific activities of L-cell nuclear and cytoplasmic fractions showed that at least 90% of pol II activity occurred in the nucleus (Table 3). Assays of nuclei from the three cell lines revealed that HeLa cells contained about 2/3 and L_6H_9 myoblasts contained only 1/5 to 1/10 of the L-cell activity (Table 3). There was somewhat variable inhibition by α-amanitin among the three lines; it was the least in HeLa cells and the most profound in L_6H_9 cells (Table 3). It remains to be established whether this

TABLE 3. Comparison of RNA polymerase activities in three cell lines

Cell type[a]	RNA polymerase activity (dpm/mg of protein per ml):		% Inhibition due to α-amanitin
	Without α-amanitin	With α-amanitin	
L-2 (NF)	30,270	10,300	66
	26,600	9,650	64
	27,810	6,700	76
HeLa (NF)	18,650	8,570	54
	17,200	9,050	47
L_6H_9 (NF)	6,160	1,200	80
	3,450	135	96
L-2 (CF)	3,100	2,550	15
	585	530	10

[a] Crude pol II preparations from the nuclear fraction (NF) or the cytoplasmic fraction (CF) were used.

variability is connected with proportionally greater pol II activity in L_6H_9 nuclei.

Repeated attempts to demonstrate nucleocytoplasmic transfer of pol II activity biochemically on fractions from infected cells yielded equivocal data. Perhaps the ambiguous results obtained were due to (i) cosedimentation of viral factories containing vaccinia virus RNA polymerase activity with the nuclear fraction or (ii) other experimental artifacts.

To ascertain whether the amount of infectious virus formed can be correlated with protein synthesis, rates of translation were determined. Vaccinia virus polypeptides were synthesized less rapidly by L_6H_9 cells than by L or HeLa cells (Fig. 5). This was determined under two conditions of pulse-labeling performed either following the conventional period of starvation (Fig. 5A) or when starvation was omitted (Fig. 5B). The differences between L_6H_9 and the other cell lines were particularly evident in cultures labeled 7 to 8 and 11 to 12 h after infection (Fig. 5A, lanes 3, 6, and 9 versus lanes 1 and 2, 4 and 5, and 7 and 8; and B, lanes 3 and 9 versus 1 and 2 and 7 and 8). Translation by uninfected L and HeLa cells labeled without prior incubation in methionine-deficient medium was greater than that by L_6H_9 cells (Fig. 5B, lanes 4 and 5 versus 6). Similar results were obtained with uninfected cultures pulse-labeled following methionine starvation (data not shown). These results are in complete agreement with the [^{35}S]methionine counts per minute incorporated into acid-precipitable material from both infected and control cultures. In controls, when the incorporation values for L cells were taken as 100% and the conditions described for Fig. 5A were used, labeling of HeLa cell proteins was 68% and that of L_6H_9 proteins was 53%, while under the experimental conditions of panel B the labeling of HeLa cell proteins was 70% and that of L_6H_9 cell proteins was 26% of that of L cells. The above-described results are interpreted to show that (i) synthesis of vaccinia

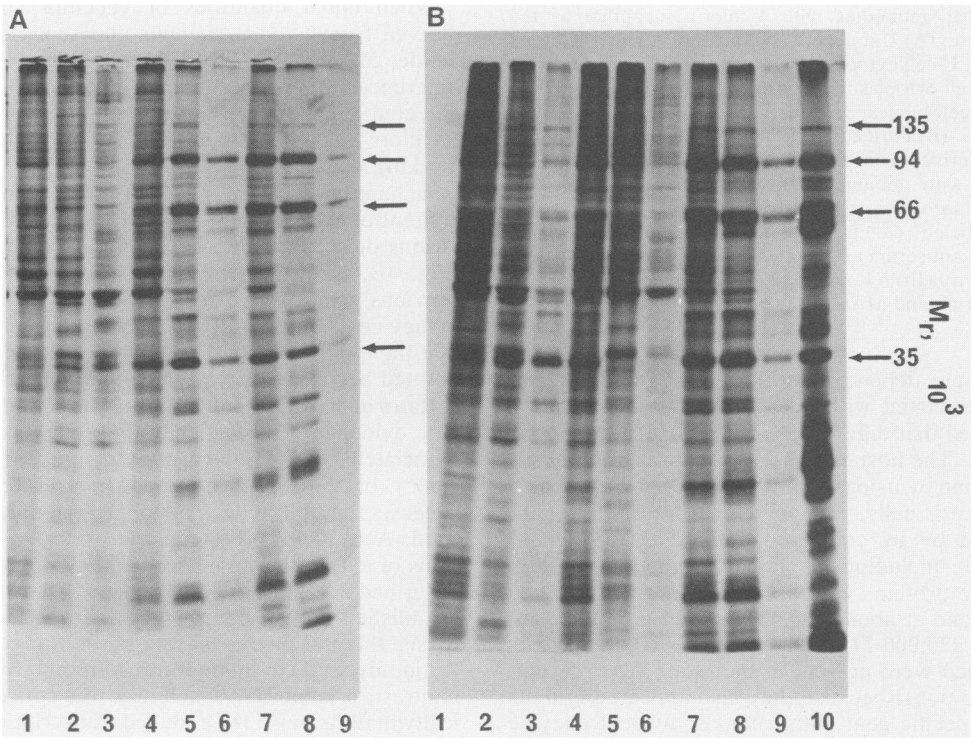

FIG. 5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of [^{35}S]methionine-labeled cellular and viral polypeptides in lysates from control and infected cells. (A) Cells inoculated with the wild-type virus at an MOI of 7 PFU per cell. Following 30 min of incubation in methionine-deficient starvation medium, the cultures were pulse-labeled as previously described (33) for 60 min at 4 to 5, 7 to 8, and 11 to 12 h after infection and harvested immediately thereafter. (B) Cultures inoculated at an MOI of 10 PFU per cell and then incubated before harvest for 60 min at 4 to 5 and 7 to 8 h postinfection in complete nutrient medium (minimal essential medium plus serum) containing [^{35}S]methionine. Each lane in panels A and B received 25 or 50 μg, respectively, of protein lysates. Lanes: 1, 4, and 7, L cells; 2, 5, and 8, HeLa cells; 3, 6, and 9, L_6H_9 cells. Lanes 1 to 3 were labeled at 4 to 5 h postinfection. Lanes 4 to 6 in panel A and 7 to 9 in panel B were labeled at 7 to 8 h postinfection. Lanes 7 to 9 in panel A were labeled at 11 to 12 h postinfection. Lanes 4 to 6 in panel B contained uninfected cells. Lane 10 of panel B contained extensively (24 h) labeled infected cells.

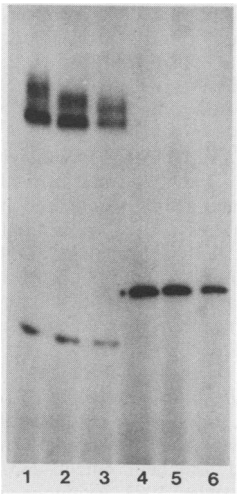

FIG. 6. Western blots comparing viral polypeptides synthesized in L, HeLa, and L_6H_9 cells during infection with IHD-J vaccinia virus. For inoculation, 3 PFU per cell was used. Cultures were harvested at 8 h postinfection. Each lane received 60 to 65 μg of lysate protein. Lanes: 1 and 4, L cells; 2 and 5, HeLa cells; 3 and 6, L_6H_9 cells; 1 to 3, reaction with MAbs H3-1.4 and I30-1.2; 4 to 6 reaction with MAb B5-3.2. Because of distortion during electrophoresis, the 28,000-M_r band in lane 1 appears at a higher-M_r position.

virus-specific polypeptides was less rapid in L_6H_9 cells than in L or HeLa cells (moreover, there was a rapid falloff in synthesis by L_6H_9 cells at 8 to 12 h postinoculation) and (ii) the translation rate in uninfected L_6H_9 cells was lower than those in the other two lines.

Differences between myoblasts and the other two lines with respect to synthesis of viral polypeptides were also evident in immunoblots with anti-vaccinia virus monoclonal antibodies (3, 32). In L and HeLa cells, about equal amounts of an early 28,000-M_r antigen and two late antigens, 32,000 and 68,000 to 85,000 M_rs, respectively, were present, and these polypeptides were less abundant in L_6H_9 cells (Fig. 6). In the case of the 68,000- to 85,000-M_r antigen, binding of MAb H3-1.4 to multiple bands revealed the presence of the 68,000-M_r polypeptide precursor and glycosylated products of the vaccinia virus hemagglutinin characterized by Shida and Dales (21). It should be noted that, despite the use of a lower inoculum of 3 PFU per cell and probing of lysates from cultures taken at 8 h after inoculation, before there is an abrupt decline in formation of infectious particles (Fig. 2), clear-cut differences were evident in the amount of vaccinia virus antigens present in L_6H_9 cells compared with the other two cell types. Therefore, the immunoblotting results combined with the pulse-labeling data indicated that synthesis of early and late viral polypeptides in L_6H_9 cells was lower than in L or HeLa cells.

DISCUSSION

The results presented here confirm and extend previous conclusions concerning the role of pol II in the replication process of poxviruses (6, 13, 14, 22, 24, 33). The correlation, now documented, among preirradiation of host cells with UV light, suppression of vaccinia virus replication, and absence of nucleocytoplasmic transfer of the pol II antigen also favors the involvement of pol II. The reason why UV stops transposition of the enzyme is uncertain but could result from UV-induced linking between pol II and the DNA template, since this type of irradiation promotes formation of cross-linked nucleic acid-protein complexes both in vitro and within living cells (27, 29, 30). By contrast, massive doses of γ irradiation, sufficient to abrogate host-specific transcription, do not inhibit vaccinia virus (22–24) or pol II migration from the nucleus (Table 1), demonstrating that transcription from host DNA is not required by the virus.

The mechanism which promotes release of pol II from the nucleus has not been elucidated. The combined results in Table 1 and Fig. 1, (i) on infection with vaccinia virus in the absence of viral DNA synthesis as affected by hydroxyurea and a DNA^- mutant, (ii) on the replication of a DNA^+ *ts* mutant which is highly inefficient at the restrictive temperature, and (iii) obtained with streptovitacin A, indicate that nucleocytoplasmic transfer of the enzyme antigen is controlled by the rates of virus-specific syntheses. More specifically, it is the efficiency of both early and late translation which may determine how much pol II is displaced into the cytoplasm. Thus, in L_6H_9 myoblasts, with lower translation rates than in L cells and slower transposition of pol II (Fig. 2D to F), less vaccinia virus was made, whether estimated in terms of particles or PFU, because the replication cycle and translation declined rapidly between 8 and 12 h postinfection. These findings are not consistent with those previously reported (13, 14), showing no arrest of pol II transfer after treatment of infected cells with inhibitors of DNA and translation.

The idea that the host may influence the efficiency of vaccinia virus replication is based on the observed differences in RNA polymerase activity among different cell types (Table 3). It was not established with our crude preparations whether the higher enzyme activity in the nuclei of L and HeLa cells than in those of myoblasts accurately reflects enzyme content. On the basis of Western blots, our inability to detect the ~200,000-M_r and ~220,000-M_r pol II antigens in L_6H_9 nuclear material when it was probed at the same concentration as that from L cells, in which these antigens were readily demonstrable (Fig. 4A), provides evidence suggestive of lower pol II content in the myoblast line. However, other explanations, such as proteolytic breakdown of the large enzyme subunits in myoblast extracts, have not been eliminated.

Occurrence of pol II antigens within minute spots in the cytoplasm and at the cell periphery (Fig. 2F) suggested that pol II is present within virions. Western blots of the purified virus also indicated an association of the host-derived pol II ~200,000-M_r antigen with vaccinia virus (Fig. 4B). This confirms the previous findings on rabbit poxvirus (13) and further implies that, after becoming concentrated in viral factories, the 200,000-M_r antigen of pol II is packaged during assembly inside cores of the progeny. Since on a per-virion basis, incorporation of the 200,000-M_r antigen was nonstoichiometric when progeny from different host cell types were compared (Fig. 4B), it is questionable whether pol II or any subcomponent is likely to have a function in transcription by the vaccinia virus RNA polymerase present in viral cores (10). We suggested previously that pol II could be involved in transcription of the vaccinia virus genome, either as the entire cell enzyme or as a hybrid cell-virus polymerase (24). We later hypothesized from additional data that transcription from the vaccinia virus genome involving pol II specifies functions which may regulate intracellular translation (33). Combined evidence from the present study suggests that mobilization of pol II into cytoplasmic foci is interconnected with rates at which vaccinia virus replication progresses. Influence of the host cell over vaccinia virus replication may

be related to the content or availability of pol II acting through some type of feedback process.

ACKNOWLEDGMENT

This research was supported by the Medical Research Council of Canada.

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