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Regulation of Herpesvirus Macromolecular Synthesis: Temporal Order of Transcription of α Genes Is Not Dependent on the Stringency of Inhibition of Protein Synthesis

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Operationally, α genes of herpes simplex virus 1 were defined on the basis of the observations that they are the earliest genes expressed in the infected cell and that the transcription, processing, and accumulation of the mRNA's in the infected cell cytoplasm can take place in the presence of inhibitors of protein synthesis, such as cycloheximide. In these studies, we translated in vitro the viral mRNA's extracted from cells infected and maintained in the presence of cycloheximide, emetine, or anisomycin. Inasmuch as all the major α proteins (no. 0, 4, 22, and 27) were translated, we conclude that the transcription of all previously defined α genes is independent of the stringency of inhibition of protein synthesis and that pre-α genes cannot be detected in such experiments.

Previous studies have shown that herpes simplex virus 1 (HSV-1) genes form at least three groups, whose expression is coordinately regulated and sequentially ordered in a cascade fashion (7, 8). The three groups identified so far and designated as α, β, and γ, are operationally defined on the basis of the temporal order and requirements for their expression. Thus, α genes are the earliest genes expressed after infection, and mRNA's specifying α gene products are made and translocated into the cytoplasm in the presence of inhibitors of protein synthesis, such as cycloheximide (5). The β genes require functional α proteins for their expression; however, their expression is independent of viral DNA synthesis (9). The γ gene products reach peak levels of synthesis only late in infection, and the increase in their rate of synthesis late in infection is dependent on viral DNA synthesis.

This report concerns the requirements for the expression of α genes. Relevant to the experimental results we report are the following observations.

(i) The four major α genes coding for the infected-cell polypeptides (ICP) 0, 4, 22, and 27 have been extensively mapped (1, 3, 9, 14, 18). The genes specifying ICPs 22 and 27 are transcribed off one strand, whereas those specifying ICPs 0 and 4 are transcribed off the complementary strand (3, 14, 18). Each α gene is transcribed from a separate promoter (14). Moreover, the sequences encoding the 5′ termini of the mRNA's are not homologous to an extent detectable by hybridization. HSV DNA is known to be transcribed by host RNA polymerase II (4). These observations raise the question as to how coordinate expression of α genes is effected.

(ii) A previous report from this laboratory has shown that a chimeric thymidine kinase (tk) gene, constructed by fusion of α ICP 4 promoter to the structural sequences of the thymidine kinase gene (a β gene), is transcribed from the α promoter and functions as an α gene (16). However, these studies also showed that α genes are positively regulated. Both the standard (β) thymidine kinase gene and the chimeric (α) thymidine kinase gene can convert Ltk^- cells to tk^+ phenotype. Whereas the induction of the β thymidine kinase in cells superinfected with tk^- virus was dependent on the function of α ICP 4 and was inhibited by cycloheximide, the induction of α thymidine kinase was independent of α ICP 4 gene, and its transcription was induced in the presence of cycloheximide. These results suggested that α gene expression is positively regulated by either a virion-associated factor or by a pre-α gene whose product is translated, in trace amounts, in the presence of cycloheximide.

(iii) Studies on the early transcription of the adenovirus genome indicated that the apparent temporal order of transcription of these genes varies with the stringency of inhibition of protein synthesis. Thus it has been reported that only a portion of one of the four major early transcriptional units is transcribed in the presence of emetine (12), and elegant studies with host-

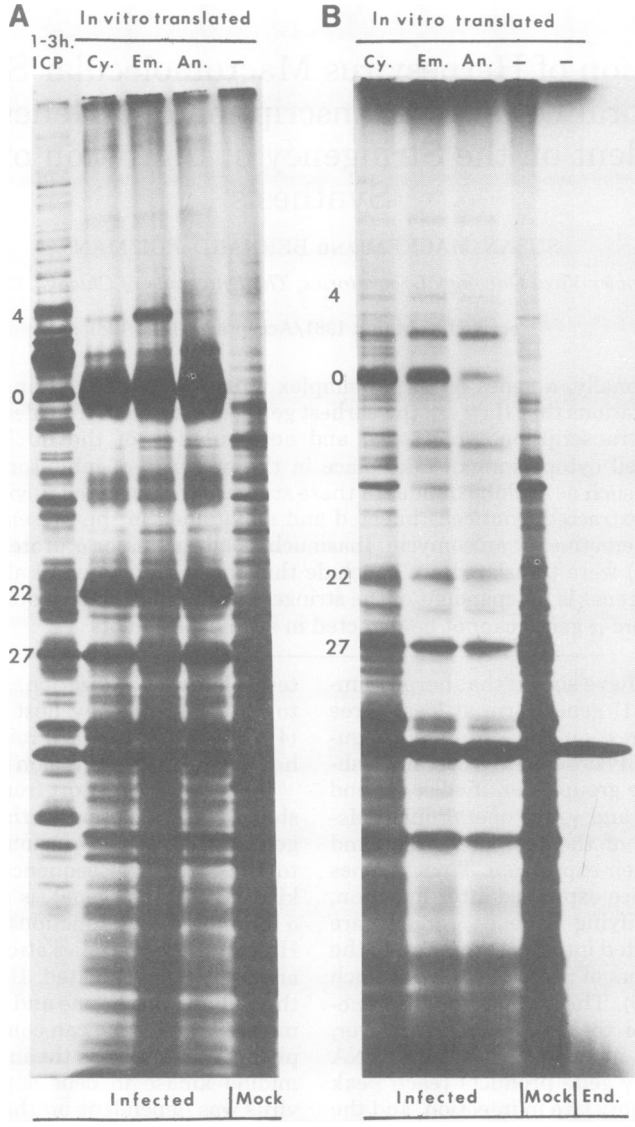

FIG. 1. *Autoradiographic images of electrophoretically separated polypeptides translated in vitro from RNA extracted from the cytoplasm of infected Vero (A) or HEp-2 (B) cell cultures. Vero or HEp-2 cells were preincubated with (i) 50 μg of cycloheximide (Cy.) per ml for 30 min, (ii) 50 μg of emetine (Em.) per ml for 10 min, or (iii) 100 μM anisomycin (An.) for 30 min and were then infected with 50 PFU of HSV-1 (F) per cell and incubated in the presence of the drug. At 5 h postinfection, total cytoplasmic RNA was extracted, and 2 μg was used to program a reticulocyte translation system (New England Nuclear Corp., Boston, Mass.) supplemented with [^{35}S]methionine, all as previously described (14). The products of translation were separated on a 9.25% sodium dodecyl sulfate-polyacrylamide gel, along with the products of translation of total cytoplasmic RNA extracted from uninfected cells (Mock) and the products made in the absence of any added RNA (End.). The procedures for electrophoresis were published elsewhere (15). α ICPs are indicated to the left of the figure. Polypeptides extracted from mock-treated HEp-2 cells labeled with [^{35}S]methionine from 1 to 3 h postinfection with 20 PFU of virus per cell are also shown for comparison. The procedures for maintaining and infecting Vero and HEp-2 cells and the properties of the HSV-1 (F) strain were described elsewhere (5, 7–9, 11, 16). In both this and preceding studies (14), the ICP 4 produced in vitro comigrated with the most rapidly migrating form of ICP 4 produced in the infected cells. This form was previously designated as ICP 4a (15). During studies with purified mRNA (14), it was observed that the efficiency of translation of ICP 4 mRNA is lower than that of other α mRNA's, possibly because of size, secondary structure, or other factors.*

range mutants have demonstrated that the function of this "pre-early" region is required for the subsequent expression of the remaining early genes (2, 10). Further studies with an even more stringent inhibitor (anisomycin) revealed that even transcription of the pre-early genes requires low levels of de novo protein synthesis (13). In the presence of this drug, the infected cell accumulates transcripts initiated from the major late promoter and containing promoter proximal regions of the major late transcriptional unit. That these promoter-proximal late transcripts form an immediate early class is also indicated by in vitro transcription (19) and in vivo pulse-labeling studies (17). Thus, studies using inhibitors of protein synthesis have been instrumental in dissecting the order of adenovirus early transcription, and the results of these experiments have been borne out by other lines of evidence, using both genetic and biochemical tools.

The evidence that α genes are coordinately expressed even though they are transcribed independently, the apparent positive regulation of chimeric genes transcribed off α gene promoters, and the studies on the adenovirus early gene transcription led us to investigate the effect of the stringency of inhibition of protein synthesis on the temporal order of transcription of the HSV-1 genome.

In these experiments Vero and HEp-2 cells were treated with cycloheximide, emetine, or anisomycin as described in Table 1, footnote *a*. The treated cells were infected and maintained in the presence of the drugs, and cytoplasmic RNA was extracted at 5 h postinfection and translated in vitro as described in the legend to Fig. 1. Because HSV contains a virion host shutoff factor (6), α polypeptides are readily discerned among the translation products of total cytoplasmic RNA. This method for assessing the presence of α gene transcripts was preferred inasmuch as it assays for functional message, whereas hybridization could also detect abortive transcription. RNAs extracted from the cytoplasm of either HSV-infected Vero or HEp-2 cells treated with the various drugs translated ICPs 0, 4, 22, and 27 (Fig. 1). The extent of inhibition of protein synthesis achieved in each instance is summarized in Table 1.

The results of these studies show that all major α genes are transcribed even under the most stringent inhibition of protein synthesis attained with anisomycin (>99% reduction) and that none requires for its transcription de novo protein synthesis greater than that which escapes inhibition in anisomycin-treated cells. The failure to detect pre-α genes could indicate either that they do not exist or that any pre-α genes responsible for regulating α transcription are functional at the RNA level, without being translated into protein.

TABLE 1. *Effect of three protein synthesis inhibitors on incorporation of labeled amino acids into Vero and HEp-2 cell protein*

Drug	Concn used	Length of preincubation[a] (min)	% Residual protein synthesis[b]	
			HEp-2	Vero
Cycloheximide	50 μg/ml	30	5.5	5.5
Emetine	50 μg/ml	10	1.1	1.8
Anisomycin	100 μM	30	0.6	1.3

[a] Time of exposure of cells to the drug before labeling with ^{14}C-amino acids to measure levels of protein synthesis and before adsorption of virus for the experiment described in the legend to Fig. 1.

[b] HEp-2 or Vero cells were preincubated in medium containing the appropriate drug for the time shown. The medium was then replaced with medium containing the drug and one tenth the normal amount of isoleucine, leucine, and valine, but supplemented with equal amounts of [^{14}C]isoleucine, leucine, and valine (5 μCi total per ml) labeled to the same specific activity. After 2 h the cells were harvested as described (15), and solubilized samples were boiled for 5 min in 5% trichloroacetic acid, chilled, and precipitated onto nitrocellulose filters, and the acid-insoluble radioactivity was measured in a scintillation counter. The residual incorporation of labeled amino acids in drug-treated cells is expressed as percent incorporation of labeled amino acids in mock-treated controls.

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