Repression of Adenovirus Early Gene Expression by Coinfection with a Temperature-Sensitive Mutant in the Immediate-Early Gene of Pseudorabies Virus

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Wild-type adenovirus was coinfecting with a mutant temperature sensitive for the immediate-early gene of pseudorabies virus. At the nonpermissive temperature, this mutant, tsG, strongly inhibited the transcription of all adenovirus early genes, including E1A. This inhibition was not observed with wild-type pseudorabies virus coinfection or with tsG coinfection at the permissive temperature. The level of repression was dependent upon the ratio of tsG to adenovirus in the infection. The results suggest that the temperature-sensitive protein may be interacting with transcription factors on the viral DNA or with the DNA itself to inhibit adenovirus transcription.

The pseudorabies virus immediate-early protein (IEP) and the adenovirus E1A protein are regulatory proteins which induce the transcription of their respective early genes (3, 14, 19, 23, 28). These proteins are also capable of activating the transcription of other genes in a transient assay. For example, we have shown that the IEP of pseudorabies virus can activate the transcription of adenovirus early genes; in fact, the IEP was a stronger activator of adenovirus genes than the homologous E1A protein (7). Plasmids encoding the IEP and E1A protein can activate the early gene E2 of adenovirus in mixed transfection assays (12). Green et al. (9) showed in similar cotransfection assays that the plasmid encoding the immediate-early gene could activate a nonviral gene, the human β -globin gene. Deletion analysis of upstream regions of E1A- and IEP-inducible genes indicates that there are no unique DNA sequences required only for induction (6, 13, 16-18). Gaynor et al. (8) demonstrated that a cellular gene, the rat preproinsulin gene, was inducible by E1A when placed in the viral chromosome. The E1A protein has been shown to activate the transcription of at least two endogenous genes, those encoding the heat shock and β -tubulin genes, but in general endogenous genes are not activated (15, 26). These studies suggest, but do not prove, that IEP and E1A do not bind to specific sites on the DNA to activate transcription.

The exact mechanism by which IEP stimulates transcription remains an interesting and open question. Some possible mechanisms are that IEP could (i) modify RNA polymerase II, (ii) increase the synthesis of transcription factors, (iii) modify a transcription factor to increase its activity, (iv) bind to a factor on the DNA, (v) bind to a specific site on the DNA. Each of these models makes a set of predictions which can be useful in designing experiments. One way to discriminate between these models experimentally is to use a temperature-sensitive mutant in the IEP gene. Our approach was to coinfect cells at the nonpermissive temperature with wild-type adenovirus and a temperature-sensitive mutant in the immediate-early gene of either pseudorabies virus or herpes simplex virus type 1 (HSV-1). We imagined

that one or more of these mutants, depending on the property of the mutant protein, would produce an observable effect on adenovirus gene expression. This effect might allow us to distinguish between at least some of the transcriptional activation models.

A temperature-sensitive mutant in the immediate-early gene of pseudorabies virus has been isolated and defined (2). This mutant, tsG, makes only immediate-early RNA at the nonpermissive temperature; at the permissive temperature this mutant is wild type for transcriptional activation of both its own genes and of adenovirus early genes in a mixed infection (7). This mutant is well characterized and can be rescued by a restriction fragment encoding part of the immediate-early gene (2).

Our results demonstrate that tsG severely inhibits adenovirus transcription, including the transcription of the E1A gene itself. The experiments we describe allow us to make two conclusions. (i) At least two models of transcriptional activation by IEP are inconsistent with our data. These models are that IEP modifies RNA polymerase II or that it acts solely to increase the production of transcription factors. (ii) The temperature-sensitive IEP is interacting with a component of adenovirus transcription. This fact will permit the use of both tsG and its IEP to explore more fully the molecular basis of transcriptional activation.

MATERIALS AND METHODS

Cells and virus. HeLa cells were maintained in suspension culture in Joklik modified minimal essential medium containing 6% newborn calf serum. Adenovirus type 5 was grown on HeLa cells in spinner culture. Pseudorabies virus was grown and plaqued on rabbit kidney cells maintained in minimal essential medium plus 5% newborn serum. tsG was a kind gift of T. Ben-Porat and A. S. Kaplan, and ts901 was kindly supplied by M. Levine. The genetic properties of tsG and ts901 have been described previously (2, 5, 25). Procedures for the infection of cells, labeling of RNA with [³H]uridine, and preparation of labeled RNA have been described previously (12).

Hybridization procedures. Plasmid DNAs were cleaved and denatured by heating to 100°C in 0.1 M NaOH for 5 min,

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FIG. 1. Western blot of the adenovirus 72K protein. HeLa cells were infected with adenovirus at 2 PFU per cell at 40°C (lanes 1 and 5) or coinfecting with pseudorabies virus or tsG. Cellular protein was isolated at 5 h postinfection, and samples were prepared for electrophoresis. Lanes: 2, 3, and 4, pseudorabies virus coinfecting at a multiplicity of infection of 0.1, 2, and 40 PFU per cell, respectively; 6, 7, and 8, tsG coinfecting at a multiplicity of infection of 0.1, 2, and 40 PFU per cell, respectively.

diluted with 20 volumes of 2 M NaCl, and loaded onto nitrocellulose filters. Procedures for DNA-RNA hybridizations have been described previously (12).

Western blot assay for the 72K protein. After electrophoresis on 10% Laemmli acrylamide gels, the gels were transferred to nitrocellulose in transfer buffer (Tris base, 3 g/liter; glycine, 14.4 g/liter; 25% ethanol) at 60 V for 3 h. The nitrocellulose was then blocked in nonfat dry milk (8.8 g/100 ml) in TBS (40 ml of 1 M Tris [pH 7.4]; 58.44 g of NaCl per 2 liters) for 0.5 h. The rabbit 72,000-molecular-weight antibody (72K protein) (a kind gift of J. R. Nevins) was then incubated with the blot at a concentration of 10 μ l in 20 ml of TBS. This incubation went overnight on a shaker at room temperature. The blot was then washed three times in TBS, and 125 I-labeled staphylococcus protein A (New England Nuclear Corp., Boston, Mass.) was added at a 1:2,000 dilution in TBS for 3 h. After several washes in TBS, the blot was air dried and exposed to X-ray film.

Labeling of cell proteins. Infected and mock-infected spinner HeLa cells were labeled in 5% dialyzed serum in spinner cell media with [35 S]methionine at 10 μ Ci/ml for 1 h. The cells were washed two times in cold phosphate-buffered saline and sonicated in sample buffer (50 mM Tris [pH 6.8], 3% sodium dodecyl sulfate, 15% glycerol), boiled for 5 min, and loaded onto an acrylamide gel.

RESULTS

We favored an hypothesis in which the IEP protein acts by binding to a transcription factor on the viral DNA. On the basis of this model, we predicted that one or more of the temperature-sensitive mutants in the IEP gene would interfere with adenovirus gene expression in mixed infections at the nonpermissive temperature. This interference would be produced by a binding of the mutant protein to a target which the adenovirus also needs for its transcription. While such an interference could be consistent with several of the models we have listed, it would be inconsistent with others.

HeLa cells were coinfecting at the nonpermissive temperature with a constant amount of adenovirus type 5 and an increasing amount of tsG. Interference of adenovirus gene expression was scored by a Western blot assay for the 72K DNA-binding protein, an E1A-inducible protein of early region E2. Figure 1 shows the level of production of the 72K protein in various coinfections with adenovirus and either wild-type pseudorabies virus or tsG, all at 40°C, the nonpermissive temperature of tsG. Lane 1 shows the amount of 72K protein produced from a wild-type adenovirus infection at 2 PFU per cell. In lanes 2 through 4 are shown the levels of 72K protein produced by coinfection with increasing amounts of wild-type pseudorabies virus. This result demonstrates that adenovirus gene expression can occur

normally at 40°C and that an early gene is inducible by the wild-type IEP at that temperature. A longer exposure of the tsG coinfection shows the amount of 72K protein produced by adenovirus infection alone (lane 5) and increasing multiplicities of tsG, from 0.1 to 40 PFU per cell (lanes 6 through 8). Whereas wild-type pseudorabies virus coinfection results in an overproduction of the 72K protein, as described previously (7), coinfection of adenovirus with tsG, the mutant in the IEP, results in a dramatic decrease in 72K protein production. We have shown previously that coinfection of tsG and dl312, an E1A deletion mutant of adenovirus, at the permissive temperature results in a stimulation of adenovirus early gene expression at the level of transcription (7). We repeated the coinfection at the permissive temperature with wild-type adenovirus in place of dl312 and again observed a stimulation similar to that of the wild-type pseudorabies virus coinfection (Fig. 1, lanes 2 through 4). Therefore, it is only the tsG coinfection at the nonpermissive temperature which produces the strong repression of E2 gene expression. The results demonstrate that the tsG IEP is not inert at the nonpermissive temperature; in fact, it is able to prevent the activation of the adenovirus E1 gene expression.

We anticipated having to search among several temperature-sensitive mutants for an observable effect on adenovirus gene expression. Having found a strong effect with tsG, we were interested in whether this effect was peculiar to tsG or whether it was a property shared by mutants in the immediate-early (ICP4) gene of HSV-1. Coinfection experiments were carried out as described in the legend to Fig. 1 with ts901, a temperature-sensitive mutant in the ICP4 gene of HSV-1 (5, 25). A similar level of repression was observed with ts901 as with tsG, indicating that at least one other herpesvirus mutant in this locus was capable of repressing adenovirus gene expression (data not shown). However, unlike tsG, ts901 has four other immediate-early genes besides ICP4, and these will be expressed at the nonpermissive temperature. We therefore continued the analysis with tsG only.

There are several possible reasons why the 72K protein is not expressed in coinfections with tsG. One very simple explanation is that the adenovirus E1A protein, which is required for inducing the transcription of the adenovirus early genes, is itself not synthesized in the tsG coinfections. In fact, the level of E1A protein was reduced by coinfection with tsG, and the reduction of E1A protein was dependent upon the multiplicity of tsG in the coinfection. (The decrease in the level of the E1A protein in a Western blot was similar to that of the 72K protein and is therefore not shown, but no E1A RNA was produced in this coinfection [see Table 2].) These observations caused us to consider the possibility that the tsG coinfection might be exerting a general inhibition of cellular function or that the tsG virus might contain unknown inhibitors. The following additional experiments eliminated these possibilities.

Although tsG, and the analogous mutants in herpes simplex virus, are generally thought to be inert with respect to host-cell functions, we examined the level of host-cell protein and host-cell RNA synthesis in the mutant-infected cells. Host-cell protein synthesis declined only slightly after tsG coinfection (Table 1), even at the highest multiplicities of 40 PFU per cell. We also examined host-cell RNA synthesis as a function of tsG infection. There was less than a twofold reduction in total RNA synthesis as a result of the highest multiplicities of tsG infection, which was much higher than needed to see a severe effect on 72K protein production.

TABLE 1. Incorporation of [³H]uridine and leucine as a function of tsG concentration^a

tsG (PFU/ml)	[³ H]uridine	[³ H]leucine
MI	1.00	1.00
1	1.10	0.96
5	0.87	0.75
20	0.73	0.76
40	0.69	0.65

^a HeLa cells were infected with tsG for 4 h at 40°C and labeled in 5% dialyzed serum plus 10 μ ci of either [³H]uridine or [³H]leucine per ml. The uridine cultures were harvested after 0.5 h, and the leucine cultures were harvested after 1 h. Triplicate aliquots were taken for trichloroacetic acid precipitation. The radioactivity is expressed as a percentage of the mock-infected (MI) value.

Similar data were obtained with poly(A)⁺ selected RNA. We interpret these as nonspecific effects since 40 PFU per cell is equivalent to 40,000 particles per cell; an infection of this magnitude is likely to cause the cell to function at less than 100% efficiency.

To show that high concentrations of tsG are not poisoning the cell in some unknown way, the adenovirus concentration was increased in a series of coinfections while the tsG concentration was kept constant. If tsG was poisoning the cell, the 72K protein would be inhibited regardless of the adenovirus concentration. On the other hand, if tsG was acting as a competitive inhibitor, we would expect the 72K protein to be produced by increasing the multiplicity of adenovirus. The 72K protein was at first repressed by coinfection of adenovirus at 10 PFU per cell and of tsG at 40 PFU per cell (Fig. 2, lane 2), but as the adenovirus multiplicity was increased to 800 PFU per cell with tsG still at 40 PFU per cell (lane 4), the 72K protein was again produced and at slightly higher levels than at 400 PFU of adenovirus alone per cell (lane 5). This indicates that tsG is not poisoning the cell; it appears that a certain ratio of temperature-sensitive IEP to adenovirus DNA template is important in the repression phenomenon.

Because there was little effect on host-cell RNA synthesis, we were able to examine the adenovirus-specific transcription in coinfections. Table 2 shows the data for two experi-

FIG. 2. Western blot of the adenovirus 72K protein. HeLa cells were infected with tsG at 40 PFU per cell and with different concentrations of adenovirus as described in the legend to Fig. 1. Lanes: 1, adenovirus at 5 PFU per cell; 2, tsG at 40 PFU per cell and adenovirus at 5 PFU per cell; 3, mock-infected cells; 4, tsG at 40 PFU per cell and adenovirus at 800 PFU per cell; 5, adenovirus at 400 PFU per cell.

TABLE 2. Pulse-labeling of infected cultures and hybridization to adenovirus DNA filters^a

Expt	Infection	cpm in RNA		
		E1A	E2	E4
1	Adenovirus	721	8,031	3,431
	Plus tsG	33	60	182
2	Adenovirus	2,777	22,376	13,395
	Plus tsG	118	189	178
	Plus WT virus	2,571	26,918	6,093

^a Cells were infected with 5 PFU of adenovirus per cell and 40 PFU of either tsG or wild-type pseudorabies virus (WT) per cell at 40°C for 4 h. The cultures were labeled for 20 min (experiment 1) or 30 min (experiment 2) and the total RNA was extracted and hybridized to filters bearing the cloned adenovirus genes.

ments in which the cells were coinfecting for 4 h, and the RNA was labeled with tritiated uridine for 20- or 30-min pulses. The counts presented are adenovirus-specific counts, obtained by hybridizing the labeled RNA to plasmid DNAs fixed to filters. Incorporation of [³H]uridine into adenovirus-specific RNA was drastically reduced by coinfection with tsG at the nonpermissive temperature. Therefore, the effect of tsG on adenovirus gene expression is at the level of transcription.

We considered one other possibility: that there might be a competition between the DNA templates of adenovirus and tsG for transcription factors. This seemed unlikely because no such inhibition was seen by the wild-type pseudorabies virus template on E1A transcription (Table 2). Still, it remained possible that the wild-type IEP could in that case cause an increase in the concentration or activity of the transcription factors. Therefore, to determine if the tsG template was inhibiting E1A transcription, the pulse experiments were repeated in the presence of cycloheximide to prevent the synthesis of the temperature-sensitive IEP. We expected transcription of all early genes in the presence of cycloheximide as reported earlier (20); the question was whether that transcription decreased in the coinfecting cells due to the added tsG template. Surprisingly, we found that the transcription actually increased slightly in both experiments when cells were coinfecting at the nonpermissive temperature (Table 3). The increase in adenovirus transcription could be due to the effect of the tsG virion protein, which is known to stimulate other genes at about this level (1, 22, 24), or perhaps the tsG template binds an inhibitor of adenovirus transcription. In either case, it is clear that the tsG template does not prevent E1A transcription through a competition for transcription factors by its DNA template.

TABLE 3. Pulse-labeling of cells infected in the presence of cycloheximide^a

Expt	Infection	cpm in RNA		
		E1A	E2	E4
1	Adenovirus	430	834	2,070
	Plus tsG	1,287	3,959	5,789
2	Adenovirus	1,046	1,679	4,464
	Plus tsG	2,848	8,389	12,827

^a Cells were infected in the presence of cycloheximide at 50 μ g/ml from the time of adsorption of the virus. Otherwise conditions were as for experiment 2 of Table 2. In this table the number of cells used for the second experiment was twice that used in the first experiment.

Finally, we examined the behavior of the tsG virus and of some temperature-sensitive mutants in the ICP4 gene of HSV-1 in mixed infections. We observed that tsG repressed gene expression from HSV-1 genes and that ts901 repressed gene expression from pseudorabies virus genes (C. Coulter, S. E. Ahlers, and L. T. Feldman, manuscript in preparation). These data also suggest that the tsG IEP is entering into a specific but nonproductive interaction with a component of transcription.

DISCUSSION

tsG, a temperature-sensitive mutant in the immediate-early gene of pseudorabies virus, represses the transcription of both the adenovirus E1A gene and the adenovirus early genes. The repression appears to be adenovirus specific in that host-cell RNA and protein synthesis are not appreciably affected. The present data do not allow us to define the molecular basis for this repression, but they do allow us to argue against certain explanations. First, it is unlikely that the repression of E1A gene transcription is caused by a competition between the adenovirus and tsG templates for limiting transcription factors. Wild-type pseudorabies virus coinfection with adenovirus resulted in wild-type levels of both E1A RNA and protein. In addition, coinfection with tsG in the presence of cycloheximide, in which the DNA would be exposed but the mutant protein would not be expressed, did not result in a repression of E1A gene transcription. Second, because the wild-type protein is not an inhibitor of E1A gene transcription, the repression is probably not similar to the E1A repression of the simian virus 40 enhancer sequences, which has been reported recently (4, 27). In addition, our previous studies showed that the wild-type IEP had no effect on the Rous sarcoma virus enhancer in transient assays (12).

The following lines of evidence suggest that tsG IEP is forming a specific but nonproductive interaction with a component of transcription. Like the wild-type IEP, the tsG IEP has an effect only on viral genes and not on endogenous cellular genes; the effect is at the level of transcription; another mutant in the HSV-1 gene, ts901, exhibits the repression phenomenon; these mutants have a similar effect on herpesvirus genes. Any explanation of the tsG repression must take into account that E1A transcription is controlled by enhancer sequences, which make it constitutively expressed (10, 11, 21, 29). Assuming that the temperature-sensitive mutant protein is blocking a step which the wild-type protein normally performs, these data allow us to argue against certain mechanisms of transcriptional activation.

(i) The IEP binds to or modifies RNA polymerase II. Our data show that tsG has a 50- to 100-fold effect on adenovirus transcription and at most a 2-fold effect on host-cell transcription. Therefore, this explanation could be true only if a certain subset of polymerases is used to transcribe newly introduced DNA.

(ii) The IEP normally functions to increase the synthesis of transcription factors needed for newly introduced DNAs such as viral DNAs or transfected DNAs. If this is the mechanism of action of IEP, it is difficult to imagine how the tsG protein is repressing. One could imagine the tsG protein not inducing the synthesis of new factors, but in that event there would be no effect on E1A gene transcription. We cannot, however, rule out a mechanism by which IEP increases the intracellular pool of a transcription factor as well as binding to the same or another factor.

Our results can best be explained by an interaction of the

temperature-sensitive IEP with transcription factors on the adenovirus DNA to physically block transcription. This explanation is consistent with the data and makes it possible to visualize how the tsG protein could inhibit so strongly the transcription of a constitutively expressed gene. If IEP normally interacts with transcription factors which are already present on the adenovirus DNA, then the temperature-sensitive IEP might remain bound to them in a nonproductive interaction. Since the temperature-sensitive IEP is greatly overproduced in infections at the nonpermissive temperature (unpublished data), coinfection of tsG at 40 PFU per cell and of adenovirus at 5 to 10 PFU per cell might result in binding of the temperature-sensitive IEP to all the transcription factors on the adenovirus genomes. Since these factors are bound to the adenovirus DNA, they might physically block transcription of the constitutively expressed E1A gene. An excess of adenovirus DNA, which we provided in the experiments shown in Fig. 2, would outcompete a given concentration of the tsG IEP because not enough of the IEP would be made to block all of the adenovirus genomes. This model therefore explains the repression of the constitutive E1A gene and the importance of the ratio of tsG protein to adenovirus DNA.

We lack sufficient information to understand the molecular basis of this repression. It is clear, however, that the temperature-sensitive form of IEP is interacting in a very strong manner with a factor or substrate which is critical for adenovirus transcription. The use of tsG in coinfections, and eventually the mutant protein itself, will thus be helpful in defining transcriptional elements in adenovirus infections.

ACKNOWLEDGMENTS

We thank Arnold Berk and Joseph Nevins for helpful discussions, and T. W. Stinson, Jr., for excellent technical assistance.

This work was supported by grants from the California Institute for Cancer Research, the Cancer Research Coordinating Committee, and the California Lung Association. S.E.A. is a trainee of the USTHS National Research Service Award GM-07104.

LITERATURE CITED

1. Batterson, W., and B. Roizman. 1983. Characterization of the herpes simplex virion-associated factor responsible for the induction of alpha genes. *J. Virol.* **46**:371-377.
2. Ben-Porat, T., P. Hoffman, L. Brown, L. Feldman, and M. L. Blankenship. 1982. Partial characterization of temperature-sensitive mutants of pseudorabies virus. *Virology* **122**:251-267.
3. Berk, A. J., F. Lee, T. Harrison, J. Williams, and P. A. Sharp. 1979. Pre-early adenovirus 5 gene product regulates synthesis of early viral messenger RNAs. *Cell* **17**:935-944.
4. Borrelli, E., R. Hen, and P. Chambon. 1984. Adenovirus-2 E1A products repress enhancer-induced stimulation of transcription. *Nature (London)* **312**:608-612.
5. Dixon, R. A. F., and P. A. Schaffer. 1980. Fine-structure mapping and functional analysis of temperature-sensitive mutants in the gene encoding the herpes simplex virus type I immediate-early protein vp175. *J. Virol.* **36**:189-203.
6. Everett, R. D. 1984. A detailed analysis of an HSV-1 early promoter: sequences involved in *trans*-activation by viral immediate-early gene products are not early-gene specific. *Nucleic Acids Res.* **12**:3037-3056.
7. Feldman, L. T., M. J. Imperiale, and J. R. Nevins. 1982. Activation of early adenovirus transcription by the herpesvirus immediate-early gene: evidence for a common cellular control factor. *Proc. Natl. Acad. Sci. USA* **79**:4952-4956.
8. Gaynor, R. B., D. Hillman, and A. J. Berk. 1984. Adenovirus early region 1A protein activates transcription of a nonviral gene introduced into mammalian cells by infection of transfection. *Proc. Natl. Acad. Sci. USA* **81**:1193-1197.

9. Green, M. J., R. Treisman, and T. Maniatis. 1983. Transcriptional activation of cloned human β -globin genes by viral immediate-early gene products. *Cell* 35:137-148.
10. Hearing, P., and T. Shenk. 1983. The adenovirus type 5 E1A transcriptional control region contains a duplicated enhancer element. *Cell* 33:695-703.
11. Hen, R., B. Borrelli, P. Sassone-Corsi, and P. Chambon. 1983. An enhancer element is located 340 base pairs upstream from the adenovirus-2 E1A capsite. *Nucleic Acids Res.* 11: 8747-8761.
12. Imperiale, M. J., L. T. Feldman, and J. R. Nevins. 1983. Activation of gene expression by adenovirus and herpesvirus regulatory genes acting in *trans* and by a *cis*-acting adenovirus enhancer element. *Cell* 35:127-135.
13. Imperiale, M. J., R. P. Hart, and J. R. Nevins. 1985. An enhancer-like element in the adenovirus E2 promoter contains sequences essential for uninduced and E1A-induced transcription. *Proc. Natl. Acad. Sci. USA* 82:381-385.
14. Jones, N., and T. Shenk. 1979. An adenovirus type 5 gene function regulates expression of other early viral genes. *Proc. Natl. Acad. Sci. USA* 75:3665-3669.
15. Kao, H. T., and J. R. Nevins. 1983. Transcriptional activation and subsequent control of the human heat shock gene during adenovirus infection. *Mol. Cell. Biol.* 3:2058-2065.
16. Kingston, R. E., R. J. Kaufman, and P. A. Sharp. 1984. Regulation of transcription of the adenovirus E1A promoter by E1A gene products: absence of sequence specificity. *Mol. Cell. Biol.* 4:1970-1977.
17. Leff, T., J. Corden, R. Elkaim, and P. Sassone-Corsi. 1985. Transcriptional analysis of the adenovirus-5 E1A promoter: absence of sequence specificity for stimulation by E1A gene products. *Nucleic Acids Res.* 13:1209-1221.
18. Murthy, S. C. S., G. P. Bhat, and B. Thimmappaya. 1985. Adenovirus E1A early promoter: transcriptional control elements and induction by the viral pre-early E1A gene, which appears to be sequence independent. *Proc. Natl. Acad. Sci. USA* 82:2230-2234.
19. Nevins, J. R. 1981. Mechanism of activation of early viral transcription by the adenovirus E1A gene product. *Cell* 26:213-220.
20. Nevins, J. R., H. S. Ginsberg, J. M. Blanchard, M. C. Wilson, and J. E. Darnell. 1979. Regulation of the primary expression of the early adenovirus transcription units. *J. Virol.* 32:727-733.
21. Osborne, T. F., D. N. Arvidson, E. S. Tyau, M. Dunsworth-Browne, and A. J. Berk. 1984. Transcriptional control region within the protein-coding portion of adenovirus E1A genes. *Mol. Cell. Biol.* 4:1293-1305.
22. Post, L. E., S. Mackem, and B. Roizman. 1980. Regulation of alpha genes of herpes simplex virus: expression of chimeric genes produced by fusion of thymidine kinase with alpha gene promoters. *Cell* 24:555-565.
23. Preston, C. M. 1979. Control of herpes simplex virus type 1 mRNA synthesis in cells infected with wild type virus of the temperature-sensitive mutant tsK. *J. Virol.* 29:275-284.
24. Preston, C. M., M. G. Cordingley, and N. G. Stow. 1984. Analysis of DNA sequences which regulate the transcription of a herpes simplex virus immediate-early gene. *J. Virol.* 50: 708-716.
25. Schaffer, P. A., V. C. Carter, and M. G. Timbury. 1978. Collaborative complementation study of temperature-sensitive mutants of herpes simplex virus types 1 and 2. *J. Virol.* 27:490-504.
26. Stein, R., and E. B. Ziff. 1984. HeLa cell β -tubulin gene transcription is stimulated by adenovirus 5 in parallel with viral early genes by an E1A-dependent mechanism. *Mol. Cell. Biol.* 4:2792-2801.
27. Velcich, A., and E. Ziff. 1985. Adenovirus E1A proteins repress transcription from the SV40 early promoter. *Cell* 40:705-716.
28. Watson, R. J., and J. B. Clements. 1980. A herpes simplex virus type 1 function continuously required for early and late virus RNA synthesis. *Nature (London)* 285:329-330.
29. Weeks, D. L., and N. C. Jones. 1983. E1A control of gene expression is mediated by sequences 5' to the transcriptional start site of the early viral genes. *Mol. Cell. Biol.* 3:1222-1234.