CONTROL OF CELLULAR AND VIRAL TRANSCRIPTION DURING ADENOVIRUS INFECTION

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I. INTRODUCTION

The use of viruses to study and elucidate the aspects of mammalian cell gene expression has stemmed from certain basic properties of viruses. Depending on the virus, they provide model systems since they often utilize host cell machinery. This is certainly the case for adenoviruses, the papovaviruses and the herpes viruses. Thus, it has been argued that what is learned from the study of these viruses is likely true for the cell as well, at least in the basic concepts. One has only to look at the discovery and subsequent elucidation of RNA splicing,[1,2] the demonstration of the mRNA 5′ end as the site of transcription initiation,[3] and the mechanism of poly(A) site formation,[4,5] all recognized first with adenovirus, as proof that such an argument is justified. But the viral systems are clearly more than just model systems. They represent in some instances "high copy genes", thus providing the advantages of amplified but easier to manipulate genes. Furthermore, they proceed through productive cycles of infection and thus in a sense provide a developmental pathway. For the more complex viruses such as adenovirus and herpes virus, this represents the coordinate control of sets of genes. Finally, a primary reason for the use of viruses to study mammalian cell gene expression is genetics. One has the availability of regulatory mutants that define genes that control gene expression. This simply is not feasible at this time in mammalian cells. As a result, a great deal of information has emerged concerning the control of viral gene expression by these regulatory genes. As an apparent added bonus, the mechanism by which some of these regulatory genes mediate their control involves the host cell and, indeed, suggests the presence of similar activities in the host cell. Thus, the viruses in this sense may be more than just a model system, but may as well lead us into pathways of regulation of cellular gene expression.

Although there are clear cases of posttranscriptional regulatory interactions during adenovirus infection,[6] the topic of this review will be limited to transcriptional regulatory events that take place in adenovirus-infected cells.

As suggested above, one of the advantages of viral systems, and in particular adenovirus, is the high expression of the viral genes in productively infected cells. This fact is of great significance when attempting to establish the events in mRNA biogenesis. To separate the contributions of primary transcription from RNA processing, one must be able to differentiate newly made RNA from processed RNA. This requires metabolic labeling, usually using ^{3}H-uridine as a precursor, for sufficiently short periods so that only nascent RNA is labeled. This was feasible in adenovirus-infected cells and allowed the establishment of the pattern of transcription of various early genes during infection.[7] More recently, it has been possible to increase the sensitivity of transcription assays through the use of isolated nuclei that can incorporate exogenous ^{32}P triphosphate. In fact, however, the validity of the isolated nuclei

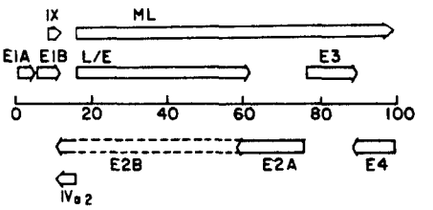

FIGURE 1. Map of the transcriptional units that function during an adenovirus productive infection. The early transcription units are E1A, E1B, E2A/B, E3, E4, and L/E. Transcription units active late in infection are ML, IX, and IVa_2.

measurements was in part established by comparing results to those obtained from in vivo measurements, again with adenovirus.[8] What became clear from these early measurements of viral transcription was the presence of control mechanisms. Early transcription units (see Figure 1) were not activated at precisely the same time and did not continue with the same kinetics.[7]

II. CONTROL OF TRANSCRIPTION INITIATION

The demonstration that there was positive control of viral transcription came from the analysis of viral mutants in the E1A gene.[10,11] These mutants were originally isolated as "host range" mutants that could replicate in an adenovirus transformed human cell line, 293,[9] but not in the conventional adenovirus host, HeLa. An examination of the infection in HeLa cells revealed a lack of expression of all early viral genes (Figure 2) despite the localization of the mutation to the E1A gene.[12,13] Thus, it was concluded that E1A mediates expression of the various other early genes. Subsequent experiments established that the level of this control was transcriptional[14] (Figure 2), although there are other suggestions of posttranscriptional effects.[15] Furthermore, one of the mutants (hrl) retained an intact E1A gene (the defect in this mutant is a single base deletion[16]), and it (E1A) was found to be expressed in infected HeLa cells, although at a lower level than in wild type.[12,14] These results thus suggested two aspects of early viral transcription control. First, there was a pre-early stage involving expression of the E1A gene, and this could occur in the absence of any other viral gene expression. Second, the product of the E1A gene was responsible for the activation of the various other transcription units at the level of transcription initiation. That the defect could be relieved by introducing the E1A defective virus into 293 cells, which express the E1A gene, demonstrated that the action of the E1A gene product was in *trans* to stimulate expression of the various other viral genes.

The reason for E1A transcription independent of early gene expression most likely is due to the enhancer elements found in the vicinity of the E1A promoter. In fact, there have been four distinct regions described as having enhancer activity, including one that is within the coding region of the E1A gene.[17-20] At least one of these, when inserted into the E2 gene that is normally dependent on the E1A protein for transcription, converts the E2 gene to E1A-independent transcription.[18]

A. Mechanism of E1A Action

Although the precise mechanism by which the E1A protein effects a transcription stimulation is still not clear, several lines of evidence suggest a potential pathway. There is a strong suggestion that the E1A protein itself is not a DNA-binding transcription factor. Instead, there are indications that E1A effects a stimulation of transcription by altering or modifying a component of the host cell. The initial indication came from experiments with cycloheximide or other inhibitors of protein synthesis. Early experiments had demonstrated

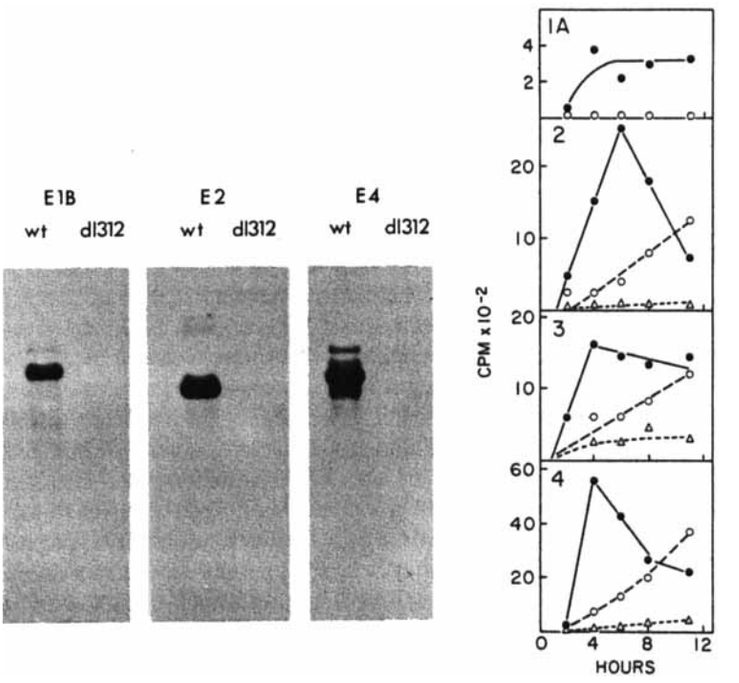

FIGURE 2. Early RNA production and transcription in the presence and absence of E1A. On the left are shown Northern blots of poly(A)+ cytoplasmic RNA from wild type (WT) infected cells (E1A+) and d1312 infected cells (E1A−). The E1B, E2, and E4 RNAs have been visualized by hybridization with specific probes. On the right are shown results of transcription rate measurements in cells infected with wild-type Ad5 (●), d1312 (△) and d1312 at a 10X multiplicity (○). (Figure on right was reproduced from Nevins, J. R., *Cell*, 26, 213, 1981. With permission.)

that the inhibition of protein synthesis had little effect on transcription in a wild-type infection when added at the time of infection.[7] Thus, there appeared to be a paradox: the E1A gene was required (as judged by the E1A mutants), but the E1A protein was not required (cycloheximide added at the time of infection did not prevent early transcription). A potential resolution of the problem came when it was shown that the inhibition of protein synthesis partially relieved the requirement for E1A in a mutant infection. Thus, these results suggested the presence of a cellular negative acting factor, which E1A appeared to counteract. Previous experiments had shown that cycloheximide added after infection increased the production of the early viral RNAs.[21-24] A reexamination of the effect of cycloheximide on early viral transcription revealed that, if added at the appropriate time in a wild-type infection, cycloheximide could in fact have a stimulatory effect. Specifically, if protein synthesis is inhibited after sufficient time has lapsed such that early viral proteins are made (including E1A), then there is a marked increase in transcription rates of early viral genes.[25] Therefore, it would appear that in the absence of E1A, the removal of a cellular protein can partially overcome the need for E1A, and in the presence of E1A, the removal can superinduce early transcription.

Further insight into the mechanism of E1A transcriptional stimulation has come from a search for other E1A-like regulators. It was found that the pseudorabies virus (a herpes virus) immediate early (IE) gene could effectively stimulate early adenovirus transcription in the absence of E1A.[26] In fact, it appeared that the herpes virus regulatory gene was more efficient at activation of early adenovirus genes than E1A. These results were confirmed, using the cloned pseudorabies regulatory gene in transient transfection assays.[18] Thus, the

isolated herpes virus immediate early gene, devoid of a viral infection, could effect a stimulation of an early adenovirus gene. And once again, the stimulation was stronger than that observed with E1A. These results appear to provide very strong evidence that the E1A or IE proteins are not direct transcription factors that recognize promoter sequences. If so, it would be difficult to reconcile the fact that the herpes virus regulatory protein could functionally interact with the genetically unrelated adenovirus promoter sequences *more* efficiently than the homologous interaction (E1A). Rather, these results argue for an intermediate common to the two regulatory proteins, and this may well be related to the cycloheximide-sensitive factor.

If the E1A protein is not a transcriptional factor, then what is it doing and is there in fact another protein that binds to the viral promoters? This second question has been approached by analyzing sequences in several early promoters that were essential for the activity of the promoter. Among the early promoters, the E2 promoter has been most extensively investigated. Deletion mutants were created in the 5′ flanking region and then assayed by transient transfection,[27,28] in vitro transcription,[27] and stable colony formation.[29] In vivo assays done under fully induced conditions (in the presence of E1A) indicate that 79 nucleotides of 5′ flanking sequences are sufficient for full promoter activity. Further deletion greatly impairs activity. The same appears to be the case for activity in vitro using extracts from HeLa cells. These results thus indicate the presence of an upstream sequence element necessary for the normal activity of this promoter. Other experiments have demonstrated that this element can function independently of its position or orientation, thus reminiscent of the enhancer elements.[28]

How does this essential sequence element relate to E1A induction? On this point, there appears to be some controversy as to the sequence requirements for induction by E1A. In one series of experiments, Kingston et al.[29] have suggested that there is no specific sequence requirement for E1A induction and specifically not the upstream sequences of the E2 promoter. Hybrid genes containing the E2 promoter and the DHFR coding region were used to transform CHO cells to DHFR(+) phenotype. Induction was scored as the increase in colonies in the presence of E1A. A gene with a promoter deleted to −18 was still induced by E1A. In contrast, Imperiale et al.[30] suggested that indeed the sequences upstream to −79 were essential for E1A induction. A mutant deleted to −59 gave only a very low level of activity in HeLa cells in the absence of E1A and was not stimulated by E1A. An intermediate result was obtained with a mutant retaining sequences to −70 in that the uninduced expression was clearly lowered, but the promoter was still capable of responding to E1A. These results would suggest that either there is a single site of protein binding for either uninduced or induced (i.e., the same protein) and that the −70 mutation impairs the site but does not eliminate it, or that there are separate sites for uninduced and induced with the induced site being located closer to the transcription start site. The reason for the discrepancy between the two results is not clearly evident, although it may relate to the nature of the assays. The assays of Imperiale et al. employed transient transfection with no pressure for expression whereas, as indicated above, the experiments of Kingston et al. measured the effect of E1A on the formation of colonies, a situation that demands that there be at least some expression of the gene.

Other early viral promoters have been examined for sequences essential for transcription, although no studies have been performed to distinguish those necessary for E1A. A study of the E1B promoter demonstrated that 135 nucleotides of 5′ flanking sequence were sufficient, but 83 nucleotides were not.[31] In vitro transcription of the E4 promoter demonstrated that 325 nucleotides of 5′ sequence (thus, the entire right terminus of the genome) were necessary for full activity.[32] Internal deletion mapping demonstrated that sequences between −140 and −40 could be deleted with no effect, indicating that the essential element was located somewhere between −140 and −325. Finally, it was demonstrated that sequences 5′ to the E3 initiaiton site as well as the E4 and E2 initiation sites were required for funciton

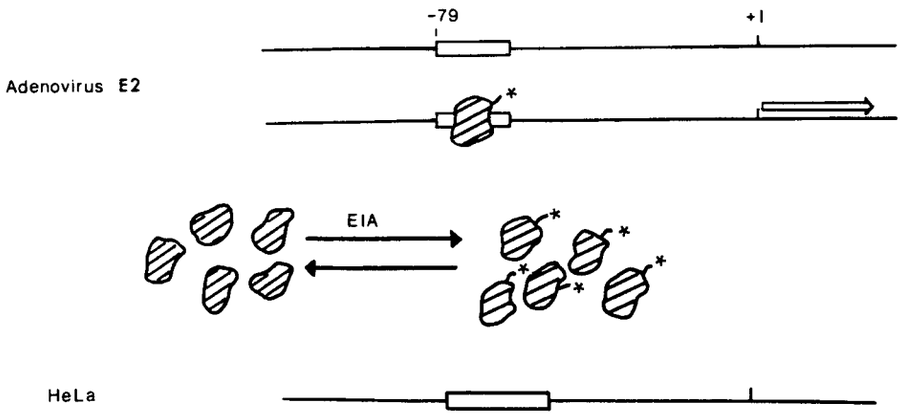

FIGURE 3. Model for the action of E1A. In the middle of the figure is schematized transcription factors of cellular origin that are in equilibrium between an inactive state and an active state (*). It is suggested that the action of E1A drives the equilibrium to the active state. An antagonistic activity would reverse the activation. The active form of the factor would have the capacity to bind to the upstream region of E1A-inducible promoters, in this case the E2 promoter, and as a result, promote transcription. If the active form of the factor were initially limiting, then such an increase would "induce" the gene. It is presumed that there are cellular genes that utilize the factor and thus would be inducible by E1A.

and E1A stimulation;[33] more recent data suggest that sequences between −80 and −105 of the E3 promoter are critical for function and that this region can transfer E1A stimulation to a heterologous gene.[91]

B. A Model for E1A Action

Although we still do not have a firm basis for suggesting possible modes of action of E1A induction, an examination of all of the relevant data does point to one model, as suggested in Figure 3, namely, that E1A is a regulatory protein that, in the simplest terms, increases the supply of a cellular transcription factor(s) that is normally limiting in the cell. This increase could be either in mass amounts or in specific activity, the latter seeming to be the most probable. The principal reason for suggesting a cellular positive acting factor derives from the analysis of promoter sequence requirements. In all of this work, there was no indication for the involvement of a negative acting factor, only positive action. Furthermore, it is clear that these promoters can be transcribed in the absence of E1A.[14] E1A appears to increase transcription from a low basal level. And, in a preliminary sense, there appears to be the possibility of the involvement of a single factor in E1A induced and uninduced transcription. Certainly, there is a precedent for the use of a cellular transcription factor by a viral promoter: the early SV40 promoter utilizes the cellular SP1 factor.[34] The model depicted in Figure 3 also suggests an equilibrium of sorts in which there is an antagonist of E1A that reverses the activation. Such an antagonist would be compatible with the earlier observation from experiments with cycloheximide.[6,25] These experiments suggested the involvement of a negative acting factor in viral transcription control. However, as stated above, there is no indication for the action of a negative factor at the level of the DNA (a repressor). Thus, one might suggest that if an antagonist in the proposed scheme were short-lived, then its removal would shift the equilibrium in the direction of E1A action, even in the absence of E1A. Furthermore, in the presence of E1A there would be a synergistic effect by removing the antagonist and thus superinduction. Therefore, a cellular regulatory activity that reversed the effect of E1A could be the negative acting component.

Analysis of viral transcription in E1A-mutant infected cells has suggested a *cis* effect in the establishment of active transcription. If cells are infected with d1312 at high multiplicities,

early transcription will take place (dependent upon the cell type; see below), but the activation is slow.[14,35] However, this slow activation is not due to an alteration in the cellular control, since if a second E1A mutant is introduced at this time, it must go through the same slow activation process. It was suggested that E1A mediates a change in the template that allows transcription.[35] However, these results might also suggest the inefficient acquisition of a cellular transcription factor in the absence of E1A, but then once such a factor has bound, the complex is stable.

Although such a model is consistent with most of the available data, there clearly is no proof at this stage. The critical tests will be to isolate the factor(s) that are utilized by these promoters both during uninduced transcription as well as during E1A induction. For instance, is the same factor utilized by the E2 promoter under induced and uninduced conditions as suggested by the model? Furthermore, how many factors are involved in E1A control? In other words, do the E2, E3, E4, and E1B promoters all utilize the same factor, or does E1A activate multiple factors? This question can be approached in the beginning by competition assays in transfection.[36-38] Potentially, such assays can determine if the E2 upstream sequence competes with the E3 upstream sequence (or E4 or E1B) for the same factor. What is the complexity of the induction? Furthermore, is the same factor utilized by cellular promoters that are induced by E1A (see below) as is utilized by the viral promoters? Finally, if the same factor is utilized in the uninduced state as in the induced state, then what is the nature of the activation?

C. E1A Activation of Cellular Transcription

If indeed there is an involvement of a cellular factor(s) in the control of viral transcription and that the role of E1A in this process is to increase or activate such cellular factor(s) as suggested above, then this would suggest two further characteristics. First, such a transcriptional factor is surely not in the cell only for the transcription of these viral genes, but rather is usually there to be utilized for the transcription of a cellular gene(s). Second, E1A mediates the activation of a cellular transcriptional factor(s) that E1A mediates, then there must be an analogous regulatory molecule similar to E1A that normally mediates this activation and that E1A simply intercedes in the pathway. Thus, a furtherance of the model would be the identification of cellular genes that were stimulated by E1A, due to an increase in the amount of the appropriate factor and the demonstration and eventual isolation of a regulatory activity functioning similarly to E1A. In fact, experiments carried out over the past several years have provided strong and direct evidence for the existence of genes that utilize similar factors in that they are activated by E1A and indirect evidence for the existence of a regulatory activity that functions in a manner analogous to E1A.

The identification of a cellular gene activated by E1A was originally made through the analysis of protein synthesis during a viral infection as it was observed that in a wild-type infection there was an increased synthesis of a protein of 70,000 mol wt.[39] Furthermore, this protein was not synthesized in increased amounts in an infection in the absence of E1A. This protein was subsequently identified as the major 70 kd heat shock protein in HeLa cells, analogous to the well-characterized gene product in *Drosophila*,[40] and it was indeed shown that the increased synthesis of this protein correlated with the activity of E1A. Subsequently, a cDNA clone was isolated complementary to the message for the 70 kd heat shock gene and was used to demonstrate activation of the gene in response to E1A and establish the fact that the activation was at the level of transcription (see Figure 4).[41] This is an important result, since it was known that E1A activation of viral gene expression was due to increased transcription initiation.[14] Thus, the mechanism for induction of the heat shock gene was the same as that for the viral genes mediated by E1A, and, furthermore, the activation of the heat shock gene during an infection exhibited kinetics that were quite similar to those seen for the various early viral transcriptional units.[41]

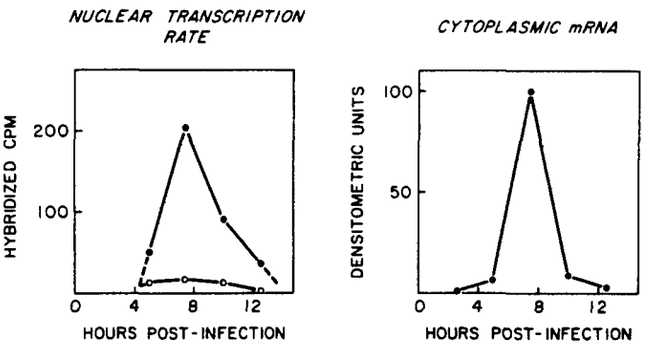

FIGURE 4. Induction of the human HSP70 gene by E1A. On the left is depicted transcription rate measurements of the HSP70 gene in cells infected with WT Ad5 (E1A+) (●) and d1312 (E1A−) (○). On the right are measurements of cytoplasmic mRNA abundance during a WT adenovirus infection. (From Kao, H.-T. and Nevins, J. R., *Mol. Cell. Biol.*, 3, 2058, 1983. With permission.)

Thus, it appears quite clear that in addition to the activation of the viral genes, E1A mediates the activation of this one particular cell gene. In addition to the heat shock gene, there is now evidence suggesting that there is also activation of the β tubulin gene in adenovirus infection.[42] Furthermore, this activation appears to be mediated by E1A as well, although in this case the activation is not nearly as dramatic as that seen with the heat shock gene. These results then suggest that the heat shock gene and the β tubulin gene may utilize a transcriptional factor that is increased in abundance or at least in activity as a result of E1A. Whether or not these cellular genes utilize the same factor as being utilized by the early viral genes or different factors that are also activated by E1A is not clear at this point and will only be answered by the isolation of such factors.

Finally, a number of recent reports have demonstrated that the E1A gene product can stimulate the transcription of a wide variety of genes in transfection assays. This was initially shown for the human β globin gene, which otherwise requires an enhancer for efficient expression in HeLa cells. However, in the presence of E1A (or the pseudorabies immediate early gene), there is efficient expression of the β globin gene.[43,44] In those same experiments, it was also found that E1A could stimulate the transcription of the early SV40 promoter from which the enhancer had been removed. Moreover, this E1A-mediated stimulation required the presence of the 21 bp repeat elements. Possibly, this suggests that E1A may mediate an effect through the SP1 factor that is known to bind to this region.[45] In contrast, the β globin promoter could be stimulated by E1A despite the removal of all sequences 5′ to the TATA element. Whether this indicates that E1A stimulation of β globin does not require a specific sequence or whether there is a sequence internal to the β globin gene that allows a response, is not clear. Interestingly, in this regard, it has been found that the β globin gene can respond to induction signals in mouse erythroleukemia cells in the absence of the 5′ upstream region,[46] suggesting the presence of an internal regulatory site. Perhaps this site allows a response to E1A in the transfection assays in HeLa cells.

Other genes have been shown to respond to E1A upon transfection including the preproinsulin gene[47] and the rabbit β globin.[48] It might thus appear from these results that the activation mediated by E1A is quite indirect and nonspecific. How might induction of such heterologous genes occur, especially given the fact that the endogenous, chromosomal counterpart is not induced?[43,44,47] First of all, the endogenous gene is not induced likely because the appropriate control sequences are not available due to chromatin structure. Thus, an important experiment would be to ask if E1A could induce the β globin gene in eryth-

roleukemia cells, either before DMSO induction or together with DMSO induction. The question of why the transfected genes are induced must await the determination of the factors involved. For instance, the transcriptional factor that the β globin gene utilizes in HeLa cells is most likely not the same as the one utilized in erythroid cells. Possibly, the β globin gene (or any gene introduced into a heterologous cell artificially) makes use of an alternate factor, one representing the "best fit". This situation surely must be analogous to the early SV40 promoter utilizing the SP1 factor, although one might assume that the SV40/SP1 interaction has evolved into a rather "good fit". If such a factor were increased greatly in concentration as a result of E1A, then there would be a stimulation even though the gene was using the factor very inefficiently. That is, if a promoter makes use of a factor, no matter how well, and that factor increases in concentration, then there will be an "induction."

It was suggested that in addition to the concept that there should be cellular genes activated by E1A, that such results also predicted the existence of a regulatory activity in cells similar to E1A. Such a regulatory activity would control the abundance of factors in a manner similar to E1A and thus would operate in the cell to control the transcription of genes that are dependent upon such factors. Evidence for such an activity has come from the analysis of the expression of the heat shock gene. It was reasoned that if the heat shock gene were utilizing a similar factor and regulatory mechanism as the early adenovirus genes, as predicted since it is activated by E1A, then if one could detect circumstances where the heat shock gene was expressed at high levels it would suggest an increased activity similar to E1A. It would then be predicted that under such circumstances there would be a lesser requirement for the viral E1A gene to activate the early viral promoters. This indeed appeared to be the case, based on the analysis of expression of the heat shock gene in various human cell lines.[49] It was observed that there were variations in the level of expression of the gene, and these variations correlated with the ability of early viral genes to be expressed in the absence of E1A, that is, from an E1A deletion mutant. This was extended to the mouse F9 teratocarcinoma cell line, which has the property that the phenotype of the cells can be altered through induced differentiation with retinoic acid and cyclic AMP.[50] It was found that indeed the uninduced F9 stem cells exhibited an E1A-like activity in the form of high expression of the heat shock gene and ability of early adenovirus genes to be expressed in the absence of viral E1A.[49] This cellular E1A-like activity disappears upon differentiation of F9 cells, since the differentiated cells show a strict requirement for the viral E1A in order for activation of the early viral genes to occur. These results then once again reinforce the idea that there is indeed a cellular regulatory activity that functions in a manner similar to E1A in that it can provide at least partial activity for the transcription of the early viral genes in the absence of the viral E1A. The idea that such an activity is similar to E1A is substantiated by the observations that the expression of the heat shock gene correlates with the ability of a cell to provide an E1A function.

What might be the role of an E1A-like cellular transcriptional regulatory gene? Once again, a clue has been provided from the study of the expression of the heat shock gene and its control in growing HeLa cells. It was found that the expression of the HSP 70 gene in sychronized HeLa cells was cell cycle regulated.[51] Specifically, the maximal time of expression was in the S/G2 phase of the cell cycle. Furthermore, an analysis of the level of cell cycle control of the HSP 70 gene demonstrated that the regulation was transcriptional. The regulation of the gene was also examined in human 293 cells where it was previously shown that the HSP 70 gene was expressed at a high level. Once again, the gene was found to be subject to cell cycle control. Expression of the E1A gene in 293 cells was also found to be cell cycle regulated and with kinetics consistent with it regulating the expression of the heat shock gene. Thus, it has been suggested that the cellular analog of E1A in HeLa cells is responsible for some aspect of regulation of cell cycle transcription and that the E1A gene takes over the same role during transformation as exhibited in the 293 cells. This is

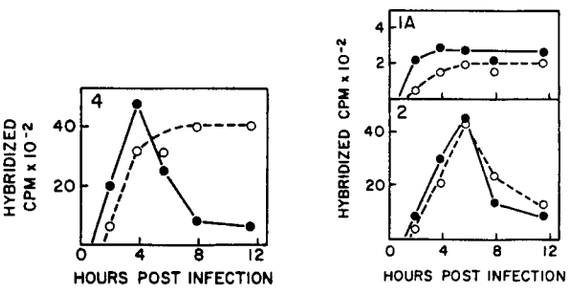

FIGURE 5. Negative regulation of the adenovirus E4 promoter by the E2 gene product. Depicted are transcription rate measurements for the E4, E1A, and E2 transcription units in a wild-type infection (●) and a ts125 (E2−) infection (○) carried out at the nonpermissive temperature. (From Nevins, J. R. and Jensen-Winkler, J., *Proc. Natl. Acad. Sci. U.S.A.*, 77, 1893, 1980. With permission.)

consistent with previous findings that demonstrated that adenovirus infection could stimulate quiescent cells into S phase as well as induce an increase in thymidine kinase and that the E1A gene was necessary for this to occur.[52-55]

D. Negative Regulation of Transcription

In addition to the positive control of transcription of viral and cellular genes during an adenovirus infection, there are also several instances of negative control of transcription. Negative control of the viral E4 transcriptional unit was originally indicated by measurements of transcription rates in the presence or absence of protein synthesis during a productive infection.[7] It was found that after activation of the E4 transcriptional unit, transcription declined to a basal level. However, in the absence of protein synthesis, there was no decline in the transcription rate of E4, suggesting the requirement of a protein to mediate the decline. The nature of the protein involved in the negative control was established through the use of a temperature-sensitive mutant in the adenovirus E2A gene that affects the 72 kd DNA binding protein.[56,57] Previous experiments had demonstrated that one particular phenotype of this mutant, termed ts125, was an overproduction of early viral RNA in cells infected at the nonpermissive temperature compared to cells infected with wild-type or cells infected with the mutant at the permissive temperature.[58] Clearly, the overproduction of the early viral RNAs could be due to an alteration of any step in mRNA biogenesis, including an effect on transcription. Using this particular mutant, transcription rates were measured from various early promoters at the permissive and nonpermissive temperature, and it was found that indeed at the nonpermissive temperature, there was no shutoff of E4 transcription as normally occurs in a wild-type infection, or in the mutant infection at the permissive temperature[59] (see Figure 5). The only effect observed, however, was with the E4 transcriptional unit, as all of the other early viral transcriptional units exhibited normal kinetics of transcription at the nonpermissive temperature. Thus, it would appear that the 72 kd DNA binding protein has a specific effect on the E4 promoter, causing a shutoff of transcription. This result has been further substantiated by a demonstration that the purified 72 kd DNA binding protein can specifically inhibit E4 transcription in vitro.[60] However, the mechanism by which the protein inhibits E4 transcription is yet to be determined. There is no indication that the 72 kd protein recognized specific sequences in double-stranded DNA. Although it has only been characterized as a single-stranded DNA binding protein, it does appear to have the ability to interact with the termini of viral DNA,[61] and it is conceivable that an interaction at the right-hand terminus of the genome inhibits the ability of polymerase to interact with the E4 promoter. However, such a mechanism has yet to be demonstrated.

Several recent reports have suggested a role of the E1A gene product in negative control of transcription. It was originally shown by van der Eb's group that the E1A gene product from Ad12 specifically shut off the expression of the gene encoding the class I major histocompatibility antigen.[62] It has been suggested that through such action, Ad12-transformed cells escape recognition by the host immune system.[63] The ability of the E1A gene to turn off the class I histocompatability gene appears to be restricted to the Ad12 E1A, since the Ad5 E1A gene in conjunction with the Ad12 E1B gene does not shut off the gene. In addition, recent reports suggest the ability of the Ad5 E1A gene product to control enhancer driven genes in a negative fashion.[64,65] The expression from a plasmid that is dependent for expression on the SV40 enhancer, the polyoma enhancer or the E1A enhancer, is inhibited by co-transfection with an E1A expressing gene. Furthermore, that this represents the action due to the E1A protein in *trans* was indicated by competition experiments where the inhibition could be relieved by a co-transfection of large amounts of competing enhancer sequence not linked to a transcribing gene.

It is difficult at this point to develop a mechanism for such negative action of the E1A gene product in light of its positive control of certain other promoters, but one in vivo aspect of this phenomenon is consistent with previous observations relating to E1A control. Specifically, as was discussed in previous sections, there appears to be a cellular activity that functions in a manner analogous to E1A that is present at high levels in the mouse F9 teratocarcinoma cell, but that disappears upon induction of differentiation in these cells.[49] It has been known for several years now that both SV40 and polyoma virus replicate only in the differentiated teratocarcinoma cells.[66,67] The restriction in viral replication in these cells is manifested at the level of expression of the early transcriptional unit. In fact, polyoma mutants have been isolated that now have the ability to grow in the F9 stem cell, and the mutation has been mapped to the enhancer sequence.[68-75] Thus, it appears quite clear in the case of polyoma that there is a restriction of transcription of the early gene that depends upon the state of differentiation of the cell and likely reflects an interaction of a factor with the enhancer element.[36,76] Thus, there is a correlation between those results and the results now being described whereby E1A in one case activates certain viral promoters and inhibits certain other viral promoters. In the case of the F9 cells, there appears to be an activity in the undifferentiated F9 cells that provides an E1A-like function to activate the early adenovirus promoters. Perhaps this same activity is responsible for an inhibitory effect on the enhancer-driven early transcription unit of polyoma of SV40. When the F9 cells are induced to differentiate, that activity disappears, thus allowing the expression of the early SV40 or polyoma gene and restricting the expression of the early adenovirus genes that normally depend upon E1A.

III. CONTROL OF TRANSCRIPTION TERMINATION

Although the control of transcription initiation is by far the best understood, and most likely is the dominant form of trancriptional control, there are, nevertheless, examples of control mediated through transcriptional termination. For instance, the decline in expression of the immunoglobulin δ heavy chain genes during B-cell maturation is due in part to transcriptional termination prior to the δ exons.[77] A similar control is seen during adenovirus infection as well as one other example of termination that may or may not be regulatory.

A. Termination Control in the Early to Late Switch

In addition to the regulation that is seen at the level of transcriptional initiation in an adenovirus infection, there are at least two instances of gene control that are mediated through transcriptional termination. The first of these relates to the transcription of the major late promoter during either an early infection or a late infection. It has been well documented

that transcription from the late promoter during late infection proceeds all the way to the very end of the genome, encompassing approximately 30 kb of transcriptional unit.[4,78] Within this transcription unit are encoded five groups of mRNAs, each RNA within the group sharing a 3′ end and thus a poly(A) site. Thus, expression of the major late transcriptional unit involves first a selection of a 3′ end [poly(A) site] out of five possible, and then upon selection of the poly(A) site, a single mRNA within that group is selected by splicing.[4]

In contrast to this situation late in infection, transcription from the same promoter early in infection proceeds only halfway through the genome, terminating somewhere between map positions 60 and 70.[79-81] Thus, in this case, transcripts only include sequences of the L1, L2, and L3 families of late mRNAs. There is some debate on this issue since the data of Shaw and Ziff[81] suggested termination shortly after L1 sequences. Akusjarvi and Persson[80] detected a drop in transcription in the L2 region, but then a rise in the L3 region, the L3 transcription being attributed to E2 transcription from the other strand. However, Nevins and Wilson[79] employed separated strands of DNA from the L3 region and detected near equimolar transcription (as compared to L1). Furthermore, recent measurements employing pulse labeling in isolated nuclei and hybridization to M13 cloned single strand fragments indicates that transcription proceeds all the way to map position 70, thus through L3, in a near equimolar fashion.[92] Regardless of the exact site of termination, it is absolutely clear that the L4 and L5 families are not transcribed early in infection. Thus, transcriptional termination clearly has a regulatory role in the sense that the L4 and L5 mRNAs simply cannot be expressed because they are not transcribed. The mechanism for this level of control is not known at this point, whether it represents a strong terminator at the position between 60 and 70 map units that then is subject to an antitermination event late in infection or whether it simply represents the state of transcription that is going on early in infection in which there is transcription coming from the opposite direction from the E2 promoter, which interferes with transcripts from the late promoter. Experiments employing superinfection of virus into late infected cells argue that genome replication is essential for expression of L4 and L5.[82]

B. Premature Termination of Late Transcription

In addition to the level of termination control just described, there is also termination shortly after the polymerase initiates transcription at the major late promoter. This was originally demonstrated through the analysis of the molarity of transcription, in pulse-labeling, in this region. Promoter proximal transcription was extramolar, indicating that transcripts often terminate shortly after they have been initiated.[83-85] More recent studies have suggested the presence of pause sites as well as termination sites within this region.[86,87] Certain pulse-labeled transcripts chase to larger species, whereas transcripts of 175 and 120 nucleotides in length do not. Furthermore, these terminated transcripts are not bound to transcriptional complexes, but rather are found in the soluble fraction. Left unclear at this time is the significance of either the premature terminations or the pausing.

IV. REPLICATION-DEPENDENT CONTROL OF INITIATION

Although the late promoter is active early in infection,[79-81] it is certainly true that the quantitative level of expression from the major later promoter is vastly different early and late in infection. Estimates have been made that the rate of transcription increases at least 100-fold[81] and possibly as much as 1000-fold[93] from the late transcription unit between early and late in infection. Given the fact there is also at least that large of an increase in the number of genomes upon replication, it is not clear whether this large increase in transcription is simply due to a greatly increased number of DNA templates or is in fact due to a qualitative difference in the late promoter between early and late in infection. Possibly, some contribution

is due to increased template number since all of the promoters active early and late (E1A and E1B) increase in activity, including heterologous cellular transcription units that have been inserted into the left end of the virus.[93]

In addition to the quantitative changes in expression from the late promoter between early and late in infection, there are changes in the expression of two other transcriptional units dependent upon replication of the genome that appear to represent "on-off" regulation. Both the transcriptional unit encoding structural protein IX and the transcriptional unit encoding protein IVa_2 appear to be inactive before DNA replication.[88] Only after the genome is actually allowed to replicate are these genes activated. Furthermore, this activation is very likely at the level of transcription.[89] It is not clear at this point if there is a specific factor requirement for these promoters as well as genome replication. What is certain is that at least genome replication is necessary, since the introduction of unreplicated templates into a late infected cell does not turn on the protein IX gene.[88] Finally, there is recent evidence that may suggest an autoregulation of the protein IX gene.[90] Overproduction of protein IX decreases the level of RNA transcripts from the protein IX promoter as well as the E1B promoter. Interestingly, there is some degree of sequence homology in the upstream regions of E1B and IX.[90]

V. FUTURE PERSPECTIVES

As should be evident from this review, the study of adenovirus transcription has elucidated many aspects of mechanisms of transcription and transcription regulation. What now remains is the definition of the factors involved in these mechanisms and how they control such events as initiation frequency, termination, and repression of initiation. Such proteins are now beginning to be isolated so the way should be clear for such approaches with the adenovirus system. Once proteins of this nature are obtained, the clear goals will be to analyze the mechanism by which they function as well as the manner in which *their* activity is regulated. What is the function of such proteins in the uninfected cell? The suggestions thus far are that these proteins may be involved in cell cycle control as well as cellular differentiation. Thus, as is often the case, the study of viral regulatory mechanisms may well accelerate our understanding of mechanisms controlling basic properties of cell gene expression.

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