± standard error), whereas decapped roots bent only 9° ± 2° (Fig. 1). Decapping had no effect on the potential for root growth, since the controls and decapped plants grew 0.80 ± 0.25 and 0.90 ± 0.26 mm, respectively. Thus, because the plant does not respond to gravity, the root cap seemed to be the site of hydrotropic sensory perception.

We suggest the following mechanism for the downward bending of emergent 'Ageotropum' pea roots. When the seed is planted, the radical and then the root grows in whatever direction it was pointed at planting. As the secondary roots appear, they also grow in random directions. By chance, some of these roots grow upward and emerge from the soil into the air (and into the light if the plants are in an illuminated area). As soon as the root cap has emerged, a sensory system in the cap detects a moisture gradient between the wet soil and the drier air. It responds to this gradient by bending and growing back into the soil. The fact that it bends downward when exposed to the light is purely fortuitous, since its behavior is the same in the dark as it is in the light as long as the moisture gradient is present. The moisture gradient of 80 percent RH (air) to about 99 percent RH (water-saturated soil) is great enough to induce hydrotropism.

Thus a model system is now available to study hydrotropism in roots, unimpeded by complications involving gravitropism, phototropism, or phytochrome. This mutation should provide a probe for the elucidation of the mechanism of hydrotropism in plant roots.

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Transcription of Class III Genes Activated by Viral Immediate Early Proteins

Abstract. *The adenovirus E1A and pseudorabies virus immediate early (IE) proteins induce transcription from transfected viral and nonviral genes transcribed by RNA polymerase II (class II genes). These proteins have now been shown also to activate transcription of transfected genes transcribed by RNA polymerase III (class III genes). As previously observed for class II genes, this stimulation of class III gene transcription was much greater for transfected genes than for the major endogenous cellular class III genes. Extracts made from cell lines stably expressing a transfected pseudorabies virus IE gene were 10 to 20 times more active in the in vitro transcription of exogenously added class III genes than extracts of the parental cell line. These results indicate that the E1A and IE proteins stimulate the expression of class III genes by a mechanism similar to the mechanism for stimulation of class II gene transcription by these proteins.*

RICHARD B. GAYNOR
Department of Hematology/Oncology, UCLA School of Medicine, Los Angeles, California 90024
LARRY T. FELDMAN
Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, California 90024
ARNOLD J. BERK
Department of Microbiology and *Molecular Biology Institute, University of California, Los Angeles*

Genetic studies have led to the discovery of viral proteins that induce viral gene transcription. In the adenoviruses, which have a duplex DNA of about 36 kilobase pairs, the largest protein (289R) encoded in the leftmost transcription unit called E1A, stimulates transcription from five viral promoters transcribed by RNA polymerase II (*1*, *2*). In the herpesviruses, which have genomes of about 150 kbp, immediate early (IE) proteins are required for transcription of an even larger set of viral promoters (*3*).

The adenovirus E1A protein, unlike well-studied positive activators of transcription in prokaryotic systems such as the adenosine 3′5′-monophosphate (cyclic AMP) binding protein of *Escherichia coli* (*4*) or sigma-like factors of the *Bacillus subtilis* phage SPO1 (*5*), is not absolutely required for the transcription of the genes under its control. After infection of HeLa cells by an Ad5 E1A deletion mutant, d1312 (*6*), transcription from early viral promoters occurs, although it is greatly delayed compared to

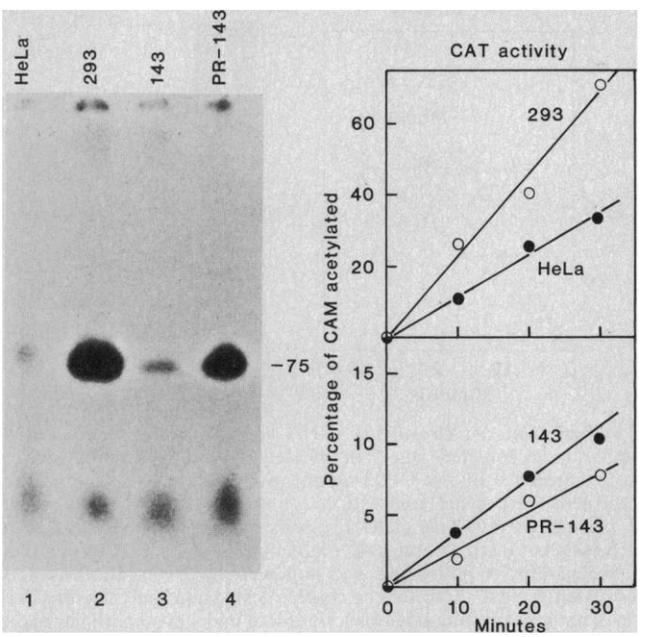

Fig. 1. Transcriptional activation of the Ad2 VA-I gene by viral early proteins. Two 100 m*M* plates of HeLa (lane 1), 293 (lane 2), 143 (lane 3), and PR-143 (line PR14) (lane 4) cells were cotransfected with 15 μg of a plasmid containing the adenovirus VA-I and VA-II genes (*28*) and 5 μg of the RSV-CAT plasmid (*23*). Total cytoplasmic RNA was harvested from one plate 48 hours after transfection (*26*), and 100 μg of RNA was analyzed by S1 nuclease analysis with a 96-bp 5′ end-labeled Bam HI–Sal I DNA fragment (*28*). Hybridized VA-I RNA protects a 75-nucleotide fragment from digestion by S1. The remaining 100 m*M* plate from each transfection was harvested and analyzed for CAT activity as described (*23*). The percentage of conversion of [^{14}C]chloramphenicol (CAM) to the acetylated form at 10, 20, and 30 minutes of reaction catalyzed by 300 μg of protein extract is shown at the right.

the time course observed after infection with wild-type Ad5 (*2*). Although the E1A proteins clearly act in *trans* to stimulate early viral transcription (*1, 7, 8*), the delayed transcriptional activation observed in d1312-infected HeLa cells is a *cis*-acting process (*9*). This observation led us to suggest that the E1A protein facilitated the assembly of viral DNA into stable transcription complexes (*9*).

Pseudorabies virus (PRV), a member of the herpesvirus group, encodes a single 180-kilodalton IE protein that is required for transcription of PRV early and late genes (*10*). This PRV-IE protein has an activity similar to that of the adenovirus E1A protein, since it can also induce the transcription of early adenovirus genes in HeLa cells infected with both PRV and d1312 (*11*). There is no significant DNA sequence homology between Ad5 and PRV. Therefore, the observation that both the Ad5 E1A protein and the PRV-IE protein can induce early Ad5 genes suggested that these proteins acted indirectly—for example, by modifying or inducing the same host-cell factor (or factors)—rather than by interacting directly with specific sequences in the Ad5 early promoter regions (*11*).

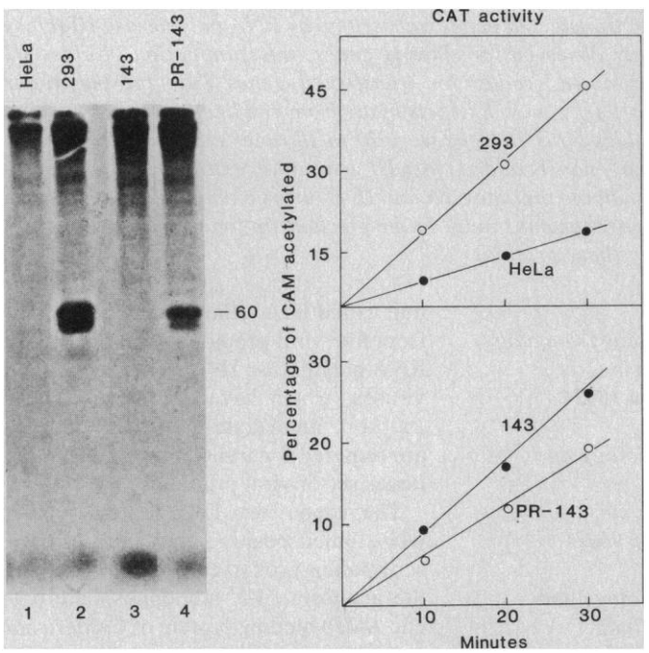

Fig. 2. Transcriptional activation of the *Drosophila* tRNA arginine gene by viral early proteins. HeLa (lane 1), 293 (lane 2), 143 (lane 3), and PR-143 cells (line PR14) (lane 4) were cotransfected with 15 μg of a plasmid containing the *Drosophila* tRNA arginine gene (*31*) and 5 μg of the RSV-CAT plasmid. Total cytoplasmic RNA was harvested 48 hours after transfection, and 100 μg of RNA was analyzed by S1 nuclease analysis with a 920-bp 5′ end-labeled Hinf I probe. Hybridizations were performed at 46°C for 12 hours; S1 digestion, electrophoresis, and autoradiography followed. Hybridized tRNA arginine protects a 60-nucleotide fragment from S1 digestion. A 100 m*M* plate from each transfection was harvested and analyzed for CAT activity. The percentage of conversion of [^{14}C]chloramphenicol (CAM) to the acetylated form at 10, 20, and 30 minutes after the reaction was initiated by addition of 300 μg of protein from each extract is shown at the right.

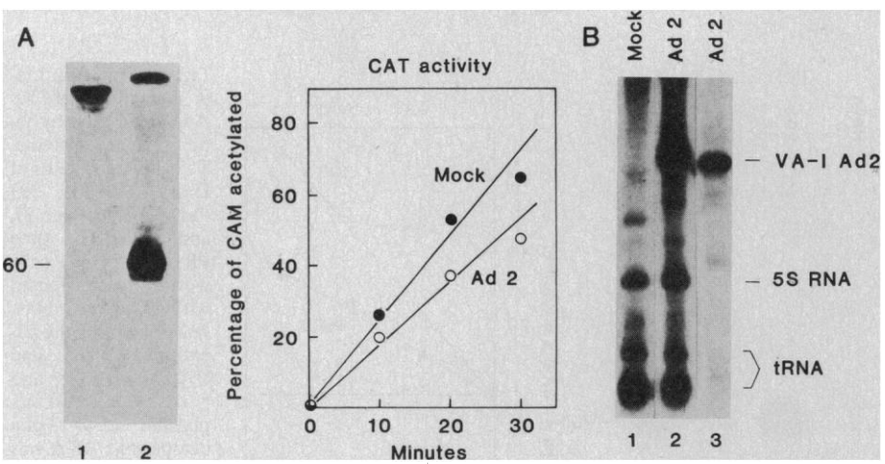

Fig. 3. (A) Transcription activation of the *Drosophila* tRNA arginine gene by adenovirus infection. HeLa cells were either mock-infected (lane 1) or infected with Ad2 at a multiplicity of infection of 100 (lane 2) in the presence of Ara-C (20 μg/ml). Six hours after infection, the medium was removed and the cells were transfected with 15 μg of the tRNA arginine plasmid and 5 μg of the RSV-CAT plasmid per 100 m*M* plate. Fresh medium containing Ara-C was added, and cytoplasmic RNA was harvested 48 hours after infection. S1 nuclease analysis was performed with a 5′ end-labeled Hinf I probe and was followed by electrophoresis and autoradiography. A 100 m*M* plate from each transfection was harvested and analyzed for CAT activity. (B) E1A protein does not greatly stimulate transcription of endogenous cellular class III genes. HeLa cells were either mock-infected (lane 1) or infected with wild-type Ad2 (lane 2) at a multiplicity of infection of 100 in the presence of Ara-C. $^{32}PO_4$ labeling was performed 34 to 36 hours after infection, and 10 μg of cytoplasmic RNA was subjected to electrophoresis on 8 percent polyacrylamide gels with 8*M* urea and analyzed by autoradiography. Lane 3 shows transcripts transcribed in vitro from Ad2 DNA by a HeLa cell S100 extract (*38*).

Since the E1A and IE proteins are nonspecific in their ability to stimulate transcription of both viral and nonviral class II genes, we asked whether these proteins could also stimulate expression of genes transcribed by RNA polymerase III. To study the effects of the E1A and PRV-IE proteins on class III genes, we examined the level of transcription of the Ad2 VA-I gene after transfection into several human cell lines. The cell line used to study the effect of the E1A protein was the 293 cell line, a human embryonic kidney cell line transformed with the left 14 percent of the adenovirus genome (*18*). The 293 cells constitutively express E1A (and E1B) proteins (*19*). HeLa cells were used as the E1A protein–negative (control) cells. The cell line used to study the effect of the PRV-IE protein was PR-143, derived from 143, a human osteogenic sarcoma cell line (*20*). The 143 cells were stably cotransformed with the plasmid pSVneo, which produced resistance to the antibiotic G418 (*21*), and a cloned gene encoding the PRV-IE protein (*22*). The PR-143 cell line produced PRV-IE protein as determined by Western immunoblot analysis and also complemented the ade-

novirus mutant d1312 for the expression of the DNA-binding protein encoded in adenovirus E2A. The parental 143 cells were used as PRV-IE protein–negative (control) cells.

Because the percentage of cells that can be transfected varies in different cell lines, a control was needed to correct for differing transfection efficiencies of the cell lines used in these experiments. A plasmid containing the long terminal repeat of Rous sarcoma virus (RSV) fused to the chloramphenicol acetyltransferase (CAT) gene (*23*) was included in each of the transfections as an internal control. E1A proteins do not further induce transcription from class II promoters closely linked to a strong viral enhancer such as that found in the RSV long terminal repeat (*8, 12*). Cotransfection into HeLa cells of a plasmid containing the Ad2 E1A gene and of the RSV-CAT plasmid results in levels of CAT expression that are similar (within a factor of 2) to single transfections with RSV-CAT (data not shown). This is in contrast to the reported suppression of transfected genes linked to the SV40 (*24, 25*) and polyoma (*24*) enhancers by cotransfection of the E1A gene. Thus, comparing the level of CAT activity produced after transfection of two different cell lines with the same amount of RSV-CAT was a measure of the transfection efficiency of the two cell lines.

The adenovirus VA-I gene is transcribed by RNA polymerase III (polIII) (*26*). During adenovirus infection, VA-I RNA is required for efficient translation of late viral messenger RNA's (mRNA's) (*27*). Plasmids containing the VA-I gene (*28*) and the RSV-CAT gene (*23*) were transfected into 293 cells, HeLa cells, PR-143 cells, and 143 cells. Cytoplasmic RNA was harvested 48 hours after transfection (*29*), and the VA-I RNA was examined by S1 nuclease analysis with an end-labeled VA-I DNA probe (*30*) (Fig. 1). Approximately 40 times more VA-I RNA was transcribed in transfected 293 cells than in HeLa cells, and about 20 times more in PR-143 cells than in 143 cells, as determined by densitometry of the 75-nucleotide S1 protected fragment (Fig. 1). CAT activity 48 hours after transfection was twice as great for 293 cells as for HeLa cells, indicating that the efficiency of transfection of the 293 cells was about twice that of the HeLa cells. But this twofold difference could not account for the 40-fold difference in the amounts of VA-I RNA transcribed in the two cell lines. The PRV-143 cells and 143 cells expressed approximately the same level of CAT activity. Thus, expression of either the E1A or PRV-IE proteins stimulated transcription of the transfected VA-I gene by a factor of approximately 20. This difference was not due to differences in transfection efficiencies between the cell lines.

Next, we asked whether the E1A and PRV-IE proteins could induce transcription of a nonviral class III gene. We chose a *Drosophila* transfer RNA (tRNA) arginine gene that has been well characterized (*31*) and produces a transcript that can be distinguished from the background of human tRNA's. A plasmid clone of the tRNA arginine gene (*31*) was cotransfected with the RSV-CAT plasmid into the same set of cells as were used in the VA-I study. Cytoplasmic RNA was harvested 48 hours after transfection and subjected to S1 nuclease analysis with an end-labeled DNA probe specific for the tRNA arginine gene. The amount of tRNA arginine transcribed in transfected 293 and PR-143 cells was at least 50 times that in HeLa and 143 cells, as determined by densitometry of the 60-nucleotide S1-protected fragment (Fig. 2). CAT activity 48 hours after transfection was three times greater in extracts of 293 cells than in HeLa cells, while the PR-143 cells and 143 cells had less than a twofold difference in the level of CAT activity, indicating that the large differences in tRNA gene transcription were not the result of differences in the efficiency of transfection. Thus, for this cellular class III gene as well as the VA-I gene, expression of either the E1A or PRV-IE proteins greatly stimulated transcription of the transfected gene.

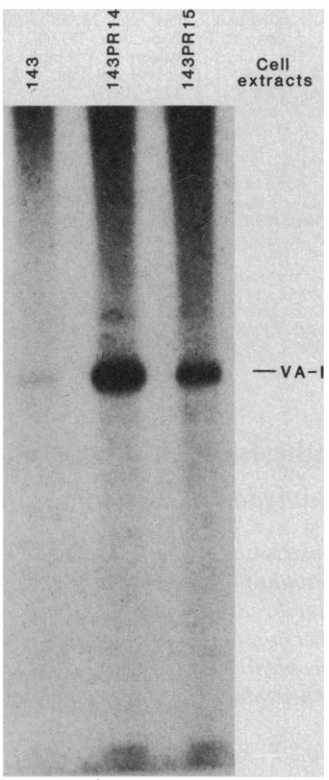

Fig. 4. Increased activity for in vitro transcription of class III genes by extracts of cells containing pseudorabies virus IE proteins. S100 extracts were prepared as described (*38*) from 143 cells or 143 cells expressing the pseudorabies virus IE protein (PR14 and PR15 cell lines). Protein (20 μg) was added to 40-μl in vitro transcription reactions (*38*) with 0.5 μg of VA-I plasmid DNA as template. Reactions were incubated for 1 hour at 30°C; RNA was extracted and analyzed by electrophoresis on an 8 percent polyacrylamide 8*M* urea gel, followed by autoradiography.

The transfected tRNA gene could also be induced by adenovirus infection of HeLa cells. HeLa cells were either mock-infected or infected with Ad2 and then cotransfected with the tRNA arginine gene and the RSV-CAT gene 6 hours after infection. Arabinosylcytosine (Ara-C) was added to the culture to prevent viral DNA replication, thereby extending the early phase of infection. Prolonged treatment of Ad2-infected HeLa cells with Ara-C also results in high E1A protein concentrations, 10 to 20 times higher than those observed in the early phase of infection of untreated HeLa cells (*32*). S1 nuclease analysis of cytoplasmic RNA isolated 48 hours after infection showed that adenovirus infection induced transcription of the transfected tRNA gene (Fig. 3A). Infection with the E1A deletion mutant d1312 failed to induce transcription of this class III gene. Thus, like the class II genes β-globin (*12, 13*) and rat preproinsulin I (*14*), the E1A proteins induce transcription of a newly introduced nonvirual class III gene as well as a viral class III gene.

E1A proteins stimulate the expression of transfected class II genes, yet do not stimulate transcription from most endogenous class II genes (*17*). Early studies of adenovirus-infected HeLa cells showed that the transcription of cellular tRNA and 5*S* ribosomal RNA (rRNA) was not stimulated by adenovirus infection and the resulting expression of E1A proteins (*33*), To confirm this result, we analyzed the transcription of endogenous cellular class III genes in adenovirus-infected HeLa cells that showed high expression of transfected class III genes. HeLa cells were infected with Ad5 or were mock-infected, treated with Ara-C as in the transfection experiments above, and labeled with $^{32}PO_4$ from 34 to 36 hours after infection, a time when E1A protein levels reach high concentrations in Ara-C–treated cells. Total cytoplasmic RNA was isolated and subjected to

electrophoresis on an 8 percent polyacrylamide gel to resolve low molecular weight RNA's that were detected by autoradiography (Fig. 3B). VA-I RNA was synthesized at high levels in the adenovirus-infected cells. However, synthesis of the major endogenous cellular class III genes, 5*S* rRNA and tRNA genes, changed little with viral infection. We did not determine whether less abundant endogenous polIII genes were induced by viral IE proteins. Expression of a reiterated class III gene is increased in SV40-transformed mouse cell lines (*34*).

From these results we conclude that for class III genes as well as for class II genes, the expression of E1A proteins does not greatly affect the transcription of the major endogenous cellular genes, even though it has significant effects on the transcription of genes introduced by transfection. Berger and Folk (*35*) also observed that transcription of transfected VA-I and tRNA genes in 293 cells and in adenovirus-infected, Ara-C–treated HeLa cells was greater than that in uninfected HeLa cells.

Several investigators have reported that gene expression after transfection can be affected by factors such as altered stability of the transfected DNA in different cell lines (*36*) and the transfection technique used (*37*). To rule out such possible artifacts of transfection experiments, we analyzed the effect of viral IE proteins on class III gene transcription by another, independent approach. S100 extracts (*38*) were prepared from 143 cells and two cotransformed 143 cell lines, PR14 and PR15, which constitutively express the pseudorabies IE protein. When these extracts were assayed for in vitro transcription activity with exogenous class III gene templates, the PR14 and PR15 cell extracts were 10 to 20 times more active than extracts of the parental 143 cells (Fig. 4). This high in vitro polIII gene transcriptional activity of PR14 and PR15 cell extracts correlates well with the stimulation of class III gene transcription in transfection experiments in which PR14 and 143 cells are compared (Figs. 1 and 2). Other experiments showed that 293 cell extracts and extracts of adenovirus-infected, Ara-C–treated HeLa cells had significantly greater polIII transcriptional activity in vitro than extracts of uninfected HeLa cells (*39*). Thus, both transfection and in vitro transcription data demonstrate that viral immediate early proteins can activate class III gene transcription.

Analysis of VA-I and tRNA gene transcription in vitro has revealed that active templates are assembled into stable transcription complexes in which at least two protein factors are stably associated with the DNA template and do not dissociate and bind to a second polIII promoter added to an in vitro reaction (*40*). The E1A and IE proteins might activate class III genes by allowing more DNA molecules to be assembled into stable transcription complexes or by modifying a component of the stable transcription complexes, in such a way that the rate of transcription initiation from each complex is increased. Further analysis of extracts prepared from cells expressing E1A and IE proteins should distinguish between these and other possibilities.

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Synthesis of Chlorophyllide b from Protochlorophyllide in *Chlamydomonas reinhardtii* y-1

Abstract. *In cells of* Chlamydomonas reinhardtii *y-1 kept in the dark, 1,7-phenanthroline stimulated the conversion of protochlorophyllide to chlorophyllide b. A membrane fraction was obtained from degreened cells that was active in this conversion only when phenanthroline was present. Untreated cells excreted protochlorophyllide, which was used as substrate for this in vitro reaction. This system may provide a clue to how chlorophyllide b is synthesized in plant cells.*

Daniel P. Bednarik
J. Kenneth Hoober*
Department of Biochemistry, Temple University School of Medicine, Philadelphia, Pennsylvania 19140

*To whom correspondence should be addressed.

Although chlorophyll a (chl a) is found in all photosynthetic plant cells, its oxidized analog, chlorophyll b (chl b), is present only in the higher plants and green algae. Both forms are essential for proper assembly of the major light-harvesting chl-protein complex in chloroplast membranes (*1*). Chlorophyllide a (chlide a) (*2*) is synthesized from protochlorophyllide (pchlide) by a reduction of pyrrole ring D catalyzed by nicotinamide adenine dinucleotide phosphate (NADPH) pchlide oxidoreductase (Fig. 1) (*3*). In most higher plants, but only a few algal strains, this reaction requires light. Synthesis of chl b, however, remains an enigma; no enzymatic activity has been identified for this process, nor has the immediate precursor been determined. It has been presumed, but not