also displaced laterally from the molecular axis. These findings may be accounted for simply when the two shortest chains—Bβ and γ—terminate independently in globular regions (β and γ domains). This assignment, as noted by Williams (*13*), follows from the location of the γ-γ cross-linking sites (*18*) at the end of the fibrinogen molecule and from the binding sites involved in fibrin formation at the COOH-terminal end of the γ chains (*19–21*). It is also consistent with other aspects of the amino acid sequence. Although the COOH-terminal sequences of these two chains are highly homologous, there is an additional internal disulfide linkage in the Bβ chains. This linkage could shift the position of the β domain closer to the center of the molecule relative to that of the γ domain.

Erickson and Fowler's study (*11*) also indicated that the COOH-terminal portion of the two longer Aα chains of native fibrinogen folds back from the end of the rod to form a small globular region (an eighth, or α, domain) located near the central region of the molecule. (Since this eighth domain is the extra central region we observe in images of shadowed native fibrinogen, but not in the protease-modified molecules, this portion of the molecule is not represented in heptad models.) The finding that this feature is seen in only some native molecules could be due to superposition with the central domain in some views, to the susceptibility of the Aα chain to proteolysis, or to some unfolding of the structure during preparation for microscopy. Thus, the COOH-terminal region of each of the three chains of fibrinogen appears to be folded independently into a globular domain.

Our suggestion that the protease-modified molecule differs from the native molecule chiefly in the loss of the COOH-terminal portion of the Aα chains is consistent with the fact that the modified molecule retains many biological functions. Cleavage in these flexible single chain regions by the enzyme from *Pseudomonas* and a variety of other enzymes (*4, 6, 8*) appears at present to be a necessary step in crystallizing fibrinogen.

A schematic representation of the folding of the polypeptide chains in fibrinogen, based on the three-dimensional heptad model, is shown in Fig. 5. Here the globular domains of the model are viewed as in Fig. 2e. The central and end domains are connected by a rodlike region shown as a three-chain α-helical coiled coil, interrupted by a small randomly folded, plasmin-sensitive domain made up of all three chains. The β and γ domains are shown for schematic purposes as random coils, since optical rotation studies indicate that the α-helical content of the molecule [about 30 percent (*22*)] can be accounted for by the coiled-coil region. In addition to the seven globular domains in the model, Fig. 5 also depicts the α domain formed by the COOH-termini of the Aα chains folding back to interact at the center of the molecule as would be seen in native fibrinogen.

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26. We thank B. Elliott and R. C. Williams for comments on the manuscript, S. Almeda for biochemical assistance, J. Black for photography, D. Hellerstein for computing, and L. Seidel for typing. Supported by an Established Investigatorship of the American Heart Association, an NIH grant (HL30954), and a Grant-in-Aid from the Pennsylvania Chapter of the American Heart Association (to J.W.W.); a postdoctoral fellowship from the Arthritis Foundation (to C.V.S.); a Biochemistry Training Grant from NIH (GM07596-07) (to E.B.); a grant from NIH (AM17346) (to C.C.); an NIH-shared instrumentation grant (3-RO1-GM21189-09S1) at Brandeis University to D. J. DeRosier; NIH instrumentation grants at the University of Pennsylvania to John Murray (RR01907), to Terrence Frey (GM28750), and Michael Summers (RR01747), and an NSF grant to Dr. Frank Pepe (PCM82-19960).

14 June 1985; accepted 5 October 1985

Repression of the Immunoglobulin Heavy Chain Enhancer by the Adenovirus-2 E1A Products

Abstract. *The products of the adenovirus-2 (Ad2) immortalizing oncogene E1A repress the activity of the SV40, polyoma virus and E1A enhancers. Evidence is presented that Ad2 infection of MPC11 plasmocytoma cells results in an inhibition of transcription of both the γ2b heavy chain (IgH) and the kappa light chain immunoglobulin genes. This inhibition is caused by the Ad2 E1A products. Furthermore, the Ad2 E1A products repress transcription activated by the immunoglobulin heavy chain enhancer in chimeric recombinants, which are either stably integrated in the genome of lymphoid cells or are present as episomes. The implications of negative regulation of cellular enhancers are discussed.*

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During adenovirus infection E1A is the first gene expressed. Early in infection it yields two messenger RNA's (mRNA's) (12*S* and 13*S*) coding for proteins of 243 and 289 amino acids, respectively. These proteins stimulate transcription of the other early adenovirus genes (*1, 2*) and of a number of cellular genes (*3*). They can also repress the activity of the SV40 and polyoma virus enhancers (*4, 5*). Enhancers are *cis*-acting promoter elements that are required for efficient transcription of some viral and cellular genes and can also activate transcription from heterologous promoters. The activity of some enhancers is restricted to particular cell types or physiological stages and therefore they may have a role in the regulation of gene expression in eukaryotes (*6*). In that expression of the E1A proteins can lead to immortalization of primary cultured cells (*7*), this oncogenic property may possibly be mediated in part by the repression of some cellular enhancers. We have therefore studied the effect of the E1A products on the activity of a well-characterized cellular enhancer, the immunoglobulin heavy

chain (IgH) enhancer (*8*), which, in contrast to the polyoma virus and SV 40 enhancers, exhibits a narrow cell specificity, being active only in lymphoid cells.

We now report here that the E1A products inhibit transcription of the immunoglobulin γ2b heavy chain gene in MPC11 plasmacytoma cells, either during adenovirus infection or after integration of a recombinant expressing the E1A products into the genome of these cells. We show that this inhibition is due to the repression of the activity of the IgH enhancer.

The MPC11 mouse plasmacytoma cell line expresses constitutively a γ2b heavy chain and a κ light chain. We used a thymidine kinase–deficient (tk^-) derivative of this cell line (*9*). To analyze the effects of the E1A products on the transcription of these genes, MPC11 cells were infected with adenovirus-2 (Ad2) at high multiplicity [100 plaque-forming units (pfu)]. Cytoplasmic RNA was extracted and nuclei were prepared from these cells 5 and 15 hours after infection. We used specific DNA probes and quantitative S1 nuclease analysis to determine the amount of RNA transcribed from the γ2b, Ad2 E1A, Ad2 E2A early, and the major histocompatibility complex (MHC) class I H2-K genes (Fig. 1). The level of the γ2b mRNA was significantly decreased 5 hours after infection (Fig. 1A, lanes 2 and 3) and was not detectable 15 hours after infection (Fig. 1A, lanes 4 and 5). Similar results were obtained when the cells were infected with adenovirus-5 (Ad5). To determine whether the E1A products were in fact responsible for these decreases, we infected the cells at high multiplicity (100 pfu) with the Ad5 mutant dl312, which lacks the E1A transcription unit (*10*). At a high multiplicity of dl312 infection the expression of the other early viral genes is delayed (2), and we therefore determined the level of E2A early mRNA to follow the course of the infection. The same level of E2A early mRNA was seen 36 hours after dl312 infection as 5 hours after Ad2 wild-type infection (Fig. 1C, lanes 3 and 7), although E1A transcripts could not be detected in dl312-infected cells (Fig. 1B, compare lanes 3, 6, and 7). Under these conditions of dl312 infection, there was no decrease in γ2b mRNA (Fig. 1A, lanes 6 and 7). Neither Ad2 nor dl312 infection affected the mRNA level of another gene expressed in MPC11 cells, the MHC class I H2-K gene (Fig. 1D).

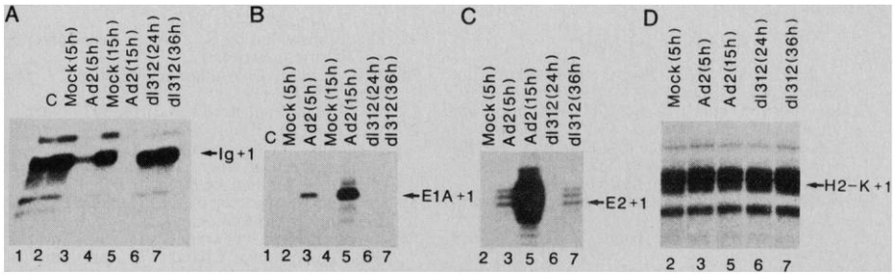

Fig. 1. Quantitative S1 nuclease analysis (*4*) of cytoplasmic RNA (10 μg) extracted from MPC11 cells either mock-infected or infected by adenovirus-2 or by the Ad2 mutant dl312 at high multiplicity (100 pfu). (Lane 1) Control cells; (lane 2) mock-infected cells, harvested after 5 hours;(lane 3) Ad2-infected cells harvested after 5 hours; (lane 4) mock-infected cells harvested after 15 hours; (lane 5) Ad2-infected cells harvested after 15 hours; (lane 6) dl312-infected cells harvested after 24 hours; (lane 7) dl312-infected cells harvested after 36 hours. The RNA was analyzed with a γ2a probe (A), an E1A probe (B), an E2A early probe (C), or an H2-K^d probe (D). These probes are single-stranded DNA fragments labeled with ^{32}P at the 5′ end. To probe the MPC11 γ2b gene transcript, we used a fragment of the γ2a constant region that is homologous to the γ2b constant region [a Bam HI fragment containing a portion of the first exon of the constant region (*27*), which yields a protected fragment of 134 nucleotides (Ig+1)]. The Ad2 E1A probe is an Eco RI–Sau 3A fragment from recombinant pE1ASV (*4*) (Fig. 3) yielding a protected fragment of 130 nucleotides (E1A+1). The Ad2 E2A early probe is a Sma I–Sau 3A fragment from recombinant pBX (*28*) giving a protected fragment of 47 nucleotides (E2+1). The mouse H2-K^d probe is an Ava I–Kpn I fragment from a complementary DNA (cDNA) clone of a MHC class I H2-K^d gene (*29*), which yields a protected fragment of 120 nucleotides (H2-K+1). The conditions for S1 mapping are described (4). When the γ2a and H2-K^d probes were used, the S1 nuclease digestion was performed at 16°C, instead of at 25°C, because of the imperfect homology between the γ2a probe and the MPC11 γ2b mRNA (*27*), and between the H2-K^d probe and the MPC11 H2-K mRNA.

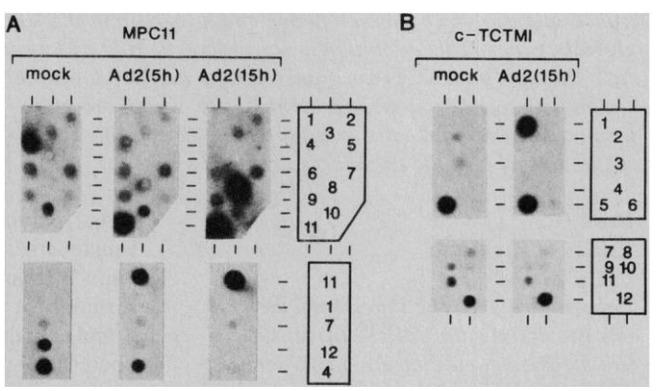

Fig. 2. (A) Nuclear run-on experiments on nuclei from MPC11 cells mock-infected or infected by Ad2, at high multiplicity (100 pfu), for 5 or 15 hours. Nuclei were prepared and incubated in the presence of [^{32}P] CTP (cytidine triphosphate) (*4*). The RNA was extracted and hybridized to nitrocellulose filters dotted with various DNA's. Hybridization and washing were as described (*2*). The following DNA's (200 ng, each) were dotted: (1) pBR322; (2) rat cDNA clone corresponding to lactate dehydrogenase (*30*); (3) mouse cDNA clone corresponding to the MHC class I H2-K^d gene (*29*); (4) mouse genomic clone corresponding to the γ2a constant region (*27*); (5) SV 40 DNA; (6) rat cDNA clone corresponding to enolase (*30*); (7) rat cDNA clone corresponding to triose phosphate isomerase (*30*); (8) Ad2 Sma I–Hind III fragment corresponding to the E2A early transcription unit; (9) rat cDNA clone D5 (*30*); (10) rat cDNA clone corresponding to glyceraldehyde phosphate dehydrogenase (*30*); (11) E1A Eco RI–Bam HI fragment from recombinant pE1ASV (extending from position −500 to +1070) (Fig. 3); (12) κ light chain genomic clone (*31*). (B) Run-on experiments with nuclei from c-TCTMI cells (legend to Fig. 5B) that have been either mock-infected or infected with Ad2 at high multiplicity (100 pfu) for 15 hours. The following DNA's (200 ng each) were dotted: (1) E1A fragment (see above); (2) γ2a constant region; (3) SV 40 DNA; (4) pBR322; (5) H2-K^d mouse genomic clone containing a repetitive sequence (*29*); (6) H2-K^d mouse cDNA clone (*29*); (7) pBR322; (8) rat actin cDNA clone (*30*); glyceraldehyde phosphate dehydrogenase cDNA; (10) lactate dehydrogenase cDNA; (11) D5 cDNA (see above); and (12) mouse retrovirus-like repetitive sequence VL30 (*30*).

To show that the specific decrease observed in γ2b mRNA accumulation after Ad2 infection is due to an inhibition of transcription, we performed a series of run-on experiments with nuclei from MPC11 cells either mock-infected or infected with Ad2 for 5 or 15 hours (Fig. 2A). The run-on experiment was done under conditions that do not allow RNA polymerase to reinitiate (2). It thus reflects the RNA polymerase density on the DNA template.

The transcription of the γ2b gene decreased 5 hours after Ad2 infection and disappeared at 15 hours (Fig. 2A, dot 4). A similar inhibition of transcription of the immunoglobulin κ light chain gene was observed (Fig. 2A, dot 12), while transcription of a number of housekeeping cellular genes remained constant (Fig. 2A, dots 2, 3, 6, 7, 9, 10). In particular, transcription of the H2-K gene was not affected by Ad2 infection (Fig. 2A, dot 3). To follow the infection

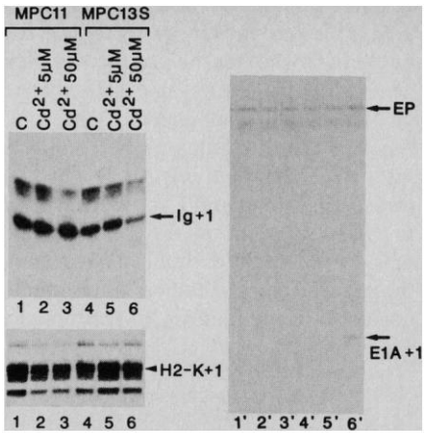

Fig. 3. Quantitative S1 nuclease analysis of cytoplasmic RNA (10 μg) from MPC11 cells (lanes 1, 2, 3; 1′, 2′, 3′) or MPC13S cells (lanes 4, 5, 6; 4′, 5′, 6′). MPC13S cells were obtained by cotransformation of the MPC11 cells with a plasmid containing the E1A 13*S* cDNA (*2*) under the control of the human metallothionein II promoter (*11, 32*) and recombinant pY3 carrying the hygromycin-resistance marker (*12*). Control (C) cells (lanes 1 and 1′, 4 and 4′); cells incubated during 15 hours with 5 μ*M* $CdCl_2$ (lanes 2 and 2′, 5 and 5′) or 50 μ*M* $CdCl_2$ (lanes 3 and 3′, 6 and 6′, respectively). The RNA was analyzed with the γ2a probe (Ig+1; lanes 1 to 6), the H2-K^d probe (H2-K+1; lanes 1 to 6), or the E1A probe (E1A+1; lanes 1′ to 6′). The DNA probes are as described in the legend to Fig. 1. EP corresponds to the full-length undigested E1A probe.

we analyzed the transcription of the Ad2 E1A and E2 transcription units (Fig. 2A, dots 11 and 8, respectively). As expected, E1A was already efficiently transcribed 5 hours after Ad2 infection, while the E2 signal was still weak. The transcription of both E1A and E2 increased between 5 and 15 hours after infection. These results parallel those obtained by analysis of cytoplasmic RNA's (Fig. 1) and demonstrate that transcription of the γ2b and κ genes is inhibited by the Ad2 E1A products.

We attempted to integrate the E1A transcription unit into the genome of MPC11 cells. We did not succeed in establishing a cell line containing the entire E1A gene, possibly because of the "toxicity" of the E1A proteins. However, transformants containing the complementary DNA (cDNA) encoding the E1A 13*S* mRNA under the control of the human cadmium-inducible metallothionein II promoter (*11*) were obtained. Among the cell lines that integrated this chimeric construction together with the recombinant pY3, which confers hygromycin-resistance (*12*), only one cell line (MPC13S) expressed a low level of 13*S* mRNA after a 15-hour exposure to 50 μ*M* cadmium (Fig. 3, lane 6′). No 13*S* mRNA could be detected in the absence of cadmium or in the presence of 5 μ*M* cadmium (Fig. 3, lanes 4′ and 5′). When RNA extracted from MPC13S cells was analyzed with the γ2b probe, a two- to threefold reduction in γ2b RNA was detected in the presence of 50 μ*M* cadmium (Fig. 3, lane 6). This decrease was observed in four independent experiments. When the same amount of cadmium (5 to 50 μ*M*) was added to the original MPC11 cells, the level of γ2b RNA remained constant (Fig. 3, lanes 1 to 3). The level of the H2-K RNA was not affected by cadmium in MPC11 cells, and was therefore used as a control in the transformed MPC13S cells, where it remained roughly constant (Fig. 3, see H2-K+1, lanes 1 to 6). The magnitude of the reduction in γ2b RNA was much less in cadmium-induced MPC13S cells than in Ad2-infected MPC11 cells (Fig. 1A), probably because of the low level of the E1A mRNA obtained after cadmium induction (about 50-fold less than 5 hours after Ad2 infection) (Fig. 1B, lane 3, and Fig. 3, lane 6′).

To ascertain whether the target for inhibition of γ2b gene transcription is the IgH enhancer, a recombinant in which the activity of the chicken conalbumin promoter is under the control of this enhancer was used (Fig. 4). The recombinant pTCT, which contains only the conalbumin promoter, and the recombinant pTCTMI (pTCT plus IgH enhancer) were transfected in MPC11 cells, either alone or in conjunction with either pE1A$^-$ (a plasmid that lacks most of the E1A coding region) or pE1ASV (a recombinant that contains the entire E1A transcription unit) (Fig. 4) (*4*). Cytoplasmic RNA was extracted 36 hours after transfection and analyzed by S1 mapping with probes corresponding to RNA initiated from the conalbumin and E1A promoters (Fig. 5A). As expected from studies of the conalbumin (Con) promoter (*13*), no RNA transcribed from the recombinant pTCT could be detected when transfected alone (*13*) or with pE1A$^-$ (Fig. 5A, Con+1, lane 1). However, in agreement with the results of others (*8*), recombinant pTCTMI, which contains the IgH enhancer, was efficiently transcribed when transfected alone or with pE1A$^-$ (Fig. 5A, lane 3). When pTCTMI was cotransfected with pE1ASV, the RNA initiated from the conalbumin promoter dramatically decreased (Fig. 5A, lanes 3 and 4), whereas no change was noticed for pTCT (Fig. 5A, lanes 1 and 2; in the presence of either pE1A$^-$ or pE1ASV, similar weak conalbumin signals were seen on a longer exposure of the autoradiogram). This decrease was not due to a competition between pTCTMI and pE1ASV promoters, since the amount of RNA transcribed from the conalbumin promoter was identical in control experiments where pTCTMI was transfected alone or with pE1A$^-$. As previously reported, the decrease in cytoplasmic RNA observed with pE1A$^-$, compared to pE1ASV (Fig. 5A, E1A+1), is not related to a lower efficiency of the E1A promoter in pE1A$^-$ but to a decreased stability of the truncated E1A$^-$ RNA (*2*).

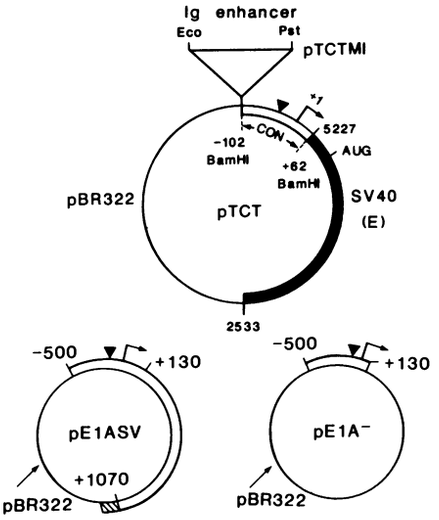

Fig. 4. Recombinant pTCT (*13*) contains the chicken conalbumin promoter linked to the SV 40 T-antigen coding sequence. Recombinant pTCTMI was derived from pTCT by inserting the Eco RI–Pst I fragment containing most of the IgH enhancer (*8*) into the Bam HI site located 102 nucleotides upstream from the conalbumin startsite (*13*). pE1ASV contains the entire Ad2 E1A transcription unit from position −500 to +1070, while pE1A$^-$ contains only the E1A promoter region from position −500 to +130 (*3*).

To demonstrate that the activity of the IgH enhancer can be repressed by the E1A products, not only on an episome (pTCTMI), but also when integrated into the MPC11 cell genome, MPC11 cells were transformed with recombinants pTCT or pTCTMI with the herpes simplex virus I thymidine kinase (tk) gene as a selection marker (*14*). These transformed cloned cells (c-TCT and c-TCTMI) were infected with Ad2, and the RNA extracted 15 hours later was analyzed by S1 nuclease mapping with probes corresponding to RNA's initiated at the conalbumin (Con+1) or at the tk (TK+1) promoters (Fig. 5B). Again, there was a decrease in RNA transcribed from the conalbumin promoter when the cell line containing pTCTMI (c-TCTMI) was infected with Ad2, whereas transcription from the tk promoter in the same cells remained unchanged (Fig. 5B, lanes 3 and 4). In contrast, infection of the cell line containing pTCT (c-TCT) did not result in any significant change in RNA transcribed from either the conalbumin or tk promoters (Fig. 5B, lanes 1

and 2). Run-on transcription assays with nuclei from c-TCTMI cells, either mock-infected or infected by Ad2, show that the above decrease was due to an inhibition of transcription activated by the IgH enhancer and initiated at the conalbumin promoter (Fig. 2B, compare spot 3 in mock- and Ad2-infected cells). This inhibition paralleled that for the endogenous γ2b gene (Fig. 2B, spot 2). Transcription of other cellular genes was not affected by adenovirus infection (Fig. 2B, spots 8, 9, 10, and 11). Transcription of two repetitive sequences remained also unchanged (Fig. 2B, spots 5 and 12). As a control for ad infection, we analyzed E1A gene transcription which was, as expected, abundant 15 hours after infection (Fig. 2B, spot 1). Thus transcription activated by the IgH enhancer can be repressed by the Ad-2 E1A products, whether the enhancer is in an extrachromosomal location or stably integrated in the genome.

The inhibition described is most likely due to repression of the IgH enhancer, since its ability to stimulate in *cis* transcription from the chicken conalbumin promoter is almost completely suppressed by the Ad2 E1A products. Repression has been observed whether the recombinant pTCTMI containing the IgH enhancer upstream from the conalbumin promoter is present in an episomal form or stably integrated in the MPC11 cell genome, indicating that the IgH enhancer can be repressed by the E1A products even in a chromosomal environment. The inhibition of transcription of the κ light chain gene in the same cells infected by Ad2 suggests that the κ light chain enhancer (*15*) is similarly repressed by the E1A products.

In contrast to the SV 40 enhancer, which exhibits very little cell-type specificity, the IgH enhancer is active only in lymphoid cells, which suggests that it is recognized by lymphoid-specific factors (*8*). This hypothesis is supported by in vivo experiments of protection against dimethyl sulfate modification (*16*) as well as by in vitro transcription experiments (*17*). In spite of their difference in specificity, the IgH and SV 40 enchancers exhibit some sequence homologies, and it is possible to observe a competition between them, both in vivo (*18*) and in vitro (*19*). They might therefore share some common elements that could be targets for the E1A-mediated repression.

Our results raise the possibility that the IgH enhancer could be negatively regulated during cellular differentiation. The existence of cellular factors able to repress enhancers is suggested by the inactivity of some viral enhancers in undifferentiated embryonal carcinoma cells (*20*), and by the possibility to restore their activity by cotransfecting increasing amounts of competitor enhancer DNA (*21*). The presence of an E1A "like" activity in EC cells has, in fact, been inferred from the ability to support growth of the adenovirus mutant, dl312, which lacks the E1A gene (*18*).

We do not know whether our results can be related to the effects of adenovirus infection in vivo. Nevertheless, it is noteworthy that Ad2 is commonly found in a latent stage in human lymphoid tissues, especially adenoids and tonsils (*22*). The herpesviruses, whose immediate early genes share immortalizing and regulatory properties with E1A (*23*), can also infect lymphoid cells and are associated with immunosuppression (*24*). It is not known whether the products of these genes can also repress the IgH enhancer or other cellular enhancers. It has been reported that the human T cell leukemia virus (HTLV) that infects T cells has transacting stimulatory activities, similar to E1A (*25*). This raises the possibility that these activities could also repress the expression of members of the immunoglobulin gene family (*26*) expressed in T cells.

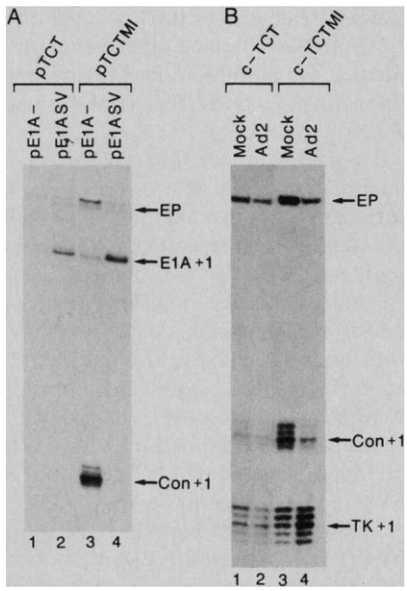

Fig. 5. Quantitative S1 nuclease mapping of 10 μg of RNA extracted from MPC11 cells either transiently (A) or stably transformed (B) with recombinants pTCT or pTCTMI. The cell lines c-TCT and c-TCTMI were obtained by stable transformation of MPC11 tk⁻ cells (*9*) with pTCT and pTCTMI plasmids, respectively; the selection marker used was the herpes simplex virus I thymidine kinase gene (*14*). (A) (Lanes 1 and 2) Cotransfection of pTCT (10 μg) with pE1A⁻ (5 μg) and pE1ASV (5 μg), respectively; (lanes 3 and 4) cotransfection of 10 μg of pTCTMI with 5 μg of pE1A⁻ and 5 μg of pE1ASV, respectively. (B) (Lanes 1 and 2) RNA from c-TCT cells that were mock-infected or infected for 15 hours with Ad2 at high multiplicity (100 pfu), respectively; (lanes 3 and 4) RNA from c-TCTMI cells either mock-infected or infected with Ad2 for 15 hours (100 pfu). The RNA was analyzed by quantitative S1 nuclease mapping with the E1A and the conalbumin probes (A) or the conalbumin and the tk probes (B). The E1A probe is described in legend to Fig. 1. The conalbumin probe (*13*) is a single-stranded Bam HI fragment that yields a protected fragment of 62 nucleotides (Con+1). The tk probe is a Bam HI–Bgl II fragment that gives a protected fragment of 49 nucleotides (TK+1) (*14*).

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32. The plasmid M13S containing the 13*S* cDNA under the control of the metallothionein promoter from Eco RI to Bgl II (*9*), was a gift of Dr. C. Goding.

33. We thank B. and C. Wasylyk for the pTCTMI recombinant; R. Breathnach, H. Diggelmann, C. Goding, C. Hauss, T. Honjo, M. Karin, K. Karjaleinen, C. Kédinger, L. Matrisian, and A. Pavirani for recombinants and viruses; and M. Acker, A. Landmann, and the members of the secretariat for their help. Supported by the CNRS (ATP 3582), the INSERM (PRC 124026), the Fondation pour la Recherche Médicale, the Ministère de la Recherche et de la Technologie (82C1283), and the Association pour le Développement de la Recherche sur le Cancer.

15 August 1985; accepted 10 October 1985