VIROLOGY 129, 393-400 (1983)

Deletion Mutants of Region E1a of AD12 E1 Plasmids: Effect on Oncogenic Transformation

J. L. BOS, A. G. JOCHEMSEN, R. BERNARDS, P. I. SCHRIER, H. VAN ORMONDT, AND A. J. VAN DER EB[1]

Department of Medical Biochemistry, Sylvius Laboratories, University of Leiden, Wassenaarseweg 72, 2333 AL Leiden, The Netherlands

Received March 18, 1983; accepted May 24, 1983

Plasmids containing the E1 region of Ad12 DNA can transform certain rodent cells into oncogenic cells. To study the role of the E1a subregion in the process of oncogenic transformation, Ad12 region E1 mutants carrying deletions in the E1a region were constructed. Deletion mutants pR7 and pR8 affect only the 13 S mRNA species encoded by region E1a, whereas deletion mutants pR11 and pR15 damage both the 12 S and 13 S E1a mRNA. All four mutants have lost their capacity to transform primary cultures of baby rat kidney cells, indicating that the E1a gene product encoded by 13 S mRNA is essential for transformation. It was further found that the mutated E1a regions of both pR7 and pR11 can induce expression of region E1b, which implies that the transformation deficiency of these mutants is not due to the inability to activate E1b expression. Surprisingly, the transforming capacity of pR7 and pR11 is restored when these mutant E1 regions are covalently coupled to the SV40 "enhancer" region. Cells transformed by these hybrids plasmids, however, were not tumorigenic in nude mice.

INTRODUCTION

Human adenoviruses have the capacity to transform certain rodent cells *in vitro* into tumor-like cells (Flint, 1980). The oncogenicity of the transformed cells depends on which serotype is used as the transforming agent. Primary rat cells transformed by adenovirus 12 (Ad12) are oncogenic after injection into syngeneic rats and into athymic nude mice, whereas cells transformed by Ad5 are nononcogenic in syngeneic rats, although they are weakly oncogenic in nude mice (Bernards *et al.*, 1983).

The transforming activity of all human adenoviruses analyzed so far has been mapped in early region 1 (E1), which stretches from about 1.5 to 11.5% of the viral genome. This region consists of two transcriptional units, E1a and E1b (see Fig. 1). In transformed cells, E1a encodes two coterminal mRNAs of 12 S and 13 S which differ in the amount of RNA removed by splicing. At least two coterminal proteins are synthesized from these mRNAs. Region E1b encodes a 22 S mRNA which directs the synthesis of two proteins, a 19- and a 60-kDa protein (for review see Flint, 1980).

In vitro mutagenesis studies with Ad12 region E1a DNA showed that the 19-kDa E1b protein and the carboxy-terminal part of the 60-kDa E1b protein are not essential for transformation. Cells transformed by such mutant plasmids carrying deletions in the genes coding for these proteins are not tumorigenic in nude mice, which demonstrates that the E1b gene products have an important role in determining the oncogenicity of transformed cells (Jochemsen *et al.*, 1982; Bernards *et al.*, 1983). This is further confirmed by our finding that the degree of oncogenicity in nude mice of cells transformed by Ad5 or Ad12 is determined by the identity of the E1b 60-kDa protein (Bernards *et al.*, 1983).

Less is known about the role of the E1a region of Ad12 in oncogenicity due to the lack of mutants of this serotype. Studies with E1a mutants of Ad5 have shown that

[1] To whom reprint requests should be addressed.

0042-6822/83 $3.00

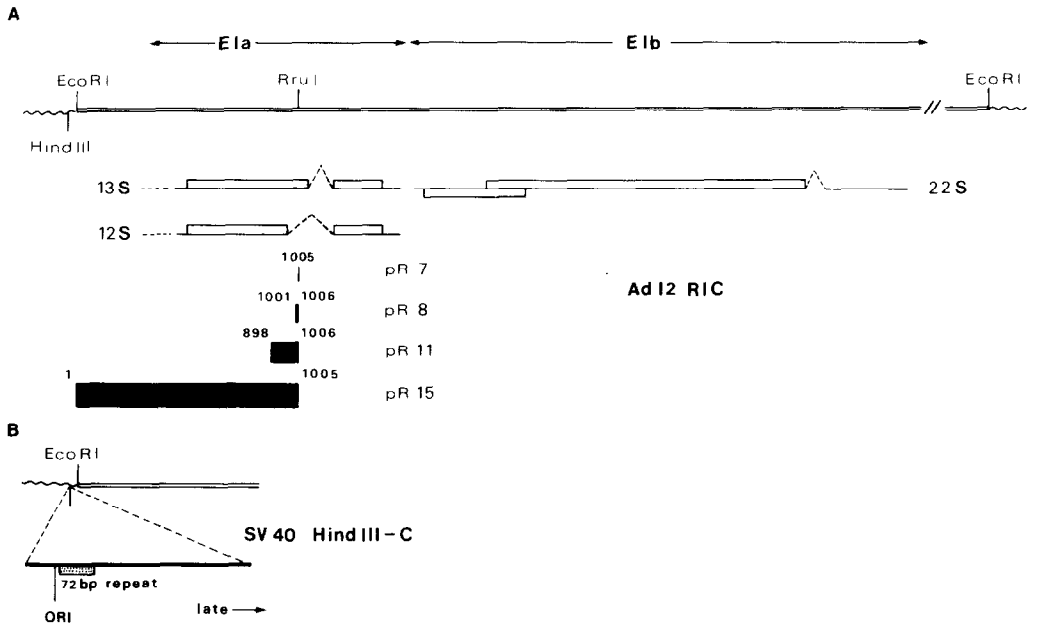

FIG. 1. Physical maps of Ad12 region E1 mutants cloned in pAT153. (A) Schematic representation of the positions of the E1a deletions. Indicated are the first and the last base pair of a deletion. (B) Schematic representation of the position and orientation of the SV40 *Hin*dIII-C fragment after insertion into Ad12 E1-containing plasmids.

E1a gene products, and especially the protein encoded by the 13 S mRNA, are involved in activation of expression of the E1b region (Berk *et al.*, 1979; Nevins, 1981). Activation of E1b expression cannot be the only function of E1a in transformation, however, since expression of region E1b alone, under control of a foreign promoter, does not result in morphological transformation (Van den Elsen *et al.*, submitted for publication). Furthermore, the Ad5 mutant hr 440 carrying a mutation in the 13 S transcript normally expresses E1b but is transformation defective, which shows that a gene product of the 13 S messenger is directly involved in transformation (Solnick and Anderson, 1982). Not all mutants in the 13 S messenger are transformation-deficient, however. Ad5 hr 1, which carries a deletion at a similar position as hr 440, can transform baby rat kidney cells (Graham *et al.*, 1978) and, at a slightly elevated temperature, also other primary rat cells (Ho *et al.*, 1982).

To extend the studies on region E1a to oncogenic adenovirus serotypes, we have constructed four E1a deletion mutants from plasmids containing the Ad12 E1 region, two of which affect the 13 S mRNA only. In this paper we report that all four mutants are transformation-deficient, indicating that a gene product of the 13 S mRNA is required for this process. However, ligation of the "enhancer" region of SV40 DNA to the mutated Ad12 E1 region resulted in the restoration of the transforming capacity of at least two of the four mutants. Although cells transformed by these hybrid DNAs normally expressed E1b tumor antigens, they were nononcogenic.

MATERIALS AND METHODS

Primary cultures of baby rat kidney (BRK) cells were prepared from kidneys of 1-week-old WAG/RIJ rats, an inbred rat strain. Transformation of these cells with recombinant plasmids was performed as described by Van der Eb and Graham (1980).

Cell labeling with [^{35}S]methionine and subsequent immunoprecipitation was per-

formed as described previously (Schrier *et al.*, 1979).

The construction of pAd12 RIC (or pRIC) has been described by Bos *et al.*, (1981). The Ad12 E1a mutants pR7, pR8, and pR11 are derived from this plasmid by deleting the *Rru*I site (AGTACT at position 1003) using standard recombinant DNA techniques. pR15 has been obtained by deleting the 1005-bp *Eco*RI–*Rru*I fragment of pRIC (see Fig. 1).

The chimeric SV40-E1 plasmids were constructed as follows: The *Hin*dIII-C fragment of SV40 was cloned into the *Hin*dIII site of pAT153. A plasmid containing the SV40 fragment in a counterclockwise orientation was used as vector to clone the *Eco*RI inserts of pRIC, pR7, pR8, pR11, or pR15. Clones in which the Ad12 segment was inserted clockwise were used in the transformation experiments.

S1 nuclease analysis was carried out as described previously (Bernards *et al.*, 1982).

RESULTS

Construction and Characterization of E1a Deletions

Figure 1 shows the physical map of early region 1 of adenovirus 12. The E1a region encodes two coterminal mRNAs of 12 S and 13 S which differ by alternative splicing. We have constructed deletions in the E1a region of plasmids containing Ad12 E1a DNA (pRIC) *in vitro* (Fig. 1A). Three mutant E1 plasmids, pR7, pR8, and pR11, were identified by the loss of the *Rru*I site and further characterized by DNA sequence analysis. pR7 has a single base pair deletion (1005) which results in a frame shift in the coding region of the 13 S mRNA only. The remaining open frame can encode a 17-kDa truncated product. pR8 has a 6-base pair deletion (1001–1006) resulting in the deletion of two amino acids of the 13 S mRNA product. In both pR7 and pR8, the coding region of the 12 S E1a mRNA is unaffected. The third mutated hybrid E1 plasmid, pR11, has a large deletion of 109 base pairs (898–1006), affecting the coding frames of both the 12 S and the 13 S mRNAs. Furthermore, it removes the donor splice point of the 12 S E1a messenger. In this case, the mutated E1a region can encode only a 15-kDa truncated product. A fourth deletion mutant of the E1a region—pR15—has been obtained by deleting the left-terminal 1005 base pairs of the viral insert of pRIC.

TABLE 1

TRANSFORMING ACTIVITY OF Ad12 REGION E1-CONTAINING PLASMIDS IN PRIMARY CULTURES OF BRK CELLS

DNA	Colonies/dish
pRIC	2
pR7	0
pR8	0
pR11	0
pR15	0
—	0

Note. Ten 5-cm dishes were transfected with 3 μg of plasmid DNA each.

The four mutated hybrid E1 plasmids were transfected into baby rat kidney (BRK) cells and transformed colonies were screened after 10 weeks. As shown in Table 1, none of the deletion mutants can transform BRK cells. Since two of the mutants were only affected in the 13 S mRNA, the result implies that a gene product of the 13 S mRNA is essential for Ad12 transformation.

Activation of E1b Expression

Region E1a is known to regulate expression of region E1b and other early regions at the level of transcription (Nevins, 1981). Thus, the absence of transforming activity of the mutated E1 plasmids might be explained by a lack of E1b expression. To test whether the mutated E1 regions have lost their capacity to activate the E1b promoter, mouse L tk^- cells were transfected with mixtures containing one of the mutant E1 plasmids and an E1b-tk chimeric plasmid. In this E1b-tk plasmid the E1b promoter is linked to the coding frame of the tk gene (Fig. 2) and recent experiments have shown that expression of the E1b-tk gene requires the presence of E1a DNA (Bos and ten Wolde-Kraamwinkel, 1983). Table 2 shows that the number of tk^+ col-

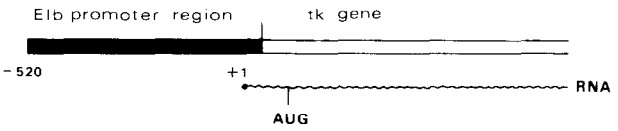

FIG. 2. Schematic representation of the E1b-thymidine kinase fusion gene pE1b-tk/-520.

onies obtained depended on the nature of the E1a mutant used for cotransfection. Very few colonies were obtained with pR8 and pR15, showing that these hybrid E1 plasmids have a defect in the activation of E1b expression. pR7, however, results in similar numbers of colonies as pRIC, while pR11 gives an even threefold increase in the number of colonies. This implies that both pR7 and pR11 can activate the E1b promoter, and thus that the transformation defect is not due to a defect in the activation of E1b transcription.

The Effect of the SV40 Origin-Promoter Region on E1 Transformation

In an attempt to obtain cells transformed by the mutated hybrid E1 plasmids, which in themselves were transformation-deficient, we have coupled the SV40 72-bp repeat region to the Ad12 E1a mutants. This was achieved by inserting the SV40 *Hin*dIII-C fragment containing the origin promoter region including the 72-bp repeat to the various mutants upstream from the E1 region (Fig. 1B). The resulting chimeric SV40-E1 plasmids (pSVRIC, pSVR7, pSVR8, pSVR11, and pSVR15) were transfected into BRK cells and screened for colony formation after 10 weeks (Table 3). Surprisingly, it was found that some of the mutated E1 plasmids transformed BRK cells in combination with the SV40 sequences. The efficiency of transformation was variable, however, and depended on the E1a deletion mutant. pSVR7 transformed with an efficiency similar to that of wild-type pRIC; pSVR8 also transformed but at a much lower level, while pSVR11, the mutant that affects the coding frames of both E1a mRNAs, transformed BRK cells with an efficiency five times higher than that of pRIC or pSVRIC. Mutant pSVR15 lacks most of the E1a region and showed no transforming activity. These results indicate that the SV40 origin promoter region can complement certain defects in the E1a region.

An interesting correlation can be made between the transformation frequency of the various mutant hybrid E1 plasmids in the presence of SV40 sequences (Table 3) and the capacity of the mutants to activate the E1b promoter (Table 2). When the capacity to induce E1b expression is high (pR11) then the transformation frequency in the presence of the SV40 sequence is also high, whereas when the capacity to induce E1b expression is low (pR8), then

TABLE 2

CONVERSION OF MOUSE L tk$^-$ CELLS BY THE E1b-tk FUSION GENE (pE1b-tk/-520) IN THE PRESENCE OF Ad12 E1 MUTANT PLASMIDS

DNA	Colonies/dish
pE1b-tk/-520	0.5
+pRIC	30
+pR7	28
+pR8	3
+pR11	101
+pR15	1

Note. Five 5-cm dishes were transfected with 0.5 μg pE1b-tk/-520 and 1 μg Ad12 plasmid DNA. See Fig. 2 for a diagram of the E1b-tk plasmid.

TABLE 3

TRANSFORMING ACTIVITY IN CHIMERIC SV40-E1 PLASMIDS IN PRIMARY CULTURES OF BRK CELLS

DNA	Colonies/dish
pRIC	2
pSVRIC	2.5
pSVR7	4
pSVR8	0.5
pSVR11	12
pSVR15	0
pSV	0

Note. Ten 5-cm dishes were transfected with 3 μg of plasmid DNA each.

the transformation frequency is also low. This correlation suggests that, to be complemented by the SV40 sequences, the E1a region should still have the capacity to activate the E1b promoter.

Characterization of Cells Transformed by Chimeric SV40-E1 Plasmids

To verify whether Ad12-specific proteins are present in cells transformed by the mutant plasmids in the presence of SV40 sequences, established cell lines were labeled with [^{35}S]methionine and extracts were immunoprecipitated with rat anti-tumor serum. As shown in Fig. 3, cells transformed by pRIC, pSVRIC, and pSVR7 (pSVR8-transformed cells have not been established) contained the normal Ad12-specific T antigens: the 55- and 19-kDa E1b proteins and a 41-kDa protein which is thought to be encoded by E1a. In addition, a 53-kDa cellular protein is found. Cells transformed by pSVR11 also contained the E1b tumor antigens but lack the 41-kDa protein. Instead, a 25-kDa protein was observed which could be a truncated E1a product. The presence of the 41-kDa protein in pSVR7-transformed cell lines and its absence in pSVR11-transformed cell lines suggests that this 41-kDa protein is encoded by the 12 S E1a messenger. The striking difference between the apparent molecular weight of this protein and its predicted value (26-kDa) has been discussed previously (Schrier *et al.*, 1979). In our experiments the product of the 13 S E1a messenger was not detected.

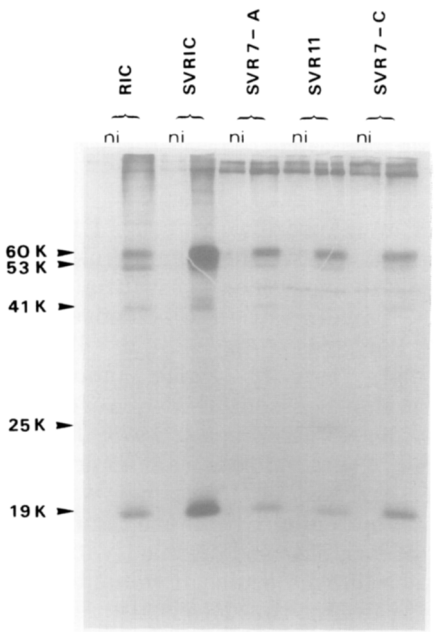

FIG. 3. Polyacrylamide gel electrophoresis of T antigens precipitated from BRK cells transformed with pRIC, pSVRIC, pSVR7 (two different cell lines), and pSVR11. Extracts of the cells labeled with [^{35}S]methionine were immunoprecipitated with normal rat serum (ni) and Ad12 anti-T serum.

We have further characterized the various E1-transformed cells by S1 nuclease analysis. The probe for E1a was a 3'-labeled *Nar*I-*Hin*dIII fragment from pRIC, which should give rise to S1 nuclease-resistant fragments of 575 and 482 nucleotides for pRIC, pSVRIC, and pSVR7 RNA (the single-nucleotide deletion in pSVR7 RNA will not be detected by S1 nuclease analysis) and to an S1 nuclease-resistant fragment of 404 nucleotides for pSVR11 RNA (see diagram, Fig. 4). The E1b-specific probe was a 3'-labeled *Hin*dIII fragment which should yield a resistant segment of 976 nucleotides. Both probes were hybridized as a mixture with cytoplasmic RNA from the various transformed cells. Figure 4 shows that all RNAs tested give the expected S1 nuclease-resistant segments. From the above results we conclude that cells transformed with the different chimeric SV40-E1 plasmids contain the expected T antigens and mRNA products.

Test for Oncogenicity

Cells transformed by Ad12 are oncogenic both in nude mice and in syngeneic rats. To identify whether deletions in the E1a region affect the oncogenic potential of the transformed cells we have injected the cells into nude mice. After a short latency period, both pRIC- and pSVRIC-transformed cells gave rise to tumors in all of the injected animals, whereas pSVR7- and pSVR11-transformed cells did not give tumors (Table 4). This implies that an intact E1a region and, more specifically, the 13 S E1a mRNA, is essential for oncogenicity of Ad12-transformed cells.

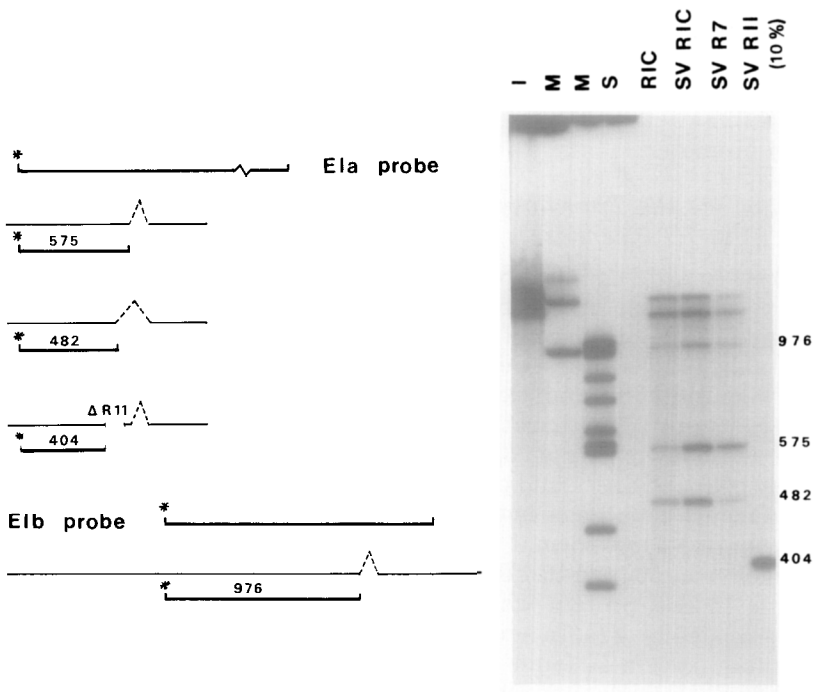

FIG. 4. S1 nuclease mapping of viral RNA in transformed cells. 3'-End-labeled DNA fragments were hybridized to total cytoplasmic RNA from transformed cells. The hybrids were treated with S1 nuclease and separated on a 5% polyacrylamide- 7 *M* urea gel. To analyze expression of region E1 RNA a 1825-bp *Nar*I-*Hin*dIII fragment (493-2318) and a 1388-bp *Hin*dIII fragment (2318-3706) isolated from pRIC were hybridized together to 25 μg RNA from cells transformed with pRIC (lane (RIC), with pSVRIC (lane SVRIC), or with pSVR7 (lane SVR7), to 2.5 μg of RNA from cells transformed with pSVR11 (lane SVR11) or to yeast tRNA (lane S). Lane I shows the untreated DNA probes; lanes M contain marker digests. Note that the amount of SVR11 RNA used is 10 times lower than the amount of RNA applied in other lanes.

DISCUSSION

Region E1a of Ad12 has several distinct roles in the process of cell transformation. First, the E1a-encoded 13 S mRNA, or its translation product, is required for initiation of transcription of the E1b region (Bos and ten Wolde-Kraamwinkel, 1983). Since E1b expression is essential for complete transformation (Jochemsen *et al.*, 1982; Bernards *et al.*, 1983), it follows that the E1a activity which induces this expression is essential also. Several lines of evidence (Berk *et al.*, 1979; Jones and Shenk, 1979) indicate that expression of other early adenovirus genes is also controlled by region E1a. If the regulatory effect of E1a is based on inactivation of repressor-like molecules, it would be reasonable to assume that E1a is also responsible for the activation of one or more cellular genes. Nevins (1982) has reported recently that Ad5 region E1a is responsible for the induction of a 70-kDa heat-shock protein in human cells. It might be that an important function of E1a in transformation is the activation of cellular "transforming"

TABLE 4

ONCOGENICITY OF Ad12 E1-TRANSFORMED CELLS IN ATHYMIC NUDE MICE[a]

Cell	Number of animals with tumors/ number of injected animals
RIC	10/10
SVRIC	6/6
SVR7-A	0/6
SVR7-C	0/6
SVR11	0/6

[a] Animals were injected with 10^7 cells/animal.

genes by a similar gene-activating function.

A second activity of the E1a region in transformation became apparent from the behavior of our E1a mutants which exhibit normal (pR7) or increased (pR11) levels of E1b induction, but which nevertheless are deficient in transformation. A similar E1a activity has recently been described in a study on the Ad5 host range mutant hr 440 which has a mutation in the E1a gene specifying the 13 S viral mRNA virus (Solnick and Anderson, 1982). This virus is transformation-deficient but expressed E1b normally. This shows that region E1a has a distinct role in morphological transformation which can be dissociated from its ability to induce E1b expression. This transforming activity also appears to be a function of the E1a 13 S mRNA or its protein product(s).

The transforming activity, which is lost in deletion mutants pR7, pR8, and pR11, is restored to a varying degree when the mutant DNAs are covalently linked to the *Hin*dIII-C fragment of SV40 (nucleotides 5171–1046). This fragment contains the origin of DNA replication and, as part of the early promoter, the 72-bp repeat or "enhancer" region. Removal of the origin of replication (*Hin*dIII–*Bgl*II; 5171–1) does not affect the phenomenon (our unpublished observation). Several effects have been described for the 72-bp repeat. For instance, in certain cases it can enhance the frequency of transformation by DNA-mediated gene transfer. It has been suggested that this effect is due to facilitated integration of cotransfected genes into the host genome (Capecchi, 1980). Since the SV40 enhancer region did not cause increased transformation frequencies for intact region E1, it seems unlikely that the effect described in this paper is caused by facilitated integration. An alternative possibility would be that the E1a deletions affect an adenovirus "enhancer" function. This is, however, unlikely, since this would imply that the deletion in pR8 does affect this enhancer element, whereas the overlapping deletion in pR11 does not (see Fig. 1).

A second property of the 72-bp repeat is that it can increase the level of activity of certain neighboring genes, such as the β-globin gene (Banerji *et al.*, 1981). Thus, increased expression of a partially defective E1a region above a certain threshold level could result in restoration of biological activity. However, we did not observe a consistent increase in the level of E1a products in cells transformed by pSVRIC or pSVR7. The elevated concentration of E1 products in one SVRIC-transformed cell line (Figs. 3, 4) is not found in SVR7-transformed cell line (Figs. 3, 4) nor in two other SVRIC-transformed lines (results not shown). Only in cells transformed by SVR11 a much higher concentration of E1a-specific RNA was found than in other transformed lines. We tentatively suggest that this may be attributed to an increased stability of the E1a 13 S mRNA due to the 109-bp deletion. A greater stability of E1a RNA might also explain the increased capacity of pR11 to induce E1b expression as compared to RIC, and the high transforming activity of pSVR11. The observation that the mutant-transformed cells are not oncogenic in athymic nude mice indicates that 13 S E1a RNA is also required for oncogenicity. Since the two genes that are specified by the E1b region (19- and 60-kDa) are also both needed for oncogenicity (Jochemsen *et al.*, 1982; Bernards *et al.*, 1983), it follows that this property requires essentially the coordinated expression of the entire E1 region.

In summary, the E1a gene which specifies the 13 S mRNA has at least three distinct functions: a gene-activating function, a role in morphological transformation which can be complemented by the SV40 enhancer region, and an activity which plays a role in the oncogenicity of transformed cells. The fact that the translation product of the 13 S E1a mRNA occurs in several distinct forms differing in their apparent molecular weights (Esche *et al.*, 1980; Esche and Siegmann, 1982) may be relevant in this respect. Alternatively, the E1a polypeptide may have different functional domains encoded by the two exons of the gene. Clearly, the gene-activating function which is still present in pSVR7 and pSVR11 would then be encoded by the first exons. The location of the second activity cannot be inferred from our results.

However, the Ad5 mutant dl 311, which has lost part of the second exon of the E1a gene, is normal in transformation (Jones and Shenk, 1979), suggesting that this activity is also located in the first domain. The same conclusion can be drawn from the Ad5 hr 1 mutant, which has a frame shift mutation close to the 3′ end of the first exon, but which can transform normally at a slightly elevated temperature (Ho *et al.*, 1982). The oncogenic activity has not yet been mapped within region E1a.

We have recently obtained evidence that region E1a may determine a fourth parameter in transformation, namely whether or not a transformed cell is recognized by the immunocompetent host as a neoplastic cell and is rejected or escapes immune surveillance and develops out into a tumor. This activity will be described elsewhere in more detail.

ACKNOWLEDGMENTS

We thank Dr. A. de Waard for a gift of endonuclease *Rru*I, Ms. H. C. ten Wolde-Kraamwinkel for technical assistance, and Ms. M. A. Veeren-Vink for typing the manuscript. This work was supported by The Netherlands Organization for the Advancement of Pure Research (ZWO) through the Netherlands Foundation for Chemical Research (SON) and the Foundation of Fundamental Medical Research (FUNGO).

REFERENCES

BANERJI, J., RUSCONI, S., and SCHAFFNER, W. (1981). Expression of a β-globin gene is enhanced by remote SV40 DNA sequences. *Cell* **27**, 299–308.

BERK, A. J., LEE, F., HARRISON, T., WILLIAMS, J., and SHARP, P. A. (1979). Pre-early adenovirus 5 gene product regulates synthesis of early viral messenger RNAs. *Cell* **17**, 935–944.

BERNARDS, R., HOUWELING, A., SCHRIER, P. I., BOS, J. L., and VAN DER EB, A. J. (1982). Characterization of cells transformed by Ad5/Ad12 hybrid early region 1 plasmids. *Virology* **120**, 422–432.

BERNARDS, R., SCHRIER, P. I., BOS, J. L., and VAN DER EB, A. J. (1983). Role of adenovirus types 5 and 12 early region 1b tumor antigens in oncogenic transformation. *Virology* **127**, 45–53.

BOS, J. L., POLDER, L. J., BERNARDS, R., SCHRIER, P. I., VAN DEN ELSEN, P. J., VAN DER EB, A. J., and VAN ORMONDT, H. (1981). The 2.2 kb E1b mRNA of human Ad12 and Ad5 codes for two tumor antigens starting at different AUG triplets. *Cell* **27**, 121–131.

BOS, J. L., and TEN WOLDE-KRAAMWINKEL, H. C. (1982). The E1b promoter of Ad12 in mouse L tk⁻ cells is activated by adenovirus region E1a. *EMBO J.* **2**, 73–76.

CAPECCHI, M. R. (1980). High efficiency transformation by direct microinjection of DNA into cultured mammalian cells. *Cell* **22**, 479–488.

ESCHE, H., MATHEWS, M. B., and LEWIS, J. B. (1980). Proteins and messenger RNAs of the transforming region of wild type and mutant adenoviruses. *J. Mol. Biol.* **142**, 399–417.

ESCHE, H., and SIEGMANN, B. (1982). Expression of early viral gene products in adenovirus type 12-infected and -transformed cells. *J. Gen. Virology* **60**, 99–113.

FLINT, S. J. (1980). Transformation by adenoviruses. *In* "Molecular Biology of Tumor Viruses" part 2, "DNA Tumor Viruses," (J. Tooze, ed.), 2nd Ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.

GRAHAM, F. L., HARRISON, R., and WILLIAMS, J. (1978). Defective transforming capacity of adenovirus type 5 host range mutants. *Virology* **86**, 10–21.

HO, Y.-S., GALOS, R., and WILLIAMS, J. (1982). Isolation of type 5 adenovirus mutants with a cold-sensitive host range phenotype: genetic evidence of an adenovirus transformation maintenance function. *Virology* **122**, 109–124.

JOCHEMSEN, H., DANIËLS, G. S. G., HERTOGHS, J. J. L., SCHRIER, P. I., VAN DEN ELSEN, P. J., and VAN DER EB, A. J. (1982). Identification of adenovirus-type 12 gene products involved in transformation and oncogenesis. *Virology* **122**, 15–28.

JONES, N., and SHENK, T. (1979a). An adenovirus type 5 early gene function regulates expression of other early viral genes. *Proc. Nat. Acad. Sci. USA* **76**, 3665–3669.

JONES, N., and SHENK, T. (1979b). Isolation of adenovirus type 5 host range deletion mutants defective for transformation of rat embryo cells. *Cell* **17**, 683–689.

NEVINS, J. R. (1981). Mechanism of activation of early viral transcription by the adenovirus E1a gene product. *Cell* **26**, 213–220.

NEVINS, J. R. (1982). Induction of the synthesis of a 70,000 dalton mammalian heat shock protein by the adenovirus E1a gene product. *Cell* **29**, 913–919.

SCHRIER, P. I., VAN DEN ELSEN, P. J., HERTOGHS, J. J. L., and VAN DER EB, A. J. (1979). Characterization of tumor antigens in cells transformed by fragments of adenovirus type 5 DNA. *Virology* **99**, 372–385.

SOLNICK, D., and ANDERSON, M. (1982). Transformation-deficient adenovirus mutant defective in expression of region 1A but not region 1B. *J. Virol.* **42**, 106–113.

VAN DER EB, A. J., and GRAHAM, F. L. (1980). Assay of transforming activity of tumor virus DNA. *In* "Methods in Enzymology" (L. Grossman and K. Moldave eds.), Vol. 65, pp. 826–839. Academic Press, New York.