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Efficient Nuclear Localization and Immortalizing Ability, Two Functions Dependent on the Adenovirus Type 5 (Ad5) E1A Second Exon, Are Necessary for Cotransformation with Ad5 E1B but Not with T24*ras*

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Expression of adenovirus type 5 E1A 12S is sufficient to immortalize primary baby rat kidney cells, but another viral or cellular oncogene, such as E1B or T24*ras*, is necessary for complete transformation. The regions of 12S sufficient for T24*ras* cotransformation have been well characterized and are located in the first exon. The second exon is dispensable for *ras* cotransformation, although it contains a region which appears to modulate the transforming phenotype. The same 12S first exon regions important in *ras* transformation are also necessary for E1B transformation. Analysis of an extensive series of second exon deletion and amino acid point mutations demonstrated that mutations affecting either the efficient nuclear localization and/or the immortalizing ability of the 12S protein also prevented cooperation with E1B. In general, the entire C-terminal half of 12S, including the nuclear localization signal, was necessary for efficient cotransformation with E1B. In addition to the differences between T24*ras* and E1B regarding 12S regions necessary for cotransformation, the characteristics of E1B-cotransformed foci differed from those of T24*ras*. The E1B foci took longer to appear and had a much slower growth rate. No hypertransformed foci were produced with E1B cotransfections, and established E1A-E1B lines exhibited minimal growth in soft agar compared with that of E1A-T24*ras* lines.

At least 80% of all solid tumors are carcinomas and are therefore of epithelial origin. Adenovirus (Ad), a DNA tumor virus, is a good system for studying epithelial cell transformation, because it can transform primary rodent kidney epithelial cells. The transforming genes of Ad are two of the early genes, E1A and E1B (for a review, see references 3 and 6). E1A encodes at least five transcripts, 13S, 12S, 11S, 10S, and 9S, through differential splicing. Two of these, 13S and 12S, encode polypeptides important for both immortalization and transformation of primary cells. These proteins are identical except for an internal 46 amino acids in 13S that are known as the transactivating domain. This region is responsible for transactivating many of the other early Ad genes as well as many cellular genes; however, it is dispensable for both immortalization and transformation. E1A alone can immortalize primary cells (18, 27) but needs E1B for complete transformation (14, 16). E1B encodes at least 4 transcripts, 22S, 14.5S, 14S, and 13S, through differential splicing. The 22S mRNA encodes the two transforming oncoproteins of E1B, 19K and 55K, from different but overlapping reading frames. The 13S mRNA also encodes the 19K protein.

The roles of 19K and 55K in transformation are still being investigated. Whether or not 19K is even necessary for transformation appears to be a controversy (1, 2, 4, 8, 12, 36). The use of viral infections versus transfections and the use of many different cell types, both primary and established lines, have contributed to the conflicting results. Some of the speculations for 19K's transforming functions include its ability to disrupt the intermediate filament, vimentin, and the nuclear lamins (37). The 19K protein has also been identified as an antiapoptotic factor, like bcl-2 (30). Recent work has suggested that the ability of 19K and bcl-2 to block apoptosis might be due to their ability to relieve p53-mediated transcriptional repression (32), suggesting another possible transforming activity.

The major transforming role attributed to the 55K protein has been its ability to bind and cytoplasmically sequester the tumor suppressor protein p53 (43). However, several 55K mutants that retain p53 binding but are still defective for transformation have been identified (41). Also, the Ad type 12 (Ad12) 55K protein does not cytoplasmically sequester p53 (44) but is quite capable of causing transformation. Both Ad5 and Ad12 55K proteins are able to inhibit p53-mediated transcriptional activation (41), which could also explain how they mediate transformation. A recent report suggests that E1B contains a transcriptional repression domain that, through its interaction with p53, is targeted to p53-activated genes (42). Although the exact transforming functions of 19K and 55K are unclear, their dependence on E1A to manifest these functions is not. Neither 19K nor 55K, individually or in tandem, has any transforming effect on primary cells or normal cell lines.

Determining the E1A regions and functions necessary for transformation has received much attention and has, thus, been quite well defined, although questions still remain. The majority of studies have utilized an activated *ras* gene for cotransformation and, therefore, do not address the requirements for E1B cotransformation. Several conserved regions located in the first exon of 12S are important for *ras* transformation (20, 21, 24, 31, 33, 39). The functions of these regions are thought to be mediated through the binding of several cellular peptides (17, 40), including pRb (38), p130, p107, cyclins A and E (13, 26), and p300 (17, 40). The induction and

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transient maintenance of DNA synthesis and proliferation are encoded by these same regions (29, 31, 33) and are necessary for both immortalization and transformation. The second exon of 12S is dispensable for cotransformation with *ras*; however, we and others have shown that it can modulate the transformation phenotype (9, 34). Mutations in certain second exon regions result in a hypertransformed phenotype, indicating a possible transformation-suppressive function for wild-type (WT) 12S. A polypeptide with a size of 48 kDa, CtBP, that binds to the region responsible for modulating transformation has been identified (7), and perhaps it mediates this function.

The E1B and Ras proteins have very different subcellular localizations and functions (see references 6, 11, and 22 and references therein). Because the mechanisms of Ras and E1B transformation are probably quite different, it is likely that their need for E1A functions may also be. It appears that both T24*ras* and E1B (35) require the same 12S first-exon-encoded regions. However, E1B seems to require second exon functions as well, because large deletions of the C terminus of 12S prevent cooperation with E1B (35). We have analyzed a series of 12S second exon deletions and amino acid point mutants to further map the necessary E1B cotransformation regions and to determine what 12S functions are needed.

We have previously demonstrated that a region encompassing nucleotides 1437 to 1488 of the Ad5 12S second exon has the ability to modulate transformation with an activated *ras* gene (9). When coexpressed with T24*ras*, 12S genes with point mutations or deletions in this area result in hypertransformation. Introduction of WT 12S into such cells suppresses this phenotype (15). This suggests the presence of a possible transformation suppressor function in WT 12S. To determine whether this phenomenon occurs with 12S-E1B cotransformation and to investigate the structural and functional contribution of the 12S second exon to E1B cotransformation, a large panel of mutants, shown in Fig. 1, was analyzed in cotransformation studies. The construction of these mutants has been described previously (9, 10).

E1A-E1B transformants are distinct from E1A-T24*ras* transformants. An E1A-independent E1B expression vector was made by cloning the genomic E1B region (nucleotides 1680 to 3600) downstream of the cytomegalovirus promoter in pcDNAI/Amp to create pCMVE1B. Cotransfection of primary baby rat kidney (BRK) cells with pCMVE1B and either WT or mutated E1A 12S genes resulted in two categories of foci. The most abundant foci were well-delineated islands of epithelial-like cells resembling immortalized foci (Fig. 2A). The second type of focus was composed of slowly growing, densely packed fibroblasts with ill-defined borders (Fig. 2C). Both types of foci were quite adherent to the culture dish and took up to 6 weeks to appear. Cotransfections involving these same E1A genes and pT24*ras* also resulted in the production of these two types of foci; however, they appeared by 2 weeks posttransfection (Fig. 2B and D). In addition to these, a third type of focus was discovered (Fig. 2F). This type of focus was made up of very refractile, loosely attached cells of an unknown cell type. These cells had the ability to detach from the dish and reseed elsewhere, indicating their ability to grow in suspension. Their rapid rate of growth, as well as their ability to detach and reattach elsewhere, enabled these foci to completely cover the dish within 3 weeks posttransfection. They also had the ability to invade the slower growing epithelial- and fibroblast-like foci, as depicted in Fig. 2F. These hypertransformed foci, which have been extensively discussed in a previous study (9), were never observed in the E1B cotransfections, indicating that they are specific to E1A-T24*ras* transformation (Fig. 3 [mutant XS3]). Another difference between E1B and *ras* cotransfections was the need for higher plasmid levels for E1B cooperation to obtain reproducible foci formation (10 μg of pCMVE1B and 5 μg of E1A plasmid compared with 2 μg of pT24*ras* and 2 μg of E1A plasmid). Neither pT24*ras* nor pCMVE1B, when transfected alone, gave rise to significant numbers of transformed foci (Fig. 3). All types of foci, from both E1B and *ras* cotransfections, could be cloned and passaged indefinitely in culture. Cell lines were established from these foci and were examined for their ability to grow in semisolid media (Fig. 4). E1A-T24*ras*-transformed cells (10^4) formed large, rapidly growing colonies when plated in soft agar. In contrast, E1A-E1B-transformed cells (10^6) formed very small, slowly growing colonies. A higher number of E1A-

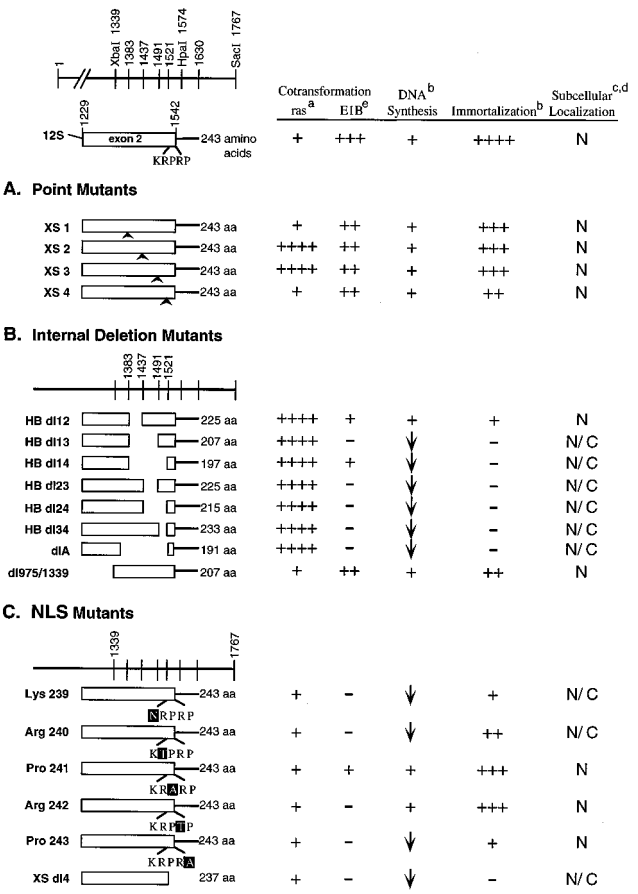

FIG. 1. Map of 12S second exon mutants and their functions. To the left are shown maps of the second exons of the WT and mutated 12S genes. All of these constructs have intact first exons. The top line, at the left, is a map indicating the salient restriction enzyme sites of the 12S second exon. The numbers above each line represent Ad nucleotide numbers. The numbers 1383, 1437, 1491, and 1521 indicate *Hpa*I restriction enzyme cleavage sites that have been individually introduced by site-directed mutagenesis (19). The lines below the top line represent transcripts. The name of each mutant is indicated at the left. The solid lines are untranslated regions, and the open boxes are the protein coding sequences. To the right is shown the number of amino acids (aa) encoded by each transcript. (A) Point mutants. (B) Internal deletion mutants. (C) NLS mutants. To the right, the properties of each mutant protein are summarized. Footnotes: a, data derived from the work of Douglas et al. (9) (+, transformation phenotype equivalent to WT 12S; ++++, hypertransformation phenotype); b, +, 0 to 5%; ++, 5 to 25%; +++, 25 to 50%; ++++, 50 to 100%; data derived from the work of Quinlan and Douglas (28); c, data derived from the work of Douglas and Quinlan (10); d, data derived from the work of Douglas and Quinlan (10a); e, data derived from this work. +, positive for the function; −, negative for the function; ↓, 20 to 50% less than the WT; N, protein is predominantly located in the nucleus; N/C, protein exhibits diffuse nuclear and cytoplasmic localization.

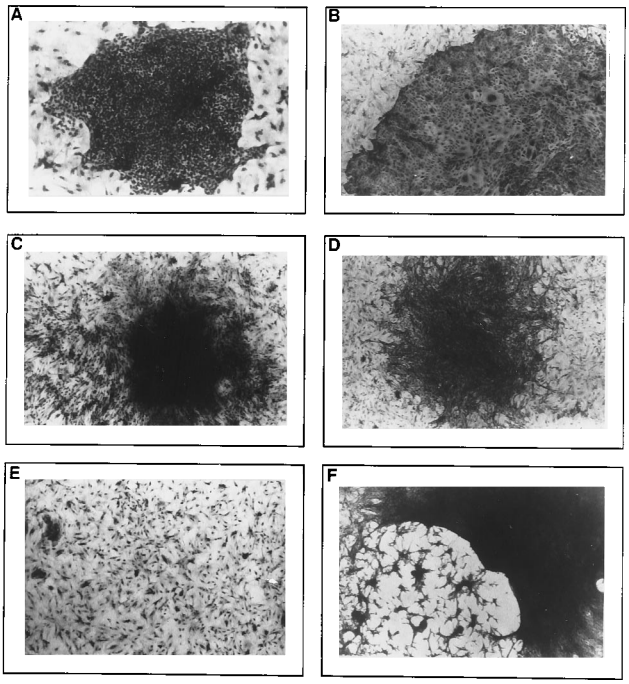

FIG. 2. Morphologies of E1B- and T24*ras*-cotransformed foci. Primary BRK cells were cotransfected with a WT 12S plasmid and either pCMVE1B or pT24*ras* 2 days after plating. For E1B cotransfections, 10 μg of pCMVE1B and 5 μg of 12S plasmid were used. For *ras* cotransfections, 2 μg of pT24*ras* and 2 μg of 12S plasmid were used. The cells were fixed with methanol and stained with Giemsa 3 weeks after transfection for *ras* cotransfections and 6 weeks after transfection for E1B cotransfections. (A) Epithelial focus from E1A-E1B cotransfection. (B) Epithelial focus from E1A-*ras* cotransfection. (C) Fibroblast focus from E1A-E1B cotransfection. (D) Fibroblast focus from E1A-*ras* cotransfection. (E) Nontransformed cells. (F) Breakaway focus invading fibroblast focus from E1A-*ras* cotransfection. Magnification, ×10.

E1B-transformed cells were used, because no colonies could be detected when less cells were plated. For comparison, a 12S immortalized line was analyzed. These cells are not transformed and did not grow in soft agar. These results indicate

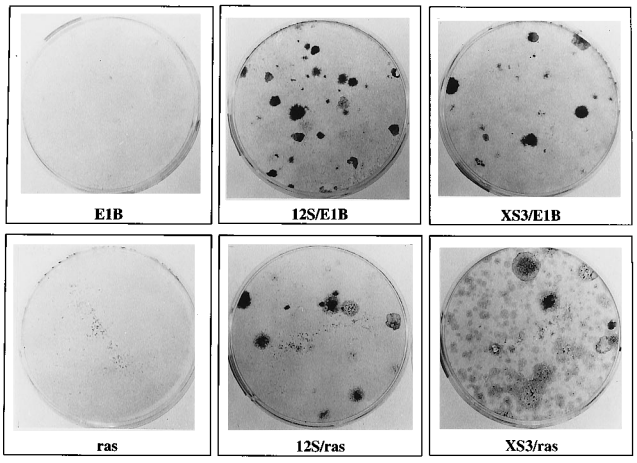

FIG. 3. Comparison of cotransformed focus-forming abilities of E1B and T24*ras*. Primary BRK cells were cotransfected with the indicated plasmids as described in the legend to Fig. 1. The cells were fixed in methanol and stained with Giemsa at 3 weeks posttransfection for *ras* and 6 weeks posttransfection for E1B.

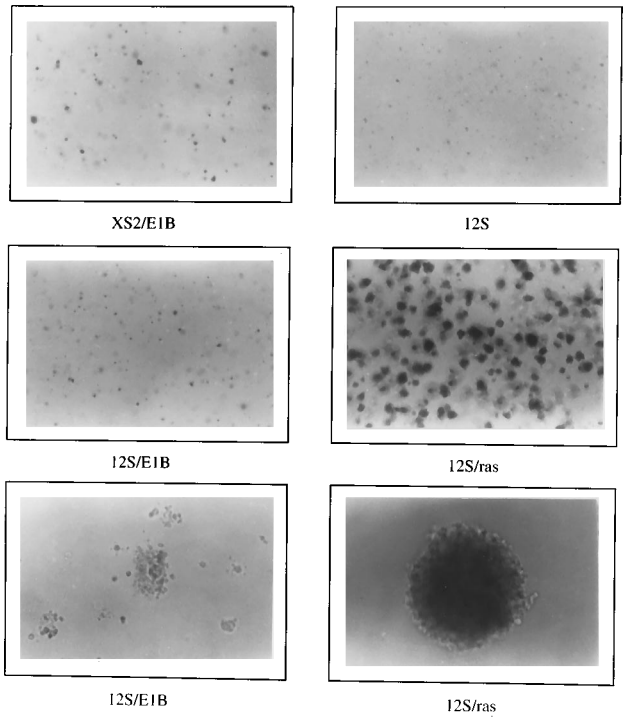

FIG. 4. Ability of E1A-E1B and E1A-T24*ras* established lines to grow in semisolid media. Cells from the indicated cell lines were counted, and 10^4 (*ras* lines) and 10^6 (E1B lines) cells were plated in soft agar as described in the text. A 12S immortalized line (10^6) was used as a negative control. The top four photomicrographs were taken at a magnification of ×10, and the bottom two were taken at a magnification of ×50.

that while both E1B and T24*ras* can cooperate with E1A to transform primary epithelial cells, the resultant phenotypes are quite different.

The immortalizing function encoded by the COOH terminus of 12S is required for cotransformation with Ad E1B. We have previously shown that the expression of the 12S second exon is required for immortalization of primary epithelial cells, although it is dispensable for cooperation with the Ras oncoprotein. To ascertain whether cotransformation with E1B requires the presence of the COOH terminus of 12S, we cotransfected primary BRK cells with pCMVE1B and a plasmid encoding WT 12S or a mutant, CT *dl*976, which encodes only an intact first exon. Transformed foci were observed only in cultures that received both WT 12S and pCMVE1B, confirming that, unlike T24ras, expression of the second exon is necessary for cooperation with E1B (35).

We have mapped the region of the second exon that is required for immortalization (28). To determine whether the same second exon region is required for cooperation with E1B, we used a series of four point mutants and eight internal deletion mutants (Fig. 1A and B, respectively) to cotransfect primary BRK cells. The results from these cotransfections are graphed in Fig. 5 as an average number of foci per dish. All of the point mutants were able to form transformed foci in the presence of E1B, which is consistent with their ability to immortalize primary BRK cells. Surprisingly, none of them gave rise to the aggressive hypertransformed phenotype exhibited by XS2 and XS3 with T24*ras* (Fig. 3). In all but one case (HB *dl*14), those mutants which could bring about immortalization (XS1 to XS4, HB *dl*12, and 975/1339) could also produce transformed foci with E1B (Fig. 1A and B). Although the

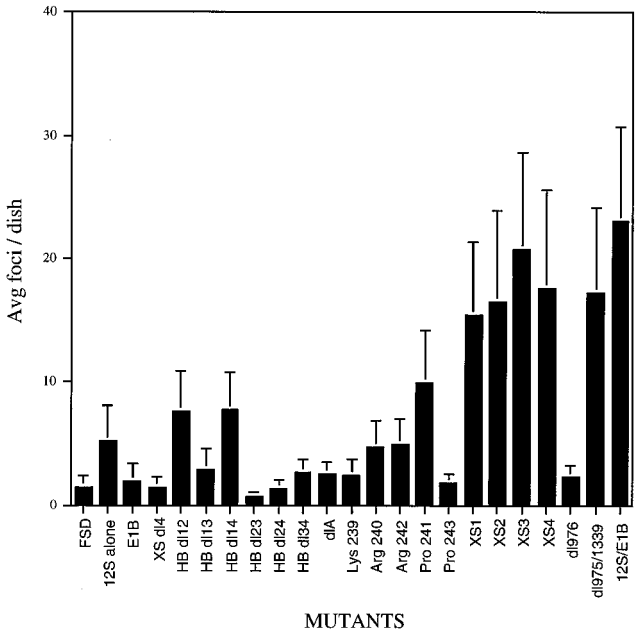

FIG. 5. Quantitation of E1B-cotransformed foci. Three dishes of primary BRK cells were cotransfected with the indicated 12S plasmid and pCMVE1B as indicated in the legend to Fig. 1. The cells were fixed with methanol and stained with Giemsa at 6 weeks posttransfection. The foci were counted and plotted as the average number of foci per dish. Two to five cotransfection experiments were performed for each mutant. FSD, fish sperm DNA; 12S alone, pLE12S alone; E1B, pCMVE1B alone. Error bars indicate standard deviations.

cotransformation ability of HB *dl*12 was significantly lower than that of WT 12S, its immortalizing ability is concomitantly reduced. Inversely, those mutants that were unable to immortalize primary BRK cells were also unable to bring about focus formation in the presence of E1B. This, again, is in contrast to the situation with T24*ras*, in which all of these mutants could enable tumorigenic transformation. In fact, many of these mutants cause hypertransformation with T24*ras* (Fig. 1) (9). Although HB *dl*14 cooperation with E1B was only about 30% as efficient as that of WT 12S, its ability to cotransform with E1B at all was surprising. Since HB *dl*14 is defective for immortalization and has a large deletion encompassing the smaller deletions of HB *dl*13, HB *dl*23, HB *dl*24, and HB *dl*34, which are all negative for E1B cotransformation, it seemed incongruous. Perhaps some conformation of this particular mutant protein enables a low level of function necessary for cooperation with E1B, but this level is insufficient for immortalization by 12S alone.

All of these mutants express levels of protein at least equivalent to that of WT 12S and retain the ability to perform functions encoded by their intact first exons, such as activation of the cell cycle and cotransformation with T24*ras*, as mentioned above (Fig. 1) (9, 28). These results indicate that expression of a region of the second exon encoded between nucleotides 1437 and 1522 is necessary for E1B cooperation. This same region is necessary for 12S to immortalize primary epithelial cells, suggesting immortalization is a prerequisite for E1B but not T24*ras* cotransformation. This region includes the region identified by Mymryk and Bayley to be involved in the induction of E2A 72-kDa protein expression (25) and thus has a role in gene expression which may be involved in immortalization. The region encoded between nucleotides 1229 and 1437 seems to increase the efficiency of both immortalization and cooperation with E1B.

It is curious, although perhaps not surprising, that these two cooperating oncogenes require such different functions from E1A 12S, especially since neither *ras* nor E1B has any observable effect on BRK cells when expressed alone. As E1A and E1B coevolved, therefore, their functions could have evolved to be mutually interdependent. On the other hand, E1A and *ras* happen to cooperate in transformation, but no limitations or codependence could be expected. As detailed above, E1B is a much less potent oncoprotein than Ras protein. In fact, BRK cells transformed by 12S and E1B are only slightly more than immortalized. This may also explain why E1B requires more of 12S to enable tumorigenesis.

Expression of the WT second exon somehow suppresses a function activated by the *ras* pathway (7, 9, 15). Many of the 12S second exon mutants enable a hypertransformed state in the presence of an activated *ras* gene compared with what is enabled by WT 12S. However, the same mutants were not only unable to cause hypertransformation but were unable to cotransform with E1B at all. Given the distribution of the mutations that enable cooperation with E1B and effect hypertransformation with T24*ras* (i.e., XS2 and XS3 can cooperate with E1B and hypertransform with *ras*, while *dl*13, *dl*23, *dl*24, etc., cannot cooperate with E1B but can hypertransform with *ras*), it seems unlikely that these functions are related. It is more likely that they are different functions encoded by overlapping regions of the second exon. Although pCtBP (7) binds to the region of E1A encoding the suppressive function (of *ras* transformation), we have not been able to detect such complexes with any WT or mutant 12S protein. The differences with respect to hypertransformation, taken together with the differential requirement of the second exon, indicate that *ras* and E1B utilize at least some different pathways, although they both require 12S first exon functions, such as activation of the cell cycle. Whether these differences are related to the ability of *ras* to activate the mitogen-activated protein kinase cascade and/or the *rac* and *rho* pathways (for reviews, see references 5 and 22) or the interaction of E1B with p53 (for a review, see reference 6) remains to be determined.

The NLS and efficient nuclear localization are necessary for cotransformation with E1B but not with T24*ras*. We have previously shown that nuclear localization is necessary for 12S immortalization but is not essential for cooperation with T24*ras* (9, 10). The nuclear localization signal (NLS) of 12S is composed of the C-terminal five amino acids of the protein (Lys-Arg-Pro-Arg-Pro) (23). Two of the basic residues, Lys-239 and Arg-240, are critical for signal function, while the characteristics of the other three amino acids, Pro-241, Arg-242 and Pro-243, are not (10, 10a). Regions upstream of the signal are also important in efficient 12S nuclear localization, probably as structural determinants (Fig. 1B) (10). It has been determined that efficient 12S nuclear localization is not necessary for T24*ras* cotransformation (9). To determine if subcellular localization or the NLS is important in E1B cotransformation, cotransfections with pCMVE1B and the mutated plasmids represented in Fig. 1C were performed. Quantitation of the transformed foci produced is shown in Fig. 5. Removal of the NLS (XS *dl*4) resulted in a loss of cooperation with E1B, suggesting it may be important in structure and/or function for E1B cotransformation. In an observation consistent with this, all of the signal mutants defective in localization were also defective in cooperation with E1B (Lys-239 and Arg-240) (Fig. 1 and 5). Similarly, other mutants defective in localization were defective in E1B cooperation, with the exception of HB *dl*14 (Fig. 1B and 5). Interestingly, however, the three efficiently localized NLS point mutants (Arg-242, Pro-241, and Pro-243) were also defective in E1B cotransformation. Other efficiently

localized proteins, such as HB *dl*12 and *dl*975/1339, and the four point mutants, XS1, XS2, XS3, and XS4, although they were able to cooperate with E1B, did so less efficiently than WT 12S. These results indicate that while efficient 12S nuclear localization may be important for E1B cotransformation, it is not sufficient. It also suggests that the NLS or region of the protein including the NLS is important in a function(s) other than just nuclear targeting. Another possibility is that E1B cooperation may be more sensitive to structural changes than the other two functions. A third possibility is that the E1B cotransformation assay itself is more stringent than the others, especially in a transfection. This would be consistent with the observation that E1B is not a very potent oncogene.

All of these results, taken together, indicate that the E1A second exon encodes several overlapping functions involved in immortalization and transformation. These results also indicate that different cooperating oncogenes require different E1A functions to effect the transformed phenotype and that E1A can participate in more than one transformation pathway.

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REFERENCES

1. **Babiss, L. E., P. B. Fisher, and H. S. Ginsberg.** 1984. Effect on transformation of mutations in the early region 1b-encoded 21- and 55-kilodalton proteins of adenovirus 5. J. Virol. **52:**389–395.
2. **Barker, D. D., and A. J. Berk.** 1987. Adenovirus proteins from both E1B reading frames are required for transformation of rodent cells by viral infection and DNA transfection. Virology **156:**107–121.
3. **Bayley, S. T., and J. S. Mymryk.** 1994. Adenovirus E1A proteins and transformation (review). Int. J. Oncol. **5:**325–444.
4. **Bernards, R., P. I. Schrier, J. L. Bos, and A. J. van der Eb.** 1983. Role of adenovirus types 5 and 12 early region 1b tumor antigens in oncogenic transformation. Virology **127:**45–53.
5. **Bokoch, G. M., and C. J. Der.** 1993. Emerging concepts in the *Ras* superfamily of GTP-binding proteins. FASEB J. **7:**750–759.
6. **Boulanger, P. A., and G. E. Blair.** 1991. Expression and interactions of human adenovirus oncoproteins. Biochem. J. **275:**281–299.
7. **Boyd, J. M., T. Subramanian, U. Schaeper, M. La Regina, S. Bayley, and G. Chinnadurai.** 1993. A region in the C-terminus of adenovirus 2/5 E1a protein is required for association with a cellular phosphoprotein and important for the negative modulation of T24-ras mediated transformation, tumorigenesis and metastasis. EMBO J. **12:**469–478.
8. **Chinnadurai, G.** 1983. Adenovirus 2 lp+ locus codes for a 19kd tumor antigen that plays an essential role in cell transformation. Cell **33:**759–766.
9. **Douglas, J. L., S. Gopalakrishnan, and M. P. Quinlan.** 1991. Modulation of transformation of primary epithelial cells by the second exon of the Ad5 E1A 12S gene. Oncogene **6:**2093–2103.
10. **Douglas, J. L., and M. P. Quinlan.** 1994. Efficient nuclear localization of the Ad5 E1A 12S protein is necessary for immortalization but not cotransformation of primary epithelial cells. Cell Growth Differ. **5:**475–483.

10a. **Douglas, J. L., and M. P. Quinlan.** Submitted for publication.

11. **Downward, J.** 1992. Regulatory mechanisms for ras proteins. Bioessays **14:** 177–184.
12. **Edbauer, C., C. Lamberti, J. Tong, and J. Williams.** 1988. Adenovirus type 12 E1B 19-kilodalton protein is not required for oncogenic transformation in rats. J. Virol. **62:**3265–3273.
13. **Fukui, Y., I. Saito, K. Shiroki, and H. Shimojo.** 1984. Isolation of transformation-defective, replication-nondefective early region 1B mutants of adenovirus 12. J. Virol. **49:**154–161.
14. **Gallimore, P. H., P. A. Sharp, and J. Sambrook.** 1974. Viral DNA in transformed cells. III. A study of the sequences of adenovirus 2 DNA in nine lines of transformed rat cells using specific fragments of the viral genome. J. Mol. Biol. **8:**49–72.
15. **Gopalakrishnan, S., and M. P. Quinlan.** 1995. Modulation of E-cadherin localization in cells expressing wild type E1A 12S or hypertransforming mutants. Cell Growth Differ. **6:**985–998.
16. **Graham, F. L., A. J. van der Eb, and H. L. Heijneker.** 1974. Size and location of the transforming region in human adenovirus type 5. Nature (London) **251:**687–691.
17. **Harlow, E., P. Whyte, B. R. Franza, Jr., and C. Schley.** 1986. Association of adenovirus early-region 1A proteins with cellular polypeptides. Mol. Cell. Biol. **6:**1579–1589.
18. **Houweling, A., P. J. van der Elsen, and A. J. van der Eb.** 1980. Partial transformation of primary rat cells by the leftmost 4.5% fragment of adenovirus 5 DNA. Virology **105:**537–550.
19. **Kunkel, T. A.** 1985. Rapid and efficient site-specific mutagenesis without a phenotypic selection. Proc. Natl. Acad. Sci. USA **82:**488–492.
20. **Lillie, J. W., M. Green, and M. R. Green.** 1986. An adenovirus E1a protein region required for transformation and transcriptional repression. Cell **46:** 1043–1051.
21. **Lillie, J. W., P. M. Loewenstein, M. R. Green, and M. Green.** 1987. Functional domains of adenovirus type 5 E1a proteins. Cell **50:**1091–1100.
22. **Lowy, D. R., and B. M. Willumsen.** 1993. Function and regulation of RAS. Annu. Rev. Biochem. **62:**851–891.
23. **Lyons, R. H., B. Q. Ferguson, and M. Rosenberg.** 1987. Pentapeptide nuclear localization signal in adenovirus E1a. Mol. Cell. Biol. **7:**2451–2456.
24. **Moran, E., B. Zerler, T. M. Harrison, and M. B. Mathews.** 1986. Identification of separate domains in the adenovirus E1A gene for immortalization activity and the activation of virus early genes. Mol. Cell. Biol. **6:**3470–3480.
25. **Mymryk, J. S., and S. T. Bayley.** 1993. Induction of gene expression by exon 2 of the major E1A proteins of adenovirus type 5. J. Virol. **67:**6922–6928.
26. **Pines, J., and T. Hunter.** 1990. Human cyclin A is adenovirus E1A-associated protein p60 and behaves differently from cyclin B. Nature (London) **346:**760–763.
27. **Quinlan, M. P.** 1994. Enhanced proliferation, growth factor induction and immortalization by adenovirus E1A 12S in the absence of E1B. Oncogene **9:**2639–2647.
28. **Quinlan, M. P., and J. L. Douglas.** 1992. Immortalization of primary epithelial cells requires first and second exon functions of adenovirus type 5 12S. J. Virol. **66:**2020–2030.
29. **Quinlan, M. P., and T. Grodzicker.** 1987. Adenovirus E1A 12S protein induces DNA synthesis and proliferation in primary epithelial cells in both the presence and absence of serum. J. Virol. **61:**673–682.
30. **Rao, L., M. Debbas, P. Sabbatini, D. Hockenbery, S. Korsmeyer, and E. White.** 1992. The adenovirus E1A proteins induce apoptosis, which is inhibited by the E1B 19-kDa and Bcl-2 proteins. Proc. Natl. Acad. Sci. USA **89:**7742–7746.
31. **Schneider, J. F., F. Fisher, C. R. Goding, and N. C. Jones.** 1987. Mutational analysis of the adenovirus E1a gene: the role of transcriptional regulation in transformation. EMBO J. **6:**2053–2060.
32. **Shen, Y., and T. Shenk.** 1994. Relief of p53-mediated transcriptional repression by the adenovirus E1B 19-kDa protein or the cellular Bcl-2 protein. Proc. Natl. Acad. Sci. USA **91:**8940–8944.
33. **Subramanian, T., M. Kuppuswamy, R. J. Nasr, and G. Chinnadurai.** 1988. An N-terminal region of adenovirus E1A essential for cell transformation and induction of an epithelial cell growth factor. Oncogene **2:**105–112.
34. **Subramanian, T., M. La Regina, and G. Chinnadurai.** 1989. Enhanced ras oncogene mediated cell transformation and tumorigenesis by adenovirus 2 mutants lacking the C-terminal region of E1a protein. Oncogene **4:**415–420.
35. **Subramanian, T., S. E. Malstrom, and G. Chinnadurai.** 1991. Requirement of the C-terminal region of adenovirus E1a for cell transformation in cooperation with E1b. Oncogene **6:**1171–1173.
36. **Telling, G. C., and J. Williams.** 1993. The E1B 19-kilodalton protein is not essential for transformation of rodent cells in vitro by adenovirus type 5. J. Virol. **67:**1600–1611.
37. **White, E., and R. Cipriani.** 1990. Role of adenovirus E1B proteins in transformation: altered organization of intermediate filaments in transformed cells that express the 19-kilodalton protein. Mol. Cell. Biol. **10:**120–130.
38. **Whyte, P., K. J. Buchkovich, J. M. Horowitz, S. H. Friend, M. Raybuck, R. A. Weinberg, and E. Harlow.** 1988. Association between an oncogene and an anti-oncogene: the adenovirus E1A proteins bind to the retinoblastoma gene product. Nature (London) **334:**124–129.
39. **Whyte, P., H. E. Ruley, and E. Harlow.** 1988. Two regions of the adenovirus early region 1A proteins are required for transformation. J. Virol. **62:**257–265.
40. **Whyte, P., N. M. Williamson, and E. Harlow.** 1989. Cellular targets for transformation by the adenovirus E1A proteins. Cell **56:**67–75.
41. **Yew, P. R., and A. J. Berk.** 1992. Inhibition of p53 transactivation required for transformation by adenovirus early 1B protein. Nature (London) **357:** 82–85.
42. **Yew, P. R., X. Liu, and A. J. Berk.** 1994. Adenovirus E1B oncoprotein tethers a transcriptional repression domain to p53. Genes Dev. **8:**190–202.
43. **Zantema, A., J. A. M. Fransen, A. Davis-Olivier, F. C. S. Raemakers, G. P. Vooijs, B. de Leys, and A. J. van der Eb.** 1985. Localization of the E1B proteins of adenovirus 5 in transformed cells, as revealed by interaction with monoclonal antibodies. Virology **142:**44–58.
44. **Zantema, A., P. I. Schrier, A. Davis-Olivier, T. van Laar, R. T. M. J. Vaessen, and A. J. van der Eb.** 1985. Adenovirus serotype determines association and localization of the large E1B tumor antigen with cellular tumor antigen p53 in transformed cells. Mol. Cell. Biol. **5:**3084–3091.