The Role of Adenovirus E1A in Transformation and Oncogenicity

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In this summary we report results on the mechanism of suppression of class-I major histocompatibility complex genes by adenovirus (Ad) E1A and the role of region E1A in transformation of primary rat cells. The results can be summarized as follows:

(1) Suppression of class-I gene activity by Ad12 is caused by the switching off of gene expression.

(2) All class-I loci are affected to the same extent.

(3) Introduction of region E1A of Ad5 in Ad12-transformed cells causes reappearance of class-I expression.

(4) A transfected class-I H-2 gene is also differentially regulated by Ad5 or Ad12 E1A.

(5) The immortalizing activity of E1A is localized in the aminoterminal part of the E1A protein.

(6) Region E1B does not seem to play a major role in transformation. Hence, region E1A is probably mainly responsible for this property.

Oncogenic transformation by adenovirus is mediated by the concerted activity of at least three viral gene products, encoded by the early regions E1A and E1B, which together constitute region E1 of the viral genome. Each of the two subregions specifies a set of coterminal mRNAs, which are translated into a number of partially overlapping proteins. In transformed cells, region E1A is expressed into two mRNAs of 12S and 13S, which specify related polypeptides of about 26 kD and 32 kD, respectively. Region E1B yields a single mRNA of 22S in transformed cells, which is translated into two unrelated proteins of about 19 kD and 55 kD (references in Pettersson and Akusjärvi 1983; Van Ormondt and Galibert 1984). Results from a number of studies have shown that region E1 contains all the information required for oncogenic transformation (although other early regions of the viral genome may also contribute to the oncogenic phenotype). The smallest DNA segment still possessing transforming activity is region E1A. Primary rodent cells transformed by this region only show a partially transformed phenotype and are nononcogenic, but they have become established into immortal cell lines (Houweling et al. 1980). Expression of E1A products in E1A-transformed cells is at least 20-fold lower than in cells transformed by E1A plus E1B, suggesting that region E1B is somehow required for high expression of region E1A (Van den Elsen 1983a,c). Conversely, region E1A is required for efficient expression of E1B, a phenomenon attributed to an E1A-mediated activation of E1B transcription (Berk et al. 1979; Jones and Shenk 1979; Bos and ten Wolde-Kraamwinkel 1983). Region E1B has no detectable transforming activity, even when it is fully expressed under the influence of a heterologous promoter (Van den Elsen et al. 1983b). Since region E1A is expressed to very low levels in E1A-immortalized cells and E1B alone does not seem to have transforming activity, it cannot be excluded that region E1A actually contains most, if not all, of the information needed for morphological transformation and that E1B is only required for full expression of E1A (Van den Elsen et al. 1983a). Although this interpretation is suggested by the data mentioned above, evidence has also been found that region E1B must have a role in determining the degree of oncogenicity of transformed cells (Bernards et al. 1983). Thus, the precise role of E1A and E1B in oncogenic transformation is still unresolved.

Recently, a new property has been identified for the E1A region of oncogenic Ad12. Cells transformed by the E1 region of this virus differ from cells transformed by the corresponding region of the nononcogenic Ad5 in that they lack expression of the class-I major histocompatibility complex (MHC) antigens on their cell membrane (Schrier et al. 1983). Class-I MHC antigens consist of a heavy chain of approximately 45 kD, which is noncovalently bound to β_2-microblogulin, the 12-kD light chain. Since killing of cells expressing foreign antigens by cytotoxic T lymphocytes (CTL) will only occur if the cells also express their class-I MHC antigens, the absence or strong reduction of these antigens on Ad12-transformed cells will render them more or less resistant to this type of cellular immune defense (cf. Bernards et al. 1983). This may explain, at least in part, why Ad12-transformed cells are as oncogenic in T-cell-deficient nude mice as in immunocompetent syngeneic rats. Since it was found that suppression of class-I MHC genes is a property of the E1A region of Ad12, it appeared of interest to study this interaction with cellular gene expression in more detail.

Subsequent work has shown that suppression of class-I genes in Ad12-transformed cells occurs at the level of mRNA accumulation, that is, the cells contain

strongly reduced amounts not only of class-I proteins but also of cytoplasmic mRNA. β_2-Microglobulin synthesis does not appear to be strongly affected. The Ad12 product responsible for the effect is encoded by the largest E1A mRNA (13S) and more specifically by its first exon, implying that the polypeptide specified by the nucleotide stretch that is unique for the 13S mRNA plays an important role in the suppression (Jochemsen et al. 1984). Interestingly, the Ad5 E1 region appears to counteract the effect of the Ad12 E1 region: BRK cells cotransformed by the Ad5 E1A and the Ad12 E1 region show no reduction of class-I expression, although both transforming regions are expressed to normal levels. This indicates that Ad5 E1A can somehow prevent Ad12 E1A from switching off expression of class-I genes. In the first part of this paper we report the results of further studies on the interaction of adenovirus genes with expression of the class-I MHC genes. In the second part results are presented of a study on the role of region E1A in oncogenic transformation in combination with the EJ *ras* oncogene.

Experimental Procedures

Transfections
All DNA transfections were performed as described by van der Eb and Graham (1980). Primary cultures of baby rat kidney (BRK) and baby mouse kidney (BMK) cells were prepared from 1-week-old WAG/RIJ rats and 1-week-old BALB/c or C57BL mice, respectively, obtained from the Radiobiological Institute, Rijswijk, The Netherlands.

RNA analysis
Standard procedures were used for S1 nuclease analysis (Bernards et al. 1982), isolation of total cytoplasmic RNA, and northern blotting analysis (Schrier et al. 1983).

Immunoprecipitation
Cell labeling with [^{35}S]methionine and subsequent immunoprecipitation was carried out as described previously (Schrier et al. 1979).

Fluorescence-activated cell sorting (FACS)
FACS analysis was performed by an Ortho Diagnostic System 50-HH and computer MP-2150.

Oncogenicity
Tumorigenicity of transformed cells was investigated by injection of 10^7 cells into adult nude mice or 4-day-old syngeneic WAG/RIJ rats.

Results

Interaction of region E1A with expression of class-I MHC genes

Ad5 E1 can restore expression of class-I genes in Ad12-transformed cells
Our previous results have shown that primary BRK cells transformed by the E1 region of highly oncogenic Ad12 have a strongly reduced expression of the class-I MHC antigens on their plasma membrane, as opposed to cells transformed by nononcogenic Ad5. When the E1A regions of both Ad5 and Ad12 are present in the same transformed cells, expression of the class-I genes is normal, indicating that Ad5 E1A can prevent the effect associated with Ad12 E1A (Bernards et al. 1983; Schrier et al. 1983). To further investigate the apparently opposite effects of Ad5 and Ad12 E1A, we have transfected class-I-negative, Ad12-transformed BRK cells with the Ad5 E1 region, in the presence of a dominant selectable marker (R.T.M.J. Vaessen, in prep.). Figure 1 shows that the level of class-I gene expression in the Ad5 E1–supertransfected cells was restored to the level found in Ad5-transformed cells. This indicated that Ad5 E1 can reactivate expression of the previously suppressed class-I genes and that the Ad12-transformed cells are intrinsically capable of forming normal amounts of class-I gene products. Experiments with hybrid Ad5/Ad12 E1A regions and mutant Ad5 E1 plasmids expressing exclusively either the 13S or the 12S E1A mRNA have shown that the dominant effect of Ad5 is a function of the product encoded by the first exon of the 13S E1A mRNA (Bernards et al. 1983; Jochemsen et al. 1984). This indicates that the class-I MHC–suppressing effect of Ad12 E1A and the opposing, dominant effect of Ad5 E1A on this suppression are functions of the same domain of the respective 13S mRNA products. It also suggests that the opposite activities may be due to a similar interaction of the E1A products with a common cellular receptor or other target and that the Ad5 product has a higher affinity for this receptor and competes out the Ad12 product.

Is the Ad12 E1A effect a general phenomenon?
To investigate whether the reduction of class-I MHC expression observed in Ad12-transformed BRK cells also occurs in cells from other organs or other species, we have transformed baby mouse kidney (BMK) cells, hamster embryo (HE) cells, rat embryo brain (REB) cells, and human embryonic retinoblast (HER) cells with Ad5 or Ad12 E1 plasmids. In all Ad12-transformed cells, a significant reduction of class-I MHC expression was observed as measured by northern blotting analysis and immunoprecipitation (not shown). The suppression was also found in cells transformed by highly oncogenic Ad31 but not in cells transformed by weakly oncogenic Ad7. Surprisingly, however, suppression was not observed in Ad12-transformed cells that were derived from established cell lines, that is, in cells that had already been established into immortal cells lines long before the Ad12 E1 region was introduced (e.g., rat 3Y1 cells; not shown). The results indicate that reduced expression of class-I MHC genes (the reduction factor varies from 4× to more than 10×) is a common feature of cells derived from a variety of animal species when they are transformed as primary or secondary cultures by highly oncogenic adenoviruses.

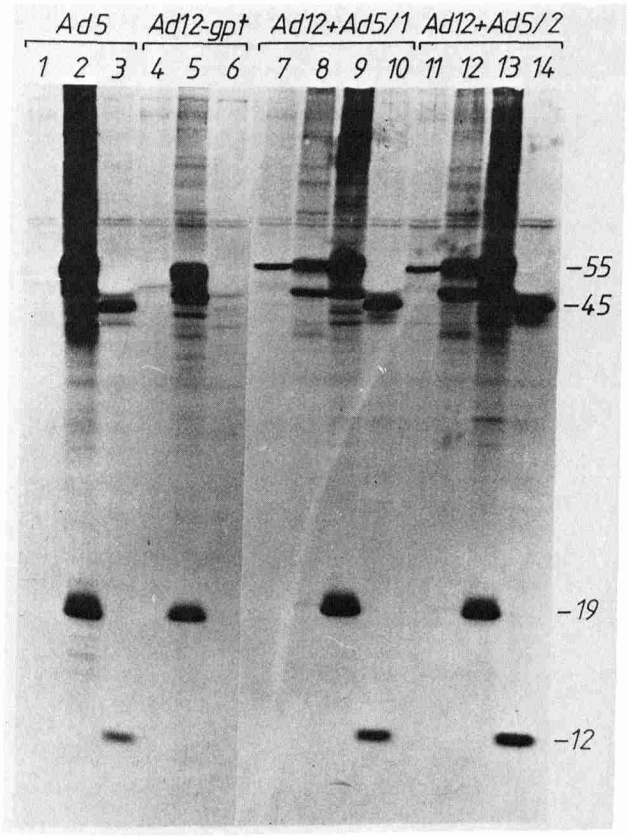

Figure 1 SDS-polyacrylamide gel electrophoresis of proteins immunoprecipitated from extracts of [^{35}S]methionine-labeled cells with normal rat serum (lanes *1*, *4*, *7*, and *11*), Ad5 anti-T serum (lanes *2*, *9*, and *13*), Ad12 anti-T serum (lanes *5*, *8*, and *12*), and a mouse monoclonal antibody against rat class-I MHC heavy chains (lanes *3*, *6*, *10*, and *14*). The following cell lines were used: Ad5 (BRK cells transformed by Ad5 E1), Ad12-gpt (Ad12 E1–transformed BRK cells transfected with pSV2gpt [Mulligan and Berg 1981]), Ad12 + Ad5 (Ad12 E1–transformed BRK cells transfected with Ad5 E1 and pSV2gpt; 1 and 2 represent different cell lines). The molecular sizes (in kD) of the adenovirus small and large T antigens (19 and 55), the class-I MHC heavy chain (45), and β_2-microglobulin are indicated.

Evidence that the Ad12 E1A-induced suppression is due to active switching-off

As mentioned above, inhibition of class-I expression by Ad12 region E1 and the activation of expression after introduction of Ad5 E1 are both functions of the first exon product of the 13S E1A mRNAs. This suggested that the suppression of class-I gene expression by Ad12 E1A may be due to an active switching-off phenomenon. However, the primary cell cultures used for the transformation assays presumably consist of heterogeneous cell populations, and it cannot be excluded that Ad5 preferentially transforms cells expressing high levels of class-I antigens, whereas Ad12 selects other cells that have low expression of class-I genes. This interpretation is supported by the observation showing that Ad12 transforms primary cell cultures with a much lower frequency than Ad5 (Bernards et al. 1982), and that Ad12 may preferentially transform cells of neural origin (Mukai 1976; Gallimore and Paraskeva 1980), which are known to express low levels of class-I antigens (Vitetta and Capra 1978; Williams et al. 1980).

To distinguish between active switching-off of MHC genes and selective transformation of cells expressing low levels of MHC antigens, we have made use of a BRK cell line transformed by a mutant Ad12 E1 region, R11. This mutant plasmid carries a 109-bp deletion in region E1A so that it can only code for a 15-kD amino-terminal truncated protein (Bos et al. 1983). The R11 mutant plasmid is defective in transformation unless the SV40 enhancer sequences are inserted upstream of the R11 region (SVR11). BRK cells transformed by the chimeric SVR11 plasmid have become immortal and express the E1B region to normal levels but are nononcogenic in nude mice. Interestingly, the SVR11-transformed cells do not show a reduction of class-I gene expression, in contrast to cells transformed by wild-type Ad12 E1. Therefore, we were interested to investigate the effect of introduction of a wild-type Ad12 E1A region on class-I expression in these cells. Transfection of an Ad12 E1 plasmid in the presence of a dominant selectable marker (pSV2neo) into SVR11-transformed cells resulted in the isolation of clones expressing both the R11 E1A and the wild-type Ad12 E1A region. One of the lines, SVR11/Ad12-C8, expressed levels of Ad12 E1A mRNAs and proteins comparable to that in Ad12 E1-transformed control cells, but the second line, SVR11/Ad12-C6, contained only small amounts of wild-type E1A mRNA and no detectable E1A proteins (Fig. 2 and Fig. 3, lanes 12 and 16). Both lines contained normal amounts of R11 E1A mRNA (Fig. 2). Interestingly, immunoprecipitation of cell extracts from the supertransfected cells with an anti–rat class-I alloantiserum showed that expression of class-I antigens was drastically reduced in the C8 line, which showed high expression of wild-type Ad12 E1A (Fig. 3, lane 16), but was not reduced in the C6 line, in which the Ad12 E1A expression was low (Fig. 3, lane 12). Similar results were obtained for class-I-specific mRNA, using northern blotting analysis. This experiment

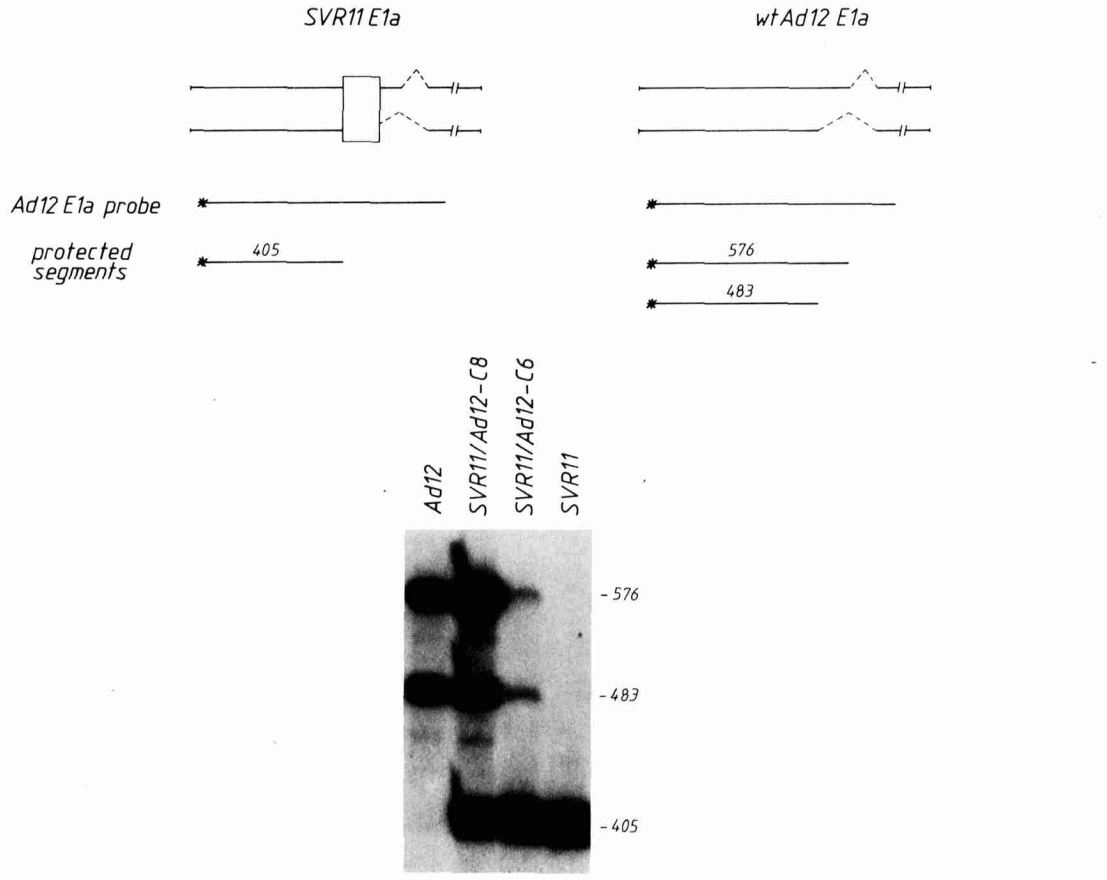

Figure 2 (*Top*) Schematic representation of the S1 nuclease analysis performed to verify the expression of wild-type Ad12 E1A mRNAs after transfection of Ad12 E1 into SVR11-transformed BRK cells. The E1A mRNAs from SVR11 and wild-type Ad12 are shown. The box in SVR11 E1A represents a 109-bp deletion. A *Nar*I-*Dde*I fragment isolated from pAd12Acc (Jochemsen et al. 1984) and 3′-end-labeled at the *Nar*I position was used as a probe. The lengths of the protected segments are indicated. The autoradiograph shows the S1-resistant fragments run on a 5% acyrlamide–7 M urea gel after hybridization of the DNA probe to 20 μg of total cytoplasmic RNA at 55°C.

indicates that expression of class-I MHC products is only reduced when wild-type Ad12 E1A is expressed at a sufficiently high level, which strongly suggests that Ad12 E1A products can indeed actively suppress class-I MHC genes.

Expression of all class-I loci is suppressed in Ad12-transformed cells

Although the level of class-I MHC antigens was strongly reduced in most Ad12-transformed primary cell cultures tested so far, the suppression was never complete since residual amounts of class-I mRNA and protein usually could be detected. To investigate whether the residual class-I mRNA and protein is due to the fact that certain class-I genes are partially suppressed and others completely, we have also investigated the expression of the individual class-I loci in Ad5- and Ad12-transformed cells from BALB/c mice. S1 nuclease analysis of cytoplasmic mRNA, using locus-specific DNA probes, showed that the level of RNA corresponding to all three loci, *K*, *L*, and *D*, is somewhat elevated in Ad5-transformed cells as compared with untransformed cells, but that the RNA concentration from all three loci is considerably reduced in the Ad12-transformed cells (not shown). Similar results were obtained for the H-2 antigens specified by the class-I genes in the same transformed cells as well as in transformed BMK cells from C57BL mice, as detected by FACS with specific alloantisera. The results obtained with the C57BL BMK cells, which lack genes of the *H-2L* locus, are presented in Figure 4. The results show that the suppression of class-I gene products in Ad12 E1-transformed cells affects all class-I loci approximately to the same extent.

Evidence that transfected class-I MHC genes are also differentially regulated by Ad5 and Ad12 region E1

In an attempt to identify which DNA sequences in or around class-I genes are involved in the inhibition of gene expression, we have first investigated whether cloned class-I genes, when introduced into Ad5- or Ad12-transformed cells, are subject to the same regulation as the endogenous genes. Ad5- and Ad12-transformed BALB/c BMK cells (*d* haplotype) were transfected with a cloned *H-2* gene of the *b* haplotype (kindly

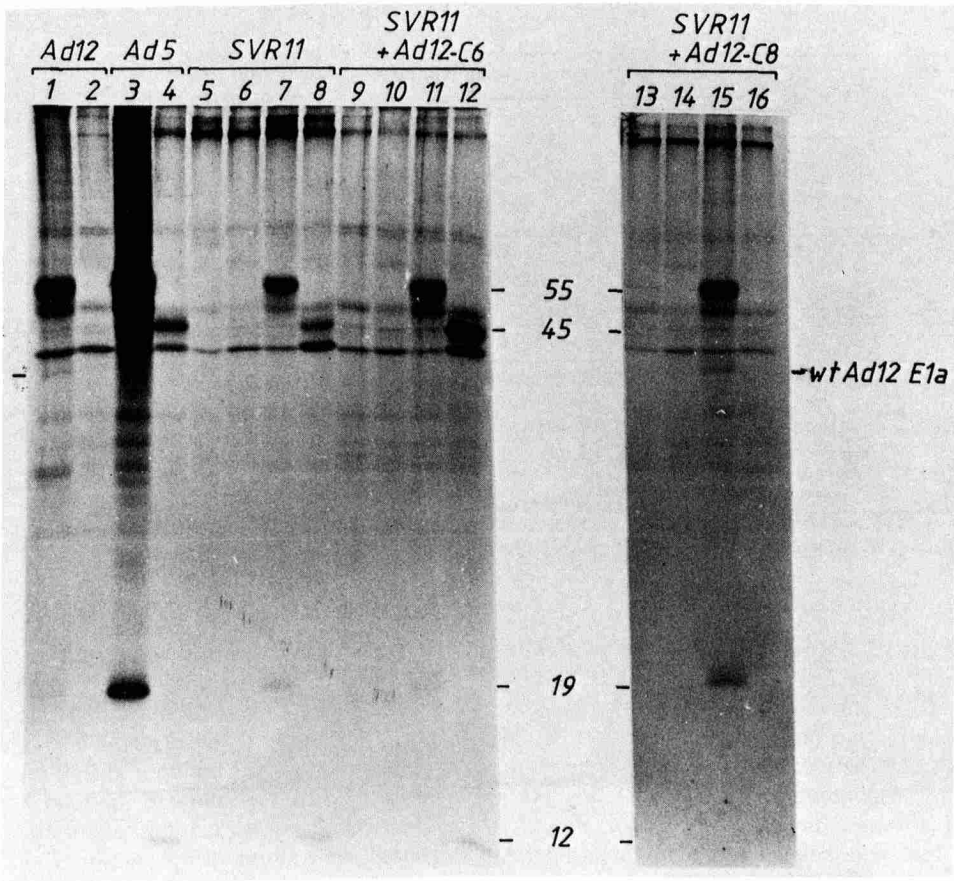

Figure 3 SDS-polyacrylamide gel electrophoresis of proteins immunoprecipitated from extracts of [^{35}S]methionine-labeled cells with normal rat serum (lanes *5*, *9*, and *13*), Ad5 anti-T serum (lanes *3*, *6*, *10*, and *14*), Ad12 anti-T serum (lanes *1*, *7*, *11*, and *15*), and a Lew anti-Wag rat alloantiserum (Schrier et al. 1983) (lanes *2*, *4*, *8*, *12*, and *16*). The following cell lines were used: Ad5 (BRK cells transformed by Ad5 E1), Ad12 (BRK cells transformed by Ad12 E1), SVR11 (BRK cells transformed by plasmid SVR11), SVR11 + Ad12 (SVR11 cells transfected with Ad12 E1 and pSV2neo; C6 and C8 refer to individual G418-resistant cell lines. wtAd12 E1A refers to wild-type Ad12 E1A protein precipitated in lanes *1* and *15*. The molecular sizes (in kD) of the adenovirus small and large T antigens (19 and 55), the class-I MHC heavy chain (45), and β_2-microglobulin are indicated.

provided by Dr. R.A. Flavell, Biogen) in the presence of a dominant selection marker. This *H-2* clone contains about 4000 bp upstream from the cap site. The expression of the transfected *H-2* gene was determined by S1 analysis using a DNA probe that specifically recognizes the transfected gene. Figure 5 shows that the transfected *H-2* gene is efficiently expressed in Ad5-transformed cells, but to a much lower level in Ad12-transformed cells. This suggests that expression of transfected genes may indeed be regulated by Ad5 or Ad12 E1A in the same way as the endogenous genes. Experiments are in progress to determine whether specific DNA sequences in the 5′ upstream region of the gene are involved in the inhibition by Ad12 E1A.

Interaction of region E1A with the T24 *ras* oncogene

To further define the role of region E1A in transformation and oncogenicity, we have studied the interaction of this region with the T24 *ras* oncogene. As was first demonstrated by Ruley (1983) and Land et al. (1983), primary rat cell cultures can be oncogenically transformed by the combination of Ad2 region E1A and the EJ *ras* oncogene. As such, the *ras* oncogene can functionally replace E1B in transformation. To investigate the effect on oncogenicity of different E1A regions in transformation with the *ras* oncogene, we have transformed BRK cultures with EJ *ras* plus either Ad5 E1A, Ad12 E1A, or the mutant Ad12 E1 region from pSVR11. As described in a previous section, R11 is an Ad12 E1 region with a 109-bp deletion in region E1A. The R11 plasmid was capable of causing transformation only when the SV40 enhancer was inserted upstream of the R11 E1 region (Bos et al. 1983).

As can be seen in Table 1, all three E1A regions could transform BRK cells in the presence of EJ *ras*. The efficiency of transformation with wild-type E1A plus *ras* was considerably lower than that of E1A plus E1B, and in the case of Ad5 E1A plus *ras* the transformation efficiency approached that of E1A alone. Analysis of the

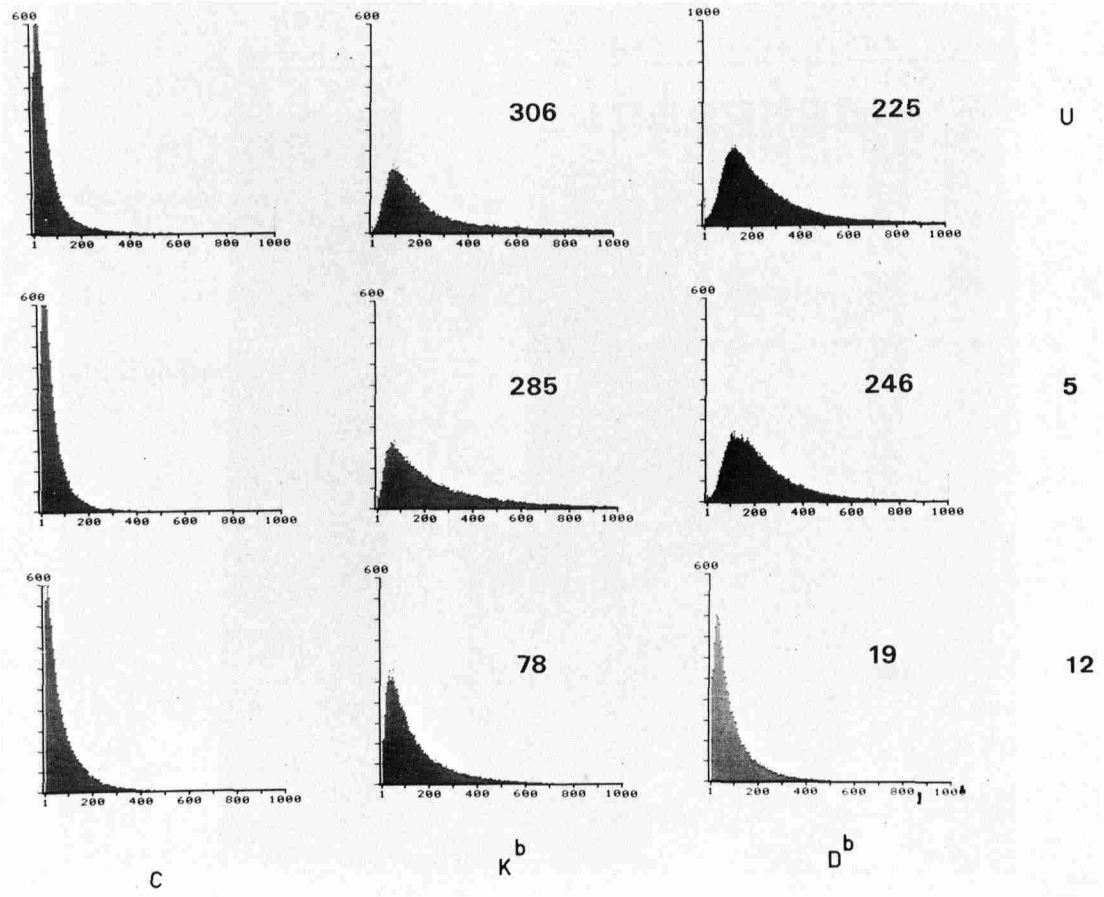

Figure 4 FACS analysis of untransformed, Ad5 E1– and Ad12 E1–transformed C57/B1 (*H-2*^{b}) BMK cells. Cells were incubated with specific anti-K^b or -D^b antiserum and fluorescent conjugate, respectively, or conjugate only (*C*). Numbers indicate the average fluorescence value for each sample, which was calculated as follows: $m_{antibody}/m_{conjugate}^{-1}$.

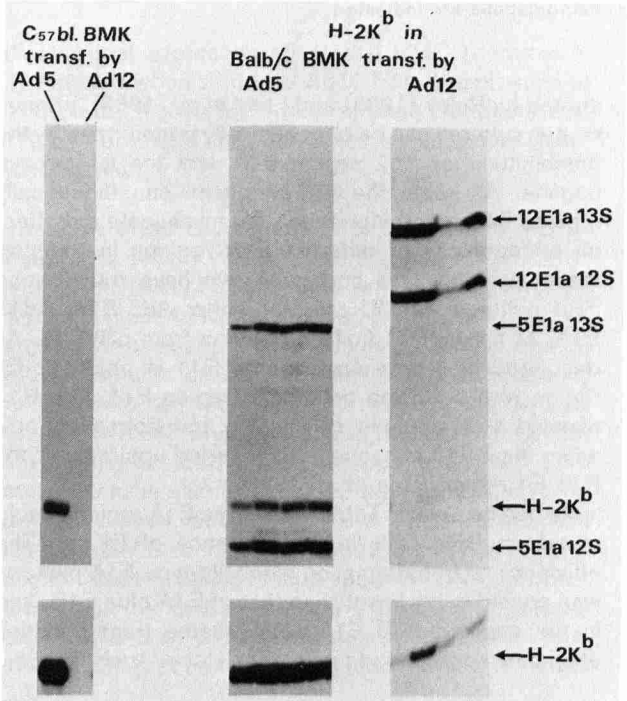

Figure 5 S1 nuclease analysis of the *H-2K*^{b} expression in supertransfected Ad5 E1– and Ad12 E1–transformed BALB/c BMK cells. As a reference, the E1A expression level in these cells was also determined. Probes: Ad5 E1A (3′-end-labeled *Hin*fI-fragment [nt 761–1372]); Ad12 E1A (3′-end-labeled *Nar*I-*Dde*I fragment (nt 1193–1255]); *H-2K*^{b} (3′-end-labeled *Sin*I-fragment [600 bp], spanning the third exon–third intron junction); C57/bl (Ad5 E1– or Ad12 E1–transformed *b* haplotype BMK cells). (*Bottom*) Longer exposure of the same gel, with only *H-2K*^{b} expression showing.

Table 1 Properties of BRK Cells Transformed by Various E1A Regions in the Absence or Presence of Either Ad12 E1B or the EJ *ras* Oncogene

DNA used for transformation	Avg. no. foci/μg genome equivalent E1A	Oncogenicity of transformed cells[a]		E1A expression	EJ *ras* expression[b]
		nude mice	syngeneic rats		
Ad5 E1A	0.36	0/12	n.d.	low	–
Ad5 E1A + Ad12 E1B	7.6	18/18 (35 days)	0/26	high	–
Ad5 E1A + EJ *ras*	0.34	15/15 (15 days)	9/9 (10 days)	low	+ +
Ad12 E1A	(0)[c]	—	—	—	–
Ad12 E1A + Ad12 E1B	0.55	23/23 (45 days)	18/18 (110 days)	high	–
Ad12 E1A + EJ *ras*	0.02	5/15 (45 days)	2/9 (90 days)	low	+ +
SVR11 E1A	0.7	0/9	ND	high	–
SVR11 E1A + Ad12 E1B	1.0	0/12	ND	high	–
SVR11 E1A + EJ *ras*	0.8	9/15 (30 days)	ND	high	+ +

Data are taken from Bernards et al. 1982, 1983; Van den Elsen et al. 1982; Bos et al. 1983; R. Bernards et al.; A.G. Jochemsen et al.; both in prep.

[a]Oncogenicity is indicated as the number of animals with tumors per the number of animals injected. The average latency period of a tumor formation is indicated in parentheses. (ND) Not done.

[b]The c-Ha-*ras* expression in the E1A + *ras*–transformed cells is comparable to the expression level in the T24 bladder carcinoma cell line.

[c]The transformation frequency of Ad12 E1A probably is below our detection level, or the immortalized cells are not distinguishable between untransformed cells. Others have reported immortalizing activity of Ad12 E1A (Gallimore et al. 1984).

expression level of E1A RNA in the transformed cells showed that the RNA concentration of wild-type E1A was very low in all cases except when region E1B was present in addition to E1A (Table 1). Apparently, the presence of region E1B is required for efficient expression of region E1A (Van den Elsen et al. 1982). Thus, the efficiency of transformation appears to be proportional to the level of E1A mRNA. Furthermore, the results summarized in Table 1 show that the EJ *ras* gene can substitute for region E1B in conferring a fully transformed phenotype to primary rat cells as well as the ability to form tumors, but that it cannot enhance the level of E1A mRNA, as region E1B can. Together, the results suggest that the major role of region E1A in the transformation with *ras* is to immortalize the primary rat cells (as it also does in the absence of other transforming genes). Morphological transformation and oncogenicity could then be functions of EJ *ras*. The question can then be asked which gene product(s) are responsible for the fully transformed phenotype when cells are transformed by E1A plus E1B and region E1A is expressed at high levels. Since we have not been able to detect any transforming activity for region E1B alone (Van den Elsen et al. 1983b), there is no clear indication that the acquisition of morphological transformation is a direct function of region E1B product(s). Therefore, there may be more reasons to believe that this property is largely a function of region E1A, provided it is expressed at a sufficiently high level (cf. Van den Elsen et al. 1983a). Major functions of E1B would then include the stimulation of expression of region E1A and a role in oncogenicity (Bernards et al. 1982, 1983). That region E1B may have no important role in transformation is also suggested by the observation that BRK cells transformed by SVR11 E1A alone or SVR11 E1A plus Ad12 E1B are very similar in phenotype and hardly appeared transformed (but nevertheless are immortalized), showing that additional expression of E1B has little or no effect. In combination with the EJ *ras* oncogene, the SVR11 E1A region can induce a fully transformed phenotype. The results with the SVR11 mutant also show that the aminoterminal part (15 kD) of the E1A protein(s) encoded by the 13S mRNA has immortalizing potential. The additional presence of the carboxyterminal part would then be required for transformation. Attempts to prove the assumption that morphological transformation is largely a function of region E1A have failed because it has not been possible to isolate E1A-transformed primary cells with a high expression of E1A. Possibly, high levels of E1A proteins are toxic in the absence of E1B protein, but other interpretations cannot be excluded.

An interesting observation was that cells transformed by Ad5 E1A plus *ras* strongly differ from cells transformed by Ad12 E1A plus *ras*. The former have a rounded-cell morphology (possibly due to the absence of fibronectin; H. van Kranen and J.L. Bos, pers. comm.), are anchorage independent, and highly oncogenic, whereas the latter have a flat appearance, adhere strongly to substrate (there is only a partial reduction of fibronectin mRNA), and are only weakly oncogenic. Thus, the cells containing the E1A region of the nononcogenic virus are much more strongly oncogenic than those containing the E1A region of the oncogenic virus. Since the expression levels of the E1A regions and of *ras*, respectively, were equal, a possible explanation for the more highly oncogenic and transformed phenotype of the cells transformed by Ad5 E1A plus *ras* may be the stronger gene expression–modulating activity of the Ad5 E1A region (cf. Bos and ten Wolde-Kraamwinkel 1983). Hence, E1A fulfills more functions in cells transformed by E1A plus *ras* than immortalization alone. If this interpretation is correct, it would imply that oncogenic transformation by adenoviruses is due to E1A-induced alterations of cellular gene expression.

Discussion

In this paper we report the results on the functional activities of the E1A regions of human adenovirus types 5 and 12 in oncogenic transformation of primary rat cell cultures. Functions that have been associated with region E1A include:

1. immortalization (Houweling et al. 1980);
2. transcriptional activation of region E1B and other early regions of the viral genome (Berk et al. 1979; Jones and Shenk 1979) and of certain cellular genes (such as the hsp70 heat-shock gene; Nevins 1979);
3. determination of the efficiency of transformation of primary rat cells (Ad5 E1A causes at least a 10× higher transformation frequency than Ad12 E1A; Bernards et al. 1982);
4. determination of the morphology of transformed colonies (Van den Elsen et al. 1983a);
5. differential modulation of class-I MHC expression (Schrier et al. 1983). The latter phenomenon provides at least a partial explanation for the fact that Ad12-transformed cells are oncogenic in immunocompetent animals.

In this paper we report results of additional experiments on the effect of region E1A on class-I MHC gene expression and on the interaction of region E1A with the EJ *ras* gene in oncogenic transformation of primary rat cell cultures. The observation that the suppressing effect of Ad12 E1A on class-I expression and the "dominant," activating effect of Ad5 E1A on class-I expression are functions of the product encoded by the first exon of the respective 13S mRNAs suggests that the two effects may be based on a similar interaction of the E1A products with a common cellular receptor or other target. The Ad5 E1A product would bind more strongly to this receptor than the Ad12 E1A product and could even compete-out the Ad12 product. The nature of this putative receptor is unknown, but possible candidates would be, for example, transcription complexes of certain genes. Evidence has indeed been presented that adenovirus E1A products directly or indirectly participate in viral gene transcription and that Ad5 E1A is a more efficient transcriptional activator than Ad12 E1A (Bos and ten Wolde-Kraamwinkel 1983). If one assumes that E1A products can also participate in the regulation of transcription of certain cellular genes, as has in fact been shown for the hsp70 gene (Kao and Nevins 1983), it is conceivable that Ad5 E1A products indeed stimulate transcription but that Ad12 products inhibit or reduce transcription due to the formation of inactive or weakly active transcription complexes. It is clear, however, that this mechanism can only be valid if suppression of class-I activity indeed occurs at the level of gene transcription. That this is still uncertain was recently suggested by nuclear run-off experiments that indicated that the rate of transcription of class-I genes is the same in Ad5- and Ad12-transformed human cells (our unpublished observations). Thus, it is still uncertain whether reduced expression of class-I genes by Ad12 E1A is caused by inhibition of class-I transcription or by a decrease in the rate of posttranscriptional maturation of mRNA (e.g., as a result of decreased stability of the primary transcripts). Our finding that a transfected genomic clone of a *H-2* gene is differentially expressed in Ad5- and Ad12-transformed cells may be of considerable help in distinguishing between these two possibilities. That the Ad12 effect is indeed due to inhibition of class-I gene expression and not to selective transformation of target cells in the primary cultures that express low levels of class-I products was indicated by the supertransfection experiment of the (class-I-positive) SVR11-transformed cells.

In the second part of this study we show that different E1A regions can cooperate with the EJ *ras* oncogene in transformation of primary rat cells. The efficiency of transformation with the combinations of these two was very low and approximately equal to that of the E1A region alone. High transformation efficiencies were only obtained when region E1B was present in addition to E1A. The frequency of transformation appeared to be correlated with the level of expression of the E1A region, that is, when E1A was expressed at high levels, the frequency of transformation was also high, and when E1A expression was low, transformation was also low. Thus, the *ras* oncogene can replace the E1B region in oncogenic transformation with E1A, but it cannot stimulate the frequency of transformation or enhance the expression of E1A. Since the effect of region E1A alone in primary cells is to cause immortalization and, at the most, partial transformation, it is reasonable to conclude that the major contribution of region E1A in the transformation with *ras* is to confer immortality to the primary cells.

Then the question arises which role should be attributed to region E1A when it is expressed at the high levels found in cells transformed by E1A and E1B. The experimental evidence obtained so far suggests that region E1A may have a major role in morphological transformation when it is expressed at high levels, because region E1B alone has no detectable transforming activity (Van den Elsen et al. 1983a) and because cells transformed by SVR11 E1A alone or by SVR11 E1A plus E1B do not differ in phenotype, and, in fact, both appear only semitransformed. Attempts to prove the assumption that high levels of E1A products alone cause full transformation have failed so far since we have not been able to isolate E1A-transformed cells with a high expression level of E1A. If this assumption will prove to be correct, the concept that E1A belongs to the *myc*-like oncogenes and E1B to the *ras*-like oncogenes may require reconsideration.

The surprising observation that cells transformed by Ad5 E1A plus *ras* appear more highly transformed and are more strongly oncogenic than cells transformed by Ad12 E1A plus *ras* implies that the role of E1A is not only to confer immortality but also to induce transformed properties, even when expressed at a low level. Since the E1A regions of the two adenoviruses have been shown to differ in their ability to stimulate transcription of the E1B gene (Bos and ten Wolde-Kraamwinkel 1983),

it is tempting to speculate that the differences in transformed phenotype just mentioned are due to differences in activity to modulate cellular gene expression.

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