REVIEW ARTICLE

Expression and interactions of human adenovirus oncoproteins

Pierre A. BOULANGER* and G. Eric BLAIR†

*Laboratoire de Virologie et Pathogénèse Moléculaires, Institut de Biologie, Faculté de Médecine, 34060 Montpellier, France, and

†Department of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT, U.K.

INTRODUCTION: CELL TRANSFORMATION BY ONCOGENIC DNA VIRUSES

Cell growth is a complex process resulting from a cascade of events regulated by both positively and negatively acting biological signals. Tumorigenic proliferation has been shown in some cases to be caused by alterations in genes encoding proteins involved in such signalling pathways (Klein, 1988). DNA tumour viruses have been extensively studied and widely used as molecular probes and models of cellular transformation. The oncogenes, i.e. tumour-inducing genes, from polyomavirus (Py), simian virus 40 (SV40) and adenovirus (Ad), are capable of inducing several distinct changes in cellular phenotype, including immortalization, secretion of growth factors, loss of contact inhibition, anchorage-independent growth and morphological transformation (Branton *et al.*, 1985). However, unlike the transforming retroviruses, the DNA tumour viruses contain oncogenes with apparently no cellular counterparts (Bishop, 1985; Branton *et al.*, 1985; Klein, 1988). Moreover, the proteins encoded by DNA viral oncogenes play an essential role in the lytic life-cycle of the virus in fully permissive host cells (Alwine *et al.*, 1977; Harrison *et al.*, 1977; Lassam *et al.*, 1978), whereas transforming oncogenes of retroviruses are not required for lytic growth (Bishop, 1982).

Adenovirus, like the other DNA tumour viruses, encodes a set of proteins that disturb cellular regulatory functions, thus reprogramming cell growth (Berk, 1986a; Branton *et al.*, 1985; Branton & Rowe, 1985). The proteins encoded by oncogenes are termed 'oncoproteins'. Certain viral oncoproteins have been found to form stable complexes with host-cell proteins which also appear to be involved in transformation. This is the case for the Py middle T–pp60^{c-src} complex (Courtneidge & Smith, 1983), the SV40 large T–p53 complex (Lane & Crawford, 1983; Linzer & Levine, 1979; McCormick & Harlow, 1980), and the adenovirus E1B–p55:p53 complex (Sarnow *et al.*, 1982a). The formation of these complexes may result in the potentiation of the transforming functions of these cellular proteins. The molecular mechanism might involve the alteration of a catalytic activity of the cellular protein, such as the protein kinase activity of pp60^{c-src} (Bolen *et al.*, 1984; Courtneidge, 1985), or a change in protein conformation and stability (e.g. p53; Oren *et al.*, 1981; Reich *et al.*, 1983).

Cellular transformation by the adenovirus oncogenes involves a very elaborate mechanism at the molecular level. It seems to occur as a sequential process, involving two blocks of genes, E1A and E1B. E1A alone has the property of immortalizing primary cells in culture (Houweling *et al.*, 1980; Ruley, 1983; Cone *et al.*, 1988). In cooperation with the adenovirus E1B gene or an activated *ras* gene, E1A stably transforms cells in culture, and these transformed cells can induce tumours in animals (Berk, 1986a; Branton *et al.*, 1985; Ruley, 1983; van den Elsen *et al.*, 1983). In addition to their role in malignant transformation, the

E1A oncoproteins are potent activators of transcription of both cellular and viral genes. This includes genes of class II and class III (reviewed in Berk, 1986b). However, E1A oncogene products have also been found to have repressor activity on transcription of genes under the control of certain viral or cellular enhancers (Borrelli *et al.*, 1984; Velcich & Ziff, 1985).

The question of the role of adenovirus oncogenes and their products in malignant transformation has given rise to an enormous literature cited in several reviews (Berk, 1986b; Branton *et al.*, 1985; Grand, 1987; Green, 1989; Kingston *et al.*, 1985; Moran & Mathews, 1987; Stillman, 1986; van der Eb & Bernards, 1984). A series of recent findings, however, has given new insights into viral carcinogenesis, and some clues have emerged which may unify its mechanism in terms of intracellular biochemical reactions. (i) Adenovirus oncoproteins were found to associate with host cell proteins, one of which was identified as an anti-oncogene product (Whyte *et al.*, 1988a). (ii) The same host cell major anti-oncogene product was also found to bind to SV40 large T antigen (DeCaprio *et al.*, 1988; Ludlow *et al.*, 1989). (iii) A domain of an E1A oncoprotein was substituted by a region of SV40 large T antigen, and the resulting chimaeric molecule retained transforming properties (Moran, 1988). The aim of this review is to present the current state of knowledge about the oncogenes and oncogene products of human adenovirus, emphasizing recent developments in understanding adenovirus-mediated oncogenesis.

STRUCTURE OF THE TRANSFORMING REGION OF THE ADENOVIRUS GENOME

Adenoviruses contain a linear, double-stranded DNA genome of approximately 36 kbp which is conventionally divided into 100 map units (m.u.) or expressed as a percentage from the left terminus (denoted 0.0%). The leftmost 11% of adenovirus DNA comprises two blocks of genes, called the E1A and E1B regions (Fig. 1). 'E' stands for 'early' since these genes are expressed at early stages of viral infection, preceding the onset of viral DNA replication. Among the blocks of early viral genes, E1A and E1B are the first ones to be transcribed. They have thus been defined as 'immediate-early' genes (reviewed in Berk, 1986b). E1A and E1B represent the transforming region of the adenovirus genome. Adenovirus-transformed cells have integrated a portion of the adenovirus genome spanning the E1A and E1B regions, and constitutively express the E1A and E1B gene products (Gallimore *et al.*, 1985b; Graham & van der Eb, 1973; van der Eb & Bernards, 1984).

Genomic organization of the E1A and E1B regions

E1A messenger RNAs have been detected as early as 30 min after infection (Berk *et al.*, 1979), and proteins are translated from three major mRNAs sedimenting at 13 S (1.1 kb), 12 S (0.9 kb) and 9 S (0.6 kb). Two additional mRNA species with

Abbreviations used: Ad, adenovirus; CTL, cytotoxic T lymphocytes; m.u., map units; NK, natural killer; TNF, tumour necrosis factor; with the aim of simplifying this text, most proteins (except post-translationally-modified E1A proteins) will be referred to as pX, X being the value, in kDa, of their apparent molecular masses in conventional SDS/polyacrylamide gels, e.g. E1Bp55 stands for E1B-55000 dalton protein.

Fig. 1. Structure and functions of the oncogenic regions E1A and E1B of human adenovirus serotype 2 (modified from Graham, 1984)

Fig. 2. Splicing events and gene products of the Ad2 E1A regions

The solid line at the top of the Figure is the left-hand end of the adenovirus linear genome, from nucleotide 500 to 1600. The Ad2 E1A mRNAs are shown as solid lines and the protein coding sequences as coloured boxes, representing the alternative reading frames. Alternative splicing occurs between one single acceptor site at nucleotide 1226 and two possible donor sites at nucleotides 1111 and 973, respectively. The 10 S and 11 S mRNAs possess two additional donor and acceptor sites: one donor site is common with the 9 S transcript, the second donor site is either common with the 12 S or 13 S species. Modified from Green *et al.* (1983) and Stephens & Harlow (1987).

respective sedimentation coefficients of 10 S and 11 S have been detected (Stephens & Harlow, 1987; Ulfendahl *et al.*, 1987). All mRNAs, except the 9 S transcript, are translated in the same reading frame. They have the same 5' and 3' ends, but they are differentially spliced (Fig. 2). Unlike the other E1A transcripts which accumulate at the early stage of infection, the 9 S mRNA is preferentially transcribed and accumulated at late stages of infection (Berk & Sharp, 1978).

The E1B region maps between 4.8 and 11.2 map units and encodes three major mRNA species, 22 S, 13 S and 9 S (Fig. 3). The E1B transcriptional unit is activated by products of the early

E1A region (Déry *et al.*, 1987), generating the two major overlapping mRNAs of 22 S and 13 S in infected cells. Both 22 S and 13 S mRNAs are derived from a common mRNA precursor by alternative splicing (Pettersson *et al.*, 1983). The 22 S mRNA is translated in two different reading frames, starting from two separate initiation codons at nucleotide 1699 and 2016 for Ad2 (Fig. 3; Bos *et al.*, 1981; Pettersson & Akusjärvi, 1983). This polycistronic message thus encodes two different proteins, of 55000 (p55) and 19000 (p19) Da.

The 9 S mRNA is transcribed from an independent promoter, and encodes a structural component of the adenovirus capsid,

Fig. 3. Map of the E1B region

E1B promoter controls a rightward transcript that starts at nucleotide 1699 (+1) in the Ad2 genome (Gingeras *et al.*, 1982). The coloured boxes represent the three alternative reading frames. Synthesis of the virion structural polypeptide IX begins at the intermediate stage of infection, from the E1B 9 S mRNA. Modified from Déry *et al.* (1987).

Fig. 4. Regulatory elements of the adenovirus E1A oncogene (a) and a tentative model for positive (promoter-mediated) and negative (enhancer-mediated) control occurring in the different blocks of adenovirus early genes (b)

(a) The nucleotide numbers, indicated over the genome line, start from the left-hand end of the Ad2 DNA sequence. (+1) refers to the start of E1A mRNA, at nucleotide +499. The ATG start codon of E1A is at nucleotide +560. Modified from Hearing & Shenk (1986). (b) On the adenovirus linear genome, the box represents the upstream enhancer elements of E1A, the diamonds the promoter of each block of early genes. Modified from Smith *et al.* (1985).

present in groups of nine hexons, which is referred to as polypeptide IX (Boulanger *et al.*, 1979; Furcinetti *et al.*, 1989). The polypeptide IX gene has been termed an intermediate gene,

since it is expressed at around 6–8 h after infection, before other structural polypeptides of the virus can be detected (Spector *et al.*, 1978). Viral DNA replication is a pre-requisite for IX

expression (Crossland & Raskas, 1983; Vales & Darnell, 1988). Although the polypeptide IX gene is present as part of the integrated viral genome in Ad-transformed cells, it is not expressed (Spector *et al.*, 1980) indicating perhaps that the structure of the IX promoter may be important for expression. A number of minor mRNAs of 14 S and 14.5 S (Virtanen & Pettersson, 1985) coding for proteins which share *N*-termini with the major p55 protein (Anderson *et al.*, 1984) have also been described but as yet no function has been ascribed to them in infection or transformation.

Regulatory elements of the E1A and E1B blocks of genes

The regulatory elements of the E1A transcriptional unit are located upstream of the E1A mRNA cap site which is at nucleotide 498 for adenovirus 2. The sequences between the left hand end of the adenovirus genome and the start site of transcription comprise (Fig. 4a): (i) the terminal repeat; (ii) an enhancer sequence and the packaging signal; (iii) a CAAT box; and (iv) a TATA motif at 30 nucleotides upstream of the cap site. A conserved TATTTATA sequence found at nucleotide 467 for Ad2 is common to all serotypes analysed. However, other TATAA-like motifs exist at other minor start sites (Osborne & Berk, 1983).

The occurrence of a *cis*-regulator of adenovirus transcription located upstream of the E1A promoter has been demonstrated (reviewed by Berk, 1986b). A refined dissection of the E1A enhancer region, using deletion mutants, suggested that it consists of two distinct functional domains. One domain (I), corresponding to the two repeated sequences around nucleotides -300 and -200, specifically regulates the E1A unit. The second domain (II), between nucleotides -218 and -250, modulates activity of all the other early transcription units (E1B, E2, E3 and E4) in *cis* (Hearing & Shenk, 1986).

In contrast to the E1A promoter, the regulatory region of the E1B transcriptional unit appears to be a rather short (60 bp) and simple eukaryotic polymerase II promoter, consisting of three adjacent elements (Wu *et al.*, 1987). (i) A sequence TGCATGGCG, between nucleotides -65 and -57 is present in the coding strand. The complementary sequence, CGCCATGCA, has a strong homology to the CAAT-box promoter element identified in other viral promoters, such as the HSV-1 TK promoter (Graves *et al.*, 1986). It might therefore be postulated that this sequence in the E1B promoter is a binding site for a CAAT-box binding factor (Berk, 1986b). (ii) A sequence G₄CG₄C, known to be the high affinity binding site for the Sp1 transcription factor (Kadonaga *et al.*, 1986), lies from nucleotides -49 to -38. (iii) Further deletions into the E1B TATA-box region (-30 TATATAAT -23) almost totally inactivated transcription (Berk, 1986b; Wu *et al.*, 1987).

The E1B promoter is stimulated 5–10-fold by E1A expression, although there is no evidence for an E1A-specific sequence. However, mutants with an interrupted TATA box were less responsive to activation by E1A (Wu *et al.*, 1987). This suggests that E1A gene products may not interact with specific sequences, but may act on the E1B promoter via host cell factors, e.g. Sp1 and TATA box-binding factors.

ROLE OF THE ADENOVIRUS TRANSFORMING REGION IN VIRAL ONCOGENESIS AND CELL TRANSFORMATION

Adenovirus-induced cell transformation and oncogenesis have been extensively analysed *in vitro*, using transfection with purified restriction fragments derived from the viral genome, or plasmids containing cloned adenovirus DNA segments. The results of these experiments can be summarized as follows.

(i) The E1A and E1B regions of the adenovirus genome contain oncogenes, and E1A encodes two major oncoproteins (Berk *et al.*, 1979; Bernards & van der Eb, 1984; van der Eb *et al.*, 1979).

(ii) Expression of the two major E1A products is required for complete and stable cellular transformation (Courtois & Berk, 1984; Houweling *et al.*, 1980; Montell *et al.*, 1984).

(iii) For the members of subgroup C of human adenoviruses (e.g. Ad2, Ad5), only one of the two major E1A oncoproteins is sufficient to induce stable transformation of the cells, provided that E1B is expressed (van der Eb *et al.*, 1979; Berk, 1986a).

(iv) The proportion of cells which are transformed *in vitro* is higher when both E1A and E1B regions are co-transfected, or are present on the same DNA fragment (Courtois & Berk, 1984; Montell *et al.*, 1984). The E1B region alone has no transforming activity, even when expressed under the control of a strong heterologous promoter (van den Elsen *et al.*, 1983).

(v) Based on these experimental data, it has been suggested that E1A acts as an immortalizing gene (analogous to *myc*), and that the E1B region (analogous to activated *ras*) may be responsible for complete and stable transformation in co-operation with E1A (Bernards & van der Eb, 1984; Gallimore *et al.*, 1985a,b).

(vi) Human adenoviruses can be classified according to their oncogenicity in newborn rodents (Graham, 1984). Thus, virus particles of subgroup A viruses (such as Ad12) induce tumours with high frequency and short latency when injected into newborn rodents whereas subgroup C viruses (e.g. Ad2 or Ad5) are non-oncogenic *in vivo*. Subgroup B viruses (e.g. Ad3 or Ad7) are weakly oncogenic. Although all adenoviruses can transform primary rodent cells in culture, the oncogenicity of transformed cells (when tested by injection into syngeneic neonatal hosts) tends to parallel the oncogenicity of the virus serotype used for transformation. Furthermore, the serotype origin of the E1A gene appears to determine the oncogenicity of adenoviruses and transformed cells; in particular, the Ad12 E1A gene confers an oncogenic phenotype upon viruses and transformed cells in which it is present (Sawada *et al.*, 1986).

(vii) *In vivo*, tumours are produced at a lower rate and with a longer latency period when cells containing only the Ad12 E1A oncogene instead of the E1A and E1B region are injected into animals (Gallimore *et al.*, 1985b).

(viii) Transformation of rat cells with the highly oncogenic adenovirus serotype 12 leads to a considerable reduction in detectable major histocompatibility (MHC) class I antigens on the cell surface (Schreier *et al.*, 1983; see below). Ad12-transformed rodent cells, with reduced expression of MHC on the surface, were found to be highly tumorigenic when injected into the syngeneic host (Tanaka *et al.*, 1985; Eager *et al.*, 1985).

(ix) As described below, cell transformation may result in part from the association (and possible inactivation) of host cell anti-oncogene products with oncogene products of viral origin, e.g. the retinoblastoma-susceptibility gene protein p105-*RB* with the E1A oncoproteins (Whyte *et al.*, 1988a, 1989) or with the SV40 large T antigen (DeCaprio *et al.*, 1988; Ludlow *et al.*, 1989).

STRUCTURE OF THE ADENOVIRUS ONCOPROTEINS

The proteins of the E1A region

The five mRNAs transcribed from the E1A transcriptional unit encode proteins of 289, 243, 217, 171 and 55 amino acids. The two most abundant species are the proteins of 289 and 243 residues translated from the 13 S and the 12 S mRNAs, respectively. They have respective theoretical molecular weights of

32000 and 26000 Da (Figs. 1 and 2). However, these two proteins appear in SDS/PAGE as multiple discrete bands ranging from 35000 to 53000 Da, with two pairs of predominant bands at 45–48.5 and 50–52 kDa (Yee & Branton, 1985). A consequence of this heterogeneity is the inaccuracy of determination of the molecular masses of the E1A gene products in SDS/PAGE. Therefore, the products of the E1A region will be referred to as 289R, 243R, 217R, 171R and 55R, 'R' standing for 'residue of amino acid'.

All E1A proteins (except the 55R, which is translated from the 9 S message) share the same *N*- and *C*-termini and are translated in the same reading frame. Differences in polypeptide chain length result from RNA splicing. The 289R and 243R differ only by 46 internal amino acids, from residues 140 to 185 (Perricaudet *et al.*, 1979). The 55R polypeptide shares its first 26 amino acids with all the other E1A proteins, but its *C*-terminal sequence of 29 residues is unique (Fig. 2).

The discrepancy between the theoretically predicted and experimentally determined molecular masses of E1A proteins might reside in their amino acid composition and sequence. Their common *C*-terminal ends are proline- and glutamic acid-rich (Feldman & Nevins, 1983; Harter & Lewis, 1978; Lupker *et al.*, 1981; Smart *et al.*, 1981; Spindler & Berk, 1984). It is now known that the occurrence of clusters of glutamic acid residues in a protein prevents the binding of SDS and results in a higher apparent molecular mass in SDS/PAGE.

However, the major reason for the heterogeneous pattern of E1A bands is the high degree of post-translational modifications in E1A proteins, which have been found to be highly phosphorylated (Gaynor *et al.*, 1982; Yee *et al.*, 1983; Yee & Branton, 1985). Treatment of the E1A 289-amino-acid product by acid phosphatase eliminated the mobility shift of the variants observed in SDS/PAGE and converted the 48/45 kDa doublet to a single band at 45 kDa (Richter *et al.*, 1988). Phosphorylation of E1A proteins has been shown to involve a Ca^{2+} -dependent, cyclic AMP-independent protein kinase (Ferguson *et al.*, 1985b; Lucher *et al.*, 1985).

The major site of E1A protein phosphorylation has been found at Ser-219 of the 289R E1A protein (Tsukamoto *et al.*, 1986). Although this serine is located in a consensus sequence for phosphorylation by cyclic AMP-dependent protein kinases, experiments with mutant CHO cells defective in these enzymes have indicated that Ser-219 was equally phosphorylated in both mutant and wild-type cells (Tsukamoto *et al.*, 1986). It must be assumed that cyclic AMP-independent protein kinases mediate phosphorylation of Ser-219.

Other phosphorylation sites have also been mapped. E1A-289R and E1A-243R are clearly phosphorylated at three major sites, on serine residues 89, 219 and 231. There may be other minor, variable sites on serine residues 96, 227, 234 and 237 (Tremblay *et al.*, 1988). Using site-directed mutagenesis, the regions responsible for the SDS/PAGE mobility shift have been mapped between residues 23 and 120, involving one or more of the six possible phosphoserines at positions 36, 63, 89 and 111 (Smith *et al.*, 1989a). In another approach, using deletion mutant analysis, it has been found that at least two serine residues, mapped between residues 86 and 120, and 224 and 289 are the sites of phosphorylation on E1A proteins, and that each phosphorylated serine can independently induce the mobility shift (Richter *et al.*, 1988).

It is generally agreed that E1A mutants which lack the major phosphoserine residues are indistinguishable from wild type E1A in transcriptional transactivation of the other early adenovirus promoters, and in replication in G_0 -arrested cells (Dumont *et al.*, 1989; Richter *et al.*, 1988; Tremblay *et al.*, 1989; Tsukamoto *et al.*, 1986). It was also initially assumed that E1A phosphorylation

was of little significance in the transformation process (Richter *et al.*, 1988; Tremblay *et al.*, 1989; Tsukamoto *et al.*, 1986). In another report, however, (Smith *et al.*, 1989a) the repression and transformation functions of E1A were altered in most serine mutants tested (i.e. Ser-63, Ser-69 and Ser-89 to Gly). Most interestingly, the Ser-96→Gly mutant gave a reproducible reduction in transformation efficiency, but repression of SV40 enhancer-stimulated transcription occurred at wild-type level (Smith *et al.*, 1989a). This suggests that transformation and repression are separable functions, as previously suggested (Velcich & Ziff, 1988).

Recent data have shown that the DNA binding activities of transcription factors are positively regulated by phosphorylation, and that the E1A 289R protein may activate some cellular kinase involved in this process (reviewed in Berk, 1989). Phosphorylation may increase the potency of an activating factor simply by contributing the net electric charge of one critical protein domain (Lech *et al.*, 1988; Ptashne, 1988). A cluster of acidic amino acids (amino acids 132–141) precedes a stretch of hydrophobic residues on the *N*-terminal boundary of the unique region of the E1A 289R protein. This structure is reminiscent of the acidic character and of the negatively charged amphipathic α -helices observed in many activating region sequences of eukaryotic transcriptional activators (Ptashne, 1988). As described below, this acidic region lies at the boundary of two conserved functional domains of the adenovirus E1A 289R proteins (Lillie & Green, 1989; Lillie *et al.*, 1987). In addition, all E1A proteins, except the polypeptide translated from the minor 9 S message, show the same unusual feature: a region of six alternating Glu-Pro residues is present between residues 189 and 200 on the Ad2 E1A 289R sequence, downstream of the major splice acceptor site at nucleotide 1226 (Gingeras *et al.*, 1982). No specific function has yet been assigned to this particular sequence, which lies on the *C*-terminal boundary of the CR3 domain (see below and Fig. 4).

The minor E1A gene products 171R and 217R, with respective apparent molecular masses in SDS/PAGE of 30 kDa and 35 kDa (Stephens & Harlow, 1987; Ulfendahl *et al.*, 1987) have also been found to be phosphorylated at Ser-219 and at additional site(s) between serine residues 227 and 237, as are the 289R and 243R proteins (Tremblay *et al.*, 1989). No specific function has yet been assigned to the three minor E1A gene products 55R, 171R and 217R (Moran *et al.*, 1986a).

Three highly conserved domains, termed conserved regions 1, 2 and 3 (CR1, CR2, CR3), have been identified, localized and their functions defined in the 289R E1A protein. These domains were first inferred from theoretical considerations of amino acid sequence homology and splicing patterns among the E1A proteins of various adenovirus serotypes (Kimelman *et al.*, 1985b; Kimura *et al.*, 1981; van Ormondt *et al.*, 1980). Genetic analysis later confirmed the role of each of these three domains in the pleiotropic activities of the E1A proteins (Lillie *et al.*, 1987; Moran & Mathews, 1987). The first conserved domain CR1 is located between residues 40 and 80 (in Ad2), CR2 between 120 and 139, and CR3 between 139 and 186 (Kimelman *et al.*, 1985b). Because of differential splicing, CR3 is unique to the 289R species (Figs. 2 and 4).

Comparative computer analysis of the amino acid sequences of SV40 large T, adenovirus E1A, *v-myc*, *c-myc* and *c-myb* oncoproteins have shown regions of high homology in their predicted secondary structure (Branton *et al.*, 1985; Figge *et al.*, 1988; Ralston & Bishop, 1983). This assumption, however, has been a matter of controversy, at least in the case of *c-myc* and E1A proteins (McLachlan & Boswell, 1985). Nevertheless, regions containing similar elements of primary structure have been identified and aligned in adenovirus E1A proteins and papovavirus large T antigens. The *C*-terminal portion of the E1A

CR2 domain consists of a stretch of acidic residues (amino acids 133–145 for Ad2/Ad5) following a serine (Ser-132), a motif also present in SV40 large T antigen (Stabel *et al.*, 1985), *myc* oncoproteins (Figge *et al.*, 1988), BK virus, lymphotropic papovavirus (LPV), and mouse polyomavirus (Moran, 1988; Pawlita *et al.*, 1985; Seif *et al.*, 1979). More precisely, this motif extends from amino acids 105 to 120 in large T antigen, and overlaps a short segment (amino acids 105–114) whose integrity is required for full transforming activity (Cheriton *et al.*, 1988; Kalderon & Smith, 1984). The same motif has been identified as a recognition site for a cellular protein kinase (Kuenzel *et al.*, 1987). On the *N*-terminal boundary of the same CR2 domain of E1A (amino acids 121–126 for Ad2/Ad5), one finds the highly conserved DLXCXE sequence, a motif also found in SV40 T antigen and in human papillomavirus type 16 E-7 gene products (Phelps *et al.*, 1988).

These observations suggest that structural similarities in oncoproteins of different origin may correspond to common biological functions. This hypothesis has been recently confirmed experimentally. A chimaeric gene was constructed (Moran, 1988), in which the coding sequence for the CR2 domain of E1A proteins was substituted with a portion of the gene encoding SV40 T antigen (from amino acid 101 to 118). The resulting chimaeric protein, which consisted of a 12 S E1A product (243R protein) with an inserted sequence from SV40 large T antigen, was biologically active and retained all the transforming properties of the wild-type E1A product (Moran, 1988). It is interesting to note that the foreign sequence introduced into the E1A gene corresponds to a domain of large T antigen located between the nuclear localization signal and the DNA-binding domain (De-Pamphilis & Bradley, 1986; Rigby & Lane, 1983; Simmons, 1986). Similarly, an acidic activating domain present in the herpes simplex virus VP16 protein, which is analogous to the acidic domains of cellular activators (Ptashne, 1988), can substitute for the transactivation domain of E1A, from amino acid 139 to 149 (Lillie & Green, 1989).

Structure of the major gene products of the E1B region

Two proteins of 19 kDa (p19, of 175 residues for Ad2/Ad5, sometimes referred to as 21 kDa) and 55 kDa (p55, of 495 residues for Ad2/Ad5, sometimes referred to as 58 kDa) which are unrelated in amino acid sequence are synthesized from the 22 S mRNA template using AUG codons in different reading frames (Bos *et al.*, 1981). The p19 protein is also translated from the 13 S mRNA and thus the level of this protein increases as infection progresses. The 13 S mRNA can also encode an 8 kDa species (p8) related to the *N*-terminal region of the p55 polypeptide, although this truncated species has not been detected in infected cells. Both p55 and p19 proteins are post-translationally modified. The p55 protein is phosphorylated on serine and threonine residues (Russell & Blair, 1978; Spindler *et al.*, 1984; Schughart *et al.*, 1985) and is also a substrate for poly ADP-ribosylation *in vitro* (Goding *et al.*, 1983). A protein kinase activity has also been suggested to be associated with the p55 species (Branton *et al.*, 1981; Lassam *et al.*, 1979; Yee & Branton, 1983) although this observation has not yet been universally accepted (Grand & Gallimore, 1984).

The p19 protein has been reported to be covalently linked to lipid, either palmitate (Grand *et al.*, 1985) or palmitate and myristate (McGlade *et al.*, 1987). Interestingly neither the Ad12 nor Ad5 p19 protein contains glycine as the *N*-terminal amino acid (Anderson & Lewis, 1980; Bos *et al.*, 1981). Myristyl-transferase has a narrow specificity for both *N*-terminal glycine and for myristic acid (reviewed by Sefton & Buss, 1987). This may explain the fact that only a minor fraction of intracellular p19 protein is fatty acylated in infected cells (McGlade *et al.*,

1987). The functional importance of post-translational modifications of the p55 and p19 protein has not yet been evaluated. However, these findings do imply that E1B proteins synthesized in prokaryotic systems may lack potentially important post-translational modifications.

BIOSYNTHESIS AND INTRACELLULAR LOCALIZATION OF THE ADENOVIRUS ONCOPROTEINS

The turnover and cellular compartmentation of E1A and E1B proteins have been extensively studied, since their localization within the cell might reflect some of their functions and role in virus pathogenesis.

E1A proteins

On the basis of pulse-chase labelling and immunoprecipitation experiments, it has been claimed that the half-life of E1A proteins is very short compared to other eukaryotic proteins (Goldberg & StJohn, 1976), 30 min in adenovirus-infected cells and 120 min in adenovirus-transformed cells (Spindler & Berk, 1984). Analysis of the amino acid sequence of E1A shows, besides an exceptionally high content of Pro (16%), several Pro-Glu-Ser-Thr (or PEST) motifs, which have been identified in many proteins with rapid turnover, leading to the 'PEST' hypothesis (Rogers *et al.*, 1986).

However, experiments using a cell line with an integrated E1A coding region placed under the control of a dexamethasone-inducible MMTV promoter have suggested that E1A proteins are very stable within the cell (Brunet & Berk, 1988). This analysis of E1A proteins was performed by immunoblotting of whole cell extracts whereas the previous study used immunoprecipitation. The observed discrepancy might originate from differences between cell systems, or from compartmentalization of the E1A proteins. Immunoprecipitation mainly detects the 'soluble' fraction of E1A, which may turn over rapidly, whereas an immunoblot of a whole cell extract reflects the E1A protein content of the total cellular pool.

The localization of E1A proteins has been analysed by subcellular fractionation, immunofluorescent staining and immunoelectron microscopy, using polyclonal or monoclonal antibodies directed against sequences common to both 289R and 243R. Both proteins appear to be mainly located in the nuclear matrix (Ferguson *et al.*, 1985b), although a small portion has been found to be associated with microtubules and intermediate filaments (Chatterjee & Flint, 1986; Grand & Gallimore, 1984). They also seem to co-localize with heat-shock protein hsp70 within the nucleus (White *et al.*, 1988b).

Immunological evidence has been presented which suggests that a fraction of E1A present in Ad5-transformed cells is located on the cell surface (Bellgrau *et al.*, 1988). In this study cytotoxic T lymphocytes (CTLs) generated against Ad5-transformed cells were able to lyse cells expressing either the 12 S or 13 S mRNA and this lysis was blocked by a polyclonal antibody against the 289R protein. However CTLs were unable to lyse cells expressing only the first exon of E1A. This suggests that a sequence present in the second exon is required either for CTL recognition or for trafficking of E1A proteins or peptides to the cell surface.

A pentapeptide sequence KRPRP is located at the *C*-terminus of both major 289R and 243R E1A proteins (Lyons *et al.*, 1987), and is responsible for transport of the E1A proteins into the nucleus. The basic nature of this sequence is similar to the karyophilic signal ¹²⁸KKRK found in SV40 large T antigen (Kalderon *et al.*, 1984). An additional karyophilic signal located in the *trans*-activation domain of the 289R protein, between residues 140 and 185, has been defined which is active only in

Fig. 5. Domain structure and protein–protein interactions of the major E1A gene products

(a) Representation of the different biological activities and functional domains of the oncoproteins 289R (13 S mRNA) and 243R (12 S mRNA). Modified from Lillie *et al.* (1987). (b) Enlargement of the unique conserved domain CR-3 of E1A-289R, and its separate functions. Modified, from Lillie & Green (1989). (c) Interactions between p105-RB and other host cell proteins with different domains of adenovirus E1A proteins. Coloured boxes represent the unique domains of the E1A-289R. Contiguous and noncontiguous contacting protein domains are underlined with thick solid lines. Modified from Whyte *et al.* (1989).

amphibian oocytes and not in somatic cells of amphibian or mammalian origin (Slavicek *et al.*, 1989). The nuclear transport and transactivation functions of bacterially-synthesized 289R

protein were not affected by 5 min of heating at 100 °C followed by assay by microinjection into mammalian cells (Krippl *et al.*, 1984).

E1B oncoproteins

The p55 protein occupies a predominantly nuclear location in infected cells (Sarnow *et al.*, 1982a; Rowe *et al.*, 1983; Blair-Zajdel *et al.*, 1985; Schughart *et al.*, 1985), and appears in granular patches in immunofluorescence. The p55 protein forms a molecular complex in infected cells with the p34 (or ORF6) protein of Ad5 early region 4 (E4) in infected cells (Cutt *et al.*, 1989; Leppard & Shenk, 1989; Sarnow *et al.*, 1984). The p55 protein also exists in complex with the cellular oncoprotein p53 in subgroup C (Ad2 and Ad5)-transformed cells (Sarnow *et al.*, 1982a; Zantema *et al.*, 1985a; Blair-Zajdel & Blair, 1988).

In subgroup C (Ad2 or Ad5)-transformed rodent cells the p55 protein co-localizes with the cellular anti-oncogene product p53 (Zantema *et al.*, 1985b; Blair-Zajdel & Blair, 1988) in the nucleus and in a perinuclear body, consistent with the presence of p55-p53 molecular complexes. This body coincides with the centrosome region of the cell (Blair-Zajdel & Blair, 1988) and the p55 and p53 proteins are found in a cluster of 8 nm filaments presumably within this body (Zantema *et al.*, 1985a), although these filaments did not correspond to any previously characterized microfilaments within mammalian cells.

In subgroup A (Ad12)-transformed cells the viral p55 and cellular p53 proteins are mainly located in the nucleus and no stable p55:p53 complexes can be detected (Blair-Zajdel & Blair, 1988; Mak *et al.*, 1988; Zantema *et al.*, 1985b). Similarly, in subgroup B (Ad7)- and D (Ad9)-transformed rat fibroblast cell lines, p53 is not complexed with viral p55 protein (M. E. Blair-Zajdel & G. E. Blair, unpublished results). Although the p55 proteins of Ad5 and Ad12 are homologous (Bos *et al.*, 1981; Grand, 1987) it may be that the binding site for p53 on the Ad5 p55 protein has been altered in the Ad12 p55 protein. Therefore the binding site for p53 on the Ad5 p55 needs to be defined. Mutants of Ad2 p55 with insertions in its central domain became phenotypically transformation-defective (Yew *et al.*, 1990), yet binding murine p53 *in vitro* (P. R. Yew & A. J. Berk, personal communication). This suggests that simple physical binding of p55 to p53 does not necessarily correlate with transformation.

It is not known what factors determine the centrosomal association of p55:p53 complexes in Ad2- or Ad5-transformed cells. A recent report has suggested that the level of Ad5 p55 expression may determine this intracellular association (van den Heuvel *et al.*, 1990). Increasing cellular levels of p55 resulted in an increased centrosomal association of the p55:p53 complex. The presence of p55 in the centrosomal structures also correlated with an increased latency period for tumorigenesis in nude mice. Thus the intracellular distribution of p55 and p53 may play an important role in tumorigenesis.

The p53 protein does not form a stable complex with p55 in Ad5-infected human cells (Sarnow *et al.*, 1982a). There is no competition for p55 between the viral E4-34 kDa protein and p53 since in human cells infected by a viral mutant (*dl* 355) which has a deletion in the C-terminal region of E4 ORF6, no complex formation between p55 and p53 could be detected (Braithwaite & Jenkins, 1989). The p55 protein, produced by *in vitro* transcription/translation from a cloned E1B template, could only associate *in vitro* with p53 present in adenovirus-transformed cells and not with p53 from normal rodent or untransformed human cells, leading to the conclusion that formation of the p55:p53 complex is determined by an, as yet, undefined property of p53. More recent work, using very mild conditions of cell lysis has shown that a complex between Ad5 p55 and p53 can be detected in infected mouse L929 cells (Braithwaite *et al.*, 1991). Only a minor fraction of cellular p53 or p55 participated in this complex, which (in contrast to the p55:p53 complex found in Ad5-transformed cells) could be readily disrupted by sodium

deoxycholate. These results again point to possible biochemical differences between the p53 protein present in normal and adenovirus-transformed cells.

The p19 protein has been shown to be located both in an intracellular membrane (probably the endoplasmic reticulum) and the nucleus (White *et al.*, 1984a; Blair-Zajdel *et al.*, 1985) where p19 is associated with the nuclear envelope and the underlying nuclear lamina. In human cells infected by Ad2 or Ad12 and in Ad12-transformed human cells, the p19 protein has also been found to be associated with the plasma membrane (Persson *et al.*, 1982; Smith *et al.*, 1989b). It is not known in which cell compartment the fatty acylated form of p19 is located (see below). Altered distribution of both p55 and p19 proteins have been reported as infection proceeds in human cells (Rowe *et al.*, 1983; White *et al.*, 1984a). In both cases immunofluorescence studies revealed that the proteins are present in a cytoplasmic (or in the case of p19, cytoplasmic membrane) location early in infection, and accumulate in the nucleus late in infection.

The p19 protein has also been found to associate with vimentin-containing intermediate filaments and the nuclear lamina, causing disruption of these structures (White & Cipriani, 1989, 1990). Such effects may have important implications for viral pathogenicity and transformation.

BIOLOGICAL FUNCTIONS OF THE ADENOVIRUS ONCOPROTEINS

The E1A oncoproteins have pleiotropic activities, including transcriptional activation, a property shared by other oncogene products (Kingston *et al.*, 1985), transcriptional repression, and, in co-operation with E1B or certain oncogene products, cell transformation (Berk, 1986a; Branton *et al.*, 1985; Grand, 1987).

Experimental approaches

Various techniques have been used to determine the biological functions of the major adenovirus oncoproteins.

(i) Micro-injection of E1A proteins (Kripl *et al.*, 1984, 1985; Ferguson *et al.*, 1984, 1985a,b), produced in prokaryotic systems (Ferguson *et al.*, 1984; Kimelman *et al.*, 1984a; Ko & Harter, 1984), using expression vectors coding for the 289R protein (Ferguson *et al.*, 1984; Kripl *et al.*, 1984) or for the 243R (Ferguson *et al.*, 1985a).

(ii) Isolation of adenovirus mutants containing either a deletion of the E1A region (*dl* 312) or lacking one of the two E1A proteins. A point mutation in the first splice donor site results in a mutant (*pm* 975) which only synthesizes a functional 289R; the deletion mutant *dl* 1500 only produces the 243R species (Montell *et al.*, 1984). Other site-specific point mutations and specifically designed anti-peptide sera have also been used to refine the topography of the functional domains of E1A proteins (Lillie *et al.*, 1986, 1987).

(iii) More recently, fusion genes have been constructed, resulting in chimaeric proteins in which functional domains can be unambiguously assigned to a particular sequence (Moran, 1986; Lillie & Green, 1989).

(iv) For the E1B proteins, the construction of point, deletion and insertion mutants in the coding regions for the p55 protein (Harrison *et al.*, 1977; Pilder *et al.*, 1986; Barker & Berk, 1987; Yew *et al.*, 1990) and the p19 protein (D'Halluin *et al.*, 1979, 1984; Subramanian *et al.*, 1984a,b; Pilder *et al.*, 1984; Takemori *et al.*, 1984; White *et al.*, 1984b) has provided a genetic approach to the contributions of the E1B proteins to infection and transformation.

Role of E1A and E1B oncogene products in the virus infectious cycle

Transcriptional *trans*-activation. The E1A gene is essential for productive infection and is involved in the positive control of all the other early regions of the viral genome (Fig. 4b). This stimulation results essentially from a *trans*-activation process, mediated by diffusible gene products (Berk, 1986b; Jones *et al.*, 1988; Nevins, 1987). Using deletions and point mutations in the E1A region, it has been demonstrated that the two major 289R and 243R E1A proteins stimulate transcription from all the early promoters (E1B, E2, E3 and E4), and even from the E1A promoter. However, transcription from the E1A promoter is stimulated by a factor of 5, compared with a 50-fold *trans*-activation for the other early promoters (Berk *et al.*, 1979; Bos & ten Wolde-Kraamwinkel, 1983; Jones & Shenk, 1979; Nevins, 1981; Osborne *et al.*, 1984).

The production of E1A proteins has been found to be partially under the control of the E1B region, but this phenomenon apparently differs according to the serotype analysed (Babiss & Ginsberg, 1984; Barker & Berk, 1987; Grand, 1987) and has not been clearly explained at the molecular level. E1B might stimulate expression of the E1A region by either a *cis*-regulatory element located in E1B, or a *trans*-regulatory mechanism involving the E1B gene products (Senear & Lewis, 1986).

Transcription of adenovirus early genes is not totally dependent upon the E1A region. A complete deletion of the E1A region, as in the Ad5 *dl* 312 mutant, is not lethal for the virus: there is a delay in the expression of all the early genes (Gaynor & Berk, 1983; Nevins, 1981). This lag can be overcome when cells are infected with very high multiplicities of the deletion mutant (Gaynor & Berk, 1983). This defective early gene *trans*-activation function of E1A can be overcome by co-infection with pseudorabies virus, a virus of the herpesviridae family. The complementing gene product is transcribed from the block of immediate early genes of pseudorabies virus (Feldman *et al.*, 1982; Imperiale *et al.*, 1983), and has been identified as the immediate early protein IEP4, a phosphoprotein of 180 kDa (Ihara *et al.*, 1983). Conversely, the adenovirus E1A proteins can activate the early genes of pseudorabies virus and other herpesviruses (Everett & Dunlop, 1984; Gorman *et al.*, 1989). A number of other unrelated viruses have been found to encode proteins with transcriptional *trans*-activating activities analogous to E1A, e.g. bovine papilloma virus (Haugen *et al.*, 1987), human papilloma virus (Phelps *et al.*, 1988), bovine leukemia virus (Chen *et al.*, 1985), HTLV-1 (Ballard *et al.*, 1988) and hepatitis B virus (Colgrove *et al.*, 1989).

Indirect versus direct E1A *trans*-activation. Since adenovirus E1A proteins were found to stimulate transcription of unrelated viral and cellular genes (Svensson & Akusjärvi, 1984), it seems at first sight unlikely that they might act directly on these viral and cellular promoters. The transcriptional *trans*-activating activity of adenovirus E1A proteins may therefore be indirect, mediated by some cellular transcription factor(s) (Berk, 1986b, 1989; Jones *et al.*, 1988; Nevins, 1987; Reichel *et al.*, 1988). Several lines of evidence support the hypothesis of an indirect, nonspecific *trans*-activation by E1A proteins, although it must also be added that some reports are in apparent conflict with this notion.

(i) E1A stimulates transcription of a wide variety of viral and cellular genes which do not share common promoter elements, or lack specific inducible elements (Berk, 1986a,b; Grand, 1987). Besides viral genes (Berk *et al.*, 1979; Imperiale *et al.*, 1983, 1984; Svensson & Akusjärvi, 1984), E1A gene products can also cause a 20-fold stimulation of transcription of transfected cellular genes, e.g. human β -globin and *c*-globin genes (Allan *et al.*,

1984). However, analysis of the effect of adenovirus E1A on several host cell genes, including the genes for pre-proinsulin and globins, suggests that stimulatory activity is restricted to newly-introduced genes, and does not occur with endogenous class II genes (Babich *et al.*, 1983; Gaynor *et al.*, 1984; Green *et al.*, 1983). This also applies to genes transcribed by polymerase III. A stimulation by E1A was observed for a tRNA gene transfected in adenovirus-infected HeLa cells, but not for the endogenous host cell genes coding for 5 S rRNA and tRNAs (Gaynor *et al.*, 1985).

(ii) Major exceptions to the rule of unresponsiveness of endogenous genes to E1A have then been reported. Whereas transcription of hsp-70 (Kao & Nevins, 1983) and β -tubulin (Stein & Ziff, 1984) is stimulated by the wild-type Ad2 E1A, and not by the deletion mutant Ad5 *dl* 312, there is no detectable stimulation of the same gene products by Ad12 (Grand & Gallimore, 1986). Cellular thymidine kinase, however, is stimulated by Ad12 early gene products (Grand & Gallimore, 1986). In addition, transcription from integrated early E2 (Kingston *et al.*, 1984) and E3 (Courtois & Berk, 1984) adenovirus promoters in stably transformed cells is stimulated by E1A.

(iii) E1A stimulation occurs for eukaryotic promoters of class II (Leong & Berk, 1986) and class III genes (Berger & Folk, 1985; Gaynor *et al.*, 1985; Hoeffler & Roeder, 1985; Yoshinaga *et al.*, 1986, 1987), transcribed by RNA polymerase II and RNA polymerase III, respectively (Berk, 1986b). Stimulation has also been observed with an insect baculovirus promoter introduced into mammalian cells (Knebel & Doerfler, 1987).

(iv) Co-transfection of plasmids containing either proto-oncogenes *c-fos*, *c-myc* or *c-Ha-ras* with an E1A-containing plasmid into HeLa and NIH-3T3 cells has shown a *trans*-activation of the *c-fos* and *c-myc*, but not the *c-Ha-ras*, promoters (Sassone-Corsi & Borelli, 1987).

(v) It has been claimed that E1A proteins do not bind directly to some specific DNA sequence (Ferguson *et al.*, 1985a), although this result has been recently contradicted by others using different assays (Chatterjee *et al.*, 1988; Ko *et al.*, 1986).

(vi) Further evidence that E1A activity is mediated by a cellular factor comes from experiments in which E1A proteins were completely depleted from 293 cell or Ad2-infected cell extracts by chromatography on a monoclonal antibody-affinity column: the E1A-depleted extracts retained their full stimulatory activity on gene transcription *in vitro* (Yoshinaga *et al.*, 1986).

It has therefore been proposed that E1A might stimulate transcription by post-translational modification(s) of endogenous cellular transcription factors, converting them from an inactive to an active form (Berk, 1986b, 1989; Hiebert *et al.*, 1989; Kovcsdi *et al.*, 1986a,b, 1987; Leong *et al.*, 1988; Reichel *et al.*, 1988). However, as described below, recent data seem to question this model and suggest that the adenovirus 289R E1A protein has a dual function characteristic of a typical cellular activator, *per se* (Lillie & Green, 1989; Ptashne, 1989).

E1A *trans*-activation and cyclic AMP. Cyclic AMP has been shown to regulate gene expression at the transcriptional level by a cascade of events involving protein kinase activities (reviewed in Rosenfeld & Barrieux, 1979). Specific cyclic AMP responsive elements (CRE) have been identified in the upstream control region of a number of cellular genes (reviewed in Roesler *et al.*, 1988), as well as viral genes (reviewed in Jones *et al.*, 1988). When a cyclic AMP-responsive cell line is infected with wild-type adenovirus, a transcriptional activation of early gene promoters (E1A, E1B, E4) occurs in the presence of dibutyryl cyclic AMP (Engel *et al.*, 1988). Cyclic AMP has been found to act synergistically with E1A, both resulting in a greater induction of transcriptional activation than with either inducer alone (Engel *et al.*, 1988).

More recent results have confirmed that a cellular transcription factor, termed ATF, which recognizes the CRE, binds to regulatory elements in several adenovirus early promoters, including E1A (Leza & Hearing, 1989; Lin & Green, 1988). In assays using mutant promoter regions, it has been found that E1A *trans*-activation of the E4 promoter can be uncoupled from cyclic AMP induction, suggesting that E1A and cyclic AMP, although being synergistic, act by independent mechanisms (Leza & Hearing, 1989). This has been confirmed by comparing the E1A- and cyclic AMP-inducible transcription from different promoter elements containing the same CGTCA motif, which is critical for the binding of a group of cellular transcription factors (ATF, CREB, E4F and EivF) and for transactivation of E1A- and cyclic AMP-inducible promoters (Lee *et al.*, 1989).

Negative regulation functions: transcriptional repression and interserotypic hierarchy of dominance. A repression phenomenon has been described in the case of certain viral or cellular promoters: E1A proteins repress transcription from SV40 and polyoma early promoters (Borelli *et al.*, 1984; Lebkowski *et al.*, 1985; Lewis & Manley, 1985; Velcich & Ziff, 1985). This repression effect does not depend upon the presence of the large T antigen, since it still occurs when the SV40 promoter is fused to other structural genes (Borelli *et al.*, 1984). Using different DNA constructs containing the 72 bp repeat enhancer sequence of SV40, these authors have provided experimental evidence that the repression effect involves interaction between enhancer elements and *trans*-acting factors, probably the E1A gene products themselves (Borelli *et al.*, 1984).

The E1A oncogene products also have the property of repressing their own expression at the transcriptional level, by acting on their own promoter (Fig. 4b), an effect that is due to both the 289R and 243R major E1A products (Borelli *et al.*, 1984; Velcich & Ziff, 1985). E1A proteins also repress their own expression in COS cells (Smith *et al.*, 1985). The E1A proteins can repress the expression of mouse immunoglobulin heavy chain in murine lymphoid cells in culture (Hen *et al.*, 1985), although surprisingly they stimulate the same enhancer sequence in the mouse NIH-3T3 fibroblast cell line (Borelli *et al.*, 1984). Repression of enhancer-driven expression of the rat insulin gene (Stein & Whelen, 1989; Stein & Ziff, 1987) and rodent muscle-specific genes (Webster *et al.*, 1988) has been reported. The reduced expression of MHC class I antigens at the surface of Ad12-transformed lines (Bernards *et al.*, 1983a,b; Bernards & van der Eb, 1984) has been attributed to a negative regulation by the Ad12 E1A gene products (Schreier *et al.*, 1983). To map the region of Ad12 E1A responsible, BRK cells were co-transfected with Ad12 E1B-containing plasmids and hybrid plasmids containing different fused segments of Ad12 and Ad5 E1A. The results obtained suggested that the regulatory activity on MHC class I antigen expression was located on the first exon of E1A, and that the E1A region of Ad5 was dominant over the E1A of Ad12 (Jochimsen *et al.*, 1984).

Point mutations and deletions in the first exon of E1A have demonstrated that the region between nucleotides 697 and 790 (CR1), and between 920 and 967 (CR2), are indispensable for *trans*-repression (Lillie *et al.*, 1987; Schneider *et al.*, 1987). The role of CR2 in this effect appeared to be a mainly co-operative one with CR1 (Schneider *et al.*, 1987).

The functions of E1A proteins can be complemented between different serotypes, but this complementation depends upon the cell line used for co-infection. Thus, Ad2 deleted in the E1A region can be complemented by wild-type Ad3 or Ad7 in KB or HEK-293 cells (Brusca & Chinnadurai, 1981). Ad3 E1A-mutants can also be complemented by wild-type Ad5 in HeLa cells (McDougall & Mautner, 1987). In contrast, Ad2 E1A and E1B

mutants can be complemented by wild-type Ad12 in HeLa cells, but not in BHK-21 cells (Rowe & Graham, 1981). The consequence of this complementation effect is the replication of the defective virus, which might suggest a lack of serotypic specificity of E1A products for their transactivation activity.

However, a hierarchy of dominance has been observed in HeLa cells co-transfected with two wild-type serotypes of adenovirus belonging to different subgroups: e.g., Ad3 appears to be dominant over Ad2, resulting in preferred transcription and translation of Ad3 mRNAs (Delsert & D'Halluin, 1984). This effect has been shown to occur at the E1A promoter level (Tibbetts *et al.*, 1986). Based on experimental data obtained with different genetic constructs, two mechanisms have been proposed to explain this dominance: (i) the Ad3 E1A promoter may be more strongly activated by E1A gene products than the Ad2 E1A promoter (Jones & Tibbetts, 1989); (ii) some Ad3 E1A gene products might repress the Ad2 E1A promoter region (Leite *et al.*, 1986). These two mechanisms are not mutually exclusive, since the two processes might occur simultaneously with a final agonistic effect.

E1A stimulation of viral and cellular DNA synthesis and host cell proliferation. Since the E1A proteins are immediate early gene products, they may play an important, although indirect, role in viral DNA replication by stimulating other early viral promoters, including the E2 promoter responsible for the control of the adenovirus DNA replication units E2A and E2B (Enomoto *et al.*, 1981; Lichy *et al.*, 1982). A mutant deleted in the E1A region, Ad5 dl 312, replicates at a low rate (Gaynor & Berk, 1983; Nevins, 1981).

Induction of cellular DNA synthesis and cell proliferation have been observed in cells expressing E1A (Kaczmarek *et al.*, 1986; Quinlan *et al.*, 1987a; Stabel *et al.*, 1985). These phenomena might result in part from the production of growth factors from infected cells (Quinlan, 1989; Quinlan *et al.*, 1987b), a function which was abolished in deletion mutants lacking the 85 N-terminal amino acids of the E1A polypeptide sequence (Zerler *et al.*, 1987). The localization of this function to the CR1 domain and its flanking N-terminal region has been recently confirmed (Smith & Ziff, 1988). Ad5 infection of quiescent normal rat kidney cells results in transcriptional activation of the p53 anti-oncogene (Braithwaite *et al.*, 1990). This effect was shown to be mediated by the 289R product of E1A. As p53 has been implicated in the regulation of normal diploid cell proliferation (Milner & Milner, 1981; Mercer *et al.*, 1982), this observation might suggest an additional pathway for Ad5 E1A-stimulated cell growth.

Further evidence which may implicate E1A expression in control of cell division is the finding that the synthesis of p34, the mammalian homologue of the yeast cdc2 protein which is involved both in the initiation of DNA synthesis and the initiation of mitosis, is stimulated during the Ad5-induced proliferation of primary baby rat kidney cells (Draetta *et al.*, 1988). Furthermore a p60 protein, which exists in complex with the cdc2 protein, can also be found in association with the E1A proteins in Ad5-infected HeLa cells (Giordano *et al.*, 1989). This p60 protein has recently been reported to be cyclin A protein (Pines & Hunter, 1990; reviewed by Lewin, 1990). Cyclins are accumulated during interphase and are rapidly degraded during mitosis. They play a key role in entry and exit from mitosis (Nurse, 1990). The complex cellular pathways involved in control of cell division may therefore be amenable to regulation by the E1A proteins.

Respective roles of the 289R and 243R E1A proteins and identification of their different functional domains. The E1A proteins have, besides cell transformation properties in co-

operation with E1B or an activated *ras* oncogene, a positive (*trans*-activator) and negative (enhancer repressor) regulatory activity on gene transcription by RNA polymerases II and III (Fig. 4b). Micro-injection of bacterially produced E1A proteins (Ferguson *et al.*, 1984) results in induction of cellular DNA synthesis in NIH-3T3 cells, an activity that is no longer observed with E1A proteins which have 169 amino acids deleted at their C-terminus (Kaczmarek *et al.*, 1986). E1A proteins having a deletion of 85 amino acids at their common N-terminus are incapable of stimulating DNA synthesis in BHK-21 cells (Zerler *et al.*, 1987). By use of two different mutants, (Ad5 *dl* 1500 which synthesizes only the 243R protein and Ad5 *pm* 975 which produces only the 289R protein), it has been shown that the 243R protein is dispensable in exponentially growing cells, but seems to be able to drive G₀-arrested cells into S phase (Montell *et al.*, 1982), a function probably important in adenovirus-induced cell immortalization (Spindler *et al.*, 1985).

A deletion between amino acids 86 and 120 in the first exon does not alter the transactivation efficiency of the E1A proteins, whereas a deletion between 121 and 150 results in a strong decrease in transactivation (Moran *et al.*, 1986b). Thus, domain 3 (unique to 289R) appeared to be essential for *trans*-activation, whereas common domains 1 and 2 did not. A deletion between residues 121 and 150 in the first exon of E1A strongly decreased its transformation capacity (Moran *et al.*, 1986b). A point mutation converting Cys¹²⁴ into a glycine residue resulted in a significant reduction of transformation efficiency in conjunction with activated *ras*, with no detectable alteration of *trans*-activation or viral replication in HeLa cells (Moran *et al.*, 1986b). This Cys¹²⁴ is not part of the putative metal-binding domain recently identified (Lillie *et al.*, 1987; Lillie & Green, 1989).

A more refined topological definition of functional domains of E1A proteins has been obtained with point mutations and synthetic peptides representing specific regions of E1A proteins (Lillie *et al.*, 1987). Induction of DNA synthesis, transcriptional repression, and malignant transformation all seem to be unambiguously confined to the CR1 and CR2 domains of the first exon, common to both 289R and 243R (Fig. 5a). The unique domain of the 289R protein appears therefore to be associated with specific functions not shared by the 243R species, in particular activation of transcription (Fig. 5a). Similar results have been obtained using mutant plasmids and deletion, linker-scanning, and frame-shift mutant viruses (Fahnestock & Lewis, 1989; Schneider *et al.*, 1987). CR1 and CR2 are therefore essential for transcriptional repression, induction of cellular DNA synthesis, cell transformation and proliferation; the CR3 domain is involved in transcriptional *trans*-activation.

Further genetic analysis, using deletion-linker insertions in the N-terminal region of E1A, which spans the CR1 domain, have shown that (i) the conserved region 1 and its N-terminal flanking region are not essential for viral growth in HeLa cells, but are required for induction of DNA synthesis and focus formation in both primary and established rodent cell lines; (ii) however, the latter two functions are not linked in infected primary rodent (BRK) cells, in which this E1A region provides two distinct functions: one required for induction of cell DNA synthesis, the other one for formation of transformed foci (Smith & Ziff, 1988).

A detailed genetic analysis by linker insertion mutagenesis in E1A has also led to the identification of more separate functions in the E1A proteins. One class of mutants have been found to be defective both in enhancer repression and in transcriptional *trans*-activation, but to have a wild-type transforming property in co-operation with an activated human *ras* oncogene, suggesting that *ras*-co-operation activity is a function distinct from positive and negative transcription regulatory functions (Velcich & Ziff, 1988). More interestingly, the mutations that inactivate enhancer

repression do not map in either domain CR1 or CR2, the two regions previously assigned to both repression and transformation. This suggests that the repression activity is dependent upon the conformational structure of the whole E1A molecule, rather than confined to a single polypeptide domain (Velcich & Ziff, 1988).

Construction of fused genes and analysis of the functions carried by the resulting chimaeric proteins have been recently used to assign specific functions with greater precision to polypeptide domains of the E1A proteins. Thus, the activity of a GAL4-CR3 fusion protein on transcription was assayed using a reporter gene placed under the control of the adenovirus early promoter E4 containing a GAL4 binding-site (Lillie & Green, 1989). The results obtained suggest that E1A functions as a typical cellular activator (Ptashne, 1988), with a DNA-binding domain, possibly interacting with a host cell transcription factor, and an activating domain, enabling the bound activator to stimulate transcription. A refined model for E1A structure-function can thus be proposed (Fig. 5b), in which both functional regions are topologically separated by the putative zinc-finger located between residues 153 and 175 (Lillie & Green, 1989).

Cellular and pseudo-cellular E1A-like activity. The E1A gene products have been shown to have a negative regulatory control on the enhancer sequences of SV40 and polyoma viruses (Borelli *et al.*, 1984; Velcich & Ziff, 1985), and of other cellular genes (Hen *et al.*, 1985). It has also been observed that the enhancer sequence of polyoma virus is inactive in undifferentiated cells, but becomes activated upon differentiation. In undifferentiated mouse F9 embryonal carcinoma (EC) cells, early adenovirus promoters present in the E1A deletion mutant Ad5 *dl* 312 (including EI1AE) are expressed at wild type levels in the absence of E1A. When F9 EC cells were induced to differentiate by treatment with cyclic AMP or retinoic acid, early promoter activity became strictly dependent upon the presence of viral E1A (Imperiale *et al.*, 1984). These observations might be explained by the hypothesis that Ad5 *dl* 312 has been complemented by a cellular E1A-like activity present in undifferentiated cells but not in their differentiated derivatives (Imperiale *et al.*, 1984). To confirm this hypothesis, a mutant in the enhancer region of polyoma virus has been constructed; this mutant fails to be inhibited by the adenovirus E1A products, as well as failing to be expressed in undifferentiated F9 EC cells (Hen *et al.*, 1986).

It is impossible to assess at present whether the F9 EC cell line expresses a cellular E1A-like protein, or whether viral E1A-like sequences have been integrated into the cell genome, by analogy with the HPV18 E-7 region found integrated and amplified in HeLa cells (Schwartz *et al.*, 1985). However, recent data on activation of the E1A-responsive adenovirus EI1AE promoter suggest that the endogenous E1A-like activity found in F9 EC cells is different from the viral E1A: in contrast to the viral E1A function, the endogenous E1A-like activity does not induce the formation of dimers of murine transcription factor E2F on the EI1AE promoter (Janssen-Durr *et al.*, 1989; Boeuf *et al.*, 1990).

E1B proteins in the adenovirus infectious cycle. It appears that the p55 protein is required for the shut-off of cellular DNA synthesis, as well as for efficient viral DNA replication and maturation of late mRNAs. Mutants in p55 are phenotypically defective for expression of late viral structural proteins, a defect which lies at a point after transcription but before transport across the nuclear membrane into the cytoplasm of infected cells (Babiss & Ginsberg, 1984; Babiss *et al.*, 1985; Barker & Berk, 1987; Leppard & Shenk, 1989; Pilder *et al.*, 1986). This transport is probably effected by the E1B-p55:E4-p34 complex since mutants in E4 ORF6 show a similar phenotype to p55 deletion

mutants: lowered levels of cytoplasmic viral mRNA and little shut-off of host protein synthesis late in infection (Halbert *et al.*, 1985).

Certain point and insertion mutants in p55 have been described which are defective for growth in human cells, and also exhibit defective, delayed replication of viral DNA (Stillman, 1986). Systematic insertional mutagenesis of p55 has recently shown that viral replication and transformation functions are not encoded within distinct polypeptide domains, although functions required for transformation could be mutationally distinguished from functions required for viral replication (Yew *et al.*, 1990). Thus in infection, it appears that the p55 protein has potential regulatory roles (perhaps in concert with the E4-p34 protein) in both post-transcriptional events in gene expression and in DNA replication.

Mutations in the E1B-p19 gene have been found to have a pleiotropic effect (reviewed by Stillman, 1986). Cell monolayers infected with p19 mutants may exhibit a large-plaque morphology (*lp* phenotype), an increased cytopathic effect (*cyt*) or an extensive degradation of cellular and viral DNA (*deg*). The mechanism for p19 mutant-induced DNA degradation might involve a cellular nuclease active at low pH (D'Halluin *et al.*, 1979, 1985). The p19 protein therefore plays an essential role in the viral replication cycle in that it is indispensable for maintaining the integrity of viral and cellular DNAs (Stillman, 1986).

Recent studies have also implicated the p19 protein in negative regulation of E1A expression. E1B p19 has a negative effect on viral gene expression in the presence of both E1A 13 S and 12 S products (White *et al.*, 1986; White & Stillman, 1987). In the absence of E1A 13 S or 12 S products, p19 has a positive effect on viral gene expression (Herrman *et al.*, 1987; White *et al.*, 1988a). E1B-p19 has also been suggested to *trans*-activate E1A in transformed rodent cells (Senear & Lewis, 1986; van den Elsen *et al.*, 1983). The p19 protein stimulates expression from early adenovirus promoters (Herrman *et al.*, 1987), as well as from the promoter of the intermediate protein IX (Vales & Darnell, 1989). It also *trans*-activates the human heat-shock hsp70 gene (Herrman *et al.*, 1987) and other enhancer-linked viral and cellular promoters (Natarajan, 1986; Yoshida *et al.*, 1987).

The observed increased level of cytoplasmic mRNA in the presence of p19 has led to the assumption that p19 exerts its effect at the transcriptional level (Herrman *et al.*, 1987; Natarajan, 1986; Senear & Lewis, 1986; Vales & Darnell, 1989; van den Elsen *et al.*, 1983; Yoshida *et al.*, 1987). HeLa cell lines containing a high copy number of stably integrated E1B-p19 genes have been recently constructed to further analyse the functions of p19 (Herrmann & Mathews, 1989). It was found that: (i) p19 indirectly increased transcription from promoters of newly introduced genes by increasing the stability of transfected plasmid DNA, and (ii) the activation of transcription from viral promoters during infection in the presence of p19 was due to increased levels of viral DNA (Herrmann & Mathews, 1989). The protective function of the E1B p19 oncoprotein therefore represents a novel mechanism of gene modulation by a viral oncogene (Herrmann & Mathews, 1989). The location of the p19 protein in the nuclear envelope region of the cell (Rowe *et al.*, 1983) would be consistent with these observations on its role in transcription and DNA stability.

Adenovirus oncoproteins and cell transformation

The E1A oncoproteins. The E1A region alone is able to immortalize nonpermissive rodent primary embryo cells (Houweling *et al.*, 1980), and, in conjunction with E1B or other oncogenes, induces a morphological transformation of these cells (Branton *et al.*, 1985; Houweling *et al.*, 1980; Ruley, 1983; van

den Elsen *et al.*, 1983; Zerler *et al.*, 1986). Both 289R and 243R E1A proteins are required for complete and stable transformation of nonpermissive rodent cells after adenovirus infection (Babiss *et al.*, 1984; Carlock & Jones, 1981; Harrison *et al.*, 1977; Ho *et al.*, 1982; Houweling *et al.*, 1980; Jones & Shenk, 1979; Montell *et al.*, 1984; Solnick & Anderson, 1982).

As described above, it is now accepted that the transforming activity of E1A proteins, in co-operation with E1B or an activated *ras* gene, can be mapped between amino acids 40 to 80 and 121 to 139 on the polypeptide sequence, i.e. in the two conserved regions CR1 and CR2 (Kuppuswamy & Chinnadurai, 1987; Lillie *et al.*, 1986, 1987; Moran *et al.*, 1986a,b; Moran & Mathews, 1987; Moran & Zerler, 1988; Murphy *et al.*, 1987; Schneider *et al.*, 1987; Smith & Ziff, 1988; Velcich & Ziff, 1988; Whyte *et al.*, 1988a; Zerler *et al.*, 1986).

Several groups have shown that adenovirus E1A oncoproteins associate with host cell proteins which were first defined by their mobility and relative molecular mass in SDS/PAGE. Polypeptides of 28 kDa (p28), 40, 50, 60, 80, 90, 105, 107, 130 and 300 kDa have thus been described (Yee & Branton, 1985; Harlow *et al.*, 1986). These complexes have been suggested to mediate some of the cellular alterations induced by E1A, since mutations in E1A which abolish its binding to the cellular p300 and p105-107 species also destroy its capacity to transform primary baby rat kidney cells in co-operation with an activated *ras* oncogene (Whyte *et al.*, 1988b, 1989).

A class of cellular genes involved in oncogenesis has been recently characterized and termed 'tumour-suppressor' genes. They are now often referred to as 'anti-oncogenes' (Fung *et al.*, 1987; Green, 1989; Hansen & Cavenee, 1988; Klein, 1988; Lee *et al.*, 1988). Inactivation of such genes by deletion or mutagenesis results in the generation of a number of different human tumours (Lee *et al.*, 1988). The association of E1A polypeptides with host cell anti-oncogene products might thus lead to inactivation of the anti-oncogene proteins. As a working hypothesis, it can therefore be postulated that one mechanism (or one step) of induction of malignant transformation by adenoviruses is the interaction of the E1A oncoproteins with host cell anti-oncogene products.

The p105 protein has now been identified as the product of the retinoblastoma susceptibility gene (*RB* gene), which belongs to this anti-oncogene family. The anti-oncogenic p105-*RB* protein has been found to be physically (and presumably functionally) associated with adenovirus E1A proteins (Egan *et al.*, 1989; Whyte *et al.*, 1988a). Results from other experiments have suggested that interaction of E1A proteins with the p107 host cell protein is also important for transformation (Dyson *et al.*, 1989a; Moran & Zerler, 1988).

The regions of E1A proteins which are physically involved in the binding to the three major cellular proteins p300, p107 and p105-*RB* have been recently mapped, using a series of deletion mutants in E1A (Whyte *et al.*, 1989). An E1A sequence between residues 1 and 76 interacts with p300, a sequence between 121 and 127 interacts with p107, and p105-*RB* binds to two non-contiguous sequences on the E1A polypeptide chain, at 30-60, and 121-127, respectively (Whyte *et al.*, 1989). Interestingly, the transforming regions of E1A proteins coincide with these protein-protein interacting domains (Fig. 5c).

The p105-*RB* protein also forms a complex with the large T antigens of SV40 and JC virus (DeCaprio *et al.*, 1988; Dyson *et al.*, 1989a; Ewen *et al.*, 1989). This result is even more relevant if one considers that a region of SV40 large T antigen, located between the DNA-binding domain and the nuclear localization peptide signal, can substitute for a transforming domain of the adenovirus E1A products (Moran, 1988). The E7 oncoprotein of human papilloma virus 16 (HPV 16) also forms a complex with p105-*RB* (Dyson *et al.*, 1989b) and the binding site on the E7

protein shows a high degree of structural similarity to Ad5 E1A (Phelps *et al.*, 1989).

The binding site on p105-*RB* for SV40 T antigen or E1A has recently been mapped (Hu *et al.*, 1990; Huang *et al.*, 1990). Two non-contiguous regions of the p105-*RB* protein (between amino acids 393 to 572 and 646 to 772) were required for E1A or T binding. Interestingly these regions of p105-*RB* are frequently deleted or mutated in naturally occurring tumour cell lines (Horowitz *et al.*, 1989; Bookstein *et al.*, 1990) yielding polypeptides unable to complex with T or E1A *in vitro*. Elucidation of the function(s) of these two regions of p105-*RB* will be awaited with great interest.

A clue to a metabolic process which may be involved in cellular transformation is the recent finding that SV40 large T antigen preferentially binds to an underphosphorylated species of the p105-*RB* (Ludlow *et al.*, 1989). Although the level of p105-*RB* protein does not alter during the cell cycle, p105-*RB* appears to be highly phosphorylated in the S and G₂/M phases but is almost all unphosphorylated in the G₀ and G₁ phases of the cycle (Buchkovich *et al.*, 1989; DeCaprio *et al.*, 1989; Chen *et al.*, 1989). Differentiation of promyelocytic HL60 cells induced by tumour promoters is accompanied by an increased level of underphosphorylated derivatives of p105-*RB* (Akiyama & Toyashima, 1990). Thus underphosphorylated p105-*RB* may be a characteristic of resting or differentiating cells, i.e. cells which are growth-suppressed. Binding of T antigen to unphosphorylated p105-*RB* may remove the growth-suppressing function of p105-*RB* and accelerate the cell towards S phase in SV40-transformed cells. The E1A proteins appear somewhat different to T in that E1A binds both to unphosphorylated and phosphorylated p105-*RB* (Dyson *et al.*, 1989). A general model of p105-*RB* growth suppression in which either phosphorylation or T antigen binding down-regulates the function of the *RB* gene product may require further elaboration in view of the more complex spectrum of E1A-p105-*RB* interactions (Cooper & Whyte, 1989; Ludlow *et al.*, 1989).

The E1B proteins. E1A can be complemented by several oncogenes substituting for E1B in the transformation process, e.g. polyoma virus middle T antigen or activated genes of the *ras* gene family (Ruley, 1983). The E1B gene products which are responsible for the E1A-co-operating effect on transformation have been identified by genetic analysis. Rodent cells can be transformed by transfection with the Hind III G fragment of Ad5 DNA, which contains the genes for the two major E1A 289R and 243R proteins, along with a truncated E1B region corresponding to the entire sequence of the p19 protein, but only the *N*-terminal portion of the p55 protein (Rowe *et al.*, 1984). The p55-defective Ad5 *hr* 6 mutant virus is incapable of viral-mediated transformation of both rat embryo cells and BRK cells, suggesting that p55 is important for the initiation of the transformation process, possibly at the level of viral DNA integration (Rowe & Graham, 1983).

Other studies on transformation of rodent cells with viral mutants in the gene coding for the p55 have confirmed that the p55 protein is necessary for efficient transformation (Babiss *et al.*, 1984; Graham *et al.*, 1977, 1978; Logan *et al.*, 1982). However, when the E1B gene was introduced (along with E1A) into rodent cells by plasmid-mediated DNA transfection, mutations which removed p55 function did not affect the number of transformed foci obtained (Rowe & Graham, 1983), suggesting that E1B-p55 is necessary for viral-mediated, but not DNA-mediated transformation. This view, however, has not been generally accepted (Bernards *et al.*, 1983). In particular, using plasmids encoding either of the major p55 or p19 products of the E1B gene, Senear & Lewis (1986) have shown that both E1B

proteins are required for transformation in co-operation with E1A. Barker & Berk (1987) have constructed point and deletion mutants in the E1B region, and have confirmed that both proteins are required for efficient transformation of rodent cells, in conjunction with E1A. However, a weak expression of the p55 protein, as in the point mutant Ad5 *pm* 2022 (having a stop signal at the second codon of the p55 coding sequence, and a re-initiation at the next AUG) is sufficient for normal growth of the mutant in permissive cells, and an efficient transformation of rodent cells (Barker & Berk, 1987).

One possible function for E1B-p55 in transformation may be an interaction with the cellular anti-oncogene product p53 (see above). The p53 is a member of the class of nuclear proteins (Bishop, 1985) which have the capacity to immortalize primary cells in culture (Jenkins *et al.*, 1984) and in this respect resemble the adenovirus E1A oncogene. Like E1A, p53 can co-operate with activated *ras* genes in the malignant transformation of primary cells (Eliyahu *et al.*, 1984; Parada *et al.*, 1984). Over-expression of p53 can convert normal rat fibroblasts into tumorigenic derivatives (Eliyahu *et al.*, 1985) and either deletions or point mutations in the mouse p53 coding sequence can activate the *ras*-co-operating function of p53 in transformation of primary rodent cells (Finlay *et al.*, 1988; Jenkins *et al.*, 1985). Normal (i.e. wild-type) p53 has the ability to suppress the transforming function of mutated p53, suggesting that normal p53 has certain characteristics of a tumour suppressor gene (Finlay *et al.*, 1989; Lane & Beach, 1990). The complex between Ad5 E1B-p55 and p53 could release cells from controlled growth in an analogous fashion to the E1A:p105-*RB* complex. As discussed above, Ad12 E1B-p55 does not appear to form a stable complex with p53 in transformed cells. The function of E1B-p55:p53 complex formation is therefore not yet clear. The binding site on mouse p53 for SV40 large T antigen has been identified (Jenkins *et al.*, 1988; Tan *et al.*, 1986) and mapped to one or two regions of amino acid sequence at internal positions in the mouse p53 protein. Interestingly the major binding site on mouse p53 for Ad5 E1B-p55 is located at the *N*-terminus, between amino acids 14 and 114 (J. Jenkins, personal communication). The weak p55:p53 complex found in Ad5-infected mouse L929 cells could not be immunoprecipitated by monoclonal anti-p53 antibody PAb242 (Braithwaite *et al.*, 1991). Since the epitope recognized by PAb242 is situated at the *N*-terminus of mouse p53 (Jenkins & Sturzbecher, 1988), the masking of this epitope in the p55:p53 complex also argues for interaction of p55 with the *N*-terminus of p53. This suggests that the structure (and therefore possibly the function) of the E1B-p55:p53 complex may be quite different to that of SV40 TAg:p53. The binding site on Ad5 E1B-p55 for p53 has not yet been reported.

The potential importance of the interaction of p53 with oncoproteins of DNA tumour viruses has been increased by the recent demonstration that the transforming E6 proteins of HPV 16 and 18 could form a complex with p53 *in vitro* (Werness *et al.*, 1990). Although the existence of E6:p53 complexes must now be confirmed *in vivo*, this work again points to common interactions among DNA tumour virus oncoproteins with cellular anti-oncogene products (namely p105-*RB* and p53) and suggests that these viruses exploit similar pathways which lead towards oncogenic transformation.

Studies on transformation by a large number of point and deletion mutants in the p19 gene have clearly shown that this protein is essential for transformation, whether by viruses or plasmid DNAs (reviewed in Stillman, 1986). In contrast, the p19 protein of Ad12 appears not to be required for transformation of primary rodent cells or viral oncogenicity (Edbauer *et al.*, 1988). The location of p19 in the nuclear envelope, the endoplasmic and plasma membranes might suggest a number of functions for p19

in cell growth and cell-cell interaction (see above). The construction of Ad2 E1B plasmids designed to express p19 in the absence of p55 allowed further conclusions about the function of p19 (White & Cipriani, 1989, 1990). In cells transiently expressing p19, or in stably transformed cells where p19 is expressed in the presence of E1A, the vimentin-containing intermediate filament network is disrupted. It is possible that anchorage-independent growth of Ad-transformed cells results from such altered organization of intermediate filaments induced by p19.

Oncogenicity of adenovirus-transformed cells. Rodent cells transformed by highly oncogenic Ad12 contain decreased levels of cell-surface major histocompatibility (MHC) class I antigens, whereas cells transformed by non-oncogenic Ad2 or Ad5 show unaltered expression of MHC class I antigens (see above). It has been proposed that cytotoxic T lymphocytes (CTLs) may be responsible for rejection of Ad2- and Ad5-transformed cells and that Ad12-transformed cells escape immune surveillance by CTLs (Bernards *et al.*, 1983). Ad12-transformed cells exhibit lowered levels of class I mRNA when compared to Ad2- or Ad5-transformed cells (Eager *et al.*, 1985; Schreier *et al.*, 1983; Vasavada *et al.*, 1986) and this down-regulation of MHC class I gene expression is exerted at the level of initiation of transcription (Ackrill & Blair, 1988; Friedman & Ricciardi, 1988). The site of modulation of class I promoter activity has not yet been identified. The MHC class I gene promoter contains an enhancer element (Kimura *et al.*, 1986; Miyazaki *et al.*, 1986) which might be a target for Ad12 E1A-mediated repression. In this respect it is interesting to note that a nuclear factor present in Ad12, but not in Ad5-transformed, cells bind to a region of the MHC class I enhancer element (Ackrill & Blair, 1989). The functional significance of this DNA-protein interaction in terms of its possible effect on MHC class I gene transcription remains to be elucidated.

Ad12-transformed cells have also been shown to be relatively resistant to cytolysis by natural killer (NK) cells, when compared to Ad2- or Ad5-transformed cells (Cook & Lewis, 1984; Cook *et al.*, 1987; Sawada *et al.*, 1985). Expression of Ad5 E1A also induces susceptibility of rodent cells to lysis by tumour necrosis factor (TNF; Chen *et al.*, 1987). The molecular basis of TNF-mediated lysis and resistance to NK cytolysis is presently unknown although data have been obtained which suggest that a fraction of the Ad5 E1A protein in the cell may be present on the cell surface (Bellgrau *et al.*, 1988; see above).

A promising recent approach to analysing the molecular pathology of adenovirus type 12 oncogenesis is the development of a transgenic mouse model system (Koike *et al.*, 1989). In order to attenuate the lytic effect of adenovirus gene expression *in utero*, the E1A promoter/enhancer of an Ad12 E1A+E1B plasmid was replaced with a mouse mammary tumour virus (MMTV) promoter/enhancer. Transgenic mice produced using this construct showed a high incidence of gastric carcinomas which developed within 3–4 months of birth. Interestingly, nine out of 11 males developed this tumour while two out of 12 females were affected, an observation which is somewhat surprising since the use of the MMTV promoter might predict the development of mammary carcinomas in females. It will be interesting to compare this transgenic model of Ad12 oncogenesis with oncogenesis induced by Ad12 virus, and also to know what molecular pathology is elicited using Ad5 E1 genes, instead of Ad12.

CONCLUSIONS AND OPEN QUESTIONS

The major E1A and E1B proteins of human adenoviruses are multi-functional proteins with a number of roles in transcription (E1A-289R and E1A-243R), DNA stability (E1B-p19), cell

transformation (E1A+E1B), post-transcriptional events and DNA replication (E1B-p55). The p55 protein also interacts with the E4-p34 protein in infected cells. The E1A-289R, E1A-243R and E1B-p55 proteins interact with anti-oncogene products in transformed cells: the p55 protein with the cellular p53, and the E1A proteins with the p105-RB. Such interactions are probably very important in mediating biological functions.

Some puzzling problems and intriguing questions can be raised concerning the adenovirus E1A and E1B oncoproteins and their biological functions.

(i) A central problem must be resolved, i.e. the reason for the existence of two overlapping oncoproteins in the early region E1A of adenoviruses. Considering the genetic economy that most viruses possess, the existence and maintenance of *two* gene products (289R and 243R) implies that both proteins have specific functions and cannot substitute for each other. Both proteins have the same *N*- and *C*-termini, and result from a different splicing event. It must therefore be assumed that the unique domain of the 289R protein possesses specific activities, and that the differential splicing of the 243R and 289R messages generates two protein molecules with significantly different conformations (and hence biological activities) in the central portion of the sequence. When analysed by a computerized program (Karplus & Schulz, 1985), the hydrophobicity/hydrophilicity plots and the motif accessibility profiles of the 289R and 243R proteins show that differential splicing introduces a drastic change in the probable conformation of the central domain of the E1A proteins (Fig. 6).

(ii) Related to this problem is the biological significance of the existence of only *one* E1A protein in the mouse adenovirus (Ball *et al.*, 1989), as well as the differential requirement for Ad12 E1A

Fig. 6. Comparative plots of the prediction of chain flexibility for E1A-289R (a) and for E1A-243R (b) sequences

The unique sequence in 289R is located between residues 140 and 185 (Perricaudet *et al.*, 1979). Computer analysis has been performed according to a method developed by Karplus & Schulz (1985). Note the drastic change in flexibility probability between 289R and 243R in the domain overlapping the splicing event. From P. Lemay (unpublished work).

proteins in cell transformation (Lamberti & Williams, 1990). In contrast to group C adenoviruses, only the large Ad12 E1A protein would be required for this process.

(iii) The functions of the two E1A oncoproteins have been analysed using adenovirus mutants defective in producing either of the two gene products (Montell *et al.*, 1982, 1984; Lamberti & Williams, 1990). It must therefore be remembered that an overproduction of the remaining protein might occur to compensate for the lack of the other one. Alternatively, the absence of one of the two E1A gene products, which under normal conditions (i.e. infection with a wild-type adenovirus) acts positively on the E1A promoter (Hearing & Shenk, 1985; Smith *et al.*, 1985), could result in a lower concentration of the other one, making the effect of the mutation difficult to interpret. In other words, the mechanisms of cellular and viral regulation by E1A might be different in a cellular context in which either of the two major E1A oncoproteins is absent. This point is supported by the fact that expression of the E1A region is regulated by its own products (Hearing & Shenk, 1985; Smith *et al.*, 1985): the mutant system would thus be totally de-regulated.

(iv) Point mutations have also been used to define the role of each domain of the E1A oncoproteins. However, a mutation in one common domain of the 289R and 243R proteins may have different structural consequences, and therefore different biological effects, when it affects a protein of 289 residues or a protein of 243 residues.

(v) Most protein post-translational modifications do not occur in prokaryotic systems. The experimental data obtained with E1A oncoproteins expressed in bacteria must therefore be carefully interpreted. In addition, such unmodified proteins have been found to be difficult to solubilize, and denaturing conditions, e.g. 2 M-potassium thiocyanate (Krippel *et al.*, 1984), or detergents (C. Keding & P. Chambon, personal communication) must be used.

(vi) The requirement for two co-operating oncogenes in human adenoviruses appears to be unique among the small DNA tumour viruses. Other DNA tumour viruses have specialized their transforming functions in one, usually multifunctional, polypeptide (e.g. SV40 large T antigen). The role of the adenovirus E1B gene and its major polypeptides, the p55 and p19, is far from clear and needs to be resolved.

It is now imperative that the major E1A and E1B proteins be prepared in large quantities and in their native, post-translationally modified state so that biological functions which are ascribed to them can be studied at the molecular level. Structural studies, e.g. crystallographic analysis of the purified proteins, also appear to be a priority for elucidating their biological roles in relation to their structure. The discovery of the E1A protein to be a zinc-finger protein with specific DNA interactions has been recently reported (Lillie *et al.*, 1987).

The proteins which interact with E1B-p55, namely E4-p34 and cellular p53, should also be prepared in quantity to study protein-protein interactions and their role in E1B protein function. Since both major E1B proteins are post-translationally modified, prokaryotic expression systems seem unsuitable. Moreover, expression of the p19 protein has been found to be inefficient in *E. coli*, probably due to poor initiation of translation (Fukui *et al.*, 1983). Therefore eukaryotic expression systems, especially yeast (Handa *et al.*, 1987), vaccinia or baculovirus vectors (Luckow & Summers, 1989), should be used for large-scale production of these important regulatory proteins. Expression of individual E1A and E1B gene products in eukaryotic systems appears therefore as an invaluable tool to answer some major unsolved questions. Two recent approaches of integrating the E1A genes under an inducible promoter (Brunet & Berk, 1988), or the cloning and expression of adenovirus proteins in

insect cells using the technology of recombinant baculovirus vectors (Patel & Jones, 1990) have already given promising results. In addition, the isolation of monoclonal antibodies directed specifically against various domains of the native, post-translationally-modified oncoproteins, and the mapping of the recognized epitope might permit the determination of the intracellular location of each E1A gene product and its cellular protein partner(s) by co-immunoprecipitation experiments.

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REFERENCES

- Ackrill, A. M. & Blair, G. E. (1988) *Oncogene* **3**, 483–487
- Ackrill, A. M. & Blair, G. E. (1989) *Virology* **172**, 643–646
- Akiyama, T. & Toyoshima, K. (1990) *Oncogene* **5**, 179–183
- Allan, M., Zhu, J., Montague, P. & Paul, J. (1984) *Cell* **38**, 399–407
- Alwine, J. C., Reed, S. I. & Stark, G. R. (1977) *J. Virol.* **24**, 22–27
- Anderson, C. W. & Lewis, J. B. (1980) *Virology* **104**, 27–41
- Anderson, C. W., Schmitt, R. C., Smart, J. E. & Lewis, J. B. (1984) *J. Virol.* **50**, 387–396
- Babich, A., Feldman, L. T., Nevins, J. R., Darnell, J. E. & Weinberger, C. (1983) *Mol. Cell. Biol.* **3**, 1212–1221
- Babiss, L. E. & Ginsberg, H. S. (1984) *J. Virol.* **50**, 202–212
- Babiss, L. E., Fisher, P. B. & Ginsberg, H. S. (1984) *J. Virol.* **52**, 389–395
- Babiss, L. E., Ginsberg, H. S. & Darnell, J. E. (1985) *Mol. Cell. Biol.* **5**, 2552–2558
- Ball, A. D., Beard, C. W., Redick, S. D. & Spindler, K. R. (1989) *Virology* **170**, 523–526
- Ballard, D. W., Boehnlein, E., Lowenthal, J. W., Wano, Y., Franza, B. R. & Greene, W. C. (1988) *Science* **241**, 1652–1655
- Barker, D. B. & Berk, A. J. (1987) *Virology* **156**, 107–121
- Bellgrau, D., Walker, T. A. & Cook, J. L. (1988) *J. Virol.* **62**, 1513–1519
- Berger, S. L. & Folk, W. R. (1985) *Nucleic Acids Res.* **13**, 1413–1428
- Berk, A. J. (1986a) *Cancer Surveys* **5**, 367–387
- Berk, A. J. (1986b) *Annu. Rev. Genet.* **20**, 45–79
- Berk, A. J. (1989) *Biochim. Biophys. Acta* **1009**, 103–109
- Berk, A. J., Lee, F., Harrison, T., Williams, J. & Sharp, P. A. (1979) *Cell* **17**, 935–944
- Berk, A. J. & Sharp, P. A. (1978) *Cell* **14**, 695–711
- Bernards, R., Schrier, P. J., Bos, J. L. & van der Eb, A. J. (1983a) *Virology* **127**, 45–53
- Bernards, R., Schrier, P. J., Houweling, A., Bos, J. L., van der Eb, A. J., Zijlstra, M. & Melief, C. M. J. (1983b) *Nature (London)* **305**, 776–779
- Bernards, R. & van der Eb, A. J. (1984) *Biochim. Biophys. Acta* **783**, 187–204
- Bishop, J. M. (1982) *Adv. Cancer Res.* **37**, 1–32
- Bishop, J. M. (1985) *Cell* **42**, 23–38
- Blair-Zajdel, M. E. & Blair, G. E. (1988) *Oncogene* **2**, 579–584
- Blair-Zajdel, M. E., Barker, M. D., Dixon, S. C. & Blair, G. E. (1985) *Biochem. J.* **225**, 649–655
- Boeuf, H., Janssen-Durr, P. & Keding, C. (1990) *Oncogene* **5**, 691–699
- Bolen, J. B., Thide, C. J., Israel, M. A., Yonemoto, W., Lipsich, L. A. & Brugge, J. S. (1984) *Cell* **38**, 767–777
- Bookstein, R., Shew, J. Y., Chen, P. L., Scully, P. & Lee, W. H. (1990) *Science* **247**, 712–715
- Borelli, E., Hen, R. & Chambon, P. (1984) *Nature (London)* **312**, 608–612
- Bos, J. L., Polder, L. J., Bernards, R., Schreier, P. I., van den Elsen, P. J., van der Eb, A. J. & van Ormondt, H. (1981) *Cell* **27**, 121–131
- Bos, J. L. & ten Wolde-Kramwinkel, H. C. (1983) *EMBO J.* **2**, 73–76
- Boulanger, P. A., Lemay, P., Blair, G. E. & Russell, W. C. (1979) *J. Gen. Virol.* **44**, 783–800
- Boulanger, P. A., Yoshinaga, S. K. & Berk, A. J. (1987) *J. Biol. Chem.* **262**, 15098–15105
- Braithwaite, A. W., Bellett, A. J. D., Blair, G. E. & McGovern, J. (1991) *Oncogene*, in the press
- Braithwaite, A. W. & Jenkins, J. R. (1989) *J. Virol.* **63**, 1792–1799

- Braithwaite, A., Nelson, C., Skulimowski, A., McGovern, J., Piggott, D. & Jenkins, J. (1990) *Virology* **177**, 595–605
- Braithwaite, A. W., Sturzbecher, H. W., Addison, C., Palmer, C., Rudge, K. & Jenkins, J. R. (1987) *Nature (London)* **329**, 458–460
- Branton, P. E., Lassam, N. J., Downey, J. F., Yee, S. P., Graham, F. L., Mak, S. & Bayley, S. T. (1981) *J. Virol.* **37**, 601–608
- Branton, P. E., Bayley, S. T. & Graham, F. L. (1985) *Biochim. Biophys. Acta* **780**, 67–94
- Branton, P. E. & Rowe, D. T. (1985) *J. Virol.* **56**, 633–638
- Brunet, L. J. & Berk, A. J. (1988) *Mol. Cell. Biol.* **8**, 4799–4807
- Brusca, J. S. & Chinnadurai, G. (1981) *J. Virol.* **39**, 300–305
- Buchkovich, K., Duffy, L. A. & Harlow, E. (1989) *Cell* **58**, 1097–1105
- Carlock, L. R. & Jones, N. C. (1981) *J. Virol.* **40**, 657–664
- Chatterjee, P. K. & Flint, S. J. (1986) *J. Virol.* **60**, 1018–1026
- Chatterjee, P. K., Bruner, M., Flint, S. J. & Harter, M. L. (1988) *EMBO J.* **7**, 835–841
- Chen, I. S. Y., Cann, A. J., Shah, N. P. & Gaynor, R. B. (1985) *Science* **230**, 570–573
- Chen, M. J., Holskin, B., Strickler, J., Gorniak, J., Clark, M. A., Johnson, P. J., Mitcho, M. E. & Shalloway, D. (1987) *Nature (London)* **330**, 581–583
- Chen, P. L., Scully, P., Shew, J. Y., Wang, J. Y. J. & Lee, W. H. (1989) *Cell* **58**, 1193–1198
- Cherington, V., Brown, M., Paucha, E., St Louis, J., Spiegelman, B. M. & Roberts, T. M. (1988) *Mol. Cell. Biol.* **8**, 1380–1384
- Colgrove, R., Simon, G. & Ganem, D. (1989) *J. Virol.* **63**, 4019–4026
- Cone, R., Grodzicker, T. & Jaramillo, M. (1988) *Mol. Cell. Biol.* **8**, 1036–1044
- Cook, J. L. & Lewis, A. M. (1984) *Science* **224**, 612–615
- Cook, J. L., May, D. L., Lewis, A. M. & Walker, T. A. (1987) *J. Virol.* **61**, 3510–3520
- Cooper, J. A. & Whyte, P. (1989) *Cell* **58**, 1009–1011
- Courtneidge, S. (1985) *EMBO J.* **4**, 1471–1477
- Courtneidge, S. & Smith, A. E. (1983) *Nature (London)* **303**, 435–439
- Courtois, G. & Berk, A. J. (1984) *EMBO J.* **3**, 1145–1149
- Crawford, L. V. (1983) *Int. Rev. Exp. Pathol.* **25**, 1–50
- Crossland, L. D. & Raskas, H. J. (1983) *J. Virol.* **46**, 737–748
- Cutt, J. R., Shenk, T. & Hearing, P. (1987) *J. Virol.* **61**, 543–552
- DeCaprio, J., Ludlow, J., Figge, J., Marsilio, E., Shew, J.-E., Lee, W.-H., Paucha, E. & Livingston, D. (1988) *Cell* **54**, 275–283
- DeCaprio, J. A., Ludlow, J. W., Lynch, D., Furukawa, Y., Griffin, J., Piwnica-Worms, H., Huang, C. M. & Livingston, D. M. (1989) *Cell* **58**, 1085–1095
- Delsert, C. & D'Halluin, J. C. (1984) *Virus Res.* **1**, 365–380
- DePamphilis, M. L. & Bradley, M. K. (1986) in *The Papovaviridae* (Salzman, N. P., ed.), vol. 1, pp. 99–246. Plenum, New York
- Déry, C. V., Herrmann, C. H. & Mathews, M. B. (1987) *Oncogene* **2**, 15–23
- D'Halluin, J. C., Allart, C., Cousin, C., Boulanger, P. & Martin, G. R. (1979) *J. Virol.* **32**, 61–71
- D'Halluin, J. C., Cousin, C., Niel, C. & Boulanger, P. (1984) *J. Gen. Virol.* **64**, 477–483
- D'Halluin, J. C., Delsert, C., Milleville, M. & Boulanger, P. (1985) *J. Gen. Virol.* **66**, 1873–1887
- Draetta, G., Beach, D. & Moran, E. (1988) *Oncogene* **2**, 553–557
- Dumont, D. J., Tremblay, M. L. & Branton, P. E. (1989) *J. Virol.* **63**, 987–991
- Dyson, N., Buchkovich, K., Whyte, P. & Harlow, E. (1989a) *Cell* **58**, 249–255
- Dyson, N., Howley, P., Mürger, K. & Harlow, E. (1989b) *Science* **243**, 934–937
- Eager, K. B., Williams, J., Breiding, D., Pan, S., Knowles, B., Appella, E. & Ricciardi, R. P. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5525–5529
- Edbauer, C., Lamberti, C., Tong, J. & Williams, J. (1988) *J. Virol.* **62**, 3265–3273
- Egan, C., Bayley, S. T. & Branton, P. E. (1989) *Oncogene* **4**, 383–388
- Eliyahu, D., Michalovitz, D. & Oren, M. (1985) *Nature (London)* **316**, 158–160
- Eliyahu, D., Raz, A., Gruss, P., Givol, D. & Oren, M. (1984) *Nature (London)* **312**, 646–649
- Engel, D. A., Hardy, S. & Shenk, T. (1988) *Genes Dev.* **2**, 1517–1528
- Enomoto, T., Lichy, J. H., Ikeda, J. E. & Hurwitz, J. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1779–1782
- Everett, R. D. & Dunlop, M. (1984) *Nucleic Acids Res.* **12**, 5969–5978
- Ewen, M. E., Ludlow, J. W., Marsilio, E., DeCaprio, J. A., Millikan, R. C., Cheng, S. H., Paucha, E. & Livingston, D. M. (1989) *Cell* **58**, 257–267
- Fahnestock, M. L. & Lewis, J. B. (1989) *J. Virol.* **63**, 1495–1504
- Feldman, L. T., Imperiale, M. F. & Nevins, J. R. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4952–4956
- Feldman, L. T. & Nevins, J. R. (1983) *Mol. Cell. Biol.* **3**, 829–838
- Ferguson, B., Jones, N. C., Richter, J. & Rosenberg, M. (1984) *Science* **224**, 1343–1346
- Ferguson, B., Krippel, B., Andrisani, O., Jones, N., Westphal, H. & Rosenberg, M. (1985a) *Mol. Cell. Biol.* **5**, 2653–2661
- Ferguson, B., Krippel, B., Jones, N., Richter, J., Westphal, H. & Rosenberg, M. (1985b) in *Cancer Cells: Growth Factors and Transformation* (Feramisco, J., Ozanne, B. & Stiles, C., eds.), pp. 265–274. Cold Spring Harbor Laboratories, New York
- Figge, J., Webster, T., Smith, T. F. & Paucha, E. (1988) *J. Virol.* **62**, 1814–1818
- Finlay, C. A., Hinds, P. W. & Levine, A. J. (1989) *Cell* **57**, 1083–1093
- Finlay, C. A., Hinds, P. W., Tan, T. H., Eliyahu, D., Oren, M. & Levine, A. J. (1988) *Mol. Cell. Biol.* **8**, 531–539
- Friedman, D. J. & Ricciardi, R. P. (1988) *Virology* **165**, 303–305
- Fukui, Y., Saito, I., Shiroki, K., Shimojo, H., Takebe, Y. & Kaziro, Y. (1983) *Gene* **23**, 1–13
- Fung, Y. K. T., Murphree, A. L., T'Ang, A., Qian, J., Hinrichs, J. & Benedict, W. F. (1987) *Science* **236**, 1657–1661
- Furcinetti, P. S., van Oostrum, J. & Burnett, R. M. (1989) *EMBO J.* **8**, 3563–3570
- Gallimore, P. H., Byrd, P. J. & Grand, R. J. A. (1985a) *Soc. Gen. Microbiol. Symp.* **37**, 125–172
- Gallimore, P. H., Byrd, P. J., Whittaker, J. L. & Grand, R. J. A. (1985b) *Cancer Res.* **45**, 2670–2680
- Gannon, J. V. & Lane, D. P. (1987) *Nature (London)* **329**, 456–458
- Gaynor, R. B. & Berk, A. J. (1983) *Cell* **33**, 689–693
- Gaynor, R. B., Tsukamoto, A., Montell, C. & Berk, A. J. (1982) *J. Virol.* **44**, 276–285
- Gaynor, R. B., Hillman, D. & Berk, A. J. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 1193–1197
- Gaynor, R. B., Feldman, L. T. & Berk, A. J. (1985) *Science* **230**, 447–450
- Gingeras, T. R., Sciaky, D., Gelinas, R. E., Bing-Dong, J., Yen, C. E. *et al.* (1982) *J. Biol. Chem.* **257**, 13475–13491
- Giordano, A., Whyte, P., Harlow, E., Franza, B. R., Beach, D. & Draetta, G. (1989) *Cell* **58**, 981–990
- Goding, C. R., Shaw, C. H., Blair, G. E. & Russell, W. C. (1983) *J. Gen. Virol.* **64**, 477–483
- Goldberg, A. L. & StJohn, A. C. (1976) *Annu. Rev. Biochem.* **45**, 747–803
- Gorman, C. M., Gies, D., McCray, G. & Huang, M. (1989) *Virology* **171**, 377–385
- Graham, F. L. (1984) in *The Adenoviruses* (Ginsberg, H. S., ed.), pp. 339–398. Plenum, New York
- Graham, F. L. & van der Eb, A. J. (1973) *Virology* **52**, 456–467
- Graham, F. L., Smiley, J., Russell, W. C. & Nairn, R. (1977) *J. Gen. Virol.* **36**, 59–72
- Graham, F. L., Harrison, T. & Williams, J. (1978) *Virology* **86**, 10–21
- Grand, R. J. A. (1987) *Biochem. J.* **241**, 25–38
- Grand, R. J. A. & Gallimore, P. H. (1984) *J. Gen. Virol.* **65**, 2149–2166
- Grand, R. J. A. & Gallimore, P. H. (1986) *EMBO J.* **5**, 1253–1260
- Grand, R. J. A., Roberts, C. & Gallimore, P. H. (1985) *FEBS Lett.* **181**, 229–235
- Graves, B. J., Johnson, P. F. & McKnight, S. L. (1986) *Cell* **44**, 565–576
- Green, M. R. (1989) *Cell* **56**, 1–3
- Green, M., Brackman, K. H., Lucher, L. A. & Symington, J. S. (1983) *Curr. Top. Microbiol. Immunol.* **109**, 167–192
- Halbert, D. N., Cutt, J. R. & Shenk, T. (1985) *J. Virol.* **42**, 30–41
- Handa, H., Toda, T., Tajima, M., Wada, T., Iida, H. & Fukasawa, T. (1987) *Gene* **58**, 123–136
- Hansen, M. F. & Cavenee, W. K. (1988) *Cell* **53**, 172–173
- Harlow, E., Whyte, P., Franza, B. R., Jr. & Schley, C. (1986) *Mol. Cell. Biol.* **6**, 1579–1589
- Harter, M. & Lewis, J. B. (1978) *J. Virol.* **26**, 736–749
- Harrison, T., Graham, F. L. & Williams, J. (1977) *Virology* **77**, 319–329
- Haugen, T. H., Cripe, T. P., Ginder, G. D., Karin, M. & Turek, L. P. (1987) *EMBO J.* **6**, 145–152
- Hearing, P. & Shenk, T. (1985) *Mol. Cell. Biol.* **5**, 3214–3221
- Hearing, P. & Shenk, T. (1986) *Cell* **45**, 229–236
- Hen, R., Borrelli, E. & Chambon, P. (1985) *Science* **230**, 1391–1394
- Hen, R., Borrelli, E., Fromental, C., Sassone-Corsi, P. & Chambon, P. (1986) *Nature (London)* **321**, 249–251
- Hen, R., Borrelli, E., Sassone-Corsi, P. & Chambon, P. (1983) *Nucleic Acids Res.* **11**, 8747–8761

- Herrmann, C. H., Déry, C. V. & Mathews, M. B. (1987) *Oncogene* **2**, 25–35
- Herrman, C. H. & Mathews, M. B. (1989) *Mol. Cell. Biol.* **9**, 5412–5423
- Hiebert, S. W., Lipp, M. & Nevins, J. R. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86**, 3594–3598
- Ho, Y. S., Galos, R. & Williams, J. (1982) *Virology* **122**, 109–124
- Hoeffler, W. K. & Roeder, R. G. (1985) *Cell* **41**, 955–963
- Horowitz, J. M., Yandell, D. W., Park, S. H., Canning, S., Whyte, P., Buchkovich, K., Harlow, E., Weinberg, R. A. & Dryja, T. P. (1989) *Science* **243**, 937–940
- Houweling, A., van den Elsen, P. J. & van der Eb, A. J. (1980) *Virology* **105**, 537–550
- Hu, Q., Dyson, N. & Harlow, E. (1990) *EMBO J.* **9**, 1147–1155
- Huang, S., Wang, N. P., Tseng, B. Y., Lee, W. H. & Lee, E. H. H. P. (1990) *EMBO J.* **9**, 1815–1822
- Ihara, S., Feldman, L. T., Watanabe, S. & Ben-Porat, T. (1983) *Virology* **131**, 437–454
- Imperiale, M. J., Feldman, L. T. & Nevins, J. R. (1983) *Cell* **35**, 127–136
- Imperiale, M. J., Kao, H. T., Feldman, L. T., Nevins, J. R. & Strickland, S. (1984) *Mol. Cell. Biol.* **4**, 867–874
- Janssen-Durr, P., Boeuf, H. & Kédinger, C. (1989) *EMBO J.* **8**, 3365–3370
- Jenkins, J. R., Chumakov, P., Addison, C., Sturzbecher, H. W. & Wade-Evans, A. (1988) *J. Virol.* **62**, 3903–3906
- Jenkins, J. R., Rudge, K. & Currie, G. A. (1984) *Nature (London)* **312**, 651–654
- Jenkins, J. R. & Sturzbecher, H. W. (1988) in *The Oncogene Handbook* (Reddy, E. P., Skalka, A. M. & Curran, T., eds.), pp. 403–423, Elsevier, Amsterdam
- Jochimsen, A. G., Bos, J. L. & van der Eb, A. J. (1984) *EMBO J.* **3**, 2923–2927
- Jones, N. & Shenk, T. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3656–3669
- Jones, N. C., Rigby, P. W. & Ziff, E. B. (1988) *Genes Dev.* **2**, 267–281
- Jones, N., Richter, J. D., Weeks, D. L. & Smith, L. D. (1983) *Mol. Cell. Biol.* **3**, 2131–2142
- Jones, S. N. & Tippetts, C. (1989) *J. Virol.* **63**, 1833–1838
- Kaczmarek, L., Ferguson, B., Rosenberg, M. & Baserga, R. (1986) *Virology* **152**, 1–10
- Kadonaga, J. T., Jones, K. A. & Tjian, R. (1986) *Trends Biochem. Sci.* **11**, 20–24
- Kalderon, D. & Smith, A. E. (1984) *Virology* **139**, 109–137
- Kao, H. T. & Nevins, J. R. (1983) *Mol. Cell. Biol.* **3**, 2058–2065
- Karplus, P. A. & Schulz, G. E. (1985) *Naturwissenschaften* **72**, 212–213
- Kimelman, D., Lucher, L. A., Brackman, K. H., Symington, J. S., Ptashne, M. & Green, M. (1985a) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 6300–6304
- Kimelman, D., Miller, J. S., Porter, D. & Roberts, B. E. (1985b) *J. Virol.* **53**, 399–409
- Kimura, A., Israel, A., LeBail, O. & Kourilsky, P. (1986) *Cell* **44**, 261–272
- Kimura, T., Sawada, Y., Shinawa, M., Shimizu, Y., Shiroki, K., Shimojo, H., Sugisaki, H., Takanami, M., Vemizu, Y. & Fujinaga, K. (1981) *Nucleic Acids Res.* **9**, 6571–6589
- Kingston, R. E., Baldwin, A. S. & Sharp, P. A. (1985) *Cell* **41**, 3–5
- Kingston, R. E., Kaufman, R. J. & Sharp, P. A. (1984) *Mol. Cell. Biol.* **4**, 1970–1977
- Klein, G. (1988) *Science* **238**, 1539–1545
- Knebel, D. & Doerfler, W. (1987) *Virus Res.* **8**, 317–326
- Ko, J. L., Dalie, B. L., Goldman, E. & Harter, M. L. (1986) *EMBO J.* **5**, 1645–1651
- Ko, J. L. & Harter, M. L. (1984) *Mol. Cell. Biol.* **4**, 1427–1439
- Koike, K., Hinckes, S. H., Isselbacher, K. & Jay, G. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86**, 5615–5619
- Kovesdi, I., Reichel, R. & Nevins, J. R. (1986a) *Science* **231**, 719–722
- Kovesdi, I., Reichel, R. & Nevins, J. R. (1986b) *Cell* **45**, 219–228
- Kovesdi, I., Reichel, R. & Nevins, J. R. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2180–2184
- Krippel, B., Ferguson, B., Jones, N., Rosenberg, M. & Westphal, H. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7480–7484
- Krippel, B., Ferguson, B., Rosenberg, M. & Westphal, H. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 6988–6992
- Kuenzel, E., Mulligan, J. A., Sommercorn, J. & Krebs, E. G. (1987) *J. Biol. Chem.* **262**, 9136–9140
- Kuppuswamy, M. N. & Chinnadurai, G. (1987) *Virology* **159**, 31–38
- Lamberti, C. & Williams, J. (1990) *J. Virol.* **64**, 4997–5007
- Lane, D. P. & Benchimol, S. (1990) *Genes Dev.* **4**, 1–8
- Lane, D. P. & Crawford, L. V. (1983) *Nature (London)* **278**, 261–263
- Lassam, N. J., Bayley, S. T. & Graham, F. L. (1978) *Virology* **87**, 463–467
- Lassam, N. J., Bayley, S. T., Graham, F. L. & Branton, P. E. (1979) *Nature (London)* **277**, 241–243
- Lebkowski, J. S., Clancy, S. & Calos, M. P. (1985) *Nature (London)* **317**, 169–172
- Lech, K., Anderson, K. & Brent, R. (1988) *Cell* **52**, 179–184
- Lee, E. Y.-H. P., To, H., Shew, J. Y., Bookstein, R., Scully, P. & Lee, W.-H. (1988) *Science* **241**, 218–221
- Lee, K. A. W., Fink, J. S., Goodman, R. H. & Green, M. R. (1989) *Mol. Cell. Biol.* **9**, 4390–4397
- Leite, J. P. G., Niel, C. & D'Halluin, J. C. (1986) *Gene* **41**, 207–215
- Leong, K. & Berk, A. J. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 5844–5848
- Leong, K. L., Brunet, L. & Berk, A. J. (1988) *Mol. Cell. Biol.* **8**, 1765–1774
- Leppard, K. N. & Shenk, T. (1989) *EMBO J.* **8**, 2329–2336
- Lewin, B. (1990) *Cell* **61**, 743–752
- Lewis, E. D. & Manley, J. L. (1985) *Nature (London)* **317**, 172–175
- Lewis, J. B. & Mathews, M. B. (1981) *Virology* **115**, 345–360
- Leza, M. A. & Hearing, P. (1989) *J. Virol.* **63**, 3057–3064
- Lichy, J. H., Field, J., Horwitz, M. S. & Hurwitz, J. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5225–5229
- Lillie, J. W. & Green, M. R. (1989) *Nature (London)* **338**, 39–44
- Lillie, J. W., Green, M. & Green, M. R. (1986) *Cell* **46**, 1043–1051
- Lillie, J. W., Loewenstein, P. M., Green, M. R. & Green, M. (1987) *Cell* **50**, 1091–1100
- Lin, Y. S. & Green, M. R. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 3396–3400
- Linzer, D. I. H. & Levine, A. J. (1979) *Cell* **17**, 43–52
- Logan, J., Pilder, S. & Shenk, T. (1982) *Cancer Cells* **2**, 527–532
- Lucher, L. A., Loewenstein, P. M. & Green, M. (1985) *J. Virol.* **56**, 183–193
- Luckow, V. A. & Summers, M. D. (1989) *Virology* **170**, 31–39
- Ludlow, J. W., DeCaprio, J. A., Huang, C. M., Lee, W. H., Paucha, E. & Livingston, D. M. (1989) *Cell* **56**, 57–65
- Lupker, J. H., Davis, A., Jochimsen, H. & van der Eb, A. J. (1981) *Virology* **37**, 524–529
- Lyons, R. H., Ferguson, B. & Rosenberg, M. (1987) *Mol. Cell. Biol.* **7**, 2451–2456
- Mak, I., Mak, S. & Benchimol, S. (1988) *Virology* **163**, 201–204
- Matsuura, Y., Possee, R. D., Overton, H. A. & Bishop, D. H. L. (1987) *J. Gen. Virol.* **68**, 1233–1250
- McCormick, F. & Harlow, E. (1980) *J. Virol.* **34**, 213–224
- McDougall, I. & Mautner, V. (1987) *J. Gen. Virol.* **68**, 1361–1371
- McGlade, C. J., Tremblay, M. L., Yee, S. P., Ross, R. & Branton, P. E. (1987) *J. Virol.* **61**, 3227–3234
- McLachlan, A. D. & Boswell, D. R. (1985) *J. Mol. Biol.* **185**, 39–49
- Mercer, W. E., Nelson, D., DeLeo, A. B., Old, C. J. & Baserga, R. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6309–6312
- Milner, J. & Milner, S. (1981) *Virology* **112**, 785–788
- Miyazaki, J. I., Appella, E. & Ozato, K. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9537–9541
- Montell, C., Courtois, G., Eng, C. & Berk, A. J. (1984) *Cell* **36**, 951–961
- Montell, C., Fisher, E., Caruthers, M. & Berk, A. J. (1982) *Nature (London)* **295**, 380–384
- Moran, E. (1988) *Nature (London)* **334**, 168–170
- Moran, E. & Mathews, M. B. (1987) *Cell* **48**, 177–178
- Moran, E. & Zerler, B. (1988) *Mol. Cell. Biol.* **8**, 1756–1764
- Moran, E., Grodzicker, T., Roberts, R. J., Mathews, M. B. & Zerler, B. (1986a) *J. Virol.* **57**, 765–775
- Moran, E., Zerler, B., Harrison, T. M. & Mathews, M. B. (1986b) *Mol. Cell. Biol.* **6**, 3470–3480
- Murphy, M., Opalka, B., Sajaczkowski, R. & Schulte-Holthausen, H. (1987) *Virology* **159**, 49–56
- Natarajan, V. (1986) *Nucleic Acids Res.* **14**, 9445–9456
- Nevins, J. R. (1981) *Cell* **26**, 213–220
- Nevins, J. R. (1987) *Microbiol. Rev.* **51**, 419–430
- Nurse, P. (1990) *Nature (London)* **344**, 503–508
- Oren, M., Maltzman, W. & Levine, A. J. (1981) *Mol. Cell. Biol.* **1**, 101–110
- Osborne, T. F., Arvidson, D. N., Tyan, E. S., Dunsworth-Browne, M. & Berk, A. J. (1984) *Mol. Cell. Biol.* **4**, 1293–1305
- Osborne, T. F. & Berk, A. J. (1983) *J. Virol.* **45**, 594–599
- Parada, L. F., Land, H., Weinberg, R. A., Wolf, D. & Rotter, V. (1984) *Nature (London)* **312**, 649–651
- Patel, G. & Jones, N. C. (1990) *Nucleic Acids Res.* **18**, 2909–2915

- Pawlit, M., Clad, A. & zur Hausen, H. (1985) *Virology* **143**, 196–211
- Perricaudet, M., Akusjärvi, G., Virtanen, A. & Pettersson, U. (1979) *Nature (London)* **281**, 694–696
- Persson, H., Katze, M. G. & Philipson, L. (1982) *J. Virol.* **42**, 905–917
- Pettersson, U. (1984) in *The Adenoviruses* (Ginsberg, H. S., ed.), pp. 205–270. Plenum, New York
- Pettersson, U. & Akusjärvi, G. (1983) *Adv. Viral. Oncol.* **3**, 83–131
- Pettersson, U., Virtanen, A., Perricaudet, M. & Akusjärvi, G. (1983) *Curr. Top. Microbiol. Immunol.* **109**, 107–123
- Phelps, W. C., Yee, C. L., Munger, K. & Howley, P. M. (1988) *Cell* **53**, 539–547
- Phelps, W. C., Yee, C. L., Munger, K. & Howley, P. M. (1989) *Curr. Top. Microbiol. Immunol.* **144**, 153–166
- Pilder, S., Logan, J. & Shenk, T. (1984) *J. Virol.* **52**, 664–671
- Pilder, S., Moore, M., Logan, J. & Shenk, T. (1986) *Mol. Cell. Biol.* **6**, 470–476
- Pines, J. & Hunter, T. (1990) *Nature (London)* **346**, 760–763
- Ptashne, M. (1988) *Nature (London)* **335**, 683–689
- Quinlan, M. P. (1989) *Oncogene* **4**, 1051–1055
- Quinlan, M. P. & Grodzicker, T. (1987) *J. Virol.* **61**, 673–682
- Quinlan, M. P., Sullivan, N. & Grodzicker, T. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3283–3287
- Ralston, R. & Bishop, J. M. (1983) *Nature (London)* **306**, 803–806
- Reich, N. C., Oren, M. & Levine, A. J. (1983) *Mol. Cell. Biol.* **3**, 2143–2150
- Reichel, R., Kovacs, I. & Nevins, J. R. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 387–390
- Richter, J. D., Slavicek, J. M., Schneider, J. F. & Jones, N. C. (1988) *J. Virol.* **62**, 1948–1955
- Rigby, P. & Lane, D. (1983) *Adv. Viral Oncol.* **3**, 31–57
- Roessler, W. J., Vandenbark, G. R. & Hanson, R. W. (1988) *J. Biol. Chem.* **263**, 9063–9066
- Rogers, S., Wells, R. & Rechsteiner, M. (1986) *Science*, **234**, 364–368
- Rosenfeld, M. G. & Barrieux, A. (1979) *Adv. Cyclic Nucleotide Res.* **11**, 205–264
- Rowe, D. T., Branton, P. E., Yee, S. P., Bacchetti, S. E. & Graham, F. L. (1984) *J. Virol.* **49**, 674–685
- Rowe, D. T. & Graham, F. L. (1981) *J. Virol.* **38**, 191–197
- Rowe, D. T. & Graham, F. L. (1983) *J. Virol.* **46**, 1039–1044
- Rowe, D. T., Graham, F. L. & Branton, P. E. (1983) *Virology* **129**, 456–468
- Ruley, H. E. (1983) *Nature (London)* **304**, 602–606
- Russell, W. C. & Blair, G. E. (1977) *J. Gen. Virol.* **34**, 19–35
- Sarnow, P., Ho, Y., Williams, J. & Levine, A. J. (1982a) *Cell* **28**, 387–394
- Sarnow, P., Sullivan, C. A. & Levine, A. J. (1982b) *Virology* **120**, 510–517
- Sarnow, P., Hearing, P., Anderson, C. W., Halbert, D. N., Shenk, T. & Levine, A. J. (1984) *J. Virol.* **49**, 692–700
- Sassone-Corsi, P. & Borelli, E. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 6430–6433
- Sawada, Y., Fohring, B., Shenk, T. E. & Raska, K. A., Jr. (1985) *Virology* **147**, 413–424
- Sawada, Y., Urbanelli, D., Raskova, J., Shenk, T. E. & Raska, K. A., Jr. (1986) *J. Exp. Med.* **163**, 563–572
- Schneider, J. F., Fisher, F., Goding, C. R. & Jones, N. C. (1987) *EMBO J.* **6**, 2053–2060
- Schreier, P. I., Bernards, R., Vaessen, R. T. M. J., Houweling, A. & van der Eb, A. J. (1983) *Nature (London)* **305**, 771–775
- Schughart, K., Bause, E. & Esche, H. (1985) *Virus Res.* **3**, 41–56
- Schwartz, E., Freese, V. K., Gissmann, L., Mayer, W., Roggenbuck, B., Stremlan, A. & zur Hausen, H. (1985) *Nature (London)* **314**, 111–114
- Sefton, B. M. & Buss, J. E. (1987) *J. Cell. Biol.* **104**, 1449–1454
- Seif, I., Khoury, G. & Dhar, R. (1979) *Cell* **18**, 963–977
- Senear, A. W. & Lewis, J. B. (1986) *Mol. Cell. Biol.* **6**, 1253–1260
- Simon, M. C., Fisch, T. M., Benecke, B. J., Nevins, J. R. & Heintz, N. (1988) *Cell* **52**, 723–729
- Simmons, D. T. (1986) *J. Virol.* **57**, 776–785
- Slavicek, J. M., Jones, N. C. & Richter, J. D. (1989) *J. Virol.* **63**, 4047–4054
- Smart, J. E., Lewis, J. B., Mathews, M. B., Harter, M. L. & Anderson, C. W. (1981) *Virology* **112**, 703–713
- Smith, C. L., Debouck, C., Rosenberg, M. & Culp, J. S. (1989a) *J. Virol.* **63**, 1569–1577
- Smith, D. H. & Ziff, E. B. (1988) *Mol. Cell. Biol.* **8**, 3882–3890
- Smith, D. H., Kessler, D. M. & Ziff, E. B. (1985) *Mol. Cell. Biol.* **5**, 2684–2696
- Smith, K. H., Gallimore, P. H. & Grand, R. J. A. (1989b) *Oncogene* **4**, 489–497
- Solnick, D. & Anderson, M. A. (1982) *J. Virol.* **42**, 106–113
- Spector, D. J., McGrogan, M. & Raskas, H. J. (1978) *J. Mol. Biol.* **126**, 395–414
- Spector, D. J., Halbert, D. N. & Raskas, H. J. (1980) *J. Virol.* **36**, 860–871
- Spindler, K. R. & Berk, A. J. (1984) *J. Virol.* **52**, 706–710
- Spindler, K. R., Eng, C. Y. & Berk, A. J. (1985) *J. Virol.* **53**, 742–750
- Spindler, K. R., Rosser, D. S. E. & Berk, A. J. (1984) *J. Virol.* **49**, 132–141
- Stabel, S., Argos, P. & Philipson, L. (1985) *EMBO J.* **4**, 2329–2336
- Stein, R. & Whelen, J. (1989) *Mol. Cell. Biol.* **9**, 4531–4534
- Stein, R. & Ziff, E. B. (1984) *Mol. Cell. Biol.* **4**, 2792–2801
- Stein, R. & Ziff, E. B. (1987) *Mol. Cell. Biol.* **7**, 1164–1170
- Stephens, C. & Harlow, E. (1987) *EMBO J.* **6**, 2027–2035
- Stillman, B. W. (1986) *Cancer Surveys* **5**, 389–404
- Subramanian, T., Kuppuswamy, M., Mak, S. & Chinnadurai, G. (1984a) *J. Virol.* **52**, 336–343
- Subramanian, T., Kuppuswamy, M., Gysbers, J., Mak, S. & Chinnadurai, G. (1984b) *J. Biol. Chem.* **259**, 11777–11783
- Svensson, C. & Akusjärvi, G. (1984) *EMBO J.* **3**, 789–794
- Takemori, N., Cladaras, C., Bhat, B., Conley, A. J. & Wold, W. S. M. (1984) *J. Virol.* **52**, 793–805
- Tan, T. H., Wallis, J. & Levine, A. J. (1986) *J. Virol.* **59**, 574–583
- Tanaka, K., Isselbacher, K. J., Khoury, G. & Jay, G. (1985) *Science* **228**, 26–30
- Tibbetts, C., Larsen, P. L. & Jones, S. N. (1986) *J. Virol.* **57**, 1055–1064
- Tremblay, M. L., McGlade, C. J., Gerber, G. E. & Branton, P. E. (1988) *J. Biol. Chem.* **263**, 6375–6383
- Tremblay, M. L., Dumont, D. J. & Branton, P. E. (1989) *Virology* **169**, 397–407
- Tsukamoto, A., Ponticelli, A., Berk, A. J. & Gaynor, R. B. (1986) *J. Virol.* **59**, 14–22
- Ulfendahl, P. J., Linder, S., Kreivi, J. P., Nordquist, K., Svensson, C., Hultberg, H. & Akusjärvi, G. (1987) *EMBO J.* **6**, 2037–2044
- Vales, L. D. & Darnell, J. E. (1989) *Genes Dev.* **3**, 49–59
- van den Elsen, P. J., Houweling, A. & van der Eb, A. J. (1983) *Virology* **131**, 242–246
- van der Eb, A. J. & Bernards, R. (1984) *Curr. Top. Microbiol. Immunol.* **101**, 23–51
- van der Eb, A. J., van Ormondt, H., Schrier, P. I., Lupker, H. J., Jochemsen, H., van den Elsen, P. J., De Leys, R. J., Maat, J., van Beveren, C. P., Dijkema, R. & De Waard, A. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **44**, 383–399
- van den Heuvel, S. J. L., van Laar, T., Kast, W. M., Melief, C. J. M., Zantema, A. & van der Eb, A. J. (1990) *EMBO J.* **9**, 2621–2629
- van Ormondt, H., Maat, J. & van Beveren, C. P. (1980) *Gene* **11**, 299–309
- Vasavada, R., Eager, K. B., Barbanti-Brodano, E., Caputo, A. & Ricciardi, R. P. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 5259–5261
- Velcich, A. & Ziff, E. B. (1985) *Cell* **40**, 705–716
- Velcich, A. & Ziff, E. B. (1988) *Mol. Cell. Biol.* **8**, 2177–2183
- Virtanen, A. & Pettersson, U. (1985) *J. Virol.* **54**, 383–391
- Webster, K. A., Muscat, G. E. O. & Kedes, L. (1988) *Nature (London)* **332**, 553–557
- Werness, B. A., Levine, A. J. & Howley, P. M. (1990) *Science* **248**, 76–79
- White, E. & Cipriani, R. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86**, 9886–9890
- White, E. & Cipriani, R. (1990) *Mol. Cell. Biol.* **10**, 120–130
- White, E. & Stillman, B. (1987) *J. Virol.* **61**, 426–435
- White, E., Blose, S. H. & Stillman, B. W. (1984a) *Mol. Cell. Biol.* **4**, 2865–2875
- White, E., Grodzicker, T. & Stillman, B. W. (1984b) *J. Virol.* **52**, 410–419
- White, E., Faha, B. & Stillman, B. W. (1986) *Mol. Cell. Biol.* **6**, 3763–3773
- White, E., Denton, A. & Stillman, B. W. (1988a) *J. Virol.* **62**, 3445–3454
- White, E., Spector, D. & Welch, W. (1988b) *J. Virol.* **62**, 4153–4166
- Whyte, P., Buchkovich, K. J., Horowitz, J. M., Friend, S. H., Raybuck, M., Weinberg, R. A. & Harlow, E. (1988a) *Nature (London)* **334**, 124–129
- Whyte, P., Ruley, E. & Harlow, E. (1988b) *J. Virol.* **62**, 257–265
- Whyte, P., Williamson, N. M. & Harlow, E. (1989) *Cell* **56**, 67–75
- Wu, L., Rosser, D. S. E., Schmidt, M. C. & Berk, A. J. (1987) *Nature (London)* **326**, 512–515
- Yee, S. P. & Branton, P. E. (1985) *Virology* **146**, 315–322
- Yee, S. P., Rowe, D. T., Tremblay, M. L., McDermott, M. & Branton, P. E. (1983) *J. Virol.* **46**, 1003–1013
- Yew, P. R., Kao, C. C. & Berk, A. J. (1990) *Virology* **179**, 795–805
- Yoshida, K., Venkatesh, L., Kuppuswamy, M. & Chinnadurai, G. (1987) *Genes Dev.* **1**, 645–658

- Yoshinaga, S. K., Boulanger, P. A. & Berk, A. J. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3585–3589
- Yoshinaga, S. K., Dean, N., Han, M. & Berk, A. J. (1986) *EMBO J.* **5**, 343–354
- Zantema, A., Fransen, J. A. M., Davis-Olivier, A., Ramakers, F. C. S., Vooijs, G. P., DeLeys, B. & van der Eb, A. J. (1985a) *Virology* **142**, 44–58
- Zantema, A., Schreier, P. I., Davis-Olivier, A., van Laar, T., Vaessen, R. T. M. J. & van der Eb, A. J. (1985b) *Mol. Cell. Biol.* **5**, 3084–3091
- Zerler, B., Moran, B., Maruyama, K., Moomaw, J., Grodzicker, T. & Ruley, H. E. (1986) *Mol. Cell. Biol.* **6**, 887–899
- Zerler, B., Roberts, R. J., Mathews, M. B. & Moran, E. (1987) *Mol. Cell. Biol.* **7**, 821–829