Definition of adenovirus type 5 functions involved in the induction of chromosomal aberrations in human cells

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Infection of human embryonic kidney cells with adenovirus type 5 (Ad5) induces aberrations (gaps and breaks) in the cell chromosomes. We have conducted a study utilizing a large number of Ad5 mutants to identify the viral functions that are responsible for the occurrence of cytogenetic damage. The results of our investigation have indicated that expression of the gene products of the Ad5 early region 1A (E1A) is necessary for the induction of chromosomal aberrations and that other early viral gene products do not appear to contribute to this phenotype. We have also shown that expression of both the major E1A gene products, the 243 amino acid and the 289 amino acid proteins, is required for induction of damage at wild-type levels, although the 289 amino acid protein appears to retain detectable activity on its own. Lastly, we have observed that deletions in the amino-terminal region of the E1A proteins and in the transactivating domain of the 289 amino acid protein prevent the occurrence of cytogenetic damage, whereas mutations elsewhere in the proteins do not affect this process.

Introduction

Chromosomal aberrations are a common feature of neoplastic cells and may play a role in the initiation and development of the neoplastic phenotype (LeBeau, 1986; Yunis, 1987). Karyotypic abnormalities are also exhibited by mammalian cells transformed *in vitro* by physical or chemical agents and by viruses (DiPaolo, 1983; Chang, 1986). Analysis of human cells transformed by simian virus 40 (SV40) or by the SV40 early region has shown that alterations in both the number and structure of the chromosomes occur as early events following expression of the viral oncogenes, and precede the onset of crisis in the transformed population (Moorhead & Saksela, 1965; Walen, 1987; Chang *et al.*, 1986). Similar observations have been reported for human 293 cells, which are transformed by adenovirus type 5 (Ad5) DNA and express only the viral early region 1 (E1). Substantial karyotypic changes have been detected in these cells prior to crisis, that is before the establishment of the immortal line (Graham *et al.*, 1977; L. Wei & S. Bacchetti, unpublished). These observations suggest that the occurrence of cytogenetic damage correlates with the expression of the viral oncogenes. As in the case of tumorigenicity *in vivo* it therefore seems possible that chromosomal rearrangements might play a role in the onset of transformation *in vitro* and/or in the progression to a fully transformed phenotype.

We are interested in investigating the mechanism by which viruses induce chromosomal aberrations in human cells. It has long been known that Ad5, as well as many other viruses, induces chromosomal damage upon short-term infection of permissive and semi-permissive cells (McDougall, 1971; Murray *et al.*, 1982*a*). Such damage consists primarily of chromatid gaps and breaks at apparently random sites throughout the cell genome (McDougall, 1971; Braithwaite *et al.*, 1983). Previous work in semi-permissive rodent cells has indicated that viruses with mutations in the E1A region are defective for induction of cytogenetic damage, whereas mutants in early region 1B (E1B) or early region 2 (E2) and DNA-negative mutants retain a wild-type (wt) phenotype (Braithwaite *et al.*, 1983). These results differ from those of recent studies with Ad12-infected human cells, in which we have observed that expression of the E1B 55K polypeptide is required for induction of chromosomal damage at both specific and random sites in the chromosomes (S. Schramayr *et al.*, unpublished).

The present study was undertaken to identify the Ad5 gene products responsible for cytogenetic damage in permissive human embryonic kidney (HEK) cells in order to assess whether they differ from those inducing damage in rodent cells and to map the relevant functions

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within these products. To this end we have assayed a large number of mutants throughout the Ad5 E1 region as well as an E2A mutant temperature-sensitive for viral DNA replication. In agreement with the results reported for semi-permissive rodent cells we have observed that expression of both of the major E1A gene products [the 289 and 243 amino acid (aa) proteins] may be necessary for the induction of damage at wt levels, and that proteins encoded by the E1B or E2A region and viral DNA replication do not participate in this process. Our data further indicated that deletions in the amino-terminal region of the E1A proteins, as well as deletions throughout the trans-activating domain of the 289 aa protein, rendered the virus incapable of inducing chromosomal aberrations, whereas deletions elsewhere in the proteins did not affect this phenotype.

Methods

Cells. HEK cells were grown in α-MEM supplemented with antibiotics and 10% foetal calf serum (FCS). The cells were obtained from embryonal kidneys by trypsinization and were used as secondary cultures. For experimental purposes the cells were seeded at $2{\cdot}5 \times 10^5$ cells/100 mm plate in α-MEM plus 10% FCS and grown for 24 h, at which time the serum concentration was reduced to 0·5%. After an additional 48 h FCS was added to 20% and the partially synchronized cells were infected 6 h later. Colcemid (0·1 μg/ml) was added 15 h after infection and the cells were harvested 4 h later. In all experiments duplicate cultures were infected with a given virus, and harvested and processed independently.

Viruses. In addition to Ad5 wt the following mutants were used in this study: *ts*125, an E2A mutant that specifies a temperature-sensitive 72K DNA-binding protein and is DNA-negative at the non-permissive temperature (38·5 °C) (van der Vliet & Sussenbach, 1975); *dl*309, which carries a deletion in the E3 gene but exhibits an essentially wt phenotype and is the parent of the E1A mutants described below (Jones & Shenk, 1979); *dl*E1,3, which lacks both the E1 and the E3 regions (Haj-Ahmad & Graham, 1986); *dl*312, an E1A$^-$ mutant (Jones & Shenk, 1979); *dl*313, with a deletion encompassing all of E1B and extending leftward to the last 69 aa in exon 2 of E1A (Jones & Shenk, 1979); *dl*50 and *dl*55, both E1B$^-$ mutants with deletions from nucleotides (nt) 1770 to 3641 (*dl*50) and from nt 1969 to 3330 (*dl*55). The deletion in *dl*55 extends rightward into the coding region of protein IX (F. L. Graham, personal communication); *dl*520, which has a deletion in the splice donor site of the 13S E1A message and specifies only the 243 aa 12S product (Haley *et al.*, 1984); *pm*975, which contains a point mutation in the splice donor site of the 12S message and specifies only the 289 aa product of the 13S message (Montell *et al.*, 1982); *dl*1101 to *dl*1109, all carrying deletions in exon 1 of E1A (Jelsma *et al.*, 1988); *dl*1110, *dl*1112, *dl*1113, *dl*1114 and *hr*1 (Harrison *et al.*, 1977; Jelsma *et al.*, 1988), which have deletions in the unique region of the 289 aa protein; *dl*1115, *dl*1116 and *sub*1117 (Jelsma *et al.*, 1988), with deletions or a substitution (*sub*1117) in exon 2 of the E1A proteins. The location in the Ad5 genome of the mutations carried by these viruses are shown in Fig. 3 and 5 and the phenotype of the E1A mutants is summarized in Table 2. All of the E1A mutant viruses, with the exception of *dl*E1,3 and *dl*312, synthesize E1A protein(s) in infected human KB cells (Rowe *et al.*, 1983; Egan *et al.*, 1988; Tremblay *et al.*, 1989). Representative mutants, among those positive or negative for induction of aberrations, were also tested for E1A expression in infected HEK cells and were found to be positive (in this study). Viruses were propagated in KB or HeLa cells at 37 °C (Ad5 wt and mutant *dl*309) or at 34 °C (*ts*125), or in 293 cells at 37 °C (E1 mutants). All virus stocks, consisting of crude cell lysates, were titrated at least twice on 293 cells, with the exception of *ts*125, which was titrated once on HeLa cells at 34 °C.

Analysis of viral proteins and viral DNA. Accumulation of E1A proteins in infected HEK cells was determined by Western blot and immunostaining. Cultures infected with 100 p.f.u./cell of Ad5 wt and mutant viruses were harvested at 19 h. Equal amounts of protein from cell lysates were electrophoresed on 9% SDS–polyacrylamide gels and proteins electrotransferred to nitrocellulose membrane using the Bio-Rad Trans-Blot Cell. Immunostaining was performed using the M73 mouse monoclonal antibody specific for E1A proteins (Oncogene Science) and alkaline phosphatase-conjugated goat anti-mouse IgG (Jackson Immunoresearch Laboratories). Production of viral DNA was determined by Southern blot hybridization of total cell DNA using Ad5 genomic DNA as a probe, labelled *in vitro* with [α-^{32}P]dCTP by nick translation.

Chromosome preparation. Colcemid-treated cells were harvested by trypsinization and incubated in 0·075 M-KCl at 37 °C for 16 min prior to fixation with methanol : acetic acid (3 : 1, v/v) for a minimum of 24 h. Chromosomes were stained with 5% Giemsa stain for 5 min at room temperature and analysed by light microscopy. The mitotic index of the cell population was determined by scoring 1000 cells and the percentage of aberrant cells, as well as the frequency of aberrations, were obtained by scoring a minimum of 200 metaphases for each experimental treatment. The values for mock-infected cells and cells infected with Ad5 wt and *dl*309 in Fig. 3 to 5 were obtained from scoring between 500 and 1000 metaphases over several experiments. Since virus titres were considered accurate within at most a factor of two, mutant viruses were taken as positive if they induced damage at a frequency within a factor of two of wt virus, and negative if this frequency did not differ more than twofold from that in mock-infected cells.

Results

Dose response, time course of induction and characteristics of cytogenetic damage

HEK cells were partially synchronized by serum starvation to maximize the mitotic index at the time of harvest and infected 6 h after release from serum depletion. The incidence of cytogenetic damage as a function of virus dose was determined by infecting duplicate cultures with 10 to 100 p.f.u. per cell of wt Ad5 for 19 h (see below). For each experimental point at least 200 metaphases were scored to determine the percentage of aberrant cells and the frequency of aberrations (expressed as aberrations/100 cells), and 1000 cells to evaluate the mitotic index. The results of these experiments are shown in Fig. 1. Over the range of 10 to 100 p.f.u./cell both the number of aberrant cells and the frequency of aberrations increased approximately linearly with dose, whereas the mitotic index decreased. Above 100 p.f.u./cell the incidence of damage increased further, but because of the reduction in mitotic index the number of metaphase cells that could be harvested was inadequate for statistically significant analysis. Since 100 p.f.u./cell

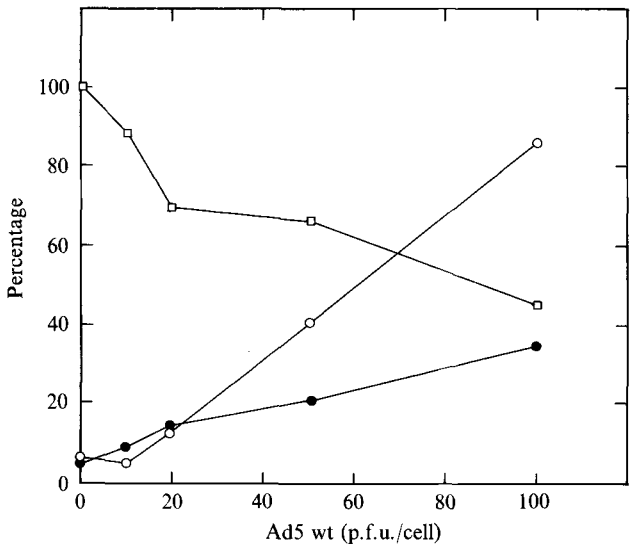

Fig. 1. Dose response of cytogenetic damage induced by Ad5 in human cells. Partially synchronized HEK cells were infected for 19 h with the indicated doses of Ad5 wt. The percentage of aberrant cells (●) and the frequency of aberrations (○) per 100 cells were determined by scoring at least 200 metaphases per treatment. Mitotic indices (□) were determined by analysing 1000 cells.

Table 1. *Time course of induction of chromosomal damage in HEK cells**

Length of infection (h)	No. aberrant cells (%)	Aberrations/100 cells
0	2	2
11	25	39
15	18	42
18	27	110
21	37	109

* The cells were infected with 100 p.f.u./cell of Ad5 wt 6 h after release from serum starvation and harvested at the time indicated. One-hundred metaphases were scored for each experimental point.

induced chromosomal damage at a significant frequency, yet reduced the mitotic index only by half, this dose was used in all subsequent experiments. The frequencies of aberrant cells and of aberrations shown in Fig. 1 were comparable to those obtained in previous studies with Ad5 (zur Hausen, 1967; Durnam *et al.*, 1986).

To determine the time course of induction of cytogenetic damage, serum-starved cells were released into the cycle by addition of 20% FCS and infected 6 h later with 100 p.f.u./cell of wt Ad5 for 11, 15, 18 and 21 h, respectively. Although damage was detectable at earlier times substantial amounts of damage were present only in cells exposed to the virus in excess of 15 h (Table 1). An interval of 19 h between infection and time of harvest

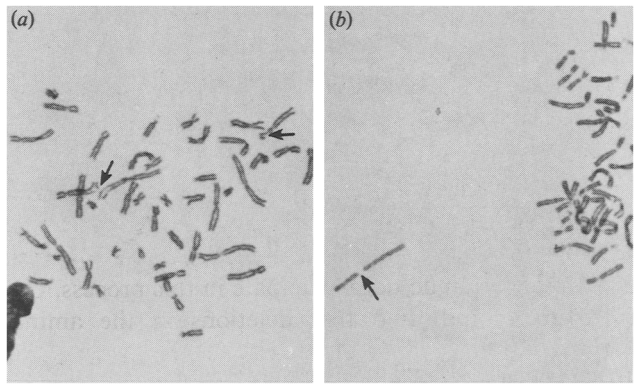

Fig. 2. Examples of cytogenetic damage induced by Ad5. Arrows indicate chromatid breaks (*a*) and the disappearance of the centromeric region (*b*).

was chosen for all subsequent experiments. Chromatid aberrations (gaps and breaks, Fig. 2*a*) were the predominant type of damage detected, but a characteristic disappearance of the centromeric region of chromosomes was also noticed at a low frequency (Fig. 2*b*). Chromosome aberrations were present only in very damaged cells (not shown). The occurrence of chromatid aberrations indicated that the damage was primarily induced in cells in the G2 phase of the cycle, in agreement with observations obtained from rodent cells (Bellett *et al.*, 1982). However, infection of G2 cells for 4 h did not induce aberrations at a significant frequency (data not shown). This suggests that a longer period of time (up to 15 h) was required for the accumulation of necessary products or the occurrence of processes of viral and/or cellular origin.

Ad5 is reported to damage the cellular genome at random sites and indeed we have observed that all of the human chromosomes appeared to be susceptible to damage by infection with this virus. However, we have also noted that virus-induced breaks overlapped almost perfectly with the known human fragile sites (Yunis, 1987) and thus their distribution may not be strictly random (D. Caporossi & S. Bacchetti, unpublished).

Effect of mutations in the E1 region

Previous studies using Ad5 infection of semi-permissive rodent cells have indicated that the occurrence of cytogenetic damage correlates with the expression of E1A gene products and does not require viral DNA replication (Murray *et al.*, 1982*b*). To map the viral functions involved in the induction of breaks in human cells we have therefore concentrated on the E1 region of the viral genome and have made use of several viral mutants defective in different functions of E1. Shown in

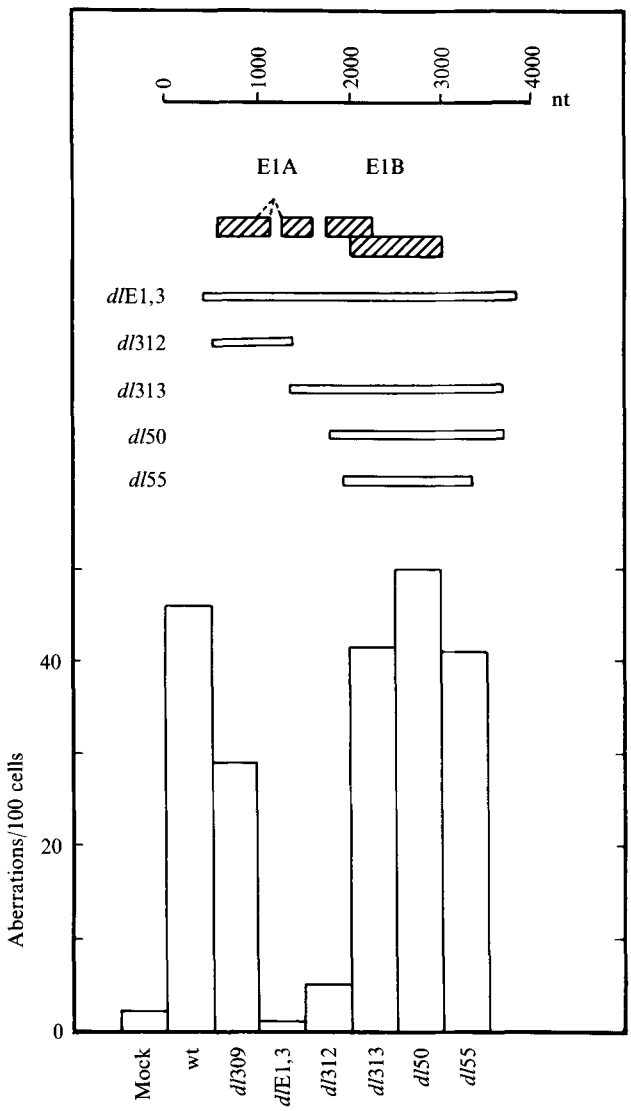

Fig. 3. Cytogenetic effects of mutants in the E1 region. The top diagrams indicate the map locations of the E1 deletions carried by the viruses and the histograms below represent the cytogenetic damage induced by each of them, expressed as frequency of aberrations per 100 cells. The experimental conditions were as for Fig. 1.

Fig. 3 are the map locations of a first set of mutants and the amount of damage (expressed as frequency of aberrations) induced by each of them in HEK cells. In mock-infected cells aberrations were only detected at a frequency of about 2/100 cells, whereas upon infection with wt Ad5 or *dl*309 the frequency increased to 30 to 50 per 100 cells. Mutant *dl*E1,3, which lacks the entire E1 region, was totally defective in this process, as was mutant *dl*312, which lacks the E1A gene. In contrast, mutants with deletion of the E1B region, such as *dl*50, *dl*55 and *dl*313 (which also lacks the last 69 aa of E1A), exhibited wt phenotypes. These results indicated that in human cells expression of E1A (precisely the first exon of

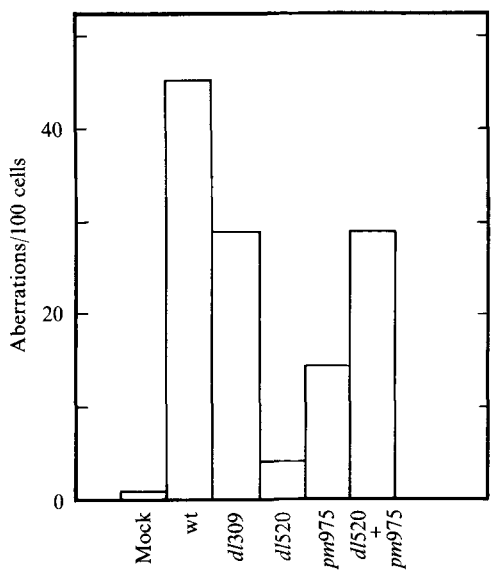

Fig. 4. Induction of aberrations by mutants specifying one or the other of the major E1A gene products. Partially synchronized HEK cells were infected with 100 p.f.u./cell of wt and mutant viruses, or coinfected with 100 p.f.u./cell of each of the *dl*520 and *pm*975 viruses.

the proteins and the 3′ portion of exon 2 up to aa 220) was necessary for the induction of aberrations and that E1B gene products did not contribute to this process.

Effect of mutations in the E1A region

To identify which E1A functions might be involved in the induction of cytogenetic damage we have assayed mutants *dl*520 (Haley *et al.*, 1984) and *pm*975 (Montell *et al.*, 1982), which specify the 243 aa and the 289 aa E1A proteins, respectively, and a set of 17 viruses with mutations spanning the entire E1A coding region (Harrison *et al.*, 1977; Jelsma *et al.*, 1988). As shown in Fig. 4 both *dl*520 and *pm*975 appeared impaired in their ability to induce breaks, compared to wt or *dl*309 viruses. However, of the two mutants *dl*520 was the most defective, inducing breaks at a frequency barely above the mock-infected level, whereas *pm*975 retained significant activity. Coinfection of HEK cells with both mutants restored the level of damage to within wt range.

The results of assays with viruses carrying mutations throughout the E1A gene are shown in Fig. 5, together with the map location of the mutations. By comparison with Ad5 wt and *dl*309 all of the mutants within exon 1 of E1A were considered proficient in the induction of damage, with the exception of *dl*1101, which consistently induced breaks at a frequency comparable to that seen in mock-infected cells, even when the m.o.i. was raised to 200 p.f.u./cell. Mutants containing deletions in the unique region of the 289 aa protein were all defective in their ability to damage chromosomes. These mutants and

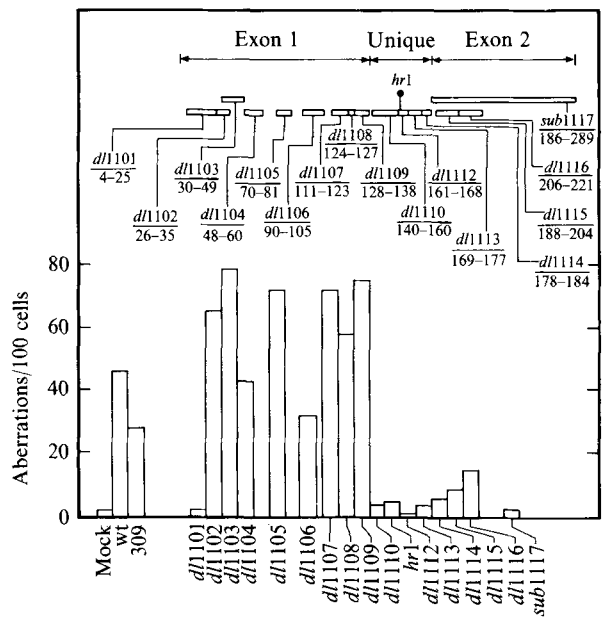

Fig. 5. Induction of aberrations by mutants within E1A. The location and extent of the deletions carried by each of the mutant viruses is indicated in the top diagram (modified from Jelsma *et al.*, 1989). Mutant *hr*1 contains a 1 bp deletion at nt 1055 (Ricciardi *et al.*, 1981). The frequency of aberrations induced by infection with the different viruses is given by the histograms below.

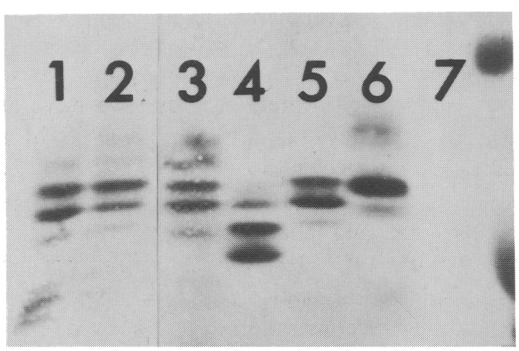

Fig. 6. Expression of E1A proteins in infected cells. HEK cells were infected with 100 p.f.u./cell of wt and representative E1A mutants, positive or negative for induction of aberrations, and harvested at 19 h. Following electrophoresis of cell lysates and electroblotting of proteins to nitrocellulose membrane the proteins were immunostained with monoclonal antibody M73 specific for E1A. (Lane 1) Ad5 wt, (lane 2) *dl*309, (lane 3) *dl*1101, (lane 4) *dl*1106, (lane 5) *dl*1112, (lane 6) *dl*1116 and (lane 7) mock-infected.

the *dl*1101 virus were positive for E1A expression in infected HEK cells (Fig. 6), indicating that their defective phenotype was related to the mutations within, rather than the absence of, these gene products. Lastly, of the mutants in exon 2 only *dl*1116 appeared to retain some activity. The deletion in this virus (aa 206 to 221) overlaps by one codon the 3′ boundary of the deletion of *dl*313, which has a wt phenotype for the induction of aberrations. Thus E1A sequences important for this process might extend to somewhere between aa 206 and 220 of exon 2.

Role of viral DNA replication in the induction of cytogenetic damage

The negative phenotype of mutants in the unique region of the 289 aa protein suggested that the requirement for E1A expression in the induction of damage might be related to the ability of this protein to activate expression of other viral genes. Although in rodent cells early viral gene products, other than E1A, and DNA replication are not required for induction of aberrations and of cell cycle alterations (Murray *et al.*, 1982*a*, *b*; Bellett *et al.*, 1985), such possibilities could not be excluded in permissive human cells, particularly in view of the fact that infection for longer than the time required for E1A expression was necessary for the detection of damage. The results shown in Fig. 4 established that E1B gene products did not participate in the clastogenic process. In addition, mutant *dl*1101, which is proficient for trans-activation, was, as expected, wt for viral DNA replication (data not shown), indicating that expression of the essential E2 gene products (polymerase, preterminal protein and DNA-binding protein) and viral DNA synthesis were not sufficient for induction of aberrations. Experiments with the *ts*125 mutant, which specifies a temperature-sensitive DNA-binding protein and is DNA replication-negative at non-permissive temperature (van der Vliet & Sussenbach, 1975), indicated that the E2A DNA-binding protein and viral DNA synthesis were also not required for the occurrence of damage. As shown in Fig. 7 a temperature of 38·5 °C was found to increase the amount of aberrations in both mock and infected cells. Nonetheless it is clear that both *dl*309 and *ts*125 retained the ability of inducing breaks at 38·5 °C, yet *ts*125 was temperature-sensitive for DNA production, as indicated by Southern blot analysis.

Discussion

The results presented in this paper show that induction of cytogenetic damage after Ad5 infection of permissive human embryonic kidney cells requires the expression of both the 243 and 289 aa E1A proteins and that sequences in the amino terminus, the unique region and the 3′ 25% of exon 2 are critical for this process. In contrast the remainder of exon 2, the E1B and E2A gene products are not required, nor is viral DNA replication necessary. In addition, the wt phenotype of mutant *dl*309, which

Virus	Aberrant cells (%)		Aberrations/100 cells	
	34 °C	38·5 °C	34 °C	38·5 °C
Mock	0	6	0	6
*dl*309	12	25	51	97
*ts*125	43	85	143	235

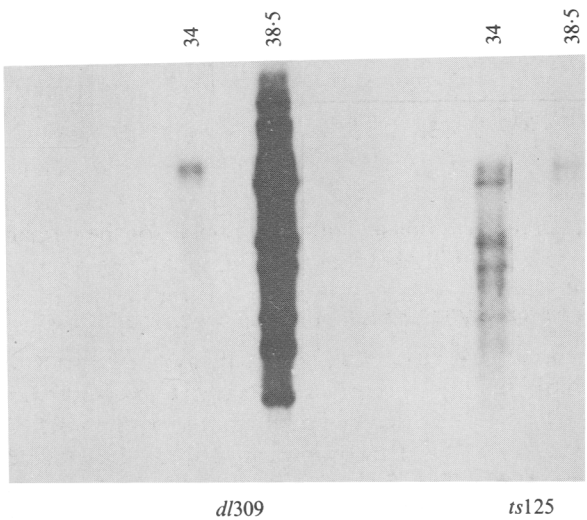

Fig. 7. Cytogenetic effects and viral DNA synthesis in *ts*125-infected cells. Partially synchronized HEK cells were infected with *dl*309 or *ts*125 and incubated at 34 °C and 38·5 °C for 19 h, at which time the cultures were harvested for chromosome preparations or for DNA analysis.

carries a deletion in the E3 region of the genome, suggests that E3 gene products are also probably dispensable for the cytogenetic effect. Thus it appears that E1A might be the only viral function required for the induction of aberrations, a conclusion tempered by the fact that we have not assayed mutants in E4. However, sufficiency of E1 gene products is indirectly supported by the observation that 293 cells, which constitutively express only this region, have numerous chromosomal abnormalities and are polyploid prior to crisis (Graham *et al.*, 1977; L. Wei & S. Bacchetti, unpublished).

Our data confirm and extend those reported for Ad5-infected rodent cells, where the ability to induce chromosomal damage has also been mapped to E1A functions. Using the same or comparable mutants to some of those we have assayed Braithwaite *et al.* (1983) have shown that expression of the first 220 aa of the 289 aa protein is required for damage induction and that two mutants in the unique region of this protein are defective in this process. We have been able to define in greater detail the critical E1A regions/functions by demonstrating that a deletion at the amino terminus of both proteins (between aa 4 and 25) as well as deletions anywhere between aa 140 and 205 to 220 in the 289 aa species prevent the induction of damage.

We have recently carried out analogous studies with a series of Ad12 E1 mutants and have obtained results that are surprisingly different from those presented here for Ad5-infected cells. The data from Ad12-infected HEK cells have indicated that expression of the E1A gene products is not sufficient for the induction of aberrations and that the E1B 55K protein is required for this effect (S. Schramayr *et al.*, unpublished). Although Ad12 damages human chromosomes primarily at specific sites (17q21–22, 1p36, 1q21 and 1q42–43) it is also capable, as is Ad5, of damaging random sites. We have observed that both specific and random effects require the expression of the major Ad12 E1B polypeptide. This suggests that different mechanisms might be involved in the induction of aberrations by the two viruses or, alternatively, that similar functions might be performed by different polypeptides in the two serotypes. Either hypothesis would be consistent with our current knowledge of the differences in the properties of the two viruses and the respective E1A and E1B gene products (Bernards & van der Eb, 1984; Branton *et al.*, 1985).

The Ad5 E1A proteins are multifunctional and distinct properties have been assigned to discrete domains of the polypeptides (Branton *et al.*, 1985; Berk, 1986). The set of E1A mutants we have assayed has been characterized in terms of ability to regulate Ad5 gene expression in trans, to cooperate with the *ras* oncogene in the transformation of rodent cells and to bind to any one of three cellular proteins (Egan *et al.*, 1988; Jelsma *et al.*, 1988, 1989). Mutants in the unique region and in exon 2 were not tested for the latter property, but were positive for enhancer repression and transformation with *ras*. However, trans-activation-negative mutants are altered in their ability to transform in collaboration with E1B, because of the requirement of E1A-mediated activation of E1B expression (Graham *et al.*, 1978; Glenn & Ricciardi, 1985). The data summarized in Table 2 indicate that the ability of E1A mutants to induce chromosomal damage correlates almost perfectly with the trans-activating ability of the 289 aa protein (and consequently with transformation by the complete E1 region), with the notable exception of mutant *dl*1101, which is trans-activation-positive, yet defective in the cytogenetic assay. A second trans-activation-proficient mutant, *dl*1116, was also consistently reduced in ability to induce aberrations compared with the *dl*309 parental virus, but in this case the reduction was within the limits of error of virus titres (see Methods). The phenotype of *dl*1101 implies that other E1A functions, in addition to trans-activation, are operational in the induction of damage, in agreement with the results obtained with *dl*520 and *pm*975, which indicated a requirement for both

Table 2. *Properties of the Ad5 EIA mutants**

	Mutant																
	*dl*1101	*dl*1102	*dl*1103	*dl*1104	*dl*1105	*dl*1106	*dl*1107	*dl*1108	*dl*1109	*dl*1110	*hr*1	*dl*1112	*dl*1113	*dl*1114	*dl*1115	*dl*1116	*sub*1117	*dl*313
Trans-activation	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+	−	+
Enhancer repression	−	+	−	−	+	+	+	+	+						+		+	
Transformation with *ras*	−	+	−	−	+	+	−	−	−			+	+	+	+	+	+	
Binding to cell proteins	±	+	±	±	±	+	±	±	±									
Cytogenetic damage	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+	−	+

* As characterized by Ricciardi *et al.* (1981) for *hr*1, Jones & Shenk (1979) for *dl*313, Jelsma *et al.* (1988, 1989) and Egan *et al.* (1988), except for their ability to induce cytogenetic damage (this study).

E1A proteins for complete activity. It should be noted that levels of E1A may be reduced in cells infected with trans-activation-defective mutants (Egan *et al.*, 1988; Tremblay *et al.*, 1989). Consequently the role of this activity in our assays may be to induce adequate levels of the 243 aa protein, in which case the latter could encode all the viral functions necessary for the induction of aberrations. Alternatively, since no other early viral genes are required, apart from possibly E4, activation of cellular genes may be of importance for the cytogenetic effect (Cheetham & Bellett, 1982; Liu *et al.*, 1985; Simon *et al.*, 1987). N-terminal mutants have not been tested for their ability to induce breaks in rodent cells, thus it is not known whether their phenotype is unique to the permissive system we have used. However this seems unlikely, since other mutants have comparable phenotypes in both mouse and human cells.

E1A functions are responsible for several alterations of the growth cycle that might be relevant to the transformation process and which require the integrity of the E1A transactivating domain (Berk, 1986; Smith & Ziff, 1988; Bellett *et al.*, 1989 and references within). However, induction of growth in G1 arrested populations, alteration in the progression of cells through the cycle and disruption of actin fibers are also prevented by mutations in conserved regions 1 and 2 (aa 40 to 80 and aa 120 to 140, respectively) of exon 1 (Bellett *et al.*, 1989). We have detected no requirement for these regions in the induction of aberrations in human cells. The lack of correlation between the ability of E1A mutants to induce cytogenetic damage and defects in other known functions could imply that damage is caused by as yet unidentified functions of E1A and occurs through mechanisms that only partially overlap with those involved in alteration of growth and morphology and, possibly, in transformation.

In rodent cells Ad5-induced cytogenetic damage is mainly chromatidic, i.e. is expressed in the G2 phase of the cycle. Chromatid gaps and breaks are also the prevalent aberrations in HEK cells, yet the cells must be infected in G1/S phase, not in G2 phase, for the damage to occur. This suggests that the Ad5 E1A gene products interfere with processes occurring early in the cycle and leading to chromosomal damage after cellular DNA replication. It has been reported that synthesis of polyamines, which occurs in G1/S phase in conjunction with DNA synthesis, is decreased by Ad5 infection, whereas cell DNA replication is enhanced (Cheetham & Bellett, 1982). Together with the fact that addition of polyamines to G2 cells protects chromosomes from breakage, these observations have suggested that polyamines, by virtue of their ability to bind to DNA, might contribute to the stability of the chromosomal structure and that their reduced level relative to levels of DNA synthesis might result in chromosomal fragility (Bellett *et al.*, 1982). Our results with human cells, including the observation that Ad5-induced breaks coincide with fragile sites, are consistent with this hypothesis.

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Note added in proof. In recent experiments we have detected some chromosomal damage in cells infected with 200 p.f.u./cell of *dl*1101, indicating that this mutant is not totally defective.

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