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DNA-binding properties of an adenovirus 289R E1A protein

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An adenovirus 2 289 amino acid (289R) E1A protein purified from *Escherichia coli* has been shown to interact with DNA by two independent methods. UV-crosslinking of complexes containing unmodified, uniformly ^{32}P-labelled DNA and purified E1A protein induced efficient labelling of the protein with covalently attached oligonucleotides, indicating that the E1A protein itself contacts DNA. Discrete nucleoprotein species were also observed when E1A protein-DNA complexes were analysed by gel electrophoresis. Although the 289R E1A protein exhibited no significant binding to single-stranded DNA or to RNA, no evidence for its sequence-specific binding to double-stranded DNA was obtained with either assay. Identification of the sites of covalent attachment of ^{32}P-labelled oligonucleotides by partial proteolysis of the crosslinked E1A protein indicated that the interaction of this protein with DNA is mediated via domain(s) in the C-terminal half of the protein. Such previously unrecognized DNA-binding activity is likely to contribute to the regulatory activities of this important adenoviral protein.

Key words: apparent association constants/DNA-binding domain

Introduction

The adenovirus E1A proteins perform several functions in adenovirus infected and transformed cells. The 289 amino acid (289R) protein is sufficient for transcriptional activation of other viral genes during productive infection (see Kingston *et al.*, 1985; Flint, 1986 for reviews) and also possesses a domain, in common with the 243R E1A protein, necessary both for repression of enhancer-driven transcription (Borrelli *et al.*, 1984; Velich and Ziff, 1985) and for transformation activities of E1A gene products (Lillie *et al.*, 1986, 1987; Moran *et al.*, 1986; Zerler *et al.*, 1986; Schneider *et al.*, 1987). Although the functions of the E1A proteins are well documented (see Kingston *et al.*, 1985; Flint, 1986; Moran and Mathews, 1987 for reviews), the molecular mechanisms by which these important viral proteins operate are poorly understood.

The lack of a consensus sequence in the promoters of genes transactivated by the E1A 289R protein has suggested that this protein interacts with DNA only indirectly, a conclusion consistent with the binding of E1A proteins to cellular proteins demonstrated by immunoprecipitation (Yee *et al.*, 1985; Harlow *et al.*, 1986) and in cell fractionation studies (Feldman and Nevins, 1983; Chatterjee and Flint, 1986). Recently, a 289R E1A protein synthesized in *Escherichia coli* has been shown to be retained on DNA-cellulose columns (Ko *et al.*, 1986) and to activate transcription from adenoviral DNA templates *in vitro* (Spangler *et al.*, 1987). Here we describe experiments to test the ability of such bacterially synthesized adenovirus 2 (Ad2) 289R E1A protein to interact directly with DNA, by UV-crosslinking of the protein bound to DNA *in vitro*, and demonstrate that a DNA-binding domain resides in the C-terminal half of this protein.

Results

UV-induced crosslinking of the 289R E1A protein to DNA

Only those proteins that bind to DNA in solution with a geometry favourable to the formation of photoadducts will become covalently attached to DNA as a result of primary photochemical reactions upon exposure of protein-DNA complexes to UV light (see Park *et al.*, 1980; Hockensmith *et al.*, 1986 for discussion). The labelling of a protein as a linear function of UV dose, by the covalent transfer of labelled nucleotides or oligonucleotides from DNA, therefore provides unequivocal evidence that the protein interacts directly with DNA. We therefore initially assessed the ability of the 289R E1A protein synthesized in *E.coli* to bind to DNA using such a label transfer assay.

The E1A protein used in these experiments was purified to apparent homogeneity (see Figure 1, Spangler *et al.*, 1987) and transactivated transcription from the Ad2 E2 early promoter *in vitro* (Spangler *et al.*, 1987). Typical results obtained when the E1A protein was mixed with an unmodified, uniformly ^{32}P-labelled Ad2 DNA fragment containing the transcriptional control regions of the E2 and E3 transcription units and exposed to UV light are shown in Figure 1. A prominent ^{32}P-labelled product migrating with similar mobility to the E1A protein was observed only after exposure of E1A protein-DNA mixtures to UV (Figure 1, lane 1). Additional ^{32}P-labelled products of crosslinking, exhibiting apparent mol. wts of ~26 kd, as well as a product of <12 kd, were observed when the background of radioactive DNA fragments was reduced by alkali treatment of the gel (Figure 1, lane 2). These appear to represent DNA-crosslinked degradation products of the E1A protein, generated before or during the experiment; similar fragments cross-reacting with anti-E1A antibodies have been observed following storage of the protein at −80°C (M.Bruner and M.L.Harter, unpublished observation). Denaturation of the E1A protein, by heating to 100°C in either the presence or the absence of sodium dodecyl sulphate inhibited such

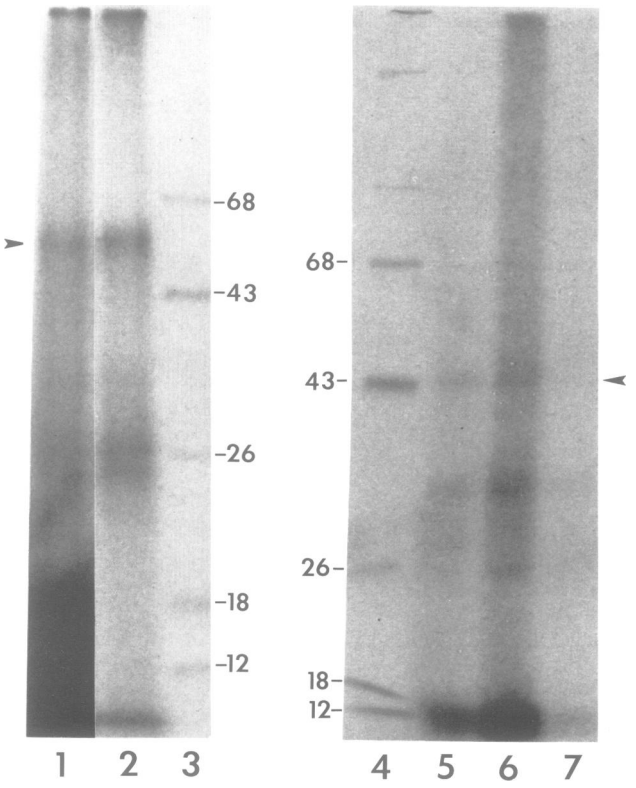

Fig. 1. UV crosslinking of the E1A 289R protein to DNA. The E1A protein was crosslinked to a ^{32}P-labelled Ad2 DNA fragment containing the E2 and E3 transcriptional control regions as described in Materials and methods for 3 min (**lanes 5** and **7**), 6 min (**lanes 1** and **2**) or 10 min (**lane 6**). The sample in lane 7 also contained 10 μg (100-fold weight excess) poly(dI.dC)·(dI.dC). Following DNase I digestion, samples were analysed by electrophoresis in 13% (lanes 1–3) or 10% (lanes 4–7) SDS–polyacrylamide gels. Lane 2 shows the autoradiogram obtained after treating the gel shown in lane 1 with 1 M NaOH at 50°C for 1 h. The arrowhead marks the position of the full-length 289R E1A protein crosslinked to ^{32}P-labelled oligonucleotides. **Lanes 3** and **4** show mol. wt standards.

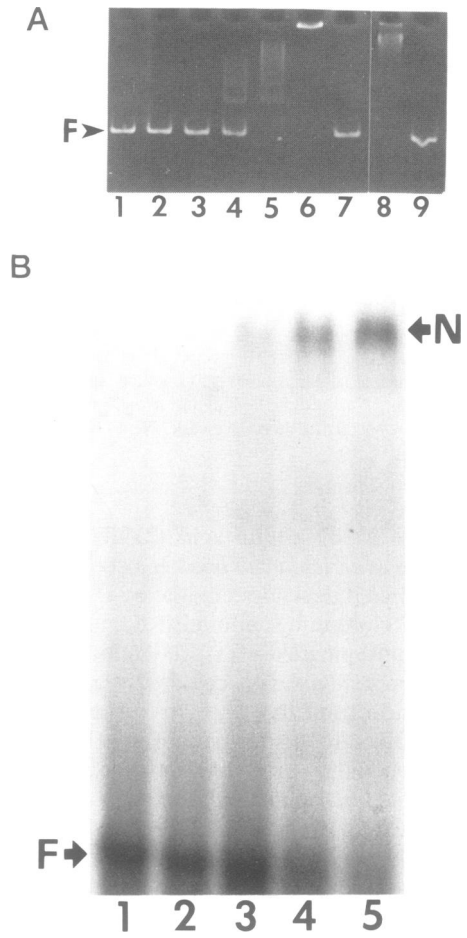

Fig. 2. Direct visualization of E1A protein–DNA complexes. (**A**) Unlabelled DNA (50 ng) of an Ad2 DNA fragment containing the E2 and E3 transcriptional control regions was incubated, under the conditions described in Materials and methods, in the absence of protein (**lane 1**), with 0.05, 0.10, 0.25, 0.50, 1.0 or 2.0 μg purified E1A protein (**lanes 2–7** respectively), with 1.0 μg E1A protein and 10 μg BSA (**lane 8**) or with 10 μg BSA (**lane 9**). The products of the reaction shown in lane 7 were extracted with phenol. Electrophoresis was as described in Materials and methods. (**B**) 50 ng ^{32}P-labelled oligonucleotide 2 (Table I) was incubated under the conditions described in Materials and methods with 0, 0.25, 0.5, 1.0 or 2.0 μg E1A protein (**lanes 1–5** respectively), prior to electrophoresis.

photolabelling of the protein (data not shown). The photoadduct(s) formed in these reactions were resistant to alkali (Figure 1, lanes 1 and 2), but did not survive conditions used to degrade DNA chemically (see Chatterjee *et al.*, 1986a,b and c), exposure to 2% diphenylamine in formic acid at 37°C (data not shown). The UV-induced ^{32}P-labelling of the E1A protein was a linear function of UV dose (Figure 1, lanes 5 and 6), indicating that the crosslinking observed was not a secondary consequence of UV irradiation. Addition of a 7-fold molar excess of BSA to reactions containing the E1A protein and labelled DNA produced neither a change in the efficiency of photolabelling of the E1A 289R protein nor crosslinking of BSA (data not shown).

The sequence specificity of binding of the bacterially synthesized E1A protein to DNA was initially examined by crosslinking of the protein to the labelled, viral DNA fragment in the presence of unlabelled, competitor DNA. Crosslinking of the E1A protein to viral DNA was eliminated when irradiations were carried out in the presence of 100-fold weight excess of unlabelled poly(dI.dC)·(dI.dC) (Figure 1, lane 7). Moreover, results very similar to those shown in Figure 1, lanes 1 and 2 were obtained when the E1A 289R protein was irradiated in the presence of uniformly ^{32}P-labelled pBR322 DNA (data not shown). The results of these crosslinking experiments therefore suggest that there are no major differences in the affinities of the E1A 289R protein for different DNA sequences.

Direct visualization of E1A protein–DNA complexes

Binding of a protein to DNA is a prerequisite for primary UV-crosslinking reactions, because of the short lifetimes of the excited bases (e.g. Harrison *et al.*, 1982; Hockensmith *et al.*, 1986). However, because the interactions mediating binding are not necessarily those that lead to efficient crosslinking of proteins to DNA, efficiencies of crosslinking cannot generally be correlated with binding affinities. As UV-crosslinking does not provide an assay well suited to quantitative interpretation of binding affinities, we next determined whether E1A protein–DNA complexes could be visualized directly in the electrophoretic mobility shift assay (Fried and Crothers, 1981; Garner and Revzin, 1981).

Table I. Binding of the E1A 289R protein to DNA oligonucleotides

Oligo-nucleotide	Sequence	Nucleo-tides	%G+C	%Pu	Apparent association constant (M^{-1})
1	mL −40 to −12	45	64.4	71.1	2.0×10^5
2	E2A −83 to −59	36	55.6	50.0	1.5×10^5
3	E2A −41 to −20	34	32.4	44.1	2.0×10^5
4	E3 +10 to +29	32	53.1	43.8	6.3×10^5

Binding of the E1A protein to each of the four oligonucleotides, corresponding to the sequences of the adenoviral genome listed in column 2 and whose length, base composition and sense-strand purine content are given in columns 3−5 respectively, was assayed as described in the legend to Figure 2B. The quantitites of DNA remaining free or bound to the E1A protein at each protein concentration tested were determined by Cherenkov counting of appropriate excised gel slices or by densitometry using a Zeineth soft laser scanning densitometer (Biomed Instruments). These values were then used to calculate apparent association constants (K_a) of E1A binding, assuming that all molecules of E1A protein (which was from the same preparation in all cases) were active in DNA binding. In the absence of definitive information about the form of the E1A protein active in DNA binding, it was assumed that the protein binds as a monomer. The values listed are these for E1A protein−DNA binding at high E1A protein concentrations (see text).

Increasing quantities of the purified E1A protein were incubated with 50 ng DNA of the same adenoviral fragment used in the cross-linking experiments and the products resolved by electrophoresis. As illustrated in Figure 2A, a series of more slowly migrating DNA−protein complexes was formed in the presence of the E1A protein (Figure 2A, lanes 1−6), but not in the presence of BSA (Figure 2A, lane 9). Such complexes were destroyed by phenol extraction prior to electrophoresis (Figure 2A, lane 7). As in crosslinking experiments, BSA neither bound to DNA nor interfered with the binding of the E1A protein (Figure 2A, lanes 8 and 9 respectively). Complexes of decreasing mobility were formed (Figure 2A, lanes 1−6) as the concentration of E1A protein in the reaction was increased, indicating that the 606-nucleotide DNA fragment contained multiple E1A protein-binding sites.

In order to analyse E1A protein−DNA binding more quantitatively and to attempt to distinguish between non-specific and specific DNA binding, similar experiments were performed with the series of ^{32}P-labelled oligonucleotides, corresponding to various transcriptional control elements or coding sequences of the Ad2 genome, listed in Table I. Typical results obtained when binding to these oligonucleotides was examined as a function of E1A protein concentration are shown in Figure 2B for oligonucleotide 2. One major E1A protein−DNA complex was formed (Figure 2B), presumably because the 36-nucleotide-long oligonucleotide 2 could accommodate only one binding site for the E1A protein. The concentration of this complex increased with E1A protein concentration. Interestingly, however, in this and all similar experiments with the oligonucleotides listed in Table I, more slowly migrating DNA−protein complexes were formed only above a certain threshold E1A protein concentration: no E1A protein−oligonucleotide 2 complex, for example, could be detected in the presence of 0.25 μg E1A protein when much longer exposures of the autoradiogram shown in Figure 2B were examined (data not shown, but see Figure 3B). Moreover, each binding reaction was biphasic: as illustrated for oligonucleotides 1 and 2 (see Table I) in Figure 3A, the concentration of DNA−protein complex formed increased dramatically when the quantity of E1A protein added to binding reactions exceeded 0.8−1.0 μg. If the oligonucleotides used in these experiments contained only a single E1A protein binding site, as the detection of a single complex (Figure 2B) suggests, the most reasonable interpretation of such binding behaviour is that an oligomeric form of the E1A protein, with greater affinity for DNA, formed as the concentration of the E1A protein in solution was increased.

The ability of the E1A protein to bind to single-stranded DNA was examined in the same fashion, with results like those shown in Figure 3B: when up to 75% of double-stranded oligonucleotide 2 (Table I) was bound by the E1A protein, no significant binding to either of the corresponding single-stranded oligonucleotides was observed (Figure 3B). Similar results were obtained with other single-stranded oligonucleotides (data not shown). Moreover, 2−2.5% of 5 ng ^{32}P-labelled pGEM RNA transcripts added to binding reactions, under conditions identical to those used for double- or single-stranded DNA oligonucleotides, was bound by quantities of the E1A protein that bound 40 ng of double-stranded DNA. Thus, the E1A protein displayed a strong preference for binding to double-stranded DNA in these experiments.

The apparent association constants of binding of the E1A protein to each of the four oligonucleotides listed in Table I was estimated from the results of titration experiments like those shown in Figures 2B and 3A. The values obtained (Table I) represent minimum estimates, for we assumed that the E1A protein−DNA complexes formed in solution were stable during electrophoresis and that all E1A protein molecules added to binding reactions were capable of binding to DNA. The latter assumption cannot be correct, because different preparations of the E1A protein do show different degrees of activity. Nevertheless, even a 10-fold underestimation of the values listed in Table I would not alter the conclusion that differences of no more than 4-fold in the apparent association constants of the protein for the oligonucleotides were measured, despite the wide variation in base composition, distribution of purine and pyrimidine residues between the two strands, length and, of course, in primary sequence among the oligonucleotides tested. Furthermore, unlabelled heterologous and homologous oligonucleotides were equally effective competitors for binding of the E1A protein to a labelled oligonucleotide (Figure 3C), as predicted from such small differences in the affinity displayed by the E1A protein for the small DNA fragments tested. These results strongly reinforce the conclusion, suggested by crosslinking studies, that the E1A protein can bind to DNA non-specifically.

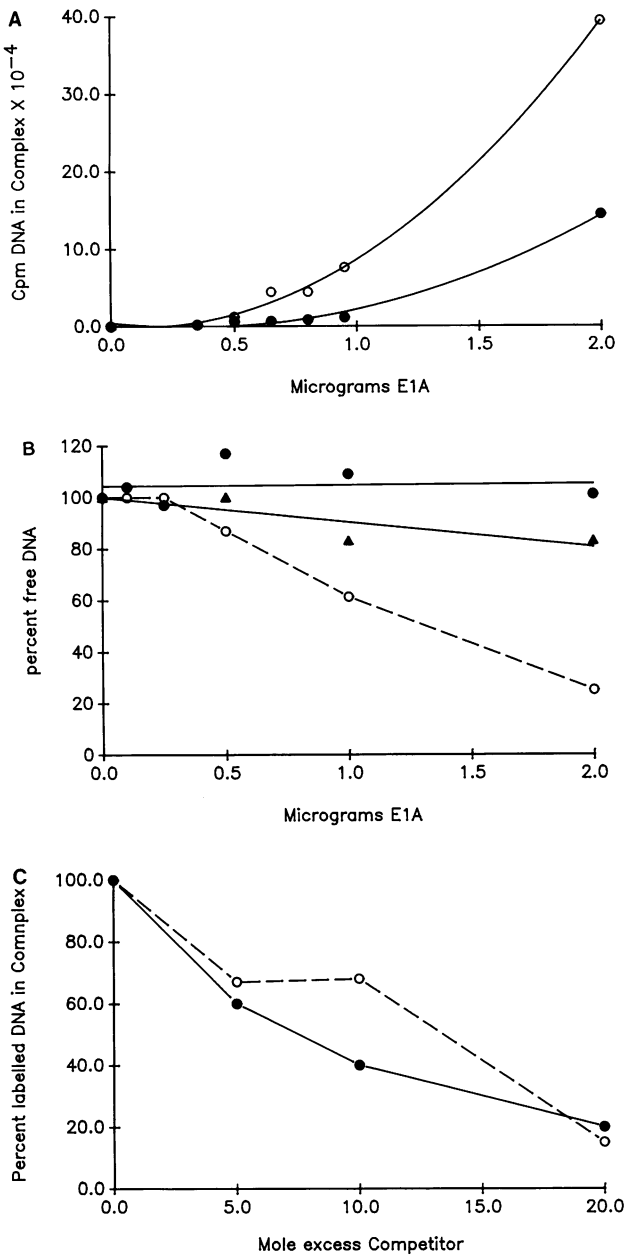

Fig. 3. Binding of the 289R E1A protein to double-stranded DNA. (**A**) Binding of the E1A protein to oligonucleotides 1 (○——○) or 2 (●——●) (see Table I) was examined as described in the legend to Figure 2B. The quantities of ^{32}P-labelled DNA entering more slowly migrating complexes with the E1A protein was determined by direct Cherenkov counting of gel slices. (**B**) Binding reactions were as described in Materials and methods and contained double-stranded oligonucleotide 2 (○-----○) and the two single-strand oligonucleotides (▲——▲ and ●——●). After binding, products were separated as described in Materials and methods and the quantities of DNA remaining free determined as described in the legend to Table I. (**C**) Binding reactions contained 50 ng ^{32}P-labelled oligonucleotide 2 (Table I), 1 μg E1A protein and the mole excess competitor oligonucleotide 2 (○-----○) or oligonucleotide 4 (●——●) indicated. Following electrophoresis and autoradiography, the quantities of DNA bound in complex were determined by densitometry and are expressed as percentage of the DNA bound in the absence of competitor.

Identification of E1A 289R protein domains that contact DNA

The label transfer method for identification of proteins that interact with DNA directly, described in a previous section, offers the opportunity to locate protein domains that contact DNA by mapping the site, or sites, within a protein at which ^{32}P-labelled nucleotides, or oligonucleotides, become covalently attached when a DNA–protein complex is exposed to UV light (Chatterjee *et al.*, 1986a,b). To investigate whether a discrete DNA-binding domain could be identified in the 289R protein, products of its crosslinking to DNA were excised from gels like those shown in Figure 1 and subjected to partial proteolysis (Cleveland *et al.*, 1977). Upon digestion with V8 protease, the full-length E1A protein crosslinked to DNA (Figure 4A, lane 1) generated ^{32}P-labelled polypeptides of ~26 kd and ~18 kd apparent mol. wt (Figure 4A, lanes 2–4). Digestion at a higher ratio of protease to crosslinked E1A protein produced only the 18-kd apparent mol. wt product (data not shown). No additional ^{32}P-labelled polypeptides of lower apparent mol. wt could be detected, even in autoradiograms made prior to alkali treatment of the gels (Figure 4A, lane 5), despite the use of gel systems designed to improve the resolution of small proteins (Thomas and Kornberg, 1978) in the experiment whose results are shown in Figure 4A, and others like it. The 26-kd E1A protein fragments recovered after crosslinking (Figure 1, lanes 1 and 2) were also cleaved to yield a ^{32}P-labelled product of ~18 kd apparent mol. wt (Figure 4A, lanes 6–8). These results therefore indicate that a single, specific region of the E1A protein, released as a protease V8 cleavage product of ~18-kd apparent mol. wt, contacts DNA.

The E1A protein is notorious for its aberrant electrophoretic mobility (see Graham, 1984), a property that is influenced by both the modification state of the protein (Harlow *et al.*, 1985) and the nature of the gel system employed (compare lanes 1–3 with 4–7, Figure 1). Thus, identification of the origin of the segment, apparent mol. wt 18 kd, of the E1A protein crosslinked to oligonucleotides cannot rely on co-migration of this product with known fragments of the protein. To circumvent this problem, and thus identify the protease V8-resistant region of the E1A protein that was crosslinked to DNA, we took advantage of the unequal distribution of both methionine and cysteine residues within the 289R E1A protein. As illustrated in Figure 4B, the C-terminal segment of the protein, encoded by the second exon common to the 289R and 243R E1A proteins (amino acid residues 186–289), contains five cysteine but no methionine residues, whereas N-terminal fragments of the protein would be enriched in methionine residues. The 289R E1A protein was therefore labelled with either [^{35}S]methionine or [^{35}S]-cysteine in an *E.coli in vitro* transcription translation system and subjected to partial proteolysis under conditions identical to those used for protease V8 digestion of the crosslinked E1A protein. The 289R E1A protein contains numerous potential protease V8 cleavage sites distributed throughout its sequence (Figure 4B). Nevertheless, the results of such partial protease V8 digestion suggest that the N-terminal half of this protein was preferentially digested under the conditions employed. Thus, the low apparent mol. wt ($\leq$6000 daltons) products of protease V8 digestion of [^{35}S]-methionine-labelled E1A protein contained a greater proportion of the label than products of apparent mol. wt $\geq$18 000 daltons (compare labelling of the products designated x and y with labelling of products designated u and v in lane 2, Figure 4C). The converse result was obtained with the E1A protein labelled with [^{35}S]cysteine: the low apparent mol. wt products of digestion, x and y, contained a small pro-

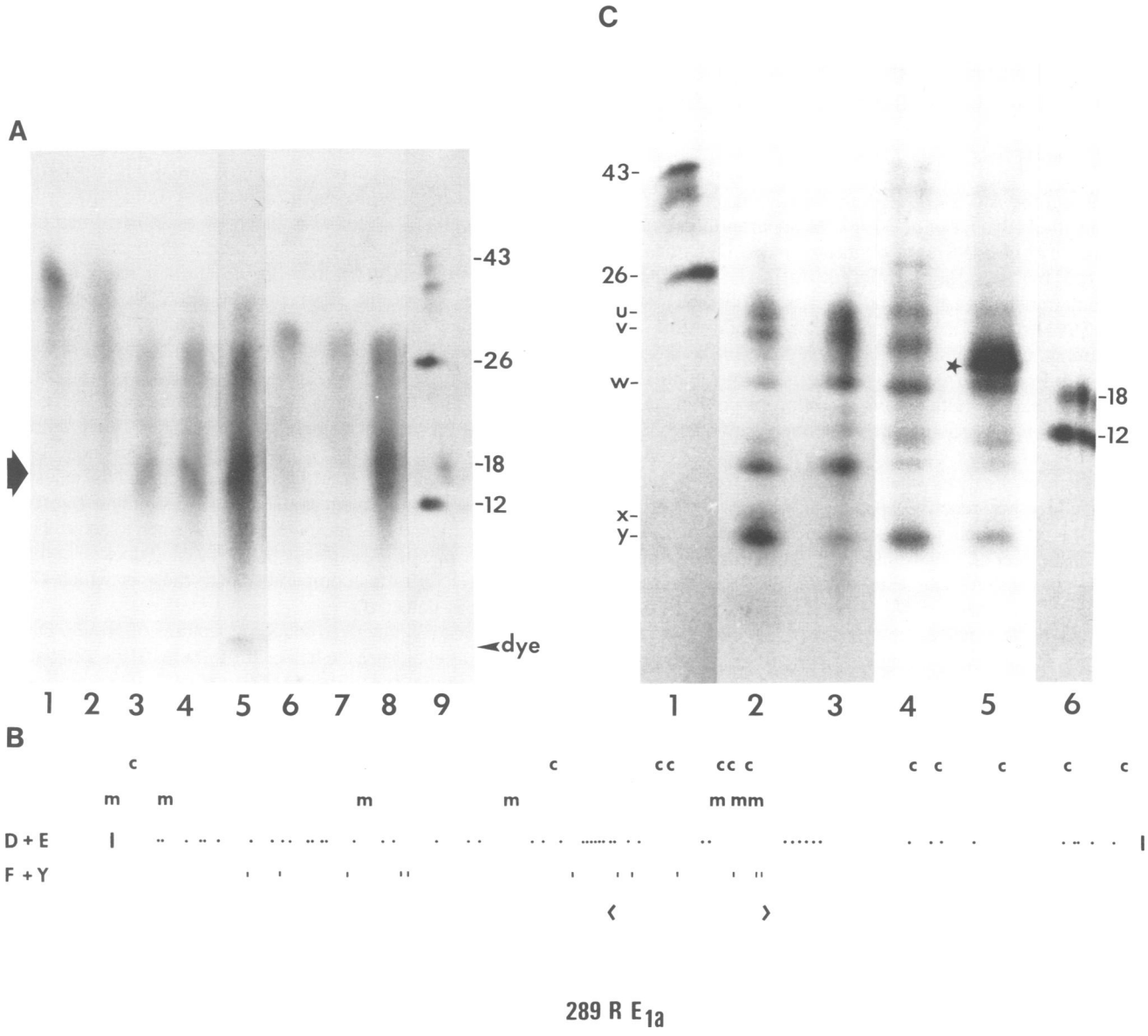

Fig. 4. Partial proteolysis of E1A proteins. (**A**) Bands corresponding to full-length (**lanes 1–5**) or fragments (**lanes 6–8**) of the 289R E1A protein crosslinked to ^{32}P-labelled oligonucleotides were excised from gels like that shown in Figure 1, and subjected to partial proteolysis with 0 (**lanes 1** and **6**), 1 (**lanes 2** and **7**), 5 (**lanes 3** and **8**) or 10 (**lane 4**) μg V8 protease. Lane 5 shows an autoradiogram of lane 3 made before alkali treatment, shown here to indicate the position of the dye-front. The arrowhead marks the protease V8-resistant fragment of the E1A protein of ~18 kd apparent mol. wt discussed in the text. (**B**) Schematic respresentation of the 289R E1A protein. The dots represent the positions of aspartic (D) or glutamic (E) acid residues. The vertical bars show the positions of phenylalanine (F) or tyrosine (Y) residues in the molecule. Cysteine and methionine residues are shown as (C) and (M) respectively. The bracket represents the 46 amino acids between positions 140 and 185 that are unique to the 289R E1A protein. (**C**) The 289R E1A protein was immunoprecipitated after labelling with either [^{35}S]methionine (**lanes 2** and **4**) or [^{35}S]cysteine (**lanes 3** and **5**) in an *in vitro* transcription–translation system (Ko *et al.*, 1986). The labelled proteins excised from polyacrylamide gels were subjected to partial proteolysis with 1 μg of V8 protease (**lanes 2** and **3**) or chymotrypsin (**lanes 4** and **5**). **Lanes 1** and **6** show mol. wt markers.

portion of the label, whereas the higher mol. wt products u, v and w were enriched (Figure 4C, lane 3). Thus, these latter products must derive from the C-terminal half of the E1A protein, for this segment contains most of the cysteine residues (Figure 4B). Similarly, the low apparent mol. wt products that were observed to be relatively enriched in methionine, but not cysteine (Figure 4C, lanes 2 and 3) must be generated from the N-terminal half of the molecule (Figure 4B). These results of partial protease V8 digestion therefore suggest that potential sites of cleavage within the C-terminal portion of the E1A protein (Figure 4B) are less accessible than those lying within the N-terminal segment.

This conclusion was confirmed by the results obtained when [^{35}S]methionine or [^{35}S]cysteine-labelled E1A protein was digested under identical conditions with chymotrypsin (Figure 4C, lanes 4 and 5): [^{35}S]cysteine-labelled E1A 289R protein yielded a unique fragment of ~18 000 daltons apparent mol. wt that was not present among the products of identical proteolysis of the [^{35}S]methionine-labelled protein (compare lanes 4 and 5, Figure 4C). This product can clearly be assigned to the C-terminal 35% of the protein, whose boundary is the potential chymotrypsin cleavage site located at amino acid 184, as this is the only region that contains cysteine, but no methionine residues (Figure 4B). These

results of partial proteolysis therefore lead us to suggest that the 289R E1A protein crosslinks to DNA via residues located in its C-terminal half, as this is the only region of the protein represented in larger products of protease V8 digestion.

Discussion

Binding of the Ad2 289R E1A protein synthesized in *E.coli* and purified (Bruner *et al.*, 1988) to apparent homogeneity (Spangler *et al.*, 1987), to double-stranded DNA has been detected by two quite different methods, UV-induced covalent transfer of labelled nucleotides, or oligonucleotides, from DNA to the protein (Figure 1) and mobility shift assays (Figure 2). The crosslinking observed cannot be the result of random collision of E1A protein molecules with DNA, for, as judged by both assays, addition of proteins that do not bind to DNA, such as BSA, neither leads to binding of BSA nor interferes with the binding of the E1A protein. Furthermore, analysis of the lifetimes of excited states of bases and other reactive species participating in primary photochemical reactions (for example, Harrison *et al.*, 1982; Hockensmith *et al.*, 1986) has established that only proteins that contact DNA with a favourable geometry can form covalent adducts when DNA–protein complexes are exposed to UV light. Finally, the transfer of labelled nucleotides, or oligonucleotides, to a single, specific fragment of the E1A protein (Figure 4) indicates that the protein must make intimate, and specific, contacts with DNA (see Paradiso *et al.*, 1979; Merrill *et al.*, 1984). It is also clear that the binding of the E1A protein detected by crosslinking cannot be a secondary consequence of UV-induced modification of the DNA and/or protein: the UV-induced labelling of this protein is a linear function of UV dose (Figure 1) and binding can be detected as the formation of slowly migrating DNA–protein complexes in reactions that were not exposed to UV light (Figure 2).

The 289R E1A protein examined here clearly exhibits a strong preference for double-stranded DNA compared to single-stranded DNA (Figure 3B) or RNA. The equally efficient crosslinking of E1A protein to adenoviral and pBR322 DNA fragments, the effective inhibition of crosslinking of the protein to labelled viral DNA by unlabelled poly(dI.dC)·(dI.dC) (Figure 1), the similar affinity of the E1A protein for different DNA sequences (Table I) and the results of competition experiments (Figure 3C) indicate that the E1A protein examined here can bind to DNA non-specifically. Our experiments do not, however, eliminate the possibility that the E1A protein can also recognize, and bind with high affinity to, specific DNA sequences or structural features that were not represented among the limited set of DNA molecules tested, or that its binding specificity might be altered by interaction with other, mammalian proteins. Binding of an Ad2–Ad5 hybrid 289R E1A protein to DNA could not be demonstrated in a previous study using a 'South–Western' assay (Ferguson *et al.*, 1985). This result is probably not surprising in view of the low apparent association constants exhibited by the E1A protein studied here (Table I): weakly bound DNA could readily re-equilibrate and dissociate from the E1A protein during the repeated washing of the filter necessary in this type of assay. In this context, it should be noted that the relatively discrete E1A–DNA nucleoprotein complexes, like those shown in Figure 2, were observed only when products of DNA-binding reactions were electrophoresed for no more than 2–3 h.

The significance of the results presented here to the molecular mechanisms by which the adenoviral E1A proteins perform their numerous functions is not yet clear. However, we believe that the DNA-binding activity of the 289R E1A protein described here is directly relevant to the functions of this protein in adenovirus infected or transformed cells. It is, for example, well established that other oncogene products synthesized in *E.coli* retain at least a subset of the activiites they display in their natural milieu; the Rous Sarcoma virus protein pp60$^{v\text{-}src}$ produced in bacteria will phosphorylate tyrosine residues of an exogenous substrate (Gilmer and Erikson, 1981); a fragment of the v-*abl* protein expressed in *E.coli* functions as an active tyrosine kinase within the bacteria (Wang and Baltimore, 1985); and bacterially synthesized reverse transcriptases of a number of retroviruses retain enzymatic activity (Tanese *et al.*, 1985; Farmiere *et al.*, 1987). Moreover, the bacterially synthesized form of the E1A protein studied here can stimulate transcription from adenoviral promoters *in vitro* when added to HeLa cell components (Spangler *et al.*, 1987) and does so at molar protein:DNA ratios comparable with those at which DNA binding is detected.

The best characterized sequence features of protein DNA-binding domains are the helix–turn–helix (Pabo and Sauer, 1984) and the Zn^{2+}-finger (Miller *et al.*, 1985) motifs. Of these, the 289R E1A protein contains one Zn^{2+}-finger motif, occupying residues 154–174 (Berg, 1986), within the 46 amino acid segment uniquely expressed in this, but not the 243R, E1A protein. It is clearly established that sequences of the first exon, common to these two E1A proteins, are not required for transactivation activity (see Lillie *et al.*, 1987; Moran and Mathews, 1987; Schneider *et al.*, 1987). Moreover, recent evidence suggests that the unique domain of the larger E1A protein can function independently to transactivate expression of viral early genes (Lillie *et al.*, 1987). The DNA-binding activity of the 289R E1A protein studied here appears to be mediated by sequences that lie in the C-terminal half of the molecule (Figure 4), which includes the transactivation and potential Zn^{2+}-finger domains (Figure 4B). It is therefore possible that the latter sequence comprises a DNA-binding domain of the 289R E1A protein and, moreover, that such binding is important to the transactivation function fulfilled by the unique segment of the 289R E1A protein. Experiments to map the DNA-binding domain(s) of the 289R E1A protein more precisely and examine its functional significance are in progress.

Materials and methods

Purification of the E1A protein

The 289R E1A protein was expressed in *E.coli* and purified to apparent homogeneity (see Figure 1, Spangler *et al.*, 1987) as described previously (Ko *et al.*, 1986; Bruner *et al.*, 1988). Both E1A preparations used in the experiments described here have been shown to stimulate transcription from the Ad2 E2 early promoter when added to whole cell extracts prepared from uninfected HeLa cells (Spangler *et al.*, 1987).

DNA-binding assays

The pHindIII H plasmid, a derivative of pBR322 containing the *Hin*dIII H fragment of Ad2 DNA inserted in the plasmid *Hin*dIII site, was digested with *Pvu*II and *Sst*I to generate a 606-nucleotide DNA fragment, nucleotides 27 028–27 634 of the Ad2 genome, containing the segment of DNA between the viral E2 and E3 cap sites. The DNA fragment was purified on

a preparative, 6% polyacrylamide gel, followed by electroelution and Eluptid-d (Schleicher and Schuell) column purification. The DNA (1 μg in a 23-μl volume) was then labelled with [α-^{32}P]dCTP (1 μCi/μl, Amersham, 400 Ci/mmol) using T4 DNA polymerase (0.06 U/μl) at 37°C for 15 min (O'Farrell *et al.*, 1980). After labelling, the DNA was purified by chromatography on a NACS Prepack (BRL) column. 100 ng of ^{32}P-labelled DNA was then incubated with 2 μg highly purified E1A protein for 30 min at 30°C in 10 mM phosphate buffer pH 7.4, containing 50 mM NaCl. Samples were irradiated with UV light from a source producing a dose of 150 mW/cm^2 for various peptides. After irradiation, the DNA was degraded with DNase I (Chatterjee *et al.*, 1986a) and the sample analysed by electrophoresis in 13% or 10% SDS–polyacrylamide gels. For mobility shift assays (Fried and Crothers, 1981; Garner and Revzin, 1981), 50 ng of unlabelled DNA fragment described in the previous paragraph or of ^{32}P-labelled oligonucleotide was incubated for 30 min at 30°C in 0.01 M Tris–HCl, pH 7.5, containing 0.06 M NaCl, 1 mM EDTA, 1 mM DDT and 5% glycerol. Samples were then subjected to electrophoresis in 4% polyacrylamide gels, cast and run in 6.7 mM Tris, 3.3 mM sodium acetate, 1 mM EDTA, pH 7.5 (DNA fragments), or in 6% gels cast and run in 0.02 M Tris–HCl pH 7.8, 0.2 mM EDTA (oligonucleotides). Following electrophoresis the DNA was stained wtih ethidium bromide and the gel photographed under UV light, or the gels were dried and exposed to Kodak X/AR or X/RP film.

Oligonucleotides were synthesized using an AB1 model 388 synthesizer and β-cyanocthyl phosphoramidates and purified by HPLC. Purified oligonucleotides were end-labelled in 0.05 M Tris–HCl, pH 7.5 containing 5 mM $MgCl_2$, 5 mM DTT, 1 mM EDTA and 1 mM spermidine using T4 polynucleotide kinase (Pharmacia) and [γ-^{32}P]ATP (1 μCi/μl, NEN, 5000 Ci/mmol). The complementary strands of the oligonucleotides were then mixed in equimolar proportions and incubated at 100°C for 3 min, 68°C for 15 min, 37°C for 30 min and room temperature for 2 h in the same buffer. The DNA was then purified on NENS50RB 20 columns.

Partial proteolysis

Partial proteolysis was performed as described by Cleveland *et al.* (1977), with minor modifications described in Chatterjee *et al.* (1986a,b).

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Note added in proof

It is interesting to note that a cysteinyl-thymine photocrosslink was shown to be labile to mild acid treatment (Paradiso *et al.*, 1979). Since all of our E1A-DNA crosslinked complex is susceptible to formic acid treatment, it is likely that the crosslinking occurs via a specific amino acid in the 289R E1A protein.