Down-regulation of human adenovirus E1a by E3 gene products: evidence for translational control of E1a by E3 14.5K and/or E3 10.4K products

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The mechanism for down-regulation of E1a expression by products encoded in the E3 transcription unit of human adenovirus types 2 and 5, that occurs in infected L929 cells, has been investigated further. We show that the phenomenon occurs in different mouse cells and also in some human cells suggesting that the observations have relevance to natural human infections. We also provide evidence that probably all viral proteins are down-regulated by E3 products, although to different extents, but that host proteins are unaffected. Whereas E1a protein levels and synthesis are reduced in the presence of E3 products, E1a protein half-life and polysomal E1a RNA levels and size distribution are not. These data suggest that E3 products down-regulate E1a protein levels by interfering with the translation of E1a-specific mRNA. Studies were additionally carried out with mutant adenoviruses containing different defects in the E3 transcription unit. Based on these studies it seems likely that the E3 14.5K and 10.4K proteins are crucially involved in E1a down-regulation. Our data are discussed in terms of strategies for immune evasion by group C human adenoviruses.

Introduction

There are at least 47 serotypes of human adenoviruses which form six distinct groups based on a number of parameters including the Mr of internal polypeptides, oncogenicity in newborn hamsters, G + C content of the genome, restriction enzyme analysis, and, where the data are available, the DNA sequence (Horwitz, 1990). In general, human adenoviruses infect the mucosal cells of the respiratory and gastrointestinal tracts, but depending on the serotype they can vary widely in tissue tropism and virulence (Horwitz, 1990). Adenoviruses usually cause mild respiratory and gastrointestinal disorders and often undergo long periods of persistent infection in man. For persistent infections to occur, adenoviruses must be capable of evading the host's immune response. Evidence from animal models suggest that early (E) region 3 of human adenoviruses might be important in this process (Ginsberg et al., 1989, 1991; X. Zhang, A. J. D. Bellett, R. Tha Hla, T. Voss, A. Müllbacher & A. W. Braithwaite, unpublished).

The E3 region of human adenoviruses is a complex transcription unit that for the group C adenoviruses is predicted to encode nine protein products, six of which have been identified using specific antisera. Although functions have not yet been assigned to all the E3 products, it appears that E3 proteins may have evolved different mechanisms to counteract the cellular immune response. One mechanism depends on the E3 19K glycoprotein (gp19K) binding non-covalently to certain class I antigens of the major histocompatibility complex (MHC) (Kvist et al., 1978). This interaction results in diminished cell surface expression of such MHC molecules (Andersson et al., 1985; Burgert & Kvist, 1985) and consequential inhibition of both the alloreactive (Andersson et al., 1987; Burgert et al., 1987) and adenovirus-specific (Rawle et al., 1989) cytolytic T (Tc) cells. A second mechanism involves the E3 14.7K, 14.5K and 10.4K products. All these proteins protect adenovirus-infected mouse cells from cytolysis by tumour necrosis factor (TNF) with the 14.7K product playing the dominant role in this process (Gooding et al., 1988, 1991a). In human cells however, complete protection from TNF also appears to require the 19K product encoded in the Elb transcription unit (Gooding et al., 1991b).

Quite apart from these strategies of evading the cellular immune response, the E3 region also has the
capacity to lower expression of Ela gene products (Zhang et al., 1991). The Ela gene encodes proteins that contain the immunodominant peptide for Tc cell recognition in the context of different murine class I MHC molecules (Mullbacher et al., 1989; Urbanelli et al., 1989; Kast et al., 1989) as well as rat class I MHC molecules (Bellgrau et al., 1988). Consistent with this finding, down-regulation of Ela protein levels causes a reduction in cytolysis by adenoavirus-specific Tc cells, but not by alloreactive Tc cells (Mullbacher et al., 1989; Zhang et al., 1991). There exists therefore at least one other mechanism by which adenoviruses can evade the immune response of the host. The molecular basis of this latter mechanism is however not understood.

In order to investigate the mechanism by which E3 products lower Ela protein levels, we have examined Ela gene expression at the levels of both mRNA and protein from cells infected with wild-type (wt) and E3 deletion mutants dl 327 and dl 355 of human adenovirus 5 (Ad 5).

Our data suggest that E3 products lower Ela gene expression by interfering with Ela protein translation. We have also used a set of other mutant adenoviruses containing defects in different E3 coding sequences to identify which E3 product(s) is responsible for Ela regulation. We report here that the E3 14-5K and/or the 10.4K products are likely to be involved in slowing the rate of Ela translation.

Methods

Cells. Mouse L929 (Commonwealth Serum Laboratories), methylcholanthrene-induced mouse fibrosarcoma (HTG), human HeLa (ATTC CCL 2), 143B TK-, Daudi, K562, monkey COS 1, and normal rat kidney (NRK) cells were routinely grown in Eagle's MEM (Flow Laboratories) supplemented with 10% fetal calf serum (Flow Laboratories) in a 37 °C humidified incubator in an atmosphere of 5% CO2.

Viruses. Growth of human wt Ad 5 and Ad 2 and E3-deficient adenoviruses has been described previously (Braithwaite et al., 1983; Bellett et al., 1985). E3 mutant adenoviruses (dl 327, dl 355, dl 801, dl 303, dl 304, dl 309 and dl 1041) used in the present study with appropriate references are described below and in brief in Table 2 of this paper. The reference for dl 327 (Thimmappaya et al., 1982) is for dl 324. Dl 327 has not itself been described, but it is 'isogenic' with dl 324 (W. Wold, personal communication).

Adenovirus titration. Human adenoviruses were titrated on HeLa cells using the immunofluorescence counting method with a monoclonal anti-Ad 5 antibody and also by using a DNA dot-blot hybridization procedure (Zhang et al., 1991).

Antibodies. Ela proteins were immunoprecipitated with the M73 (Whyte et al., 1988) monoclonal antibody (Oncogene Science). The E1b 55K product was immunoprecipitated with a polyclonal serum from tumour-bearing hamsters (H14b) and was provided by Dr E. Blair, Leeds University, U.K. E3 gp19K was immunoprecipitated with a rabbit anti-peptide serum provided by Dr W. Wold, St Louis, Miss., U.S.A. (Wold et al., 1985). To immunoprecipitate the late antigen hexon from Ad 5, a rabbit polyclonal serum was used (Braithwaite & Jenkins, 1989). The p53 tumour suppressor protein was immunoprecipitated using the p5-specific monoclonal antibody PAB 421 (Harlow et al., 1981). Class I MHC H-2Kb molecules were immunoprecipitated using a monoclonal antibody that was specific for H-2Kb (clone 11-4.1, Becton Dickinson). Normal mouse serum (NMS) was used as a non-specific control in most immunoprecipitations.

Immunoprecipitation analysis. Forty h after infection or at indicated times, cells (usually about 4 x 106 to 5 x 106 per 80 cm2 tissue culture flask) were pulse-labelled with 200 μCi [35S]methionine/cysteine (ICN Trans35S-label) for about 1 h (or as indicated) in 3 ml methionine-free medium (Flow Laboratories), following a methionine depletion period of 1 h in this medium. Cells were washed twice with cold (4 °C) PBS and lysed in 3 to 4 ml of modified RIPA buffer (10 mM-Tris-HCl pH 8, 150 mM-NaCl, 1 mM-EDTA, 1% NP40) plus 0.1% SDS. Cell lysates were cleared at 12,000 g for 20 min and extensively preabsorbed with Protein A-Sepharose beads (Pharmacia) and NMS. The cleared lysates were incubated with specific antibodies overnight at 4 °C, during which time immune complexes were adsorbed onto Protein A-Sepharose beads. Complexes were washed in modified RIPA buffer and resuspended in 30 μl of sample buffer (Laemmli, 1970) containing bromophenol blue. Samples were boiled to disrupt complexes and proteins were separated by electrophoresis on 12% SDS-polyacrylamide gels. Gels were then fixed in 47% methanol/10% acetic acid, enhanced using Amplify (Amersham) and then dried under vacuum, before exposure to Kodak Xar-5 film.

Isolation of polysomal RNA. Polyribosomes were isolated using the magnesium precipitation procedure described by Palfynter (1974) and RNA was extracted by the procedure of Mechler & Rabbitts (1981). In brief, adenovirus-infected and mock-infected L929 cells were Dounce-homogenized on ice in a buffer containing 25 mM-NaCl, 5 mM-MgCl2, 1 mg/ml heparin, 2% Triton X-100, and Tris-HCl pH 7.5. Cell debris was removed by centrifugation at 27,000 g for 5 min then an equal volume of buffer containing 0.2 M-MgCl2 was added to the supernatant and incubated at 4 °C for 1 h. This was then layered over 4 ml of 1 M-sucrose and centrifuged at 27,000 g for 10 min. The pellet containing polyribosomes was then resuspended in 20 mM-HEPES buffer and mixed with an equal volume of hot (100 °C) buffer containing 20 mM-Tris-HCl pH 7.4, 40 mM-EDTA, 0.2 M-NaCl, and 1% SDS and was then incubated at 100 °C for 2 min. The mixture was rapidly cooled to 30 °C and protease K was added to a final concentration of 0.5 mg/ml and then the mixture was incubated for 10 min. Polysomal RNA was then extracted with phenol/chloroform and precipitated with 300 mM-sodium acetate. RNA concentration was determined using a spectrophotometer.

Slot-blot analysis of polysomal RNA. Twenty μg of each RNA sample was glyoxylated for 1 h at 50 °C and then diluted 10-fold with 10 mM-sodium phosphate buffer pH 6.7, and loaded down under vacuum onto a Zeta-Probe membrane (Bio-Rad) using a Bio-Rad Hybri-Slot manifold. The manifold was then disassembled and membrane filters were baked at 80 °C for 2 h. Filters were then prehybridized and hybridized with radiolabelled Ela- and α-tubulin-specific probes (Zhang et al., 1991) and after washing and drying the membrane filters were exposed to X-ray film at ~70 °C. Radiolabelled probes were prepared by oligo-priming (Feinberg & Vogelstein, 1983) of Ela DNA (0 to 1571 bp of Ad 5), Ad 5 genomic DNA, and human α-tubulin cDNA (Cowan et al., 1983).

Northern analysis of polysomal RNA. Twenty μg of polysomal RNA was glyoxylated as described above and then subjected to electrophoresis through a gel containing 1% agarose in 10 mM-sodium phosphate buffer pH 6.7. RNA was then transferred to a Zeta-Probe membrane using the alkaline blotting procedure (according to the Bio-Rad manual's instructions) and then hybridized to an Ela-specific...
Human adenovirus Ela down-regulation

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(a) wt Ad 5 dl 327

(b) Mock wt Ad 5 dl 327

Fig. 1. E3 products down-regulate adenovirus proteins but do not affect host proteins. L929 cells were mock-infected, infected with wt Ad 5 or infected with dl 327. Forty hours post-infection cells were labelled with [35S]methionine/cysteine, extracts were prepared and immunoprecipitations, SDS–PAGE and fluorography were carried out as described in Methods. (a) Results from an analysis of selected adenovirus proteins are presented showing over-expression of all adenovirus proteins tested. Hexon C1 and C2 represent sequential immunoprecipitations with a hexon-specific antiserum. (b) Results from an analysis of the two cellular proteins, p53 and H-2Kk, are presented showing that expression of these proteins is unaffected by E3 gene products. Details of antibodies used can be found in Methods.

Results

E3 products lower the expression of adenovirus proteins but do not affect cellular proteins

We have previously reported that Ela protein levels are about 10 times higher in mouse L929 cells infected with the E3 deletion mutants dl 327 and dl 355 as compared with those of L929 cells infected with wt Ad 5 (Zhang et al., 1991). Over many experiments the relative levels of Ela proteins vary considerably but range from about fivefold to greater than 12-fold more Ela in mutant compared to wt Ad 5-infected cells. Similar results were obtained when Ela levels in wt Ad 2-infected cells were compared with the Ad 2 E3 deletion mutant dl 801 (Ad 2 is another member of the group C adenoviruses), but not when compared with the hybrid virus rec 700 (Zhang et al., 1992) in which the E3 region from Ad 2 is cloned into an Ad 5 genome lacking its own E3 region (Wold et al., 1986). The reason for this apparent serotype specificity is unclear at this time).

In the experiment described below, we have examined whether E3 products can lower expression of two host proteins and other viral proteins in addition to Ela.

L929 cells were mock-infected or infected with wt Ad 5 or mutant dl 327. About 40 h post-infection cells were radiolabelled, extracts were prepared, and immunoprecipitations were performed using specific antibodies. Protein levels encoded by the Ela, Elb (55K), E3 (gp19K) and hexon encoded by the late transcription unit were measured, as well as two cellular proteins, class I MHC H-2Kk and the tumour suppressor protein p53. Immunoprecipitates were then analysed by SDS–PAGE and fluorography.

The results (Fig. 1a–b) show that E3 gp19K was expressed in wt Ad 5-infected cells but not in mutant virus-infected cells which confirms the genotypes of the viruses. Results in Fig. 1(a) also show that Ela and Elb 55K proteins were expressed to higher levels in dl 327 mutant-infected cells as compared with wt Ad 5-infected cells. Measurement of the levels of these proteins showed them to be 10- and eightfold higher in the mutant-infected cells. However, hexon appeared to be expressed only about twofold more in mutant as compared with wt Ad 5-infected cells. Other viral early (the DNA-binding protein encoded in the E2a gene) and late region proteins (fibre) were also found to be over-expressed in E3 mutant-infected cells, but none as profoundly as those encoded in the E1 region (data not shown). However, when the levels of the cellular proteins p53 and H-2Kk...
Table 1. An E3-defective adenovirus over-expresses E1a proteins in both human and mouse cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Species</th>
<th>Relative E1a expression*</th>
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<tr>
<td>L929</td>
<td>Mouse</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>HTG</td>
<td>Mouse</td>
<td>8-10</td>
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<tr>
<td>HeLa</td>
<td>Human</td>
<td>~ 1</td>
</tr>
<tr>
<td>143B</td>
<td>Human</td>
<td>~ 5</td>
</tr>
<tr>
<td>Daudi</td>
<td>Human</td>
<td>&gt; 10</td>
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<tr>
<td>K562</td>
<td>Human</td>
<td>~ 1</td>
</tr>
<tr>
<td>COS</td>
<td>Monkey</td>
<td>~ 1</td>
</tr>
<tr>
<td>NRK</td>
<td>Rat</td>
<td>~ 1</td>
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* E1a levels were determined by immunoprecipitation analysis of radiolabelled cell extracts using M73 monoclonal antibody and laser densitometry as described in the text. Levels of E1a proteins were determined for each cell type after infection with either wt Ad 5 or an E3 mutant. The densitometric value for E1a proteins from mutant-infected cells was divided by the corresponding value for wt Ad 5-infected cells to give the relative E1a expression value.

E3 products lower expression of E1a proteins in several but not all cell lines

To determine whether E3 mutant viruses expressed higher levels of E1a products than wt Ad 5 in cells other than mouse L929 cells, a set of experiments was performed to determine the relative levels of E1a proteins in a variety of cell lines infected with wt Ad 5 and mutant viruses. The experiments were done using immunoprecipitation analysis and laser scanning densitometry as described previously (Zhang et al., 1991; see also Fig. 1). The results are presented in Table 1.

The data show that several cell types infected with wt Ad 5 expressed less E1a protein than the same cells infected with an E3 mutant. No difference was observed in human HeLa cells, human K562 cells, monkey COS cells or in NRK cells. The finding that there is no difference in E1a expression between wt and mutant Ad 5-infected HeLa cells is consistent with the absence of any difference in virus yields when HeLa cells are used as the propagation line for adenoviruses (data not shown).

Thus, increased expression of E1a products in E3 mutant-infected cells compared with wt Ad 5-infected cells is not universal but is found in cells that are either permissive (human) or non-permissive (mouse) for human adenovirus replication.

E1a protein levels are constitutively down-regulated by E3 products in adenovirus-infected mouse cells

To determine whether low E1a expression was a transient phenomenon in wt Ad 5-infected L929 cells the kinetics of E1a expression were examined in wt Ad 5-, dl 355- and dl 327-infected cells. The kinetics of E3 gp19K expression were also measured, as these served as a marker of the expression of the E3 transcription unit. This analysis was also performed by using metabolic labelling of proteins followed by immunoprecipitation, SDS–PAGE and quantification by densitometric measurements.

The results (Fig. 2) show that E1a protein levels were very low at 12 h post-infection in all three virus-infected cells. However, by 24 h post-infection E1a products had increased substantially in dl 327- and dl 355-infected cells reaching a peak at around 48 h post-infection and had declined a little by 72 h post-infection. In contrast, E1a levels from wt Ad 5-infected cells declined after 12 h post-infection and remained at barely detectable levels throughout the remainder of the experiment.

E3 gp19K expression in wt Ad 5-infected cells increased between 12 and 24 h post-infection and then slowly declined over time.

We conclude that E1a protein levels are constitutively down-regulated in wt Ad 5-infected L929 cells, an effect that we attribute to a product encoded in the E3 region.

E3 products do not affect transport or processing of E1a-specific mRNA

We have previously shown that the levels of total E1a-specific mRNA were no different between wt Ad 5- and dl 327-infected cells under the same conditions in which differential E1a protein levels were detected (Zhang et al., 1991). This result suggests that E3 products do not interfere with transcription of the E1a gene, a result which is consistent with the fact that E3 proteins are non-nuclear. The possibility still exists however that E3 products might interfere with transport of E1a mRNA to ribosomes and/or processing of E1a mRNA.

To determine whether E1a mRNA transport or processing was being affected by E3 products, L929 cells were mock-infected or infected with wt Ad 5, dl 327 or dl 355. Forty hours later polysomal RNA was extracted and analysed for E1a mRNA content. Polysomal RNA should represent the amount of total RNA which is translated (Darnell et al., 1986) and hence is correctly processed (Chow et al., 1977). If there was less E1a
Fig. 2. Kinetics of Ela protein expression and E3 19K product expression in wt Ad 5 and E3 mutant-infected mouse L929 cells. Cells were infected with wt Ad 5, dl 327 or dl 355 and at indicated times cells were pulse-labelled with [35S]methionine/cysteine and immunoprecipitation and SDS-PAGE analysis was carried out as described for Fig. 1 and in Methods. (O) Ela expression in wt Ad 5-infected cells; (●) E3 19K product expression in wt Ad 5-infected cells; (■) Ela expression in dl 327-infected cells; (▲) Ela expression in dl 355-infected cells.

mRNA present in this fraction in wt Ad 5-infected cells as compared to E3 mutant-infected cells, then effects of E3 products on processing or transport could be concluded.

The first part of this experiment was performed using the slot-blot technique. Results presented in Fig. 3(a) show that both Ela and adenovirus-specific probes hybridize to RNA molecules from wt Ad 5-, dl 327- and dl 355-infected cells but not to RNA from mock-infected cells. More importantly, the experiment demonstrates that the levels of both Ela and virus transcript in general (Ad 5 probe) do not differ between samples. As a loading control, the levels of the cellular gene α-tubulin were examined. These levels did not vary among any of the samples. This experiment suggests that E3 products do not affect transport of viral mRNA to the ribosomes because the viral RNA that is associated with the ribosomes (polysomal RNA) is present in equivalent amounts in cells infected with wt Ad 5 and E3-deficient viruses.

In the second part of this experiment, another set of polysomal RNAs was examined for Ela mRNA size distribution using Northern analysis. The Ela probe hybridized to transcripts from wt Ad 5- and dl 327-infected cells but not to the mock-infected cells (Fig. 3(b)). RNA molecules over a broad size range from approx. 1.0 kb to 0.5 kb were detected. This is consistent with previous data on the size distribution of Ela mRNA which was obtained using Northern blotting (Nelson, 1990). The overall levels of Ela mRNA in dl 327-infected cells were found to be similar to those observed for wt Ad 5-infected cells, as was also found by slot-blot analysis. Furthermore, there is no obvious difference in the pattern of hybridization suggesting that E3 products do not affect Ela mRNA processing.

We conclude that E3 products do not interfere with the transport of Ela RNA to the ribosomes since there are equivalent levels of Ela mRNA in mutant- and wt-infected cells, nor do they appear to interfere with
mRNA processing as determined by size class distribution using Northern analysis. Therefore, E3 products probably regulate the levels of Ela protein expression by some other mechanism.

**E3 products do not affect the half-life of Ela proteins**

The above data and a previous publication (Zhang et al., 1991) have shown that the regulation of Ela levels by E3 proteins does not appear to be due to E3 regulation of the levels of Ela mRNA. Rather these results suggest that E3 products might regulate Ela protein levels by altering the rate of protein synthesis or stability. This latter possibility was examined using a pulse-chase experiment.

L929 cells were separately infected with wt Ad 5 or dl 327 and 40 h later they were pulse-labelled with [35S]methionine, then chased with medium containing an excess of unlabelled methionine for the indicated times (Fig. 4). Cells were then lysed and Ela proteins were immunoprecipitated as described previously. The levels of immunoprecipitated Ela proteins were measured by densitometric scanning and the values obtained were plotted against chase time with the line of best fit being calculated by regression analysis. Results (Fig. 4) show that although Ela protein levels were lower in wt Ad 5-infected cells as compared with dl 327-infected cells at every time point examined, there is no significant difference in the decay of the Ela proteins' label, which showed a half-life of about 70 min in each case.

We conclude from these data that E3 products do not affect Ela protein stability.

![Fig. 4. E3 gene products do not affect the half-life of Ela proteins in infected L929 cells. Cells were infected with wt Ad 5 or dl 327 and 40 h later they were pulse-labelled for 30 min then chased in medium containing 20 mM-unlabelled methionine for indicated lengths of time. Immunoprecipitations were carried out as described in Methods followed by laser densitometry. Values of Ela protein levels thus obtained were then plotted against chase time. The slopes of the lines obtained by this procedure showed that the decay time of Ela proteins in wt Ad 5 (○) and dl 327 (○) infected cells were identical with a half-life in each case estimated to be about 70 min.](image)

**E3 14.5K and/or the E3 10.4K proteins appear to be responsible for lowering Ela expression**

The E3 mutants that have been tested so far for their effects on Ela regulation are substantially defective in E3 protein expression. From sequence information mutant dl 327 should express only the 12.5K and the 3.6K proteins (Thimmapaya et al., 1982), dl 355 should express

<table>
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<tr>
<th>Relative Ela expression and E3 product expression by different E3 adenovirus mutants*</th>
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<tr>
<td><strong>Adenovirus</strong></td>
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</tr>
<tr>
<td>wt Ad 5</td>
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<td>wt Ad 2</td>
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<td>dl 327†</td>
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<td>dl 303</td>
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<td>dl 1041</td>
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* The data summarized here are from experiments carried out in L929 cells and each done at least three times.
† E3 product expression in these mutant viruses is taken from various literature sources which are cited in the text.
‡ The E3 7.5K and 3.6K products are predicted to exist but have not yet been detected in virus-infected cells (W. Wold, personal communication).
§ E3 7.1K in Ad 5 infections is 6.7K in Ad 2 infections (W. Wold, personal communication). E3 7.1K might be altered in dl 309 (Jones & Shenk, 1979).
¶ E3 12.5K has been detected in dl 327-infected cells by immunoprecipitation, but the E3 3.6K has not (A. Mullbacher, H. Hayder, R. Tha Hla & A. W. Braithwaite, unpublished).
† DL 801 is an Ad 2 mutant. All other mutants in this table are Ad 5 mutants.
of Ela protein as compared with cells infected with wt Ad 5.

Collectively, the results from studies with these mutants exclude all but two proteins, E3 14.5K and 10.4K, from regulating Ela levels. We therefore suggest that the 14.5K and/or the 10.4K are the likely candidate proteins involved in lowering Ela levels during wt adenovirus infection of L929 cells and presumably of other cell types as well.

**Discussion**

This paper reports an examination of the molecular basis of down-regulation of Ela by E3 proteins in group C adenoviruses. We show that E3 products present in wt Ad 5 cause all virus proteins to be expressed at considerably lower levels than those in cells infected with the E3-deficient virus dl 327, but that host proteins (p53 and class I MHC H-2K^k^) are unaffected by E3 products (Fig. 1). Other experiments demonstrate that whereas Ela and other viral protein levels are affected by E3 products, Ela half-life, and virus RNA levels and transport to ribosomes are not (Fig. 3 and 4). Moreover, results from Northern analysis also suggest that Ela mRNA processing is not affected by E3 products although RNase protection experiments would be required to exclude this possibility formally. However, there are two lines of evidence that contradict the proposal that E3 products affect RNA processing. Firstly, all the E3 products that have been identified are non-nuclear and secondly, preliminary experiments using a cloned Ela cDNA in transfection experiments have demonstrated that an E3 expression plasmid inhibits expression of Ela protein (data not shown), even though no RNA processing is required from this Ela cDNA template.

Taken together, these data suggest that E3 products are likely to down-regulate Ela and other viral protein levels through interference with the translation of adenovirus-specific mRNA.

Experiments were also performed using a series of E3 mutants defective in expression of different E3 proteins. A summary of the data and the defects in all the E3 virus mutants is shown in Table 2. These data show that all mutants expressed high levels of Ela proteins as compared with wt Ad 5/2-infected cells, but that host proteins (p53 and class I MHC H-2K^k^) are unaffected by E3 products (Fig. 1). Other experiments demonstrate that whereas Ela and other viral protein levels are affected by E3 products, Ela half-life, and virus RNA levels and transport to ribosomes are not (Fig. 3 and 4). Moreover, results from Northern analysis also suggest that Ela mRNA processing is not affected by E3 products although RNase protection experiments would be required to exclude this possibility formally. However, there are two lines of evidence that contradict the proposal that E3 products affect RNA processing. Firstly, all the E3 products that have been identified are non-nuclear and secondly, preliminary experiments using a cloned Ela cDNA in transfection experiments have demonstrated that an E3 expression plasmid inhibits expression of Ela protein (data not shown), even though no RNA processing is required from this Ela cDNA template.

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Taken together, these data suggest that E3 products are likely to down-regulate Ela and other viral protein levels through interference with the translation of adenovirus-specific mRNA.
both of these proteins is required for E1a down-regulation.

The E3 14.5K and 10.4K proteins are both cytoplasmic membrane-bound proteins as determined from cell fractionation studies (Tollefson et al., 1990a, b). The 10.4K protein is immunoprecipitated as well as 14.5K suggesting that the two exist in complex in vivo and this is consistent with the genetic data indicating that they function in concert (Tollefson et al., 1991). These proteins have two reported functions (for review see Wold & Gooding, 1991). One is in protection against cytolysis by TNF and the second is in down-regulating cell surface expression of the receptor for epidermal growth factor.

Recent biochemical evidence together with amino acid sequence data suggest that both E3 10.4K and 14.5K proteins are integral membrane proteins with C-terminal residues extending into the cytoplasm (Krajceti et al., 1992a, b). For the E3 14.5K product the N terminus would be exposed on the luminal side of the endoplasmic reticulum (ER). One form of E3 10.4K protein is in a similar configuration to E3 14.5K whereas the second form has both N and C termini exposed on the cytoplasmic side of the ER.

The evidence presented in this paper suggests that E1a mRNAs are less efficiently translated in the presence of E3 proteins than in their absence, and that this ‘regulation’ of translation is controlled by E3 14.5K and 14.5K proteins. Given their location in the membrane, a model of this regulation could involve E3 10.4K and 14.5K C-terminal domains, present on the cytoplasmic side of the ER, binding together and in turn interacting directly with the nascent E1a protein thus slowing the rate of E1a protein elongation. This model allows selectivity for E1a and consequential effects on other viral proteins without affecting expression of cellular proteins. Alternatively, the E3 10.4K and 14.5K products could affect the translation independently of all viral mRNA molecules by interaction with a factor required for translation of viral but not cellular mRNAs. We have no data at this time to distinguish between these two possibilities.

In addition to these general mechanisms, the observation that differential viral protein expression occurs only in some cell types argues that there must be another (presumably cellular) factor involved. Otherwise, differential expression would be observed in all cell types. Experiments are in progress to define further the viral and cellular components necessary for regulating E1a protein expression.

Most of our studies have focused on the use of L929 cells; however, the data in Table 1 show that differential expression of E1a proteins between wt Ad 5 and E3 mutant viruses does occur in other cell types. Importantly, it occurs in mouse cells expressing different class I MHC alleles, including those in which class I MHC cell surface expression is down-regulated through binding of E3 gp19K (HTG cells, which express H-2Kd and H-2Dd, both of which interact with E3 gp19K). The differential expression of E1a also occurs in human cells, which are the natural host for the adenoviruses under study. These data suggest that the down-regulation of E1a proteins by E3 14.5K and 10.4K that we have studied in adenovirus-infected L929 cells has wider implications for the ability of adenoviruses to evade immune surveillance.

Evocation of T cell-mediated immune surveillance by adenoviruses appears to focus on limiting the epitope recognized by CD8+ cytotoxic T cells. The epitope consists of a trimolecular structure comprising the highly polymorphic class I MHC heavy chain, B2-microglobulin and a peptide derived from the E1a antigen. Unlike the down-regulation by E3 gp19K by specific binding to the class I MHC heavy chain, down-regulation of the CD8+ T cell immunodominant E1a antigen is not constrained by the polymorphism of the target protein. Immu- logical evidence from studies using CD8+ cytotoxic T cell assays (A. Müllbacher, H. Hayder, R. Tha Hia & A. W. Braithwaite, unpublished), appear to demonstrate that E1a down-regulation is the more widely employed strategy for immune evasion by group C human adenoviruses as exemplified by studies with Ad 5.

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