(Pharmacia). The v-Rel–containing fractions were passed over an immunoaffinity column that contained an antibody to v-Rel (anti-pp59$^{v\text{-}rel}$). pp40 was eluted from the column in 1M $MgCl_2$. The purified protein was analyzed on SDS-PAGE, electrotransferred to nitrocellulose, and digested in situ with trypsin. The tryptic peptides were separated by high-performance liquid chromatography (HPLC), and two tryptic peptides yielded the following sequences: QAAGDAAFLNFQNNLSQTPLHLAVITDQAEIAEHLL and EPPARPHAWAQQLTEDGDTFLHLAIIHEE. The protein was sequenced by the Harvard Microchemistry Facility. The peptide sequences were used to generate partially degenerate guessmers. The resulting 44-nt probes had the following sequences: TTCCT(GC)AACTTCCAGAACAACCT(GC)(TA)G(TC)CAGACCCCCCT(GC)CACCT and TGGGC(GC)CAGCAGCAGCTGAC(CA)GAGGA(TC)GGCGA(CT)ACCTT(TC)CTGCACCT. These oligonucleotides were end-labeled with T4 polynucleotide kinase and used to screen cDNA libraries.

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28. Proteins electroblotted onto nitrocellulose were processed by tryptic digestion, and the resulting peptides were separated with narrow bore reversed-phase HPLC. Two of the peptide peaks were successfully sequenced with initial yields of 7 and 3 pmol.
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30. The templates for in vitro transcription–translation were obtained by linearizing the plasmids with the appropriate restriction enzymes. The linearized templates were then transcribed with T3, T7, or SP6 RNA polymerases. The purified RNAs were translated in a wheat germ extract with [^{35}S]methionine to obtain labeled proteins that were quantitated by SDS-PAGE and fluorography. The quantitation was done to ensure that equivalent amounts of proteins were used in the assays. The electrophoretic mobility shift assay was performed in 0.25× tris-borate-EDTA (1× TBE: 0.089 M tris, 0.089 M boric acid, 0.02 mM EDTA) to allow greater resolution of the homo- and heteromeric complexes.

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Cell Cycle Regulation of Histone H1 Kinase Activity Associated with the Adenoviral Protein E1A

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Several cellular proteins form stable complexes with the proteins encoded by the adenovirus early region 1A (E1A) gene in extracts derived from adenovirus infected or transformed cells. Two of the cellular proteins that bind to E1A have been identified; one, a 105-kilodalton protein (pRb), is the product of the retinoblastoma gene, and the other, a 60-kilodalton protein, is a human cyclin A. Two other proteins that bind E1A have now been shown to be related to p34^{cdc2}. This E1A complex displayed histone H1–specific kinase activity; the kinase activity was modulated during the cell division cycle, and association of pRb with E1A apparently was not required for this activity.

THE *CDC2* GENE ENCODES A 34-kD protein kinase (p34^{cdc2}) (*1*). In the fission yeast, *Schizosaccharomyces pombe*, *cdc2* is required in the G_1 stage of the cell cycle before DNA replication has begun and in G_2 at the beginning of mitosis (*2*). A *cdc2* homolog encodes a component of the maturation-promoting factor (MPF) of *Xenopus* eggs (*3*). The mammalian *cdc2* product is a protein kinase and is required for mitosis (*4*) and for the transition from G_1 to S phase (DNA replication) (*5*). The p34^{cdc2} protein activates the replication function of the SV40 T antigen (*6*). At least two complexes of mammalian p34^{cdc2} have been identified, the p62 (cyclin B)-p34^{cdc2} complex and the p60 (cyclin A)-p34^{cdc2} complex. The amount of p62-p34^{cdc2} complex correlates with a cell cycle–dependent histone H1 kinase activity that is most active in metaphase. The p60-p34^{cdc2} complex acts as a histone H1 kinase in interphase and metaphase (*7, 8*). Human p34^{cdc2} can form a complex with a 13-kD protein that probably is the product of a homolog of the fission yeast *suc1*$^+$ gene, a gene that rescues some but not all temperature-sensitive mutant alleles of *cdc2* in fission yeast (*9*), and is essential for cell cycle progression (*10*). The p13^{suc1} protein is a subunit of the p34^{cdc2} protein kinase in mammalian cells and in the budding yeast, *Saccharomyces cerevisiae* (*11*).

The E1A proteins are the first viral gene products synthesized after cells are infected with adenovirus. The E1A polypeptides function in both cellular transformation and regulation of gene expression (*12, 13*). For example, co-transfection of the E1A gene with another adenovirus early region gene product, E1B, or with an activated Ha-*ras* oncogene results in morphogenic transformation of either primary cells or proliferating tissue culture cells (*13*). Other activities of E1A include the induction of G_0-arrested mammalian cells to progress to DNA synthesis (*14*), the ability to induce tumor necrosis factor (TNF) sensitivity (*15*), and the prevention of transforming growth factor (TGF) β–mediated inhibition of cell growth (*16*).

Three regions, designated domains 1, 2, and 3, are highly conserved among E1A proteins from different adenovirus serotypes (*17*). Two sets of nuclear phosphoproteins arise from differential splicing of the E1A transcripts. The resulting proteins consist of 289 or 243 amino acids and are both post-translationally modified. The larger protein contains an internal sequence of 46 amino acids that has been identified as conserved domain 3 and appears to be sufficient for E1A-mediated transactivation of transcription (*18*).

The E1A conserved domain 2 and the NH_2-terminal region, including conserved domain 1 both contribute to transforming activity (*17, 18, 19*). Sites within these domains of E1A are required for complex formation with several specific cellular proteins (*20*). Two of these proteins have been identified; one of 105 kD (pRb) is the product of the retinoblastoma gene (*21*); and one of 60 kD (p60) is associated with only certain isoforms of p34^{cdc2} and is encoded by a cyclin A gene (*8, 22*). We now show that two cellular proteins in the E1A immune complex are either products of the human *cdc2* gene or a related gene, and that E1A immune complexes containing these proteins have a histone H1 kinase activity.

The cdc2-associated polypeptide, p60, is the same p60 protein that interacts with the adenovirus E1A protein (*8*). Four specific isoforms of p34^{cdc2} were present in immune complexes (*8*), isolated with a monoclonal antibody to p60 (C160). Two of these isoforms were recognized by an antiserum to the COOH-terminal portion of human p34^{cdc2} (G6). All four isoforms were recognized by an antibody to *S. pombe* p34^{cdc2} (G8) (*23*) and by p13-Sepharose. Proteins

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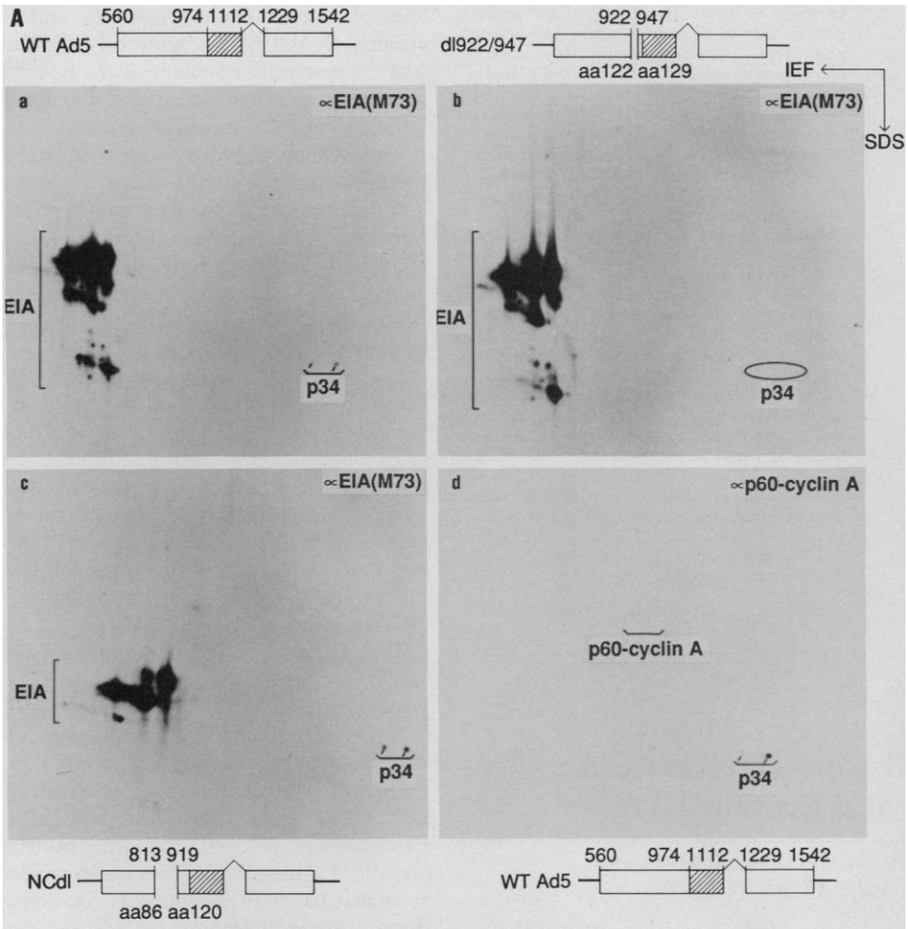

Fig. 1. Two-dimensional gel analysis of E1A-p34 and p60 (cyclin A)-p34 complexes. (**A**) HeLa cells were infected with various adenoviruses and extracts of those cells were immunoprecipitated with the indicated antibodies as follows: Wild-type adenovirus 5 (WT Ad5), M73 antibody (a); *dl* 922/947 recombinant adenovirus, M73 (b); NC*dl* recombinant adenovirus, M73 (c); WT Ad5, C160 (d). Spots corresponding to E1A, p34, and p60 (cyclin A) are indicated. Autoradiography was for 8 days at −70°C with a screen. (**B**) Two-dimensional gel analysis of E1A-p34 complexes using another antibody to E1A and a different cell line. The complexes were isolated by immunoprecipitating E1A from HeLa cells infected with Ad5 with the monoclonal antibody to E1A (M58) (a), E1A from the 293 cell line with M73 (b), proteins from HeLa cells infected with WT Ad5 with Pab 416 (c), and proteins from 293 cells with Pab 416 (d). Autoradiography was for 6 days at −70°C with a screen.

immunoprecipitated with antibody to E1A (anti-E1A) from cells expressing E1A were separated by two-dimensional gel electrophoresis; two spots were detected that appeared to be the same as the two isoforms of $p34^{cdc2}$ associated with p60 that were not recognized by the G6 antibody but that were bound by the G8 antibody and p13-Sepharose. To further investigate the relation of these proteins to $p34^{cdc2}$, we infected HeLa cells with three different recombinant adenoviruses (*24*), and then labeled the cells with [^{32}P]orthophosphate. Lysates from infected cells were immunoprecipitated with the monoclonal antibody to E1A (M73) or with C160. The M73 immune complexes from cells infected with wild-type adenovirus (Fig. 1A) contained two ^{32}P-labeled proteins that comigrated with the $p34^{cdc2}$ isoforms present in the C160 immune complex (Fig. 1A). [Only two of the four isoforms associated with p60 (cyclin A) were detected by labeling with ^{32}P for the interval used in these experiments.] HeLa cells infected with the deletion mutant NC*dl*, a deletion of residues between domains 1 and 2, revealed the same two p34 isoforms in the M73 immune complex (Fig. 1A). This deletion does not affect the transforming properties of E1A (*18*). In contrast, infection of HeLa cells with the deletion mutant *dl* 922/947, a domain 2 deletion, resulted in a decrease in the amount of p34 associated with E1A (Fig. 1A). A longer exposure of the autoradiograph revealed that the amount of ^{32}P detected in each of the p34 spots in the *dl* 922/947 pattern was approximately 20% of that in the wild-type pattern (Fig. 1A). The association of p60 (cyclin A) with the *dl* 922/974 E1A products is similarly reduced (*19*). The *dl* 922/947 mutant does not form a complex with pRb (*20*) and lacks transforming activity (*19, 21*).

The same two p34 isoforms that were associated with M73 and C160 immune complexes were immunoprecipitated with a second monoclonal antibody to E1A (M58), indicating that they coprecipitate with E1A and were not recognized directly by the monoclonal antibodies (Fig. 1B). The same isoforms were precipitated from the human carcinoma cell line 293 with the M73 antibody (Fig. 1B). These cells constitutively express E1A (*25*). Similar immunoprecipitations from HeLa cells infected with recombinant E1A adenoviruses encoding the 289– or 243–amino acid forms revealed the same two p34 spots. A control monoclonal antibody, Pab 416, which recognizes SV40 T-antigen, was used to immunoprecipitate proteins from either adenovirus-infected HeLa cells or the 293 cells (Fig. 1B). No isoforms of p34 were detected in the resultant immune complexes. Therefore, the coprecipitation of these p34 molecules is dependent on their interaction with E1A or with cellular proteins bound to E1A.

To further establish the relation of the p34 isoforms associated with E1A to products of the human *cdc2* gene, adenovirus-infected HeLa cells were labeled with [^{32}P]-orthophosphate and extracts were prepared. These extracts were immunoprecipitated with the G6 antibody (*7*) or were exposed to p13-Sepharose beads. The two spots that are associated with p13-Sepharose comigrated on two-dimensional gels with the $p34^{cdc2}$ spots in the M73 immune complex (Fig. 2A). However, the G6 antibody did not immunoprecipitate these forms of p34. The two isoforms associated with E1A are present in uninfected HeLa cells and can be recovered by p13-Sepharose from extracts of these cells (*23*). The structure of the two p34 isoforms associated with p13-Sepharose and M73 immune complexes was compared to that of $p34^{cdc2}$ immunoprecipitated with G6. The peptides resulting from digestion of each isoform with *N*-chlorosuccinamide (NCS) were identical (*26*) (Fig. 2B).

The histone H1 kinase activity of the $p60\text{-}p34^{cdc2}$ complex is increased when isolated from HeLa cells at the interphase or G_2-M phase of the cell cycle (*8*). We therefore assayed the histone H1 kinase activity in the M73 immune complexes from unin-

fected, wild-type adenovirus-infected, and *dl* 922/947–infected HeLa cells. Little or no phosphorylation of H1 occurred when the substrate was incubated with the immune complex from uninfected cells (Fig. 3). Phosphorylation of H1 did result from incubation with the M73 immune complex from cells infected with wild-type virus; less phosphorylation was detected when the immune complex from *dl* 922/947–infected cells was incubated with histone H1. This result is consistent with the lower amount of $p34^{cdc2}$ isoforms in the complex with the *dl* 922/947 protein (Fig. 1A) and does not reflect a difference in the amount of E1A itself in the immune complex. M73 immune complexes from cells infected with wild-type virus contain cellular proteins of 100 to 150 kD that are phosphorylated in the in vitro kinase assay. These proteins do not associate with the *dl* 922/947 form of E1A (*21*) and were therefore not phosphoylated in reactions containing *dl* 922/947 immune complexes. The H1 kinase activity in M73 immune complexes from HeLa cells infected with wild-type virus or NC*dl* was greater than that in complexes from cells infected with *dl* 922/947 (Fig. 3B). The H1 kinase activity in M73 immune complexes from the N*cdl* infected cells was greater than that in complexes from cells infected with wild-type virus. Again, the kinase activity was proportional to the amount of the specific p34 isoforms in such complexes (Fig. 1A). Complexes immunoprecipitated with Pab 416 from HeLa cells infected with wild-type virus had one fifth of the H1 kinase of the M73 immune complexes (*23*). The H1 kinase activity in Pab 416 immune complex from the 293 cells is less than one seventh of that in M73 complex (Fig. 3B) as expected because isoforms of p34 were not detected in such immune complexes (Fig. 1B). The domain 2 mutant pm928, in which cysteine was substituted for glycine (*18*), did associate with p34 isoforms in infected HeLa cells (Fig. 4). The immune complex containing the pm928 form of E1A had greater histone H1 kinase activity than the wild-type E1A complex (Fig. 3B). In addition to p34, the

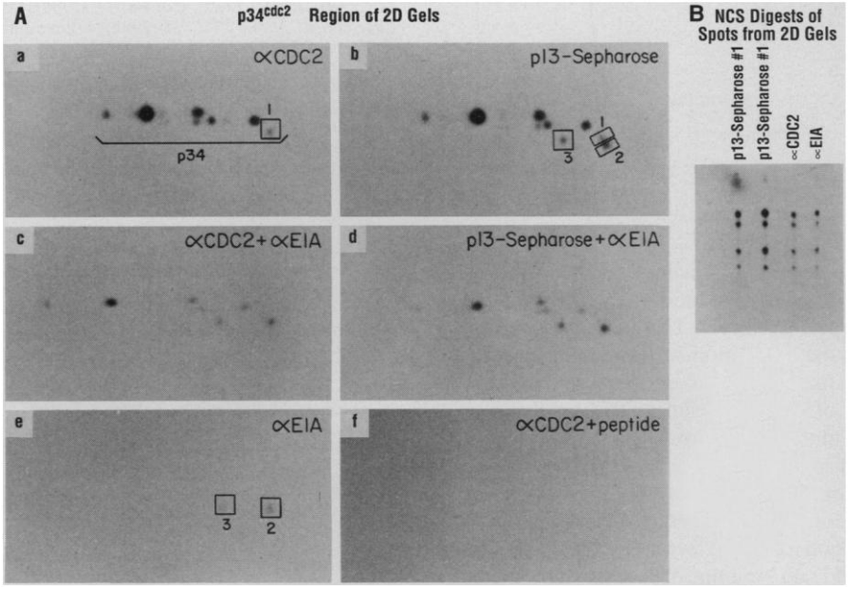

Fig. 2. Characterization of different isoforms of p34. (**A**) Two-dimensional gel analysis of the different p34 isoforms. The p34 isoforms precipitated form HeLa cells (infected with WT Ad5) with an antiserum to $p34^{cdc2}$ (G6) (a); p34 precipitated from HeLa cells (infected with WT Ad5) with p13-Sepharose beads (b); mixture of proteins from precipitates shown in (a) and (e), (c); mixture of proteins from precipitates shown in (b) and (e), (d); HeLa cells infected with WT Ad5 and immunoprecipitated with M73 (e), immunoprecipitation with G6 of HeLa cells infected with WT Ad5, performed in the presence of 100 nmol of the antigenic carboxyl-terminal cdc2 peptide (f). Autoradiography was for 6 days at −70°C with screen. (**B**) NCS digest of p34 spots from two-dimensional gels. Spot 1 from p13-Sepharose (see Fig. 2A, panel b), lane 1; spot 2 from p13-Sepharose, lane 2; anti-$p34^{cdc2}$ spot 1 (see Fig. 2A, panel a), lane 3; p34 spot 2 in anti-E1A precipitate (Fig. 2A, panel e), lane 4. Autoradiography was for 11 days at −70°C with screen. In all cases the spots represent phosphoproteins.

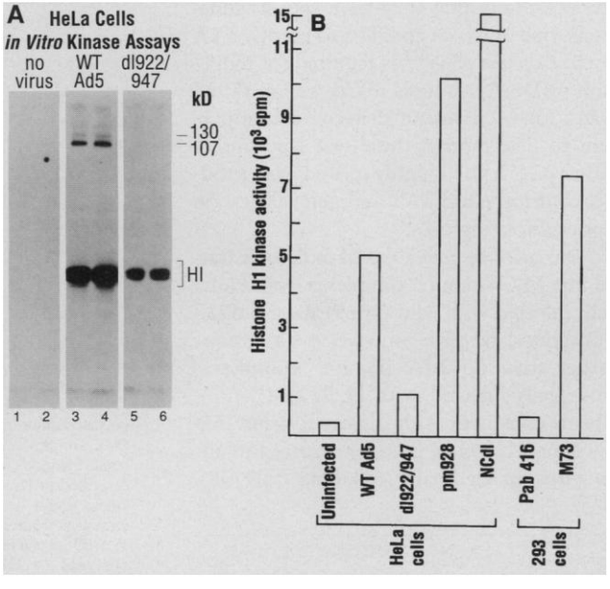

Fig. 3. Histone H1 kinase assays of HeLa cells after infection with different recombinant adenoviruses. (**A**) Assays were performed as described (*24*) and contained M73 immunoprecipitates from uninfected cells (lanes 1 and 2), from cells infected with WT Ad5 (lanes 3 and 4), or from HeLa cells infected with mutant *dl* 922/947 (lanes 5 and 6). (**B**) Duplicate reactions were normalized with respect to the protein concentrations of the extracts from HeLa cells that were uninfected, or infected with WT Ad5, *dl* 922/947, or pm928. Single reactions, repeated at least two times, for the 293 cell cultures were similarly normalized. Histone H1 kinase activity is expressed as ^{32}P incorporated (10^3 cpm). Duplicate analysis agreed to within ±5% of the mean. Previous measurements (*8*) of H1 kinase activity in complexes of E1A were performed without Mn^{2+} in the preparation of extracts and showed lower levels of the kinase activity.

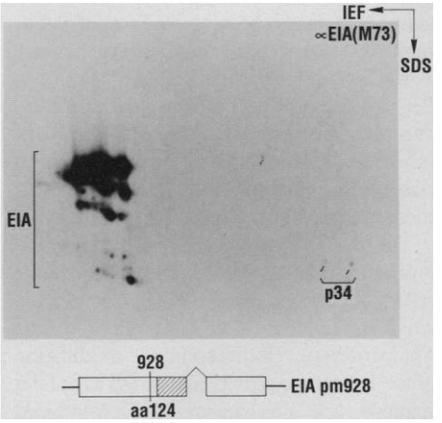

Fig. 4. The pm928 E1A mutant protein binds p34, p60 (cyclin A), but not pRb. Proteins immunoprecipitated with antibody to E1A (M73) from cells infected with pm928 recombinant adenovirus analyzed on two-dimensional gels. Spots corresponding to E1A and p34 are indicated. Autoradiography was for 5 days at −70°C with screen.

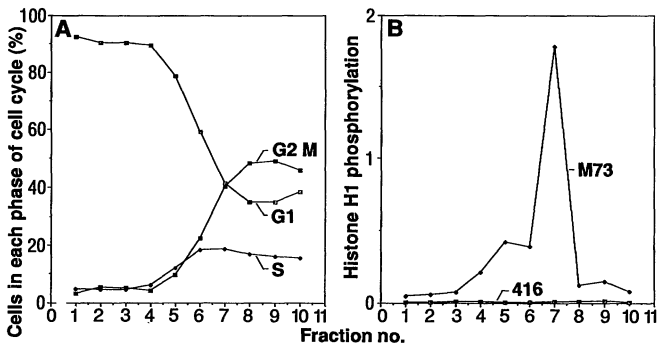

Fig. 5. Histone H1 kinase activity of the E1A immune complex through the cell division cycle. Centrifugal elutriation (**A**) and E1A-immune complex histone H1 kinase activity (**B**) of 293 cells. Fractions containing cells were collected by centrifugal elutriation and were fixed and stained with propidium iodide and analyzed by flow cytometry. The remaining cells in each fraction were extracted, and portions containing 250 μg of protein were immunoprecipitated with either M73 or Pab 416 and assayed for histone H1 kinase activity.

pm928 associated with p60 (cyclin A); however association with pRb was not detected (*19, 20, 23*).

The histone H1 kinase activity precipitated with the antibodies to p60 displays a distinctive pattern of cell cycle regulation in mammalian cells. The $p34^{cdc2}$-p60 (cyclin A) complex has properties distinct from those of the $p34^{cdc2}$-p62 (cyclin B) complex in HeLa cells (*7, 8*). We therefore studied the E1A-immune complex histone H1 kinase activity in populations of the 293 cells enriched at different stages of the cell division cycle by centrifugal elutriation. Portions of cell lysates containing equivalent amounts of protein were immunoprecipitated with either Pab 416 or M73 (Fig. 5). An enrichment of cells was achieved (Fig. 5A). At the transition from a population containing predominantly cells in G_1 to one containing more S phase cells (between fractions 3 and 4), the histone H1 kinase activity in the E1A immune complex was increased.

It is not clear whether the p34 isoforms associated with E1A are products of the *cdc2* gene. They may represent products of a gene highly related to the human *cdc2* gene such as the Eg1 cDNA from *Xenopus laevis* (*27*). Perhaps the unique isoforms contain different phosphorylated amino acids or some other modification that affects their migration in the two-dimensional gels and blocks their recognition by the G6 antibody.

The $p34^{cdc2}$-p60 (cyclin A) complex immunoprecipitated with C160 from HeLa cells had maximal histone H1 kinase activity when isolated from interphase cells (*7, 8, 23*). The pattern of cell-division cycle modulation of the H1 kinase activity associated with E1A suggests one mechanism of E1A-induced transformation may be its association with cell cycle regulatory proteins prior to induction of DNA synthesis. The *dl* 922/947 deletion mutant (deletion in domain 2) is defective for oncogenic and mitogenic activity and binds less strongly to p34 and p60 (cyclin A).

The pm928 mutant that apparently does not bind pRb (*17*) maintains full histone H1 kinase activity and binds the isoforms of p34 and p60 (cyclin A). Therefore, it is possible that the association of the pm928 product with these proteins contributes to the induction of viral and cellular DNA synthesis by this mutant E1A protein (*28*). The pm928 E1A complex might phosphorylate pRb during G_1 and thereby contribute to induction of DNA synthesis by inhibiting suppression of DNA synthesis by pRb. This would represent one of perhaps many mechanisms that permit the mutant protein to be as mitogenic as wild-type E1A. The physical association of E1A with pRb would represent an additional component of transformation phenotype with other effects on cellular growth control.

The extensive, rapid post-translation modifications of E1A (*29*) may be due at least partly to the presence of p34 in the E1A complex. The resolution of the same two specific forms of p34 in both the E1A and p60 (cyclin A) immune complex raises the possibility that they have specific functions, one of which could be to modify E1A itself. Because $p34^{cdc2}$ is required for induction of DNA synthesis (*5*) as well as G_2-M transition (*4*) in mammalian cells it is important to determine if these two isoforms of either $p34^{cdc2}$ of a highly related gene product contribute to E1A's mitogenic effects on nonproliferating cells.

Note added in proof: We have demonstrated that M73 immune complexes from HeLa cells infected with wild-type virus or pm928 phosphorylate pRb in vivo to a greater extent than do EIA immune complexes, from cells infected with *dl* 922/947 (*37*). This is consistent with the observation that phosphorylation of pRb is increased in adenovirus-infected baby rat kidney cells (*38*).

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24. HeLa cells and 293 cells were grown at 37°C on 100-mm plates in Dulbecco's modified Eagle's medium (DMEM) with 100 μl/ml of penicillin and 100 μ/ml streptomycin, supplemented with fetal bovine serum (10%). The E1A mutants have been described (*20, 21*) with the exception of NC*dl* provided by E. Moran (*18*). HeLa cells were infected with 100 plaque-forming units per cell of each adenoviral stock. Infection progressed for 1 hour in DMEM without serum, and the medium was removed and replaced with DMEM containing fetal bovine serum (10%). At 13 hours after infection HeLa cells were labeled with [^{32}P]orthophosphate (2 mCi/ml) (Amersham) in DMEM without phosphate for 4 hours at 37°C. After the labeling period, the cells were rinsed in phosphate-buffered saline (PBS) and lysed (*8*). Cells were lysed in 1 ml of a buffer containing 50 mM tris-HCl (pH 7.4), 0.25 M NaCl, 0.1% Triton X-100, 5 mM EDTA, 50 mM NaF, 0.1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, *N*-tosyl-L-phenylalanine chloromethyl ketone (50 mg/ml), and leupeptin (10 mg/ml). Protein concentration in each extract was determined (*30*). M58 and M73 monoclonal antibodies were prepared from BALB/c mice immunized with a bacterial trpE-E1A protein (*31*). M73 recognizes an epitope within the second exon of E1A molecules whereas M58 is specific for the product of the first exon (*32*). E1A was immunoprecipitated using the M73 and M58 as described (*31*). Pab 416 was used as a control (*33*). C160 was described (*8*). Preparation of the G6 antiserum (*7, 8*) and p13-Sepharose (*30*) have been described. Two-dimensional gels consisted of a (pH 3.5 to pH 10) gel in the isoelectric focusing (pH 3.5 to pH 10) in the first dimension and an SDS-polyacrylamide gel (10%) in the second dimension (*35*). Samples for such gels were prepared (*8*). *N*-chlorosuccinamide cleavage of $p34^{cdc2}$ was performed as described in Draetta *et al.* (*11*). Histone H1 protein kinase assays were performed as described (*8*). Immunoprecipitates were incubated for 10 min at 30°C in a 50-μl reaction mixture containing 50 mM tris-HCl (pH 7.4), 10 mM $MgCl_2$, 5 mM $MnCl_2$, 1 mM dithiothreithol, histone H1 (50 μg/ml), 10 μCi of [γ-^{32}P]ATP, and 1 μM ATP. Reactions were stopped by addition of Laemmli sample buffer (*36*) and run on an SDS-polyacrylamide gel (12%). After autoradiog-

raphy, radioactivity in gel pieces was determined by scintillation counting. Approximately 1.5 × 10^8 of the 293 cells were centrifuged (JE-10X rotor, Beckman J-6M) at 1200 rpm (pump speed, 80 ml/min). The elutriation buffer (EB) was PBS containing 0.3 mM EDTA, fetal calf serum (1%), and glucose (0.1%). Cell fractions were collected in 500 ml by increasing the pump speed by 20 ml/min in a stepwise manner. The fractionated cells were centrifuged, washed, and counted with trypan blue dye exclusion. Approximately 75% of the cells were recovered, of which more than 90% were viable. Fractionated cells (1 × 10^6) were fixed 1 hour or overnight in 80% ethanol and then washed twice in PBS containing fetal calf serum (1%). Fixed cells were stained in PBS containing propidium iodide (PI) (4 μg/ml) and ribonuclease (1 μg/ml) at 37°C for 30 min. Stages of the cell cycle were assessed by analysis of stained cells with a Coulter EPICS C flow cytometer. The remaining cells in each fraction were extracted, immunoprecipitated, and assayed for histone H1 kinase activity.

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Requirement of Microfilaments in Sorting of Actin Messenger RNA

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Specific messenger RNAs (mRNAs) can be sequestered within distinct cellular locations, but little is known about how this is accomplished. The participation of the three major cellular filaments in the localization of actin mRNA was studied in chicken embryo fibroblasts. Movement of actin mRNA to the cell periphery and maintenance of that regionalization required intact microfilaments (composed of actin) but not microtubules or intermediate filaments. The results presented here suggest that actin-binding proteins may participate in mRNA sorting.

A FUNDAMENTAL QUESTION IN CELL biology concerns how molecules are targeted to their sites of function. A number of works have described the sequence-specific sorting of mRNA to distinct cellular regions (*1*). Often these mRNAs are sequestered in cellular locations where their corresponding proteins function (*2*), implying that localized synthesis may be a means to regionalize some proteins. Our studies address the mechanism of actin mRNA sorting to the actin-rich ruffling edges of motile chicken embryo fibroblasts (CEFs).

Information for the localization of β-actin mRNA is encoded in the nucleic acid sequence of the mRNA and not the amino acid sequence of the nascent actin polypeptide (*3*). In *Drosophila*, a sequence in the 3′ untranslated region of *bicoid* mRNA is necessary for its localization in the embryo (*4*). Therefore, a mechanism exists in cells to transduce nucleic acid information into spatial information. It is likely that this mechanism involves cellular filament systems. Cytoplasmic motor proteins have been described that implicate both microtubules and actin filaments in intracellular transport (*5*). Furthermore, evidence exists that a direct cytoskeletal interaction with mRNA (*6*) appears to be necessary for translation (*7*). In *Dictyostelium discoideum*, the translation elongation factor EF-1a was shown to be an actin-binding protein (*8*).

We have focused on the functions of the three cellular filament systems in the movement of actin mRNA to the cell periphery of CEFs, as well as the subsequent maintenance of that localization. Movement and maintenance of localization were distinguished experimentally with the use of spreading cells. In cells in suspension, actin mRNA is uniformly distributed; however, when these cells are transferred to tissue culture plates and allowed to spread, actin mRNA relocalizes to the cell periphery in minutes (*3*). Maintenance of this localization was studied in cells that had completed spreading and had peripherally localized their actin mRNA.

In order to correlate the spatial relation between actin message and cellular filaments, we visualized both simultaneously. A method was developed for detection of mRNA and specific proteins that preserved the intracellular location of the mRNA as well as the antigenicity of the protein (*9*). The three major filament systems—microtubules (MTs), intermediate filaments (IFs),

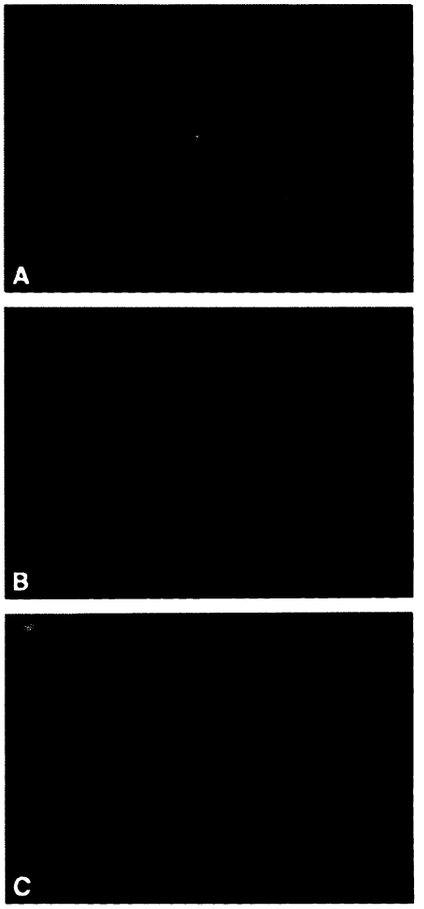

Fig. 1. Distribution of actin mRNA in relation to each of the three major cellular filament systems in CEFs. The simultaneous visualization of mRNA and protein was achieved by in situ hybridization with a digoxigenin-labeled actin cDNA probe in conjunction with mouse antibodies to tubulin, vimentin, and actin; detection was achieved with a rhodamine-conjugated antibody to digoxigenin (red) and fluorescein-conjugated secondary goat antibodies to mouse protein (green). (**A**) Actin mRNA, red; MTs, green; (**B**) actin mRNA, red; IFs, green; and (**C**) actin mRNA, red; MFs, green. All images were single exposures photographed with a Zeiss ×63 infinity-corrected objective with standard epifluorescence optics. A dual-wavelength filter set was used that allowed simultaneous visualization of rhodamine and fluorescein without optical shift.

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