Ela Induces the Expression of Epithelial Characteristics

Steven M. Frisch

La Jolla Cancer Research Foundation, La Jolla, California 92037

Abstract. Cells closely resembling epithelia constitute the first specific cell type in a mammalian embryo. Many other cell types emerge via epithelial–mesenchymal differentiation. The transcription factors and signal transduction pathways involved in this differentiation are being elucidated. I have previously reported (Frisch, 1991) that adenovirus Ela is a tumor suppressor gene in certain human cell lines. In the present report, I demonstrate that Ela expression caused diverse human tumor cells (rhabdomyosarcoma, fibrosarcoma, melanoma, osteosarcoma) and fibroblasts to assume at least two of the following epithelial characteristics: (*a*) epithelioid morphology; (*b*) epithelial-type intercellular adhesion proteins localized to newly formed junctional complexes; (*c*) keratin-containing intermediate filaments; and (*d*) down-regulation of non-epithelial genes. Ela thus appeared to partially convert diverse human tumor cells into an epithelial phenotype. This provides a new system for molecular analysis of epithelial-mesenchymal interconversions. This effect may also contribute to Ela's tumor suppression activity, possibly through sensitization to anoikis (Frisch, S. M., and H. Francis, 1994. *J. Cell Biol.* 124:619–626).

THE Ela gene of adenovirus has been extensively investigated for diverse reasons. When introduced into various human tumor cell lines, it acts as a tumor suppressor gene, not by killing tumor cells, but by converting them into a nontransformed phenotype (Frisch, 1991). It regulates the transcription of several cellular genes positively or negatively (Shenk and Flint, 1991; Flint and Shenk, 1990; Jones et al., 1988; Braithwaite et al., 1991) and represses a large number of promoters in transient transfection assays (Rochette-Egly et al., 1990). It facilitates the transformation of primary rodent cells by oncogenes such as *ras* (Ruley, 1983). It blocks the differentiation in culture of pheochromocytoma cells into neurons (Maruyama et al., 1987) or myoblasts into myotubes (Webster et al., 1988). In addition, Ela complexes with proteins such as retinoblastoma protein, p107 and p130, thereby interfacing with the cell cycle machinery via cyclins and the transcription factor E2F (reviewed in Moran, 1993; Dyson and Harlow, 1992).

The epithelial cell is characterized by its morphology, extensive intercellular interactions through cell adhesion molecules (Ekblom et al., 1986; Grunwald, 1991; Schmidt et al., 1993), cell polarity (Rodriguez-Boulan and Nelson, 1992), keratin-containing intermediate filaments (Steinert and Roop, 1988), and synthesis of basement membrane proteins. The epithelial-specific intercellular adhesions consist of adherens junctions, tight junctions, and desmosomes. The major proteins involved forming these structures are: cadherins and catenins (adherens junctions; reviewed in Takeichi, 1991; Geiger and Ayalon, 1992), ZO-1, cingulin, and occludin (tight junctions; reviewed in Citi, 1993), desmoplakin, desmoglein, desmocollin, and plakoglobin (desmosomes; reviewed in Koch et al., 1991; Buxton et al., 1993). These adhesions are critical for forming selectively permeable epithelial sheets, for establishing cellular polarity and for regulating pattern formation in epithelial tissues (reviewed in Schmidt et al., 1993; Birchmeier and Birchmeier, 1993; Grunwald, 1991).

The results reported here show that the expression of Ela in certain nonepithelial cells induced the expression of genes encoding various epithelial cell-specific adhesion proteins and the formation of the appropriate intercellular adhesions. Other epithelial features, such as synthesis of keratin-containing intermediate filaments, were induced in some of the cell lines as well. As such, Ela appeared to be capable of reprogramming gene expression to produce a partial or complete epithelial phenotype, depending upon the original cell type.

Materials and Methods

Cell Lines

The tumor cell lines used in this study, A204 rhabdomyosarcoma, HT1080 fibrosarcoma, A2058 melanoma, and Saos-2 osteosarcoma and RD rhabdomyosarcoma were obtained from American Type Culture Collection (Rockville, MD) and subcloned before use. MCF-7 cells were from American Type Culture Collection and were used without subcloning. The Li-Fraumeni fibroblast cell line 172 (Bischoff et al., 1990) was obtained from Dr. Michael Tainsky (M. D. Anderson Cancer Center, Houston, TX). It had been passaged until loss of the wild-type allele of p53 was achieved (M. Tainsky, personal communication). HaCat cells were from R. Fusenig (German Cancer Center, Heidelberg, Germany). Construction of Ela derivatives of the H4 subclone of HT1080 (Frisch and Francis, 1994) and of A2058 melanoma cells (Frisch, 1991) were described. The Ela derivatives of the other cell lines were constructed using methods described previously (Frisch and Francis, 1994) with the retrovirus that contained the 12 S form of adenovirus-5 Ela under control of the SV-40 early promoter.

Address all correspondence to S. M. Frisch, 10901 N. Torrey Pines Rd., La Jolla, CA 92037. Ph.: (619) 455-6480 (437). Fax: (619) 455-0181.

Western Blotting

Western blots were performed using Immobilon filters (Millipore Corp., Bedford, MA), horseradish peroxidase-conjugated secondary antibodies (Boehringer Mannheim Biochemicals, Indianapolis, IN) and ECL detection (Amersham Corp., Arlington Heights, IL). Primary antibodies were from the following sources: anti-Ela clone M73 (Oncogene Science Inc., Manhasset, NY), anti-desmoplakin polyclonal antibody NW6 (Kathleen Green, Northwestern University, Evanston, IL), or anti-desmoglein monoclonal antibody (Pamela Cowin, New York University, New York), anti-desmoglein monoclonal antibody clone 3.10 (BioDesign, Kennebunkport, Maine), anti-ZO-1 polyclonal antibody no. 7445 (James Anderson, Yale University, New Haven, CT), anti-pan-cadherin polyclonal antibody C3678 (Sigma Chemical Co., St. Louis, MO), anti-E-cadherin (DECMA-1; Sigma), anti-desmin (Oncogene Science; clone DE-B1). In all Western blots, equal protein loading of the SDS-PAGE gels was confirmed by Amido black staining of the blots after development with the ECL reagent.

Northern Blotting

Northern blotting was performed using formaldehyde-agarose gels and nitrocellulose (Schleicher & Schuell, Keene, NH) filters on RNAs (12 μg) that were prepared by guanidine thiocyanate/cesium chloride gradients. Equal RNA loading was verified by photographing the ethidium bromide-stained gels prior to transfer. cDNA probes were made random-primer labelling (Strategene Corp., La Jolla, CA) of restriction fragments in low-melt agarose gel slices. Probes were from the following sources: human desmoplakin (pDPI/II; K. Green, Northwestern University, 1,800-bp HindIII fragment), human desmoglein-2 (W. Franke, German Cancer Research Institute, Heidelberg; phDsg2.pc2a, 800-bp EcoRI fragment), human plakoglobin (W. Franke, pHPGCa2.1, 1540 bp SacI fragment), E-cadherin (pCO5.3; David Rimm, Yale University), human keratin 18 (pK189; R. Oshima, La Jolla Cancer Research Foundation; EcoRI/BamHI 1,078-bp fragment), human keratin 8 (pK812; R. Oshima; XhoI/ScaI 1,496-bp fragment), human fibronectin (pBS70K; Alex Morla, La Jolla Cancer Research Foundation; EcoRI/XbaI 592-bp fragment), glyceraldehyde-phosphate-dehydrogenase (pGAPDH;[1] ATCC, 800-bp XbaI/PstI fragment), tyrosinase (pKS-E1-3.4-HR2.1; M. Spritz, Yale University; 2.1-kb HindIII-EcoRI fragment).

Immunofluorescence

Cells were grown on 2 cm gelatin-coated coverslips. Fixation was either with cold methanol (1 min for desmoplakin or desmoglein, 10 m for keratins) or with 1% paraformaldehyde for other proteins (10 m) followed by 10 min permeabilization in 0.1% Triton X-100. Images were photographed on a Zeiss Axiophot microscope using the 63× lens. Antibodies were as described above; anti-keratin 18 was monoclonal CK5 (R. Oshima). Sodium-potassium ATPase was localized by staining with 5 μg/ml anthroyl-ouabain (Sigma Chemical Co.) in complete growth medium for 30 min, followed by three washes in medium and fluorescent microscopy using a UV filter (G. Fortes, University of California at San Diego, San Diego, CA, personal communication).

Other Microscopy

Domes were documented on a Zeiss LSM410 laser scanning microscope with confocal fluorescence of laser scanning bright field and images recorded digitally on disk. Final magnification shown in photographs was 365× (1 cm = 63 m). For electron microscopy, cells were fixed in modified Karnovsky's fixative (2% paraformaldehyde; 1.5% glutaraldehyde in 0.1 M cacodylate, pH 7.4) for 1–2 h at 4°C, postfixed in 1% OsO_4 in the same buffer for 1 h at room temperature (RT); transferred to 1% tannic acid in 0.02 M cacodylate buffer for 30 min at RT and stained enblock in 1% aqueous uranyl acetate for 1 h at RT. Fixed cells were dehydrated in graded ethanol and flat-embedded in epoxy resin. Thin sections were cut parallel to the culture dish and stained with uranyl acetate and lead citrate and viewed with a Hitachi HU12-A electron microscope.

1. *Abbreviations used in this paper*: aa, amino acid; DSG2, desmoglein-2; FB, 172 fibroblast; FS, HT1080 fibrosarcoma; OS, Saos-2 osteosarcoma; pGAPDH, glyceraldehyde-phosphate-dehydrogenous; RM, A204 rhabdomyosarcoma; RM2, RD rhabdomyosarcoma; RT, room temperature.

Electrical Resistance

Cells (2×10^5) were plated onto Falcon filter inserts (25 mm) that had been precoated for 1 h with 100 μg/ml Matrigel (Collaborative Research, Lexington, MA). 2 d later, electrical resistance across the filters was measured using an epithelial voltohmeter (World Precision Instruments, Sarasota, FL).

Results

Ela Expression Causes Conversion to an Epithelioid Morphology

A retrovirus that transduces the 12 S (243-amino acids [aa]) form of adenovirus-5 Ela (Frisch, 1994) was used to generate stable Ela-expressing cell lines from the following parental cells: HT1080 fibrosarcoma (FS), A204 rhabdomyosarcoma (RM), RD rhabdomyosarcoma (RM2), Saos-2 osteosarcoma (OS), and fibroblasts spontaneously immortalized by deletion of p53 (Li-Fraumeni fibroblasts; FB); Ela derivatives of A2058 constructed by transfection were described (Frisch, 1991). Expression of Ela protein in G418-selected clones was verified by Western blotting (Fig. 1 *a*). All of the Ela-expressing cell lines adopted an epithelioid morphology (Fig. 1 *b*). Because of the large variation in Ela expression levels amongst different G418-resistant clones, individual clones were analyzed instead of pooling together large numbers of clones, which could have introduced the risk of unpredictable selection for a particular Ela expression level.

Expression of Epithelial-type Cell Adhesions of Ela-expressing Cell Lines

The morphologic properties of the Ela-expressing cell lines suggested that they had assembled epithelial-type junctional complexes. This was investigated by electron microscopy, immunofluorescence, Western blotting, and Northern blotting.

Desmosome formation requires the expression and assembly of demosplakin, desmoglein, desmocollin, plakoglobin and other proteins (Koch et al., 1991; Buxton et al., 1993).

Ela-expressing cell lines were subjected to immunofluorescence using anti-desmoplakin and desmoglein antibodies. Punctate pericytoplasmic structures closely resembling normal desmosomes were detected with desmoplakin antibody in Ela-expressing fibrosarcoma and rhabdomyosarcoma (Fig. 2) but neither in parental nor in Ela-expressing derivatives of other parental cell lines. In the fibrosarcoma cells, some perinuclear staining was also observed, which night reflect the binding of desmoplakin to intermediate filaments (Green and Jones, 1990). Although the desmosome density was lower in the Ela/FS than in the Ela/RM cells, well-formed desmosomes of double-leaflet structure (such as those arrowed) were readily detectable.

To determine whether Ela induced the expression of genes encoding desmosomal proteins, Northern blots were performed. The desmoplakin gene was induced by Ela to various extents in all of the tumor cell lines examined (although very weakly in the osteosarcoma cells) as well as in fibroblasts (Fig. 3). Expression of desmoglein-2 (DSG2), which is the form ubiquitously expressed in desmosome-bearing cells (Koch et al., 1991; Buxton et al., 1993), was induced by Ela in both of the rhabdomyosarcoma, melanoma, and

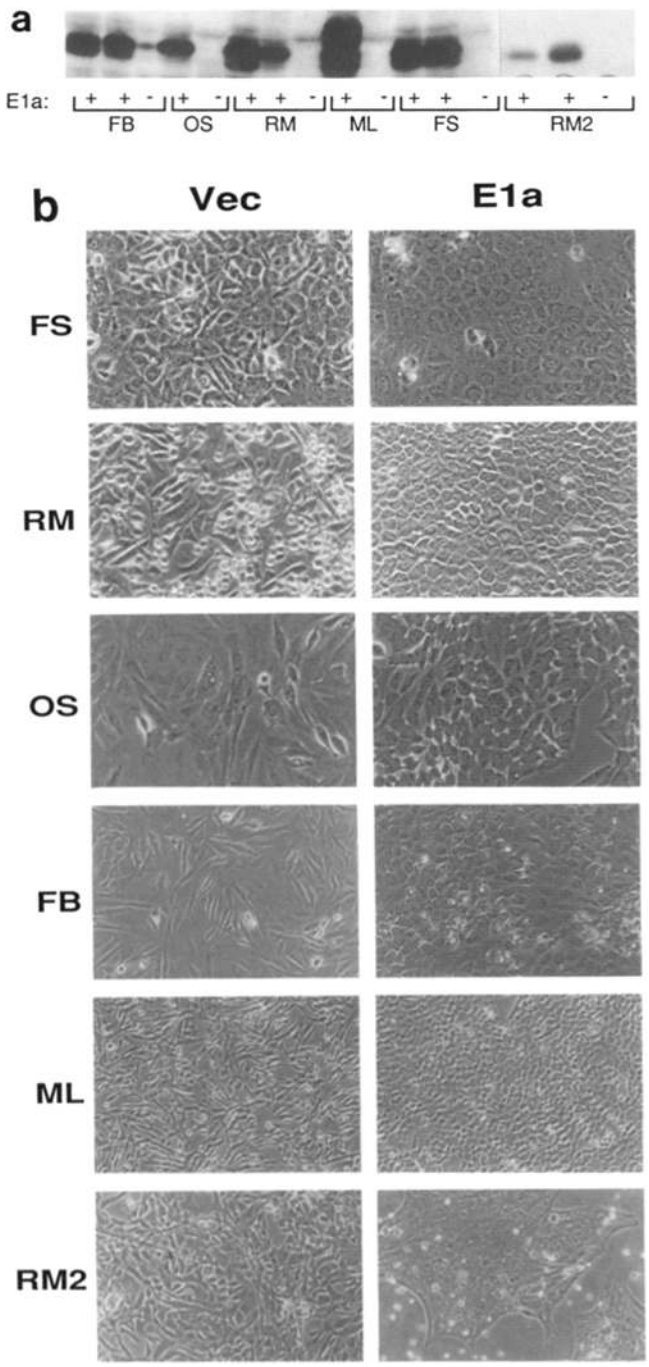


Figure 1. Expression of Ela and its effect on cell morphology. (*a*) Expression of Ela protein in human tumor cells and fibroblasts infected with Ela retrovirus. Tumor cells were infected with the Ela-containing retrovirus, 4LNSX/8 (described in Frisch and Francis, 1994) or by transfection in the case of the ML cells (Frisch, 1991). After selection of G418-resistant clones, Ela expression was tested by Western blotting using anti-Ela antibody M73. Cell lines are abbreviated throughout as follows: fibroblasts (*FB*), osteosarcoma (*OS*), rhabdomyosarcoma (*RM* or *RM2*), melanoma (*ML*), or fibrosarcoma (*FS*). (*b*) Morphologies of representative Ela-expressing clones and "empty vector-infected" (control) cell lines were documented by phase-contrast microscopy.

fibroblast cell lines (Fig. 3 *a*). (Western blotting revealed low-level DSG2 protein expression in the fibrosarcoma cells in the presence or absence of Ela; data not shown.) Confirmation that desmoplakin and desmoglein proteins of correct molecular weight were induced by Ela was obtained by Western blotting lysates of the most highly induced clone, Ela/RM No. 4.2 (Fig. 3 *b*). Another desmosomal protein gene, desmocollin 3, was also induced by Ela in the RM and FS cells.

The plakoglobin gene, whose protein is found both in desmosomes and adherens junctions (where it is associated with cadherins) was induced by Ela in all of the cell lines examined (Fig. 3) (except in RM2, where it was constitutively expressed; data not shown). Overall, the fibrosarcoma and RM cells had the highest desmosomal protein expression levels and the detection of desmosomes by immunofluorescence was optimal in these cell lines.

Electron microscopic analysis of the Ela-expressing RM cells revealed junctional structures, some of which resembled desmosomes (Fig. 4). These occurred in virtually every field; they could not be detected in the cells infected with control retrovirus. Although the electron microscopy alone was not of sufficient resolution to unequivocally identify these cell structures as desmosomes, this interpretation was consistent with the immunofluorescence data.

Electron microscopy (Fig. 4) also revealed the presence of extended electron-dense membranous junctions in the Ela/FS cells, as well as occasional desmosome-like structures. The resolution of the electron microscopy did not permit a distinction between zonulae adherens versus tight junctions (Stevenson et al., 1988). However, they were detected only in the Ela-expressing derivative cell lines, and could not be detected in the cells infected with control retrovirus.

Further evidence for tight junctions was provided by immunofluorescent and Western blot analysis of the tight junction protein ZO-1 (Stevenson et al., 1986). This revealed weak, diffuse fluorescence in rhabdomyosarcoma cells, which was induced by Ela to a level and staining pattern similar to that found in epithelial cells (Fig. 5; Stevenson et al., 1986, 1988), indicating the presence of tight junctions. Pericytoplasmic ZO-1 staining was found in the parental fibrosarcoma cells in a punctate pattern, consistent with the published observation that ZO-1 is expressed in some non-epithelial cell lines (Howarth et al., 1992). Ela caused the ZO-1 pattern staining to become more continuous in the fibrosarcoma cells, correlating with the incidence of putative tight junctions observed by electron microscopy.

Certain epithelial cells with well-formed tight junctions are reportedly capable of forming "domes". These structures result from the basolateral localization of sodium-potassium ATPase, resulting in uptake of water beneath the cell layer to equilibrate the osmotic pressure. Confluent cultures of Ela-expressing rhabdomyosarcoma cells were examined for dome formation by phase-contrast and confocal microscopy (Fig. 6). Dome formation was observed at a frequency of about 200 domes in 5×10^6 cells. No domes were observed in the empty vector-infected parental cells. Thus, Ela-expressing cells were apparently capable of forming tight junctions and polarizing to an extent that allowed dome formation.

Direct evidence for the basolateral localization of the sodium-potassium ATPase was obtained by staining con-

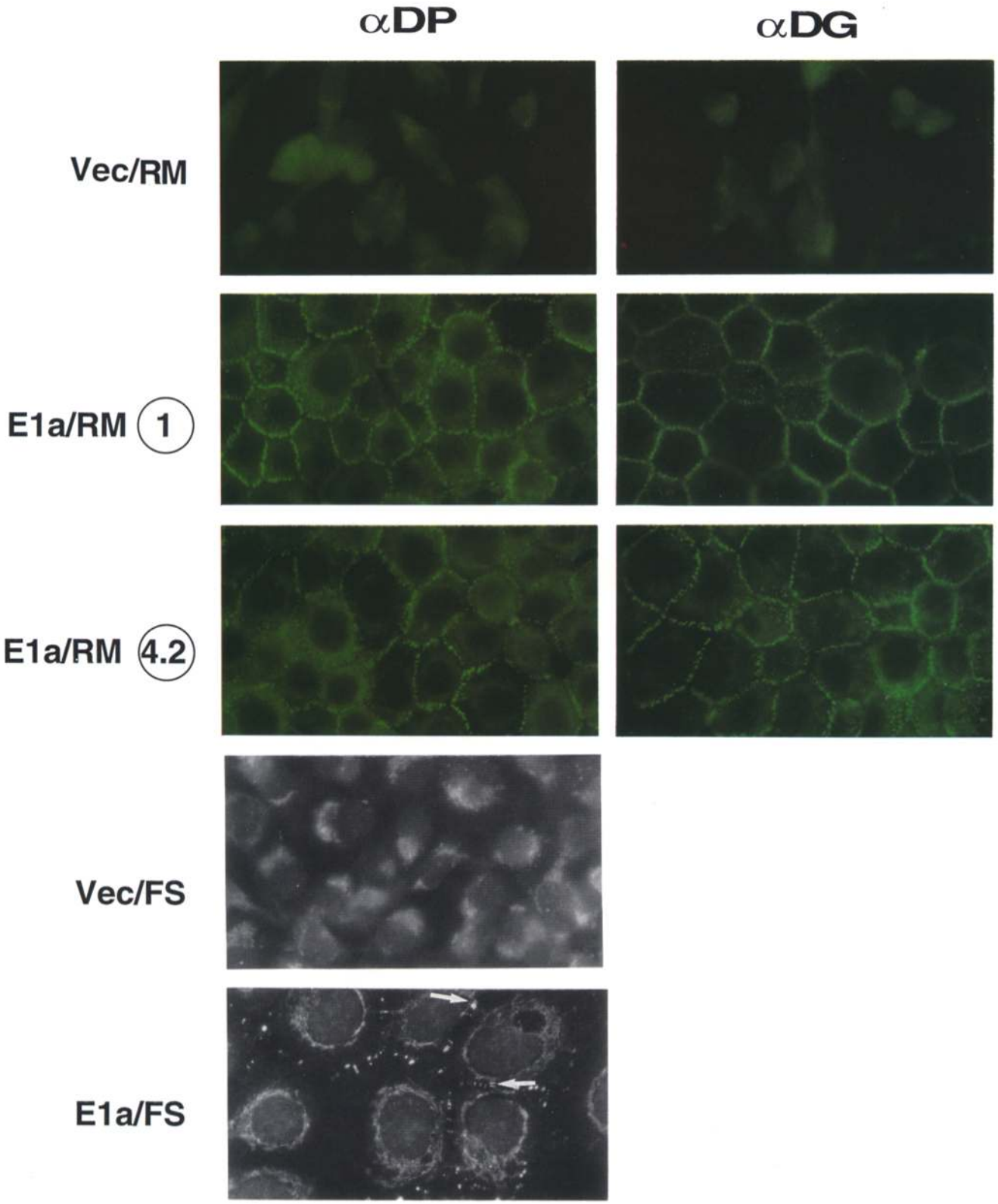


Figure 2. Assembly of desmosomes in Ela-expressing RM and FS cell lines, (*Ela/RM* or *Ela/FS*), or control cell lines (*vec/RM*, *vec/FS*). These were detected by immunofluorescence using anti-desmoplakin (*α-DP*) or anti-desmoglein (*α-DG*) antibodies, as described in Materials and Methods. Particularly well-formed desmosomes in the Ela/FS cells are arrowed.

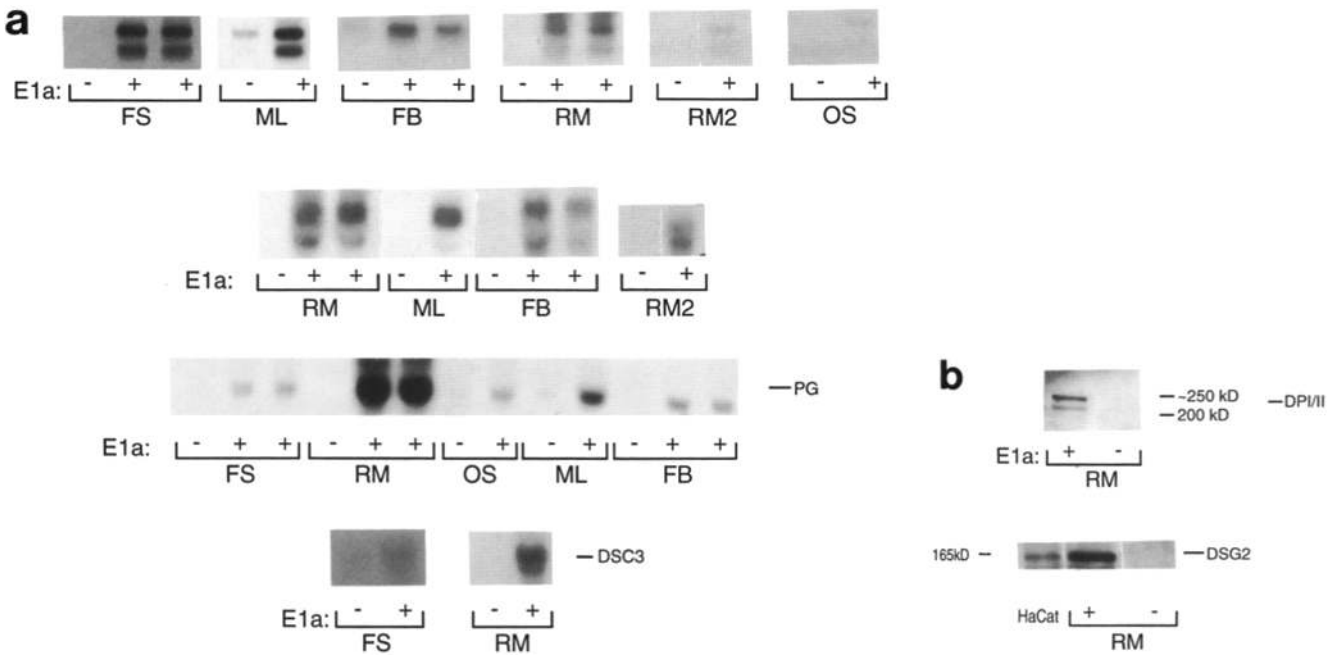


Figure 3. Expression of desmosomal proteins in Ela-expressing cell lines. RNAs from vector-infected (*Ela*−) or Ela retrovirus-infected (*Ela*+) cell lines were probed on Northern blots (*a*) for desmoplakin (*DP*), desmoglein-2 (*DSG2*), plakoglobin (*PG*) or desmocollin-3 (*DSC3*) expression. To confirm expression of the corresponding protein of the correct molecular weight, Western blots (*b*) of indicated protein lysates were probed with DP or DSG2 antibodies, as described in Materials and Methods. Molecular weights were calculated relative to rainbow markers (Amersham Corp.). Protein from a human keratinocyte line HaCat was also run as a control.

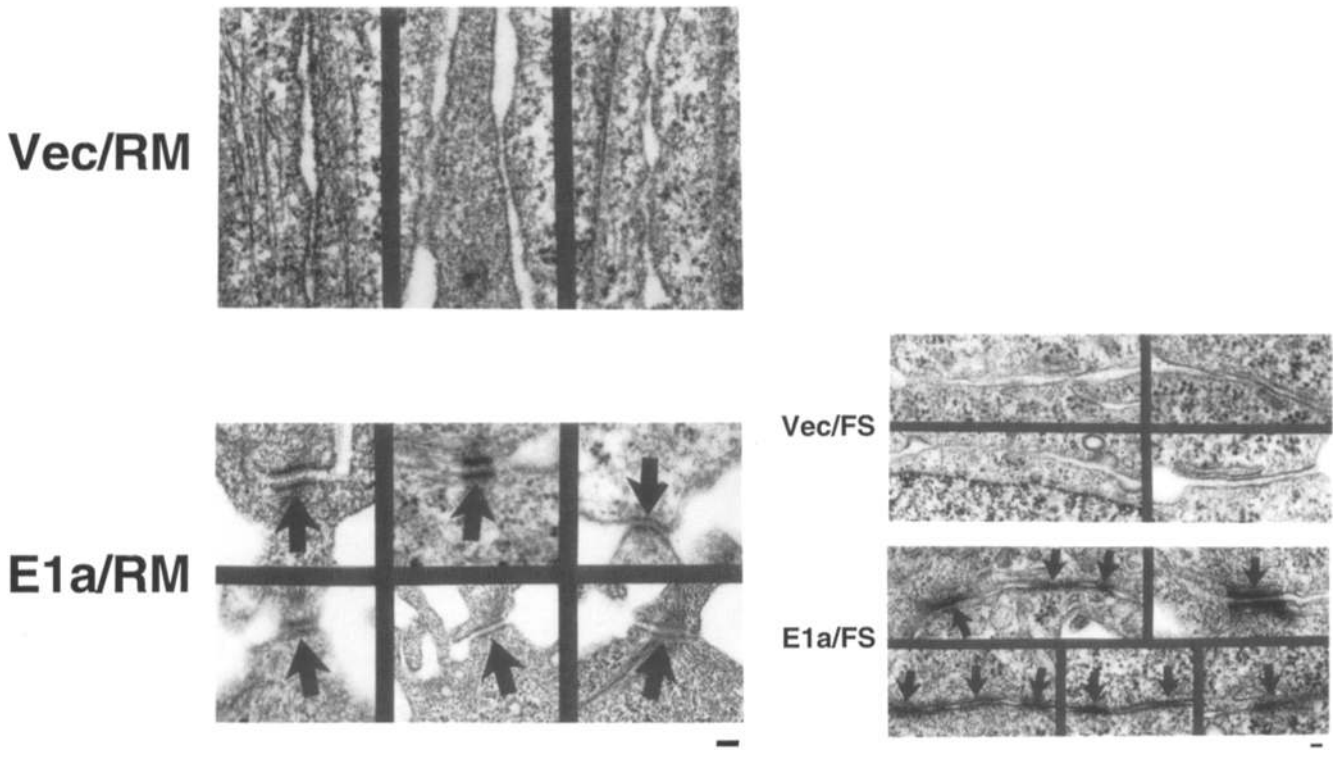


Figure 4. Electron microscopic analysis of cell–cell adhesions in parental tumor cells (*vec/FS*, *vec/RM*) and Ela-expressing (*Ela/FS*, *Ela/RM*) derivative cells. Electron microscopy was carried out as described in Materials and Methods. The positions of electron-dense putative adhesional structures are arrowed. Bars, 1 μm.

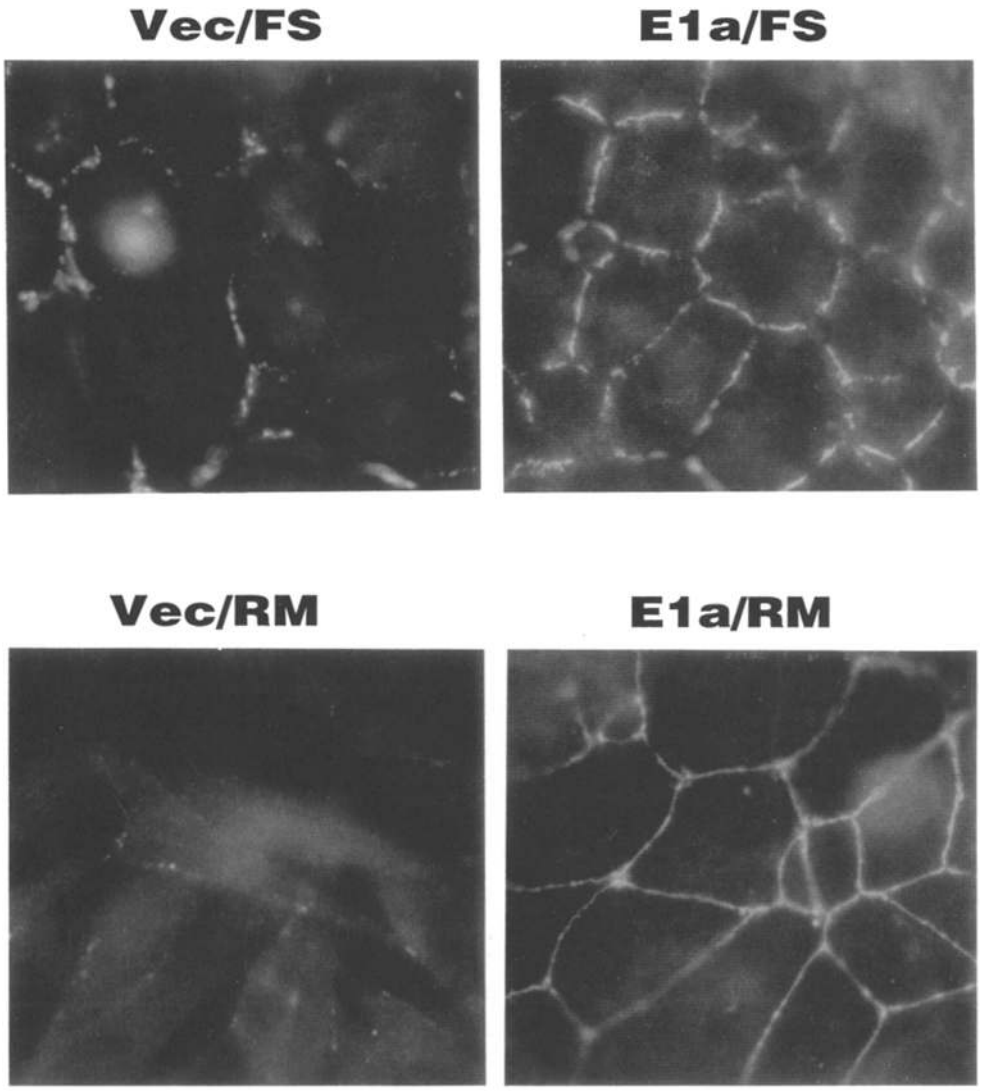


Figure 5. Analysis of the expression and localization of the tight junction protein ZO-1. (*Left*) Expression of ZO-1 protein in rhabdomyosarcoma cells and Ela derivatives: analysis by Western blotting. (*Right*) Immunofluorescent localization of the tight junction protein ZO-1 in control (*vec/FS*, *ve/RM*) and Ela-expressing (*Ela/FS*, *Ela/RM*) cells.

fluent monolayers of RM cells with anthroyl-ouabain (Fig. 6). While the parental cells showed diffuse cytoplasmic distribution, the Ela/RM cells showed the lateral staining pattern that is characteristic of epithelial cells.

Functionality of the tight junctions was also tested by measuring the electrical resistance across confluent sheets of Ela-expressing cells, which were grown on matrigel-coated filter inserts. The empty vector-infected rhabdomyosarcoma cells had no measurable electrical resistance above filter background. Ela/rhabdomyosarcoma clone No. 1 and MDCK (control) epithelial cells had resistances of 250Ω/cm² and Ela/rhabdomyosarcoma clone No. 4.2 had a resistance of 204 Ω/cm². Ela induced the formation of tight junctions that could block ionic flow as efficiently as those in authentic epithelial cells.

Homotypic interactions between cadherins on adjacent epithelial cells can initiate junctional complex formation (e.g., Gumbiner et al., 1988; Geiger and Ayalon, 1992). Because of the large number of cadherin family members (Geiger and Ayalon, 1992) protein lysates of the Ela-expressing cell lines were initially probed with an antibody that recognizes all cadherins through a 21 aa conserved cytoplasmic epitope. Ela induction of a cadherin band was observed only in the RM cells (Fig. 7). This band was identified as E-cadherin by reaction with an E-cadherin–specific antibody (Fig. 7). A Northern blot probed with an E-cadherin probe confirmed

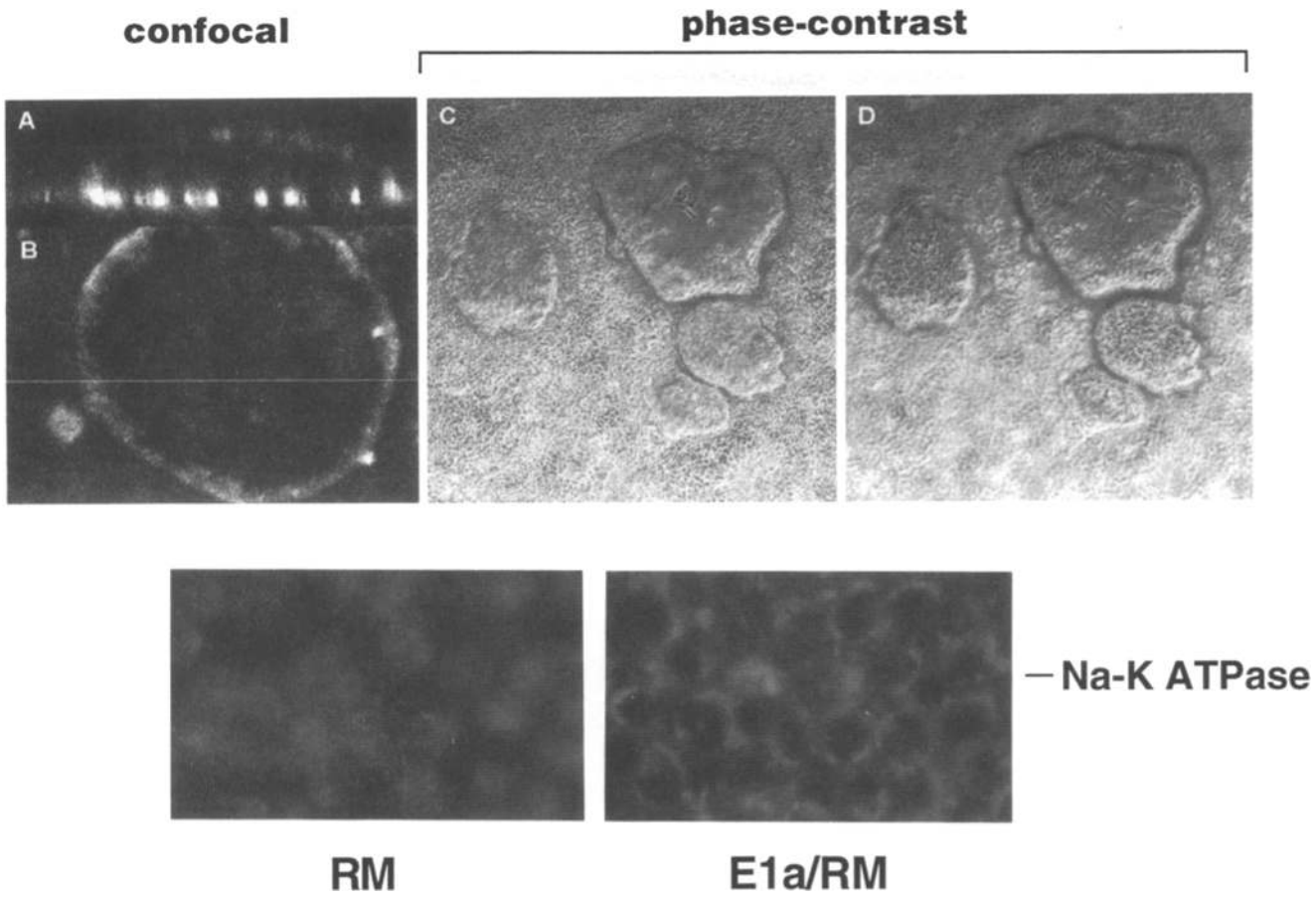


Figure 6. Dome formation and polarized distribution of Na-K ATPase in Ela-expressing RM cells. (*a*) Domes were identified by phase contrast microscopy as depicted in *C* and *D*; these represent different focal planes where cells around the top rim of the dome (*C*) or at the bottom surface (*D*) were in focus. *A* and *B* show confocal microscopy of a fluorescently labeled dome, viewing the xy plane (*B*) or along the xz plane (*A*). (*b*) The distribution of sodium-potassium ATPase in confluent cultures of RM or Ela/RM cells was visualized by staining with anthroyl-ouabain, followed by fluorescence microscopy, as described in Materials and Methods.

that E-cadherin mRNA was induced by Ela in the RM (Fig. 7) but not in the FS or RM2 cell lines (data not shown).

Induction of Other Epithelial Markers and Effects on Non-epithelial Markers

Because simple epithelial cells contain intermediate filaments composed of keratins type 8 and 18 (Oshima et al., 1983), Northern blots were probed to determine whether Ela induced these genes. Ela induced keratin 18 expression in RM and melanoma cells and induced keratin 8 expression in the RM cells (Fig. 8 *a*). Based on these results, keratin-containing intermediate filaments were expected to be found

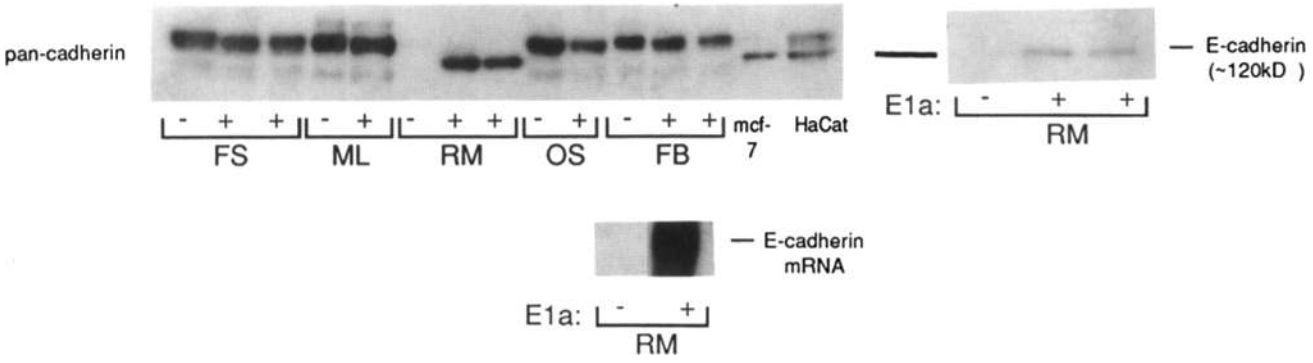
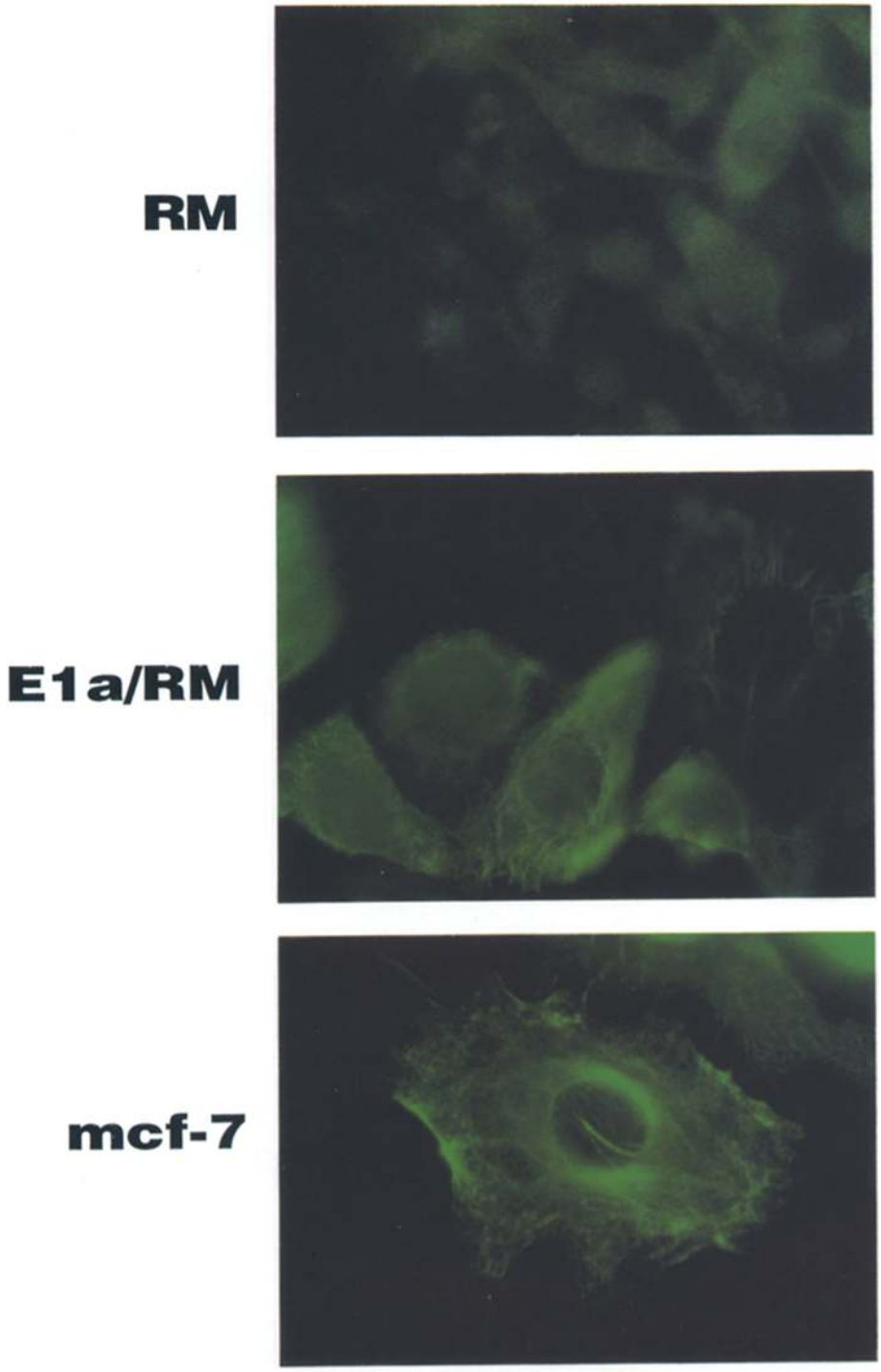


Figure 7. Expression of cadherins in Ela-expressing cell lines. (*Top*) A Western blot containing protein lysates from parental (−) or Ela-expressing (+) cell lines was probed with pan-cadherin antibody or E-cadherin–specific antibody, as described in Materials and Methods; lysates from mammary carcinoma cell line mcf-7 and from the keratinocyte cell line HaCat were used as positive controls. (*Bottom*) The Northern blot, containing RNAs from parental (−) or Ela-expressing (+) RM cells was probed with an E-cadherin–cDNA probe.



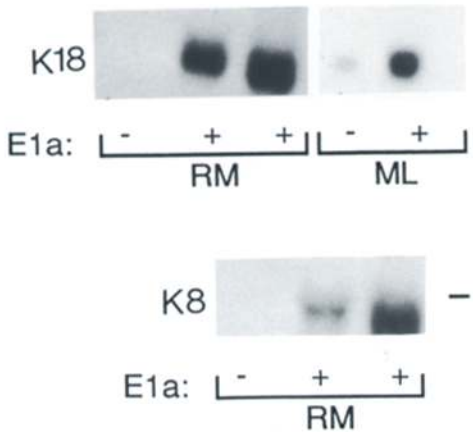


Figure 8. Expression of keratins in Ela-expressing cell lines. (*Left*) The Northern blot, containing RNAs from parental (−) or Ela-expressing (+) RM or ML cells was probed with human keratin 18 (*K18*) or human keratin 8 (*K8*) probes. the expression levels were similar to those seen in HeLa cell control (data not shown). (*Middle*) Ela/RM clone 4.2, vec/RM (negative control) or mammary carcinoma mcf-7 cells were stained with anti-K18 antibody CK5 and photographed on a fluorescence microscope.

in the Ela-expressing rhabdomyosarcoma cells. Immunofluorescent antibody staining confirmed the presence of these intermediate filaments (Fig. 8 *b*).

Ela did not induce gene expression globally. The gene for the glycolytic enzyme GAPDH was unaffected by Ela expression (Fig. 9), except in the osteosarcoma cells, where it was slightly induced.

To determine whether Ela repressed the expression of certain nonepithelial genes, RM2 cells were probed for desmin protein, cardiac α-actin mRNA and myoD mRNA (Fig. 9). The expression of these muscle-specific gene products was repressed by Ela. The melanocyte-specific tyrosinase gene was down-regulated in the melanoma cells as well. Ela also down-regulated the fibronectin gene, which is usually expressed at higher levels in mesenchymal than in epithelial cells.

Discussion

Programmed interconversions between epithelial cells and mesenchymal cells occur during development (Hay, 1992). An important goal is to identify the regulatory molecules and mechanisms involved in these interconversions. This may also contribute to an understanding of how the epithelial phenotype is compromised by oncogenic transformation. Because Ela is a viral gene, it is not normally involved in these interconversions. However, analysis of them may be facilitated by the use of Ela, as demonstrated by the results presented herein.

The adenovirus Ela gene was previously shown to be a tumor suppressor gene in various human cell lines (Frisch, 1991). Intuitively, a tumor suppressor gene might be expected to revert tumor cells to their pre-transformed phenotype. However, as reported herein, cell lines expressing Ela did not resemble their corresponding parental phenotypes; for example, melanoma cells that expressed Ela (Frisch, 1991) did not morphologically resemble melanocytes. Instead, the morphologies of Ela-expressing cell lines constructed in this laboratory, as well as those depicted photographically in the literature (Maruyama et al., 1987; Taylor et al., 1993) resembled that of epithelial cells, regardless of the parental cell type. The morphologic effects were clearly due to Ela expression rather than selection of spontaneously occuring variants for the following reasons: (*a*) all of the parental cell lines were subcloned prior to introduction of Ela; (*b*) after infection with the empty retrovirus vector and G418 selection, no epithelioid colonies were observed; (*c*) every clone that expressed Ela had an epithelioid morphology; (*d*) colonies of epitheloid morphology were observed within 3–5 d after infection, rendering the hypothetical requirement for secondary stochastic changes unlikely; and (*e*) microinjection of Ela expression plasmids into HT1080 induced desmosomes which were detectable by immunofluorescence two days after injection (data not shown).

The combined data in this report suggest that Ela is capable of programming several aspects of the epithelial phenotype. The effects of Ela appeared to reflect an epithelial-specific programming event rather than generalized activation of transcription. The 243-aa Ela protein used in this study is not a general activator of transcription (Shenk and Flint, 1991; Berk, 1986; Braithwaite et al., 1991). It did not affect the expression of a housekeeping gene (GAPDH), and it repressed the expression of non-epithelial genes, indicating the specificity of Ela's effects.

Certain genes have been shown previously to cause the loss of epithelial characteristics. Oncogenes such as c-*fos* (Reichmann et al., 1992) and v-*src* (Warren and Nelson,

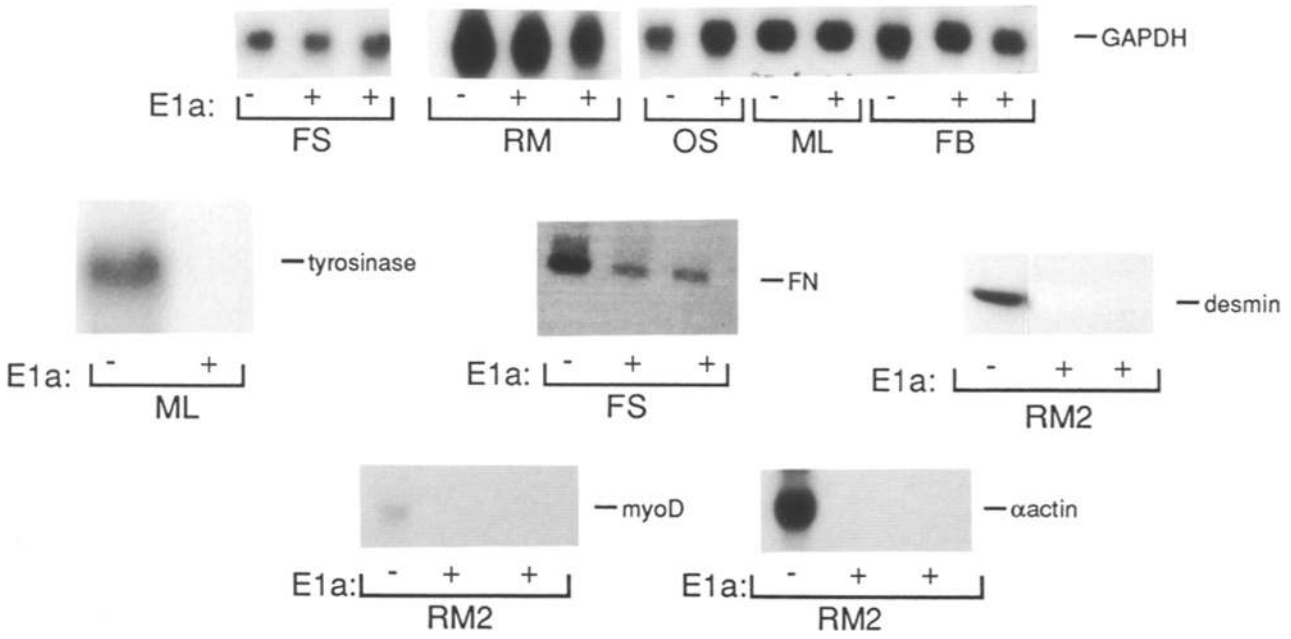


Figure 9. Expression of housekeeping (*GAPDH*) or non-epithelial-specific genes (*tyrosinase*, *desmin*, cardiac *α-actin*, fibronectin [*FN*], *myoD*) in Ela-expressing cell lines. Western blots (*desmin*) or Northern blots (other panels) were reacted with the anti-desmin antibodies or the appropriate cDNA probes as described in Materials and Methods.

1987), protein kinase C activators (Ojakian, 1981), and scatter factor (Schmidt et al., 1993; Behrens et al., 1991) can each cause epithelial cells to lose their intercellular adhesions and polarity. This activity of scatter factor, acting through the c-*met* receptor, may play an important role in the normal epithelial–mesenchymal interactions. Disruption of E-cadherin interactions, either indirectly through c-*met*, or directly (by the use of anti-E-cadherin antibodies) causes the transient breakdown of most junctional complexes and loss of epithelial morphology.

Ectopic expression of E-cadherin in fibroblasts causes cell–cell aggregation, formation of epithelial-type cellular sheets and polarized distribution of certain cell surface markers (Nagafuchi et al., 1987; Edelman et al., 1987; NcNeill et al., 1990). The de novo induction of epithelial gene expression by E-cadherin has not been reported. However, it is interesting that the RM cell line—the only cell line in which E-cadherin expression had been induced by Ela—showed the most comprehensive induction of other epithelial genes. Conceivably, the presence of E-cadherin could promote epithelial gene induction by Ela in this cell line. It will be of interest to perform the appropriate transfection experiments to address this possibility.

Developmentally, mesenchymal–epithelial transitions occur during nephrogenesis, in which the product of the WT-1 tumor suppressor/transcription factor gene has been implicated, although the transfection of this gene has failed to initiate the process in cultured cells (Rauscher, 1993). The 12 S form of Ela used in this study did not induce expression of WT-1 (data not shown).

Epithelialization directed by two genes has very recently been reported. The extracellular signaling molecule encoded by Wnt-1 causes PC12 pheochromocytoma (neural tumor) cells to adopt an epithelial morphology and elevated expression of plakoglobin and E-cadherin (Bradley et al., 1993). Interestingly, the retinoic acid–induced neuronal differentiation of P19 embryonal carcinoma cells involves an epithelioid intermediate (Schmidt et al., 1992).

Epithelialization of fibroblasts has been demonstrated in one rather complex system (Tsarfaty et al., 1994). When 3T3 cells are cotransfected with the genes encoding scatter factor and the scatter factor receptor, c-*met*, and then injected into mice, tubular structures form whose cells express epithelial cell adhesion molecules. This epithelialization may involve a complex interplay of matrix, growth factor, and cell-associated signals provided by the injected mouse.

Ela thus appears to be the first protein capable of conferring an extensive epithelial commitment upon mesenchymal cells in culture, especially the rhabdomyosarcoma cells. Genes encoding epithelial cell adhesion molecules responded to Ela to varying degrees in the various cell lines; however, cell type-specific responses of a given gene to a transcription factor have been widely reported in the literature.

By what mechanism could Ela induce an epithelial phenotype? Myogenic conversion by the muscle-specific transcription factor myoD involves the direct activation of muscle-specific promoters. However, Ela is not a DNA-binding protein. In addition, because no epithelial-specific transcription factor has been indentified to date, a different paradigm should probably be invoked for epithelial conversion by Ela. The "proto-epithelial" cell (Fleming and Johnson, 1988) emerges at around the time of compaction of the mammalian eight-cell embryo, being the earliest identifiable embryonic cell type. It possesses intercellular adhesions involving E-cadherin, tight junctions, and cytokeratin filaments. All primary mesenchymal cells arise by differentiation from these proto-epithelial cells; some of this mesenchyme will convert into epithelia, which can in some cases be the precursor of secondary mesenchyme (e.g., skeletal muscle) (Hay, 1990; Fleming and Johnson, 1988; Birchmeier and Birchmeier, 1993).

It may prove useful therefore to conceptualize the epithelial cell as a "default phenotype" for mammalian cells. Under this model, epithelial gene expression involves only the interplay of ubiquitous transcription factors. Tissue-specific transcription factors both activate appropriate tissue-specific gene expression and repress epithelial-type gene expression (e.g., muscle creatine kinase and keratin 18, respectively). The model predicts that epithelial-type genes are under repression in non-epithelial cells. A relevant example of this is the epithelial-specific promoter of the human papilloma virus p97 gene. Its epithelial transcription is driven purely by "ubiquitous" activator proteins, including the factor NF-1/CTF. Fibroblasts express both NF-1/CTF and an additional NF-1 family member (called NF-1X) that represses transcription through the NF-1 sites of the promoter (Apt et al., 1993).

The default phenotype model suggests that the epithelial conversion effects of Ela may largely reflect de-repression of epithelial promoters resulting from inhibition of the expression or transcriptional activities of non-epithelial transcription factors. Analysis of the interactions of Ela with cellular proteins will reveal the first step of the process. The NH_2 terminus of Ela interacts with several potentially global transcriptional regulators. These include: (*a*) p300, a nuclear DNA-binding phosphoprotein (reviewed in Moran, 1993); (*b*) Dr-1, which itself interacts with TATA box binding protein (Inostroza et al. 1992); (*c*) helix-loop-helix domains of (at least) myogenin and E12 (Taylor et al., 1993) which may be involved in cell differentiation. The role of retinoblastoma protein binding to Ela is unclear in this context. Although this protein regulates the transcription factor E2F, the known target genes for this factor are ubiquitously expressed, cell cycle-related genes such as c-*myc* and DHFR (reviewed in Nevins, 1992).

Although the significance of this work is primarily in the molecular biology of cell differentiation, it also may be informative to consider a new function of Ela for adenovirus. Adenovirus has evolved to infect human airway epithelial cells. However, it it infects a non-epithelial cell (e.g., tonsillar fibroblast) a gene such as Ela could be of substantial benefit to the virus. By altering the transcriptional machinery of the cell to (even modestly) activate epithelial-type promoters, Ela would presumably stimulate the transcription of adenovirus promoters even in the originally inappropriate host cell type. This would allow some probability of viral life cycle completion.

In summary, the conversion into an epithelial phenotype caused by Ela provides a new system for investigating the intracellular mechanisms by which epithelial and mesenchymal cells interconvert during development (reviewed in Hay, 1990). Because the phenotypic effects of Ela are mediated by its interactions with a discrete set of cellular proteins

(Dyson and Harlow, 1992; Moran, 1993), it may be possible to use this phenomenon to molecularly analyze the epithelial–mesenchymal transition, albeit run in reverse. It also may provide a conceptual framework for understanding the diversity of transcriptional effects of E1a: by altering a master switch that converts a mesenchymal cell to an epithelial cell, the transcription of a large number of genes could be affected.

The tumor suppressive effect of E1a may result in part from its induction of intercellular adhesion molecules (Tsukita et al., 1993), which may provide a new approach for understanding and controlling cancer. In particular, epithelial cells containing junctional complexes are sensitive to anoikis, a form of apoptosis induced by the disruption of cell–matrix interactions (Frisch and Francis, 1994; Ruoslahti and Reed, 1994).

Finally, it is interesting that the cells of the mouse morula or blastocyst contain an E1a-like functional (i.e., transcriptional) activity (Suemori et al., 1988). This activity disappears upon implantation of the embryo. Conceivably, there is a cellular gene functionally analogous to E1a that maintains the epithelial phenotype during early development.

In addition to those who provided clones and antibodies listed in Materials and Methods, I would like to thank Drs. E. Ruoslahti, H. Baribault, and R. Oshima for critical reading of the manuscript, Dr. D. Herzlinger (Cornell University, New York, NY) for helpful discussions, Dr. H. Chang (The Scripps Research Institute) for electron microscopy, Dr. M. Schibler (La Jolla Cancer Research Foundation) for significant help with other microscopy, Dr. G. Fortes (University of California at San Diego) for protocols on anthroyl-ouabain staining, Dr. M. Hasham for artwork, and Dr. Z. Werb for teaching me the importance of looking at cells.

Although no explicit funding for this project was available, funds were borrowed from National Institutes of Health grant number R29 GM44573-05.

Received for publication 4 April 1994 and in revised form 17 August 1994.

References

Apt, D., T. Chong, Y. Liu, and H. Bernard. 1993. Nuclear factor I and epithelial-cell-specific transcription of the human papillomavirus 16. *J. Virol.* 67:4455–4463.

Behrens, J. 1993. The role of cell adhesion molecules in cancer invasion and metastasis. *Breast Cancer Res. Treat.* 24:175–184.

Behrens, J., K. Weidner, U. Frixen, J. Schipper, M. Sachs, N. Atakaki, Y. Saikuhara, and W. Birchmeier. 1991. The role of E-cadherin and scatter factor in tumor invasion and cell motility. *In* Cell Motility Factors. I. Goldberg, editor. Birkhauser Verlag, Basel. 109–126.

Behrens, J., U. Frixen, J. Schipper, M. Weidner, and W. Birchmeier. 1992. Cell adhesion in invasion and metastasis. *Semin. Cell Biol.* 3:169–178.

Berk, A. 1986. Adenovirus promoters and E1a transactivation. *Annu. Rev. Genet.* 20:45–79.

Bischoff, F., S. Kim, S. Pathik, G. Grant, M. Siciliano, B. Giovanella, L. Strong, and M. Tainsky. 1990. Spontaneous abnormalities in normal fibroblasts from patients with Li-Fraumeni cancer syndrome: aneuploidy and immortalization. *Cancer Res.* 50:7979–7984.

Birchmeier, C., and W. Birchmeier. 1993. Molecular aspects of mesenchymal-epithelial interactions. *Annu. Rev. Cell Biol.* 9:511–540.

Blau, H., and D. Baltimore. 1991. Differentiation requires continuous regulation. *J. Cell Biol.* 112:781–783.

Bradley, R., P. Cowin, and A. Brown. 1993. Expression of Wnt-1 in PC12 cells results in modulation of plakoglobin and E-cadherin and increased cellular adhesion. *J. Cell Biol.* 123:1857–1865.

Braithwaite, A., C. Nelson, and A. Bellet. 1991. E1a revisited: the case for multiple cooperative trans-activation domains. *New Biol.* 3:18–26.

Buxton, R., P. Cowin, W. W. Franke, D. R. Garrod, K. J. Green, I. A. King, P. J. Koch, A. I. Magee, D. A. Rees, J. R. Stanley, et al. 1993. Nomenclature of the desmosomal cadherins. *J. Cell Biol.* 121:481–483.

Citi, S. 1993. The molecular organization of tight junctions. *J. Cell Biol.* 121:485–489.

Dyson, N., and E. Harlow. 1992. Adenovirus E1a targets key regulators of cell proliferation. *Cancer Surv.* 12:161–195.

Edelman, G. 1988. Morphoregulatory molecules. *Biochemistry.* 27:3533–3543.

Edelman, G., B. Murray, R. Mege, and B. Cunningham. 1987. Cellular expression of liver and neural cell adhesion molecules after transfection with their cDNAs results in specific cell-cell binding. *Proc. Natl. Acad. Sci. USA.* 84:8502–8506.

Ekblom, P., D. Vestweber, and R. Kemler. 1986. Cell-matrix interactions and cell adhesion during development. *Annu. Rev. Cell Biol.* 2:27–47.

Fleming, T., and M. Johnson. 1988. From egg to epithelium. *Ann. Rev. Cell Biol.* 4:459–485.

Flint, J., and T. Shenk. 1990. Adenovirus E1a protein: paradigm viral transactivator. *Annu. Rev. Genet.* 23:141–161.

Frisch, S. M. 1991. Antioncogenic effect of adenovirus E1a in human tumor cells. *Proc. Natl. Acad. Sci. USA.* 88:9077–9081.

Frisch, S. M., and H. Francis. 1994. Disruption of epithelial cell-matrix interactions induces apoptosis. *J. Cell Biol.* 124:619–626.

Geiger, B., and O. Ayalon. 1992. Cadherins. *Annu. Rev. Cell Biol.* 8:307–332.

Geiger, B., T. Volberg, D. Ginsberg, S. Bitzur, I. Sabanay, and R. Hynes. 1990. Broad spectrum pan-cadherin antibodies reactive with the C-terminal 24 amino acids of N-cadherin. *J. Cell Sci.* 97:607–614.

Green, K., and J. Jones. 1990. Interaction of intermediate filaments with the cell surface. *In* Cellular and Molecular Biology of Intermediate Filaments. R. Goldman and P. Steinert, editors. Plenum Press, New York. 147–174.

Grunwald, G. 1991. The conceptual and experimental foundations of vertebrate emybronic cell adhesion research. *In* A Conceptual History of Modern Embryology. S. Gilbert, editor. Plenum Press, New York. 129–158.

Gumbiner, B., B. Stevenson, and A. Grimaldi. 1988. The role of the cell adhesion molecule uvomorulin in the formation and maintenance of the epithelial junctional complex. *J. Cell Biol.* 107:1575–1587.

Hay, E. D. 1990. Epithelial-mesenchymal transitions. *Dev. Biol.* 1:347–356.

Heasley, L., S. Benedict, J. Gleavy, and G. Johnson. 1991. Requirement of the E1a domain 1 for inhibition of PC12 cell neuronal differentiation. *Cell Reg.* 2:479–489.

Howarth, A., M. Hughes, and B. Stevenson. 1992. Detection of the tight junction-associated protein ZO-1 in astrocytes and other nonepithelial cell types. *Am. J. Physiol.* 262: C461–C469.

Inostroza, J., F. Mermelstein, I. Ha, W. Lane, and D. Reinberg. 1992. Dr1, aTATA-binding protein-associated phosphoprotein and inhibitor of class II gene transcription. *Cell.* 70:477–489.

Jones, N., P. W. J. Rigby, and E. Ziff. 1988. Transacting protein factors and the regulation of eukaryotic transcription: lessons from studies on DNA tumor viruses. *Genes & Dev.* 2:267–281.

Koch, P., M. Goldschmidt, M. Walsh, R. Zimbelmann, and W. Franke. 1991. Complete amino acid sequence of the epidermal desmoglein precursor polypeptide and identification of a second type of demosglein gene. *Eur. J. Cell Biol.* 55:200–208.

Koch, P., M. Goldschmidt, R. Zimbelman, R. Troyanovsky, and W. Franke. 1992. Complexity and expression patterns of the desmosomal cadherins. *Proc. Natl. Acad. Sci. USA.* 89:353–357.

Maruyama, K., S. Schiavi, W. Huse, G. Johnson, and H. Ruley. 1987. Myc and E1a oncogenes alter the responses of PC12 cells to nerve growth factor and block differentiation. *Oncogene.* 1:361–367.

McNeill, H., M. Ozawa, R. Kemler, and W. J. Nelson. 1990. Novel function of uvomorulin as an inducer of cell surface polarity. *Cell.* 62:309–316.

Moran, E. 1993. DNA tumor virus transforming proteins and the cell cycle. *Curr. Opin. Gen. Dev.* 3:63–70.

Nagufuchi, A., Y. Shirayoshi, K. Okazaki, K. Yasuda, and M. Takeichi. 1987. Transformation of cell adhesion properties by exogenously introduced E-cadherin cDNA. *Nature (Lond.).* 329:341–343.

Nevins, J. 1992. E2F: a link between the Rb tumor suppressor protein and viral oncoproteins. *Science (Wash. DC).* 258:424–429.

Nilles, L., D. Parry, E. Powers, B. Angst, R. Wagner, and K. Green. 1991. Structural analysis and expression of human desmoglein: a cadherin-like component of the desmosome. *J. Cell Sci.* 99:809–821.

Ojakian, G. 1981. Tumor promoter-induced changes in the permeability of epithelial tight junctions. *Cell.* 23:95–103.

Oshima, R., W. Howe, F. Klier, E. Adamson, and L. Shevinsky. 1983. Intermediate filament protein synthesis in preimplantation mouse embryos. *Dev. Biol.* 99:447–455.

Rauscher, F., III, 1993. The WT-1 gene product: a developmentally regulated transcription factor in the kidney that functions as a tumor suppressor. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 7:897–903.

Reichmann, E., H. Schwarz, E. Deiner, I. Leitner, M. Eilers, J. Berger, M. Busslinger, and H. Beug. 1992. Activation of an inducible c-fosER fusion protein causes loss of epithelial polarity and triggers epithelial-fibroblastoid cell conversion. *Cell.* 71:1103–1116.

Rochette-Egly, C., C. Fromental, and P. Chambon. 1990. General repression of enhanson activity by the adenovirus-2 E1a protein. *Genes & Dev.* 4:137–150.

Rodriguez-Boulan, E., and W. J. Nelson. 1989. Morphogenesis of the polarized epithelial phenotype. *Science (Wash. DC).* 245:718–725.

Ruoslahti, E., and J. Reed. 1994. Anchorage dependence, integrins and apoptosis. *Cell.* 77:477–478.

Ruley, H. 1983. Adenovirus early region 1A enables viral and cellular transforming genes to transform primary cells in culture. *Nature (Lond.).*

304:602–606.
Schmidt, J., J. Brugge, and W. J. Nelson. 1992. pp60^{src} tyrosine kinase modulates P19 embryonal carcinoma cell fate by inhibiting neuronal but not epithelial differentiation. *J. Cell Biol.* 116:1019–1033.
Schmidt, J., P. Piepenhagen, and W. J. Nelson. 1993. Modulation of epithelial morphogenesis and cell fate by cell to cell signals and regulated cell adhesion. *Semin. Cell Biol.* 4:161–173.
Shenk, T., and J. Flint. 1991. Transcriptional and transforming activities of the adenovirus E1a proteins. *Adv. Cancer Res.* 57:47–85.
Sonnenberg, E., D. Meyer, K. Weidner, and C. Birchmeier. 1993. Scatter factor and its receptor, the c-met tyrosine kinase, can mediate a signal exchange between mesenchyme and epithelia during mouse development. *J. Cell Biol.* 123:223–225.
Steinert, P., and D. Roop. 1988. Molecular and cellular biology of intermediate filaments. *Annu. Rev. Biochem.* 57:593–625.
Stevenson, B., J. Siliciano, M. Mooseker, and S. Goodenough. 1986. Identification of ZO-1: a high molecular weight polypeptide associated with the tight junction in a variety of epithelia. *J. Cell Biol.* 103:755–766.
Stevenson, B., J. Anderson, and S. Bullivant. 1988. The epithelial tight junction: structure, function and preliminary biochemical characterization. *Mol. Cell Biochem.* 83:129–145.
Suemori, H., S. Hashimoto, and N. Nakatsuji. 1988. Presence of the adenovirus E1a-like activity in preimplantation stage mouse embryos. *Mol. Cell Biol.* 8:3553–3555.
Takeichi, M. 1991. Cadherin cell adhesion receptors as a morphogenetic regulator. *Science (Wash. DC).* 251:1451–1455.
Taylor, D., V. Kraus, J. Schwarz, E. Olson, and W. Kraus. 1993. E1a-mediated inhibition of myogenesis correlates with a direct physical interaction of E1a and bHLH proteins. *Mol. Cell Biol.* 13:4714–4727.
Tsarfaty, I., S. Rong, J. Resau, S. Rulong, P. da Silva, and G. Vande Woude. 1994. The met proto-oncogene mesenchymal to epithelial conversion. *Science (Wash. DC).* 263:98–100.
Tsukita, S., M. Itoh, A. Nagafuchi, S. Yonemura, and S. Tsukita. 1993. Submembraneous junctional plaque proteins include potential tumor suppressor molecules. *J. Cell Biol.* 123:1049–1053.
Uehara, Y., and N. Kitamura. 1992. Expression of a human HGF/SF cDNA in mdck cells influences cell morphology, motility and anchorage-independent growth. *J. Cell Biol.* 117:889–894.
Warren, S., and W. J. Nelson. 1987. Nonmitogenic morphoregulatory action of pp60^{src} on multicellular epithelial structures. *Mol. Cell Biol.* 7:1326–1337.
Webster, K., G. Muscat, and L. Kedes. 1988. E1a products suppress myogenic differentiation and inhibit transcription from muscle-specific promoters. *Nature (Lond.).* 332:553–557.