Integrin-mediated interactions between cells and the extracellular matrix play a fundamental role in the development and function of a variety of tissues by triggering intracellular signals that regulate gene expression. In this study, mouse mammary epithelial cells plated on tissue culture plastic were shown to dramatically up-regulate the steady state levels of mRNA encoding the α3, α5, αv, α2, α2, α2, α2, and β1 integrin subunits, in contrast to cells cultured on a basement membrane matrix or cells in vivo. This pattern of expression was also observed in a mouse mammary epithelial strain, CID-9 and in other mouse cell lines such as MMTE cells and K1735-M2 melanoma cells. The control of integrin expression was mediated at different levels in different cell types. In K1735-M2 cells, transcription of the β1 integrin gene was influenced by the substratum, although the levels of integrin protein remained similar. In mammary epithelial cells, the rates of β1 integrin gene transcription were similar, but mRNA and protein levels were higher in cells cultured on plastic than those on basement membrane. For both cell types, the rate of integrin protein turnover was nearly identical in cells cultured on either substrate. Our results demonstrate that extracellular matrix controls the expression of β1 integrin subunits and that this regulation is exerted at both transcriptional and post-transcriptional levels.

During tissue formation, maintenance, and remodeling, extracellular matrix (ECM) has an invaluable role not only in promoting cell motility and anchorage, but also in inducing cell activation and differentiation. It is becoming increasingly clear that cell-matrix interactions, through specific adhesion receptors, trigger biological responses similar to those transduced by growth factors, hormones, or cytokines (1, 2).

We previously showed that interactions between mammary cells and the ECM could regulate the expression of ECM molecules themselves (3). In an in vivo environment, mammary epithelial cells interact with a basement membrane, but on an inadequate substratum in tissue culture, such as on a plastic surface, the cells attempt to recreate their basement membrane by transcribing and translating genes coding for ECM proteins such as fibronectin and laminin (3). We have now asked whether the type of cell-matrix interactions also induce a similar regulation in the expression of cell surface β1 integrin receptors for ECM components. Any matrix-induced changes in the levels and patterns of integrin subunits might alter the way that microenvironmental signals are perceived, trigger the expression of new sets of genes, and modify cell phenotype.

We therefore examined the mRNAs coding for different sub-units of the β1 integrin family in mammary epithelial cells and compared their expression levels in cells cultured either on a plastic surface or on a laminin-enriched reconstituted basement membrane matrix. We then assessed whether changes in the amounts of mRNA were reflected at the protein level.

Our results demonstrate that the nature of the substratum controls the expression of integrins at both the mRNA and protein levels. The ECM-dependent modulation of β1 integrin expression was not ligand-specific since the levels of mRNA encoding a wide variety of α chains of the β1 integrins were altered. This regulation was not restricted to mouse mammary epithelial cells but also applied to other cell types, such as melanoma cells, and cells from other species including epithelial cells from normal human breast. We argue that this pattern of integrin gene expression reflects a general control mechanism activated by cells in an inappropriate environment, which is required for the establishment of sufficient cell-matrix interactions to maintain survival and differentiation.

### MATERIALS AND METHODS

#### Antibodies

The rat mAb GoH3 directed against the mouse integrin α6 subunit (4) was purchased from Serotec (Oxford, UK). The anti-mouse β6 chain rat mAb 346-11A was from Dr. E. F. Reichardt, University of California, San Francisco, CA; α2 (Dr. E. Russelholt, La Jolla Cancer Research Center, CA); and α6 (Dr. V. Quaranta, Scripps Research Institute, La Jolla, CA). A synthetic peptide corresponding to the C-terminal amino acids of mouse integrin β1 chain, with an extra N-terminal cysteine residue, was coupled to keyhole limpet hemocyanin (Sigma, Poole, UK) through the N-terminal cysteine, using maleimidobenzoyl succinimide ester (Pierce and Warriner Ltd, Chester, UK) (6). Following dialysis, 1 mg of the conjugated peptide was emulsified in Freund’s adjuvant (Sigma) and injected into rabbits at multiple sites. The antibodies were assayed after three injections by enzyme-linked immunosorbent assay, immunoprecipitations, and immunofluorescence staining. Specific antibodies were purified after ammonium sulfate precipitation, by affinity chromatography on cyogen bromide-activated, Sepharose-immobilized peptide, and finally by affinity chromatography on protein A-Sepharose.

Plasmid DNA Probes—pEMBL-P and pEMBL-D, carrying, respectively, the proximal and distal promoter regions of the human β1 chain gene, were ligated into the SmaI site of the polylinker of the plasmid pEMBL8CAT and were from Dr. F. Altzuda (7) (University of Turin, Torino, Italy); pSV2neo was from Dr. C. Schmidhauser (ETH-Zentrum, Zürich, Switzerland); pSV2CAT and pRSVCAT were the kind gift of M. Berwaer (University of Liège, Liège, Belgium); pHELp contains a 828-bp polymerase chain reaction DNA fragment of murine αv, inserted in the EcoRV site of pKs and was from Dr. V. Quaranta (Scripps Research Institute, La Jolla, CA); pDra7c.1 contains 443 bp of the rat αv chain and was from Dr. S.L. Goodman (University of Erlangen-Nürnberg, Erlangen, Germany); STIA contains a 2.9-kb fragment of the mouse β1 chain inserted in the EcoRI site of pUC13 and was from Dr. S. Tominaga (8) (Jichi Medical School, Tochigi-Ken, Japan); plas-
mids containing cDNA polymerase chain reaction fragment of guinea pig α1c, α2c and α3c, inserted in pK5+ were from Dr. D. Sheppard (University of California, San Francisco, CA). Polymerase chain reaction-generated cDNA fragments of mouse α1c, α2c and α3c, inserted in pKS were from Dr. R. J. Pyteida (University of California, San Francisco, CA). A plasmid containing a 2.2-kb fragment of the hamster α1c chain insert was isolated from the EcoRI site of the pTH171 plasmid was from Dr. T. Tsuji (10) (University of Tokyo, Tokyo). A plasmid containing a 4.1-kb fragment of rat GAPDH inserted in pBR 322 was from Dr. M. Cross (Paterson Institute, Manchester, UK). The probe for mouse β-casein, pBC-1, is a subclone derived from a plasmid from Dr. J. Rosen (Baylor College of Medicine, Houston, TX).

Cell Culture and Transfection—Mouse mammary epithelial cells were prepared from midpregnant CD-1 mice as described previously (3), and primary or first passage cultures were used. The mouse mammary epithelial milk-producing strain COMMA 1D-9 (or CID-9) was maintained as described elsewhere (11). K-1735 M2 (or M2) and MMTE cell lines were cultured in DMEM medium (Life Technologies Ltd., Paisley, Scotland) with minimal essential medium nonessential amino acids, 10% fetal calf serum (Life Technologies), and glucose at 4.5 g/liter. Primary human breast luminal epithelial cells were obtained directly from biopsy and cultured as mouse mammary cells.

To induce differentiation in the mammary cultures, cells were first plated on tissue culture plastic or on EHS matrix (prepared as described previously) (12, 13) for 2 days in DMEM/F-12 medium containing 5 μg/ml G418 (Life Technologies) at 1 mg/ml. After the selection was started 36 h after transfection by addition of 25% glycerol in 1× Hanks’ balanced salts (Sigma), and 3 μg/ml gentamicin (Sigma) and 5% FCS, then washed three times with serum-free DMEM/F-12 medium and cultured in differentiation medium, DMEM/F-12 supplemented with 5 μg/ml insulin, 1 μg/ml hydrocortisone (Sigma), and 3 μg/ml prolactin (Sigma), as previously described (13, 14).

To establish cells stably expressing CAT under the control of integrin promoters, CID-9 cells were transfected as described previously with some minor changes (15). M2 cells were plated in DMEM, 5% FCS at 8 × 104 cells/85-mm dish 24 h prior to the transfection. They were cotransfected by incubation for 16 h with calcium phosphate precipitates containing 30 μg of the different plasmids and 3 μg of pSV2neo. The cells were shocked with 25% glycerol in 1× Hanks’ balanced salts for 5 min. This selection was started 36 h after transfection by addition of G418 (Life Technologies) at 1 mg/ml. After 2 weeks, the G418-resistant clones were pooled and expanded.

RNA Isolation and Northern Blotting—Total RNA was isolated using the guanidinium thiocyanate method (16). 10 μg of total RNA were fractionated on 1% formaldehyde-agarose gels, and blotted to Hybond-N membrane (Amersham International, Little Chalfont, UK) as described elsewhere (17). Membranes were then probed with cDNA fragments labeled with [α-32P]dCTP (DuPont NEN Ltd., Stevenage, UK) by random dioxiguanosine primer synthesis.

CAT Assay—Cells cultured on EHS matrix were washed and harvested by treatment with 1% dispase for 30 min at 37°C (11). Cells cultured on plastic were detached with trypsin-EDTA, washed three times with phosphate-buffered saline, and suspended in 0.25 M Tris-Cl, pH 7.8. A cell extract was prepared by three cycles of freeze-thawing followed by centrifugation at 14,000 × g for 5 min. The total protein concentration in these lysates was estimated using the Bradford assay (18). Membranes were then probed with 32P-labeled RNA fragments labeled with [α-32P]dCTP (DuPont NEN Ltd., Stevenage, UK) by random dioxiguanosine primer synthesis.

Changes of Morphology and Cell Migration—To assess whether the expression of β1 integrin subunit transfected into CID-9 cells on EHS matrix, the spreading in culture was determined. Cells were plated at 0.5 × 104 cells/cm2 on EHS matrix or tissue culture plastic. Primary mouse mammary epithelial cells and CID-9 cells were cultured for 2 days in medium containing 5% FCS, then for 2 further days in differentiation medium. MMTE and M2 cells were cultured for 2 days in serum-containing medium followed by 1 day in serum-free medium. These images are phase contrast micrographs (bar = 1 mm).

**FIG. 1.** Influence of substrata on cell morphology and spreading in culture. Cells were plated at 0.5 × 104 cells/cm2 on EHS matrix or tissue culture plastic. Primary mouse mammary epithelial cells and CID-9 cells were cultured for 2 days in medium containing 5% FCS, then for 2 further days in differentiation medium. MMTE and M2 cells were cultured for 2 days in serum-containing medium followed by 1 day in serum-free medium. These images are phase contrast micrographs (bar = 1 mm).
grins might be regulated by the type of ECM with which cells interact, we evaluated the levels of integrins subunit mRNAs both in primary mouse mammary epithelial cells, cultured under differentiation conditions, and in several cell lines. For all of these studies, we utilized tissue culture plastic and a reconstituted basement membrane (EHS matrix). All of the cell types plated on plastic showed a flattened appearance and grew to form homogenous monolayers (Fig. 1). Cells cultured on top of EHS matrix were rounder and tended to gather in colonies. The colonies that formed were either multicellular clusters (CID-9 cells, primary mammary epithelial cells, M2 cells), or alternatively the colonies sent out processes and displayed a lacelike pattern (MMTE cells). These differences presumably reflect variations in adhesion with neighboring cells or with the ECM.

In our initial studies on integrin expression, we focused on mammary epithelial cells. These cells differentiate and synthesize milk proteins in the presence of lactogenic hormones, but differentiation requires β₁ integrin-mediated interaction with the basement membrane (13). By using cDNA probes to most of the α subunits that form heterodimers with the β₁ chain, we found that primary cultures of mammary epithelial cells express α₂, α₃, α₅, α₆, α₇, and αᵥ (Fig. 2A). α₄ and α₉ were undetectable (not shown). The expression of α₁ was very low and was probably due to contamination of our cultures by myoepithelial cells, which in humans are known to express the α₁ subunit strongly (19). We did not examine α₄ mRNA, but no α₄ protein was detectable in mammary epithelial cultures. The spectrum of integrin mRNA expression compares well with our subsequent protein analysis and defines for the first time the α

![Fig. 2. Expression of integrin subunit mRNAs in mouse mammary epithelial cells in culture and in vivo.](attachment:image.png)

Total cellular RNA (10 µg/ lane) was isolated from cultured primary mammary epithelial cells (A), mouse mammary gland at different stages of development (B), and cultured CID-9 cells (C), electrophoresed, transferred to Hybond membrane, and hybridized with α₁, α₂, α₃, α₅, α₆, α₇, and β₁ integrin, and β-casein probes. Even loading of each gel was verified by ethidium bromide staining (see B for representative example). In each case, standard RNA size markers were included in the gel. A, primary mammary epithelial cells cultured on EHS matrix (E) or plastic (P) for 4 days in serum-containing medium and 2 more days in differentiation medium. Up-regulation of integrin mRNA in primary cells cultured on plastic was continuous and sustained at day +1 and +2 in differentiation medium; there were also no changes of integrin mRNA expression in cells plated on EHS matrix between day +0 and +2 in differentiation medium. Note that β-casein expression was higher in cells cultured on the EHS matrix. B, integrin expression was assessed in virgin mouse mammary gland (V), 14.5-day midpregnant mouse mammary gland (Pr), and purified mammary epithelial cells isolated from a midpregnant mouse mammary gland (To). Integrin mRNA levels in vivo were compared with levels of the mRNA in culture, in cells plated on EHS matrix (E) and plastic (P). C, CID-9 cells cultured as for primary mammary epithelial cells. Total RNA was harvested after a 2-day culture in differentiation medium. Note that the integrin chain mRNA intensities shown in the autoradiographs are not representative of their absolute cell content since the probes for the different integrin subunits used were of different lengths, and were of variable activities, and the autoradiographs were exposed for different times.
Control of Integrin Expression by Extracellular Matrix

When we compared the mRNA levels of the α subunits and the β₁ chain in cells cultured on the plastic and basement membrane substratum, we found that the cells responded to plastic by up-regulating expression of these integrin subunits (Fig. 2A). This was particularly dramatic for the α₂, α₃, and β₁ subunits, although the integrin mRNA levels were so low in cells cultured on EHS matrix that they could not satisfactorily be quantitated with a densitometer. The response was likely to represent an up-regulation, since the integrin mRNA levels both in mammary gland tissue isolated at different stages of development and in purified alveolar epithelial (T₁₀) cells isolated directly from the mammary gland were low and comparable to the levels in cells cultured on EHS matrix (Fig. 2B). The integrin subunit expression profile and response to ECM were similar in a mammary epithelial cell strain, CID-9, that retains its ability to differentiate after stimulation with base-

ment membrane and lactogenic hormones (Fig. 2C). In contrast to the integrin expression pattern, both primary mammary cells and CID-9 cells expressed higher levels of the milk protein gene, β-casein, on the basement membrane matrix, as expected (11, 14) (Fig. 2A, A and C).

In order to determine whether this pattern of integrin regulation was specific for differentiating mammary epithelial cells or not, we examined the dependence on ECM of integrin expression in one other mouse epithelial cell type, MMTE, and one other cell line of nonepithelial lineage, the melanoma cell line K1735-M2 (or M2) (Fig. 3). In MMTE cells, the influence of substrata was dramatic, notably for the α₂, α₃, and β₁ subunits, and was serum-independent (Fig. 3A). The majority of integrin mRNAs tested were also up-regulated in M2 cells cultured on plastic (Fig. 3B). These results indicate that this substratum-dependent control of integrin expression was not confined to mammary epithelial cells either of a differentiating phenotype (primary cultures and CID-9) or of a tumor phenotype (MMTE), but was also seen in cells of a completely different lineage (M2).

Transcriptional Regulation of β₁ Integrin by Different Substrata—Having shown that the steady state levels of integrin mRNAs were increased by culture on plastic, we next asked whether this was due to increased transcriptional activity of the integrin promoter by CAT assay and nuclear run-on. M2 cells were transfected with vectors encoding the CAT reporter gene under the control of β₁ integrin or SV40 promoter sequences (Fig. 4A). Stably transfected clones were pooled, and their levels of CAT expression were assessed after plating the cells on different substrata. Our results show that transcription from the human β₁ integrin distal promoter was more efficient in cells cultured on plastic than on basement membrane (Fig. 4B). The promoter sequence more proximal to the start site did not show any appreciable expression of CAT enzyme in M2 cells cultured on either substrata, indicating that either this region does not contain an ECM-regulated transcription binding site or contains a silencer sequence to outweigh transcriptional activation, as shown for the α₁b integrin subunit (20).

To confirm that transcription was regulated by cell-matrix interactions, nuclear run-on assays were performed. Our results show that transcription from the endogenous β₁ integrin promoter in M2 cells was increased by culture on a plastic substratum (Fig. 4C), suggesting that a matrix-dependent control element lies within the promoter sequence for this integrin. However, in contrast to M2 cells, β₁ integrin transcription in mammary epithelial cells was not up-regulated on plastic, as assessed either by CAT assay (Fig. 4D) or by nuclear run-on (Fig. 4E). This suggests that the ECM-dependent transcription machinery in mammary epithelial cells is different from that in M2 cells.

Thus, at least in some cell types, part of the ECM-dependent control of integrin expression occurs at the transcriptional level. The difference between rates of transcription and levels of β₁ integrin mRNA suggests that additional controls occur at the level of mRNA stability, although we have not been able to assess this owing to the negligible quantity of integrin mRNA present in any of the epithelial or melanoma cell cultures on EHS matrix.

ECM-dependent Control of β₁ Integrin Subunit Expression at the Protein Level—Since the mRNA levels for α and β integrin subunits were increased in cells cultured on plastic, we asked whether the changes in expression were reflected at the level of protein synthesis. Immunoprecipitations with antisera that recognize mouse β₁ integrins established that both primary cultures of mammary epithelial cells and the CID-9 cell strain expressed α₂, α₃, α₃, and α₄ subunits but not α₁ or α₄. The
Control of Integrin Expression by Extracellular Matrix

**Fig. 4. Substratum effect on transcriptional regulation of β1 integrin.**

A, schematic representation of the constructs containing the human β1 integrin promoter regions. pEMBL-D carries the distal promoter contained in a 630-bp PstI-Apal fragment spanning nucleotides 842-1471 of a 4.5-kb genomic clone containing the 5'-flanking region of the β1 gene, and pEMBL-P carries the proximal promoter contained in a 360-bp Apal fragment spanning nucleotides 1471-1828. Nomenclature for nucleotide numbering is as in Cerella et al. (7). Black squares, GC-rich regions; black triangle, potential octamer binding site; black circle, potential AP-1 binding site. B and D, CAT activity in M2 cells (B) and CID-9 cells (D) transfected with the constructs pEMBL-D, pEMBL-P, and pSV2-CAT and cultured for 2 days on EHS (white columns) or plastic (gray columns). Cells were harvested, and 10 μg of total cell protein was assayed for CAT activities as described under "Materials and Methods." In each case 1 μg of the same protein extract was separated by SDS-PAGE and analyzed by silver staining to confirm accurate estimation of protein concentrations. In this study, only the distal promoter sequence and proximal promoter sequence were used since the complete promoter sequence and proximal promoter were shown to have the same activity as that of the distal region (7). The increased activity of the SV40 promoter in CID-9 cells cultured on plastic is similar to that noted previously (42). The transcription activity assessed by nuclear run-on. 5 μg of β1 integrin and GAPDH cDNAs were immobilized on nylon membrane using a multwell blot apparatus. The cDNAs were probed with equal counts of trichloroacetic acid-precipitated, 32P-labeled nuclear transcripts from nuclei of M2 cells (C) and primary mammary cells (E) cultured under the same conditions as for the CAT assays. Relative transcription activities in cells cultured on plastic (gray columns) are shown as a percentage of transcription activities in cells cultured on EHS taken equal to 100% (white columns).

**Fig. 5. Characterization of β1 integrin subunits in mammary epithelial cells.** Mouse mammary epithelial cells were cultured for 2 days on plastic, and steady state-labeled with Tran35S-label for 24 h. Rabbit antisera against synthetic peptides corresponding to C-terminal sequences in subunits revealed that the β1 integrin subunit was synthesized at higher amounts of trichloroacetic acid-precipitable newly synthesized proteins were used for immunoprecipitations. We could not examine the relative abundance of surface-labeled integrin due to the harsh procedure required to dissociate cells from EHS matrix. An anti-β1 integrin peptide antibody that precipitates the β1 chain together with its associated α subunits was present in this cell type. The α subunit was also present in mammary cells and was precipitated by both α-specific and β-specific monoclonal antibodies (Figs. 5 and 6B).

To determine whether ECM regulated the expression of any integrin subunits at the protein level, cells cultured on different substrata were steady state-labeled and equal amounts of trichloroacetic acid-precipitable newly synthesized proteins were used for immunoprecipitations. The 90–93-kDa band was shown to be the β1 chain precursor by a pulse-chase experiment (not shown) and was not immunoprecipitated with the anti-α3 antiserum. Comparison of the levels of subunits in the anti-α3 and the anti-β1 integrin immune precipitates indicated that the major subunit to associate with β1 was α5 (Fig. 5). This suggests that α5β1 is one of the most abundant β1 integrins present in this cell type. The α chain was also present in mammary cells and was precipitated by both α-specific and β-specific monoclonal antibodies (Figs. 5 and 6B).
showed that expression of $\alpha_6$ and $\beta_1$ protein was also up-regulated on plastic (Fig. 6B). Scanning densitometry and PhosphorImager analysis demonstrated that the up-regulation varied from 3-fold ($\beta_1$ chain) to 5-fold ($\alpha_6$ and $\beta_4$ chains). A 3-fold up-regulation of integrin synthesis was also observed, for both the $\alpha_6$ and $\beta_4$ chains, in primary cultures of luminal epithelial cells isolated from normal human breast (Fig. 6C). This result suggests that matrix-dependent control of $\beta_1$ integrin expression is conserved across species and may therefore be of widespread importance.

Although we found that different cell types cultured on plastic dishes all showed increased levels of integrin mRNA, this was not always reflected at the protein level. For example, in M2 cells, which expressed $\alpha_3$, $\alpha_2$, and $\alpha_4$ integrin subunits (Fig. 7A), as well as $\alpha_4$ (not shown), integrin protein synthesis was either similar on the different substrata ($\beta_1$ and most of the $\alpha$ subunits), or for the $\alpha_3$ subunit, was 3 times lower on plastic than on basement membrane matrix (Fig. 7B).

Evaluation of $\beta_1$ Integrin Protein Turnover in Cells Cultured on Plastic or EHS Matrix—Since no strict correlation was found between the differential regulation of integrin protein and mRNA expression, we asked whether the turnover rate of these receptors in mammary epithelial cells was higher on plastic than on EHS matrix. First passage mammary epithelial cells were steady state-labeled, and $\beta_1$ integrins were immunoprecipitated after varying times of a cold chase (Fig. 8A). The kinetics of integrin turnover was very similar on both substrata and the half-life of both the $\alpha$ and $\beta$ subunits was close to 24 h.
(Fig. 8B). In addition, we performed analogous experiments with CD1-9 and M2 cells and found that the rates of \( \alpha \) and \( \beta \) integrin subunit turnover were similar on both plastic and EHS matrix. However, since the absolute values of both \( \alpha \) and \( \beta \) subunits were higher in mammary epithelial cells cultured on plastic (Fig. 8A), it follows that the rates of integrin subunit degradation were also higher under these conditions.

Together, our results indicate that the amount of integrin subunits expressed by cells is controlled at the transcriptional and post-transcriptional levels, but the mechanisms employed appear to be cell type-specific.

**DISCUSSION**

Our study establishes that ECM controls the expression of \( \beta_1 \) integrins in mammary epithelial cells. When these cells were cultured on a basement membrane matrix, integrin mRNA levels were comparable to those in the mammary gland. By contrast, culturing cells on a plastic substratum triggered a dramatic and sustained expression of the mRNA coding for various \( \beta_1 \) integrin subunits. It is likely that multiple mechanisms operate since only a proportion of the increase in mRNA was reflected at the level of protein synthesis. This control of integrin expression was also observed in epithelial cells from normal human breast and in cells from other lineages such as melanoma cells, which suggests that it stems from a general matrix-dependent regulatory mechanism. However, not all of the transcribed mRNAs behaved like those of integrin subunits since the amount of \( \beta \)-casein mRNA was reduced, not increased, in mammary cells cultured on plastic, and we have recently shown that the mRNA levels of some transcription factors required for mammary differentiation (2) are not regulated by matrix.2

This work extends previous studies where we have shown that the expression of ECM molecules themselves are controlled by the type of interactions between mammary cells and their substratum (3). In the absence of a suitable ECM, these cells up-regulate expression of laminin both at the mRNA and protein levels, but once the cells interact with a basement membrane, laminin synthesis is suppressed (3). Since mammary epithelial cells normally interact with a basement membrane, laminin synthesis is suppressed (3). Since mammary epithelial cells normally interact with a basement membrane in vivo, it is possible that, when they are deprived of an interaction with this type of matrix, as occurs on tissue culture plastic, they increase the levels of both ECM protein and integrin receptor in order to maximize their chances of establishing further contacts with the ECM. Precisely why this should be so important to mammary cells is not clear at this time, but it may reflect a generic wound repair response that often occurs when cells are placed in tissue culture. This type of control has been discussed in relation to the wound response of keratinocytes, where \( \beta_1 \) integrin receptors are greatly increased following removal of skin to tissue culture (21); here it has been proposed that a shift in expression of \( \alpha_5\beta_2 \) integrin in stable contacts between hemidesmosomes and the underlying dermis, to an expression of \( \beta_1 \) heterodimers in migratory keratinocytes, is critical for wound repair (22, 23). Alternatively, and perhaps more appropriately for mammary epithelium, it may represent an effort to maintain extracellular survival signaling, since mammary epithelial cells, either in vivo or in culture, undergo apoptosis in the absence of appropriate ECM, and survival is maintained only by cell interactions with basement membrane.3

Substratum-dependent control on integrin gene expression has been noted previously in other cell systems. In addition to the already mentioned for keratinocytes, integrin mRNA levels were increased rapidly in osteosarcoma and cervical carcinoma cell lines that were deprived of adhesion to any substratum (24). This regulation is likely to be different to that reported here since in our study increased integrin expression occurred when cells formed intact monolayers on tissue culture plastic, most likely through a fibronectin and/or vitronectin interaction, rather than in the complete absence of any ECM. However, the well documented change in adhesion receptors that occurs during terminal differentiation of keratinocytes (as opposed to the wound response) may be relevant (25). In this instance, reduced transcription of integrin subunits and transport of functionally active heterodimers to the cell surface correlates with the expression of differentiation markers. Although this may be mechanistically related to the reduction of integrin expression in mammary epithelial cells induced to differentiate by culture on EHS matrix, in the latter case integrins are present at the cell surface, and indeed functional integrins are required for basement membrane to induce casein production (13).

One possible effect of the increased expression of both ECM protein and receptor in the mammary system is TGF-\( \beta \). In addition to substratum-dependent control of ECM and integrin, we previously demonstrated that TGF-\( \beta_1 \) mRNA levels were low in mammary epithelial cells cultured on a basement membrane matrix but were up-regulated in cells on plastic (26). Since TGF-\( \beta_1 \) is known to increase the expression of various ECM proteins as well as integrins (27–29), it might behave as an autocrine regulator when cells are placed in an inappropriate culture environment. This is an attractive mechanism, but it is likely to represent only part of the story since first, TGF-\( \beta \) has previously only been shown to increase integrin mRNA expression by a small amount but we detected a very dramatic ECM-dependent up-regulation of integrin mRNA (27, 28), and second, TGF-\( \beta \) is a normal contaminant of EHS matrix preparations (30), but there were only very low levels of integrin mRNA in cells cultured on this substratum.

Because the integrin expression pattern that we have reported is not restricted to normal mammary epithelium, but also applies to tumor lines and cells from other developmental lineages, an alternative explanation for its regulation is that the effector mechanism is a general one. Changes in cell shape, for example, represent such a mechanism, and morphological alterations are evident in our cultures. It has long been proposed that shape contributes to the phenotype of a cell by regulating gene expression (31, 32), but it is still unclear whether it acts directly through cytoskeleton and alteration of nuclear structure, for example via tensile forces (33), or indirectly through adhesion receptor-directed second messenger signals such as focal contact associated kinases (1) or cadherins. Since cells cultured on EHS matrix are more cohesive than those on plastic, cadherin-mediated signals may indeed be responsible for integrin mRNA and protein down-regulation, as has been shown in differentiating keratinocytes (34).

The intracellular mechanism of substratum-dependent control on integrin gene expression is complex, and since it occurs at the transcriptional and post-transcriptional levels, appears to be multifactorial. In addition, mammary epithelial cells and M2 cells respond differently to ECM, with only the latter showing altered transcription of the \( \beta_1 \) integrin gene, indicating that the pathways triggered in response to signals from the matrix are cell type-dependent. The low level of transcriptional activation by basement membrane in mammary epithelial cells suggests that the dramatic up-regulation of steady state integrin mRNA is very likely accounted for by an increase of inte-

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2 T. G. Burdon, C. J. Watson, and C. H. Streuli, unpublished data.
3 S. Pullan, J. Wilson, A. Metcalfe, G. Edwards, N. Goberdhan, J. Tilly, J. A. Hickman, C. Dive, and C. H. Streuli, manuscript submitted for publication.
The discrepancy between the up-regulation of integrin mRNA and protein levels in this system suggests that post-translational controls are operative. This is especially evident in mRNA and protein levels in this system suggests that post-gran transcript stability.

In summary, this study extends our understanding of ECM-dependent gene regulation. We have now shown that expression of ECM proteins (3) and their integrin receptors, and cytokines such as TGF-β1 (26), can all be regulated by the type of matrix with which cells interact. We propose that ECM-dependent regulation of all these proteins is part of a general mechanism whereby normal cells react to an inappropriate substrate and attempt to reestablish suitable cell-matrix interactions necessary for maintaining survival and for progressing through a differentiation program. It is significant that the only cell type in our study that did not conform to this pattern was the transformed, highly metastatic M2 cells. The lack of integrin up-regulation at the protein level in these cells cultured on plastic might be related to their anchorage-independent growth (40) and very likely results from the diversification that takes place during tumor progression when clones with traits most adapted for immortality and metastasis are selected (41).

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Control of Integrin Expression by Extracellular Matrix

26801