Induction of Cell Scattering by Expression of β1 Integrins in β1-deficient Epithelial Cells Requires Activation of Members of the Rho Family of GTPases and Downregulation of Cadherin and Catenin Function

Clotilde Gimond,* Arjan van der Flier,* Sanne van Delft,* Cord Brakebusch,† Ingrid Kuikman,* John G. Collard,* Reinhard Fässler,‡ and Arnoud Sonnenberg*

*Division of Cell Biology, The Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands; and ‡Lund University Hospital, Section of Experimental Pathology, Lund S-22185, Sweden

Abstract. Adhesion receptors, which connect cells to each other and to the surrounding extracellular matrix (ECM), play a crucial role in the control of tissue structure and of morphogenesis. In this work, we have studied how intercellular adhesion molecules and β1 integrins influence each other using two different β1-null cell lines, epithelial GE11 and fibroblast-like GD25 cells. Expression of β1A or the cytoplasmic splice variant β1D, induced the disruption of intercellular adherens junctions and cell scattering in both GE11 and GD25 cells. In GE11 cells, the morphological change correlated with the redistribution of zonula occludens (ZO)-1 from tight junctions to adherens junctions at high cell confluency. In addition, the expression of β1 integrins caused a dramatic reorganization of the actin cytoskeleton and of focal contacts. Interaction of β1 integrins with their respective ligands was required for a complete morphological transition towards the spindle-shaped fibroblast-like phenotype. The expression of an interleukin-2 receptor (IL2R)-β1A chimera and its incorporation into focal adhesions also induced the disruption of cadherin-based adhesions and the reorganization of ECM–cell contacts, but failed to promote cell migration on fibronectin, in contrast to full-length β1A. This indicates that the disruption of cell–cell adhesion is not simply the consequence of the stimulated cell migration. Expression of β1 integrins in GE11 cells resulted in a decrease in cadherin and α-catenin protein levels accompanied by their redistribution from the cytoskeleton-associated fraction to the detergent-soluble fraction. Regulation of α-catenin protein levels by β1 integrins is likely to play a role in the morphological transition, since overexpression of α-catenin in GE11 cells before β1 prevented the disruption of intercellular adhesions and cell scattering. In addition, using biochemical activity assays for Rho-like GTPases, we show that the expression of β1A, β1D, or IL2R-β1A in GE11 or GD25 cells triggers activation of both RhoA and Rac1, but not of Cdc42. Moreover, dominant negative Rac1 (N17Rac1) inhibited the disruption of cell–cell adhesions when expressed before β1. However, all three GTPases might be involved in the morphological transition, since expression of either N19RhoA, N17Rac1, or N17Cdc42 reversed cell scattering and partially restored cadherin-based adhesions in GE11-β1A cells. Our results indicate that β1 integrins regulate the polarity and motility of epithelial cells by the induction of intracellular molecular events involving a downregulation of α-catenin function and the activation of the Rho-like G proteins RhoA and Rac1.

Key words: β1 integrins • cadherins • epithelial cells • Rho-like GTPases • migration
Cells also form adhesive contacts with proteins in the surrounding extracellular matrix (ECM) via different types of proteins, mostly of the integrin family. Integrins are heterodimeric transmembrane receptors, formed by the noncovalent association of an α and a β subunit. 18 α and 8 β subunits have been identified so far, giving rise to a family of >20 different dimers. Dimers containing the β1 integrin subunit constitute the most abundantly expressed integrin subfamily. A nitroblue inhibition studies and disruption of the β1 subunit gene by homologous recombination have demonstrated the critical role of β1 integrins in development, cell differentiation, migration, and the assembly of the ECM proteins (for reviews see Brakebusch et al., 1997; Sakai et al., 1998). Four alternative cytoplasmic variants, β1A–D, of the β1 subunit have been described (for review see de Melker and Sonnenberg, 1999). Whereas β1A is ubiquitously expressed, the β1D isoform is muscle-specific (van der Flier et al., 1995; Zhidkova et al., 1995). In nonmuscle cells, transfected β1D was found to be localized in focal contacts and to activate both focal adhesion kinase and mitogen-activated protein kinase, similarly to β1A (Belkin et al., 1996). However, we have shown recently that β1A and β1D are not functionally equivalent during embryonic development or in adult tissues (Baudoin et al., 1998).

Adhesive interactions between different cells or between a cell and the surrounding ECM can be either stable or dynamic. An example are the epithelium-mesenchyme transitions (EMT), which occur during specific stages of embryonic development but also under certain pathological conditions. EMT are morphogenetic events characterized by the loss of epithelial polarity, the disruption of intercellular adhesions, and the acquisition of a migratory, mesenchymal cell phenotype. Previous studies have indicated a role for integrins in the downregulation of cadherin activity during neural crest cell EMT (Monier-Gavelli and D’uband, 1997) and in the loss of epithelial polarity in breast tumor cells (W.eaver et al., 1997; Faraldo et al., 1998), suggesting the existence of cross-talk mechanisms between different types of adhesion molecules.

Small GTPases of the Rho family are regulators of the actin cytoskeleton (Mackay and Hall, 1998). Microinjection of RhoA, Rac1, or Cdc42 in fibroblasts triggers the formation of stress fibers, lamellipodia, or filopodia, respectively (Ridley and Hall, 1992; Ridley et al., 1992). Recent studies have implicated Rho-like GTPases in the regulation of cadherin-mediated adhesion and in epithelial cell motility. Dominant negative Rac blocks lamellipodia formation induced by hepatocyte growth factor in MDCK cells (Ridley et al., 1995), and constitutively active Rac and Cdc42 stimulate the motility of mammary carcinoma cells (Keeley et al., 1997). In addition, activation of phosphatidylinositol3 kinase by the α6β4 integrin stimulates Rac-dependent migration of colon carcinoma cells (Shaw et al., 1997). In contrast, other studies have suggested a role for small GTPases in the establishment and maintenance of epithelial intercellular adhesions (Braga et al., 1997; Hordijk et al., 1997; Takaishi et al., 1997; Zhong et al., 1997). Recently, Tiam1-Rac signaling has been shown to promote either cell–cell adhesion or the migration of epithelial cells in a matrix-dependent manner (Sander et al., 1998), suggesting that integrin-mediated adhesion plays a role in the control of intercellular adhesions through the regulation of Rho-like GTPases. Studies using dominant negative or constitutively active mutants of Rho-like GTPases have suggested a role of integrins in the regulation of Rho, Rac, and Cdc42 activation (Chong et al., 1994; Renshaw et al., 1996; Schwartz et al., 1996; Barry et al., 1997; Clark et al., 1998). In addition, cell adhesion activates p21-activated kinase (PAK), a downstream effector of Rac and Cdc42 (Price et al., 1998). Finally, Ren and co-workers have recently demonstrated the regulation of Rho by cell adhesion in fibroblasts, using an assay to detect GTP-bound Rho (Ren et al., 1999).

In this study, we have used two β1-deficient cell lines, an epithelial cell line, GE11, which we isolated for this study, and the previously described fibroblast-like GD 25 cell line (Fässler et al., 1995) to study the functional relationship between integrins, cadherins, and Rho-like GTPases. Expression of either of the splice variants, β1A or β1D, in these two cell lines induced the disruption of intercellular adhesions and cell scattering. This was accompanied by a decrease of cadherins and α-catenin protein levels as well as their redistribution from the cytoskeleton-associated fraction to the soluble fraction. Overexpression of α-catenin inhibited the disruption of cell–cell adhesions by β1 in GE11 cells and prevented cells from scattering. We also found that expression of β1 integrins in GE11 and GD 25 cells resulted in the activation of both RhoA and Rac1. Experiments performed with dominant negative or active mutants showed that both RhoA and Rac1 were required but not sufficient for the phenotypic conversion induced by β1 integrins.

Materials and Methods

Antibodies and Adhesive Ligands

The mouse anti-α3A and anti-α3B antibodies (29A 3 and 54B 3; de Melker et al., 1997) and the rat anti-α6 antibody (Goh 3; Sonnenberg et al., 1997) have been described previously. The rabbit polyclonal anti-synthetic peptide derived from the cytoplasmic domain of the α1, α2, and α5 integrin subunits (Effili et al., 1991; Hirsch et al., 1994) were kindly provided by Dr. G. Tarone (Universita di Torino, Torino, Italy). The rat anti-α5 antibody (MBA 5; Fehlner-Gardiner et al., 1996) was the generous gift of Dr. B. M. Chan (J. P. Robarts Research Institute, London, Ontario, Canada). The rat polyclonal antibody recognizing β5 (K emperman et al., 1997) was kindly supplied by Dr. E. Roos (The Netherlands Cancer Institute, Amsterdam, The Netherlands). The rabbit polyclonal antibody against the cytoplasmic domain of β1A (U 19) was a kind gift of Dr. U. Mayer (Max Planck Institute for Biochemistry, Martinsried, Germany). The mouse anti-human β1 (TS2/16) and the mouse anti-human β3 (K20) antibodies were obtained from the ATCC and Biotrend, respectively. The hamster anti-β3 and the rat anti-α4 antibodies were purchased from Pharmingen. The mouse mAb against vinculin (VIIF 5; Glukhova et al., 1990), rabbit anti-vinculin (Geiger, 1979), anti-keratin (TROMA 1; Bru-let et al., 1980), and the anti-desmoplakin (11-5F; Parrish et al., 1987), and the anti–NCA M–1 (Moolenaar et al., 1990) antibodies were kindly pro-

Abbreviations used in this paper: ECM, extracellular matrix; EMT, epithelium-mesenchyme transitions; ES, embryonic stem; GST, glutathione S-transferase; IL 2R, interleukin-2 receptor; PAK, p21-activated kinase; ZO, zona occluden.

1. Abbreviations used in this paper: ECM, extracellular matrix; EMT, epithelium-mesenchyme transitions; ES, embryonic stem; GST, glutathione S-transferase; IL 2R, interleukin-2 receptor; PAK, p21-activated kinase; ZO, zona occluden.
vided by Dr. M. Glukhova (Institut Curie, Paris, France), Dr. B. Geiger (The Weissenmann Institute of Science, Rehovot, Israel), Dr. R. Kemler (Max Planck Institute for Immunobiology, Freiburg, Germany), Dr. D. Garrod (University of M. anchester, Manchester, U.K.), and Dr. R. Michailides (The Netherlands Cancer Institute, Amsterdam, The Netherlands), respectively. The mouse anti-interleukin-2 receptor (IL2Rα) (TB30) was a gift of Dr. R. van Lier (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands). The rabbit anti-pan-cadherin, the rabbit anti-α-catenin, and the mouse anti-talin (8D4) antibodies and rhodamine-labeled phalloidin were obtained from Sigma Chemical Co. The mouse anti-β-catenin and mouse anti-γ-catenin antibodies were purchased from Transduction Laboratories, and the rabbit anti-ZO-1 and the rabbit anti-occludin antibodies were obtained from Santa Cruz Biotechnology, and mouse anti-Rac1 antibody from Transduction Laboratories. Secondary antibodies used were: FITC-conjugated goat anti-mouse antibody (Jackson ImmunoResearch Laboratories), Texas red-conjugated goat anti-mouse and anti-rabbit antibodies (Molecular Probes), FITC-conjugated rabbit anti-hamster antibody (Nordic Immunological Laboratories), and FITC-conjugated goat anti-rabbit antibody (Zymed). Fibronectin was purchased from Sigma Chemical Co., and laminin-1 from Collaborative Biomedical Products.

**GD 25 Cells and the Establishment of GE11 Cells**

The previously described fibroblast-like GD 25 cell line (Fassler et al., 1995) was obtained by in vitro differentiation and immortalization of βJ-knockout embryonic stem (ES) cells. For the establishment of epithelial-like GE11 cells, β1-null ES cells were grown, injected into blastocysts, and transferred into foster mice as reported earlier (Fassler and Moyer, 1995). Pregnant females were killed at around embryonic day 10.5 and β1-null chimeric embryos removed and subsequently freed of membranes. A β1-embryonic embryos in trypsin/EDTA, they were broken up by pipetting embryos repeatedly using Pasteur pipettes. Cells were plated on gelatin-coated tissue culture dishes overnight, incubated with SV-40 large T transducing retrovirus (Fassler et al., 1995) for 1 h at 37°C, and immediately selected at high concentration of G418 (1 mg/ml). A 2 h more days in medium, cells were trypsinized, counted, and distributed over 96-well plates and cultured in selection medium. 10 96-well plates were prepared with 3 cells/well, 10 plates with 6 cells/well, and 10 plates with 12 cells/well. Cells in these 96 wells were cultured in the presence of G418. A 3 wk in culture, 14 wells contained polarized cells which had formed small colonies (GE1-GE14). They were trypsinized and expanded. Whereas 13 clones stopped growing during the expansion period, one clone (GE11) continued to grow and was clearly polarized. A though all cells of clone GE11 showed the typical morphology, cells were stained strongly for β1-catenin in their cytoplasm, suggesting that GE11 cells were clonal even before the limiting dilution experiment.

**Generation of Retroviral Expression Constructs**

A cDNA encoding full-length human β1A (nucleotides 17–3096; Argraves et al., 1987) was obtained by screening an human α-phage keratinocyte library with two β1 oligonucleotide probes (nucleotides 8–25 and nucleotides 2357–2389) and subsequently cloned into pUC18. A Kozak consensus sequence was introduced by PCR, and the Kozak-containing human β1A cDNA was then ligated into the retroviral LZR-S-IRE5-zeo expression vector, a modified LZR5 retroviral vector conferring resistance to zeocin (Kinsella and Nolan, 1996; van Leeuwen et al., 1997). A full-length cDNA encoding the cytoplasmic domain of β1A by a β1D reverse transcription PCR product (van der Flier et al., 1995) in the LZR-S-IRE5-zeo retroviral vector. The IL2Rα encoding sequence of the PCMVL-IL2Rα vector (Lambeau et al., 1992), which was kindly provided by Dr. K. Yamada (National Institutes of Health, Bethesda, M.D.), was cloned into the retroviral LZR-S-IRE5-zeo vector. The IL2Rα-β1A chimeric construct was obtained by cloning the β1A cytoplasmic domain into the LZR-S-IL2Rα-IRE5-zeo vector. Myc epitope-tagged dominant negative N17Cdc42, N17Rac1, N19-RhoA, and dominant active V12Cdc42, V12Rac1 were cloned in the LZR-S-IRE5-zeo vector (van Leeuwen et al., 1997; Stam et al., 1998). The mouse α(E)-catenin cDNA (Herrenknecht et al., 1991) was kindly provided by Dr. R. Kemler (Max Planck Institute for Immunobiology, Freiburg, Germany) and was cloned in the same vector.

**Retroviral Transduction and Cell Culture**

Phoenix packaging cells (Kinsella and Nolan, 1996) were transfected with retroviral constructs as described previously (Stam et al., 1998) to produce culture supernatants containing virus. Then, 3 × 10^6 GE11 or GD25 cells were infected with virus, cells were cultured for 8 h in 1 ml of cell-free Phoenix supernatant in the presence of 10 μg/ml DOTAP (Boehringer Mannheim). Cells were then cultured in fresh DME medium supplemented with 10% FCS and penicillin/streptomycin (GIBCO-BRL Life Technologies). ZEocin (0.2 mg/ml; Invitrogen) was added to the culture medium 48 h after transduction.

Expression of the β1 subunit or the IL2Rα-β1 chimera was determined by FACS® analysis. Expression of the mutant forms of Rho-like GTPases was checked by immunoblotting as described previously (Michiels et al., 1997), using a HRP-conjugated goat anti-mouse antibody (Amersham Pharmacia Biotech). Immunoreactive proteins were visualized using enhanced chemiluminescence as described by the manufacturer (Amersham Pharmacia Biotech).

**Immunofluorescence and Flow Cytometry**

Cells were grown on coverslips in DME, 10% FCS, fixed in 2% paraformaldehyde for 15 min, and permeabilized in PBS containing 0.2% Triton X-100 for 5 min. Cells were blocked in PBS, 2% BSA for 1 h, and incubated with primary antibodies for 1 h at room temperature. After washing in PBS, cells were incubated in the presence of FITC- or Texas red-conjugated secondary antibodies or in the presence of rhodamine-labeled phalloidin for 1 h. Preparations were then washed in PBS, mounted in Vectashield (Vector Laboratories Inc.), and analyzed with a confocal Leica TCS NT microscope.

For flow cytometry and cell sorting, cultured cells were trypsinized, washed twice in PBS, 2% FCS, and incubated with primary antibodies for 45 min at 4°C. Cells were then washed in PBS, incubated with FITC-conjugated secondary antibodies for 45 min at 4°C, washed again, and analyzed in a FACScan® by Lysys II software (Becton Dickinson) for determination of integrin expression levels. Cells were sorted on a FACStar Plus® (Becton Dickinson).

**Transwell Migration Assays and In Vitro Wound Healing**

For the Transwell migration assay, 3 × 10^3 or 10^4 cells in DME, 0.5% BSA were seeded in the upper compartment of 8-μm Transwells (Costar) previously coated with 10 μg/ml fibronectin on the lower side of the insert, and allowed to migrate for 2 h at 37°C. Cells in the upper chamber were removed with a cotton swab, cells on the lower side of the filter were fixed in methanol and stained with crystal violet. The number of cells that had migrated was counted on photographs taken from the filters. For each filter a total of three different 5-mm² fields were photographed to obtain an average cell count.

For in vitro wound healing assay, cells were seeded for 2 h in DME, 10% FCS. A cell sector spreading, a cross was scratched in the cell monolayer to analyze wound closure and facilitate the localization of the same spot in the same cell in the same time. Cells were photographed at the indicated time points (magnification 500×1).

**Detergent Solubility Assay**

Subconfluent cell cultures were lysed for 10 min on ice in 1% Triton X-100, 50 mM Tris, pH 7.6, 150 mM NaCl, 2 mM EDTA in the presence of protease inhibitors and the lysates were centrifuged at 14,000 g for 15 min to obtain the soluble protein fraction. The pellet (the cytoskeletal, insoluble fraction) was resuspended in L-ammonium sample buffer. For detection of total protein samples, cells were treated with radio immunoprecipitation assay (RIPA) lysis buffer. Samples were adjusted to 50 μg of total proteins, separated by SDS-PAGE, and transferred to polyvinylidene difluoride membranes (Immobilon-P; Millipore). The membranes were incubated for 1 h with anti-pan-cadherin, anti-α-catenin, or anti-β-catenin antibodies and then further incubated for 1 h with HRP-conjugated secondary antibodies. Immunoreactive proteins were visualized using enhanced chemiluminescence.
RhoA, Rac1, and Cdc42 Activity Assays

The biochemical activity assays were performed essentially as described previously (Sander et al., 1998). For the RhoA activity assay, a glutathione S-transferase (GST) fusion protein of the Rho effector protein rhotekin (Reid et al., 1996) was employed. For the Rac1 and Cdc42 assays, we used a GST fusion protein of the binding domain PAK1b, which binds Cdc42 and Rac in the GTP-bound form only. The GST-rhotekin or GST-PAK1b precoupled to Sepharose-glutathione beads (Amersham Pharmacia Biotech) were used to precipitate GTP-bound RhoA, Rac1, or Cdc42 from cleared lysates of cells.

For each measurement, two T75 flasks of subconfluent GE11 or GD25 cells were lysed for 5 min at 4°C in 1% NP-40, 50 mM Tris, pH 7.4, 10% glycerol, 100 mM NaCl, 2 mM MgCl2, in the presence of protease inhibitors. Lysates were clarified by centrifugation and the appropriate GST fusion protein was added for 30 min at 4°C, followed by three washes in lysis buffer. The beads were boiled in Laemmli sample buffer and protein samples were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes (Millipore). The blots were probed with anti-RhoA, anti-Rac1, or anti-Cdc42 antibodies and developed by enhanced chemiluminescence.

Results

Expression of β1A or β1D in β1-deficient Cells Induces the Disruption of Intercellular Adhesions and Cell Scattering

GE11 cells were isolated from a β1 integrin subunit knockout chimeric embryo aged 10.5 d postcoitum and their ontogeny is unclear. They grow in epithelial colonies (Fig. 1), contain keratin 8, no desmoplakin, and express the neural cell adhesion molecule NCAM-1. Electron microscopic studies showed that GE11 cells are polarized, have microvilli at their apical surface, and organized tight junctions, but do not assemble desmosomes (data not shown). Together, these observations suggest that GE11 cells are epithelial cells of neural origin, possibly the neuroepithelium. The previously described GD25 cells were obtained by in vitro differentiation and immortalization of β1-knockout ES cells (Fässler et al., 1995). GD25 cells have a more fibroblastic phenotype than GE11 cells, although they maintain intercellular adhesions (Fig. 1).

We have expressed two cytoplasmic splice variants of the β1 integrin subunit, β1A and β1D, in GE11 and GD25 cells, by retroviral transduction. FACS® analysis revealed that 90–95% of the cells express β1 at their plasma membrane 24 h after the start of retroviral transduction (data not shown). Stable transfectants were selected with zeocin. Immunoprecipitation experiments showed that GE11 cells expressed the β3 and β5 subunits in association with αv (data not shown). GE11-β1A cells express several integrins of the β1 family, including α3β1, α5β1, α6β1, and α4β1 at low levels, but not α1β1 or α2β1 (data not shown).

Infection of cells with the empty retroviral vector (GE11- and GD25-control) did not alter the epithelial phenotype of GE11 cells or the intercellular adhesions in GD25 colonies (Fig. 1). In both cell types, the expression of the β1A integrin subunit resulted in disruption of cell-cell contacts and dissociation of cell colonies. Cells originally present in epithelial cell colonies separated from them and assumed a morphology resembling that of motile fibroblasts. These morphological changes were detected as early as 24 h after the start of retroviral infection (Fig. 1). Similar effects, although somewhat less pronounced, were observed after the expression into GE11 and GD25 cells of the muscle-specific β1D splice variant (Fig. 1). The lower levels at which β1D was expressed (50% of those of β1A) probably account for this less pronounced phenotype.

Because the effect of β1 expression was most dramatic on intercellular adhesions in GE11 cells, we concentrated...
our studies on stable GE11 cells expressing β1A (GE11-β1A). The distribution of various proteins associated with the actin cytoskeleton and with intercellular adhesions was analyzed in GE11 and GE11-β1A cells (Fig. 2). In GE11-control cells, actin filaments were organized in heavy peripheral bundles, which ran parallel to the outer membrane of cells at the periphery of the epithelial cell colonies. Actin filaments were also present in cortical bundles under the plasma membrane, along cell–cell boundaries, and in stress fibers at the cell basis, where they were attached to the plasma membrane at sites of focal contacts (Fig. 2). In GE11-β1A cells, peripheral bundles of actin filaments were absent and stress fibers crossed the entire cell. Interacellular staining of cadherins, typical of epithelial cells, was observed in GE11-control cells, and no staining was found at the free cell border at the periphery of colonies (Fig. 2). α-, β-, and γ-catenins had a similar localization (data not shown). In contrast, cadherins and catenins were more diffusely distributed over the membrane of GE11-β1A cells, and although there were some residual adherens junctions at high confluency, these proteins were also found in regions of the plasma membrane that were not in contact with other cells (Fig. 2, medial plane). In GE11-control cells, vinculin was found in regions of cell–cell contacts, where it was colocalized with cadherins (Fig. 2, medial plane). In contrast, although GE11-β1A cells developed cell–cell contacts at high confluency, vinculin and cadherins were not colocalized in these cells (Fig. 2, medial plane). Using interference reflection microscopy, we found that the number and size of focal contacts were different in GE11-control and GE11-β1A cells (data not shown), and this was confirmed by the distribution of vinculin (Fig. 2, basal plane) and talin (data not shown) at the basal surface of the cells. Typically, focal contacts were small and numerous in GE11-control cells, and distributed over the entire basal cell surface. However, at the periphery of the colonies they were more concentrated at the outer region of the cell, thus forming a characteristic interrupted ring-like structure. In GE11-β1A cells, focal contacts were thick, and appeared to be arranged in long streaks frequently found at the end of actin stress fibers (Fig. 2). In confluent GE11-β1A cells, focal contacts were also found between two cells and sometimes on their apical surface, as seen by staining for talin and vinculin. Electron microscopic analysis revealed that this was likely to be due to the presence and assembly of secreted ECM proteins between cells and on their apical surface (data not shown). A nuchor specialized membrane domain involved in intercellular adhesion of epithelial cells is the tight junction. A marker of tight junctions is ZO-1, but in cell types lacking these structures, such as fibroblasts or cardiac muscle cells, ZO-1 is colocalized with cadherins at adherens junctions (Yonemura et al., 1995). Upon expression of the β1A integrin subunit, ZO-1 became redistributed from tight junctions to the adherens junctions formed by GE11-β1A cells at high confluency (Fig. 2). This relocalization was correlated with the transition from polarized epithelial cells to fibroblast-like cells. A nother marker of tight junctions, occludin, was also found at the apical lateral border of GE11 cells, and became diffusely distributed in GE11-β1A cells (data not shown). Furthermore, EM showed that tight junctions present in GE11-control cells were no longer present in GE11-β1A cells (data not shown).

Finally, we have found that β1 and the endogenous β3 integrin subunits were both present in focal contacts formed by GE11-β1A cells in the presence of FCS (data not shown).
In conclusion, these results show that the expression of the β1 integrin subunit in GE11 cells induces a reorganization of the actin cytoskeleton and of focal contacts, accompanied by the disruption of both cadherin-based intercellular adhesions and tight junctions in epithelial cell colonies. However, adherens junctions of the type formed by fibroblasts (Yonemura et al., 1995) can assemble in the presence of β1 integrins at high cell confluency.

**Disruption of Intercellular Adhesions and Cell Scattering Is Dependent on β1 Integrin–Ligand Interactions**

When GE11 cells were cultured on plastic in the presence of FCS, the mere expression of the β1 subunit was sufficient for inducing the disruption of intercellular adhesions and the dissociation of cell colonies. Because GE11-β1A cells express several integrins that can bind to fibronectin and vitronectin present in FCS, we investigated whether the change in morphology was due to the expression of β1 or whether it was triggered by the interaction of β1 integrins with their ligands. Although GE11-β1A cells expressed the laminin receptor α6β1, we have generated GE11 cells expressing α6β1 at higher levels by coexpression of the human α6 integrin subunit in GE11-β1A cells and by further selection by FACS®. The overexpression of α6 in GE11-α6β1A cells resulted in a strong decrease of the percentage of cells expressing the fibronectin receptor α5β1 as well as in a decrease of its average expression levels (Fig. 3 A), probably because α6 associated with most of the available β1 subunit. Although these cells could spread, they poorly scattered and developed strong cell-cell adhesions when cultured on fibronectin (Fig. 3 B), suggesting that the expression of all fibronectin-binding β1 integrins (α5β1 as well as αvβ1) was reduced. However, scattering was induced when they were cultured on laminin-coated dishes (Fig. 3 B). Together, these results indicate that the interaction of β1 integrins with their ligand is required for the disruption of cadherin-based cell-cell adhesion and cell scattering. They also show that several integrins of the β1 family, which bind to various ECM proteins (α5β1 or αvβ1 to fibronectin, and α6β1 to laminin), can trigger the described morphological transition.

**Expression of IL2R-β1A Chimera Induces the Disruption of Cadherin-based Cell–Cell Adhesions and Remodels Focal Contacts**

The localization of a particular integrin in focal contacts is regulated by its α subunit and requires the binding of the integrin to ligand (Briesewitz et al., 1993). In contrast, when chimeric molecules containing the extracellular and transmembrane domains of the human IL2R and the cytoplasmic domain of the β1A integrin subunit were expressed at relatively low levels, they were colocalized with endogenous integrins in focal contacts and were able to transduce signals leading to the phosphorylation of the focal adhesion kinase (LaFlamme et al., 1992; Akiyama et al., 1994). These properties indicate that IL2R-β1 chimerae mimic endogenous ligand-occupied integrins. We have analyzed the effects of an IL2R-β1A chimera on the morphology of GE11 cells. Fig. 4 shows that expression of the IL2R alone did not either alter the morphology of GE11 cell colonies or cause any changes in the subcellular distribution of actin, α-catenin, or ZO-1 (Fig. 4, upper panels). Expression of the IL2R-β1A chimera, on the contrary, induced the disruption of most intercellular adhesions. A few small epithelial-like colonies remained and cell-cell adhesions had a tendency to reform, although they did not appear to be as stable as those between GE11-control cells. IL2R-β1A expression induced an alteration of the peripheral bundles of actin filaments and changes in the localization of cadherins, catenins, and ZO-1 (Fig. 4, lower panels), similar to the full-length β1A subunit. In addition, the IL2R-β1A chimera promoted cell spreading and induced a redistribution of vinculin: the ring of focal adhesions at the periphery of the colonies was no longer assembled in GE11-IL2R-β1A cells and was replaced by thick and long streaks of vinculin at the base of cells. The arrows in Fig. 4 indicate vinculin-positive rings in cells in which IL2R-β1A was not expressed. In cells expressing IL2R-β1A, the chimera was colocalized with vinculin (Fig. 4) and the endogenous β3 subunit (data not shown) in focal contacts. Together these results indicate that IL2R-β1A induces both the disruption of intercellular adhesions and the reorganization of the cytoskeleton, thus mimicking the effects of full-length β1A.

---

**Figure 3.** Scattering of GE11 cells is dependent on β1 integrin-ligand interaction. (A) Expression of β1, α5, and α6 integrin subunits in GE11-β1 and GE11-α6β1 cells overexpressing the α6β1 integrin, as determined by FACS® analysis. Both the percentage of cells expressing the different subunits and their expression levels in positive cells in mean fluorescence (arbitrary units) are indicated. (B) Phase-contrast microscopy of GE11-α6β1 grown on plastic in the presence of FCS (FN/VN) or on laminin-1 substrate (LN-1).
pates in the formation of new adhesion structures into which β3 is eventually recruited will be discussed.

The Expression of β1, but Not That of IL2R-β1, Enhances Cell Migration

To quantify potential changes in the motility of GE11-β1A cells as compared with that of GE11-control cells, we have performed migration experiments using fibronectin-coated Transwells. As shown in Fig. 5A, although GE11-control cells are able to migrate to some extent on this substrate, the expression of β1A strongly increased cell motility. The expression of IL2R-β1A failed to enhance cell migration in any of the conditions tested.

In addition, we tested random cell migration using an in vitro wound healing system. Fig. 5B shows that GE11-β1A cells spread fast when grown under standard conditions on plastic, and that they migrate into the introduced wound. GE11-β1D cells showed similar migration kinetics (data not shown). In contrast, GE11-control cells maintained stronger cell–cell adhesions and spread and migrated more slowly.

Together, these results show that the motility of GE11-β1 cells is increased. The fact that IL2R-β1A is sufficient to trigger the disruption of intercellular adhesions, although it does not increase cell motility, indicates that cell migration is not simply the cause of the disruption of cell-cell adhesions.

Expression of β1 Integrins in GE11 Cells Induces a Decrease in Cadherin and α-Catenin Protein Levels and Their Redistribution to the Detergent-soluble Fraction

Because the expression of β1 integrins altered the integrity of intercellular adherens junctions, we compared the cadherin and α- and β-catenin protein levels as well as their detergent solubility in GE11-control, and GE11-β1A and -β1D cells. Immunoblotting of total cellular proteins revealed that GE11-β1A cells contained smaller amounts of cadherin and α-catenin than their control counterparts (Fig. 6). The amount of β-catenin also appeared to be reduced in GE11-β1A and GE11-β1D cells, but to a lesser extent than the amounts of cadherin and α-catenin. Results of a detergent solubility assay, using 1% Triton X-100,
indicated that the ratios between the insoluble and soluble fractions of cadherin and α-catenin were reduced in GE11-β1A cells as compared with GE11-control cells. Thus, smaller amounts of these two proteins are associated with the detergent-insoluble cytoskeletal fraction in GE11-β1A cells. Although the amount of β-catenin is reduced in GE11-β1A cells, its distribution in the cytoskeletal and soluble fractions is apparently not affected by β1 expression. This might be due to the existence of a detergent-insoluble pool of β-catenin in the nucleus of GE11-β1A cells, although we could not confirm this hypothesis by immunofluorescence. These results indicate that β1 integrins might cause the disruption of intercellular adhesions by inducing a down-regulation of cadherin and/or catenin function. Moreover, immunofluorescence studies performed on cells that were first permeabilized in Triton X-100 and subsequently fixed in paraformaldehyde showed stainings for cadherins and catenins similar to those obtained with paraformaldehyde-fixed cells without prior solubilization (data not shown). This suggests that the redistribution of cadherins and α-catenin from the Triton-insoluble to the -soluble fraction upon β1 expression is due to a reduced number of cell–cell adhesions rather than a decrease in their rigidity.

**Overexpression of α-Catenin in GE11 Cells Prevents the Morphological Change Induced by β1 Integrins**

By providing a link between cadherin complexes and the cytoskeleton, α-catenin plays a major role in the establishment and maintenance of cadherin-based adhesions (Rödiger, 1998). Moreover, α-catenin has been implicated in the formation of tight junctions (Watabe-Uchida et al., 1998). Because we have found that both the protein level
and the detergent solubility of α-catenin were affected by β1 integrins in GE11 cells, we have investigated the role of this protein in the β1-induced phenotypic changes. First, α-catenin was retrovirally overexpressed in GE11 cells (GE11-α-catenin cells). The effect of subsequent β1 expression in GE11-α-catenin cells was less pronounced than in GE11 cells or in cells previously transduced with the empty LZRS vector. Several clones were isolated in which the expression of β1 and α-catenin was analyzed by FACS® and immunoblotting, respectively. Fig. 7 shows the results obtained with three of them. The expression levels of α-catenin were higher in GE11-control cells and in cells from clones 3, 4, and 9, than in GE11-β1A cells (Fig. 7 A). In addition, β-catenin and cadherin protein levels were greater in GE11-control cells and in the three GE11-α-catenin-β1A clones than in GE11-β1A cells. Thus, when α-catenin is overexpressed in GE11 cells, protein levels of β-catenin and cadherin are not decreased by the expression of β1 integrins.

Although the surface expression levels of β1 integrins in clones 3 and 9 were similar to those in GE11-β1A cells (Fig. 7 B), cells from these clones remained clustered in
epithelial cell colonies (Fig. 7 C), indicating that overexpression of α-catenin prevented the β1-induced morphological change. In spite of β1 expression, cells from clone 9 exhibited the peripheral bundles of actin filaments and the vinculin-positive ring found in GE11-control cells but not in GE11-β1A cells. In addition, these cells presented well-developed intercellular adhesions containing both vinculin and cadherins (Fig. 7 D). However, results obtained with clone 4 showed that a further increase of β1 levels could overcome the inhibitory effect of α-catenin and allow cell scattering.

These results suggest that there is a tight balance between intercellular adhesion and β1-mediated attachment to ECM proteins, which can be regulated at the level of α-catenin.

**Expression of β1 Integrins Induces Activation of RhoA and Rac1**

Small G proteins of the Rho family are involved in the regulation of the actin cytoskeleton, the turnover of focal adhesions, in cell migration, and in the assembly of intercellular adhesions (Mackay and Hall, 1998). Therefore, we have studied their role in the cell scattering induced by β1 integrins. GST-rhotekin and GST-PAK fusion proteins were used to precipitate the active form (GTP-bound) of RhoA, and of Rac1 and Cdc42, respectively, from cell lysates of stably transfected GE11 cells expressing either β1A, β1D, IL2R, or IL2R-β1A. The total amounts of GTPases (guanosine diphosphate (GDP)- and GTP-bound) were measured in parallel in the same lysates. Increased amounts of GTP-RhoA were precipitated from the lysates of cells expressing either β1A or β1D, when compared with vector controls (Fig. 8). Experiments performed with transduced GD25 cells confirmed the activation of RhoA by β1A, and to a lesser extent, by the β1D integrin splice variant. Similarly, the expression of either splice variant of the β1 integrin subunit induced the activation of Rac1 in both GE11 and GD25 cells. Fig. 8 also shows that the activation of RhoA and Rac1 mediated by IL2R-β1A was less efficient than that observed with full-length β1A. Finally, in contrast to RhoA and Rac1, the activity of Cdc42 was not significantly increased upon expression of β1 integrins. These data demonstrate that expression of either the β1A or the β1D integrin splice variant activates RhoA and Rac1 in both GE11 and GD25 cells.

**Dominant Negative Mutants of Small G Proteins of the Rho Family Inhibit and Reverse the β1-Induced Phenotypic Changes and Migration**

Next, we investigated whether the activation of RhoA and Rac1 is required for the phenotypic conversion induced by β1 integrins. We first transduced dominant negative mutants of RhoA, Rac1, and Cdc42 in GE11 cells, and 2 d later, we introduced β1A. Because the same retroviral vector was used for both proteins, the clones expressing β1A could not be selected with antibiotics. Therefore, we isolated the cells expressing β1 at high levels by FACS® analysis and sorting. In the second step, several individual clones were isolated from this β1-positive cell population. The expression of both N17Rac1 (Fig. 9 A) and β1 (data not shown) was finally measured in these clones. We show that even when β1 was strongly expressed at the cell surface, substoichiometric amounts of N17Rac1 inhibited the disruption of cell–cell adhesion and cell scattering (Fig. 9 B). It has been reported previously that amounts of RhoA or Rac1 mutants well below those of endogenous RhoA and Rac1 levels caused changes in the cellular organization (Jou and Nelson, 1998), presumably because their ability to rapidly bind and dissociate from guanine-exchange factors is impaired by the mutation. The β1-positive clones that displayed a more fibroblast-like phenotype did not express N17Rac1 at detectable levels (data not shown). Fig. 9 C shows that N17Rac1 was associated with the entire plasma membrane of GE11-N17Rac1-β1A cells, but that it was especially concentrated in regions of cell–cell contacts. Cadherins and catenins remained present in intercellular junctions (Fig. 9 C). Pictures taken at both the basal and medial plane of the cell show that the β1A subunit was found in both focal contacts and in regions of cell–cell contacts (Fig. 9 C). This indicates that N17Rac1 prevented β1-mediated disruption of cadherin-based adhesion and further scattering.

We have not been successful in isolating GE11 clones expressing detectable levels of dominant negative forms of RhoA and Cdc42, probably because these constructs have a toxic effect. This was most obvious for N19RhoA, of which expression induced cell rounding and detachment 2 d after the retroviral transduction. Therefore, we followed another approach in which the dominant negative mutants were expressed subsequently to the β1 integrin subunit. A II three RhoA, Rac1, and Cdc42 mutants inhibited cell scattering, but the morphology of the cells in the colonies was different from that of untransfected GE11.
Rho-like GTPases was not due to a decrease in the levels of surface expression of β1 integrins. Finally, we have found that both N17Rac1 and N17Cdc42, but not N19RhoA, were enriched at cell–cell contacts (Fig. 10 A). This localization of N19RhoA (Jou and Nelson, 1998) and N17Rac1 (Takaishi et al., 1997; Jou and Nelson, 1998) in epithelial cells has been described previously.

It is now well-established that RhoA plays a role in actin stress fiber and focal contact formation (Mackay and Hall, 1998). As expected, strong expression of N19RhoA inhibited the formation of actin stress fibers in GE11 cells (Fig. 11). Moreover, in those cells that developed stable intercellular adhesions, vinculin remained diffusely distributed, indicating that focal contact formation is inhibited by N19RhoA (Fig. 10, lower panel). These results suggest that under conditions in which the formation of stress fibers and focal contacts is inhibited by N19RhoA, GE11-β1A cells can still form intercellular adhesions.

To determine whether the activation of Rac1 or RhoA by β1 integrins was sufficient for inducing the morphological change, dominant active mutants of RhoA (V14RhoA) or Rac1 (V12Rac1) were retrovirally expressed in GE11 cells. Although V14RhoA cells appeared to be more contracted and V12Rac1 cells displayed larger lamellipodia, neither of the constitutively active mutants separately nor when they were combined induced cell scattering (data not shown). Taken together, these results suggest that Rac, RhoA, and Cdc42 are required but not sufficient for the morphological changes induced by the expression of the β1A integrin subunit.

**Discussion**

In this study we have shown that the expression of either β1A or β1D integrin splice variants in two different β1-deficient cell lines, GE11 and GD25 cells, induces the disruption of cell-cell adhesions followed by cell scattering. This phenotypic conversion, which depends on the interaction of β1 integrins with their respective ligands, is accompanied by the reorganization of the actin cytoskeleton and focal contacts, and an increased ability of cells to migrate. However, loss of cell–cell adhesions and the reorganization of the cytoskeleton does not require cell migration, since the expression of an IL2R-β1 chimera had both these effects on cell morphology without stimulating cell motility.

Disruption of cell–cell adhesions by β1 integrins was correlated with a decrease in cadherin and α-catenin protein levels and with their redistribution from the cytoskeleton-associated fraction to the soluble fraction. When the levels of α-catenin were increased in GE11 cells by retroviral transduction, the β1-induced phenotypic changes did not occur, suggesting an important role for this catenin in the regulation of cadherin-based adhesions by β1 integrins.

We have also found that the activity of RhoA and Rac1, but not that of Cdc42, was enhanced upon expression of β1A, β1D, or IL2R-β1A in both GE11 and GD25 cells. These findings suggest that activation of these two Rho-like GTPases by β1 integrins contributes to the loss of cell-cell adhesions. Indeed, expression of either N17Rac1 or N19RhoA prevented the morphological transition in-
duced by β1 integrins. However, additional β1-induced intracellular signals are required for the phenotypic change, since constitutively active mutants of either RhoA or Rac1 or both could not induce the disruption of cell–cell contacts in GE11 cells.

**Effects of the Interaction of β1 Integrins with Their Respective ECM Ligands**

The β1-deficient GD25 cells, which have been described previously as fibroblast-like cells (Wennerberg et al., 1996), develop intercellular adhesions but scatter upon expression of the β1 integrin subunit. GE11 cells, on the other hand, displayed several features of a simple epithelium, including their morphology, polarization, the presence of apical microvilli and tight junctions, and the expression of keratin-8. Both adherens and tight junctions were disrupted upon the expression of full-length β1A. The interaction of β1 integrins with their ECM ligand appeared to be required for the disruption of cell–cell adhesions, since GE11 cells expressing αβ1 integrin, scattered only when laminin-1 was provided as a substrate.

The IL2R-β1A chimeras are distributed to focal contacts, triggers phosphorylation signals independently of binding to the ligand, and functions as a constitutively active integrin when expressed at relatively low levels (LaFlamme et al., 1992, Akiyama et al., 1994). The expression of IL2R-β1A in GE11 cells caused the disruption of intercellular adhesions and a dramatic reorganization of the ECM cell adhesion structures, similar to those induced by the intact β1 subunit. The characteristic ring of focal contacts at the periphery of the colonies of GE11 cells was replaced by more prominent, streak-like focal adhesions in which IL2R-β1A was colocalized with β3 integrins. The incorporation of IL2R-β1A into preexisting β3-containing focal contacts, might lead to the recruitment of larger amounts and/or other cytoskeletal proteins, ultimately causing the remod-
eling of these adhesion structures. However, it is also possible that IL2R-β1A participates in the formation of complexes of cytoskeletal proteins at the basal membrane in a ligand-independent manner. β3 integrins could eventually be incorporated in these complexes and stabilize them by interactions with ECM proteins. Such ligand-independent formation of cytoskeletal complexes by the cytoplasmic domain of integrins have been reported previously (Martín-Bermudo and Brown, 1996; Homan et al., 1998; Nevers et al., 1998). Although the expression of IL2R-β1A led to the disruption of intercellular adhesions, it did not promote cell migration, probably because the ligand-binding domain of the β1 integrin subunit is not present in the chimera. This indicates that the disruption of cell–cell adhesion is not merely the consequence of the stimulated cell migration.

In many epithelial cells, and in particular those forming desmosomes, the mere expression of β1 integrins is not sufficient to induce cell scattering. However, it has been shown previously that specific ECM–integrin interactions may lead to such changes in MDCK cells (Sander et al., 1998). In addition, EMT, which occurs during normal development and in epithelial tumorigenesis, is often correlated with an increase in the expression of β1 integrins and functional studies using inhibitory antibodies or chimeric proteins containing the cytoplasmic domain of β1 have indicated a role for these receptors in EMT (Monier-Gavelle and Duband, 1997; Weaver et al., 1997; Faraldo et al., 1998; Kii et al., 1998). We show here that expression of the β1 integrin subunit induces an EMT-like transition in GE11 cells. However, it is likely that β1 integrins are involved in some but not all stages of EMT, because keratin 8, an epithelial marker, remained expressed in GE11-β1 cells, whereas vimentin, a mesenchymal marker, was expressed in both GE11-control and GE11-β1 cells (data not shown).

Monier-Gavelle and Duband (1997) have suggested previously that β1 integrins are not involved in the regulation of cadherin activity during EMT of neural crest cells. Rather, they might potentiate αvβ3-mediated cell migration by an unknown mechanism. In contrast, our results suggest a requirement for β1-mediated effects for the regulation of cadherin activity, at least in some cell types. αvβ3 has been shown previously to be involved in cell migration, and it is not clear why it does not efficiently support the motility of GE11 cells, given the strong homology between the cytoplasmic domains of β1 and β3 subunits. One possibility could be that the surface levels of αvβ3 are too low to induce migration.

What Are the Molecular Mechanisms Responsible for the Disruption of Intercellular Adhesions Induced by β1 Integrin Clustering?

Our results suggest that a decrease in cadherin and α-catenin protein levels, together with their redistribution from the cytoskeleton-associated, Triton X-100-insoluble to the -soluble fraction play a major role in the β1-induced phenotypic changes. This was further supported by our finding that high levels of α-catenin expressed by retroviral transduction could inhibit cell scattering. Cadherin protein levels were increased in GE11-α-catenin-β1A cells as com-
pared with those in GE11-β1A cells, probably because overexpression of α-catenin stabilizes cadherin-based adhesions and prevents protein degradation. In addition, because α-catenin is also involved in the formation of tight junctions between epithelial cells (Watabe-Uchida et al., 1998), its downregulation by β1 integrins might be responsible for the disassembly of these structures between GE11 cells.

Although our results clearly show that intercellular adhesions can be regulated by β1 integrins via α-catenin, the molecular mechanisms of this regulation remain to be elucidated. Integrin ligation triggers multiple intracellular events, among them the activation of various protein kinases (Hows et al., 1998). Although the physiological relevance of cadherin phosphorylation in the regulation of adherens junctions has been questioned previously (Takeda et al., 1995), other studies have shown that changes in cadherin phosphorylation correlated with the regulation of cadherin-based adhesions (for reviews see Daniel and Reynolds, 1997; Monier-Gavelle and Duband, 1997; Serres et al., 1997; Calautti et al., 1998; Oaza and Kelm, 1998; Soler et al., 1998). We have not observed any changes in the tyrosine phosphorylation of cadherins in GE11-β1A stable transfectants versus GE11-control cells (our unpublished data).

Phosphorylation generally occurs on β- and γ-catenins and not on α-catenin, leading to their dissociation from the cytoskeleton. However, if the disruption of intercellular adhesions by β1 integrins would be the result of β- or γ-catenin phosphorylation, we would not expect that α-catenin could compensate for such an effect, which suggests another type of regulation by β1 integrins. It is also possible that β1-induced phosphorylation of α-catenin binding proteins other than β- or γ-catenin contributes to the disruption of intercellular adhesions.

Alternatively, overexpression of α-catenin might stabilize adherens junctions by compensating for the redistribution of structural proteins. While many components of intercellular adherens junctions and focal contacts are specific to one or the other structure, other proteins such as vinculin and α-actinin, are found in both complexes. Previous studies have suggested that α-actinin and vinculin play an important role in the establishment and maintenance of intercellular adhesions. Notably, vinculin, which shares homology with α-catenin (Rodiger, 1998), mediates anchorage of the cadherin complexes to the actin cytoskeleton in certain cell types by direct binding to β-catenin (Hazar et al., 1997). In addition, the binding of vinculin to α-catenin might provide alternative and possibly stronger links between cadherins and the actin cytoskeleton (Wiest et al., 1998). The redistribution of vinculin from adherens junctions to well-developed focal contacts that we observed in GE11 cells upon β1 expression might thus contribute to the disassembly of cell-cell adhesions and overexpression of α-catenin might compensate for this redistribution. However, this hypothesis implies that the amount of vinculin in the cell is a limiting factor, which does not seem to be the case, since it was found to reassociate with intercellular junctions in GE11-α-catenin-β1A cells.

Our data show that in addition to α-catenin, Rho-like small G proteins also play a major role in the regulation of cell scattering by β1 integrins. Recently, cell adhesion was found to regulate the activity of RhoA (Ren et al., 1999) and that of the Rac1 and Cdc42 downstream effector, PAK (Price et al., 1998). We show in this report that the stable expression of β1A or β1D in either β1-deficient GE11 or GD25 cells induces an increase in RhoA and Rac1 activity that is correlated with enhanced cell motility. That the stimulation of RhoA and Rac1 activity by IL2R-β1A does not correlate with increased cell migration is due to the absence of the ligand-binding domain of the β1 integrin subunit in the IL2R-β1A chimera. Results of experiments performed with dominant negative mutants of RhoA, Rac1, and Cdc42 indicated that all three GTPases are involved in GE11 cell scattering induced by β1 expression. In addition, our data confirm previous findings that Rho-like GTPases regulate the formation of focal contacts and focal complexes (Hotchin and Hall, 1995; Clark et al., 1998). Together, these results indicate that RhoA and Rac1 act simultaneously upstream and downstream of integrins, and that positive feedback mechanisms probably regulate the relationship between integrins and Rho-like GTPases.

The finding that β1 integrin-mediated adhesion activates both RhoA and Rac1 is consistent with the changes in the organization of the cytoskeleton and in cell behavior that we have observed in GE11 cells. Upon β1 expression, GE11 cells developed extensive lamellipodia and started to migrate. Whereas Rac1 stimulates the formation of lamellipodia and small focal complexes at the leading edge of migrating cells, RhoA stimulates the formation of new focal contacts and regulates the interaction of myosin-based motors with actin filaments to generate contractile forces required for cell motility. Although previous work suggested that cell adhesion induces the activation of the Cdc42 and Rac downstream effector PAK (Price et al., 1998), β1 integrins did not significantly activate Cdc42 in our cells. Nevertheless, basal levels of Cdc42 activity seem to be required for scattering of GE11 cells, since expression of N17Cdc42 in GE11-β1 cells inhibited their scattering and partially restored intercellular adhesions. A role for Cdc42 in cell migration has been documented for correct cell polarization during migration, but not for random migration (Allen et al., 1998; Nobes and Hall, 1999). Alternatively, the effects of N17Cdc42 might be mediated by unspecific inhibition of the Rac1 signaling pathway, by binding to guanine-nucleotide exchange factors common to both Rac1 and Cdc42.

Immunofluorescence microscopy revealed that cadherins and catenins are redistributed to intercellular adhesions in GE11-β1A cells upon expression of dominant negative mutants of RhoA, Rac1, and Cdc42. This indicates that in these cells, Rho-like GTPases are not strictly required for the assembly of cadherin-based adhesions. These observations are in contrast to previous studies that reported decreased staining intensity of cadherins and catenins induced by N17Rac1 or the RhoA inhibitor C3 in keratinocytes (Braga et al., 1997), M D C K cells (Takaishi et al., 1997), and a mammary epithelial cell line (Zhang et al., 1997). However, other investigators showed that dominant negative mutants of Rho-like GTPases affect cadherin distribution in M D C K at low, but not at high cell density (Jou and Nelson, 1998). Finally, inhibition of these GTPases had no effect on the organization of adhe-
ens junctions in intestinal epithelial cells (Nusrat et al., 1995).

These discrepancies might reflect specific regulation mechanisms in different cell types, but also different methods used for the expression of dominant negative mutants, i.e., microinjection of recombinant proteins versus inducible expression systems. A reasonable explanation is that the amounts of dominant negative mutants of Rho-like GTPases expressed by retroviral transduction are sufficient to prevent the migration of cells derived from the originally transduced single cell, which results in the formation of small epithelial-like colonies but still allows cell–cell contacts to be formed.

When expressed before β1, N17Rac1 was able to prevent GE11 cell scattering. Expression of N17Rac1 did not appear to affect the distribution of cadherins and catenins, and cells remained tightly attached to each other even in the presence of high levels of β1. This suggests that Rac1 activation is required not only for cell migration but also for the disruption of cadherin-based adhesions in these cells. However, expression of either constitutively active RhoA, Rac1, or their simultaneous expression was not sufficient for dissociating the cells in GE11 colonies, suggesting that β1 integrins trigger additional types of signals involved in the disruption of cell-cell adhesions. Moreover, the phenotypic reversal induced by the dominant negative mutants of small GTPases was only partial, and the expression of dominant negative mutants of Rho-like GTPases Rho and Rac are required for the establishment of cadherin-dependent cell–cell contacts. J. Cell Biol. 137:1421–1431.

Brakebusch, C., E. Hirsch, A. Potocnik, and R. Fässler. 1997. Genetic analysis of β1 integrin function: confirmed, new and revised roles for a crucial family of cell adhesion molecules. J. Cell Sci. 110:2895–2904.

Bräsewitz, R. A., K. E. M. Arca, et al. 1993. Ligand-dependent and -independent integrin focal contact localization: the role of the αc chymotrypsin domain. Mol. Biol. Cell. 4:593–604.

Brieu, P., C. Babinet, R. Kemler, and F. Jacob. 1980. Monoclonal antibodies against trophectoderm-specific markers during mouse blastocyst formation. Proc. Natl. Acad. Sci. USA. 77:4113–4117.

Calautti, E., S. Cabodi, P.L. Stein, M. Hatzfeld, N. Kedersha, and G. Paolo Dotti. 1998. Tyrosine phosphorylation and src family kinase control keratinocyte–cell adhesion. J. Cell Biol. 141:1449–1465.

Chong, L.D., A. Traynor-Kaplan, G.M. Bokoch, and M.A. Schwartz. 1997. The small GTP-binding protein Rho regulates a phosphatidylinositol 4-phosphate–sensitive kinase in mammalian cells. Cell. 79:207–213.

Clark, E.A., W.G. King, J.S. Brugge, M. Symons, and R.O. Hynes. 1998. Integrin-mediated signals regulated by members of the Rho family of GTPases. J. Cell Biol. 142:573–586.

Daniel, J.M., and A.E. R. Ekins. 1997. Tyrosine phosphorylation and cadherin/catenin function. Bioessays. 19:883–891.

de Melker, A.A., and A. Sonnenberg. 1999. A histogenic and histogenic role of integrin β1 in localization of β1 integrin focal contacts to the plasma membrane. J. Cell Biol. 132:211–226.

de Melker, A.A., L.M.T. Sterk, G.O. Delwel, D.L.A. Faas, H. Daams, J.J. Weening, and A. Sonnenberg. 1997. The A and B variants of the α3 integrin subunit: tissue distribution and functional characterization. Lab. Invest. 76:547–563.

Defilippi, P., A. Van Hinsbergh, A. Bertolotto, P. Rossino, L. Silengo, and G. Tarone. 1991. Differential distribution and modulation of expression of αvβ3 integrin on human endothelial cells. J. Cell Biol. 114:855–863.

Faraldo, M.M., M.A. Deugnier, M. Lukashev, J.P. Thiery, and M. Glukhova. 1990. Developmental regulation of the α1216 integrin by the tyrosine kinase Csk. J. Cell Biol. 110:2895–2904.

Feinberg, A.P. 1979. A 130K protein from chicken gizzard: its localization at the terminal barbed end of thin filaments from skeletal and smooth muscle during mouse development. J. Cell Biol. 89:999–1008.

Fässler, R., M. Pfaff, J. Murphy, A. Noegel, S. Johansson, R. Timpl, and R. A. Breslow. 1997. Lack of β1 integrin gene in embryonic stem cells affects morphogenesis, adhesion, and migration but not integration into the inner cell mass of blastocysts. J. Cell Biol. 128:979–988.

Fehlner-Gardiner, C.C., S.U. Nival, G.C. Ballestrem, and B.M. Chan. 1996. Differential utilization of VLA-4 (α4β1) and -5 (α5β1) integrins during the development of mouse bone marrow-derived mast cells. Differentiation. 60:317–325.

Geiger, B. 1979. A 130K protein from chicken gizzard: Its localization at the terminal barbed end of thin filaments from skeletal and smooth muscle during mouse development. J. Cell Biol. 89:999–1008.

Glukhova, M.A., M.G. Frid, and V.E. Koteliansky. 1990. Developmental changes in expression of contractile and cytoskeletal proteins in human aortic smooth muscle. J. Biol. Chem. 265:13042–13046.

Gumbiner, B.M. 1996. Cell adhesion: the molecular basis of tissue architecture in morphogenesis. Cell. 94:351–365.

Hazar, R.B., L. Kang, S. Roe, P.I. Borgen, and D.L. Rimm. 1997. Vinculin is associated with the E-cadherin adhesion complex. J. Biol. Chem. 272:32448–32453.

Herrenknecht, K., A. van der Flier, and R. Fässler. 1997. Genetic analysis of β1 integrin function: confirmed, new and revised roles for a crucial family of cell adhesion molecules. J. Cell Sci. 110:2895–2904.

Hirch, E., D. Gullberg, F. Balzac, F. A. Itruda, L. Silengo, and G. Tarone. 1994. αvβ3 integrin subunit is predominantly located in nervous tissue and skeletal muscle during mouse development. In. Dev. Dyn. 210:118–120.

Homan, S.M., A.M. Mercuro, and S.E. LaFlamme. 1998. Endothelial cells assemble a distinct αvβ4-containing vimentin-associated structures: roles for integrin β3 tyrosine phosphorylation and the β3 cytoplasmic tail. J. Cell Sci. 111:217–228.

Horváth, P., R. Tanaka, R.A. Van der Kammen, V. Mioli, K. Mittermayer, G. Geiger, B. 1979. A 130K protein from chicken gizzard: its localization at the terminal barbed end of thin filaments from skeletal and smooth muscle during mouse development. J. Cell Biol. 89:999–1008.

Horn, G., N. A., and A. Hall. 1995. The assembly of integrin adhesion complexes

Gimond et al. Cross-talk between β1 Integrins and Cell–Cell Adhesion Molecules 1339
requires both extracellular matrix and intracellular rho/rac GTPases. J. Cell Biol. 133:1857-1865.

Hove, A., A.E. Aplin, S.K. A lahari, and R.L. Juliano. 1998. Integration signaling and cell growth control. Curr. Opin. Cell Biol. 10:220–231.

Jou, T.S., and W.J. Nelson. 1998. Effects of regulated expression of mutant RhoA and Rac1 small GTPases on the development of epithelial (MDCK) cell polarity. J. Cell Biol. 142:85–100.

Keeley, P.J., J.K. Westwick, C.J. Der, and L.V. Parise. 1997. Cdc42 and Rac1 induce integrin-mediated cell motility and invasiveness through PI3K. Nature 390:652–635.

Kempner, H., Y.M. Wijnands, and E. Roos. 1997. On integrins on HT-29 colon carcinoma cells: adhesion to fibronectin is mediated solely by small amounts of αvβ6 and αvβ6 is codistributed with actin fibers. Exp. Cell Res. 234:156–164.

KI, S.H., C.E. Krull, G. Cann, D. Clegg, and M. Bronner-Fraser. 1998. The αv subunit of integrin is important for neural crest cell migration. Dev. Biol. 202:29–42.

Kinsella, T.M., and G.P. Nolan. 1996. Episomal vectors rapidly and stably produce high-titer recombinant retrovirus. Hum. Gene Ther. 7:1405–1413.

LaFlamme, S.E., S.K. Akiyama, and K.M. Yamada. 1992. Regulation of fibronectin receptor distribution. J. Cell Biol. 117:437–447.

Mackay, D.J., and A. Hall. 1998. Rho GTPases. J. Biol. Chem. 273:20685–20688.

Martin-Bermudo, M.D., and N.H. Brown. 1996. Intracellular signals direct integrin localization to sites of function in embryonic muscles. J. Cell Biol. 134:217–226.

Michiels, F., J.C. Stamm, P.L. Nordick, R.A. van der Kamm, L. Ruitu Van Stalle, C.A. Feltkamp, and J.G. Collard. 1997. Membrane localization of Tiam1 is required for Rac-mediated membrane ruffling and JNK activation. J. Cell Biol. 137:387–398.

Monier-Gavelle, F., and J.L. Duband. 1997. Cross-talk between adhesion molecules: control of N-cadherin activity by intracellular signals elicited by β1 and β3 integrins in migrating neural crest cells. J. Cell Biol. 137:1663–1681.

Moellenar, C.E., E.J. Muller, D.J. Schol, C.G. Fidler, E. Bock, D. Bitter-Suermann, and R.J. Michalides. 1990. Expression of neural cell adhesion molecule-related sigaIoglycoprotein in small lung cancer and neuroblastoma cell lines H69 and CHF-212. Cancer Res. 50:1102–1106.

Nieves, M.G., R.Q. Schapweld, L.C. Oomen, L. Fontao, D. Geerts, and A. Sonnenberg. 1998. Ligand-independent role of the β4 integrin subunit in the formation of hemidesmosomes. J. Cell Sci. 111:1659–1672.

Nobes, C.D., and A. Hall. 1999. Rho GTPases control polarity, protrusion, and adhesion during cell movement. J. Cell Biol. 144:1225–1244.

Nusrat, A., M. Giry, J.R. Turner, S.P. Colgan, C.A. Parkos, D. Carnes, E. Lemicher, P. Boucot, and J.L. Madara. 1995. Rho protein regulates tight junctions and peri-junctional actin-organization in polarized epithelia. Proc. Natl. Acad. Sci. USA. 92:10629–10633.

Ozawa, M., and R. Kemler. 1998. Altered cell adhesion activity by pervanadate due to the dissociation of α-catenin from the E-cadherin-catenin complex. J. Biol. Chem. 273:6166–6170.

Parrish, E.P., P.V. Steart, D.R. Garrard, and R.O. Weller. 1987. βcatenin: a monomeric actin-binding protein. J. Biol. Chem. 262:1157–1161.

Price, L.S., J. Leung, M.A. Schwartz, and G.M. Bokoch. 1998. Activation of Rac and Cdc42 by integrins mediates cell spreading. Mol. Biol. Cell. 9:1863–1871.

Reid, T., T. Furuyashiki, T. Ishizaki, G. Watanabe, N. Watanabe, K. Fujisawa, N. Morii, P. Madaule, and S. Narumiya. 1996. Rhotekin, a new putative target for Rho bearing homology to a serine/threonine kinase, and rhophilin in growth factor responses by Ras, Rac and Rho in MDCK cells. Mol. Cell. Biol. 16:1110–1122.

Ridley, A.J., J.O. Pim, and A. Hall. 1995. Regulation of scatter factor/hepatocyte growth factor responses by Ras, Rac and Rho in MDCK cells. Mol. Biol. Cell. 15:1110–1122.

Ridley, A.J. 1998. Vinculin and α-catenin: shared and unique functions in adhesion junctions. Bioessays. 20:733–740.

Sonnenberg, A.H., J. Bienens, W. Birchmeier, and S. Tsukita. 1995. V-src kinase shifts the cadherin-based cell adhesion system from the strong to the weak state and α-catenin is not required for the shift. J. Cell Biol. 131:1839–1847.

Takai, K., T. Sasaki, H. Kotani, H. Nishioka, and Y. Takai. 1997. Regulation of cell-cell adhesion by rac and rho small G proteins in MDCK cells. J. Biol. Chem. 139:1047–1059.

Takeda, H., A. Nagafuchi, S. Yonemura, S. TakaIta, J. Behrens, W. Birchmeier, and S. Tsukita. 1995. V- src kinase shifts the cadherin-based cell adhesion system from the strong to the weak state and α-catenin is not required for the shift. J. Cell Biol. 131:1839–1847.

van der Flier, A., I. Kuikman, C. Grangeasse, M. Haftek, Y. Durocher, B. Ducleo, and D. Schmitt. 1997. Hyperphosphorylation of β-catenin on serine-threonine residues and loss of cell-cell contacts induced by calcylulin A and okadaic acid in human epidermal cells. Exp. Cell Res. 231:163–172.

Shaw, L.M., I.R. Rabinovitch, H.H.F. Wang, A. Toker, and A.M. Mercurio. 1997. A novel pleiotropic protein kinase. Nature. 393:213–217.

Takeishi, K., T. Sasaki, H. Kotani, H. Nishioka, and Y. Takai. 1997. Regulation of cell-cell adhesion by rac and rho small G proteins in MDCK cells. J. Biol. Chem. 163:137–146.

Wennerberg, K., L. Lohikangas, D. Gullberg, M. Pfaff, S. Johansson, and R. Fässler. 1996. Restoration of adhesion to fibronectin in a null mouse fibroblastic cell line by viral expression of cDNA for β1 integrins. J. Cell Biol. 137:1857–1865.

Wu, P.J., J.K. Westwick, I.P. Whitehead, C.J. Der, and L.V. Parise. 1997. Cdc42 and Rac1 induce integrin-mediated cell motility and invasiveness through PI3K. Nature 390:652–635.