Journal of Cell Science 103, 943-951 (1992)
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A chimeric N-cadherin/β_1-integrin receptor which localizes to both cell-cell and cell-matrix adhesions

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Summary

To study the molecular mechanisms involved in formation of cell contacts, we have transfected cultured cells with a chimeric cDNA encoding the cytoplasmic and transmembrane domains of β_1 integrin and the extracellular region of N-cadherin and determined the subcellular distribution of the chimeric molecule. We show that the chimeric receptor associates preferentially with cell-matrix focal contacts, suggesting that its distribution is directed by its β_1 integrin segment, presumably via interactions of the cytoplasmic domain with cytoskeletal elements characteristic of focal contacts. Transfected cells which expressed relatively high levels of the cadherin/integrin chimera underwent an apparent epithelialization and contained the molecule both in cell-matrix and cell-cell contacts. Location in cell-cell contacts indicates competence of the cadherin extracellular domain to participate in formation of cell-cell junctions using a foreign cytoplasmic domain. Labeling of these cultures for talin, which is normally associated only with matrix adhesions, revealed specific labeling along the newly formed intercellular junctions. This suggests that the local association of talin with these sites is induced by the cytoplasmic tail of β_1 integrin receptor presented by the chimeric protein. These results suggest that the formation of adherens-type junctions is driven by the cooperative interactions of the relevant adhesion molecules (cadherins and integrins) both with the respective extracellular ligands and with the cytoskeleton.

Key words: cell adhesion, adherens functions, cadherin, integrin.

Introduction

Adherens type junctions (AJ) comprise a family of cellular contacts characterized by their association, within the cells, with the actin-containing microfilament network (Geiger, 1982; Geiger et al., 1985a, 1987, 1990a,b; Burridge et al., 1988). Two major subfamilies of microfilament-associated adhesions have been described in cells and tissues. These are (1) intercellular AJ such as the *zonula adherens* found in polar epithelia and (2) cell-matrix contacts including focal contacts of cultured cells and similar adhesions to the basement membrane. Each type of AJ contains a set of cytoskeletal proteins linked via submembrane plaques to specific transmembrane receptors (Geiger et al., 1987, 1990a,b; Burridge et al., 1988). Recent studies have shown that many cytoskeletal components of both cell-cell and cell-matrix AJs are molecularly similar (actin, α-actinin), whereas some of the junctional plaque proteins and the transmembrane receptors of cell-cell and cell-matrix AJ contain distinct classes of molecules (Geiger et al., 1985b, 1990a,b). Thus, intercellular AJ contain plakoglobin, catenins and cadherins (Magee and Buxton, 1991) while matrix adhesions contain talin and members of the integrin family (Turner and Burridge, 1991). Furthermore, it has been shown that both cadherins and integrins are encoded by multigene families which are expressed in a cell-type specific manner (Takeichi, 1988, 1991; Hynes, 1987, 1992) and mediate selective interactions with other cells or extracellular matrices. The assembly of AJ has been thought to be initiated by the interaction of the relevant surface recep-

tors with the respective "extracellular ligands", thus nucleating the local assembly of the plaque and cytoskeletal elements of the junction (Geiger et al., 1984a,b; Geiger and Ginsberg, 1991; Dejana et al., 1988; Singer et al., 1988; Fath et al., 1989). However, evidence has also been presented that the cytoplasmic domains of both cadherins (Nagafuchi and Takeichi, 1989; Ozawa et al., 1989, 1990) and integrins (Solowska et al., 1989; Hayashi et al., 1990; Marcantonio et al., 1990) are necessary for stable association of transmembrane adhesion receptors with cell junctions. These data suggest that interactions of the cytoplasmic domains with plaque and/or cytoskeletal proteins also play crucial roles in junction assembly.

To explore these ideas further, we have transfected different cells, which contain no, or low levels of, cadherins, with cDNA encoding a chimeric receptor consisting of the cytoplasmic and transmembrane moieties of β_1 integrin (Tamkun et al., 1986) and the extracellular domain of N-cadherin (Hatta et al., 1988). Immunolocalization of the chimeric receptor using N-cadherin-specific antibodies (anti-A-CAM; Volk and Geiger, 1984) revealed that the chimera associated with focal contacts (characteristic of cell-matrix interactions) in the transfected cells. However, transfectants that expressed relatively high levels of the cadherin/integrin chimera formed, in addition, extensive cell-cell contacts in which the chimeric molecule was present. Interestingly, these novel contact sites contained the plaque protein talin, which is normally associated only with the cell-matrix focal contacts. These results indicate first that recruitment of transmembrane proteins to focal contacts occurs, at least in part, via the cytoplasmic moiety of the β_1 integrin subunit. Conversely, intercellular adhesions, triggered by the cadherin extracellular domain of the chimeric receptors, can recruit talin to the contact sites, most likely via the integrin β_1 cytoplasmic tails localized in these regions. Our results lead to a view of AJ formation driven jointly by interactions of adhesion receptors (cadherins, integrins, etc.), both with their extracellular ligands and with the cytoskeleton in a form of two-dimensional transmembrane-coupled assembly reaction.

Materials and methods

Molecular genetic techniques

Unless otherwise indicated, the molecular genetic techniques employed here were carried out according to Sambrook et al. (1989). Most enzymes were purchased from Boehringer Mannheim (FRG). The preparation of the construct consisted of the following stages. (a) Isolation of the 2.15 kbp *Hpa*II fragment of chicken N-cadherin cDNA, cloned in BlueScript vector (Hatta et al., 1988). The enzyme cuts 7 nucleotides downstream from the initiator ATG and in the 5th ectodomain, close to the transmembrane sequence. (b) Cloning of the *Hpa*II fragment into the *Bgl*II site of pECE vector(Ellis et al., 1986), using a *Bgl*II/*Hpa*II linker. This linker also reconstructs the sequences encoding the first 3 amino acids. (c) Isolation of the *Hae*III-*Xba*I (840 bp) fragment of chicken β_1 integrin cDNA in pGEM1 vector (Tamkun et al., 1986). The *Hae*III site is located in the extracellular domain of the β_1 chain, close to the transmembrane sequence, while the *Xba*I site is located in the polylinker, beyond the termination codon. (d) Ligation of the *Hae*III-*Xba*I β_1 integrin fragment into the *Sma*I-*Xba*I sites of the pECE vector containing the N-cadherin. Orientations and proper ligations were all verified by direct sequencing using suitable oligonucleotides. The final product consists of 5′ N-cadherin sequences and 3′ β_1 integrin sequences linked by 12 amino acids contributed by the linker and the vector. Transfection of cDNA (including cDNAs encoding the intact N-cadherin, truncated N-cadherin from which most of the cytoplasmic domain was deleted and β_1 integrin) into CHO and 3T3 cells was carried out using the calcium phosphate method and transfectants were selected with 600 μg/ml G-418 (GIBCO Labs, USA). Sequencing of DNA was carried out using the Sequenase kit (US Biochemicals, USA).

Immunological techniques

Antibodies used in the present study include monoclonal anti-N-cadherin (A-CAM), clone ID-7.2.3 (Volk and Geiger, 1984), which specifically reacts with the chicken protein. Anti-β_1 integrin was a rabbit antibody (R-363) prepared against the C-terminal 36 amino acids, corresponding to the cytoplasmic domain of the β_1 integrin (Marcantonio and Hynes, 1988). Control rabbit serum was anti-cytokeratin (Gigi et al., 1982), which does not react specifically with CHO and 3T3 proteins. Vinculin antibodies were prepared in rabbits (Geiger, 1979) and talin antibodies were kindly supplied by Keith Burridge, UNC, Chapel Hill. Secondary fluorescent antibodies were purchased from Jackson Labs. USA. Immunofluorescence microscopy was carried out using an Axiophot microscope (Zeiss, FRG), equipped for fluorescein and rhodamine labeling. Immunoblotting analysis following SDS-polyacrylamide gel electrophoresis was performed according to Towbin et al. (1979). Immunoprecipitation with anti-integrin was performed using R-363 antibodies and *Staphyloccus* A (Pansorbin, Sigma, USA).

Results

Construction of N-cadherin/β_1 integrin chimeric cDNA

The transfection of cells with chimeric receptors for domain analysis rather than the truncation of the respective regions was used here in view of previous results indicating that cadherins or integrin β_1 subunits, missing the intracellular domain are not fully functional (Nagafuchi and Takeichi, 1989; Ozawa et al., 1990; Solowska et al., 1989; Marcantonio et al., 1990; Hayashi et al., 1990). The chimeric cDNA was prepared by isolating a 2.15 kbp *Hpa*II fragment of chicken N-cadherin cDNA corresponding to most of the ectodomain, and ligating it to the *Bgl*II site of the pECE vector(Ellis et al., 1986). For ligation to the vector we used a synthetic *Hpa*II/*Bgl*II linker, which also reconstructed the N-terminal 3 amino acids encoded by sequences located 5′ to the *Hpa*II site (Fig. 1). The *Hae*III/*Xba*I fragment of chicken β_1 integrin cDNA, encoding the cytoplasmic and transmembrane regions, was then isolated and ligated into the *Sma*I/*Xba*I sites of the vector as shown in Fig. 1. The switch site was thus located in the extracellular domains of the molecules close to transmembrane segment.

Expression of N-cadherin/β_1 integrin chimeric receptors in cells

After the correct construction was verified by direct sequencing, the entire chimeric cDNA was transfected into either CHO or 3T3 cells (Graham and Van der Eb, 1973) in conjunction with pSV2-neo plasmid (Southern and Berg,

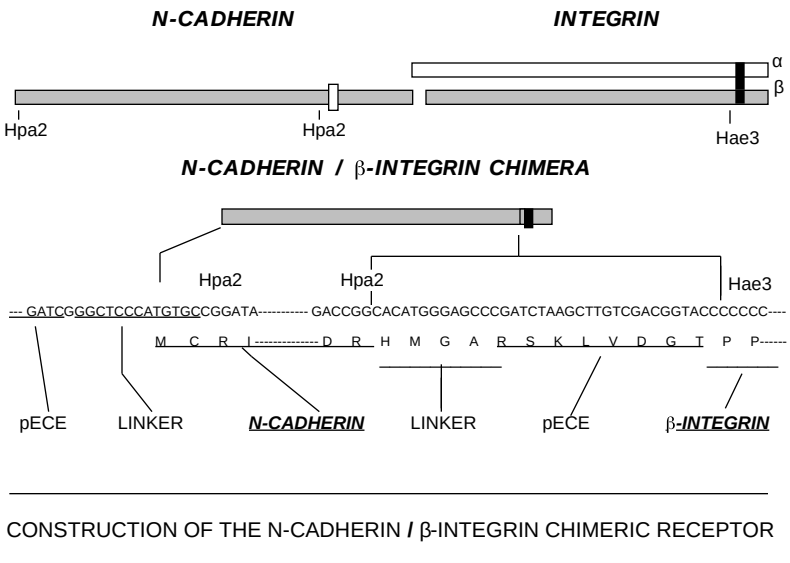

Fig. 1. The construction of N-cadherin/β_1 integrin chimeric cDNA for transfection into eukaryotic cells (for details, see text).

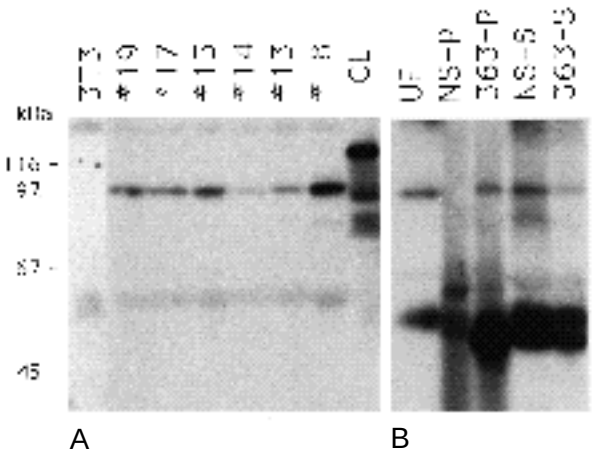

Fig. 2. Western blot analysis of the cadherin/integrin chimeric receptor in transfected 3T3 cells. (A) Immunoblotting of several 3T3 clones transfected with the chimeric cDNA as well as neo-transfected controls (3T3) using anti-chicken N-cadherin antibody reactive with the extracellular domain (ID 7.2.3). For comparison we examined chicken lens cells (CL), which contain relatively large amounts of intact N-cadherin (135 kDa apparent molecular mass). Note that there are considerable variations in the level of expression of the ~100 kDa chimera. Quantitative densitometric analysis indicated that the amounts of N-cadherin/integrin chimera in the different clones (relative to clone no. 14, taken here as reference) were: no. 19, × 5.9; no. 17, × 4.7; no. 15, × 7.5; no. 13, × 3.0, no. 8, × 18.8. (B) Immunoprecipitation of the chimeric protein with anti b_1 integrin antiserum (R-363, reactive with the cytoplasmic domain) followed by immunoblotting with anti N-cadherin (ID 7.2.3) antibody. As control, anti-keratin was used, instead of anti-integrin. The lanes on the gel are as follows: (1) unfractionated cell lysate, mixed with anti-integrin antibodies (UF); (2) fraction immunoprecipitated with the irrelevant (non-specific) antibody (NS-P); (3) fraction immunoprecipitated with anti-integrin antibody (363-P); (4) supernatant fraction left after immunoprecipitation with the irrelevant antibody (NS-S); (5) supernatant fraction left after immunoprecipitation with anti-integrin antibody (363-S). Note that anti-integrin effectively immunoprecipitates the cadherin immunoreactivity, confirming that both epitopes are present on the same molecule.

1982). The expression of the molecule in the transfected cells was monitored by both immunoblotting analysis and immunofluorescent staining with chicken N-cadherin (A-CAM)-specific antibody, reactive with the extracellular domain of the molecule.

Different stably transfected clones expressed greatly different levels of the 100 kDa chimeric protein as determined by quantitative densitometric scanning of the immunoblot autoradiograms. Thus, expression of the molecule in clone no. 8 was nearly 20-fold higher than that found in clone no. 14, with intermediate values detected in other clones (Fig. 2a). The expressed molecules were mostly present at the cell surface, as concluded from their sensitivity to extracellular proteases (data not shown). To verify that the molecule recognized by the anti-N-cadherin antibody contained the cytoplasmic tail of the β_1 integrin subunit, we immunoprecipitated the molecule from total cell lysates, with an antibody (R-363) directed against a synthetic peptide corresponding to the C terminus of chicken β_1 integrin (Marcantonio and Hynes, 1988). The immunoprecipitates were then examined by immunoblotting with anti-N-cadherin. As shown in Fig. 2B, the anti-β_1-integrin antibody effectively immunoprecipitated an N-cadherin-reactive 100 kDa molecule from the cells transfected with the chimeric cDNA, confirming that the two moieties are indeed associated with the same molecules. Notably, anti-integrin antibodies did not co-precipitate N-cadherin from cells transfected with N-cadherin cDNA (not shown).

Subcellular distribution of the chimeric receptor

To localize the cadherin-integrin chimera in the transfected cells, we carried out indirect immunofluorescence labeling with anti-chicken N-cadherin. This antibody did not react with non-transfected cells either by immunoblotting (Fig. 2A) or in immunofluorescence (Fig. 3E) assays.

In the transfected 3T3 cells, two distinct patterns of distribution of the chimeric protein were noted. The first, which was characteristic of transfectants expressing relatively low or intermediate levels of the cadherin-integrin chimera, consisted of a typical focal contact distribution

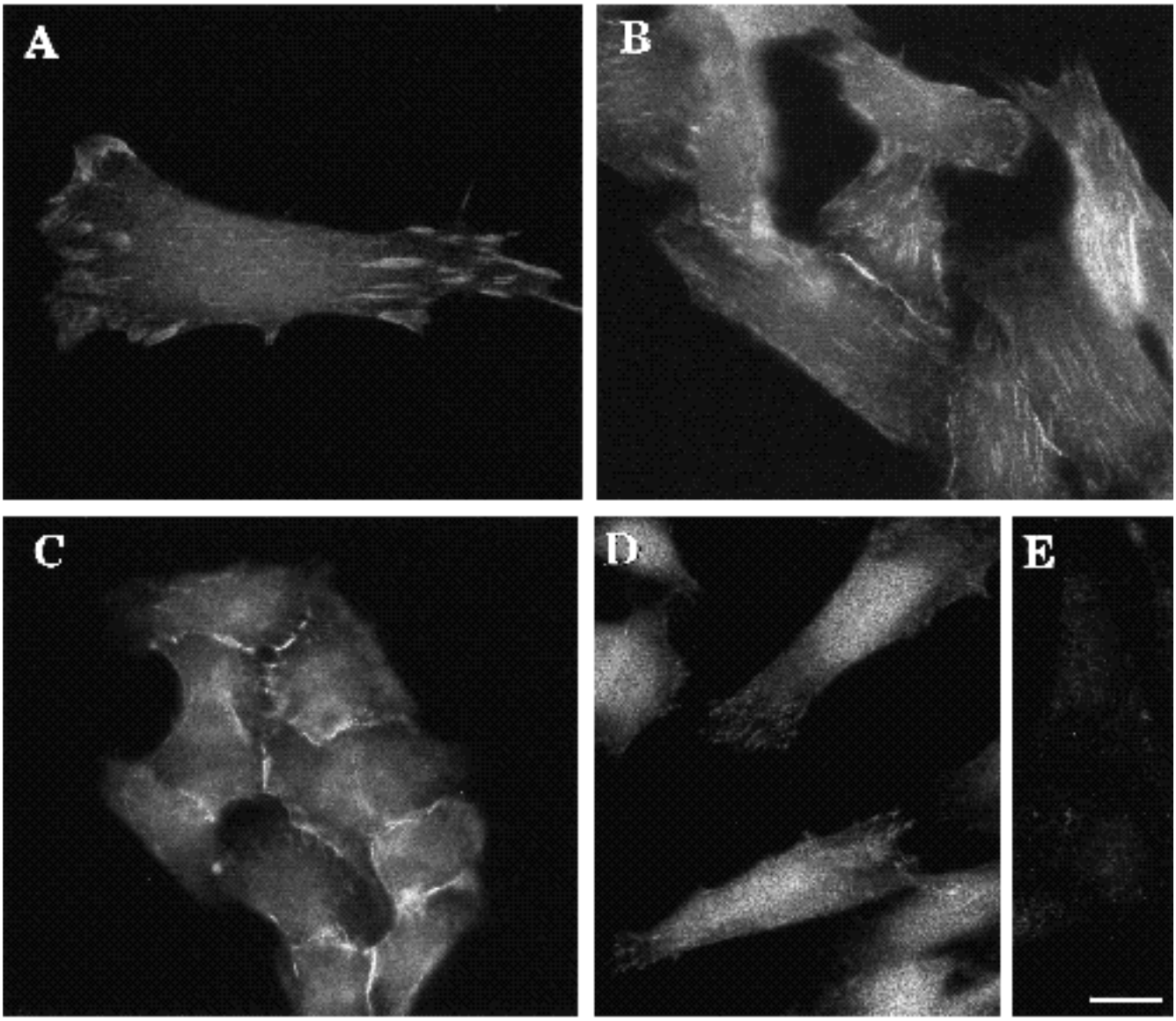

Fig. 3. Immunofluorescence localization of the cadherin/integrin chimera in transfected 3T3 (A-C) and CHO (D,E) cells. The labeling was carried out with anti-chicken cadherin monoclonal antibody (ID-7.2.3), which does not react with mammalian cadherins (see E and Fig. 2, lane 3T3). The transfected cell lines selected for labeling were clones no. 17 (A) expressing moderate levels of the chimeric protein and no. 8 (B and C) expressing relatively high levels. Transfected CHO cells are shown in D. Note that N-cadherin labeling in the transfected cells is mainly associated with focal contacts, often displaying "needle-eye" pattern (see text). In clone no. 8, an additional prominent intercellular labeling is apparent, which, in some dense areas throughout the culture, becomes predominant (C). Bar, 10 μm.

(Fig. 3A and B). The ventral patches labeled with the anti-N-cadherin antibodies were localized at the termini of stress fibers (Fig. 4A and B), in apparent association with vinculin (Fig. 4C and D). It was, however, noted that the cadherin-integrin chimeric receptor often had a needle-eye-shaped distribution along focal contacts, as previously reported for integrins in some cells (Damsky et al., 1985), and distinct from plaque components such as vinculin and talin (Geiger, 1979; Burridge and Connell, 1983, and see Fig. 4), which were uniformly distributed throughout the contact site. Preliminary studies in which 3T3 and CHO cells were transfected with cDNA encoding either β_1 integrin or N-cadherin indicated that the respective proteins were associated exclusively with focal contacts or cell-cell adhesions (Geiger et al., 1992).

In 3T3 or CHO cells expressing especially high levels of the chimeric receptor, an additional distribution of the molecule at regions of cell-cell contact became apparent (Fig. 3C). In addition, cell-matrix adhesions were invariably positive in these cells. It is noteworthy that these cells have also assumed a more epithelioid morphology than the cells expressing lower levels or no chimeric molecules, as well as cells containing a truncated N-cadherin from which the cytoplasmic domain was deleted or an intact β integrin. These morphological effects as seen in CHO cells, which normally show only limited intercellular adhesion, are

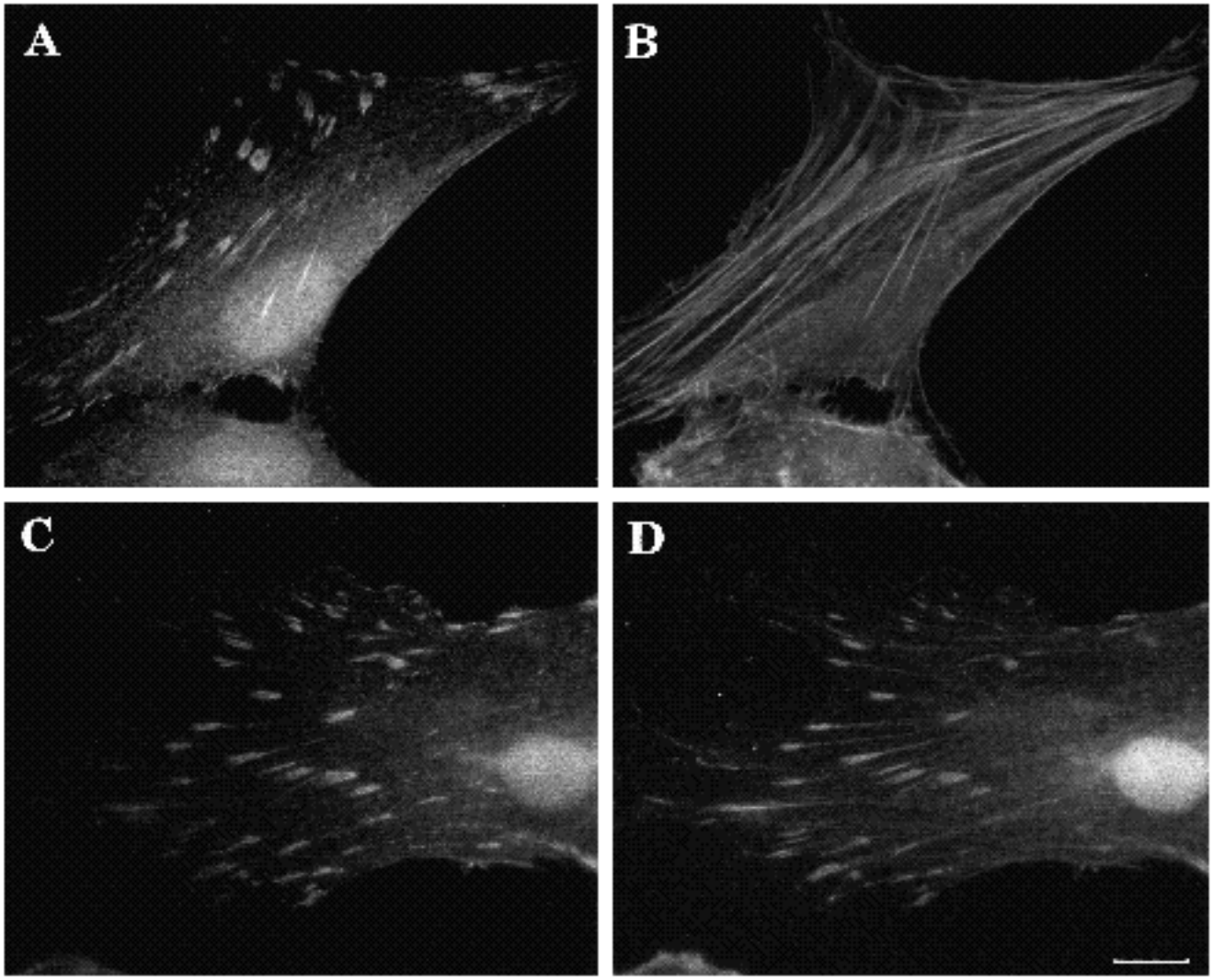

Fig. 4. Double immunofluorescence labeling of transfected 3T3 cells (clone no. 17) with anti N-cadherin (A and C) and either phalloidin (B) or rabbit anti-vinculin (D). Note that the chimeric receptor is largely associated with vinculin-positive focal contacts at the termini of actin-containing stress fibers. Bar, 10 mm.

shown in Fig. 5. The typical CHO morphology (Fig. 5A) is unaffected by transfection of truncated cadherin (Fig. 5D) but, when either intact cadherin (Fig. 5B) or the chimera (Fig. 5C) are expressed at relatively high levels, epithelioid clusters appear, which are characterized by elongated cell appositions (arrows in Fig. 5B and C). In contrast, when exogenous β_1 integrin is expressed, the cells flatten and show ruffled membranes but elongated cell appositions were not evident (Fig. 5E).

Therefore, the N-cadherin/β_1 integrin chimera can become concentrated at both cell-cell and cell-matrix contacts whereas the parent molecules each can associate with only one type of contact.

Redistribution of talin to cell-cell junctions

The cadherin-integrin chimeric molecules contain the C-terminal cytoplasmic tail of the β_1 subunit of integrin, which normally participates in the linkage to the cytoskeleton at cell-matrix AJ. Since this cytoplasmic domain is ectopically localized in cell-cell contact sites of the cells expressing high levels of the chimeric molecule (Fig. 3B and C), we examined the subcellular localization, in these cells, of talin, which is known to interact with the cytoplasmic region of β_1 integrin (Horwitz et al., 1986). Talin is normally associated exclusively with cell-matrix AJ and is absent from intercellular junctions (Geiger et al., 1985b). Double labeling of the transfected 3T3 cells with anti-N-cadherin and anti-talin antibodies indicated that talin was colocalized with the cadherin-integrin chimera not only in focal contacts (Fig. 6C) but also along the intercellular adhesions (Fig. 6A,D and B,E respectively). It is noteworthy that intercellular adhesions in control cells expressing intact N-cadherin did not contain talin (not shown but see Geiger et al., 1985b, 1990).

Therefore, the N-cadherin/β_1 integrin chimera contains sufficient information, presumably in the β_1 integrin cytoplasmic domain, to target talin to cell-cell junctions from which it is normally excluded.

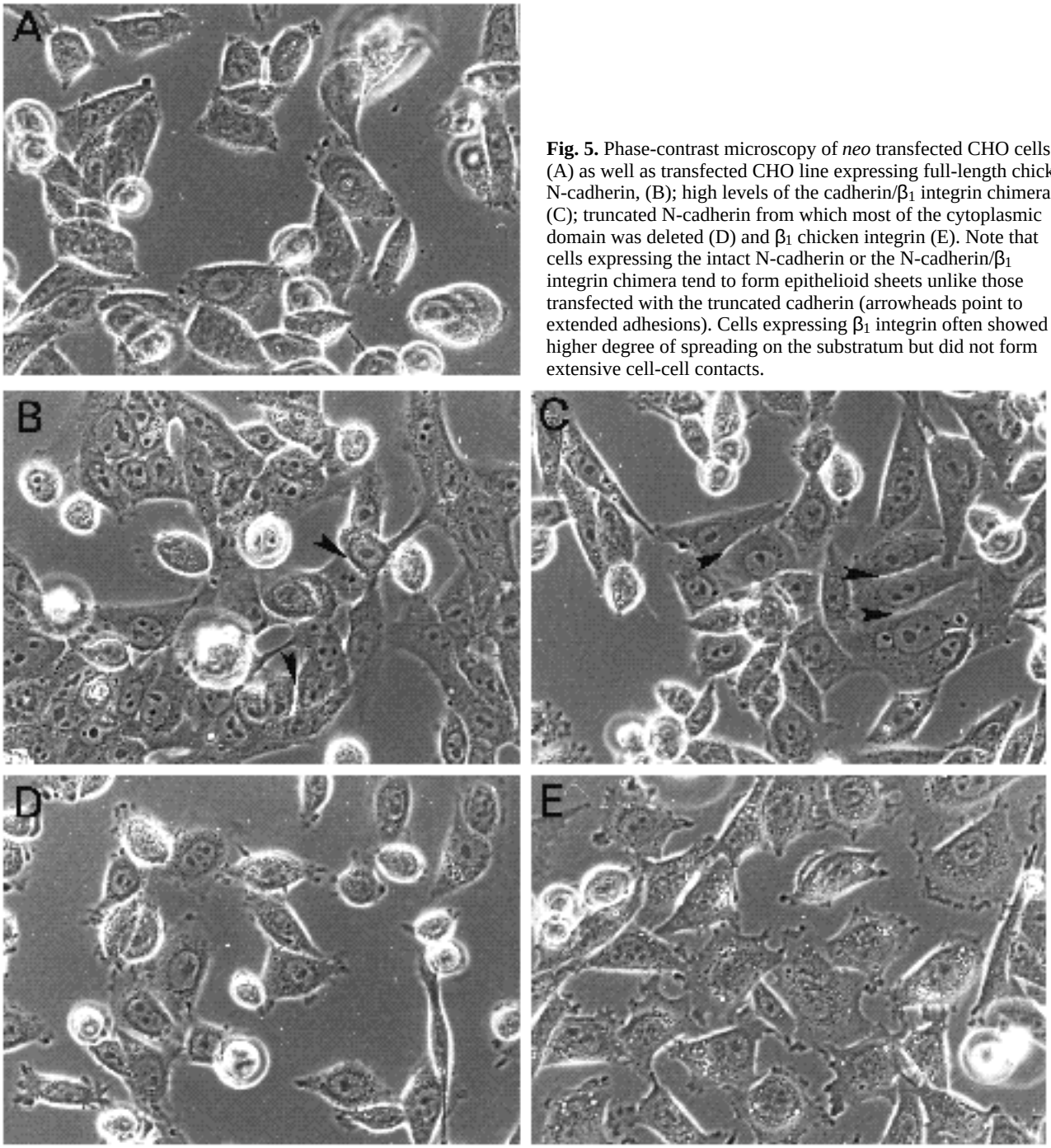

Fig. 5. Phase-contrast microscopy of *neo* transfected CHO cells (A) as well as transfected CHO line expressing full-length chicken N-cadherin, (B); high levels of the cadherin/β_1 integrin chimeras, (C); truncated N-cadherin from which most of the cytoplasmic domain was deleted (D) and β_1 chicken integrin (E). Note that cells expressing the intact N-cadherin or the N-cadherin/β_1 integrin chimera tend to form epithelioid sheets unlike those transfected with the truncated cadherin (arrowheads point to extended adhesions). Cells expressing β_1 integrin often showed higher degree of spreading on the substratum but did not form extensive cell-cell contacts.

Discussion

As discussed in the Introduction, the transmembrane and submembranous plaque components of cell-cell and cell-matrix junctions are distinct. Cadherins and their associated cytoskeletal plaque proteins, catenins and plakoglobin, are concentrated in cell-cell adherens junctions but are absent from cell-matrix focal contacts. In contrast, many integrins are concentrated in focal contacts together with the characteristic cytoskeletal protein, talin. While some integrins have been observed associated with the lateral faces of epithelial cells (Larjava et al., 1990; Carter et al., 1990; Marchisio et al., 1991) they do not become concentrated in cell-cell adherens junctions and talin is not found there.

The segregation of cadherins and integrins in cells is presumably a function of their molecular interactions. The relative contributions of the interactions of these transmembrane adhesion receptors with extracellular components and with the cytoskeleton remain uncertain. Some data favor a key role for extracellular interactions while others implicate crucial interactions of the cytoplasmic domains. The behavior of the N-cadherin/β_1 integrin chimera, which we report here, provides further insight into these issues and leads to a picture in which *both* extracellular *and* intracel-

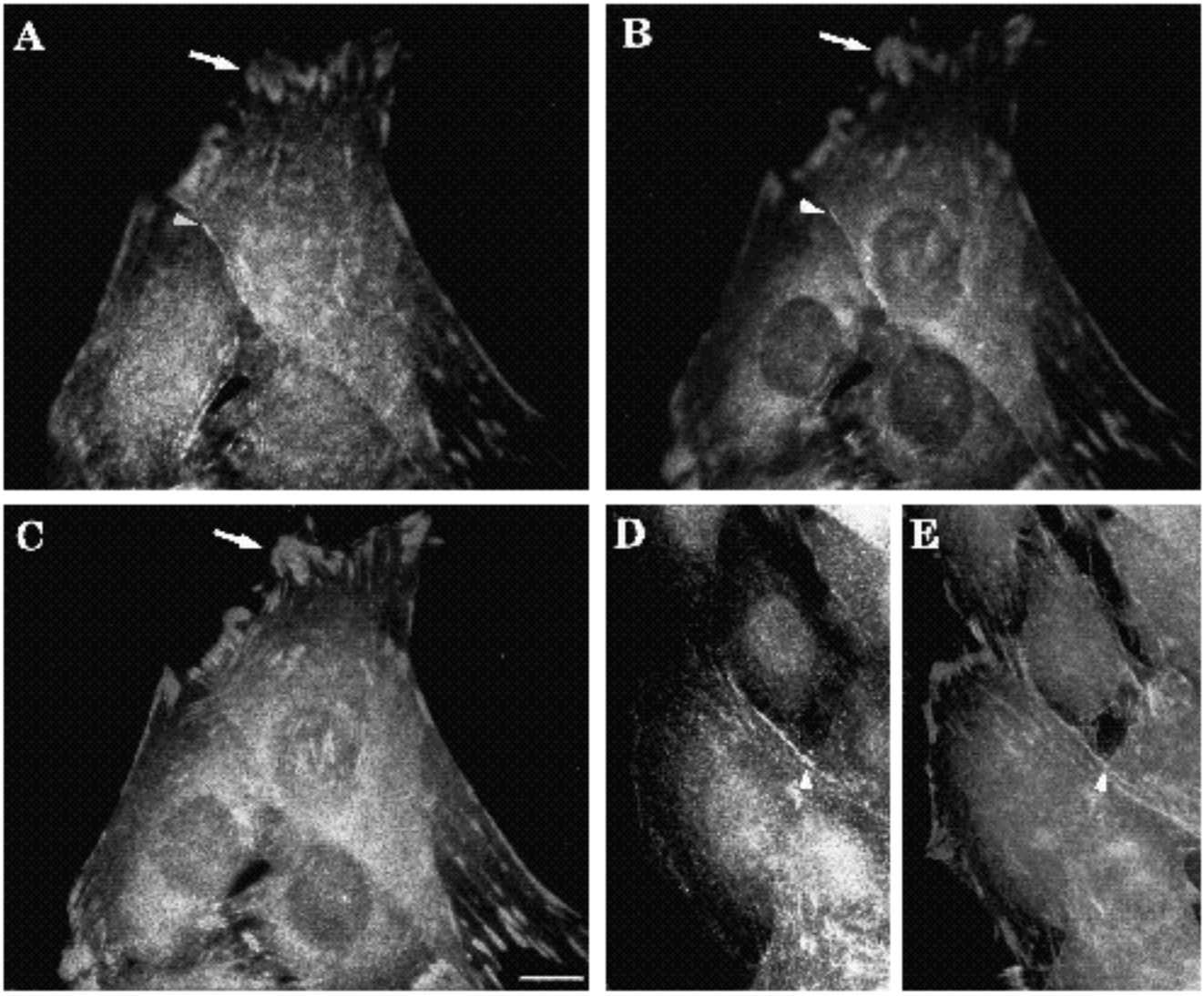

Fig. 6. Double immunofluorescence labeling of transfected 3T3 cells (high expressors, clone no. 8) with anti chicken N-cadherin (A and D) and anti-talin (B, C and E). Note that in these cells, which contain chimeric molecules in both focal contacts and cell-cell contacts (matched arrows in A and B; see Fig. 3), talin becomes associated with both adhesions. (A and B) were taken at the same dorsal focal plane, whereas (C) shows talin at a ventral focal plane in the same cells. (D and E) show another example of colocalization of the chimera (D) with talin (E) at cell-cell junctions (arrowhead). In non-transfected cells or in cells transfected with a cadherin cDNA talin is never found in association with cell-cell adhesions (Geiger et al., 1985).

lular interactions play important roles in the behavior and distribution of cadherins and integrins. Several separable conclusions can be drawn.

Earlier work has shown that extracellular interactions of integrins can target them to focal contacts (Dejana et al., 1988; Singer et al., 1988; Fath et al., 1988). Other experiments show that the β_1 cytoplasmic domain is *necessary* for this targeting to focal contacts (Solowska et al., 1989; Hayashi et al., 1990; Marcantonio et al., 1990). The localization of the N-cadherin/β_1 integrin in focal contacts (Figs 3,4,6) shows that the β_1 integrin transmembrane plus cytoplasmic segment is *sufficient* to target the chimera to focal contacts. Other interpretations are unlikely for several reasons. Since cadherins are never found in focal contacts, the N-cadherin extracellular domain cannot be responsible for the concentration of the chimera in focal contacts. Equally, since the association of integrins in αβ dimers relies on the N-terminal globular domains (Hynes, 1992), a contribution of associated integrin α subunits is extremely unlikely. We have been unable to rule it out absolutely, since the chicken-specific anti-N-cadherin antibody functions poorly in immunoprecipitation (unpublished results). Therefore, the concentration of the chimera in focal contacts appears to be driven by the β_1 integrin segment, presumably by interactions of the cytoplasmic domain with focal contact cytoskeletal components. A recent paper (LaFlamme et al., 1992), which appeared while this paper was being prepared for publication, also indicates that the β_1 cytoplasmic domain is *sufficient* to target transmembrane proteins to focal contacts.

The recruitment of integrins or of the chimera to focal contacts via interactions of the β_1 integrin cytoplasmic

domain raises several further implications. First, there must be excess binding sites for this domain in the submembranous plaque of the focal contact. Assembly of the plaque is a cooperative process involving two or more sites in the vinculin molecule (Bendori et al., 1989) and possibly in talin also (Nuckolls et al., 1990; Rees, Critchley and Hynes, unpublished data). α-Actinin, which is a dimer, can also bind to β_1 integrin cytoplasmic domain (Otey et al., 1990). Therefore, during assembly of focal contacts, involving these three molecules as well as many others, it is plausible that excess binding sites for β_1 integrin cytoplasmic domain could be available, allowing accretion of additional integrin or, in this case, of the cadherin/β_1 integrin chimera. Such an accretion process could be responsible for the normal growth and stabilization of focal contacts following their initiation by interactions with the extracellular matrix. This model requires an explanation of the fact that β_1 integrins do not concentrate in focal contacts unless their extracellular ligand is present. In an intact αβ integrin, the β_1 cytoplasmic domain must either be in an unfavorable conformation or be occluded by the a cytoplasmic domain unless the integrin binds ligand and the β_1 cytoplasmic domain becomes available for binding to the plaque constituents. In the chimeras described here and by LaFlamme et al. (1992) the β_1 cytoplasmic domain appears free to interact. It is, however, important to emphasize that the present results do not imply that association of integrins with the cytoskeleton is sufficient to induce the assembly of cell adhesions. It appears rather likely that interactions with the extracellular matrix play indispensible role in the initiation of focal contact formation, as well as in their maintenance and modulation.

Which plaque proteins are the most likely sites for interaction with β_1 cytoplasmic domains? Since vinculin and α-actinin are also in other locations in the cell, these two proteins appear not to be good candidates (at least on their own), even though α-actinin does bind to the β_1 cytoplasmic domain (Otey et al., 1990). Talin (Geiger et al., 1985), which also binds to β_1 integrins (Horwitz et al., 1986; Tapley et al., 1989), appears a better candidate, since it is normally concentrated only at focal contacts. This conclusion is supported by the observation that talin can also cosegregate with the N-cadherin/β_1 integrin chimera to cell-cell junctions (Fig. 6) as will be discussed further below.

We turn first to a consideration of the association of the chimera with cell-cell junctions. Earlier work has shown clearly that truncated cadherins lacking all or parts of their cytoplasmic domains fail to concentrate in cell-cell adherens junctions and also fail to bind cadherins (Nagafuchi and Takeichi, 1989; Ozawa et al., 1989, 1990). Those results suggested that stable concentration of cadherins in adherens junctions required cytoskeletal interactions as well as cell-cell interactions mediated by the extracellular domains. The observations that the N-cadherin/β_1 integrin chimera can concentrate at cell-cell junctions when expressed at sufficient levels (Figs 3,6) and, furthermore, can lead to cosegregation of talin to those junctions (Fig. 6) lead to an extension of the earlier interpretation. It appears that the requirement for a cytoskeletal association of cadherins can be met by a foreign cytoplasmic domain interacting with a cytoskeletal component normally absent from cell-cell adherens junctions. In this case, the requirement appears to be met by a β_1 integrin-talin interaction but one could imagine that others would also suffice, such as an interaction between the cytoplasmic domain of N CAM and fodrin (Pollenberg et al., 1987). In any event, it seems clear that assembly of cadherins in cell-cell adherens junctions requires interactions both at the extracellular surface and on the cytoplasmic face, just as for focal contact assembly.

The cosegregation of talin with the chimera clearly indicates that the β_1 cytoplasmic domain contains sufficient information to *direct* binding of talin although the results do not demonstrate direct binding and do not preclude the existence of another intervening or coassociating protein. The ectopic targeting of talin via the N-cadherin/β_1 integrin chimera provides an *in vivo* assay for further investigation of such interactions of the β_1 cytoplasmic domain.

In summary, the results presented here, together with earlier data, lead to three conclusions. (1) The cytoplasmic domain of the β_1 integrin appears to be both necessary and sufficient to target transmembrane receptors to focal contacts. (2) Stable association of cadherins with cell-cell contacts requires cytoskeletal association but this requirement can be met either by the cadherin cytoplasmic domain or by an exogenous β_1 integrin cytoplasmic domain, both of which can mediate interactions with the actin-based cytoskeleton. (3) The β_1 integrin cytoplasmic domain contains a binding site for talin (and/or associated proteins) that is sufficient to target talin to particular points in the cell.

We thank Dr. J. Weston for critically reading the manuscript and for his excellent comments. This study was supported in part by grants from the Revson Foundation from the Minerva and the DKFZ-NCRD German-Israeli Binational Program and from the National Institutes of Health (RO1 CA 17007). B.G. holds the E. Neter Chair for Cell and Tumor Biology.

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(Received 25 August 1992 - Accepted 17 September 1992)