A chimeric N-cadherin/β1-integrin receptor which localizes to both cell-cell and cell-matrix adhesions

BENJAMIN GEIGER*, DANIELA SALOMON

Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel

MASATOSHI TAKEICHI

Department of Biophysics, Faculty of Science, Kyoto University, Kyoto, Japan

and RICHARD O. HYNES

Howard Hughes Medical Institute and Center for Cancer Research, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

*Author for correspondence

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 β1 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 β1 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 β1 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 β1 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, transficients 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 β1 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 β1 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 kb HpaII 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 HpaII fragment into the BglII site of pECE vector (Ellis et al., 1986), using a BglIII/HpaII linker. This linker also reconstructs the sequences encoding the first 3 amino acids. (c) Isolation of the HaeIII-XbaI (840 bp) fragment of chicken β1 integrin cDNA in pGEM1 vector (Tamkun et al., 1986). The HaeIII site is located in the extracellular domain of the β1 chain, close to the transmembrane sequence, while the XbaI site is located in the polylinker, beyond the termination codon. (d) Ligation of the HaeIII-XbaI fragment into the Sma-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′ β1 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 β1 integrin) into CHO and 3T3 cells was carried out using the calcium phosphate method and transficients 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-β1 integrin was a rabbit antibody (R-363) prepared against the C-terminal 36 amino acids, corresponding to the cytoplasmic domain of the β1 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 Axiohot 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 Staphylococcus A (Pansorbin, Sigma, USA).

Results

Construction of N-cadherin/β1 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 β1 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 HpaII fragment of chicken N-cadherin cDNA corresponding to most of the ectodomain, and ligating it to the BglII site of the pECE vector (Ellis et al., 1986). For ligation to the vector we used a synthetic HpaII/BglII linker, which also reconstructed the N-terminal 3 amino acids encoded by sequences located 5′ to the HpaII site (Fig. 1). The HaeIII-XbaI fragment of chicken β1 integrin cDNA, encoding the cytoplasmic and transmembrane regions, was then isolated and ligated into the SmaI/XbaI 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/β1 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,
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 β1 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 β1 integrin (Marcanonio and Hynes, 1988). The immunoprecipitates were then examined by immunoblotting with anti-N-cadherin. As shown in Fig. 2B, the anti-β1-integrin antibody effectively immunoprecipitated the cadherin immunoreactivity, confirming that both epitopes are present on the same molecule.

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 transfecants expressing relatively low or intermediate levels of the cadherin-integrin chimera, consisted of a typical focal contact distribution
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 β1 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. 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.
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 \( \beta_1 \) integrin is expressed, the cells flatten and show ruffled membranes but elongated cell appositions were not evident (Fig. 5E).

Therefore, the N-cadherin/\( \beta_1 \) 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 \( \beta_1 \) 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 \( \beta_1 \) 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/\( \beta_1 \) integrin chimera contains sufficient information, presumably in the \( \beta_1 \) integrin cytoplasmic domain, to target talin to cell-cell junctions from which it is normally excluded.
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/β1 integrin chimera, which we report here, provides further insight into these issues and leads to a picture in which both extracellular and intracel-
Cellular 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 β1 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/β1 integrin in focal contacts (Figs 3, 4, 6) shows that the β1 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 β1 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 β1 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 β1 integrin cytoplasmic domain is likely.

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).
from cell-cell adherens junctions. In this case, the requirement appears to be met by a β1 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 β1 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/β1 integrin chimera provides an in vivo assay for further investigation of such interactions of the β1 cytoplasmic domain.

In summary, the results presented here, together with earlier data, lead to three conclusions. (1) The cytoplasmic domain of the β1 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 β1 integrin cytoplasmic domain, both of which can mediate interactions with the actin-based cytoskeleton. (3) The β1 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 Revesion Foundation from the Minerva and the DFKZ-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.

References


(Received 25 August 1992 - Accepted 17 September 1992)