Prototypical Type I E-cadherin and Type II Cadherin-7 Mediate Very Distinct Adhesiveness through Their Extracellular Domains

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Using a dual pipette assay that measures the force required to separate adherent cell doublets, we have quantitatively compared intercellular adhesiveness mediated by Type I (E- or N-cadherin) or Type II (cadherin-7 or -11) cadherins. At similar cadherin expression levels, cells expressing Type I cadherins adhered much more rapidly and strongly than cells expressing Type II cadherins. Using chimeric cadherins, we found that the extracellular domain exerts by far the dominant effect on cell adhesivity, that of E-cadherin conferring high adhesivity, and that of cadherin-7 conferring low adhesivity. Type I cadherins were incorporated to a greater extent into detergent-insoluble cytoskeletal complexes, and their cytoplasmic tails were much more effective in disrupting strong adherent junctions, suggesting that Type II cadherins form less stable complexes with β-catenin. The present study demonstrates compellingly, for the first time, that cadherins are dramatically different in their ability to promote intercellular adhesiveness, a finding that has profound implications for the regulation of tissue morphogenesis.

Adhesive interactions, so essential to multicellular life, are mediated by a diversity of cell surface receptors. Prominent among them are the cadherins, calcium-dependent adhesion molecules central to tissue development and morphogenesis (1–3). The growing superfamily of cadherins is subdivided into five families: classical Type I cadherins, atypical Type II cadherins, desmosomal cadherins, protocadherins, and seven-pass transmembrane cadherins (4, 5). Classical Type I and desmosomal cadherins are found primarily in tissues where a high degree of cell cohesion is required for tissue integrity. Other types of cadherins are expressed in situations where cells are more motile, and intercellular interactions are more transitory (6–9). It is increasingly clear that cadherins contribute to other cellular functions, including cell signaling, proliferation, differentiation, segregation, and migration (10–17).

Predictably, the pattern of cadherin expression during development is complex. For example, development of the neural crest involves epithelial to mesenchymal transitions, cell migration, cell aggregation, and cell differentiation (18), each of which is associated with tightly regulated, differential expression of Type I and II cadherins. Premigratory cells of the avian neural crest express first N-cadherin, and then they down-regulate N-cadherin and express Type II cadherin-6B, but later down-regulate it and induce expression of the Type II cadherin-7 as they migrate throughout the embryo (19, 20). Another Type II cadherin, cadherin-11, is similarly induced in migrating neural crest cells of rat and Xenopus embryos (21–23). Cell grafting experiments in vivo verify that expression of cadherin-7 correlates with cell dispersion and migration along migratory pathways, whereas that of N-cadherin fosters strong intercellular cohesion and failure to migrate (6).

Regulation of cellular adhesion can be achieved in a variety of ways (for review see Ref. 24). The simplest, altering the number of cadherins on the cell surface, has been clearly shown to dictate the pattern of cell sorting in vitro (25–27). Changes in the cadherins expressed on cells during morphogenesis and under pathological conditions are also well documented (1–3). Such differential expression would be even more effective if each cadherin promoted intercellular adhesion that is qualitatively or quantitatively unique.

To test this possibility, we developed a dual pipette assay that quantifies precisely and reproducibly the force required to separate pairs of adherent cells (28, 29). In earlier work we focused on E-cadherin that mediates rapid initial adhesion subsequently strengthened by cytoskeletal remodeling and nectin modulation (28, 30). Here we compare four different cadherins. The results indicate that Type I cadherins (E and N) promote much stronger adhesiveness than Type II cadherins (7 and 11), the difference being attributable primarily to the extracellular domain of the molecules.

MATERIALS AND METHODS

Antibodies and Reagents—NCD-2 and CCD7–1 mAbs were a generous gift from M. Takeichi and S. Nakagawa (Kyoto University, Japan). The polyclonal antibody recognizing an epitope common to N- and E-cadherin and the ECCD-2 mAb were from Takara Biomedicals. mAb against cadherin-11 (clone 5B2H5) was obtained from Zymed Laboratories. ACAM and DECA-1 mAb directed against N- and E-cadherin, respectively, were from Sigma. 7D6 mAb directed against chicken E-cadherin was from Developmental Studies Hybridoma Bank. mAbs

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against α-catenin, β-catenin, or phosphorylated tyrosine (clone PY20) were from Transduction Laboratories. mAb against α-tubulin and secondary antibodies coupled to horseradish peroxidase were from Amer sham Biosciences. Secondary antibodies coupled to Texas Red, Cy3, and fluorescein isothiocyanate were from Jackson ImmunoResearch Laboratories.

Cell Lines and Stable Transfections—The Ecad and Ncad are S180 transfected clones expressing chicken E-cadherin and N-cadherin, respectively, and were previously described (6, 26). The E14, E38, E41, and E58 clones, named according to their level of E-cadherin expression, were previously described by Chu et al. (28). Clones with different levels of cadherin-7 or cadherin-11 were obtained by stable transfection of S180 cells with pMiwccad7 or pMiwmcad11, respectively (20, 31), together with pAG60, as previously described (28). Transient transfections of MDCK cells with constructs containing the cytoplasmic tails of cadherins were carried out using Lipofectamine (Invitrogen).

Construction of Chimeric Cadherins and Cytoplasmic Tails of Cadherins Fused to GFP—pMiwNcad, pMiwcad7, and pBSSKcad7 were a generous gift from M. Takeichi. The pCE2 and pCE-Ecad plasmids were described by Boyer et al. (32). We produced plasmids coding for chimeric EE7, E77, 7EE, and 77E cadherins (Fig. 1B), in which the extracellular, transmembrane, and cytoplasmic domains of E-cadherin and cadherin-7 were exchanged, as described in supplemental Fig. S2. Constructs coding for GFP fused in frame with the cytoplasmic tails of N-cadherin and cadherin-7 (GFP-Ncadcyto and GFP-cad7cyto, respectively) were produced as indicated below. The list of primers used is shown in Fig. 1A. The cytoplasmic domain of N-cadherin was amplified by PCR from pMiwcNcad using P16 and P17 primers. Amplification of the cadherin-7 cytoplasmic domain was performed from pMiwcad7 using P18 and P19 primers. The corresponding inserts were digested with either BglII and XbaI or BglII and BamHI and subcloned into pEGFPC1 (Clontech). The integrity of each construct was verified by analysis of the DNA sequence.

Cell Dissociation and Aggregation Assays—The cells were maintained in Dulbecco’s modified Eagle’s medium with 10% fetal calf serum, and confluent cultures were routinely passaged by treatment with 0.05% trypsin/0.02% EDTA in phosphate-buffered saline. For separation force (SF) measurements and cell aggregation assay, cell dissociation was performed in TC buffer (0.01% trypsin/10 mM calcium) (20). Prior to SF measurements, the cells were resuspended in working medium (CO2-independent medium; Invitrogen) supplemented with 1% fetal calf serum and used immediately. Pre-existing doublets (doublets never disrupted by the dissociation procedure used to prepare cell suspensions) were allowed to sit for one additional hour in the assay dish before use to be certain that they were not mitotic pairs. Cadherin-dependent aggregation assays were performed as described elsewhere (6).

FIGURE 1. Wild type and chimeric cadherins used in this study. A, primers used for the construction of sequences encoding the chimeric cadherins and the GFP-tagged cytoplasmic domain of cadherins. The positions of restriction sites are underlined. B, schematic representation of the wild type cadherins and chimeras carrying the extracellular, transmembrane, or cytoplasmic domain exchanged between E-cadherin and cadherin-7.
Preparation of Cell Extracts and Immunoblotting—Extraction of cell monolayers and Western blot analysis were performed as described previously (6) with a mixture of antibodies directed against β-catenin and α-tubulin or cadherin, followed by ECL detection (Amersham Biosciences). Quantitative analysis was done by the ImageQuant program on a representative Western blot from three independent experiments. The α-tubulin content was used to normalize for the protein level of each lane. The levels of β-catenin and E-cadherin of Ecad cells were taken as the 100% standard for comparison with all other transfected clones. For Western blot analysis of tyrosine-phosphorylated proteins, the cell extracts were subjected to SDS-PAGE, transferred to a nitrocellulose membrane, incubated with specific antibodies, and reacted with ECL detection antibodies. Quantitative analysis was done using the ImageQuant program on a cooled CCD camera (Hamamatsu C5985). Image acquisitions were controlled by a Power Macintosh work station operating IP-Lab software. Cadherin expression was analyzed by flow cytometry, as previously described (28).

Measurement of Separation Forces between Cells—The micromanipulation technique was previously described in detail by Chu et al. (28, 29). Briefly, SF were measured on the heating stage of a Leica inverted epifluorescence microscope equipped with a cooled CCD Hamamatsu C5985 or Nikon Coolpix 5000 camera. Image acquisition was controlled by a Power Macintosh work station operating IP-Lab software. Two cells were gently aspirated, one on the tip of each pipette, and were brought into contact for a designated period of time through the use of micromanipulators. At the end of the designated time, the pipettes were moved apart in an effort to detach the adherent cells from one another. A doublet pulled intact from the left pipette was moved back to the orifice of that pipette, where the aspiration was then increased. The cycle was repeated with discrete increments in aspiration, the magnitude of which were measured with a pressure sensor (Validyne; model DP103-38; 0–50000 Pa), until the level reached in the left pipette was sufficient to pull the doublet apart. Aspiration values recorded for each of the last two cycles in the series (P_{n-1} and P_n) were used to calculate the SF for each doublet using the equation: $SF = \frac{\pi d^2}{2} (P_{n-1} + P_n)/2$, where d is the internal diameter of left pipette. The results for 30–50 measurements were used to obtain the mean SF for a specific contact time in at least three independent experiments. The mean SF is referred to as the SF values indicated in the figures presented.

RESULTS

Cadherin and β-Catenin Expression in Transfected Cells—Parental S180 cells do not express detectable levels of cadherins or β-catenin and
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![Figure 3. Co-immunoprecipitation of α-catenin and β-catenin together with different cadherins.](image)

**Kinetics of the SF Measured for Different Cadherin-expressing Cells—** Using our dual pipette assay, we first determined the time course of SF for cells expressing the four different cadherins. We had previously observed that Ecad cells adhere rapidly and then strengthen their adhesion with time of contact (Ref. 28 and Fig. 4F). Ncad doublets (194 nN) was much greater than that obtained for Ncad doublets (74 nN), even though cadherin expression in the latter was ~115% that in the former. Similar results were obtained with a second, comparable N-cadherin expressing clone (not shown).

**Measurement of SF in Long Term Adherent Doublets—** The maximal SF was determined using doublets never disrupted by the TC dissociation procedure (pre-existing doublets). In every case, the SF for pre-existing doublets was significantly higher than that obtained for doublets formed after 60 min of contact (Fig. 4F). For cells expressing Type I cadherins, the SF for pre-existing doublets were much more similar (SF Ncad/SF Ecad = 0.86) than those of 60-min doublets (SF Ncad/SF Ecad = 0.40). For pre-existing doublets expressing Type II cadherins (cadherin-7 or cadherin-11) were much weaker than those of Type I cadherins (about 50 nN) and were indistinguishable from those of pre-existing doublets of parental S180 cells lacking cadherins.

**Fixed Time Point Comparison of SF for Cells Expressing Different Cadherins—** A fixed time point comparison of cells expressing different cadherins yielded corroborating results (Table 1). The SF at 30 min for Ecad doublets (194 nN) was much greater than that obtained for Ncad doublets (74 nN), even though cadherin expression in the latter was ~115% that in the former. Similar results were obtained with a second, comparable N-cadherin expressing clone (not shown).

**Incorporation of Cadherins into Junctional Complexes—** We next investigated the subcellular distribution of cadherins by analyzing detergent-soluble and -insoluble fractions extracted from confluent monolayers of Ecad, Ncad, and cad7 cells. All three cadherins were found in both fractions (Fig. 5A), but their distribution between the two fractions varied considerably with the type of cadherin: 34 and 33% of the E- and N-cadherin, respectively, but only about 6% of cadherin 7, was found in the detergent-insoluble fraction of the corresponding cell extracts. The results for cadherin-11 were similar to those for cadherin-7 (not shown). Post-extraction immunolabeling of cells with anti-β-catenin revealed that cell-cell junctions of Ncad cells were much more resistant to detergent treatment than those of cad7 cells. The majority of junctional staining remained in Ncad cells after detergent treatment (Fig. 5B and D), whereas most staining was lost in cad7 cells (Fig. 5, C and E). These results suggest that Type II cadherin-7 is incorporated much less readily than Type I E- or N-cadherin into the detergent-insoluble cytoskeleton complex.

**E-cadherin, N-cadherin and cadherin-7 co-precipitated with β- and α-catenin (Fig. 3). Tyrosine phosphorylation of β-catenin is known to modulate cadherin function (35, 36), but Ecad, cad7, and Ncad cells displayed similar levels of tyrosine-phosphorylated β-catenin (Fig. 5F), suggesting that the reduced adhesiveness of cad7 cells does not involve changes in the tyrosine phosphorylation of β-catenin.

**Transient expression of Type I cadherin cytoplasmic tails can disrupt adherent junctions in highly cohesive cells and interfere with β-catenin localization (37). We have therefore compared the ability of the cytoplasmic domain of the different cadherins to affect intercellular adhesion. Constructs containing GFP fused to the cytoplasmic tails of each

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**Figure 3.** Co-immunoprecipitation of α-catenin and β-catenin together with different cadherins. Autoradiogram showing the cadherin-catenin immunocomplexes prepared from metabolically labeled Ncad, Ecad, and cad7 cell extracts. IP, immunoprecipitation.
cadherin were transiently expressed in MDCK cells, and their effect on the organization of adherens junctions was determined (Fig. 5, G and J).

Expression of a Type I cadherin tail (E- or N-) resulted in an increased cytoplasmic pool of β-catenin and a significant decrease in the localization of β-catenin at cell-cell contacts (Fig. 5, G and H, arrows, and Ref. 37). Of the 110 cells examined, more than 90% displayed accumulation of β-catenin in the cytoplasm, and at least 55% showed much reduced staining for β-catenin at cell-cell junctions (Fig. 5 J). By contrast, expression of the Type II cadherin-7 cytoplasmic tail affected neither junctional organization nor β-catenin distribution. β-Catenin was localized at cell-cell contacts in more than 70% of the cells (Fig. 5I), and only about 50% of them displayed accumulation of β-catenin in the cytoplasm (Fig. 5J).

SF Measurements on Cell Doublets Expressing Chimeric Cadherins—
We constructed expression vectors coding for chimeric cadherins where the extracellular, transmembrane or cytoplasmic domain were

### TABLE 1

| Cells | Estimated cadherin content | SF at 30 min |
|-------|---------------------------|--------------|
| Ncad  | 115 %                     | 74.9 ± 7.8   |
| Cad7  | 79 %                      | 8.8 ± 0.9    |
| Cad11 | 39 %                      | 3.5 ± 0.5    |
| Ecad  | 100 %                     | 194.1 ± 14.9 |
| E58   | 58 %                      | 53.4 ± 1.3   |
| E38   | 38 %                      | 19.9 ± 2.4   |
| E14   | 14 %                      | 8.5 ± 1.4    |
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FIGURE 5. Fractionation of cadherins between Triton X-100-soluble and insoluble fractions, level of tyrosine-phosphorylated β-catenin, and disruption of MDCK cell junctions by overexpression of cytoplasmic tails of cadherins. A, distribution of cadherins into the Triton X-100-soluble and insoluble fractions of Ecad, Ncad, and cad7 cell extracts. B–E, effect of detergent treatment on adherens junctions. Monolayers of Ncad cells (B and D) or cad7 cells (C and E) were either untreated (B and C) or treated (D and E) with CSK buffer for 10 min on ice, fixed, and then labeled with an antibody directed against β-catenin (in green). The bar represents 20 μm. F, Western blot analysis of total and tyrosine-phosphorylated β-catenin (PhY) levels in Ecad, Ncad, and cad7 cell extracts. G–J, effect of the cytoplasmic domains of different cadherins on the organization and level of β-catenin in MDCK cells. GFP-tagged cadherin cytoplasmic tails of E-cadherin (G), N-cadherin (H), and cadherin-7 (I) were transfected into MDCK cells and the cell cultures immunostained for β-catenin (in red). The arrows in G and H point to a decrease in β-catenin staining at cell-cell junctions in cells transfected with N-cad and E-cad tails but not in cells transfected with the cad7 tail (J). J, quantitative analysis of the effect of cadherin tail expression on the organization of junctional complexes in MDCK cells and the staining intensity of β-catenin. The patterns of β-catenin and GFP co-staining were used to distinguish three categories of cells: those showing a clear increase in the intensity of β-catenin staining in the cytoplasm, either with (gray bars), or without (black bars) a concomitant decrease in junctional staining, and those showing a weak increase in β-catenin staining and no effect on junctional staining (white bars).

exchanged between E-cadherin (prototypical Type I cadherin) and cadherin-7 (prototypical Type II cadherin). These two cadherins were chosen because they fostered the greatest measurable difference in SF in our assay. Stably transfected S180 cells expressing EE7, E77, 77E, or 7EE chimeric cadherins (Figs. 6 and 7) were selected and compared with clones producing either wild type E-cadherin (EEE) at different levels or cadherin-7 (777) (Table 1).

Western blot and FACS (Fig. 6, B and D, respectively) analysis revealed that E77-cl10 and EE7-clK1 expressed cadherin at levels slightly higher than EEE-E58 cells. 7EE-cl4 and 77E-cl1 cells expressed cadherin levels similar to EEE-E38 cells (Fig. 7, B, asterisks, and D). All chimeric cadherins localized at cell-cell contacts, indistinguishable from the wild type cadherin (Figs. 6C and 7C).

SF measurements were performed on doublets of cells expressing each of the chimeric cadherins (Figs. 6, E and F, and 7E). 30-min doublets of EEE-E58, EE7-clK1, and E77-cl10 cells (SF of 52.5, 65, and 44 nN, respectively) all exhibited SF significantly higher than that of cad7 cells (777-cl29, Fig. 6E), although cad7 cells expressed higher levels of cadherin than of those clones (Table 1). The kinetics of SF measured for EE7 and E77 clones exhibited strong initial SF that increased in intensity with time (Fig. 6F), similar to those displayed by E58 cells expressing wild type E-cadherin. By contrast, the SF for 7EE-cl4 and 77E-cl1 doublets, at 30 min, were under the lowest measurement limit of our technique (<1 nN). After 90 min of contact, 7EE-cl4 and 77E-cl1 doublets displayed the very weak SF of 3.1 and 2.0 nN, respectively, as compared with the 40.2 nN obtained for 90 min E38 doublets (Fig. 7E). Interestingly, E77-cl10 and 77E-cl1 expressing the cadherin-7 transmembrane domain displayed slightly lower SF (44 and 2.0 nN, respectively) than the corresponding clones EE7-clK1 and 7EE-cl4 (65 and 3.1 nN, respectively) expressing the E-cadherin transmembrane domain.

DISCUSSION

Cells Expressing Different Cadherins Differ in Their Adhesiveness—We previously studied the strength, kinetics, and regulation of E-cadherin-mediated intercellular adhesion (28, 30). Here, we compare the adhesivity conferred upon cells by four different cadherins: E- and N-cadherin, (prototypical Type I cadherins) and cadherin-7 and -11 (prototypical Type II cadherins). All four cadherins supported intercellular adhesion in gyratory aggregation assays (supplementary Fig. S1). In the dual pipette assay Ecad, Ncad, and cad7 cells showed a similar kinetic curve: rapid initial adhesion, an early phase showing increased in force with time of contact, and a later plateau phase (Fig. 4). Despite the comparable levels of cadherin expression and the similar shape of the curve generated by both Type I cadherins, the values for Ecad cells were significantly higher than those for Ncad cells. The differences between Type I and Type II cadherins were even greater. For cad7 cells, the early linear phase took much longer than for cells expressing Type I cadherins, and the maximum force level reached was much lower. Only the lowest expressor of E-cadherin (E14) yielded an SF in the range of those displayed by cad7 and cad11 cells.

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Taken together, the data indicate that different cadherins are intrinsically different in their ability to confer adhesive properties on S180 cells, and, in particular, Type I cadherins confer much greater cell adhesiveness than Type II cadherins. Additional support for this view was provided by the results obtained with pre-existing doublets where the forces measured for doublets transfected with Type II cadherins were much weaker (approximately one seventh) than those for doublets transfected with Type I cadherins.

It has been previously reported that fibroblastic L cells transfected with different Type II cadherins (cadherin-6 to -12 or cadherin-14) formed aggregates in suspension similar in size to those formed with cells expressing E-cadherin (38). In that study, the authors used a long term aggregation assay (48 h) because the trypsin sensitivity of Type II cadherin-6 and -14 rendered the short term gyratory assays useless. By contrast, the Type II cadherins used in our study (cadherin-7 and -11) are resistant to trypsin proteolysis (6, 20, 39), and suspensions of Ecad, Ncad, cad7, and cad11 cells can all form aggregates in short term gyratory assays (supplementary Fig. S1). Even so, aggregates formed by Ecad or by Ncad cells after 2 h of gyratory culture were characteristically of a larger size and more cohesive than those formed with cad7 or cad11 cells (not shown).

The dual pipette assay and aggregation assay yield mutually supportive results, but they are not equally sensitive. In the aggregation assay, Ecad cells and cad11 cells were, respectively, 95 and 39% aggregated after 1 h, whereas the dual pipette assay yielded SF of 200 and 7.4 nN for 60-min Ecad and cad11 doublets. Similarly, although EEE-cl58, EE7ck1, E77cl10, and cad7 clones expressing approximately the same level of cadherin formed aggregates of similar size after 24 h in suspension, the kinetics of their adhesiveness revealed by the dual pipette assay showed that cad7 cells were much less adhesive than the other clones (Fig. 6F). These results therefore indicate that the two assays give different, yet complementary information characterizing intercellular adhesiveness.

A Possible Role for the Cadherin Extracellular Domain in Controlling the Differences in Cell Adhesiveness by Various Cadherins—We show here that chimeric cadherins possessing the extracellular domain of cadherin-7 confer low adhesivity to cells, whereas those possessing the extracellular domain of E-cadherin confer high adhesivity. This suggests that the inefficiency of cadherin-7 to promote high cellular adhesivity results from intrinsic properties of the cadherin-7 extracellular domain, not its intracellular domain, a view consistent with the immunoprecipitation data showing that, similar to E- and N-cadherin, cadherin-7 co-precipitated with β- and α-catenin (Fig. 3).

The cadherin-7 extracellular domain exerts its effect on cell adhesiveness probably by preventing cadherins from forming stable homophilic

7 O. Eder, unpublished data.
adhesions (40, 41). This view gains support from results showing that cell-cell contacts mediated by cadherin-7 have a higher turnover rate than those mediated by N-cadherin (6). A failure to dimerize or oligomerize could also prevent cadherin-7 from being rapidly incorporated into higher order molecular complexes strongly connected to the cytoskeleton, as suggested by the reduced incorporation of this cadherin into the detergent-insoluble fraction (Fig. 5). Because cytoskeleton recruitment and remodeling are essential for the evolution of the strong adhesions mediated by E-cadherin (28), a defect in the ability of cadherin-7 to contribute to larger molecular complexes could account for its much weaker ability to confer cellular adhesiveness. This may prove to be a consistent difference between Type I and Type II cadherins.

**Possible Mechanisms Controlling the Differences in Cell Adhesiveness Modulated by Different Cadherins: the Role of the Transmembrane and Cytoplasmic Domains**—We observed that the presence of the cadherin-7 transmembrane domain in chimeric cadherins slightly reduced cell adhesivity relative to that observed with chimeric cadherins containing the E-cadherin transmembrane domain (Figs. 6 and 7). Huber and co-workers (52) have shown that the transmembrane domain plays a role in lateral associations of E-cadherin molecules. Our results suggest that the transmembrane domain of cadherin-7 is less effective in this function, contributing to a reduced cis-dimerization and, in turn, reduced adhesiveness (as discussed above for the extracellular domain).

Cadherins transduce adhesion-dependent signals through catenin-mediated association with the cytoskeleton (53–55). The differential partitioning of Type I and Type II cadherins into the detergent-soluble and -insoluble cell-cell contacts (Fig. 5) indicates that Type I cadherins may be more readily stabilized by association with the cytoskeleton than their Type II counterparts. Previous results employing transient trans-
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REFERENCES

1. Takeichi, M. (1995) *Curr. Opin. Cell Biol.* 7, 619–627
2. Tepass, U. (1999) *Curr. Opin. Cell Biol.* 11, 540–548
3. Wheelock, M. J., and Johnson, K. R. (2003) *Annu. Rev. Cell Dev. Biol.* 19, 207–235
4. Yagi, T., and Takeichi, M. (2000) *Genes Dev.* 14, 1169–1180
5. Nollet, F., Kools, P., and van Roy, F. (2000) *J. Mol. Biol.* 299, 551–572
6. Dufour, S., Beauvieux-Jouveau, A., Delouveé, A., and Thiery, J. P. (1999) *J. Cell Biol.* 146, 501–516
7. Pishvaian, M. J., Feltes, C. M., Thompson, P., Bussemakers, M. J., Schalken, J. A., and Byers, S. W. (1999) *Cancer Res.* 59, 947–952
8. Sano, K., Tanhara, H., Heimark, R. L., Ohata, S., Davidson, M., St. John, T., Taketani, S., and Suzuki, S. (1993) *EMBO J.* 12, 2249–2256
9. Suzuki, S. C., Inoue, T., Kimura, Y., Tanaka, T., and Takeichi, M. (1997) *Mol. Neurosci.* 9, 433–447
10. Chen, H., Paradis, N. E., Fedor-Chaiken, M., and Brackenbury, R. (1997) *J. Cell Sci.* 110, 345–356
11. Murase, S., and Schuman, E. M. (1999) *Curr. Opin. Cell Biol.* 11, 549–553
12. Steinberg, M. S., and McMurti, P. M. (1999) *Curr. Opin. Cell Biol.* 11, 554–560
13. Toynanovskv, S. M. (1999) *Curr. Opin. Cell Biol.* 11, 561–566
14. Gottiardi, C. J., Wong, E., and Gumbiner, B. M. (2001) *J. Cell Biol.* 153, 1049–1060
15. Stockinger, A., Eger, A., Wolf, J., Beug, H., and Fois, R. (2001) *J. Cell Biol.* 154, 1185–1196
16. Price, S. R., De Marco Garcia, N. V., Ranscht, B., and Jessell, T. M. (2002) *Cell* 109, 205–216
17. Conacci-Sorrell, M., Simcha, I., Ben-Yedidia, T., Blechman, J., Savagner, P., and Ben-Ze’ev, A. (2003) *J. Cell Biol.* 163, 847–857
18. Le Douarin, N. M., and Kalcheim, C. (1999) *The Neural Crest*, 2nd Ed., Cambridge University Press, Cambridge
19. Duband, J.-L., Volberg, T., Sabanay, I., Thiery, J. P., and Geiger, B. (1988) *Development* 103, 325–344
20. Nakagawa, S., and Takeichi, M. (1995) *Development* 121, 1321–1332
21. Simonnete, L., Kitagawa, M., Suzuki, S., and Thiery, J. P. (1995) *Cell Adhies. Commun.* 3, 115–140
22. Vallin, J., Thiery, J. P., and Broders, F. (1998) *Mech. Dev.* 75, 183–186
23. Borchers, A., David, R., and Wedlich, D. (2001) *Development* 128, 3049–3060
24. Gumbiner, B. M. (2000) *J. Cell Biol.* 148, 399–403
25. Steinberg, M. S., and Takeichi, M. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 206–209
26. Friedlander, D. R., Mege, R.-M., Cunningham, B. A., and Edelman, G. M. (1989) *Proc. Natl. Acad. Sci. U. S. A.* 86, 7043–7047
27. Duguay, D., Foty, R. A., and Steinberg, M. S. (2003) *Dev. Biol.* 253, 309–323
28. Chu, Y. S., Thomas, W. A., Eder, O., Pincet, F., Perez, E., Thiery, J. P., and Dufour, S. (2005) *J. Cell Biol.* 167, 1183–1194
29. Chu, Y. S., Dufour, S., Thiery, J. P., Perez, E., and Pincet, F. (2005) *Phys. Rev. Lett.* 94, 028102
30. Martinez-Rico, C., Pincet, F., Perez, E., Thiery, J. P., Shimizu, K., Takai, Y., and Dufour, S. (2005) *J. Biol. Chem.* 280, 4753–4760
31. Kimura, Y., Matsuoka, H., Inoue, T., Shimamura, K., Uchida, N., Ueno, T., Miyazaki, T., and Takeichi, M. (1995) *Dev. Biol.* 169, 347–358
32. Boyer, P., Dufour, S., and Thiery, J. P. (1992) *Exp. Cell Res.* 201, 347–357
33. Bauer, A., Lickert, H., Kemler, R., and Stappert, J. (1998) *J. Biol. Chem.* 273, 28314–28321
34. Ozawa, M., and Kemler, R. (1998) *J. Biol. Chem.* 273, 6166–6170
35. Balsamo, J., Leung, T., Ernst, H., Zanin, M. K., Hoffman, S., and Lilien, J. (1996) *J. Cell Biol.* 134, 801–813
36. Rouza, S., Miravet, S., Piedra, J., Garcia de Herreros, A., and Dunach, M. (1999) *J. Biol. Chem.* 274, 36734–36740
37. Simcha, I., Kirkpatrick, C., Sadot, E., Shtutman, M., Polevoy, G., Geiger, B., Peifer, M., and Ben-Ze’ev, A. (2001) *Mol. Biol. Cell* 12, 1177–1188
38. Shimoyama, Y., Tsujimoto, K., Itajima, M., and Naito, M. (2000) *Biochem. J.* 349, 159–167
39. Kawaguchi, J., Takeishi, S., Kashima, T., Imai, T., Machinami, R., and Kudo, A. (1999) *J. Bone Miner. Res.* 14, 764–775
40. Yap, A. S., Brieher, W. M., Prusczy, M., and Gumbiner, B. M. (1997) *Curr. Biol.* 7, 308–315
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41. Troyanovsky, R. B., Sokolov, E., and Troyanovsky, S. M. (2003) *Mol. Cell. Biol.* 23, 7965–7972
42. Shan, W. S., Tanaka, H., Phillips, G. R., Arndt, K., Yoshida, M., Colman, D. R., and Shapiro, L. (2000) *J. Cell Biol.* 148, 579–590
43. Tamura, K., Shan, W. S., Hendrickson, W. A., Colman, D. R., and Shapiro, L. (1998) *Neuron* 20, 1153–1163
44. Nose, A., Tsuji, K., and Takeichi, M. (1990) *Cell* 61, 147–155
45. Boggon, T. J., Murray, J., Chappuis-Flament, S., Wong, E., Gumbiner, B. M., and Shapiro, L. (2002) *Science* 296, 1308–1313
46. Pertz, O., Bozic, D., Koch, A. W., Fauser, C., Brancaccio, A., and Engel, J. (1999) *EMBO J.* 18, 1738–1747
47. Ahrens, T., Lambert, M., Pertz, O., Sasaki, T., Schulthess, T., Mege, R. M., Timpl, R., and Engel, J. (2003) *J. Mol. Biol.* 325, 733–742
48. Sivasankar, S., Gumbiner, B., and Leckband, D. (2001) *Biophys. J.* 80, 1758–1768
49. Chappuis-Flament, S., Wong, E., Hicks, L. D., Kay, C. M., and Gumbiner, B. M. (2001) *J. Cell Biol.* 154, 231–243
50. Zhu, B., Chappuis-Flament, S., Wong, E., Jensen, I. E., Gumbiner, B. M., and Leckband, D. (2003) *Biophys. J.* 84, 4033–4042
51. Patel, S. D., Chen, C. P., Bahna, F., Honig, B., and Shapiro, L. (2003) *Curr. Opin. Struct. Biol.* 13, 690–698
52. Huber, O., Kemler, R., and Langosch, D. (1999) *J. Cell Sci.* 112, 4415–4423
53. Vestweber, D., and Kemler, R. (1985) *EMBO J.* 4, 3393–3398
54. Kemler, R. (1993) *Trends Genet.* 9, 317–321
55. Ben-Ze'ev, A., and Geiger, B. (1998) *Curr. Opin. Cell Biol.* 10, 629 – 639
56. Sadot, E., Simcha, I., Shlutman, M., Ben-Ze’ev, A., and Geiger, B. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 15339–15344
57. Stewart, D. B., Barth, A. I., and Nelson, W. J. (2000) *J. Biol. Chem.* 275, 20707–20716
58. Gumbiner, B. M. (1996) *Cell* 84, 345–357
59. Thoumine, O., Ott, A., and Louvard, D. (1996) *Cell Motil. Cytoskeleton* 33, 276–287
60. Yamamoto, A., Mishima, S., Maruyama, N., and Sumita, M. (1998) *Biomaterials* 19, 871–879
61. Hohen, G., Huang, W., Thoma, B. S., LeBaron, R. G., and Athanasiou, K. A. (2002) *Ann. Biomed. Eng.* 30, 703–712
62. Wu, C. C., Su, H. W., Lee, C. C., Tang, M. J., and Su, F. C. (2005) *Biochem. Biophys. Res. Commun.* 329, 256–265