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The Body Language of Cells: The Intimate Connection between Cell Adhesion and Behavior

Minireview

Michael W. Klymkowsky* and Brian Parr†*
*Molecular, Cellular and Developmental Biology
University of Colorado, Boulder
Boulder, Colorado 80309-0347
†Molecular and Cellular Biology
Harvard University
Cambridge, Massachusetts 02138

A simple touch between organisms can convey a wide range of different messages, from affection to hostility. Touch appears critical for both social intercourse and the achievement of normal developmental milestones. The analogy of the cells in a metazoan to the individuals in a society has been, perhaps, overused. Yet, in a fundamental way, it is extremely apt. For metazoan cells the importance of touching (and the associated "juxtacrine signaling") as a source of information in both embryonic morphogenesis and the maintenance of tissue integrity and organ function is becoming increasingly obvious. Cells touch one another through a number of different surface molecules; among the most intriguing are the cadherins and their associated proteins. Together with juxtacrine signals, most notably those involving proteins of the Wnt family, these proteins generate a range of adhesive and signaling interactions between neighboring cells.

Juxtacrine signaling requires that cells be brought into close apposition. A major class of cell–cell adhesion junctions (AJs) are those mediated by the cadherins. As cells approach one another and touch, cadherins begin to cluster and connect, through their cytoplasmic domains and associated proteins (catenins), with the cytoskeleton. Within 20 s of cell–cell contact, discrete AJs, characterized by cytoplasmic plaques and associated cytoskeletal fibers, are evident (Heaysman and Pegrum, 1973) (Figure 1).

The first step in the assembly of an AJ (Figures 2A and 2B) is the interaction between the extracellular domains of cadherins on neighboring cells. The extracellular portion of cadherins typically consists of five tandem repeats of an ~110 amino acid homology domain, the cadherin repeat. X-ray crystallographic studies of the isolated N-terminal domain of N-cadherin, together with deductions based on the crystal packing of polypeptides (Shapiro et al., 1995), suggest that these cadherin domains stack on one another and that this stacking is stabilized by Ca^{2+} positioned at the interface between domains. Lateral interactions between the stacks of cadherin repeats lead to the formation a cadherin dimer. Interactions between cells involve the N-terminal cadherin repeat domain and are proposed to generate a "cadherin zipper" whose strength depends on the number of cadherin molecules involved. It is not known whether the cadherin dimer exists prior to the formation of the cadherin zipper and whether simple dimerization generates signals in a manner similar to the signaling induced by the dimerization of other membrane receptors.

Prior to their clustering, cadherins associate with the cytoplasmic proteins β-catenin or plakoglobin (PKG) through their cytoplasmic domains (Hinck et al., 1994); it is not clear, however, whether all cadherin molecules are associated with catenins prior to clustering. β-Catenin and PKG are closely related to one another and to the product of the Drosophila segment polarity gene *armadillo* (*arm*) (see Peifer, 1995). β-Catenin binds to the tail domain of nondesmosomal cadherins, whereas PKG (sometimes called γ-catenin) binds to both desmosomal and nondesmosomal cadherins (Cowin, 1994). The clustered cadherin–β-catenin/PKG complex appears to act as a nucleus for the formation of a cytoplasmic "plaque" composed of other catenins (Figure 2C). If cadherin clustering generates a signaling response (see above), it seems likely that the association of β-catenin/PKG with cadherins could act to enhance this signal.

At adherens junctions, the plaque associated with cadherin tail domains mediates the "end-on" anchoring of microfilaments via the vinculin-like α-catenin polypeptide and α-actinin (Knudsen et al., 1995). At desmosomes, cadherins interact with other polypeptides (e.g., desmoplakins) that mediate an en passant interaction with intermediate filaments. In the absence of functional catenins, AJs do not form, presumably because the cadherin zipper is not adequately stabilized by the assembly of the cytoplasmic plaque.

In addition to linking cadherins and thereby stabilizing AJs, β-catenin and PKG also mediate interactions between cadherins and other membrane receptor proteins, notably the epidermal growth factor (EGF) receptor (Hoschuetzky et al., 1994) and c-ErbB (Kanai et al., 1995). There are also indications that N-cadherin interacts with fibroblast growth factor receptors during neurite outgrowth (Williams et al., 1994). How these interactions modulate receptor activity is unclear. It is firmly established, however, that β-catenin and PKG mediate the effects of several juxtacrine signaling systems, most notably those involving Wnt proteins. The diffusion of secreted Wnts is severely limited through their binding to extracellular matrix and cell surfaces; this effectively restricts the effects of Wnt signals to the immediate neighbors of the Wnt-secreting cell. Wnt signaling plays a key role in patterning invertebrate and vertebrate embryos during processes such as the determination of segment polarity in Drosophila, primary axis formation in Xenopus, and limb development in mice and chickens (Moon, 1993; Perrimon, 1994; Yang and Niswander, 1995; Parr and McMahon, 1995).

In Drosophila, the segment polarity gene *wingless* (*wg*) encodes a Wnt polypeptide. Genetic studies (see Perrimon, 1994; Peifer, 1995) have identified a number of the gene products involved in the Wnt signaling pathway. The β-catenin/PKG homolog arm appears to play a key role in

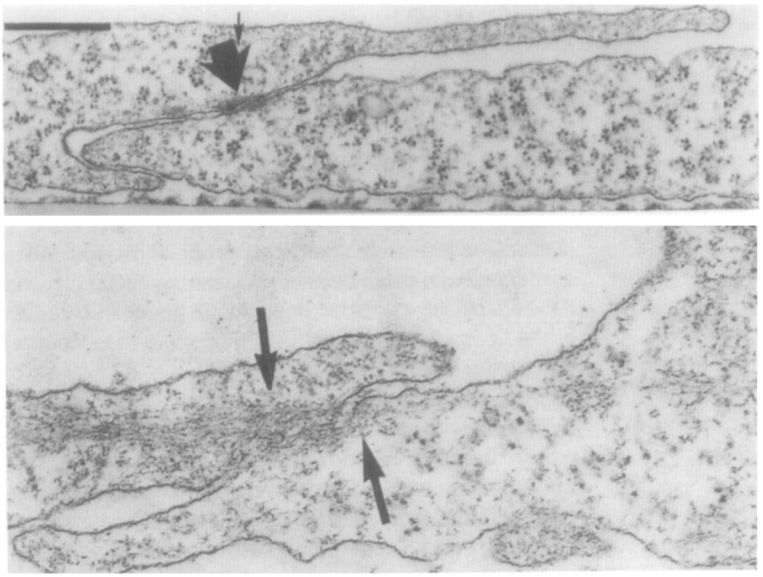

Figure 1. Cells Touch and Rapidly Assemble an AJ

In a pair of electron micrographs, taken from Heaysman and Pegrum (1973), we see the assembly of adherens junctions 20 s (top) and 60 s (bottom) after two cells touch one another.

transmitting the wg signal. In response to wg released by a neighboring cell, the dishevelled (dsh) protein inhibits the activity of the *shaggy/zeste-white 3* gene product. As a consequence, the level of cytoplasmic arm protein is increased and arm function is activated.

A recent series of papers using Xenopus as a model system indicates that many of the components of the wg pathway are present and conserved in vertebrates (Sokol et al., 1995; He et al., 1995; Pierce and Kimelman, 1995). Injection of *Wnt* mRNA into Xenopus embryos induces the formation of a secondary neural axis due to the formation of ectopic dorsal mesoderm (see Moon, 1993). If vertebrate Wnts employ the same signaling pathway as wg, then manipulating the level or activity of vertebrate homologs of wg pathway components should either mimic or inhibit Wnt effects. McCrea et al. (1993) found that injection of antibodies against β-catenin lead to the induction of a secondary neural axis. The ability of anti-β-catenin antibodies to induce dorsal mesoderm suggested a role for β-catenin in the process, although it was unclear whether β-catenin acted in a "positive" or "negative" manner. This uncertainty was removed by the subsequent demonstration that the overexpression of β-catenin (Funayama et al., 1995) or PKG (Karnovsky and Klymkowsky, 1995) leads to neural axis duplication, while an antisense-induced decrease of β-catenin levels leads to an inhibition of normal neural axis formation (Heasman et al., 1994). Based on these studies, it appears that the injection of anti-β-catenin antibody produces an effective increase in the intracellular concentration or activity (or both) of β-catenin.

Mutational analysis indicates that the central region of β-catenin or PKG is both necessary and sufficient to induce neural axis duplication (Funayama et al., 1995; Karnovsky and Klymkowsky, 1995). Surprisingly, overexpressed β-catenin and PKG concentrate in the nuclei of Xenopus blastomeres (Funayama et al., 1995; Karnovsky and Klymkowsky, 1995). Nuclear localization of exogenous β-catenin/PKG correlates with the axis duplication ability of mutant polypeptides, and suppression of PKG's nuclear localization, by the coexpression of a desmoglein tail polypeptide, suppresses PKG's axis duplication effect.

Our understanding of the Wnt pathway is still incomplete. Nevertheless, it is possible to generate a testable model for Wnt signaling (Figure 2E). As originally proposed by Peifer et al. (see Peifer, 1995), we suggest that Wnt signaling depends on an unidentified factor X. Upon reception of a Wnt signal, interactions between β-catenin/PKG and factor X alters the activity or intracellular localization (or both) of factor X, resulting in changes in gene expression. Based on the nuclear localization of β-catenin and PKG in the Xenopus injection experiments (see above), together with the inhibition of dorsal mesoderm induction seen following the down-regulation of β-catenin (Heasman et al., 1994), we argue that factor X–β-catenin and factor X–PKG complexes are responsible for regulating patterns of gene expression. Whether β-catenin/PKG binding inhibits or activates factor X remains to be determined.

In this model, regulation of cytoplasmic β-catenin/PKG levels is crucial. The form of PKG not bound to cadherin appears to turn over quickly (Kowalczyk et al., 1994). β-Catenin and PKG both form a soluble (not cadherin-associated) complex with the adenomatous polyposis coli (APC) tumor suppressor protein. In the SW480 colorectal cancer cell line, which lacks functional APC, levels of cytoplasmic β-catenin are elevated; reintroduction of functional APC leads to a decrease in cytoplasmic β-catenin (Munemitsu et al., 1995), suggesting that APC may play a role in controlling the cytoplasmic level of β-catenin/PKG. It is interesting that studies in cultured cells and Drosophila embryos show that exposure to Wnt signals leads to an increase in cytoplasmic β-catenin/PKG (see Peifer, 1995), which should, in our model, lead to an increase in the amount of factor X bound by β-catenin/PKG. Other studies suggest that Wnt pathway activation may effect β-catenin/PKG turnover by altering their phosphorylation. There is a highly conserved GSK3 consensus phosphorylation site located in the N-terminal domain of β-catenin/PKG, and inhibition of GSK3 activity in response

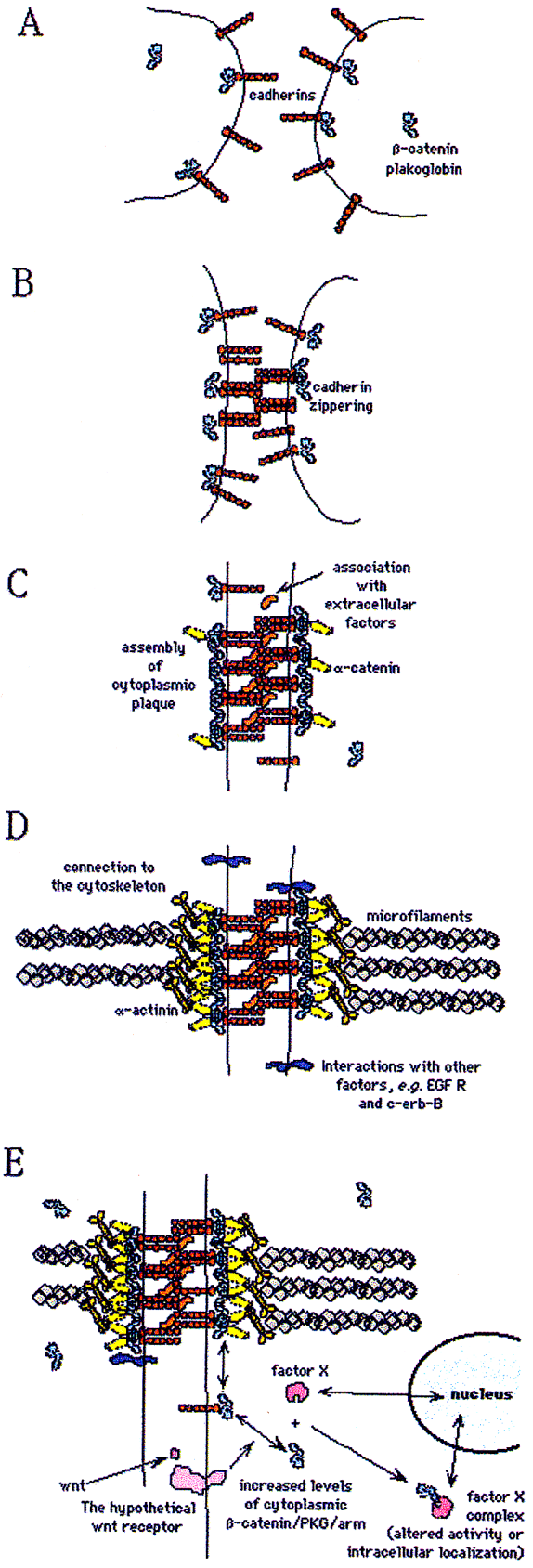

Figure 2. A Schematic of AJ Assembly and Wnt Signaling

A schematic of the assembly of the cadherin-based AJ. Two cells, with the same type of cadherin on their surfaces, approach one another (A); once they touch (B), the cadherins begin to assemble a cadherin zipper, which is stabilized (C) by the association of extracellular factors (as is likely the case in desmosomes) and cytoplasmic factors. Assembly of the cytoplasmic plaque continues with the association of accessory proteins (D) that stabilize the plaque and connect it to microfilaments (assembly of an intermediate filament–associated desmosome is likely to be quite similar). Surface receptors, such as the EGF receptor, also associate with, and may modify, the junctional complex. Wnt signals, presumably acting through a specific receptor (E), lead to an increase in the level of cytoplasmic β-catenin/PKG, the exact size of the cytoplasmic pool being determined by factors such as cadherin binding and catenin turnover. Increased levels of cytoplasmic β-catenin/PKG could act to liberate the hypothetical factor X, which then moves to the nucleus and alters gene expression. Alternatively, cytoplasmic β-catenin/PKG could alter the activity of factor X by forming a complex with it.

to a Wnt signal could alter the ability of β-catenin/PKG to interact with cadherins, APC, or other cellular factors.

It is clear that β-catenin/PKG modulates both cell adhesion and Wnt signaling, but is there any obligate connection between the two processes? It has been proposed that wg signaling via cytoplasmic arm during segmentation of the Drosophila embryo does not affect cell adhesion (Peifer, 1995); this interpretation is complicated by the presence of maternal arm mRNA in the early embryo. On the other hand, Heasman et al. (1994) showed that increasing cadherin levels in Xenopus embryos seemingly inhibits the signaling ability of β-catenin. Similarly, Bradley et al. (1993) found that Wnt1 signaling can increase adhesion in mammalian cell cultures, apparently by increasing levels of cadherin and catenins required for AJ formation. The amount and localization of β-catenin/PKG in any particular cell type, determined by the amount of cadherin, the number and extent of AJs, and the stability of cytoplasmic forms of β-catenin and PKG, together with the types of Wnt receptors expressed by the cell, will combine to determine its responsiveness to Wnt signaling. Unfortunately, our lack of knowledge about the nature of Wnt receptors significantly affects our model making abilities.

As complex as the above story sounds, it does not end here. There is a suggestion, based on genetic interactions in Drosophila, that wg signaling interacts with another juxtacrine signaling pathway, that mediated by the Notch (N) transmembrane protein (Couso and Arias, 1994). Interestingly, the interaction between N and its ligands triggers changes in the binding of the cytoplasmic domain of N to various cytoplasmic factors, including the Suppressor of Hairless (Su(H)) protein. This change is proposed to release Su(H), enabling it to enter the nucleus and regulate gene expression (Artavanis-Tsakonas et al., 1995). The careful reader will recognize the similarity to this pathway to the one proposed for Wnt signaling (see above).

Thus, a growing number of signaling systems appear to need the cadherin and catenin proteins for their activity. It is clear that juxtacrine signaling in the first place requires the close apposition of cells. By acting to attach cells to one another, AJs permit juxtacrine signaling. In turn, juxtacrine signals can modulate AJ components and feed back in complex information loops. This reminds us that the first

touch between cells can influence the entire course of their subsequent relationship.

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