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Secret Handshakes: cell-cell interactions and cellular mimics

Daniel J. Cohen^{1,^,*} and W. James Nelson^{1,2,#}

¹Department of Biology, Stanford University, Stanford, CA 94305, USA

²Department of Molecular and Cellular Physiology, Stanford University, Stanford, CA 94305, USA

Abstract

Cell-cell junctions, acting as ‘secret handshakes’, mediate cell-cell interactions and make multicellularity possible. Work over the previous century illuminated key players comprising these junctions including the cadherin superfamily, nectins, CAMs, connexins, notch/delta, lectins, and eph/Ephrins. Recent work has focused on elucidating how interactions between these complex and often contradictory cues can ultimately give rise to large-scale organization in tissues. This effort, in turn, has enabled bioengineering advances such as cell-mimetic interfaces that allow us to better probe junction biology and to develop new biomaterials. This review details exciting, recent developments in these areas as well as providing both historical context and a discussion of some topical challenges and opportunities for the future.

Introduction

Underlying complex, coordinated, multicellular behaviors is a key cellular decision made at each physical contact. For each interaction, cells classify the contacting object either as ‘not-cell’ (e.g., extracellular matrix [ECM]) or ‘cell’ which is further classified based on the cell type (e.g., epithelia vs. muscle). The type of classification dictates subsequent cellular behaviors (e.g. focal adhesion formation with ECM via integrins vs. cell-cell adhesion via cadherins or other junctional proteins), and the net result of those decisions across a tissue affect the spatial organization and function of cells, the establishment of homeostasis, healing of an injury, or even the invasion and metastasis of cancer cells. In this review, we focus on juxtacrine interactions that arise specifically via mechanical contacts between cells. After providing historical context and reviewing recent, biological findings, we discuss how our growing understanding of cell-cell adhesion and recognition is being parlayed into powerful new tools to study and manipulate cellular behaviors.

[#]Manuscript correspondence: W. James Nelson, Department of Biology, The James H. Clark Center, W200B, 318 Campus Drive, Stanford University, Stanford, CA 94305, Phone: (650) 725-7596, Fax: (650) 498-5286, wjnelson@stanford.edu. [^]Correspondence after publication: Daniel J. Cohen: danieljc@stanford.edu, Department of Mechanical and Aerospace Engineering, Princeton University, 111 Hoyt Laboratory, William Street, Princeton, NJ 08544.

^{*}Present address: danielcohen@princeton.edu

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From locks and keys to secret handshakes

While questions of how cells recognize and attach to other cells have played a central role in Biology for over a century, the players involved remained unknown until relatively recently. Despite the first experiments demonstrating species-specific cell-cell recognition and adhesion performed by Wilson in 1907 (1), it would not be until 1977 that the first vertebrate cell-cell adhesion protein, N-CAM, was identified (Edelman et al, (2)), and not until 1981 that the cadherins were discovered (Takeichi et al, (3)). Since then, the list of players implicated in cell-cell recognition and adhesion has grown to include the cadherin superfamily comprising classical, atypical- and proto-cadherins (4–7), nectins (8, 9), CAMs (9, 10), Connexins (11, 12), Notch/Delta (13, 14), Lectins (15, 16), and eph/Ephrin (17, 18) (Fig. 1). Such a rich palette of adhesion proteins has the potential to provide radically different effects upon cell-cell contact, from pure repulsion to pure adhesion and everything in between.

The earliest models for how cell-cell recognition occurs relied on the ‘Lock-and-Key’ framework proposed by Fischer (1897) and further developed by Ehrlich (1900) in which cell recognition depended on unique ligand/receptor pairs (19). Holtfreter and Townes (1955) expanded on this to propose that cell-type specific adhesion molecules could mediate adhesion and tissue patterning in a process called ‘Selective Affinity’ (20). Steinberg’s ‘Differential Adhesion’ hypothesis (1965) reflected an alternative framework requiring only differential relative adhesion between cell types (21). In essence, Selective Affinity posits that cell types A and B must have different adhesion proteins in order to separate into unique tissues, while Differential Adhesion posits that cell types A and B can have the same adhesion protein but will sort out uniquely so long as the level of the adhesive protein is different in a cell type-specific manner.

Since these models were proposed, a sea change has occurred in terms of how we think about of cell-cell recognition, adhesion and tissue sorting mechanisms. As none of the original models were correct in all particulars, we have had to broaden our understanding of what recognition and adhesion mean. For instance, we now know that: 1) one adhesion protein can support both heterotypic and homotypic interactions (e.g., cadherins) (22, 23); 2) different recognition and adhesion modules can be multiplexed to guide cellular responses (e.g. cadherins and ephrins) (18, 24); and 3) even the geometry of how these adhesion proteins are presented can affect how they are interpreted (e.g., 3D presentation of cadherin, and junction size and cell shape in Notch signaling) (25, 26). In light of this, our modern framework of cell-cell recognition and adhesion includes elements of the Lock-and-Key, Selective Affinity, Differential Adhesion, and more recent Differential Interfacial Tension models (27). This new synthesis reflects the fact that cell-cell contacts are complex, multivariable systems where the type, level, cross-interactions, and spatial presentation of the cell-cell interaction proteins are integrated to determine the adhesion and recognition response—akin to a cellular ‘secret handshake’.

The cell-cell adhesome: complementary and contradictory cues

A given cell type can be characterized by the complement of proteins in the cell-cell adhesome (see Fig. 1). As each protein modulates downstream effectors in unique ways, combinatorial presentation of cell-cell adhesion proteins can result in complex interactions between cells with different phenotypic outcomes beyond that of simple adhesion or repulsion (18, 22, 28, 29). Even different members of the same family of adhesion proteins expressed in the same cells can contribute different effects. For instance, recent work in epithelial cells expressing both E- and P-cadherin showed that P-cadherin levels tracked the absolute level of intercellular tension, whereas E-cadherin levels responded to the rate of change of intercellular tension (28). Hence the co-presentation of multiple recognition and adhesion proteins can have profound effects on tissue properties and organization. The following case studies highlight this in the specific contexts of complementary and contradictory interactions at the cell-cell junction.

Recent work by Katsunuma et al (29) demonstrates how the presentation of complementary adhesive proteins plays out in the olfactory epithelium due to the contributions of two different cadherins (E- and N-cadherin) and two different nectins (nectins 2 and 3). The olfactory epithelium is a monolayer of supporting epithelial cells studded at regular intervals with olfactory sensory cells (Fig. 2A). There are fewer sensory cells than support cells and, *in vivo*, the olfactory epithelium takes on the appearance of a regularly spaced, hexagonal array of sensory cells completely surrounded by support cells. The support and sensory cells possess distinct adhesomes: sensory cells primarily express two cell-cell adhesion proteins (nectin-2 and N-cadherin), and support cells express 4 cell-cell adhesion proteins (nectin-2 and nectin-3; E- and N-cadherin). Together, these proteins mediate three combinations of cell-cell interaction: (1) support cell homotypic adhesion mediated by E-cadherin and nectin-3; (2) sensory-support cell heterotypic adhesion mediated by nectin-2, nectin-3, and N-cadherin; and (3) sensory cell homotypic adhesion mediated by nectin-2 and N-cadherin. Computational modeling and *in vitro* mixing assays demonstrated that varying the relative adhesion strengths of each of these interactions altered the final tissue pattern, either two contiguous, segregated tissues, or a mixed checkboard configuration found in the auditory epithelium (30), or the hexagonal configuration of the olfactory epithelium. The key interaction that dictated these outcomes as heterotypic nectin-2/nectin-3 adhesion between support and sensory cells that resulted in sensory cell intercalation into the epithelium. Together, these results demonstrate how complementary adhesomes synergize to produce complex cell/tissue patterns.

More surprising, perhaps, than the synergy between complementary adhesion proteins is the synergy that can arise between contradictory cell-cell interactions to form a precise, stable interface between cells in different, contiguous tissues. This was demonstrated by Taylor et al (18) who explored how opposing adhesive (N-cadherin) and repulsive (eph/Ephrin) interactions (see Fig. 1) contribute to border sharpening at a tissue interface. Here, two distinct tissues expressing combinations of N-cadherin and eph2B/ephrin2B were allowed to migrate and collide with each other and the properties of the resulting interface were studied. The results demonstrated that while ephrin-mediated repulsion was necessary for a sharp border, cadherin-mediated cell adhesion tempered the extent of the repulsion and served to

improve border sharpening (Fig. 2B). Specifically, N-cadherin adhesion increased the amount of time opposing cells at the border spent in contact with each other, which, coupled with the mechanical pressure built up at the interface, balanced the cellular rearrangements and repulsion induced by eph/Ephrin repulsion. Such precise tissue patterning resulting from tuning the relative strengths of opposing repulsive and adhesive behaviors between cells exemplifies the benefits of multiplexed junctional interactions.

These two examples—complementary and opposing interactions—illustrate the sophistication of physical cell-cell interactions and how underlying complexity can give rise to sophisticated behaviors that might not occur with simpler, single-protein homotypic interactions. The myriad possible interactions between different adhesomes emphasizes the versatility and power of local cell-cell interactions to shape and drive complex, multicellular behaviors.

Mimicking existing handshakes: engineering cell-mimetic materials

Fundamental research on cell adhesion biology has had a transformative impact on the field of biomaterials—the interface between cells and materials. Found from the petri dish to the operating room, commercial biomaterials target the integrin/ECM interaction by promoting integrin binding at the tissue-material interface. This trend was spearheaded over a generation ago with the advent of the RGD integrin-binding peptide, where any material coated in RGD could mimic the ECM (31, 32). We believe that a similar paradigm shift is occurring due to the introduction of cell-cell junction fusion proteins that should allow us to make cell-mimetic materials that can integrate with tissue in ways distinct from existing biomaterials. These proteins, consisting of the extracellular domain of a junctional protein (e.g. cadherin) fused to a conjugation tag domain (e.g. Fc) were first used to coat glass surfaces or microbeads to study cadherin recruitment and mechanotransduction (Fig. 3A,B) (33–38). Today, these fusion proteins exist for every adhesion protein class represented in Fig. 1, including: 2D micropatterned ECM/cadherin substrates to interrogate integrin/cadherin crosstalk (39), eph/Ephrin-conjugated hydrogels to improve β -cell culture (40), synaptogenic surfaces to refine neuronal cell culture (41), and cadherin-conjugated soft gel surfaces to explore how cadherin adhesions modulate cellular perception of both substrate and contact rigidity (42, 43).

While rigidly linking the right fusion protein to a stiff glass surface is a valuable reductionist assay, knowledge of native cell-cell junctions makes it clear that the context in which the proteins are presented matters. Teasing apart these interactions and identifying the minimum essential components needed to recapitulate specific cellular interactions has been difficult. However, the growing synergy between cell-cell junction biology and bioengineering has led to a recent, concerted effort to better understand the key features of cell-cell junctions needed to recapitulate these behaviors (25, 44, 45). For instance, Biswas et al recently explored what would happen when cells contacted cadherin fusion proteins that were presented within an engineered lipid bilayer substrate instead of on a rigid glass surface (44). Notably, they found that high bilayer fluidity hindered the stability of cadherin *trans* interactions, and a relatively low bilayer fluidity slowed cadherin mobility enough to improve the dwell time and increase cell adhesion stability.

Cell-mimetic surfaces are traditionally produced in a 2D configuration (e.g. patterned proteins on a glass slide), but native cell-cell junctions occur in a 3D context between cells and are often spatially orthogonal to the matrix plane (e.g. the glass slide, native ECM). Two recent studies noted this and developed complementary approaches to explore the effects of 3D cell-cell junctions at the cell and tissue level, respectively. At the cellular scale, Li et al cultured hepatocyte (liver cell) doublets within microwells in which the basal surface was coated with ECM and the sides were coated with either ECM or E-cadherin fusion protein (45). While hepatocyte function depends on both ECM- and cell-cell adhesion, this assay demonstrated that the ECM plays a more critical mechanical role in guiding hepatic luminal geometry than was previously realized. Shifting to the tissue scale, Cohen et al created a 3D biomimetic junction comprised of a 2D ECM surface to which 3D vertical walls (barriers) coated with E-cadherin fusion protein were affixed (Fig. 3C) (25). Here, the goal was to determine the minimum cues that prompted a healing tissue colliding with the barrier to undergo contact-inhibition-of-locomotion and stably heal with that barrier. Significantly, this 3D biomimetic junction proved to be minimally sufficient to recapitulate wound healing—cells within a healing epithelium that encountered the biomimetic junction not only formed stable, 3D E-cadherin junctions with it, but also matured and developed proper apical-basal polarity guided by the combinatorial presentation of E-cadherin and a 3D obstacle. As these examples demonstrate, refining our ability to mimic cellular handshakes will improve our ability to dissect and interrogate cell-cell junctions, in turn advancing both cell junction biology and future cell-mimetic interfaces.

Handshake agreements: future work with cell-cell recognition and adhesion

The incredible complexity of cell junction biology and the importance of being able to control aspects of the junction emphasize the synergy between biology and engineering. For instance, the 2D cell-mimetic surface assays that have been developed using single fusion proteins can now be expanded to explore the full range of secret handshake interactions by co-patterning cocktails of different proteins (e.g. cadherins, ephrins, nectins, etc.). This approach has already shown success in teasing apart the relative contributions of different desmosomal cadherins (46), and is easily scalable to explore how different protein levels, pattern geometries, and combinations of adhesion proteins affect cell behaviors. Similarly, ECM stiffness and 2D/3D environments can be iterated in a systematic fashion to provide a much richer understanding of junctional organization and mechanics.

Fundamental biological knowledge from these assays will, in turn, be invaluable in the growing area of mammalian synthetic biology that has been limited by a lack of good tools to manipulate cell-cell interactions (47). This challenge is slowly being met, one example being a recent study demonstrating the first engineered, synthetic Notch/delta pathways that induced programmable gene activation patterns within a tissue (48). Such tools that take advantage of natural self-assembly and patterning within tissues are poised to give us unprecedented control over how we grow and engineer complex tissues.

Finally, combined advances in biophysics, protein biology, and peptide chemistry are poised to improve our abilities to design cell-mimetic interfaces. Advanced biophysical approaches are already yielding deep knowledge of protein-level mechanics of junctional proteins such

as cadherin bond dynamics (49, 50). This knowledge, combined with a structural understanding of the protein-protein interactions may help us design new junctional protein mimics with greater stability, bond strength, etc. At present, junctional proteins need to be produced using biological expression vectors and protein purification approaches which limits their widespread use. However, given the success and ubiquity of the RGD integrin-binding peptide, peptide mimics of junctional proteins such as N-cadherin (e.g. HAVDI peptides (51, 52)) may provide an alternative approach to full length proteins. All of these predictions are feasible if we maintain the handshake between biology and technology that already brought us to this point but is only recently coming into its own.

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Interaction Protein	Model	Function	Refs.
Cadherin superfamily mechanical coupling, sorting		calcium (in)dependent-adhesion mechanoadhesion and signaling cell sorting; tissue integrity downstream: catenin complex, actin	(4-7)
Nectins (n1-4) mixing, cadherin modulation		cell-cell intercalation homotypic and heterotypic tissue borders and patterning downstream: afadin, catenins	(8-9)
CAMs (Ep-/N-/L1/Syn-) adhesion, immunity		calcium-independent adhesion adhesion, NCAM first CAM discovered neurite outgrowth downstream: varied	(9-10)
Connexins (e.g. Cx43) ion, signaling exchange		gap junction proteins open/closed conformations electrical coupling (ions) neural function, cardiac coupling	(11, 12)
Notch/Delta patterning, signalling		contact-mediated signalling neuron and blood vessel develop. cell fate regulation C-terminal Notch cleaved on binding	(13, 14)
Lectins (sugar binding) adhesion, self/non-self		earliest identified adhesion module multiple binding modes and types adhesion, immune modulation blood group markers	(15, 16)
eph/Ephrin (A/B) repulsion, sorting		4-way signaling/reverse signaling homeostasis, cell segregation angiogenesis, migration, neurogen. downstream: RTK (eph), PDZ domain	(17, 18)

Figure 1.
summary of key cell-cell interaction proteins

Figure 2. case studies of complementary and contradictory multiplexed cell-cell interactions

(Left) schematic of sensory cell sorting within the olfactory epithelium as described in (29).

The three classes of cell-cell interaction are ordered in descending adhesion strength, and the resulting pattern is shown on the right.

(Right) schematic of how contradictory cues of repulsion (ephrins) and adhesion (cadherins) can synergize to produce a stable, sharp border between tissues without allowing cell mixing, as described in (18).

Figure 3. Case studies of E-cadherin-based cell-mimetic interfaces

(Left) 2D co-presentation of both ECM and E-cadherin:Fc (fusion protein) patterned on glass as in (33–38). (Center) Microbeads coated with E-cadherin:Fc to interrogate junctional mechanics. (Right) Generic schematic of 3D biomimetic junctions recapitulating native cell-cell junction biology as used in (25, 45).