Holding on by a thread: Evidence for filament linkage to hyperadhesive cell-cell contacts

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Introduction

Cell-cell adhesions are crucial for maintaining tissue structure and function, mediating interactions between neighboring cells (1). These adhesive interactions are essential for normal development, tissue organization, and immune response. Their failure is also at the center of many diseases such as epidermolysis bullosa, cancer invasion, and metastasis. Different types of cadherins are responsible for the initial contact and cohesion between cells (classical cadherins that form adherens junctions) and for strong reinforced adhesions (desmosomal cadherins that form desmosomes) (2). Both types of cadherins link to the cytoskeleton inside the cell, creating a physical scaffolding that extends within and between cells. While adherens junctions are typically associated with actin filaments, desmosomes connect to intermediate filaments (3).

Adherens Junctions, Desmosomes, & Hyperadhesive Desmosomes

Each of the junctional types makes a unique contribution to overall intercellular adhesion. The earliest mechanism of forming cell-cell contacts involves classical cadherins that form adherens junctions. Desmosomes typically form later, in more mature, polarized tissues. In even more stabilized tissues, some desmosomes mature into 'hyperadhesive desmosomes', particularly strong adhesions that uniquely capture calcium (Ca^{2+}) ions in a way that is resistant to EGTA chelation. Previous work has found hyperadhesive desmosomes in mouse embryos but not until E13, well after early developmental events of gastrulation and neurulation (4). Others have also used cell cultures to demonstrate distinct dynamics of both the adhesion proteins (2) and the associated actin and keratin cytoskeleton networks (3) when transitioned between low and normal Ca^{2+} conditions. Here, stemming from prior observations of strong cohesiveness in the ectoderm, we examined whether hyperadhesive desmosomes exist in early gastrulating frog embryos and how these adhesions may be connected to cytoskeletal networks. Understanding the mechanisms underlying cellular junctions illuminates fundamental cellular processes and informs our understanding of pathological diseases.

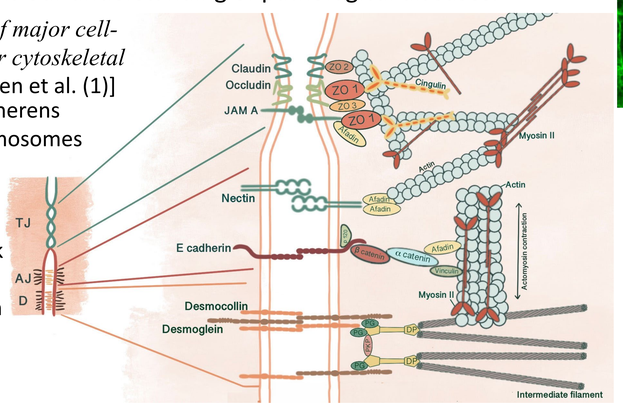

Figure 1. *Illustration of major cell-cell adhesions and their cytoskeletal connections.* [From Ceren et al. (1)] Tight junctions (TJ), adherens junctions (AJ) and desmosomes (D) are together responsible for most intercellular adhesion. All three adhesions link to the cytoskeleton to control the distribution of different filaments throughout the cell.

Methods

Ectodermal animal caps were dissected from *Xenopus laevis* embryos at stage 10 (gastrula) using an eyebrow knife, then transferred via glass pipettes. Animal caps (8 caps/condition) were exposed to either 1x MBS (a simple saline solution containing Ca^{2+} and Mg^{2+}) or Ca^{2+} / Mg^{2+} -free solution with EGTA. EGTA was used due to its strong chelating properties ensuring that any desmosomes remaining after 90 minutes were hyperadhesive in character. Animal caps were then fixed overnight at 4°C in either 100% methanol or 37% formaldehyde. Immunofluorescence was performed on the methanol-fixed caps to detect keratin intermediate filaments and rhodamine-conjugated phalloidin was used to detect actin filaments in formaldehyde-fixed caps. Samples were mounted on a glass depression slide and observed on an Olympus BX3 microscope equipped with fluorescence. Images were acquired at 100x magnification.

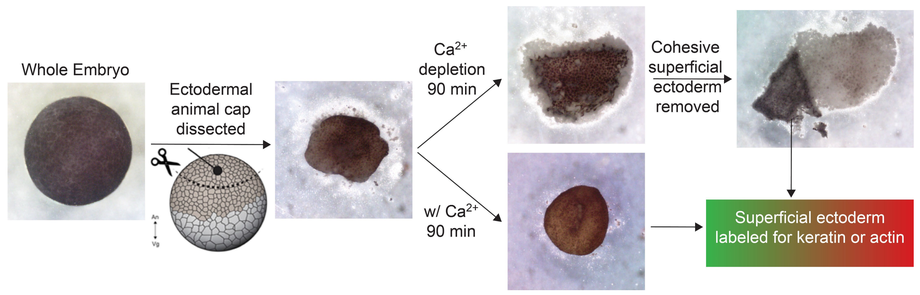

Figure 2. *Illustration representing the workflow of ectoderm isolation, Ca^{2+} depletion, and fluorescent labeling of keratin intermediate filaments and actin filaments.*

Results

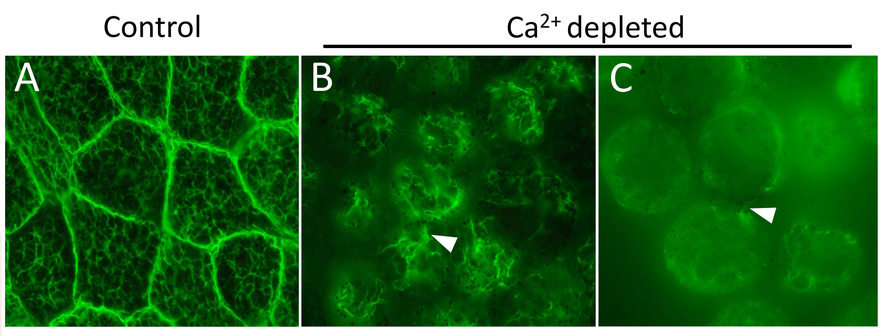

Figure 3. *Keratin filaments of ectodermal layer observed by immunofluorescence. (A) Control cells in 1xMBS, (B and C) cells in Ca^{2+} / Mg^{2+} -free solution with EGTA.* Keratin intermediate filaments in ectodermal animal caps treated in 1xMBS for 90 min (A: Control) remained intact and cortical, indicative of association with cell-cell adhesions. Intermediate filaments were also seen extending throughout the cell near the apical membrane. Ectodermal caps exposed to Ca^{2+} / Mg^{2+} -free solution with EGTA (B and C: Ca^{2+} depleted) exhibited collapse of the keratin filament network toward the middle of the cells. Only keratin filaments associated with hyperadhesive desmosomes remain near cell-cell interfaces where fine tethers could be seen (arrowheads).

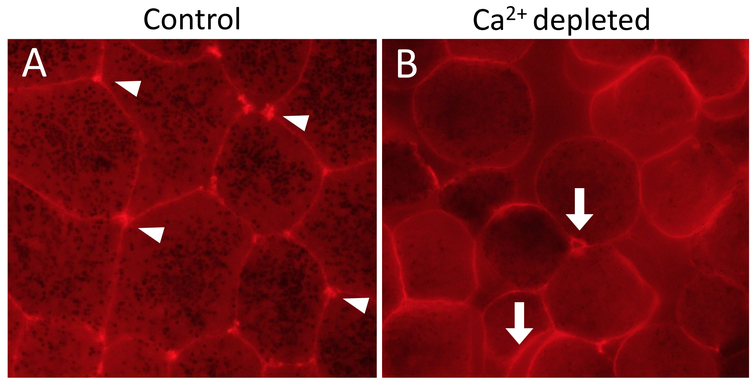

Figure 4. *Actin filaments of ectodermal layer observed by fluorescence microscopy. (A) Control cells in 1xMBS, (B) cells in Ca^{2+} / Mg^{2+} -free solution with EGTA.* Actin filaments were cortically located in control cells (A: Control), localizing to cell-cell interfaces. Particularly high concentrations of actin filaments were localized at the vertices where more than two cells met (arrowheads). Exposure of ectodermal cells to Ca^{2+} / Mg^{2+} -free solution with EGTA for at least 90 minutes (B: Ca^{2+} depleted) induced a loss of the polygonal cellular shape and the general rounding of cells, suggestive of a loss of interfacial junctional tension. Gaps formed at multicellular junctions, highlighting the likely weakening of adherens junctions (arrows).

Conclusion

- Hyperadhesive desmosomes are present in the embryo by the onset of gastrulation, a timeframe far earlier than anticipated.
- Hyperadhesive desmosomes are likely linked to keratin intermediate filaments and these associations persist even when Ca^{2+} is withdrawn.
- Actin filaments remain cortical despite weakened cell-cell adhesions but are no longer concentrated at vertex junctions.
- Ca^{2+} -dependent adhesions are essential for the normal cortical organization of both actin and intermediate filaments.

References

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