Formation and maturation of desmosomal cell-cell adhesions during early vertebrate embryo development

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Introduction

Cell-cell adhesions are the fundamental basis of all multicellular organisms. Desmosomes are a type of attachment that cells of vertebrate animals use to spot-anchor to other cells. Desmosomes are composed of strong elastic intermediate filament cytoskeleton inside of the cell that connects to the internal portion of transmembrane receptor proteins, so-called desmosomal cadherins, whose extracellular domains attach to neighboring cells. Importantly, cell-cell connections between cadherins require calcium ions, and the progressive maturation of desmosomes into a hyperadhesive state involves increasing affinity to this calcium. These anchoring junctions between cells are necessary for normal embryonic development and defects are associated with congenital skin and heart pathologies. Despite their importance, relatively little is known about when and how desmosomes first form during development (1,2). In this study, embryos of the African clawed frog *Xenopus laevis* were used to investigate when desmosomes first appear during early vertebrate development. Histological examination of *X. laevis* embryos between Nieuwkoop-Faber stages 2-13 was performed to track the formation and maturation of both desmosomes and the associated cytoskeleton. The maturation process of cell-cell adhesions was functionally tested using calcium-depletion assays. We found that cell-cell adhesions undergo dramatic strengthening and reinforcement during blastula stages, coinciding with the emergence of a superficial layer of ectodermal epithelia. Further strengthening of cell-cell adhesions occurs throughout gastrulation as the epithelium becomes increasingly differentiated toward an epidermal lineage. These functional changes are indicative of an increased abundance of desmosomes and changes in their maturation state, occurring earlier in development than previously recognized.

Hyperadhesive Desmosomes

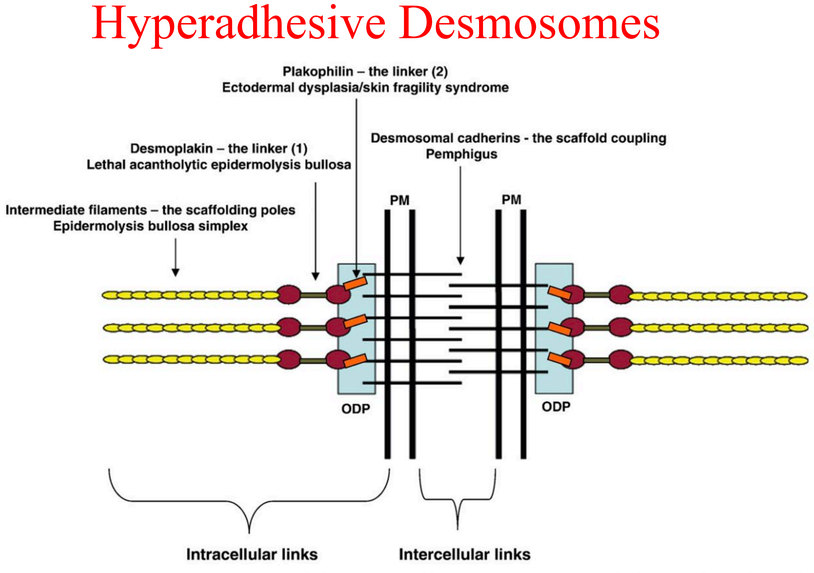

Figure 1. Desmosome structure and the impact of key components to pathologies (from Garrod and Chidgey (1))

Hyperadhesive desmosomes are resistant to calcium depletion. This much stronger state of desmosomes is adopted sometime after the initial formation of the calcium-sensitive, weaker affinity state. Not much is known about the mechanisms that facilitate this transition. The desmosomal cadherins, desmocollin and desmoglein, require calcium to form cohesions, a conserved feature of all cadherins. However in the hyperadhesive state, it is hypothesized that desmosome cohesion is protected by the adhesion's quasi-crystalline configuration and/or the molecular trapping of Ca^{2+} ions. Determination of desmosome hyperadhesion is made by challenging tissues to remain intact for at least 90 minutes following a combination of Ca^{2+} removal and exposure to strong calcium chelators, such as EGTA (3).

Methods

For calcium depletion assays, ectodermal animal caps were dissected from *X. laevis* embryos between Nieuwkoop-Faber stages 6-10 (late cleavage-early gastrula) using an eyebrow knife and transferred via glass pipette. Animal caps (10 caps/condition) were exposed to either 1x MBS (a simple saline solution containing Ca^{2+}), Ca^{2+} free MBS (calcium-depletion), or Ca^{2+} free MBS containing 3mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA). EGTA was used due to its strong chelating properties ensuring that any desmosomes remaining after 90 minutes were hyperadhesive in character. For histology, hematoxylin and eosin stained *X. laevis* embryo slides were obtained from Carolina and Thompson Biological Labs. Slides were examined on an Olympus CX41 microscope.

Results

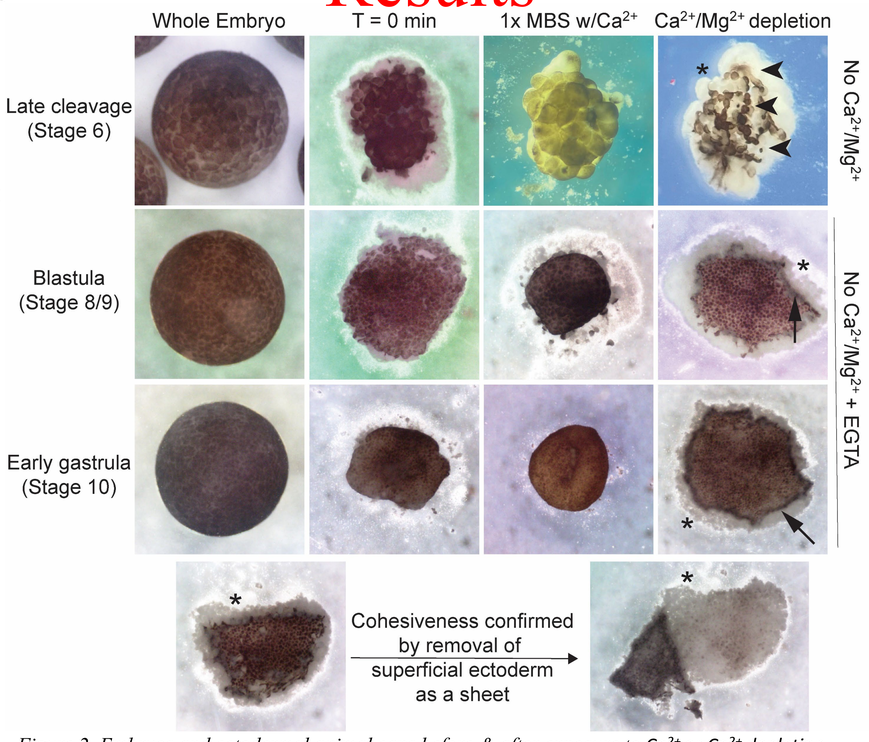

Figure 2. Embryos and ectodermal animal caps before & after exposure to Ca^{2+} or Ca^{2+} depletion

Animal caps from all stages examined remained intact in 1x MBS solution, and furthermore maximized cell cohesions as cells compacted into a spherical cluster. In stage 6 (late cleavage) animal caps, both the pigmented superficial ectoderm (arrowheads) and the yolk-filled deep cells (*) were effectively dissociated in 90-min by simply the removal of Ca^{2+} and Mg^{2+} from solution. This indicates that all cell-cell adhesions at this early stage pre-blastula are Ca^{2+}-dependent. Deep ectoderm cells of blastula and gastrula caps similarly dissociated in response to removal of Ca^{2+} (*). Surprisingly, the pigmented superficial ectoderm of all blastula and gastrula ectodermal caps remained mostly intact even in the more stringent Ca^{2+}-depleted EGTA solution. The center region of the caps appeared to be more tightly cohesive and contractile than the sides, often leading to curling of the marginal tissue inwards towards the center (arrows). These results strongly suggest that hyperadhesive desmosomes form in the blastula embryo, prior to gastrulation and much earlier during embryonic development than previously thought (1). Hyperadhesive desmosomes may be necessary in the superficial ectoderm at these early stages to stabilize the overall embryo structure as it experiences significant stresses due to rapid proliferation and morphogenesis associated with development.

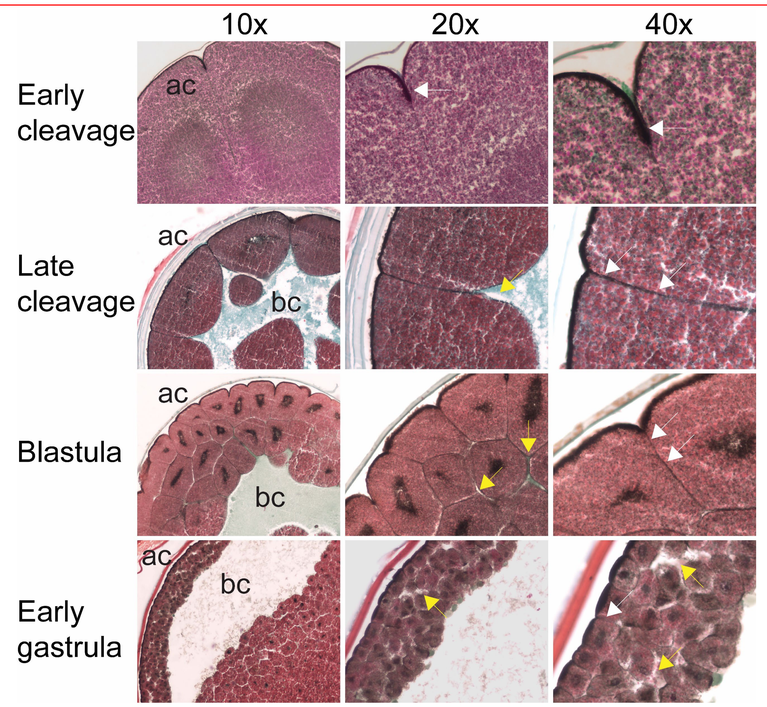

Figure 3. Histological images of X. laevis embryos from early cleavage through the early morphogenetic stages of gastrulation

Cell-cell interfaces between cells at the presumptive ectodermal animal cap region (ac) are well-defined from early stages and form extended tight juxtaposition, notable in the pigmented superficial layers (white arrows). Underneath these cells, the blastocoel (bc) first forms during late cleavage stages and looser association between deep cells of the ectodermal animal cap is indicated by gaps (yellow arrows), reflective of their less stable adhesions.

Conclusion

- The formation of calcium-dependent desmosomes, as well as the transition to hyperadhesive calcium-independent desmosomes occurs much earlier and faster than previously recognized
- The pre-gastrula presence of hyperadhesive desmosomes suggests that they are needed to help stabilize the superficial ectodermal epithelia due to the high stresses these cells experience during proliferation and morphogenetic events of early embryonic development

References

1. Garrod, David, and Martyn Chidgey. "Desmosome Structure, Composition and Function." *Biochimica et Biophysica Acta (BBA) - Biomembranes*, 1778(3), Mar. 2008: 572–587, doi: 10.1016/j.bbamem.2007.07.014.

2. Baker, Patricia C., and Thomas E. Schroeder. "Cytoplasmic Filaments and Morphogenetic Movement in the Amphibian Neural Tube." *Developmental Biology*, 15(5), May 1967: 432–450, doi:10.1016/0012-1606(67)90036-x.

3. Garrod, David R. "The assay that defines desmosome hyper-adhesion." *The Journal of Investigative Dermatology,* 133(2), 2013: 576-7. doi:10.1038/jid.2012.275.