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Myoblast Fusion: Playing Hard to Get

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In *Drosophila* myoblast fusion, the fusing cell invades another by actin-enriched protrusion. In this issue of *Developmental Cell*, Kim et al. (2015) examine the myoblast fusion mechanism from the perspective of the "receiving" cell and report that fusion depends on the ability of this cell to stiffen its actomyosin cortex.

Much of the current understanding of cell-to-cell fusion that generates and regenerates our skeletal muscles has originated from work on myogenesis in *Drosophila* (Onel and Renkawitz-Pohl, 2009; Sens et al., 2010). Prior to fusion, binding between adhesion molecules specific for each of the two types of muscle cells, founder cells and fusion-competent myoblasts, establishes an adhesive structure between the cells. Within this "fusogenic synapse" (Sens et al., 2010), an "attacking" fusion-competent myoblast inserts its finger-like actin-rich protrusions into a "receiving" founder cell, and one of these protrusions evolves into a fusion pore joining the volumes of the two cells.

In this issue of *Developmental Cell*, Kim et al. (2015) explored myoblast fusion in *Drosophila* embryo and fusion between cultured cells expressing Sns, a *Drosophila* protein that organizes invading protrusions in the fusion-competent myoblasts, and *C. elegans* fusogen EFF1, discovered and characterized by the group of B. Podbilewicz in Technion, Israel. Genetic screening identified myosin II (MyoII) as a player in myoblast fusion. MyoII is a molecular motor protein that reversibly crosslinks actin filaments and generates contractile mechanical stresses in cytoskeleton that control cell shape, adhesion, and migration. The mechanical force applied by the protrusion of the invading cell induced accumulation of the activated MyoII in the receiving cell to the fusogenic synapses in both myoblast and cultured cell systems. MyoII binding and contractile activity increased the stiffness of the actomyosin cortex under the plasma membrane of the receiving cell. Compared with normal fusion-promoting invasive protrusions, those invading cells with reduced MyoII activity were wider and longer and did not promote cell-cell fusion.

The mechanisms by which protrusion into a receiving cell with stiffened cortex promotes fusion are yet to be understood. At the same time, studies on well-characterized membrane fusion processes, including those mediated by viral and intracellular proteins, have identified a conserved pathway of membrane rearrangements in fusion and generated qualitative ideas about the physical forces driving fusion. A local approach of two membrane bilayers is followed by a merger between their contacting leaflets (hemifusion) and then opening and expansion of a fusion pore (Chernomordik and Kozlov, 2008). Myoblast fusion apparently proceeds by the same fusion-through-hemifusion pathway (Leikina et al., 2013). In terms of physics, the early fusion stages from hemifusion through nascent pore formation represent a sequence of local bending deformations and topological remodeling of the involved membrane monolayers. It has been suggested that the elastic energy of these fusion intermediates is provided by relaxation of the membrane-bending stresses pre-accumulated in the fusion site through its deformation by fusion proteins (Chernomordik and Kozlov, 2008). The fusion pore expansion representing a large-scale membrane rearrangement is apparently driven by in-plane (lateral) tension, which exists or is generated and maintained in the fusing membranes throughout the fusion reaction (Chernomordik and Kozlov, 2008).

How could the interplay between the invading protrusion and the resisting actomyosin cortex provide membrane bending stresses and lateral tension within the fusogenic synapse, and what could the importance of cortex stiffening by MyoII be?

Let us start with the latter question. It is sensible to assume that the primary force driving both the membrane bending and the tension comes from the actin bundles polymerizing within the growing invading finger. The force developed by actin filaments polymerizing against an obstacle depends on the resistance provided by the obstacle, and hence on the polymerization rate (Mogilner, 2006). The larger the resistance (the lower the polymerization rate), the larger the polymerization force. Provided that the actomyosin cortex opposes the invading finger elongation, cortex stiffening by MyoII-mediated contractility can be necessary to guarantee generation of sufficiently large invasion forces by the actin bundles within the finger.

Returning then to the first question, to accumulate pre-existing bending stresses sufficient for driving the early fusion stages, membranes at the fusion site have to be bent to curvature radii of a few tens of nanometers (McMahon et al., 2010). The surfaces of the invading fingers and specifically of their end-caps are bent, but their *average* curvature radii are of the order of ~500 nm, much larger than those required for fusion. However, the *local* curvature radii of the "attacking" and "receiving" membranes covering the end-caps of the invading fingers may be small enough to drive fusion. Indeed, at nanometer scale, the finger end-cap region can be seen as two opposing membranes pushed against each other by the actin bundles on one side and the spots of interaction between the actomyosin complex and the membrane on the other (Figure 1). The dimensions of cortex-membrane anchors such as

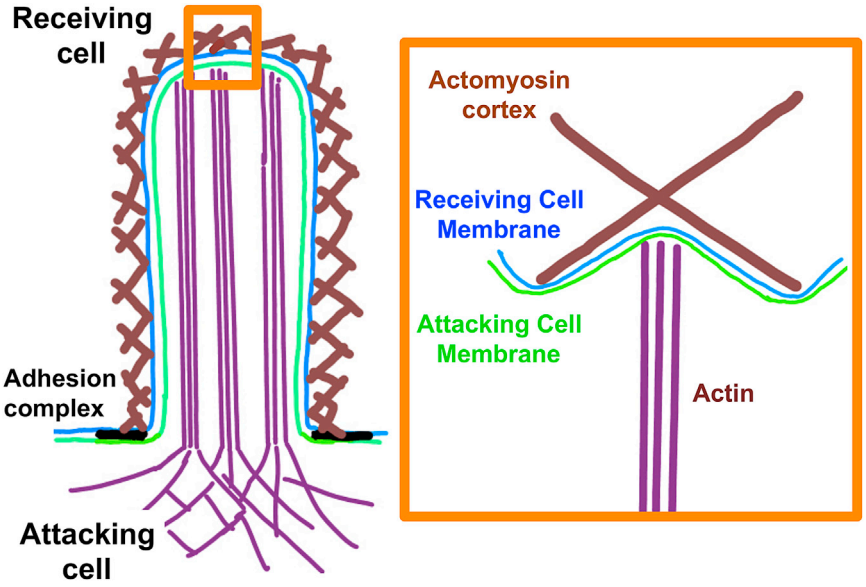

Figure 1. Schematic Representation of a Fusogenic Synapse with an Invading Finger of the "Attacking" Cell and an Actomyosin Cortex of the "Receiving" Cell

Left: Large-scale view. Right: Small-scale view of a fragment of the invading finger end-cap with the opposing membranes squeezed and locally bent between the pushing actin bundle and the resisting actomyosin cortex.

ezrin-radixin-moesin and the cross-sectional dimensions of actin filaments are ~10 nm. As a result of sufficiently strong pushing, the membranes are expected to bend around the actin bundle tips and the cortex-membrane anchors, adopting curvature radii similar to the dimensions of the latter, i.e., being of the order of 10 nm. These regions of strong local bending can facilitate hemifusion and nascent pore formation.

Finally, could the finger invasion also contribute to the membrane tension required for the fusion pore expansion? Generation or increase of membrane tension in the fusogenic synapse may develop only dynamically, i.e., during finger elongation, provided that redistribution of membrane lipids to this region from the surrounding cell membrane is impeded. A straightforward reason for that could be an effective friction between the lipid bilayers covering the growing finger and membrane-associated protein structures (Schweitzer et al., 2014) such as a ring of adhesion proteins around the finger base and/or membrane anchors of the actomyosin cortex. For instance, such friction can be based on membrane-bound septins that interact with actomyosin cortex (Beise and Trimble, 2011). The friction, and hence the related membrane tension, may be enhanced by increase in the density of membrane anchors of the actomyosin cortex resulting from cortex contraction by cross-linking or MyoII activity. Protrusion-generated or constituent tension in the fusing membranes may explain why, in contrast to many well-characterized fusion processes, myoblast fusion almost never stalls at a stage of a small fusion pore (Leikina et al., 2013), suggesting that in this case there is a significant tension in plasma membranes that rapidly expands early membrane connections.

In spite of their mechanistic importance, invading podosomes are, most likely, not the whole story in myoblast fusion. The search for proteins that initiate this fusion process and, perhaps, are enriched in the deformed membranes of the fusogenic synapse is still going. That said, there is no doubt that the exciting conclusion of Kim et al. (2015) that this fusion depends on the ability of the seemingly passive receiving cell to resist an invasion by an attacking cell (Kim et al., 2015), along with a recent study documenting the importance of MyoII in self-contact-induced fusion of epithelial cells (Sumida and Yamada, 2015), will motivate a search for similar mechanistic motifs in cell fusion stages of fertilization, osteoclast formation, and other important cell-to-cell fusion processes.

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