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understanding of cytokinesis will require that we think 'outside of the cell' and incorporate a role for the ECM in cell division.

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Cell–Cell Fusion: A New Function for Invadosomes

Podosomes are cytoskeletal-based structures involved in extracellular matrix remodeling and cellular motility. A new study now implicates podosomes in pore formation during myoblast fusion.

Bong Hwan Sung and Alissa Weaver*

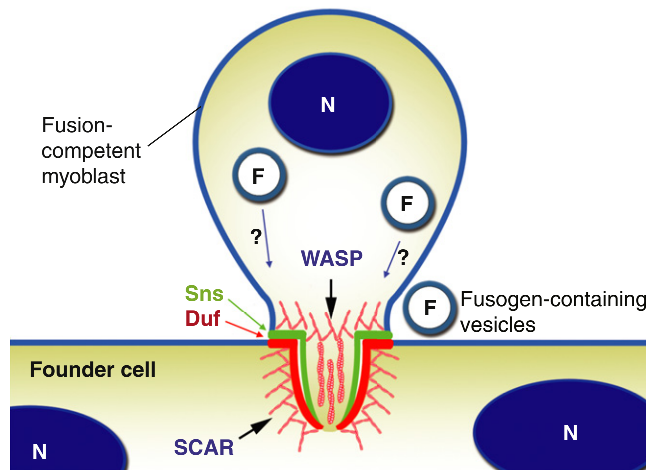
Cell–cell fusion is a highly regulated event that is critical for many physiological and pathological events, including fertilization, muscle development, and immune response. During skeletal muscle development, fusion of muscle cells generates multinucleate and functional muscle fibers and aberrant fusion has been implicated in dystrophic muscle diseases [1,2]. Recently, a number of groups have studied myoblast fusion during body wall muscle formation in *Drosophila melanogaster* as a genetically tractable *in vivo* model system to study cell–cell fusion [1]. In a new study published in the *Journal of Cell Biology*, Sens *et al.* [3] investigated the role of actin assembly in formation of the fusion pore during *Drosophila* myoblast fusion. Interestingly, they find that an invasive, actin-rich, podosome-like structure is used by fusion-competent myoblasts (FCMs) to adhere to and fuse with muscle founder cells.

Previously, it was known that actin filaments accumulate transiently at the site of myoblast fusion [4–6], dependent on signaling from heterotypic adhesion molecules and downstream regulators of branched actin assembly, including Rac, SCAR and WASP [7]. Furthermore, both the SCAR and WASP complex activators of the branched-actin-nucleating Arp2/3 complex were known to be essential for myoblast fusion [5,6,8–10]. However, the nature of the fusion structure and the roles of individual actin regulators were poorly understood.

To determine whether the prominent actin accumulations at pre-fusion sites were unique to a muscle cell subtype, Sens *et al.* [3] expressed GFP–actin under the control of FCM-specific or founder-cell-specific promoters and costained for all actin filaments in embryos with fluorescent phalloidin. Interestingly, the large actin foci were exclusively found in FCM cells and were associated with a deformation in the founder cell membrane. Transmission electron microscopy studies showed finger-like FCM cell protrusions apparently invading into the founder cells at the site of cell–cell fusion.

Invasive, actin-rich, finger-like protrusions have been well characterized in cells that invade or remodel tissue and are termed invadopodia in cancer cells and podosomes in normal cells (or collectively, invadosomes) [11,12]. However, a role in cell–cell fusion has not been previously described, and their main function is thought to be degradation of extracellular matrix (ECM), in part due to active trafficking of ECM-degrading proteinases to sites of protrusion formation (Figure 1). The myoblast structures observed by Sens *et al.* [3] seemed to be a potential variation of podosomes, as they were morphologically similar by electron microscopy and even had adhesion ring structures, albeit cell–cell rather than cell–ECM adhesions. If these structures really were podosomes, the new data revealed that podosomes might be more versatile than previously appreciated and also that they are formed *in vivo* during developmental 'invasions'.

To determine whether the FCM actin-rich protrusions resembled podosomes at the molecular level, Sens *et al.* [3] manipulated the



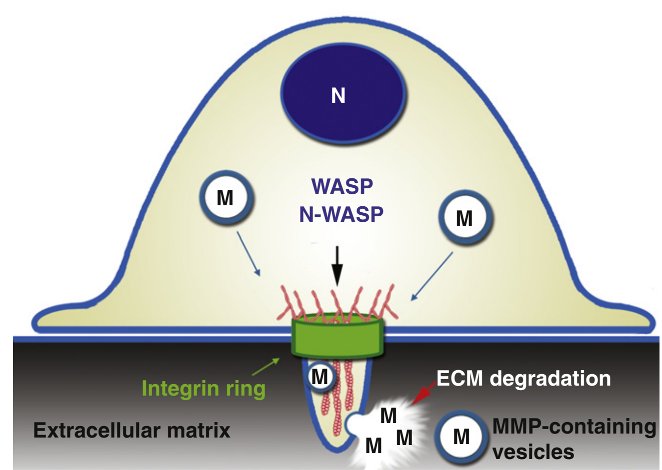


Figure 1. Comparison of myoblast podosome with ECM-degrading podosome.

(A) Actin assembly is required on both the fusion-competent myoblast and founder cell membranes for initiation of fusion pore formation. Although WASP and SCAR have redundant functions in the formation of actin foci in FCM podosome structures, WASP is essential for invasion of FCM podosomes into founder cells and SCAR induces the formation of a thin actin layer at the pre-fusion site in founder cells. Similar to traditional podosomes (B), myoblast podosomes have an adhesion ring; however, the molecular components are immunoglobulin (Ig) super-family cell–cell adhesion receptors found in the FCM (Sns ring) and founder cells (Duf ring). Fusogen-containing vesicles may be trafficked to podosomes to promote cell fusion. (B) WASP/N-WASP promotes formation of branched-actin-rich, ECM-degrading podosomes. Rings containing integrins and focal adhesion proteins are formed around ECM-degrading podosomes. Matrix metalloproteinases (MMPs) are embedded in vesicles, transported to podosomes, and secreted to induce ECM degradation. N, nucleus.

SCAR and WASP regulators of branched-actin assembly and determined the effects on F-actin foci formation and myoblast fusion. Loss of both protein complexes in *scar,sltr* double mutants led to loss of the FCM actin focus, verifying that it is a branched-actin-based structure. However, the actin focus was present in single *scar* or *sltr* mutants, suggesting compensation of one branched-actin regulator for the other. More interesting, however, was the finding that, despite continued formation of the F-actin foci on the FCM side of the pre-fusion site, loss of WASP but not SCAR complexes led to defective formation of invasive protrusions and lack of FCM invasion into founder cells. By contrast, loss of SCAR affected actin assembly at the founder cell membrane side of the pre-fusion site. As WASP, but not SCAR, homologues are known to be essential for podosome formation and function in mammalian cells, these data support the concept that the invasive myoblast protrusions are similar or identical to podosomes.

Sens *et al.* [3] suggest that actin assembly is required on both the FCM and founder cell membranes for initiation of fusion pore formation. In FCM cells, WASP activity promotes formation of actin-rich, invasive podosomes that protrude into founder cells and allow extensive membrane contact at the pre-fusion site. In founder cells, SCAR promotes formation of a thin actin layer at the pre-fusion site and is necessary for cell fusion. These two actin structures may allow close enough apposition and/or curvature of the membranes for fusion to initiate. They may also serve as docking sites for vesicles containing fusogenic factors, such as lipid rafts (Figure 1) [13,14]. Notably, Golgi-derived vesicles with electron-dense rims have been observed to move toward muscle-cell contact sites in *Drosophila* embryos [5]. Moreover, invadopodia in cancer cells have been shown to be dependent on the lipid raft protein caveolin-1 [15], and both podosomes and invadopodia are sites of active vesicle trafficking [11,12]. Since both WASP and SCAR are essential for myoblast fusion, the authors were not able to dissect further the individual roles of these actin regulators in later events. However, they did analyze the structure of the fusion site in wild-type embryos by a high-pressure freezing/ freeze substitution electron microscopy preparation and found a single macrofusion pore filled with ribosomes and other organelles but not actin. Thus, after fusion pore initiation, rapid disassembly of actin and pore expansion is likely to occur.

Overall, the study by Sens *et al.* [3] provides an elegant example of the versatility of actin-based structures for cellular invasion processes *in vivo*. Although a previous study had shown that leukocytes use podosomes as adhesion and invasion structures during transcytosis of endothelial cells [16], podosomes had not been previously identified as mediators of direct fusion of two cells. Indeed, the dogma in the field has been that podosomes are structures that mediate ECM adhesion and degradation. A final novel contribution of this paper is the identification of podosomes in an *in vivo* setting, evidence for which has so far been limited [17,18], potentially due to their small size and transient nature [11,19]. Future studies should shed further light upon the adaptability of these invasive structures.

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Neuroscience: What We Cannot Model, We Do Not Understand

To understand computations in neuronal circuits, a model of a small patch of cortex has been developed that can describe the firing regime in the visual system remarkably well.

William S. Anderson[1] and Gabriel Kreiman[2,3,4,*]

Circuits of neurons in the brain are very complicated: because of the multiple non-linearities, different types of neurons, complex dendritic geometries, diverse connectivity patterns and dependencies on learning and development, the cerebral cortex and other neuronal circuits constitute the most complex systems ever studied by science. Perhaps not surprisingly, the computational power that emerges from such circuits is astounding; neuronal networks are responsible for diverse cognitive phenomena such as seeing, smelling, remembering, planning and so on.

To understand how function emerges from ensembles of neurons and their interactions, we need a rigorous interplay of theoretical work and experimental approaches capable of listening to the activity of neurons. This synergy of theory and neurophysiology is beautifully illustrated in recent work by Rasch *et al.* [1]. These authors took a courageous approach using computational models to describe the activity in a local 5 x 5 mm patch of neocortex with an impressive set of 35,000 neurons and ~4 million synapses. They focused on primary visual cortex, one of the most studied parts of cortex and the first stage in the hierarchical cascade of processes that convert the retinal input into our visual perceptions. The Logothetis lab used multiple microwire electrodes to measure the activity of neurons in primary visual cortex of anesthetized monkeys while the monkeys watched a natural scene movie. The authors then ‘presented’ the same movie to their model to explore its fidelity and quantitatively compare the computational output and the neurophysiological one.

To compare the circuit *in silico* and *in vivo*, one must consider what aspects of the complex neuronal ensemble responses one aims to explain. Instead of trying to predict the detailed spiking activity of every single neuron as done in many other studies (for example [2,3]), Rasch *et al.* [1] defined a ‘firing regime’ that is characterized by several properties of the neuronal responses. These properties included the firing rate, distribution of interspike intervals, variability in spike counts over time, degree of burst firing and degree of synchronization in the network. The authors use these inter-related properties to define the state of the network.

Another important aspect that the theorist must consider when thinking about such network models is the large number of parameters that arise as a consequence of the complexity in the circuitry. The modeler needs to make decisions about the number and type of neurons, their distribution and connectivity, the type of ionic channels they are embedded with and their corresponding characteristics. Some of these decisions may be constrained by experimental data; others may require more guesswork. Parameters are our enemies. It is extremely difficult from a computational viewpoint to systematically characterize the