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Review

Role and organization of the actin cytoskeleton during cell-cell fusion

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ABSTRACT

Cell-cell fusion is a ubiquitous process that underlies fertilization and development of eukaryotes. This process requires fusogenic machineries to promote plasma membrane merging, and also relies on the organization of dedicated sub-cortical cytoskeletal assemblies. This review describes the role of actin structures, so called actin fusion foci, essential for the fusion of two distinct cell types: *Drosophila* myoblast cells, which fuse to form myotubes, and sexually differentiated cells of the fission yeast *Schizosaccharomyces pombe*, which fuse to form a zygote. I describe the respective composition and organization of the two structures, discuss their proposed role in promoting plasma membrane apposition, and consider the universality of similar structures for cell-cell fusion.

Contents

1. Introduction

The fusion of eukaryotic cells underlies many critical developmental events, from fertilization during sexual reproduction to formation of functional multinucleated syncytia that underlie mycelia, muscle or bone formation. In all events of cell-cell fusion, three major conceptual steps can be defined: First, signalling occurs between the two partner cells, to induce cell differentiation. Second, the cells polarize towards each other for cell-cell adhesion. Finally, a fusion machinery is assembled at the site of cell-cell contact to promote plasma membrane merging. This fusion machinery may be composed of two main parts: a specific actin structure that promotes membrane juxtaposition [2], and fusogenic proteins that drive membrane fusion [3]. This notion comes from work in several organisms, each contributing distinct information to the process, and from reconstitution experiments in heterologous cells [69]. In particular, the strong contribution of the actin cytoskeleton to cell fusion comes primarily from work in two distinct model systems: *Drosophila* myoblasts, which fuse to form muscles, and fission yeast gametes, which fuse to form the zygote.

This review describes and compares the actin cytoskeletal structures that are put in place to promote cell-cell fusion in these two organisms, and discusses the generality of these observations.

2. Fission yeast

We recently described that a specialized actin structure, named the actin fusion focus, underlies cell-cell fusion in fission yeast *Schizosaccharomyces pombe* to form the zygote during sexual repro-

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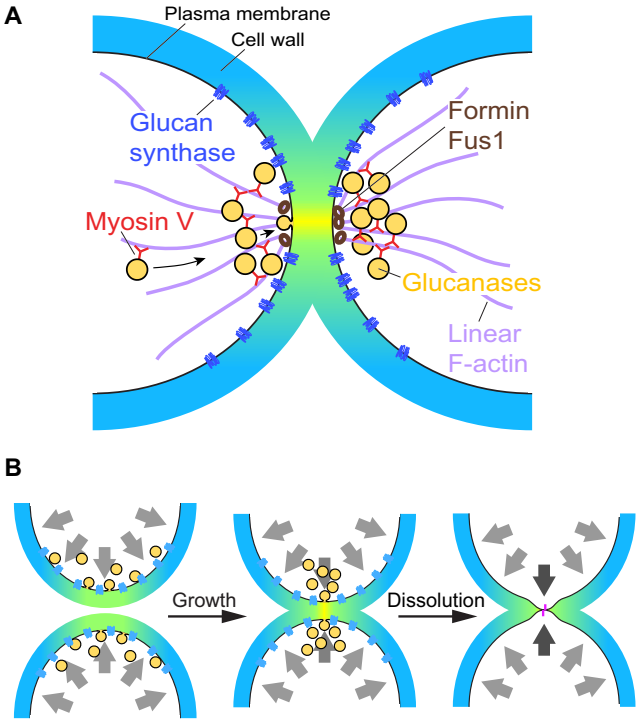

Fig. 1. The actin fusion focus in the fission yeast.
(A) Schematic representation of the organization of the actin cytoskeleton and cell wall remodelling enzymes. The formin Fus1 (brown) nucleates linear actin filaments (purple) from a restricted location at the plasma membrane. This allows the delivery of secretory vesicles by type V myosins (red). These secretory vesicles carry glucanases (yellow), which serve, upon secretion, to hydrolyse in the cell wall the bonds linking the glucan chains together. In contrast to the focused delivery of glucanases, transmembrane glucan synthases (blue) occupy a broader area at the plasma membrane. This leads to a differential of synthesis and hydrolysis activities in the cell wall, represented by the blue to yellow colour gradient, respectively. Strong hydrolytic activity at the center of the zone leads to cell wall digestion to bring the plasma membranes into contact.
(B) Progression of the partner yeast cells through the fusion process. Before formation of the actin fusion focus, the glucanases and glucan synthases are likely co-distributed, leading to a balance of activities (green cell wall) driving polarized cell expansion. Upon formation of the fusion focus, focalization of glucanases favors cell wall hydrolysis in the center of the contact zone. Turgor pressure applies force that is resisted by the stiff cell wall (grey arrows). This force drives cell expansion at zones of cell wall remodelling. This same force likely becomes effective (darker grey arrows) to promote plasma membrane juxtaposition upon local cell wall disassembly. Unidentified fusogens (pink) may also be required for plasma membrane merging.

duction [24]. Cell fusion occurs upon pairing of M (minus) and P (plus) haploid yeast cells, which express distinct pheromone and cognate GPCR receptors [45]. In these cells, the fusion focus forms 1–2 h before cell fusion and disassembles shortly after plasma membrane merging. This structure depends on a dedicated formin-family protein, Fus1, which is expressed specifically during this life stage [56], nucleates linear actin filaments [65], and forms an intense dot at the fusion site [24,55]. The fusion focus also depends on the two fission yeast type V myosin motors, both of which also accumulate at this same focal point [20,24]. The live-cell super-resolution imaging of F-actin, localization of the F-actin nucleator that associates with filament barbed ends [65] and of motor proteins that move towards actin filaments barbed ends, all indicate that the fusion focus in yeast forms an aster-like structure [24]: actin filaments originate from a spatially restricted zone close to the plasma membrane upon Fus1-dependent nucleation. By moving on these actin tracks, myosin motors accumulate where the filaments originate from, transporting cargoes to this location, and also contributing to the focalization of the structure (Fig. 1A).

Besides formin and type V myosins, several other actin-binding proteins (ABPs) associate with the fusion focus. These include profilin (Cdc3), tropomyosin (Cdc8), calmodulin (Cam2) and coronin (Crn1) [20,30,38,55]. Work in other biological contexts and in vitro suggests that the localization of several of these ABPs may be intimately linked to formin and myosin V functions. For instance, tropomyosins were shown to promote the filament elongation activity of several mammalian and yeast formins [71,74], to specifically decorate linear actin filaments and do so in an isoform-specific manner [32,70], and to promote the processivity of type V myosin motors [10,11,29]. Similarly, the small G-actin-binding protein profilin promotes formin-dependent actin assembly, by competing with Arp2/3-dependent assembly for limited G-actin [61,72]. Competition with Arp2/3-dependent nucleation activity, which leads to formation of branched actin networks, may be particularly important. Indeed, the formin-dependent actin focus is overlaid by 'actin patches' [24,54], well-described Arp2/3-dependent structures that mark sites of endocytosis [33]. Thus, clear delineation from Arp2/3 activity may be particularly important for the formation of the formin-dependent fusion focus because of their overlapping distributions.

One major function of the fusion focus in yeast is to promote local cell wall degradation (Fig. 1). Indeed, yeast cells are encased in a rigid cell wall that protects the cell against lysis. The cell wall is composed primarily of glucan polymers [14]. For cell morphogenesis during polarized growth, the cell wall has to be locally remodelled, and the growth zone geometry can be predicted from the zone of the plasma membrane where exocytosis occurs [1]. Exocytic vesicles carry transmembrane glucan synthases [5,12,13,50], which promote the formation of α and β bonds linking glucan subunits into polymers. However, it is noteworthy that the spatial distribution of glucan synthases is not a direct predictor of cell morphology [1]. Exocytic vesicles also carry glucanases, which are secreted proteins that promote the hydrolysis of the bonds linking glucan subunits into polymers [15,44,63]. Thus, it is likely that a balance between hydrolysis and re-synthesis of glucan cross-links permits local cell wall expansion for growth. For cell fusion, the walls of the partner cells have to be locally digested to allow plasma membrane contact. The glucanases, which are essential for this process, are targeted at the fusion focus, where they are likely secreted, whereas the glucan synthases remain broadly distributed along the zone of cell-cell contact [24]. This differential distribution suggests that hydrolysis and synthesis reactions are now unbalanced, with hydrolysis preferentially occurring in the center of the zone, thus driving local cell wall disassembly (Fig. 1B). In summary, actin filaments, formin and myosin V promote the local secretion of vesicles containing glucanases that degrade the cell wall at the contact site between mating gametes. Vesicle delivery may also contribute to local membrane expansion to help bring plasma membranes into close contact.

3. Myoblasts

A dense F-actin structure, also known as the actin focus, underlies the fusion of *Drosophila* myoblast cells. Myotubes are generated through an asymmetric fusion process between two distinct cell types: a fusion-competent myoblast (FCM) and a founder cell (FC). Upon cell fusion, the FCM cell fate is lost and the incoming FCM nucleus acquires FC/myotube identity [35]. New rounds of fusion now take place between additional FCMs and the now nascent myotube. These two cell types express distinct surface adhesion molecules essential for cell-cell recognition and fusion. Several adhesion molecules are involved: in the FCM, the protein Dumbfounded (Duf) plays the major role; in the FC/myotube, the protein Sticks and Stones (Sns) is the critical one. These surface molecules