Developmental Cell
Article

A Critical Function for the Actin Cytoskeleton in Targeted Exocytosis of Prefusion Vesicles during Myoblast Fusion

Sangjoon Kim,[1,2] Khurts Shilagardi,[1,2] Shiliang Zhang,[1,2] Sabrina N. Hong,[1] Kristin L. Sens,[1] Jinyan Bo,[1] Guillermo A. Gonzalez,[1] and Elizabeth H. Chen[1,*]
[1] Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
[2] These authors contributed equally to this work.
*Correspondence: echen@jhmi.edu
DOI 10.1016/j.devcel.2007.02.019

SUMMARY

Myoblast fusion is an essential step during muscle differentiation. Previous studies in *Drosophila* have revealed a signaling pathway that relays the fusion signal from the plasma membrane to the actin cytoskeleton. However, the function for the actin cytoskeleton in myoblast fusion remains unclear. Here we describe the characterization of *solitary* (*sltr*), a component of the myoblast fusion signaling cascade. *sltr* encodes the *Drosophila* ortholog of the mammalian WASP-interacting protein. Sltr is recruited to sites of fusion by the fusion-competent cell-specific receptor Sns and acts as a positive regulator for actin polymerization at these sites. Electron microscopy analysis suggests that formation of F-actin-enriched foci at sites of fusion is involved in the proper targeting and coating of prefusion vesicles. These studies reveal a surprising cell-type specificity of Sltr-mediated actin polymerization in myoblast fusion, and demonstrate that targeted exocytosis of prefusion vesicles is a critical step prior to plasma membrane fusion.

INTRODUCTION

Cell-cell fusion is critical to the development and physiology of multicellular organisms, and is involved in a variety of biological processes such as fertilization, myogenesis, placenta development, bone remodeling, immune response, tumor metastasis, and aspects of stem cell-mediated tissue regeneration (reviewed by Chen and Olson, 2005). Thus, a mechanistic understanding of this process is not only important for fundamental biology but may also provide a basis for its manipulation in therapeutic settings.

Compared to our understanding of intracellular organelle fusion and virus-cell fusion, much less is known about the underlying mechanisms of cell-cell fusion. Recent studies in the genetically amenable fruit fly *Drosophila* have begun to provide significant insights into this process (reviewed by Abmayr et al., 2003; Chen and Olson, 2004). In *Drosophila* embryos, development of the segmentally repeated somatic muscles requires fusion between muscle founder cells and fusion-competent myoblasts. While different subsets of founder cells express different selector genes, all fusion-competent cells are specified by a single transcription factor, Lame duck (Lmd)/Myoblast incompetent (Duan et al., 2001; Ruiz-Gomez et al., 2002). During myoblast fusion, muscle founder cells attract the surrounding fusion-competent cells, which recognize, attach, and fuse with founder cells to form multinucleated syncytia. Following syncytia formation, the nucleus of the fusion-competent cell adopts the same transcriptional profile as that of the founder cell with which it has fused. Myoblast fusion takes place in two phases, with the first phase yielding bi- or trinucleated muscle precursors, followed by a second phase of additional rounds of fusion that give rise to muscle fibers with distinct sizes (Rau et al., 2001). At the ultrastructural level, myoblast fusion involves several characteristic steps (Doberstein et al., 1997). Upon cell adhesion, paired vesicles with an electron-dense margin form along the juxtaposed plasma membranes between founder and fusion-competent cells. These vesicles then presumably resolve into elongated electron-dense plaques along the apposing membranes, followed by the formation of membrane discontinuity (fusion pores), which ultimately leads to the complete fusion of the two cells. The importance of these intermediate structures (vesicles, plaques, and pores) in myoblast fusion is highlighted by the observation that various mutants arrest fusion at different steps along this path (Doberstein et al., 1997).

Further insights into the molecular mechanisms of myoblast fusion came from the elucidation of a signaling cascade from transmembrane receptors to the actin cytoskeleton (reviewed by Abmayr et al., 2003; Chen and Olson, 2004). In founder cells, two immunoglobulin (Ig) domain-containing transmembrane receptors, Dumbfounded (Duf)/Kirre and Roughest (Rst)/IrreC, are expressed and play redundant roles during myoblast fusion (Ruiz-Gomez et al., 2000; Strunkelnberg et al., 2001). Two parallel pathways mediate signal transduction from the fusion receptors to the cytoskeleton. First, a founder cell-specific

adaptor protein Antisocial (Ants)/Rols7 physically links Duf and the cytoskeleton-associated protein Myoblast city (Mbc) (Chen and Olson, 2001; Menon and Chia, 2001; Rau et al., 2001). Mbc, as its human ortholog DOCK180, likely functions as a guanine nucleotide exchange factor (GEF) for the small GTPase Rac (Brugnera et al., 2002; Erickson et al., 1997). Consistent with this, *Drosophila* Rac is essential for myoblast fusion (Hakeda-Suzuki et al., 2002). A second pathway downstream of the Duf receptor involves Loner, a different GEF, and its target, the small GTPase Arf6 (Chen et al., 2003). The Loner-Arf6 module is required for the proper localization of Rac, thus converging into the Ants → Mbc → Rac pathway at the level of Rac (Chen et al., 2003). Signal transduction in fusion-competent cells is relatively less characterized. Two fusion-competent cell-specific receptors, Sticks and stones (Sns) and Hibris (Hbs), have been identified (Artero et al., 2001; Bour et al., 2000; Dworak et al., 2001). However, intracellular proteins that mediate signal transduction from membrane receptors to the actin cytoskeleton in fusion-competent cells have yet to be uncovered.

The Wiskott-Aldrich syndrome protein (WASP) family of proteins, including WASP, neural-WASP (N-WASP), and WASP family verprolin homologs (WAVEs) 1–3, are important regulators of the actin cytoskeleton (reviewed by Miki and Takenawa, 2003). In mammalian cells, binding of the small GTPase Cdc42 activates WASPs by releasing them from an autoinhibitory conformation. WAVEs, on the other hand, are activated by Rac, another Rho family small GTPase. Both WASPs and WAVEs feed into the Arp2/3 complex, a direct regulator of actin polymerization (reviewed by Stradal and Scita, 2006). Previous studies in *Drosophila* have implicated the Mbc → Rac → WAVE pathway in myoblast fusion, as mutations in *mbc*, *rac*, or the WAVE-associated protein Kette result in myoblast fusion defects (Hakeda-Suzuki et al., 2002; Rushton et al., 1995; Schroter et al., 2004). However, the precise role of the actin cytoskeleton in myoblast fusion remains mysterious. Specifically, how could organization of the intracellular actin cytoskeleton affect plasma membrane dynamics and fusion?

In this paper, we describe the characterization of a component of the myoblast fusion signaling cascade. This component, which we named Solitary (Sltr), is the *Drosophila* ortholog of the human WASP-interacting protein (WIP). We show that Sltr is a fusion-competent cell-specific protein that is recruited to sites of fusion by the transmembrane receptor Sns. Sltr is a positive regulator of actin polymerization and is required for F-actin accumulation at sites of fusion. Electron microscopy analysis suggests that the actin cytoskeleton is involved in targeted exocytosis of prefusion vesicles at sites of fusion, thus revealing a critical step prior to plasma membrane merger.

RESULTS

Myoblast Fusion Defect in *sltr* Mutant

The *solitary* (*sltr*) allele *S1946* was isolated in a genetic screen for fusion-defective mutants. Homozygous *sltr* mutant embryos contain many mononucleated myosin heavy chain (MHC)-positive myocytes (Figures 1A and 1B). This lack-of-fusion phenotype is further confirmed by electron microscopy, which reveals myoblast clusters containing single founder cells surrounded by multiple unfused competent cells (see Figure S1 in the Supplemental Data available with this article online). Dmef2, which marks the nuclei of all somatic muscle cells (Lilly et al., 1994; Nguyen et al., 1994), is similarly expressed in wild-type and *sltr* embryos, suggesting that muscle cell fate is properly specified in *sltr* embryos (Figures 1H and 1I). Thus, the fusion phenotype of *sltr* embryos does not result from a secondary consequence of defective muscle cell fate determination. In late-stage embryos, unfused myoblasts are seen attached to elongated muscle precursors (Figures 1D and 1E), suggesting that the *sltr* mutation does not affect the recognition or adhesion between founder and fusion-competent cells, but blocks a later step in myoblast fusion.

Myoblast fusion takes place in two phases, with the first phase yielding bi- or trinucleated muscle precursors, followed by a second phase of additional rounds of fusion that give rise to muscle fibers with distinct sizes (Rau et al., 2001). To determine which phase of fusion is defective in *sltr* embryos, we examined the expression of two transcription factors, Krüppel (Kr) and Even skipped (Eve), which mark different subsets of muscle founder cells (Carmena et al., 1998; Ruiz-Gomez and Bate, 1997). In wild-type embryos, Kr staining is initially detected in bi- or trinucleated muscle precursors at stage 13 (Figure 1J), evolves into multinucleated clusters at stage 14 (Figure 1L), and is absent from mature muscle fibers (Ruiz-Gomez and Bate, 1997). The characteristic pattern of Kr expression remains the same in *sltr* mutant embryos, except that each Kr-positive "cluster" is mononucleated at stage 13 (Figure 1K) but contains one to three nuclei at stage 14 (Figure 1M). These results suggest that the fate of Kr-positive founder cells is properly specified at stage 13, although these cells complete the first phase of fusion to form bi- or trinucleated syncytia at a later stage compared to wild-type. Similar results were obtained with Eve-positive founder cells. While stage 14 Eve-positive dorsal acute muscle 1 (DA1) contains approximately ten nuclei in each cluster (Figure 1F), its counterpart in *sltr* mutant embryos is mostly binucleated (Figure 1G). We conclude that *sltr* is required for the second phase of fusion to form multinucleated syncytia.

Due to its low resolution, direct visualization of myoblasts with MHC antibody (Figures 1B–1E) cannot unambiguously exclude the possibility that small fusion pores might have formed between the founder cells and the surrounding fusion-competent cells in *sltr* mutant embryos. We devised a green fluorescent protein (GFP) diffusion assay to directly test this possibility. When GFP was expressed in *sltr* mutant embryos via the founder cell-specific *rP298-GAL4* driver, we found that the GFP signal was completely retained within the Kr-positive founder cells (Figures 1N–1N″). Given the small size of GFP (a β-barrel structure with a diameter of 3.2 nm and a length