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The Arf-GEF Schizo/Loner regulates N-cadherin to induce fusion competence of *Drosophila* myoblasts

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ABSTRACT

Myoblast fusion is a key process in multinucleated muscle formation. Prior to fusion, myoblasts recognize and adhere to each other with the aid of cell-adhesion proteins integrated into the membrane. Their intracellular domains participate in signal transduction by binding to cytoplasmic proteins. Here we identified the calcium-dependent cell-adhesion protein N-cadherin as the binding partner of the guanine-nucleotide exchange factor Schizo/Loner in *Drosophila melanogaster*. N-cadherin was expressed in founder cells and fusion-competent myoblasts of *Drosophila* during the first fusion phase. Our genetic analyses demonstrated that the myoblast fusion defect of *schizo/loner* mutants is rescued in part by the loss-of-function mutation of *N-cadherin*, which suggests that Schizo/Loner is a negative regulator of N-cadherin. Based on our findings, we propose a model where N-cadherin must be removed from the myoblast membrane to induce a protein-free zone at the cell–cell contact point to permit fusion.

Introduction

Cell–cell fusion is an important type of membrane fusion that occurs during development, e.g., fertilization, myogenesis and osteoclast formation (Chen et al., 2007; Oren-Suissa and Podbilewicz, 2007, 2010). Similar to intracellular vesicle fusion, the separate lipid bilayers of the two plasma membranes must merge and unite. Fusion of most biological membranes is preceded by intermediate stages of fusion: hemifusion, stalk and fusion pore formation (reviewed by Chernomordik and Kozlov, 2008; Marsden et al., 2011).

But how are the two membranes brought into proximity so that fusion can occur? Diverse transmembrane proteins essential for cell–cell fusion might fulfil this function. Some of these also possess fusogenic characteristics, meaning that they are expressed in fusing cells and that their ectopic expression can induce fusion of cells that normally do not undergo fusion (see Oren-Suissa and Podbilewicz, 2007). Biological membranes are normally packed with membrane proteins. However, it is believed that after the initial contact, the fusion zone becomes protein-free and the transmembrane proteins that initiated the contact move radially away (reviewed by Martens and McMahon, 2008).

During embryonic skeletal muscle development, mononucleated myoblasts fuse to form multinucleated muscle fibres. The fusion of myoblasts is also essential during postnatal development for muscle growth and repair. During this process, muscle stem cells, so-called satellite cells, on the muscle fibres become activated, differentiate and fuse. Similar to skeletal muscles, *Drosophila melanogaster* muscles arise through the fusion of mononucleated myoblasts. Many genes required for myoblast fusion in *Drosophila* have been identified (reviewed by Chen and Olson, 2004; Önel and Renkawitz-Pohl, 2009; Gildor et al., 2010; Haralalka and Abmayr, 2010; Rochlin et al., 2010; Önel et al., 2011). Recent studies indicate that some aspects of the molecular mechanism of fusion are conserved between *Drosophila* and vertebrates (Pajcini et al., 2008; Srinivas et al., 2007; Nowak et al., 2009; Sohn et al., 2009; Vasyutina et al., 2009).

Drosophila myoblast fusion depends on two distinct myoblast types: founder cells (FCs), which determine muscle identity, and fusion-competent myoblasts (FCMs). Specific transmembrane proteins of the immunoglobulin (Ig) superfamily mediate the recognition and adhesion of both myoblast types. The Ig-domain proteins Dumbfounded (Duf; also known as Kirre) and its paralog Roughest (Rst; also known as IrreC) function redundantly in FCs (Ruiz-Gomez et al., 2000; Strünkelnberg et al., 2001). Duf heterotypically binds the Ig-domain protein Sticks and Stones (Sns) expressed on FCMs (Bour et al., 2000). In the absence of Sns, fusion of many FCMs is severely disrupted. However, in some muscle groups, the Sns paralog Hibris (Hbs; Artero et al., 2001;

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Dworak et al., 2001) can partially compensate for the loss of Sns (Shelton et al., 2009).

The Ig-domain proteins form a ring-like structure at cell–cell contact points (Kesper et al., 2007; Sens et al., 2010; reviewed by Rochlin et al., 2010 and Önel et al., 2011). The diameter of the ring structure ranges between 1 and 5 μm, with 5 μm being the diameter of a myoblast (Kesper et al., 2007). The Ig-domain proteins serve as a platform for the assembly of a transient cell-adhesion complex, called Fusion-Restricted Myogenic-Adhesive Structure (FuRMAS). The FuRMAS shares structural features with other transient adhesion structures, such as podosomes, invadopodia and the immunological synapse (Kesper et al., 2007; Önel and Renkawitz-Pohl, 2009; Sens et al., 2010; Haralalka et al., 2011). A prominent feature of the FuRMAS is its F-actin-rich core that depends on Arp2/3-based actin polymerization (Kesper et al., 2007; Kim et al., 2007; Massarwa et al., 2007; Richardson et al., 2007; Schäfer et al., 2007; Berger et al., 2008; Gildor et al., 2009).

The cadherin family has been implicated in adhesion and fusion of mammalian myoblasts (reviewed by Krauss, 2010). Neuronal (N-) cadherin (Duband et al., 1987; Hatta et al., 1987; Fredette et al., 1993) and muscle (M-) cadherin (Donalies et al., 1991; Moore and Walsh, 1993; Irintchev et al., 1994) are expressed in developing and regenerating muscles. However, fusion can still occur in myoblasts mutant for N- or M-cadherin (Charlton et al., 1997; Hollnagel et al., 2002). These observations have led to the proposal that N-cadherin and M-cadherin are redundant. The *Drosophila* genome encodes three classical cadherins, referred to as epithelial (E-) cadherin (Shotgun), N-cadherin (CadN) and N-cadherin2 (CadN2) (Hill et al., 2001), but no M-cadherin. The function of these three cadherins in fusion of myoblasts has not been assessed.

In the present study, we identified *Drosophila* N-cadherin as an interaction partner of the guanine-nucleotide exchange factor (GEF) Schizo/Loner by using a yeast two-hybrid screen. We showed that *schizo*/*loner* and *N-cadherin* interact genetically. Surprisingly, the major fusion deficit observed in *schizo*/*loner* mutant embryos was in part rescued by the loss of *N-cadherin*. A similar rescue of *schizo*/*loner* was observed in embryos that express the activated form of the d-Arf1-GTPase. Based on our previous findings that Schizo/Loner serves as a regulator via Dynamin-dependent endocytosis during commissure formation (Önel et al., 2004), we blocked endocytosis by expressing dominant-negative Dynamin in myoblasts, but we failed to generate a *schizo*/*loner*-like phenotype. Thus, we propose that N-cadherin is regulated in a Schizo/Loner- and d-Arf1-dependent manner that does not require Dynamin.

Results

Identification of N-cadherin as a Schizo/Loner interaction partner

To investigate the function of Schizo/Loner during myoblast fusion, we used the *schizo* (*siz*) alleles siz^{C1-28} and siz^{U112} identified

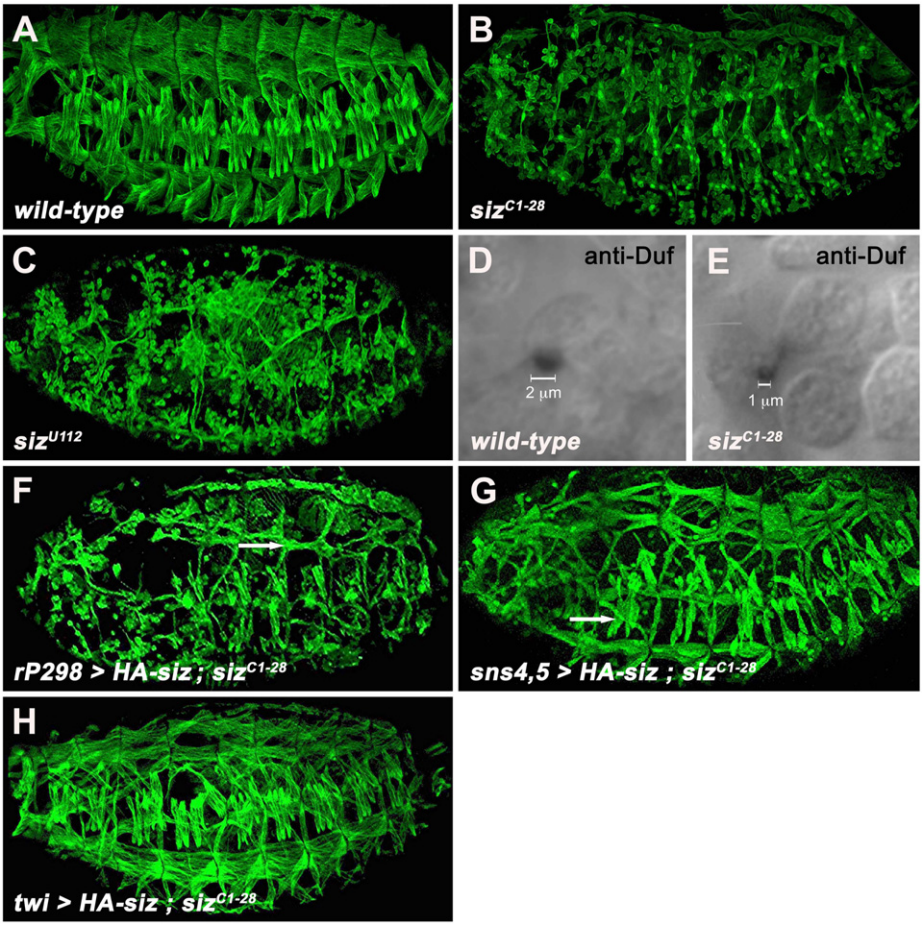

Fig. 1. Schizo/Loner function is required in both FCs and FCMs. (A–C and F–H) Lateral view of stage 15/16 embryos stained with anti-β3-Tubulin to show unfused myoblasts and multinucleated muscles. (A) Wild-type embryo. (B) Homozygous stage 15 siz^{C1-28} and (C) stage 16 siz^{U112} mutant embryos showing a strong myoblast fusion defect. (D) Localization of the cell-adhesion protein Duf at cell–cell contact points in wild-type embryos and (E) siz^{C1-28} mutant embryos. (F) Partial rescue of the siz^{C1-28} mutant muscle phenotype by specific expression of *schizo* in FCs and (G) in growing myotubes/FCMs. (H) Full rescue of the siz^{C1-28} mutant phenotype with *schizo* transcription driven by *twist*-GAL4.