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N-ethylmaleimide sensitive factor is required for fusion of the *C. elegans* uterine anchor cell

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Abstract

The fusion of the *Caenorhabditis elegans* uterine anchor cell (AC) with the uterine-seam cell (utse) is an excellent model system for studying cell–cell fusion, which is essential to animal development. We obtained an egg-laying defective (Egl) mutant in which the AC fails to fuse with the utse. This defect is highly specific: other aspects of utse development and other cell fusions appear to occur normally. We find that defect is due to a missense mutation in the *nsf-1* gene, which encodes *N*-ethylmaleimide-sensitive factor (NSF), an intracellular membrane fusion factor. There are two NSF-1 isoforms, which are expressed in distinct tissues through two separate promoters. NSF-1L is expressed in the uterus, including the AC. We find that *nsf-1* is required cell-autonomously in the AC for its fusion with the utse. Our results establish AC fusion as a paradigm for studying cell fusion at single cell resolution and demonstrate that the NSF ATPase is a key player in this process.
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Introduction

The enclosure of cells' contents by phospholipid membranes is central to life, and eukaryotic cells also contain internal organelles that are bounded by membranes. Intracellular vesicular transport establishes these phospholipid boundaries and requires the fission and fusion of membranes. The well-known proteins required for vesicular fusion include NSF (*N*-ethylmaleimide-sensitive factor), SNAPs (soluble NSF attachment proteins), and SNARE (SNAP receptor) complexes. SNAREs are membrane proteins that are specific to either the vesicular or target membrane and assemble with one another during membrane fusion (Bennett, 1995; Clary et al., 1990; Malhotra et al., 1988). NSF is broadly required for intracellular membrane fusion and functions by binding to SNARE complexes through SNAPs (Clary et al., 1990; reviewed in Whiteheart and Matveeva, 2004). This leads to the disassembly of the SNARE complexes, permitting the reutilization of individual SNAREs (Sollner et al., 1993).

Although many different SNAPs and SNAREs have been identified, some with tissue-specific expression, each organism contains only one or two *NSF* genes. In general, NSF appears to be particularly abundant in the nervous system. *Drosophila* contains two paralogous *NSF* genes, which have some redundant functions. In addition, one of these is specific to the nervous system at the adult stage (*dNSF1*), while the other functions in the mesoderm in larvae (*dNSF2*), one of whose fates is syncytial muscle (Golby et al., 2001; Pallanck et al., 1995). In addition, mouse seems to make two *NSF* transcripts from a single *NSF* gene, probably through alternative splicing (Puschel et al., 1994). The transcripts are largely found in the nervous system.

Recently, NSF has also been reported to associate with non-SNARE receptors and influence their transport. Examples include the GluR2 subunits of the AMPA receptors (AMPARs) (Hanley et al., 2002; Huang et al., 2005; Lee et al., 2002) and β2-adrenergic receptors whose recycling might also involve another interaction of NSF with β-arrestins 1 and 2 (Cong et al., 2001; reviewed in Shenoy and Lefkowitz, 2003). In addition,

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NSF also binds a number of intracellular constituents including small GTP-binding proteins of the Rab family. In at least some cases described above, NSF may promote disassembly of complexes, such as AMPAR-PICK1 (protein interacting with C-kinase-1) (Hanley et al., 2002) and γ-SNAP-Gaf-1/Rip11 (Tani et al., 2003; reviewed in Whiteheart and Matveeva, 2004), similar to its role with SNAREs.

Just as transporting vesicles fuse, the plasma membranes that separate cells can also fuse (Fig. 1A). Cell–cell fusion is critical to animal development, including sperm–egg fusion during fertilization and cell–cell fusion during formation of placenta, muscles, and bones (reviewed in Shemer and Podbilewicz, 2000). In addition, it has been shown that stem cell and tumor cell fusion is important for stem cell transdifferentiation and cancer progression, respectively (Camargo et al., 2003; reviewed in Chen and Olson, 2005; reviewed in Duelli and Lazebnik, 2003). Intensive research has revealed several fusogenic proteins used by enveloped viruses for entry into host cells as well as their mechanisms of function (reviewed in Hernandez et al., 1996). Much less is known about the molecular mechanisms of cell–cell fusion in multicellular organisms. In general, viral fusogenic proteins each contain a hydrophobic stretch that can associate with target membranes. However, there are no consensus sequences shared among virus families (reviewed in Hernandez et al., 1996).

To date, several candidates for cell–cell fusion proteins in multicellular organisms, such as *Caenorhabditis elegans*, *Drosophila*, mouse, and human, have been proposed (reviewed in Potgens et al., 2002; Schultz and Williams, 2005 and Stein et al., 2004). Izumo (Inoue et al., 2005), tetraspanin protein family members, such as CD9 (Hemler, 2003), and ADAM (a disintegrin and metalloprotease) protein family members including fertilins and cyritestin have been implicated in sperm–egg fusion. Izumo and CD9 have been shown to be necessary for the fusion event, while ADAMs are suggested to be involved in sperm–egg binding rather than in their fusion. Several immunoglobulin superfamily members, such as Duf, Rst, Sns, and Hbs, have been proposed to be essential adhesion molecules for myoblast fusion, an essential aspect of muscle formation (reviewed in Chen and Olson, 2004, 2005; Taylor, 2000). In addition, human endogenous retrovirus envelope proteins, Syncytins have been shown to be fusogenic and involved in trophoblast fusion (Blaise et al., 2003; Dupressoir et al., 2005; Mi et al., 2000; reviewed in Potgens et al., 2002). All of the above observations strongly imply that a diversity of proteins are involved in cell–cell fusion and suggest that the full range of fusogenic proteins and/or fusion mechanisms may not yet be known.

The *eff-1* gene in *C. elegans* has recently been found to be required for epithelial cell fusion (Mohler et al., 2002). EFF-1 is an integral membrane protein that, like viral fusion proteins, contains a short hydrophobic sequence (Mohler et al., 2002). Ectopic expression of EFF-1 causes cells that would not normally fuse to do so (del Campo et al., 2005; Shemer et al., 2004), suggesting that this protein is sufficient to induce cell fusion. While *eff-1* is required broadly for *C. elegans* cell fusions, it appears not to function in every fusion-fated tissue. For instance, sperm–egg fusion does not appear to require EFF-1 (del Campo et al., 2005). In addition, *eff-1* mutants do not show defects in the process of AC/utse fusion discussed below.

The reproductive system of the *C. elegans* hermaphrodite is comprised of a number of tissues whose development is coordinated through cell–cell interaction. The uterine anchor cell (AC) plays a central role in the development of this system. The AC/VU (Ventral Uterine precursor cell) decision, which is completed through LIN-12/Notch and LAG-2/Delta lateral signaling, ensures that only a single cell in each hermaphrodite uterus becomes an AC (Greenwald et al., 1983; Kimble, 1981; Seydoux and Greenwald, 1989; Wilkinson et al., 1994). Subsequently, the AC initiates vulval development by inducing

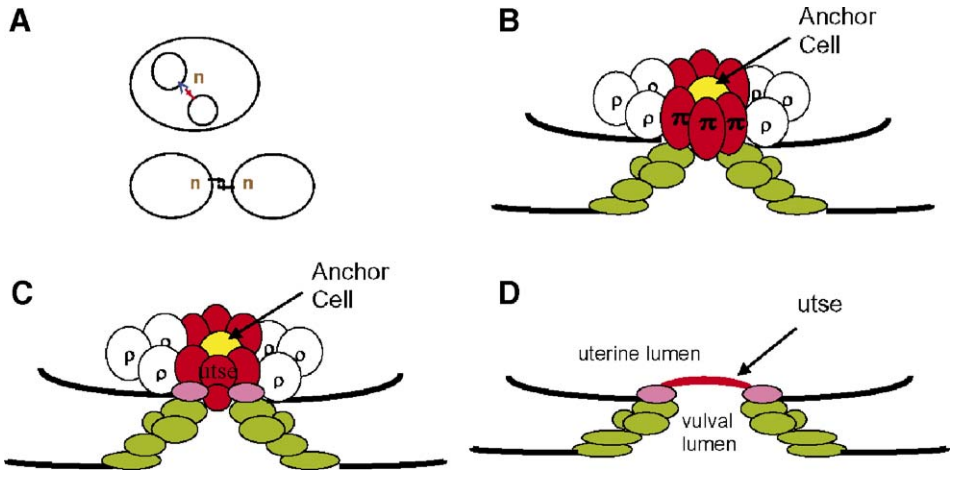

Fig. 1. Membrane fusion and uterine–vulval development. (A) Intra- (upper) vs. inter- (lower) cellular membrane fusion. The ovals and the circles indicate cells and vesicles, respectively. red t: t-SNARE, blue v: v-SNARE, brown n: NSF, green f: cell fusogens. Cell fusogens functioning in AC/utse fusion are currently unknown. Following vesicle-to-plasma membrane or vesicle-to-vesicle contact, the cytoplasmic localization of NSF enables it to disassemble SNAREs. In cell–cell fusion, NSF would need to be outside the cell in order to disassemble fusogens. (B–D) uterine–vulval development during the L3 to L4 stage. Modified from Newman et al. (2000). (B) The AC induces the adjacent six VU granddaughters to adopt π cell fates (red) and others become ρ cells (white). (C, D) π cells divide once to produce 12 π cell daughters. Eight of them (red) fuse together to form the utse to which the AC fuses and the rest become uv1 cells (pink).

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