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A Fringe-modified Notch signal affects specification of mesoderm and endoderm in the sea urchin embryo

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Abstract

Fringe proteins are *O*-fucose-specific β-1,3 *N*-acetylglucosaminyltransferases that glycosylate the extracellular EGF repeats of Notch and enable Notch to be activated by the ligand Delta. In the sea urchin, signaling between Delta and Notch is known to be necessary for specification of secondary mesenchyme cells (SMCs). The *Lytechinus variegatus* Fringe homologue is expressed in both the signaling and receiving cells during this first Delta–Notch signal. Perturbation of Fringe expression through morpholino antisense oligonucleotide (MO) injection results in fewer SMCs but also causes decreased and delayed archenteron invagination. Partial endoderm specification occurs but expression of some endoderm genes is compromised. The data are consistent with a Fringe-requiring Notch signal as one upstream component of archenteron morphogenesis. Finally, *Fringe* perturbations result in more severe phenotypes than those previously reported for Notch dominant-negative (LvN^{neg}) injections or reported here for Notch MO (NMO) injections. Injecting a combination of LvN^{neg} and NMO results in a more severe phenotype than either treatment alone, and this combination phenocopies the *fringe* MO embryos. Taken together, the results show that Fringe is necessary both for maternal and zygotic Notch signals, and these Notch signals affect specification of mesoderm and endoderm.

Keywords: Sea urchin; Fringe; Notch; Specification; Gastrulation; Morphogenesis; Morpholino antisense oligonucleotide

Introduction

The Notch signaling pathway is important for the processes of cell specification and boundary formation throughout development in many organisms. Originally, identified and characterized in the wing disc of *Drosophila melanogaster*, Notch signaling has since been identified as essential for many aspects of development including axis formation, germ layer specification, organogenesis, and cellular differentiation (Artavanis-Tsakonas et al., 1990; Fehon et al., 1991; Jacobsen et al., 1998; Mello et al., 1994; Rones et al., 2000; Sherwood and McClay, 1999). The Notch receptor is activated through interaction with a Delta, Serrate, or Lag-2 type ligand (Fehon et al., 1990; Rebay et al., 1991), and this interaction leads to the cytoplasmic release of the Notch intracellular domain which translocates to the nucleus and acts as a transcription cofactor (Baron, 2003; Iso et al., 2003; Lai, 2002; Schroeter et al., 1998).

Notch plays an early and central role in endomesoderm specification in the sea urchin (Sherwood and McClay, 1999, 2001). Vegetal blastomeres initiate endomesoderm specification at about 4th cleavage {Logan, 1999 #2157} {Oliveri, 2003 #3066}. Delta is expressed by micromeres at the vegetal pole shortly after the 6th cleavage. This expression is coincident with the experimentally timed reception of the Notch-activating ligand by veg2 cells, located just above the micromeres (Fig. 1; McClay et al., 2000; Oliveri et al., 2002; Sweet et al., 1999; Sweet et al., 2002). The first Delta–Notch signal results in specification of pigment cells and blastocoelar cells (Sweet et al., 2002). Delta is expressed a second time, this time in the veg2 tier of cells, and this later expression results in specification of

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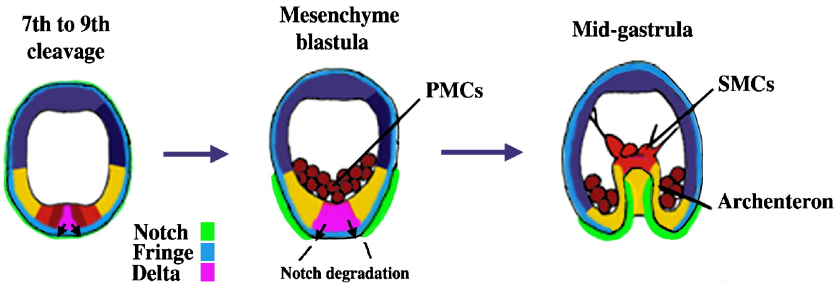

Fig. 1. Overview of sea urchin morphogenesis and the role of Notch signaling. This panel represents development of the sea urchin embryo from the blastula stage through the midgastrula stage. Cells with an ectoderm fate are represented in blue, endoderm in yellow, and mesoderm in red. Maternal Notch (green) surrounds the blastula on the apical surface of all cells. Notch is lost from the vegetal plate after being activated there by Delta from the micromeres (arrows in blastula). The SMCs then express Delta in a broader pattern at the vegetal plate and presumably expand the cleared SMC region (arrows at mesenchyme blastula). At mesenchyme blastula, Notch is up-regulated on the apical surface of endoderm. Fringe is expressed by all cells of the embryo at the blastula stage, then it is lost first from the ingressed PMCs, and later Fringe is lost from the SMCs and finally from the endoderm as it invaginates.

more blastocoelar cells and pharyngeal muscle. Presumably, this is a consequence of a second Delta–Notch signal between veg2 progeny (Sweet et al., 2002). Delta continues to be present in veg2 progeny until the mesenchyme blastula stage, where it has the potential to signal across the SMC/endoderm boundary (Oliveri et al., 2002; Sherwood and McClay, 2001; Sweet et al., 2002). Support for a possible Delta–Notch signal across the SMC/endoderm boundary currently is largely circumstantial. Antibody staining shows Delta on the SMC side of the boundary and Notch on the endoderm side (Sherwood and McClay, 1997; Sweet et al., 2002). Injection of N^{act} into one animal blastomere at the eight-cell stage results in an ectopic gut or increased contribution to the existing gut, suggesting that added Notch signaling augments endoderm (Sherwood and McClay, 2001). Additionally, perturbation analyses showed that animal caps from embryos injected with N^{act} develop into embryoids with differentiated endoderm, while control animal caps gave rise only to unspecified Dauer blastulae (Sweet et al., 2002). While the underlying signaling and its consequences are not understood in detail relative to the endomesoderm gene regulatory network (Davidson et al., 2002), it is clear that Notch signaling somehow impinges on endoderm specification.

From the above, it appears as though Notch signaling during sea urchin cleavage occurs in a cascade of activation events, beginning with the micromeres and continuing cell-by-cell through the mesoderm, and perhaps into the endoderm (Sherwood and McClay, 1999, 2001; Sweet et al., 2002). The challenge is to parse out these sequential Notch signaling events to determine the function of each. One approach to meet this challenge is to examine modifiers of Notch signaling that are known to regulate Notch activation both spatially and temporally. Fringe is one such modifier that glycosylates extracellular EGF-like repeats on Notch, functioning as an *O*-fucose specific β-1,3 *N*-acetylglucosaminyltransferase (Haltiwanger and Stanley, 2002; Moloney et al., 2000). Fringe glycosylation potentiates activation of Notch by Delta and blocks any Notch activation by Serrate (Fleming et al., 1997; Irvine and Wieschaus, 1994; Panin et al., 1997). Thus, Fringe contributes to the spatial and temporal patterns of Notch activation signals by determining where each ligand can activate Notch.

Here, *Lytechinus variegatus fringe* is identified and its function studied to better understand the timing and role of Notch activation in germ layer specification. Fringe is required for activation of Notch during SMC specification. Fringe is also required for a Notch signal that directly or indirectly activates archenteron invagination while not appearing to disrupt expression of some genes in the endoderm gene regulatory network. The evidence also suggests a delay in oral–aboral ectoderm specification, most likely due to a delayed reception of required vegetal signals.

Materials and methods

Fringe cloning and sequence comparison

Degenerate primers were designed against peptides derived from ClustalX alignments (Chenna et al., 2003) to amino acid sequences from various Fringe homologues. A PCR product was obtained using degenerate primers on reverse transcribed RNA from multiple timepoints of *L. variegatus* development. The product was cloned into pGEM-T and sequenced by the Duke University Comprehensive Cancer Center DNA Analysis Facility (http://www.cancer.duke.edu/dna/Sequencing).

Library screening and sequence analysis

A λ-ZAP-II *L. variegatus* midgastrula cDNA library was screened with the product of degenerate primer PCR. The probe was ^{32}P-dCTP labeled with Rediprimer II random prime labeling kit (Amersham-Pharmacia Biotech). Screens were performed as previously described (Gross et al., 2003). Positive clones were excised using ExAssist into pBS-II plasmids, which were transformed, purified, and sequenced.