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Dorsoventral patterning of the postembryonic mesoderm requires both LIN-12/Notch and TGF-beta signaling. **Marisa Foehr**, Jun Liu. Department of Molecular Biology & Genetics, Cornell University, Ithaca, NY.

The *C. elegans* post-embryonic mesodermal lineage arises from a single precursor cell, the M mesoblast, which will diversify to generate distinct dorsal and ventral cell types. The dorsal daughter of M gives rise to a subset of body wall muscles and two non-muscle coelomocytes, whereas the ventral daughter of M gives rise to two sex myoblasts in addition to a subset of body wall muscles. Mutations in the *C. elegans* Schnurri homolog *sma-9* cause ventralization of the M lineage. We have previously shown that SMA-9 antagonizes the Sma/Mab TGF-beta signaling pathway to promote dorsal M lineage fates (Foehr et al., 2006). Interestingly, loss-of-function mutations in the Notch receptor homolog *lin-12* cause dorsalization of the M lineage (Greenwald et al., 1983), an exact opposite phenotype of *sma-9* mutants. We have found that while LIN-12 protein is present in both the dorsal and ventral M lineage cells, the ligands for LIN-12, LAG-2 and APX-1, are asymmetrically localized in cells adjacent to ventral M-derived cells, and they function redundantly in promoting ventral M lineage fates. To investigate how LIN-12/Notch signaling interacts with SMA-9 and the Sma/Mab TGF-beta pathway in regulating M lineage patterning, we generated double and triple mutant combinations among *lin-12*, *sma-9* and *dbl-1* (the ligand for the Sma/Mab TGF-beta pathway) and examined their M lineage phenotypes. Our results suggest that the LIN-12/Notch pathway and the Sma/Mab TGF-beta pathway function independently in regulating dorsoventral patterning of the M lineage, with LIN-12/Notch required for ventral M lineage fates, and SMA-9 antagonism of TGF-beta signaling required for dorsal M lineage fates. Our work provides a model for how combined Notch and TGF-beta signaling regulates the developmental potential of two equipotent cells along the dorsoventral axis.

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OSM-11 activates LIN-12 Notch signaling during C. elegans vulval development. Hidetoshi Komatsu[1,2], Michael Y. Chao[1,2,3], Jonah M. Larkins-Ford[1], Heather M. Dionne[1], Tim Tucey[1], James Q. White[1], Khursheed Wani[1], Mike Boxem[1,4], **Anne Hart[1,2]**. 1) Ctr Cancer Research, Massachusetts General Hosp, Charlestown, MA; 2) Department of Pathology, Harvard Medical School; 3) Department of Biology, California State University San Bernardino; 4) Center for Cancer Systems Biology and Department of Cancer Biology, Dana-Farber Cancer Institute and Harvard Medical School, Department of Medicine.

A critical regulatory event in many developmental cell fate decisions is Notch receptor activation by conserved DSL domain ligands (i.e. Delta, Serrate and LAG-2). Here, we define a synergistic role in Notch receptor activation for the OSM-11 protein family. Originally identified by Jim Thomas and colleagues, *osm-11* and *osm-7* were predicted to encode soluble hypodermal proteins of unknown function whose loss perturbs response to high osmolarity, defecation and imparts dramatic resistance to osmotic stress. Here we present a developmental analysis detailing the role of OSM-11 in LIN-12 Notch receptor activation during *C. elegans* vulval development.

Complete loss of *osm-11* perturbs vulval development with changes in the expression of the secondary cell fate marker expressions *egl-17* and *lip-1*, as well as alterations in vulval cell fate specification. Genetic epistasis indicates that *osm-11* normally increases *lin-12* activity during vulval cell fate specification. *osm-11* acts synergistically with the *dsl-1*, which ecodes a *C. elegans* DSL domain Notch ligand, in these cell fate decisions.

OSM-11 is a secreted protein and lacks the DSL domain found in canonical Notch ligands. However, OSM-11 shares a conserved motif with *Drosophila* and vertebrate Notch ligands. Consistent with a direct role for OSM-11 in Notch receptor activation, we find that OSM-11 binds to the LIN-12 Notch receptor extracellular domain and that a vertebrate homolog of OSM-11, DLK1 (Deltalike 1), can functionally substitute for OSM-11 during vulval morphogenesis. Combined, these studies predict that OSM-11 and homologous *C. elegans* proteins act as co-activators with previously defined DSL-domain ligands to activate Notch receptors during vulval development.

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A New Class A SynMuv Mutation is a *lin-3(gf)* Allele That Causes Increased *lin-3* mRNA Levels. **Adam Saffer**, Bob Horvitz. HHMI, Dept. Biology, MIT, Cambridge, MA 02139 USA.

The expression of *C. elegans* vulval cell fates requires an RTK/Ras signaling pathway and is antagonized by the synthetic multivulva (synMuv) genes. synMuv genes can be grouped into two redundant classes: A and B. Animals with mutations in one class are non-Muv, whereas animals with mutations in both classes are Muv. Many class B proteins have homologs that act in chromatin remodeling and transcriptional repression. The class A synMuv genes might also function in transcription, as four of the five class A proteins contain zinc-finger or zinc-finger-like motifs, and the two class A proteins tested are nuclear.

In a screen for new class A synMuv alleles we isolated the mutation *n4441*. *n4441* is dominantly Muv in combination with class B synMuv mutations but not as a single mutant or in combination with class A synMuv mutations. Therefore, *n4441* causes a dominant class A synMuv phenotype. This observation could be explained if *n4441* were a mutation in a synMuv target gene that abolished class A synMuv-mediated repression. We determined that *n4441* is an allele of *lin-3*, which encodes an EGF ligand that controls vulval cell fates. Loss-of-function mutations in *lin-3* cause a vulvaless phenotype, whereas overexpression of *lin-3* causes a Muv phenotype. Cui *et al*. (Dev. Cell 10, 667-672, 2006) recently showed that the two classes of synMuv genes redundantly repress *lin-3* expression. Using real-time RT-PCR, we found that *lin-3(n4441)* , like other class A synMuv mutations, synthetically causes an increase in *lin-3* mRNA levels when combined with a class B synMuv mutation. *lin-3* has multiple transcription start sites several kb apart. Because there are multiple *lin-3* transcripts and the *n4441* mutation is closest to the start of the *lin-3a* transcript, we tested whether the synMuv genes might specifically regulate *lin-3a*. Preliminary evidence shows that in a *lin-3(n4441); class B* double mutant, the *lin-3a* transcript is upregulated more than is total *lin-3* relative to wild-type levels, suggesting that the *n4441* mutation might cause derepression of the *lin-3a* transcript specifically.

Because a mutation in *lin-3* can entirely recapitulate the class A synMuv phenotype, *lin-3* is likely to be the major functionally relevant target of the class A synMuv genes in vulval development. Based on these data, we propose that the class A synMuv proteins bind to the *lin-3* promoter at the site of the *n4441* mutation and repress transcription of *lin-3a*. Currently, we are determining the exact DNA sequences in the *lin-3* promoter required for class A synMuv-mediated repression and testing whether the class A synMuv proteins directly bind to the *lin-3* promoter.