Developmental Biology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

# The forkhead transcription factor UNC-130/FOXD integrates both BMP and Notch signaling to regulate dorsoventral patterning of the *C. elegans* postembryonic mesoderm

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ARTICLE INFO

*Keywords:*
*unc-130*
FOXD
*unc-129*
Mesoderm
M lineage
SMA-9
Schnurri
LIN-12
Notch
BMP
Dorsoventral patterning
Sex myoblast (SM)
Coelomocyte (CC)

ABSTRACT

The proper development of a multicellular organism requires precise spatial and temporal coordination of cell intrinsic and cell extrinsic regulatory mechanisms. Both Notch signaling and bone morphogenetic protein (BMP) signaling function to regulate the proper development of the *C. elegans* postembryonic mesoderm. We have identified the *C. elegans* FOXD transcription factor UNC-130 as a major target functioning downstream of both BMP signaling and Notch signaling to regulate dorsoventral patterning of the postembryonic mesoderm. We showed that *unc-130* expression in the postembryonic M lineage is asymmetric: its absence of expression in the dorsal side of the M lineage requires the antagonism of BMP signaling by the zinc finger transcription factor SMA-9, while its expression in the ventral side of the M lineage is activated by LIN-12/Notch signaling. We further showed that the regulation of UNC-130 expression by BMP signaling and Notch signaling is specific to the M lineage, as the ventral expression of UNC-130 in the embryonically-derived bodywall muscles was not affected in either BMP pathway or Notch pathway mutants. Finally, we showed that the function of UNC-130 in the M lineage is independent of UNC-129, a gene previously shown to function downstream of and be repressed by UNC-130 for axon guidance. Our studies uncovered a new function of UNC-130/FOXD in the *C. elegans* postembryonic mesoderm, and identify UNC-130 as a critical factor that integrates two independent spatial cues for the proper patterning and fate specification of the *C. elegans* postembryonic mesoderm.

## 1. Introduction

The proper development of a multicellular organism requires precise spatial and temporal coordination of cell intrinsic and cell extrinsic regulatory mechanisms. A small number of highly conserved signaling pathways are utilized repeatedly to mediate a variety of developmental processes in metazoa. Understanding how these different signaling pathways are integrated and interpreted to regulate specific cell fate decision events is critical to our ability to dissect the regulatory circuit underlying normal animal development. The *C. elegans* postembryonic mesoderm provides a model to dissect how different signaling pathways are integrated to regulate the diversification and fate specification of multiple cell fates from a single pluripotent precursor cell.

In *C. elegans*, all the non-gonadal postembryonic mesodermal cells are derived from a single precursor cell, the M mesoblast, which is born during embryogenesis (Sulston and Horvitz, 1977). During postembryonic development of a hermaphrodite animal, the M cell first divides dorsoventrally to generate two daughter cells, M.d and M.v. During subsequent larval development, these two cells further divide to produce distinct dorsal and ventral cell types (Fig. 1A-B). The dorsal cell, M.d, divides three more times to produce six bodywall muscles (BWMs) and two non-muscle coelomocytes (CCs), while the ventral cell, M.v, divides four more times to produce eight BWMs and two multipotent sex myoblasts (SMs), which further migrate and divide to produce eight vulval muscles (VMs) and eight uterine muscles (UMs).

Previous work has shown that the dorsoventral asymmetry in the M lineage is regulated by two different signaling pathways. The LIN-12/Notch pathway is required for specifying the ventral SM fate, while antagonism of the BMP pathway is required for specifying the dorsal CC fate (Foehr et al., 2006; Foehr and Liu, 2008; Greenwald et al., 1983). On the ventral side, three redundant Notch ligands, LAG-2, APX-1 and DSL-1, are expressed in the ventral hypodermal cells directly adjacent to the ventral M lineage cells (Foehr and Liu, 2008). They act together to activate the LIN-12 receptor in the ventral M lineage (Foehr and Liu, 2008; Levitan and Greenwald, 1998; Wilkinson and Greenwald, 1995). Loss of LIN-12/Notch signaling results in a ventral-to-dorsal fate transformation in the M lineage, thus the loss of

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https://doi.org/10.1016/j.ydbio.2017.11.008
Received 26 October 2017; Received in revised form 13 November 2017; Accepted 15 November 2017

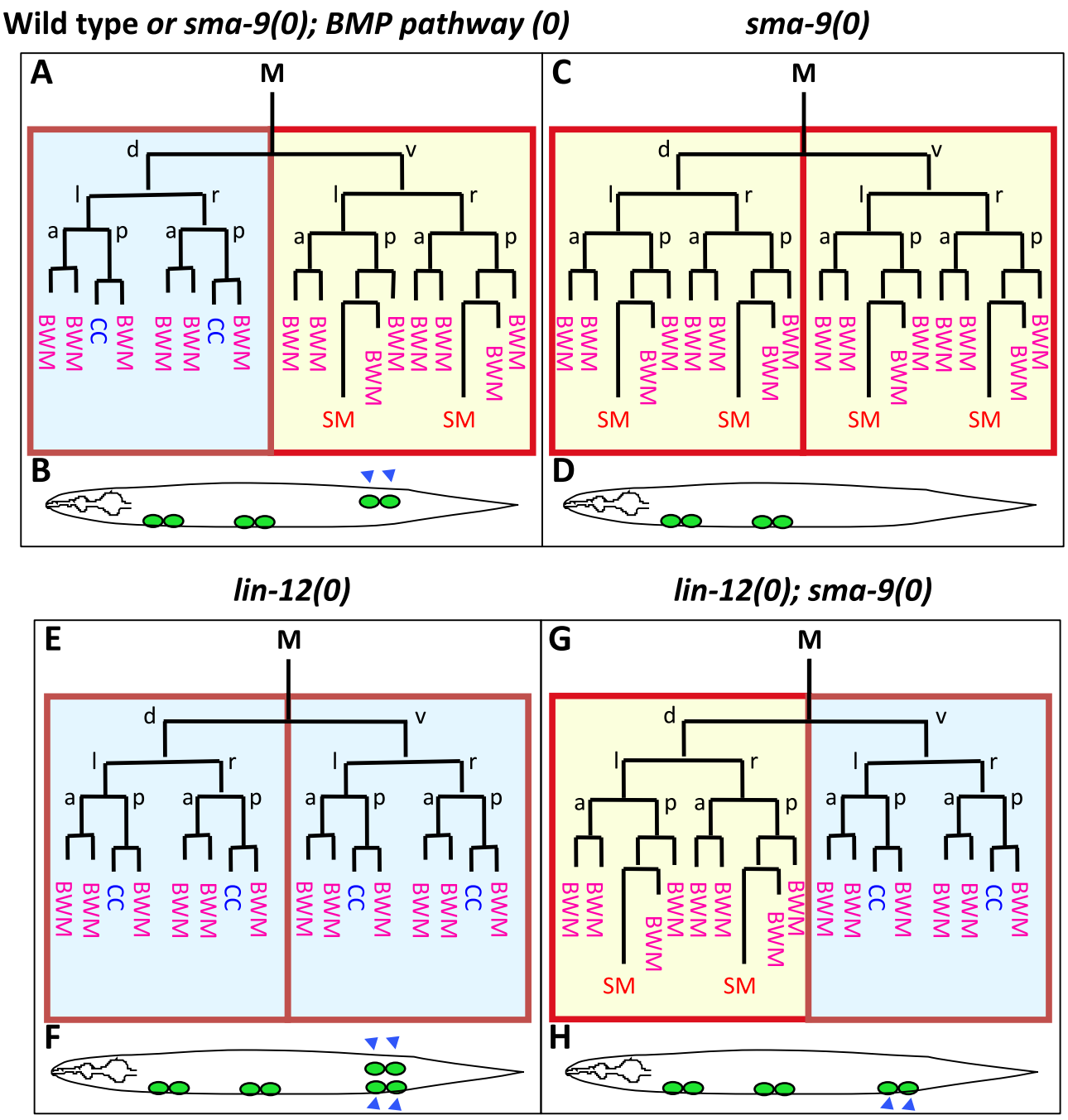


**Fig. 1. SMA-9, BMP signaling and LIN-12/Notch signaling regulate M lineage dorsoventral patterning.** Diagrams showing the M lineage (A-D) and the locations of CCs (E-H) in wild-type or *sma-9(0); BMP pathway(0)* (A, E), *sma-9(0)* (B, F), *lin-12(0)* (C, G), and *lin-12(0); sma-9(0)* (D, H) animals. Blue arrowheads point to the two M lineage-derived CCs. BWM: body-wall muscle, CC: coelomocyte, SM: sex myoblast. d: dorsal, v- ventral, l: left, r: right, a: anterior, p: posterior. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

the SM fate and gain of the CC fate (Fig. 1E-F) (Foehr and Liu, 2008; Greenwald et al., 1983). Specification of the dorsal M lineage fate requires the zinc finger-containing protein SMA-9, whose *Drosophila* homolog Schnurri is known to act as a transcriptional repressor (Liang et al., 2003; Marty et al., 2000; Pyrowolakis et al., 2004). In *C. elegans*, SMA-9 has also been shown to exhibit transcriptional repressor activity (Liang et al., 2007). In the M lineage, *sma-9(0)* mutants exhibit a dorsal-to-ventral fate transformation, thus the loss of the CC fate and gain of the SM fate (Fig. 1C-D) (Foehr et al., 2006). We have previously obtained genetic evidence showing that SMA-9 specifies the dorsal M lineage fate by antagonizing the DBL-1/BMP-like signaling pathway. Mutations in core components of the BMP pathway specifically suppress the M lineage defect of *sma-9(0)* mutants (Fig. 1A-B) (Foehr et al., 2006; Liu et al., 2015). Based on double and triple mutant analysis between *sma-9(0), lin-12(0)* and BMP pathway mutations, we proposed a model for how LIN-12/Notch and DBL-1/BMP pathways act independently to regulate dorsoventral patterning in the M lineage (Foehr and Liu, 2008). However, no major target genes have been identified that serve as a node to integrate and interpret these signaling inputs.

In this study, we report our finding that the forkhead transcription factor UNC-130 acts downstream of both SMA-9-BMP signaling and LIN-12/Notch signaling to integrate these spatial cues for the proper patterning of the M lineage. UNC-130 is a conserved transcription factor that belongs to a family of proteins containing a forkhead DNA-binding domain. It specifically falls into the FOXD subclass of forkhead