Developmental Biology 419 (2016) 273–284

Contents lists available at ScienceDirect

Developmental Biology

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

Drosophila FoxL1 non-autonomously coordinates organ placement during embryonic development

Caitlin D. Hanlon, Deborah J. Andrew *

Department of Cell Biology, The Johns Hopkins University School of Medicine, 725 N. Wolfe St., Baltimore, MD 21205-2196, United States

ARTICLE INFO

Article history:
Received 16 August 2016
Received in revised form
7 September 2016
Accepted 8 September 2016
Available online 13 September 2016

Keywords:
Drosophila
Embryogenesis
Fox proteins
FoxL1
Germ cells
Hemocytes
Malpighian tubules
Migration
Salivary gland
Somatic muscle

ABSTRACT

Determining how organs attain precise positioning within an organism is a crucial facet of developmental biology. The Fox family winged-helix transcription factors are known to play key roles in development of multiple organs. *Drosophila* FoxL1 (aka Fd64A) is dynamically expressed in embryos but its function is completely uncharacterized. FoxL1 is expressed in a single group of body wall - muscles in the 2nd and 3rd thoracic segments, in homologous abdominal muscles at earlier stages, and in the hindgut mesoderm from early through late embryogenesis. We show that FoxL1 expression in T2 and T3 is in VIS5, which is not a single muscle spanning the entire thorax, as previously published, but is, instead, three individual muscles, each spanning a single thoracic segment. We generate mutations in *foxL1* and show that, surprisingly, none of the tissues that express FoxL1 are affected by its loss. Instead, loss of *foxL1* results in defects in salivary gland positioning and morphology, as well as defects in the migration of hemocytes, germ cells and Malpighian tubules. We also show that FoxL1-dependent expression of secreted Sema2a in T3 VIS5 is required for normal salivary gland positioning. Altogether, these findings suggest that Drosophila FoxL1 functions like its mammalian counterpart in non-autonomously orchestrating the behaviors of surrounding tissues.

1. Introduction

Organ specification is typically mediated through the activation of a unique combination of autonomous factors. Tissue-specific transcription factors trigger signaling cascades and downstream effectors that induce cell differentiation and organ specialization (Chung et al., 2014; Cleveland et al., 2012; Loganathan et al., 2016; Rankin and Zorn, 2014; Zaret, 2016). A critical part of this process is ensuring that organ-specific products are synthesized at the right levels at the right time and that organs acquire their final correct size, shape and position in the developing animal. Whereas many aspects of organ development may be entirely tissue autonomous, all organs become dependent on surrounding tissues to provide spatial cues for refinements in cell fate, to coordinate cell division and growth, and/or to regulate changes in organ shape and position. These cues give an individual organ proprioception within the developing organism: a sense of space relative to the other organ systems. Consequently, the movement and placement of each organ is coordinated with its neighbors, leading to optimal function throughout the life of the organism. A wide range of signaling molecules such as Wnts, BMPs, Notch ligands, EGFs, FGFs, Ephrins and Semaphorins are necessary for inter-tissue communication during development. The transcription factors that activate these signaling molecules - as well as the machinery that allows for an appropriate cellular response in the target tissues - are key to regulating and coordinating developmental processes both during embryogenesis and at later stages for tissue homeostasis.

The Fox family of transcription factors plays key roles in development in all higher organisms (Hannenhalli and Kaestner, 2009; Weigel and Jackle, 1990). Fox (forkhead box) proteins are members of a well-conserved family of winged-helix DNA binding proteins with many diverse functions, from the regulation of organ development and growth, to vocal learning (Haesler et al., 2004; Lee and Frasch, 2004). There are 19 Fox genes in *Drosophila*, 44 in mouse, and 50 in human (Jackson et al., 2010). Despite their wide range of activities in development, relatively few Fox family members have been well characterized. Fork head (Fkh), the homologue of the vertebrate FoxA proteins, was the first Drosophila Fox gene cloned (Weigel et al., 1989). *fkh* is expressed in the embryonic salivary gland (SG) from the earliest stages of placode formation through the life of the organ (Myat and Andrew, 2000). *fkh* is an excellent example of a gene that functions largely intrinsically to control multiple aspects of organ development. Loss of *fkh* results in death of the embryonic SG and when the glands are kept alive by removing apoptotic activator genes, the SGs in *fkh* mutants do not invaginate. Although *fkh* cannot specify glands by

* Corresponding author.
E-mail address: dandrew@jhmi.edu (D.J. Andrew).

http://dx.doi.org/10.1016/j.ydbio.2016.09.007
0012-1606/

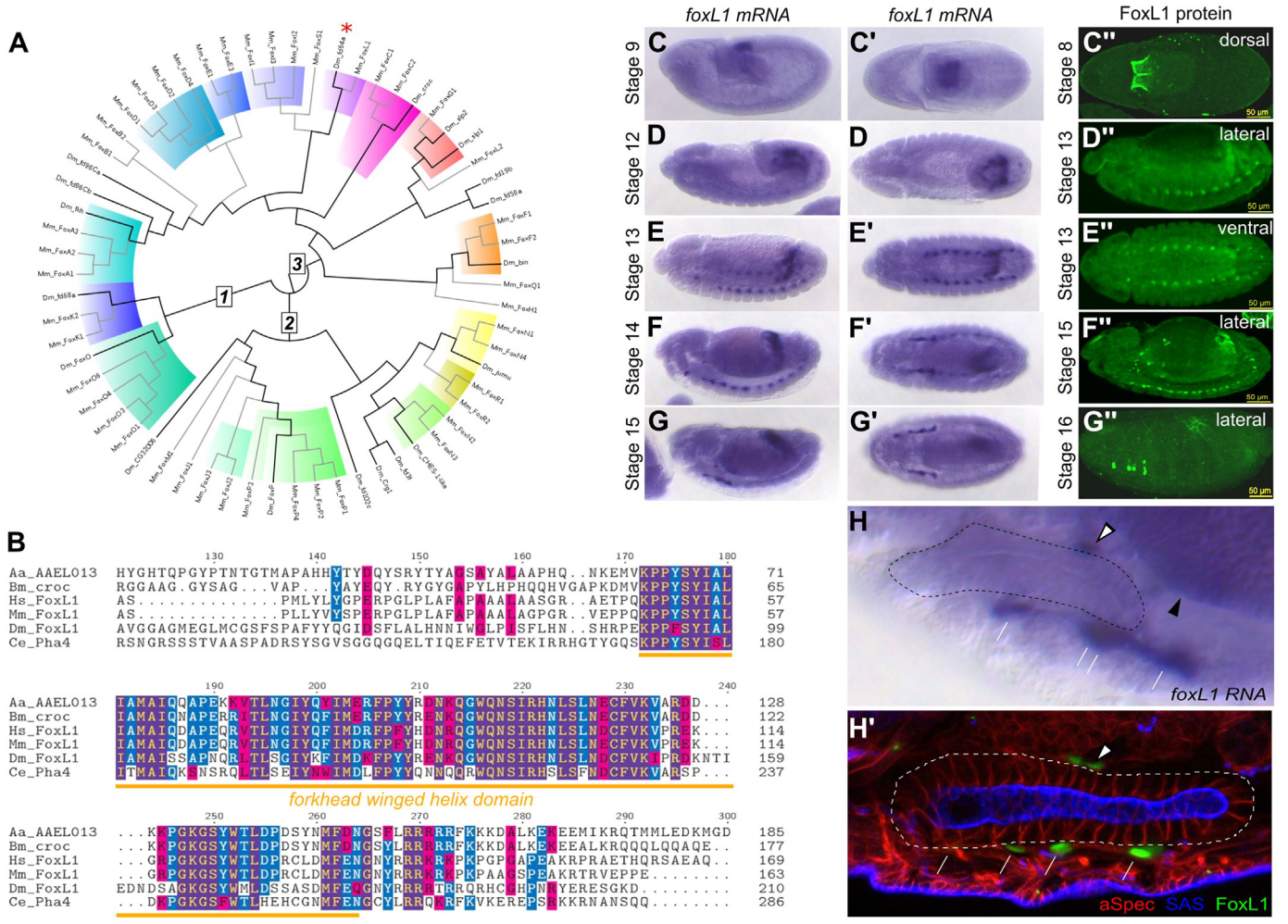

Fig. 1. Drosophila *foxL1* is dynamically expressed during embryogenesis (A) Rooted Phylip tree analysis reveals that *fd64A* (asterisk) is most similar to mouse FoxL1 (purple shading). (B) A ClustalX based line-up of representative invertebrate and mammalian members of the FoxL1 family indicate that the region of most similarity includes the winged-helix DNA binding domain. (C-G and C′-G′) *foxL1* mRNA is detected in cells near the hindgut and a subset of cells in the ventral-lateral region of each segment from T1-A8. (C″-G″) Staining with FoxL1 antiserum reveals nuclear staining in the same cells in which *foxL1* mRNA is detected. (H, H′) High magnification images reveal *foxL1* mRNA and protein in cells and nuclei directly contacting the SG at embryonic stage 15 (white arrows and arrowheads) as well as low levels of *foxL1* mRNA in some VM cells (black arrowheads). FoxL1 (green nuclear), αSpectrin (red lateral membrane), SAS (blue apical membrane).

itself, it does maintain its own expression and expression of many other genes that implement the SG cell fate (Maruyama et al., 2011). *fkh* is also expressed in the hindgut, Malpighian tubules, and proventriculus (among other tissues), and induces expression of SG specific gene products only when co-expressed with the SG-specific bHLH transcription factor Sage (Fox et al., 2013). In mammals, FoxA is known as a pioneer transcription factor because it binds chromatin early to provide accessibility to other tissue-specific transcription factors (Zaret and Carroll, 2011). Whether Drosophila Fkh functions similarly remains to be determined.

The 18 other *Drosophila* Fox genes show dynamic expression throughout development (Lee and Frasch, 2004). One of these genes, *fd64a*, has a robust embryonic expression pattern and has not been characterized. The closest mouse homologue of Fd64a is FoxL1 (Fig. 1A), which is expressed in the mouse mesenchyme and is necessary for proper development and organization of the gut epithelia (Katz et al., 2004). FoxL1 regulates gut morphology non-autonomously by altering the amount of Syndecan-1, Perlecan, and EphrinB secreted by the mesenchyme, which in turn regulates the amount of Wnt/β-catenin in the gut epithelium (Perreault et al., 2001; Takano-Maruyama et al., 2006). FoxL1 is also necessary for correct gut function, as loss of *foxL1* causes a decrease in acid secretion due to reduction in SNAP25 expression (Kato et al., 2004). *Drosophila fd64a* is expressed in a subset of the somatic musculature, close to the developing midgut, hindgut, and SG. Based on its intriguing expression pattern, we decided to fully characterize the role of *fd64a* in embryogenesis.

Here, we identify the embryonic tissues in which *foxL1* mRNA and protein are expressed. We show that *foxL1* is expressed in several muscles that have not been well described, including muscle 33 (also known as ventral intersegmental 5 – VIS5), as well as in an undescribed population of homologous abdominal muscles, and in the hindgut visceral mesoderm. Our phenotypic characterization reveals non-autonomous functions for *foxL1* in multiple embryonic tissues, a conclusion supported by our microarray data. The musculature of *foxL1* null embryos appears normal, but the SGs, hemocytes, germ cells, and Malpighian tubules show defects in migration and placement. We show that FoxL1 affects SG positioning – at least in part – through activation of a secreted signal. We conclude that *foxL1*'s primary role in embryonic development is to provide signaling cues from the mesoderm/musculature to nearby tissues.