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A role of the LIN-12/Notch signaling pathway in diversifying the non-striated egg-laying muscles in *C. elegans*

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ABSTRACT

The proper formation and function of an organ is dependent on the specification and integration of multiple cell types and tissues. An example of this is the *Caenorhabditis elegans* hermaphrodite egg-laying system, which requires coordination between the vulva, uterus, neurons, and musculature. While the genetic constituents of the first three components have been well studied, little is known about the molecular mechanisms underlying the specification of the egg-laying musculature. The egg-laying muscles are non-striated in nature and consist of sixteen cells, four each of type I and type II vulval muscles and uterine muscles. These 16 non-striated muscles exhibit distinct morphology, location, synaptic connectivity and function. Using an RNAi screen targeting the putative transcription factors in the *C. elegans* genome, we identified a number of novel factors important for the diversification of these different types of egg-laying muscles. In particular, we found that RNAi knockdown of *lag-1*, which encodes the sole *C. elegans* ortholog of the transcription factor CSL (*C*BF1, *S*uppressor of Hairless, *L*AG-1), an effector of the LIN-12/Notch pathway, led to the production of extra type I vulval muscles. Similar phenotypes were also observed in animals with down-regulation of the Notch receptor LIN-12 and its DSL (Delta, Serrate, LAG-2) ligand LAG-2. The extra type I vulval muscles in animals with reduced LIN-12/Notch signaling resulted from a cell fate transformation of type II vulval muscles to type I vulval muscles. We showed that LIN-12/Notch was activated in the undifferentiated type II vulval muscle cells by LAG-2/DSL that is likely produced by the anchor cell (AC). Our findings provide additional evidence highlighting the roles of LIN-12/Notch signaling in coordinating the formation of various components of the functional *C. elegans* egg-laying system. We also identify multiple new factors that play critical roles in the proper specification of the different types of egg-laying muscles.

Introduction

One of the fascinating questions in developmental biology is how functional organs form from cell types of diverse origins. The *Caenorhabditis elegans* hermaphrodite egg-laying system requires the proper specification, differentiation and functional integration of four major components: the uterus, the vulva, the egg-laying muscles attached to the vulva, and the egg-laying neurons that innervate the vulval muscles (Sulston and Horvitz, 1977; Kimble and Hirsh, 1979; White et al., 1986; Li and Chalfie, 1990). The proper formation and function of the egg-laying systems allow eggs that are stored in the uterus to be released through the vulva by the contraction of the egg-laying muscles, which are innervated by the egg-laying neurons. The molecular mechanisms underlying the development of the vulva, the uterus, and the egg-laying neurons have been relatively well understood (Sternberg, 2005; Gupta et al., 2012; Schafer, 2005). In contrast, very little is known about how the different types of egg-laying muscles are specified.

The egg-laying muscles are descendants of the multipotent sex myoblasts (SMs), which are derived from the postembryonic mesoderm lineage, the M lineage (Sulston and Horvitz, 1977, Fig. 1A–C). The M lineage arises during embryogenesis from a single M mesoblast cell. During hermaphrodite post-embryonic development the M mesoblast undergoes two waves of proliferation. The first wave of proliferation occurs during the L1 larval stage, where the M cell produces fourteen striated body wall muscle (BWMs) cells, two non-muscle coelomocytes (CCs) on the dorsal side, and two SMs on the ventral side. The SMs then migrate to the future vulval region, where they undergo another wave of proliferation during the L3 larval stage to produce sixteen non-striated sex muscles or egg-laying muscles of four different types: four type I and four type II vulval muscles (VM1s, VM2s), as well as four type I and four type II uterine muscles (UM1s, UM2s).

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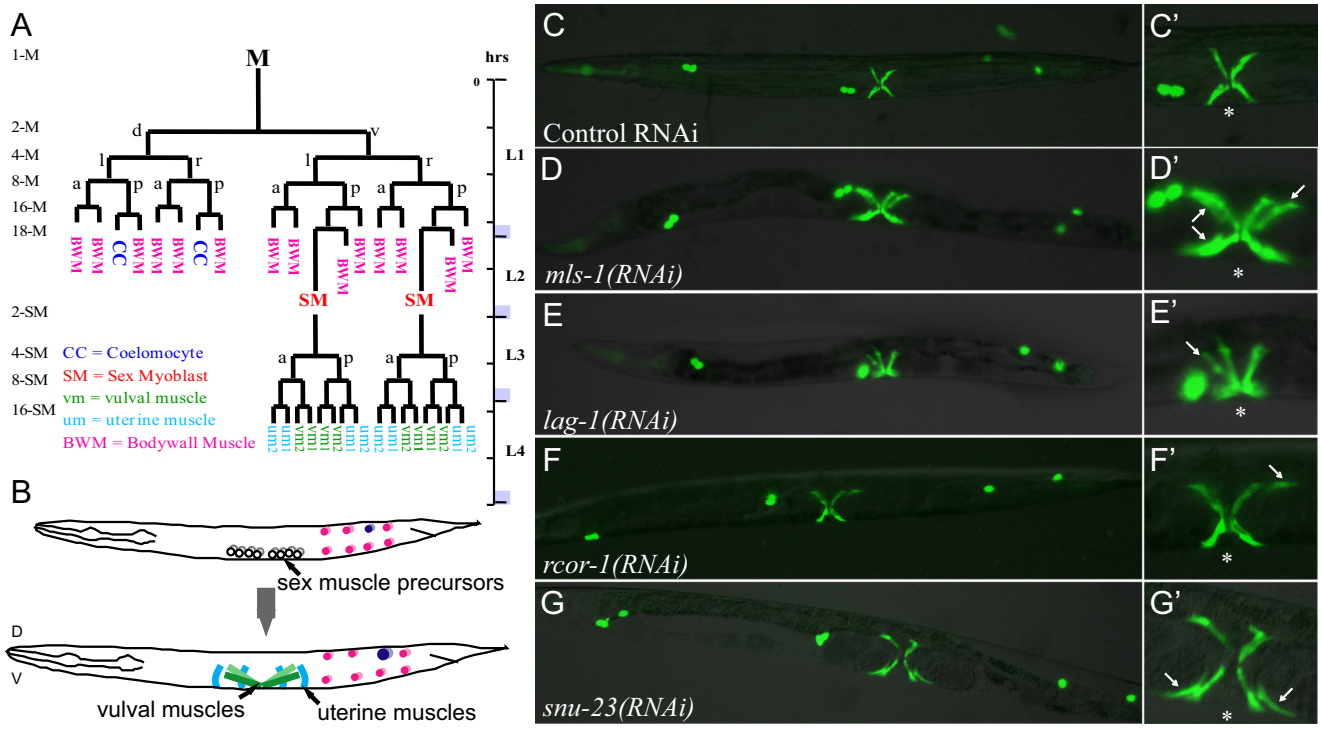

Fig. 1. An RNAi screen identified factors important for sex muscle development in *C. elegans*. (A) The *C. elegans* postembryonic mesodermal lineage, the M lineage, in wild-type hermaphrodites. Indicated on the left are the corresponding stages of M lineage development referenced within the text. Bar on the right indicates larval stages and shading indicates molt. a. anterior, p. posterior, d. dorsal, v. ventral., l. left, r. right. (B) Schematics of L4 and adult hermaphrodites with their M lineage cells highlighted. (C–G) Adult hermaphrodites visualized with an intrinsic *CC::gfp* and *egl-15::gfp*, with the corresponding region expressing *egl-15::gfp* shown in (C'–G'). Asterisks indicate the presumptive vulval region. Arrows indicate additional *egl-15::gfp*-expressing type I vulval muscle-like cells. Control RNAi with empty vector L4440 (C), *mls-1(RNAi)* (D), *lag-1(RNAi)* (E), *rcor-1(RNAi)* (F), and *snu-23(RNAi)* (G).

The sixteen non-striated sex muscles exhibit different morphology, location and function. The eight uterine muscles (UMs) wrap around the uterus, while the eight vulval muscles (VMs) are associated with the vulva and organized into two layers: the four VM1s are attached to the body wall subventrally, while the VM2s are attached to the body wall more ventrally. Only VM2s are directly innervated by the HSNs (hermaphrodite specific neurons) and VC4/5 (ventral type C 4/5) egg-laying neurons, while the remaining VM1s and UMs are connected together with VM2s by gap junction (White et al., 1986). Defects in the proper specification and development of the sex muscles do not affect the viability of the hermaphrodite as embryos can hatch inside the mother and continue to develop and reproduce, making it possible to study the mechanisms involved in the specification of these cells via various genetic manipulations.

Previous studies have identified two highly conserved transcription factors critical for sex muscle fate specification. The T-box transcription factor MLS-1/TBX1 acts as a fate determinant of uterine muscles (Kostas and Fire, 2002): loss of *mls-1* leads to the fate transformation of UMs to VMs, while forced expression of *mls-1* throughout the M lineage can convert other M lineage cells to adopt the fate of UMs. The TALE homeodomain protein UNC-62/MEIS also plays a role in sex muscle specification (Jiang et al., 2009): while knocking down *unc-62* causes early M lineage defects, a partial loss-of-function allele of *unc-62*, *ku234*, leads to the conversion of UMs and VM2s to VM1s. How these two factors function to specify the particular types of sex muscles is not well understood.

We set out to identify additional factors important in sex muscle specification by interrogating the putative transcription factors in the *C. elegans* genome using RNAi. By screening through over 70% of the predicted transcription factors, we identified a number of factors not previously known to function in sex muscle specification. In particular, we uncovered a role for the key effector and the sole CSL (*C*BF1, *S*uppressor of Hairless, *L*AG-1) protein of the Notch pathway in VM2 fate specification.

Materials and methods

C. elegans strains

Strains were cultured and handled as described by Brenner (Brenner, 1974). All analyses were conducted at 20°C, unless stated otherwise. The reference wild-type strain was LW0081 [*ccIs4438* (*intrinsic CC::gfp*) III; *ayIs2*(*egl-15::gfp*) *IV*; *ayIs6*(*hlh-8p::gfp*) *X*] (Jiang et al., 2005). The M lineage-specific reporters used include *arg-1p::gfp*(*ccIs4443*) that labels both type I and type II vulval muscles (Kostas and Fire, 2002), *rgs-2p::gfp*(*vsIs4*) that preferentially labels uterine muscles (Dong et al., 2000), *egl-15::gfp*(*ayIs2*) that labels type I vulval muscles (Harfe et al., 1998a), *hlh-29p::gfp* (*TLM908*) that labels type II vulval muscles (McMiller et al., 2007, kindly provided by Dr. Casonya Johnson), *hlh-8::gfp*(*ayIs6*) that labels all undifferentiated M lineage cells (Harfe et al., 1998b) and an *intrinsic CC::gfp*(*ccIs4438*) that has a nuclear-localized GFP under the control of a coelomocyte-specific enhancer from the *hlh-8* upstream region (Harfe et al., 1998b; Kostas and Fire, 2002). Additional transcriptional reporters used include *arIs88[apx-1p::lacZ, dpy-20(+), ceh-22::gfp]*, *arEx481[dsl-1p::lacZ, dpy-20(+), ceh-22::gfp]* (Chen and Greenwald, 2004), and *asIs131[lag-2p::2xnls-yfp::unc-54 3'UTR]* (Zhang and Greenwald, 2011), which were all kindly provided by Dr. Iva Greenwald. The strain LW0683 [*rrf-3* (*pk1426*); *ayIs6*(*hlh-8::gfp*); *ayIs2*(*egl-15::gfp*); *ccIs4438* (*intrinsic CC::gfp*)] (Amin et al., 2009) was used to visualize M lineage cells in the RNAi screen. Mutant alleles included LG III, *lin-12* (*n676n930ts*), *lin-12*(*wy750*); *LG IV, dsl-1*(*ok810*); *LG V, unc-62*