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Endoderm development in *Caenorhabditis elegans*: The synergistic action of ELT-2 and -7 mediates the specification→differentiation transition

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ABSTRACT

The transition from specification of cell identity to the differentiation of cells into an appropriate and enduring state is critical to the development of embryos. Transcriptional profiling in *Caenorhabditis elegans* has revealed a large number of genes that are expressed in the fully differentiated intestine; however, no regulatory factor has been found to be essential to initiate their expression once the endoderm has been specified. These gut-expressed genes possess a preponderance of GATA factor binding sites and one GATA factor, ELT-2, fulfills the expected characteristics of a key regulator of these genes based on its persistent expression exclusively in the developing and differentiated intestine and its ability to bind these regulatory sites. However, a striking characteristic of *elt-2(0)* knockout mutants is that while they die shortly after hatching owing to an obstructed gut passage, they nevertheless contain a gut that has undergone complete morphological differentiation. We have discovered a second gut-specific GATA factor, ELT-7, that profoundly synergizes with ELT-2 to create a transcriptional switch essential for gut cell differentiation. ELT-7 is first expressed in the early endoderm lineage and, when expressed ectopically, is sufficient to activate gut differentiation in nonendodermal progenitors. *elt-7* is transcriptionally activated by the redundant endoderm-specifying factors END-1 and -3, and its product in turn activates both its own expression and that of *elt-2*, constituting an apparent positive feedback system. While *elt-7* loss-of-function mutants lack a discernible phenotype, simultaneous loss of both *elt-7* and *elt-2* results in a striking all-or-none block to morphological differentiation of groups of gut cells with a region-specific bias, as well as reduced or abolished gut-specific expression of a number of terminal differentiation genes. ELT-2 and -7 synergize not only in activation of gene expression but also in repression of a gene that is normally expressed in the valve cells, which immediately flank the termini of the gut tube. Our results point to a developmental strategy whereby positive feedback and cross-regulatory interactions between two synergistically acting regulatory factors promote a decisive and persistent transition of specified endoderm progenitors into the program of intestinal differentiation.

Introduction

Two central challenges facing metazoan embryos are imbuing progenitor cells that arise from a single fertilized egg with distinct properties and activating their ensuing differentiation into tissues with unique functions. For development to succeed, the differentiation program must ensure a rapid and robust transition from specification, coordinate the proper patterning of cells in organ systems, and lock down the terminally differentiated state of all cells. Understanding how these biological switches are controlled is pivotal to our understanding of animal development.

An effective model system for illuminating the mechanisms at the interface between the programs of specification and differentiation is provided by the *Caenorhabditis elegans* endoderm. As revealed over a century ago, the endoderm in nematodes arises exclusively from one blastomere, the E cell, in the early embryo (Boveri, 1893, 1899). Through a determinate pattern of 4–5 rounds of cell division, E gives rise to the 20 cells of the intestine, the sole endoderm-derived organ (Sulston et al., 1983). These 20 cells are organized into an epithelial tube consisting of 9 intestinal rings, or "ints," with four cells forming int1 and two in each of the remaining rings (Leung et al., 1999). The intestine comprises the midgut of the *C. elegans* alimentary tract, connecting to the pharynx (foregut) and rectum (hindgut) by interfacing with sets of valve cells on either termini of the gut tube. The differentiating intestine arising from the E lineage must coordinate with its neighbors to engender a functional digestive organ system.

The well-described regulatory pathway for endoderm links early maternal genes through a series of intermediary regulators to

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terminal structural proteins and enzymes comprising the intestine. A maternal transcription factor, SKN-1 (Bowerman et al., 1993, 1992), initiates the transcriptional cascade for endoderm development. Its immediate zygotic targets are two redundant genes encoding the atypical GATA transcription factors MED-1 and -2 that specify both E and its sister, the mesoderm-producing MS cell (Broitman-Maduro et al., 2005; Maduro et al., 2007, 2001). In the E lineage, the MEDs directly activate expression of two GATA factor-encoding genes, *end-1* and *end-3*, which are redundantly required to specify the entire endoderm (Maduro et al., 2005; Zhu et al., 1997). Removal of both genes causes E to adopt the fate of its cousin, C, a progenitor of mesectoderm (Bowerman et al., 1993; Maduro et al., 2005; Zhu et al., 1997). While *end-1* and *-3* are expressed only until the E4 and E8 cell stages, respectively (Baugh et al., 2003; Maduro et al., 2007), they activate expression of another GATA factor, *elt-2*, which maintains its own expression throughout development (Fukushige et al., 1998, 1999; Hawkins and McGhee, 1995; Maduro and Rothman, 2002). ELT-2 also binds directly to transcriptional regulatory elements of genes encoding structural components and enzymes of the differentiated gut (Fukushige et al., 1998, 1999; Hawkins and McGhee, 1995). This transcriptional cascade appears to be a conserved mechanism for endoderm specification and differentiation across metazoa. In *Drosophila*, for example, the SERPENT GATA factor, which specifies endoderm in the embryo, activates dGATAe, whose expression persists through adulthood and which initiates gene expression for terminal gut differentiation (Murakami et al., 2005; Okumura et al., 2005). GATA factors have also been found to specify endoderm throughout the vertebrates, implying a pan-triploblastic mechanism for endoderm formation (Murakami et al., 2005; Okumura et al., 2005; Shivdasani, 2002).

The first known terminal marker of intestinal differentiation in *C. elegans*, the GES-1 gut esterase, was identified nearly 25 years ago (Edgar and McGhee, 1986). Subsequent studies revealed that GATA-type regulatory sequences are required for endoderm-specific expression of the *ges-1* gene, leading to identification of the endoderm-specific ELT-2 GATA factor based on its ability to bind these sequences (Fukushige et al., 1998, 1999; Hawkins and McGhee, 1995). Comprehensive transcriptional profiling of isolated embryonic and adult intestines revealed that a common element linking all gut-expressed genes is an extended TGATAA-like consensus binding site sequence, which, in some cases, has been shown to be essential for gut-specific expression, suggesting that gut-specific differentiation is broadly controlled by GATA factors (McGhee et al., 2009; McGhee et al., 2007; Pauli et al., 2006). Confirming that it acts in gut formation or function, deletion of *elt-2* results in an obstructed gut at the anteriormost intestinal rings, resulting in L1 larval lethality. While two other GATA factors, ELT-7 and ELT-4, are also expressed in the developing endoderm (Baugh et al., 2003; Fukushige et al., 2003; this study), ELT-4 shows no discernible function *in vivo* or *in vitro* (Fukushige et al., 2003) and no phenotype is apparent in *elt-7(0); elt-4(0)* double mutants, leading to the suggestion that ELT-2 is the dominant, and perhaps sole required regulator of intestinal differentiation (McGhee et al., 2009, 2007). However, such a conclusion conflicts with the observation that the gut in *elt-2(0)* mutants appears morphologically as fully differentiated as that of wild-type worms, with a complete lumen, well-developed brush border, and characteristic rhabditin granules throughout all gut cells (Fukushige et al., 1998) (this work). Moreover, transcription of *ges-1* and other genes is robustly activated in *elt-2* mutant embryos (McGhee et al., 2009). These observations make it clear that other factor(s) likely function to mediate the critical specification-to-differentiation transition during endoderm development.

Here we report that ELT-7, acting with ELT-2, is a key component of the intestinal developmental program, explaining how gut differentiation is initiated. *elt-7* is activated by the END-1/3 GATA factors, is first expressed before *elt-2*, and is sufficient to activate gut differentiation in ectopic lineages. We find that *elt-7(0)*; *elt-2(0)* double-knockout mutants fail to express a number of markers of gut differentiation, including GES-1, and are profoundly defective in gut differentiation in a regionalized manner, revealing an apparent underlying all-or-none differentiation switch. Finally, we find that ELT-7 and ELT-2 also synergize to repress transcription of a gene whose expression is normally limited to the valve cells flanking the gut tube, suggesting that activation of gut differentiation acts to exclude differentiation of non-gut cell types of the digestive tract. Our findings suggest a model in which the auto- and cross-regulatory action of ELT-2 and -7 initiates and locks down gut differentiation, thereby directing the transition from specification of endoderm fate to the persistent differentiated state of the intestine.

Materials and methods

elt-7 reporter constructs

Several different *elt-7*::GFP reporter constructs containing 1 kb or more of upstream sequence between the predicted translation start sites of *elt-7* and neighboring predicted protein-coding region C18G1.9 were created. (Oligonucleotide sequences are available on request.) The largest construct contained 2647 bp upstream of the *elt-7* ATG, which includes almost 90% of C18G1.9. Another construct was made by fusing GFP to the amino terminus of the entire *elt-7* protein coding region with 1 kb of upstream and 660 bp of downstream sequence, which includes the entire 3′-UTR found in a NEXTDB cDNA clone (Kohara, http://nematode.lab.nig.ac.jp/) plus an additional 500 bp. The transcriptional fusion reporters shown in this paper, containing 1 kb of upstream sequence, produced expression patterns identical to all other reporter constructs tested.

Ectopic expression of GATA factors

Gravid adult animals (for embryonic heat shock) or larvae growing on agar plates were incubated at 34 °C for 30 minutes. After incubation, adults were allowed to lay embryos for 2 hours before being removed from plates. Heat-shocked embryos or larvae were then placed at 20 °C overnight and were observed the following day.

Genetics

All genetic manipulations were performed according to standard techniques (Ahringer, 2006). Two methods were used to generate *elt-7(−)*; *elt-2(−)* double mutants and the phenotypes seen with both approaches were indistinguishable. (1) RNAi knockdown of *elt-7* transcripts was performed on strain JR2531 *elt-2(ca15); wEx1527 [sur-5*::*GFP, elt-2(+)]* mothers using standard injection or feeding procedures (Fire et al., 1998; Timmons and Fire, 1998) and *elt-2(ca15)* homozygotes were identified as non-GFP-expressing embryos or larvae. (2) Strain MS851 *elt-2(ca15); irEx404 [unc-119*::*CFP, elt-2(+)]* was crossed with strain FX840 *elt-7(tm840)* to generate the double-mutant strain JR3295 *elt-7(tm840)*; *elt-2(ca15)*; *irEx404 [unc-119*::*CFP, elt-2(+)]* and homozygous double mutants were identified as those not expressing the CFP marker. The *ca15* deletion removes the entire *elt-2* coding sequence (Fukushige et al., 1998). The *tm840* deletion removes exons two and three, including the first 22 amino acids of the DNA-binding domain of *elt-7* (Supp. Fig. 1) (WormBase Web site, http://www.wormbase.org, release WS213, 31 May 2010). The *elt-2(+)* rescuing array is transmitted to ~75% of MS851 offspring and to ~90% of JR3295 offspring.

Immunofluorescence analysis

Embryos and L1 larvae were fixed and stained for immunofluorescence by methanol/acetone fixation on slides. Embryos were