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Partially compromised specification causes stochastic effects on gut development in *C. elegans*

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ABSTRACT

The *C. elegans* gut descends from the E progenitor cell through a series of stereotyped cell divisions and morphogenetic events. Effects of perturbations of upstream cell specification on downstream organogenesis have not been extensively investigated. Here we have assembled an allelic series of strains that variably compromise specification of E by perturbing the activation of the gut-specifying *end-1* and *end-3* genes. Using a marker that allows identification of all E descendants regardless of fate, superimposed with markers that identify cells that have adopted a gut fate, we have examined the fate of E lineage descendants among hundreds of embryos. We find that when specification is partially compromised, the E lineage undergoes hyperplasia accompanied by stochastic and variable specification of gut fate among the E descendants. As anticipated by prior work, the activation of the gut differentiation factor *elt-2* becomes delayed in these strains, although ultimate protein levels of a translational ELT-2::GFP reporter resemble those of the wild type. By comparing these effects among the various specification mutants, we find that the stronger the defect in specification (i.e. the fewer number of embryos specifying gut), the stronger the defects in the E lineage and delay in activation of *elt-2*. Despite the changes in the E lineage in these strains, we find that supernumerary E descendants that adopt a gut fate are accommodated into a relatively normal-looking intestine. Hence, upstream perturbation of specification dramatically affects the E lineage, but as long as sufficient descendants adopt a gut fate, organogenesis overcomes these effects to form a relatively normal intestine.

1. Introduction

Organogenesis is an essential part of metazoan development. During this process, progenitor cells activate gene regulatory networks to drive specification, regulate mitotic divisions, direct cell migration and morphogenesis to form the appropriate shape of an organ or tissue, and activate terminal tissue-specific genes. In the face of sources of gene expression variation, robustness of organ formation is generally assured by several mechanisms. Embryonic gene networks include feed-forward and autoregulatory loops that enforce downstream gene activation and maintain cell identity (Davidson, 2010). Organ size can be regulated by global and local mechanisms that influence cell proliferation and cell size (Hariharan, 2015; Irvine and Harvey, 2015; Patel et al., 2017). Experimental perturbations of the earliest steps in organ specification, therefore, might not always have consequences for later organ development, depending on the extent to which compensatory mechanisms can overcome their effects.

In the nematode *C. elegans*, embryonic patterning must be especially robust because the animal lacks compensatory mechanisms to replace missing somatic cells. In particular, the gut (intestine) is clonally generated from a single embryonic cell called E (Fig. 1A). In wild-type animals, the E cell undergoes a stereotyped pattern of cell divisions to produce 20 (and rarely, 21 or 22) cells that form the larval intestine (Fig. 1B; Sulston et al., 1983). During embryogenesis, the developing gut primordium undergoes morphogenesis in a highly reproducible way with only minor animal-to-animal variation (Asan et al., 2016). Because the E founder cell generates only one tissue, and development occurs in a highly predictable way from one embryo to the next, the intestine has been a good model for investigating the relationship among specification, cell fate and the cell lineage (Boeck et al., 2011; Maduro, 2017; Sulston et al., 1983).

The gene regulatory network that drives E specification has been a focus of study for more than 25 years (Fig. 1C). The most critical zygotic regulators for specifying gut are the paralogous and redundant END-1 and END-3 GATA transcription factors, as loss of both genes together results in a complete failure to specify endoderm (Maduro et al., 2005a; Owraghi et al., 2010). The absence of gut is sometimes compatible with normal development of the rest of the embryo,

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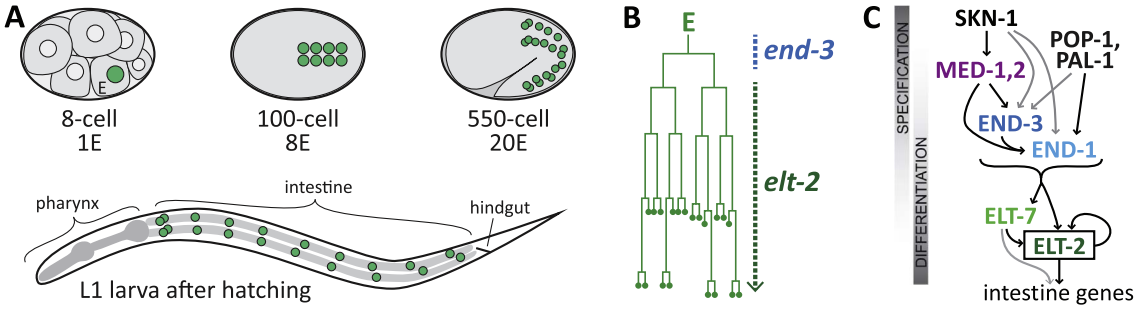

Fig. 1. Endoderm specification and E lineage development. (A) The locations of the E lineage nuclei at various stages in normal development, modified from (Maduro, 2015). Overall embryogenesis takes ~12 h at 25 °C (Sulston et al., 1983). (B) Cell division pattern of the wild-type E progenitor cell, after (Sulston et al., 1983). The vertical axis is time and a horizontal line indicates a cell division. The approximate time of transcription of *end-3* and *elt-2* is shown to the right of the diagram. (C) Simplified pathway showing hierarchy of transcription factors, modified from (Maduro, 2015). The endoderm specification strains described in this work perturb the overall contributions made by the MED-1,2 and END-1,3 regulators in a way that does not affect other lineages. Embryos are ~50 µm long and the larva is ~200 µm long. Dorsal is at top and anterior is to the left.

resulting in a larva with an absent gut but relatively normal morphogenesis (Maduro, 2009; Owraghi et al., 2010). Normal expression of the *end* genes occurs in the early E lineage, but only transiently, as their primary function is to activate expression of *elt-2* and *elt-7*. Expression of *elt-2* and *elt-7* is then maintained by positive autoregulation throughout the remainder of development and adulthood (Fukushige et al., 1998; Sommermann et al., 2010). ELT-2 is the predominant factor maintaining intestinal fate, as loss of *elt-2* alone results in embryos that contain a malformed gut, while loss of *elt-7* alone has no apparent phenotype though its loss can synergize with loss of *elt-2* (Fukushige et al., 1998; Sommermann et al., 2010). All four of END-1, END-3, ELT-2 and ELT-7 form a group of 'endodermal GATA factors' that have similar transcription factor activities, in that any of one of them is sufficient to specify the gut when overexpressed ectopically, and ELT-2 under the control of the *end-1* promoter can even specify gut in the E lineage in the absence of the other three genes (Du et al., 2016; Maduro et al., 2005a; Sommermann et al., 2010; Wiesenfahrt et al., 2015; Zhu et al., 1998). Parallel maternal and zygotic inputs upstream of END-1, END-3, ELT-2 and ELT-7 contribute to timely activation of *end-1* and *end-3* in the early E lineage (Maduro et al., 2005b, 2001; Shetty et al., 2005). These upstream factors are also required for specification of other early embryonic cells, hence their perturbation results in embryos that arrest with abnormal morphogenesis (Bowerman et al., 1992; Hunter and Kenyon, 1996; Lin et al., 1995).

An important question in development is how developing embryos cope with the stochastic nature of gene expression. Expression variation due to intrinsic and extrinsic factors occurs in many systems (Blake et al., 2003; Colman-Lerner et al., 2005; Holloway et al., 2011; Raj et al., 2010). The branched, redundant architecture of the zygotic gut specification network in *C. elegans* makes gut specification robust, but it also allows the construction of strains that have partial defects in gut specification in which the inherent variability of gene expression becomes apparent (Maduro et al., 2015, 2007). In such backgrounds, which we call "Hypomorphic Gut Specification" or HGS strains, gut development becomes highly stochastic: The number of gut nuclei that are made, as identified by expression of an *elt-2*::GFP transcriptional reporter, varies from none to over 30 (Maduro et al., 2015, 2007). We have interpreted these results to mean that gut specification is not strictly an all-or-none phenomenon at the level of the E blastomere, and proposed that the variable number of gut nuclei in HGS embryos is likely to be the result of two effects occurring simultaneously: One is an increase in the number of cells made by E, and the other is a stochastic adoption of gut fate among those descendants (Maduro, 2015).

Here, we examine gut specification mutant strains for their effect on the E lineage, from mid- to late embryogenesis. We use a two-reporter strategy that allows us to visualize, at any embryonic stage, all descendants of the E cell as well as those E descendants that have committed to a gut fate. We find that all HGS strains produce extra cell divisions within the E lineage, and that the stronger the defect in gut specification, the lower the proportion of cells that commit to a gut fate. Surviving HGS adults have more similar numbers of gut nuclei across all HGS strains, consistent with a required minimum number of gut cells for survival past the first larval stage. We find that expression of the terminal regulator ELT-2 is delayed in HGS embryos, proportional to the severity of the specification defect, but that final levels of ELT-2 expression are essentially normal. Finally, using a marker that enables visualization of cell membranes in the gut primordium, we find that the developing gut can accommodate extra cells produced by the E lineage, both in HGS strains as well as in a previously described mutant that dramatically increases cell divisions with the E lineage without affecting cell specification. Together, these results show that timely activation of gut specification is critical for establishing the correct pattern of cell divisions within the E lineage and the full commitment of all E descendants to an intestinal fate. We also find that variability in the pattern of cell divisions with the E lineage can be accommodated during gut development, as long as sufficient E descendants adopt a gut fate.

2. Materials and methods

2.1. Strains and worm handling

The wild-type control was N2. All strains were grown on *E. coli* OP50 and maintained at 20–22 °C and observed at 23–25 °C except as noted. Mutations and transgenes were as follows. *LG I*: *cdc-25.1(rr31), irSi10 [end-3(MED sites mutated) + Cb-unc-119], irSi13 [end-3(+) + Cb-unc-119(+)]. LG II*: *irSi7 [end-1(MED sites mutated), Cb-unc-119(+)], irSi9 [end-1(+), Cb-unc-119(+)], irSi12 [end-1,3(+), Cb-unc-119(+)]. LG III: med-2(cxTi9744). LG IV*: *him-8(e1489), him-8(me4), irIs98 [Cb-unc-119(+), end-1(MED sites mutated), end-3(MED sites mutated), irSi24 [pept-1/opt-2::mCherry::H2B, Cb-unc-119(+)], itIs37 [Cb-unc-119(+), pie-1::H2B::mCherry]. LG V*: *end-1(ok558), end-3(ok1448), end-3(ir62), end-3(ir64), oxTi389 [Cb-unc-119(+), eft-3::H2B::dTomato], stIs10116 [Cb-unc-119(+), his-72::H2B::mCherry], zuIs70 [end-1::GFP::CAAX], irIs133 [elt-2::mCherry::H2B, unc-119::CFP, rol-6D]. LG X*: *med-1(ok804), rrIs1 [unc-119(+), elt-2::NLS::GFP::lacZ], wIs84 [rol-6D, elt-2::NLS::GFP::lacZ]*. Unmapped: *stIs10064 [Cb-unc-119(+), end-3::HIS-24::mCherry::let-858_3′UTR], gaIs290 [elt-2::ELT-2::TY1::EGFP::3xFLAG]*. Extrachromosomal arrays: *irEx697 [elt-2*::mCherry::H2B, *unc-119::CFP, rol-6D]*. Mutations and transgenes were combined using standard crosses. The *cdc-25.1(rr31)* strain was grown at 23–25 °C. Because of the close proximity of *zuIs70* to *end-3* (< 2 map units), we made a *de novo* null mutation (*ir64*) in *end-3* in a *zuIs70* strain using CRISPR/Cas9-mediated mutagenesis (Arribere et al., 2014) with guide RNAs with targeting regions 5′-aaacacgtgaaatttagag-3′ and 5′-tcgggaaacgaaattgtgg-3′, which delete 1.1 kbp of the *end-3*