Developmental Biology 340 (2010) 209–221

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

Roles of the Wnt effector POP-1/TCF in the *C. elegans* endomesoderm specification gene network☆

Melissa Owraghi [1], Gina Broitman-Maduro, Thomas Luu, Heather Roberson, Morris F. Maduro *

Department of Biology, University of California, 2121A Genomics Building, Riverside, Riverside, CA 92521, USA

ARTICLE INFO

Article history:
Received for publication 22 July 2009
Revised 23 September 2009
Accepted 25 September 2009
Available online 7 October 2009
doi:10.1016/j.ydbio.2009.09.042

Keywords:
C. elegans
Endomesoderm
Specification
POP-1
Gene network

ABSTRACT

In *C. elegans* the 4-cell stage blastomere EMS is an endomesodermal precursor. Its anterior daughter, MS, makes primarily mesodermal cells, while its posterior daughter E generates the entire intestine. The gene regulatory network underlying specification of MS and E has been the subject of study for more than 15 years. A key component of the specification of the two cells is the involvement of the Wnt/β-catenin asymmetry pathway, which through its nuclear effector POP-1, specifies MS and E as different from each other. Loss of *pop-1* function results in the mis-specification of MS as an E-like cell, because POP-1 directly represses the *end-1* and *end-3* genes in MS, which would otherwise promote an endoderm fate. A long-standing question has been whether POP-1 plays a role in specifying MS fate beyond repression of endoderm fate. This question has been difficult to ask because the only chromosomal lesions that remove both *end-1* and *end-3* are large deletions removing hundreds of genes. Here, we report the construction of bona fide *end-1 end-3* double mutants. In embryos lacking activity of *end-1*, *end-3* and *pop-1* together, we find that MS fate is partially restored, while E expresses early markers of MS fate and adopts characteristics of both MS and C. Our results suggest that POP-1 is not critical for MS specification beyond repression of endoderm specification, and reveal that Wnt-modified POP-1 and END-1/3 further reinforce E specification by repressing MS fate in E. By comparison, a previous work suggested that in the related nematode *C. briggsae*, *Cb*-POP-1 is not required to repress endoderm specification in MS, in direct contrast with *Ce*-POP-1, but is critical for repression of MS fate in E. The findings reported here shed new light on the flexibility of combinatorial control mechanisms in endomesoderm specification in *Caenorhabditis*.

Introduction

Combinatorial control, achieved through spatiotemporally precise activation of genes in regulatory networks, is a central mechanism by which cells choose appropriate pathways of cell specification in metazoan development. Elucidation of such networks allows an appreciation for the complexity of the instructions encoded in the genome that bring about embryogenesis from the zygote. The endomesoderm specification network in the nematode *C. elegans* is a model for such networks in general. While an exhaustive compendium of protein–DNA interactions has yet to be made, many years of forward and reverse genetics, transcriptome analysis and protein–DNA studies have revealed a core network that includes motifs found in many other similar networks, such as feed-forward loops and autoregulation (Maduro, 2006). Evolutionary comparisons of this network have begun to be performed in related nematodes, and important similarities and differences have been identified (Coroian et al., 2005; Lin et al., 2009).

In *C. elegans*, the 4-cell stage blastomere EMS is an endomesoderm precursor: its anterior daughter, MS, generates some 80 cells that are primarily mesodermal, which includes a portion of the body muscles, many posterior cells of the pharynx, and four embryonically-derived coelomocytes (Fig. 1A) (Sulston et al., 1983). Its posterior sister E is the sole endoderm progenitor and generates 20 intestinal cells. Specification of MS and E involves the participation of two pathways that work in parallel (Fig. 1B): the SKN-1 pathway assigns endomesodermal identity to the EMS daughters, while the Wnt/β-catenin asymmetry pathway directs the two cells to adopt different fates (Bowerman et al., 1992; Lin et al., 1995; Maduro and Rothman, 2002; Mizumoto and Sawa, 2007; Rocheleau et al., 1997; Rocheleau et al., 1999; Thorpe et al., 1997).

Endomesoderm specification begins with maternal SKN-1, a bZIP/homeodomain transcription factor that is present in the nuclei of EMS and its sister P_2 at the 4-cell stage (Bowerman et al., 1993). Loss of SKN-1 leads to mis-specification of MS all the time, and E most (~70–80%) of the time (Bowerman et al., 1992). In addition to the MS-derived posterior portion of the pharynx, mutants for *skn-1*

☆ This article was accepted in 2009 to celebrate the 50th anniversary of Developmental Biology.

* Corresponding author. Fax: +1 951 827 4286.
E-mail address: mmaduro@ucr.edu (M.F. Maduro).
[1] Graduate Program in Cell, Molecular and Developmental Biology, UC Riverside, USA.

0012-1606/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2009.09.042

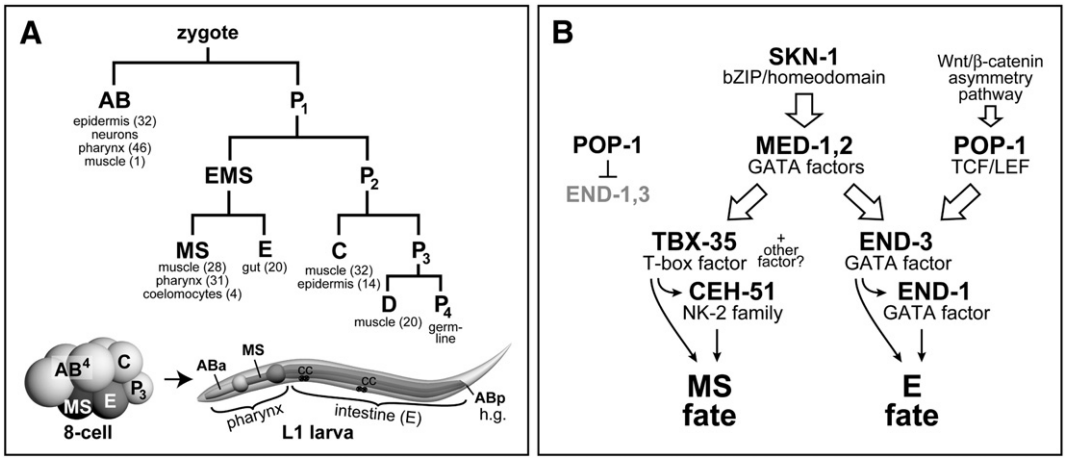

Fig. 1. The early *C. elegans* lineage and an abbreviated version of the endomesoderm gene regulatory network. (A) An abbreviated lineage shows the origin of the six founder cells and tissues made by each, with numbers of cells for gut, pharynx, muscle and coelomocytes in brackets (Sulston et al., 1983). Diagrams of an 8-cell stage embryo and larva show the lineal origins of the components of the digestive tract: Foregut/pharynx, midgut, and hindgut/rectum (h.g.). The assignment of ABa to 'anterior' pharynx and MS to 'posterior' pharynx is convenient but not strictly correct (Priess et al., 1987). On the larva, coelomocytes are indicated by 'CC'. (B) A flow diagram depicts a simplified version of the endomesoderm gene regulatory network, showing combinatorial control through the SKN-1 and Wnt/β-catenin pathways (Broitman-Maduro et al., 2009; Maduro, 2008).

also lack the anterior portion of the pharynx, which is specified by a GLP-1/Notch-dependent cell–cell interaction between MS and descendants of the AB founder cell (Mello et al., 1994; Priess et al., 1987). Within EMS, SKN-1 activates expression of a presumptive ligand for this interaction and the *med-1,2* divergent GATA factor gene pair (Lowry et al., 2009; Maduro et al., 2001). Loss of *med-1,2* together results in a penetrant mis-specification of MS, and low-penetrance mis-specification of E, owing to parallel inputs into E specification (Broitman-Maduro et al., 2009; Goszczynski and McGhee, 2005; Maduro et al., 2007; Maduro et al., 2001). Within the early MS lineage, the MEDs activate the T-box gene *tbx-35*, and TBX-35 (perhaps in combination with another factor) activates the NK-2 homeobox gene *ceh-51* (Broitman-Maduro et al., 2009). Loss of *tbx-35* and *ceh-51* together results in a penetrant mis-specification of MS that resembles the MS phenotype of *med-1,2* mutants (Broitman-Maduro et al., 2009). TBX-35 and CEH-51 are hypothesized to activate further pathways that lead to specification of pharynx, muscle and other tissues made by MS. Pharynx specification involves activation of a gene network with PHA-4/FoxA at the top (Gaudet and Mango, 2002), and which includes the pharynx muscle-specific regulator CEH-22/Nkx2.5 (Okkema and Fire, 1994). Muscle specification occurs via a three-way collaboration of HND-1/Hand, HLH-1/MyoD and UNC-120/Srf (Fukushige et al., 2006) and activation of a muscle gene network (Roy et al., 2002).

Within the early E lineage, the MEDs, SKN-1, the Wnt effector POP-1/TCF, and PAL-1/Caudal contribute to endoderm specification through activation of the GATA factor genes *end-1* and *end-3*. Expression of *end-3* occurs slightly earlier than *end-1* and there is evidence that END-3 also contributes to *end-1* activation (Baugh et al., 2003; Maduro et al., 2007). Downstream of *end-1,3*, the principal target is the GATA factor gene *elt-2* (Fukushige et al., 1998), which activates a network of targets for intestinal development (McGhee et al., 2009; McGhee et al., 2007; Pauli et al., 2006).

For both MS and E, loss of function of SKN-1, the MEDs, ENDs or TBX-35/CEH-51 causes adoption of a C-like fate by the mis-specified blastomeres. In contrast, mutations in the Wnt/β-catenin asymmetry pathway, which makes MS and E different, result in either an MS to E transformation (a 'Pop' phenotype) or the reverse, an E to MS transformation (a 'Mom' phenotype). Specification of E requires a cell–cell interaction between EMS and its sister P_2 (Goldstein, 1992). This interaction involves overlapping Wnt/MAPK/Src pathways and ultimately results in differential modification of the nuclear effector POP-1/TCF (Bei et al., 2002; Lin et al., 1998; Lin et al., 1995; Meneghini et al., 1999; Rocheleau et al., 1997; Rocheleau et al., 1999; Shin et al., 1999; Thorpe et al., 1997). This modification results in the nuclear export of POP-1 in E (a phenomenon called 'POP-1 asymmetry'), lowering its concentrations relative to the divergent β-catenin SYS-1, and permitting it to function as an activator in E (Huang et al., 2007; Maduro et al., 2002; Phillips et al., 2007). Within MS, the high nuclear levels of POP-1 allow it to repress *end-1* and *end-3*, while in E, POP-1 contributes to activation of (at least) *end-1* (Maduro et al., 2005b; Maduro et al., 2002; Shetty et al., 2005). Mutations in the upstream Wnt/MAPK/Src components result in a Mom phenotype, while loss of *pop-1* is epistatic to Mom mutants and results in a Pop phenotype. POP-1 and the Wnt/β-catenin asymmetry pathway form a switch that is used multiple times in *C. elegans* development (Kaletta et al., 1997; Lin et al., 1998; Mizumoto and Sawa, 2007).

Outside of the EMS lineage, multiple parallel pathways block activity of SKN-1 or promote its degradation (Bei et al., 2002; Lin, 2003; Maduro et al., 2001; Mello et al., 1992; Page et al., 2007; Shirayama et al., 2006). For example, the CCCH zinc finger protein PIE-1 blocks activity of SKN-1 in P_2: loss of *pie-1* function results in an ectopic mis-specification of the P_2 daughters as MS- and E-like (Mello et al., 1992). Similarly, a gain-of-function mutation in *oma-1*, which encodes a zinc finger protein similar to PIE-1, results in ectopic mis-specification of the C blastomere as an EMS-like cell, due to increased concentrations of SKN-1 in C (Lin, 2003).

The POP-1 switching system in *C. elegans* participates in the MS/E decision primarily by repressing endoderm specification in MS, although it makes a weaker contribution to endoderm specification in E (Lin et al., 1995; Shetty et al., 2005). In the related nematode, *C. briggsae*, analysis of the *Cb-pop-1* and *Cb-skn-1* RNAi phenotypes led to the conclusion that *Cb*-POP-1 contributes to MS and E specification primarily as an essential activator of the *Cb-end* genes in E, with a parallel function in MS specification with *Cb*-SKN-1 (Lin et al., 2009). This observation, along with other observations regarding expression of MS factors in *pop-1*-depleted embryos (Broitman-Maduro et al., 2006; Broitman-Maduro et al., 2009), prompted us to examine the role of *C. elegans* POP-1 in MS outside of repression of endoderm specification.

Here, we describe the construction of chromosomal *end-1 end-3* double mutants, and from a fully penetrant gut defect, conclude that these two genes are completely essential for endoderm specification in *C. elegans*. We test the requirement for POP-1 in MS specification outside of repression of the *end*s, and find that loss of *pop-1* function in *end-3* single mutants, and *end-1,3* double mutants, results in an apparent restoration of some aspects of MS specification to the MS