Biochimica et Biophysica Acta 1789 (2009) 250–260

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

Structure and evolution of the *C. elegans* embryonic endomesoderm network

Morris F. Maduro *

Department of Biology, University of California, Riverside, CA 92521, USA

ARTICLE INFO

Article history:
Received 6 February 2008
Accepted 29 July 2008
Available online 6 August 2008

Keywords:
C. elegans
Endomesoderm
Cell fate specification
Gene network
Development

ABSTRACT

The specification of the *Caenorhabditis elegans* endomesoderm has been the subject of study for more than 15 years. Specification of the 4-cell stage endomesoderm precursor, EMS, occurs as a result of the activation of a transcription factor cascade that starts with SKN-1, coupled with input from the Wnt/β-catenin asymmetry pathway through the nuclear effector POP-1. As development proceeds, transiently-expressed cell fate factors are succeeded by stable, tissue/organ-specific regulators. The pathway is complex and uses motifs found in all transcriptional networks. Here, the regulators that function in the *C. elegans* endomesoderm network are described. An examination of the motifs in the network suggests how they may have evolved from simpler gene interactions. Flexibility in the network is evident from the multitude of parallel functions that have been identified and from apparent changes in parts of the corresponding network in *Caenorhabditis briggsae*. Overall, the complexities of *C. elegans* endomesoderm specification build a picture of a network that is robust, complex, and still evolving.

1. Introduction

Triploblastic animals begin life as a single cell, which after many rounds of mitosis will ultimately consist of a multitude of genetically equivalent cells. By adulthood, the majority of these will have selected a particular pathway of differentiation, each expressing a subset of the genes in the organism's genetic complement that uniquely defines its type. At some point in embryogenesis, precursor cells become specified, and acquire transcriptional differences that set them apart from their neighbors. These differences will instruct their descendants as to their ultimate cell type, or at least restrict their choices until a later decision is made.

In the nematode, *Caenorhabditis elegans*, cells acquire these differences very early, as seen in the stereotyped cleavage patterns that are the hallmark of its nearly-invariant cell lineage [1]. The point of sperm entry sets the posterior of the embryo, defining one of the three embryonic axes (reviewed in [2]). The first division produces a larger cell, AB, and a smaller posterior cell, P_1. Following division of AB and P_1, the embryo consists of the anterior and posterior daughters of AB (ABa and ABp, respectively), and the two daughters of P_1, called EMS and P_2 (Fig. 1). EMS, situated ventrally, is an endomesoderm precursor: it will divide to produce a posterior daughter, called E, and an anterior daughter, MS. The E cell will clonally generate the 20 larval cells of the midgut (endoderm), while MS generates many cells that are primarily mesodermal, which includes most cells in the posterior half of the pharynx, and many of the animal's body muscles. The remaining portion of the pharynx is made by the anterior daughter of AB (ABa). Because many cells in the *C. elegans* lineage undergo anterior–posterior divisions to produce daughters that will acquire different fates [1], specification of MS and E makes a good platform for examining mechanisms that may operate throughout much of the animal's development.

Work over the past 15+ years has identified multiple factors that specify the *C. elegans* endomesoderm. Essentially, there are two pathways that converge on EMS specification: the SKN-1/MED-1,2 pathway assigns an endomesodermal fate to EMS, while the Wnt/β-catenin asymmetry pathway makes E different from MS [3,4]. Although the pathways that lead to MS and E specification look superficially like a simple cascade, the network contains much subtlety, crosstalk, redundancy, and flexibility. This review will examine the genes that specify MS and E, how deployment of their developmental programs is restricted to the appropriate lineages, and how the overall network may be evolving. A diagrammatic summary of the information flow in the network is presented in Fig. 1, and a summary of the relevant genes is given in Table 1.

2. The endomesoderm network

The rapid development of *C. elegans* is considered derived within the phylum, and the rapid divisions in the early embryo are proposed to be correlated with the use of maternal factors to drive much of the early cell specification events [5–7]. Screens for maternal embryonic lethals, in which arrested embryos lack one or more major tissue types but still contain many differentiated nuclei, led to the identification of multiple factors, including the gene *skn-1* [8].

* Tel.: +1 951 827 7196; fax: +1 951 827 4286.
E-mail address: mmaduro@ucr.edu.

1874-9399/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbagrm.2008.07.013

2.1. Getting it all started: Maternal SKN-1 specifies EMS

Embryos from *skn-1(−)* mothers undergo a developmental arrest and lack pharynx 100% of the time, while endoderm is absent in approximately 70% of embryos [8]. Pharynx originates from descendants of both MS and ABa [1]; the absence of AB- derived pharynx in *skn-1* mutant embryos is attributed to the failure of a GLP-1/Notch-mediated cell induction that normally occurs between the MS cell and descendants of ABa [8,9].

Antibody staining shows that SKN-1 protein is present in the EMS and P_2 nuclei at the 4-cell stage, placing it in the correct time and place to directly act in EMS specification [10]. As discussed below, SKN-1 is blocked in P_2 due to the function of another maternal gene, *pie-1* [11–13]. The *skn-1* locus encodes a transcription factor that has domains similar to those found in bZIP and homeodomain proteins [14]. As its expression normally disappears during the MS and E cell cycles [10], SKN-1 is likely to be a factor that initiates a zygotic gene cascade that will specify MS and E. The observation that many *skn-1(−)* embryos still make endoderm points to the existence of parallel pathways that are capable of contributing to gut specification in the absence of SKN-1; these pathways will be discussed later.

2.2. Zygotic specification of endoderm by END-1 and END-3

Mutagenic screens for penetrant zygotic mutations that resulted in the absence of endoderm identified only a large genomic region on chromosome V, named the 'Endoderm Determining Region' or EDR [15]. EDR-deficient [EDR(Df)] embryos lack endoderm and show a transformation of E to a C-like cell [15]. Within a 30-kbp region located within the EDR, two GATA factor genes, *end-1* and *end-3*, were identified that could individually restore endoderm development to EDR(Df) embryos, suggesting that they share overlapping function [15,16]. Consistent with this, overexpression of either *end-1* or *end-3* can reprogram non-endodermal cells into gut precursors [16,17]. Transgene fusion reporters for both genes are also expressed in the early E lineage, though expression goes away after several cell divisions [16,18]. Hence, *end-1* and *end-3* are clearly paralogous, likely having arisen from an ancient gene duplication [16].

Two observations suggest that *end-1* and *end-3* have diverged somewhat, which might account for their maintenance [19]. First, while a null mutation of *end-1* has no discernible phenotype, mutation of *end-3* results in a weak endoderm specification defect, and in those embryos making endoderm, the number of gut cells frequently

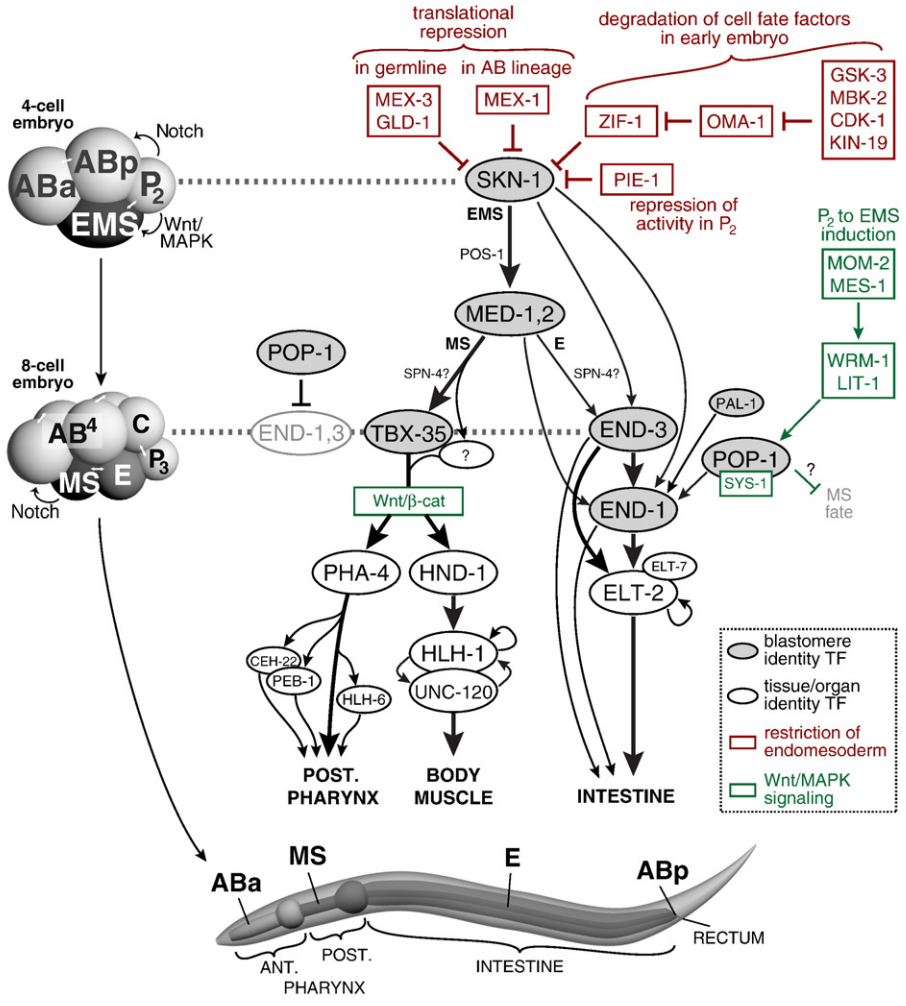

Fig. 1. The *C. elegans* endomesoderm gene regulatory network. Ovals represent transcription factors, while rectangles indicate other types of proteins. Question marks denote hypothesized co-regulators or functions. Arrows denote direct regulatory interactions. Thicker arrows denote stronger inputs as defined by the phenotype of loss of the input, while thinner arrows denote weaker parallel, autoregulatory or feed-forward inputs. Overlapping transcription factor symbols denote common function and are not meant to imply physical interaction. Diagrams of the *C. elegans* embryo (4-cell and 8-cell stages), and anatomy of the digestive tract in a larva are shown, after Ref. [3], with anterior to the left, and dorsal upwards. Sections of the digestive tract are labeled with the name of the blastomere whose descendants contribute to that region. The association of 'anterior pharynx' with ABa, and 'posterior pharynx' with MS is in reality not a precise distinction [9] and is shown here as such for simplicity. GLP-1/Notch-dependent cell–cell interactions are shown as 'Notch' with an arrow; the MS-to-AB induction actually occurs later than shown [9]. Only some of the pharynx and intestinal regulators are shown as examples; for more comprehensive descriptions see references [77,75].