Molecular Cell, Vol. 7, 475–485, March, 2001, Copyright ©2001 by Cell Press

Restriction of Mesendoderm to a Single Blastomere by the Combined Action of SKN-1 and a GSK-3β Homolog Is Mediated by MED-1 and -2 in *C. elegans*

Morris F. Maduro,* Marc D. Meneghini,†§ Bruce Bowerman,† Gina Broitman-Maduro,* and Joel H. Rothman*‡

*Department of Molecular, Cellular, and Developmental Biology and Neuroscience Research Institute
University of California
Santa Barbara, California 93106
†Institute of Molecular Biology
University of Oregon
Eugene, Oregon 97403

Summary

The endoderm and much of the mesoderm arise from the EMS cell in the four-cell *C. elegans* embryo. We report that the MED-1 and -2 GATA factors specify the entire fate of EMS, which otherwise produces two C-like mesectodermal progenitors. The *med*s are direct targets of the maternal SKN-1 transcription factor; however, their forced expression can direct SKN-1-independent reprogramming of non-EMS cells into mesendodermal progenitors. We find that SGG-1/GSK-3β kinase acts both as a Wnt-dependent activator of endoderm in EMS and an apparently Wnt-independent repressor of the *med*s in the C lineage, indicating a dual role for this kinase in mesendoderm development. Our results suggest that a broad tissue territory, mesendoderm, in vertebrates has been confined to a single cell in nematodes through a common gene regulatory network.

Introduction

Early in embryogenesis of all bilaterian animals, three concentric germ layers are created, each giving rise to distinct organs and systems. The establishment of separate germ layers with unique developmental potential is critical to the formation of organs and systems. In mammals, the innermost germ layer, or endoderm, produces internal organs, including the intestine, liver, pancreas, and lungs, while mesoderm engenders the circulatory, endocrine, excretory, skeletal, and muscle systems and other organs. In many animals, endoderm and some or all of the mesoderm arise from a single anlage, the mesendoderm, which is subsequently organized into the individual germ layers by regulatory mechanisms that apportion unique differentiation pathways to spatially distinct embryonic regions (e.g., Osada and Wright, 1999; Warga and Nüsslein-Volhard, 1999; Angerer and Angerer, 2000; Kimelman and Griffin, 2000).

Mesendoderm in the nematode *C. elegans* arises from the ventral-most blastomere, the EMS cell, at the four-cell stage (Sulston et al., 1983). A maternally contributed transcription factor, SKN-1, is required to specify the identity of EMS; in *skn-1* mutants, EMS adopts a fate reminiscent of that of its sister, P_2, which produces ectoderm and muscle (Bowerman et al., 1992). While *skn-1* transcripts are distributed throughout the early embryo (Seydoux and Fire, 1994), SKN-1 protein accumulates to high levels only in the two posterior blastomeres of the four-cell embryo, EMS and P_2, by a process requiring the maternal MEX-1 protein and various of the PAR proteins (Mello et al., 1992; Bowerman et al., 1993, 1997; Figure 1). Though SKN-1 is present at comparable levels in both EMS and P_2, it is able to activate transcription in EMS only, owing to a general transcriptional inhibitor, PIE-1, that is differentially segregated to the P_2 cell during the first two cell divisions (Mello et al., 1996; Tenenhaus et al., 1998; Batchelder et al., 1999). Paradoxically, while the anterior daughter of P_2, the C cell, like EMS, contains significant levels of SKN-1 and no PIE-1 protein, it nonetheless does not produce mesendoderm.

Subdivision of the mesendoderm into two germ layer types occurs immediately upon division of EMS to produce its daughters, the mesodermal (MS) and endodermal (E) progenitors (Sulston et al., 1983). While the E cell is the sole progenitor of the intestine (Sulston et al., 1983), the MS cell gives rise to much of the mesoderm, including the posterior portion of the pharynx feeding organ, which appears to be homologous to the vertebrate heart (Haun et al., 1998). This subdivision of the mesendoderm results from an inductive interaction in EMS that causes its posterior daughter to become an endoderm progenitor instead of an MS-like cell (Schierenberg, 1987; Goldstein, 1992, 1993, 1995). Two convergent signaling systems, a Wnt-type and a MAP kinase pathway, participate in the interaction that polarizes EMS and activates endoderm development (Rocheleau et al., 1997, 1999; Thorpe et al., 1997; Meneghini et al., 1999). These signals allow endoderm development to occur by inactivating the endoderm-repressive activity of the Lef-1-like POP-1 protein (Lin et al., 1995, 1998; Rocheleau et al., 1997; Thorpe et al., 1997), possibly converting it to an activator of endoderm (J. Kasmir et al., unpublished observation).

While the greatest variety and number of mesodermal cells derive from MS, some mesoderm is also produced by nonmesendodermal founder cells (i.e., the AB, C, and D founder cells; Sulston et al., 1983). Unlike the mesoderm from MS, which is made cell autonomously, AB mesoderm is secondarily induced by a signal from MS involving the Notch-like receptor, GLP-1 (Priess and Thomson, 1986; Priess et al., 1987). The C and D blastomeres produce only a single mesodermal cell type, body muscle; PAL-1, a CAUDAL-like homeoprotein, is required maternally for the muscle from these cells (Hunter and Kenyon, 1996).

Here we report that two immediate targets of maternal SKN-1, *med-1* and *-2*, which encode GATA-type transcription factors, specify the mesendoderm in *C. elegans*. Further, we demonstrate mechanisms that direct mesendoderm development exclusively to the EMS lin-

‡To whom correspondence should be addressed (rothman@lifesci.ucsb.edu).
§Present address: Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143.

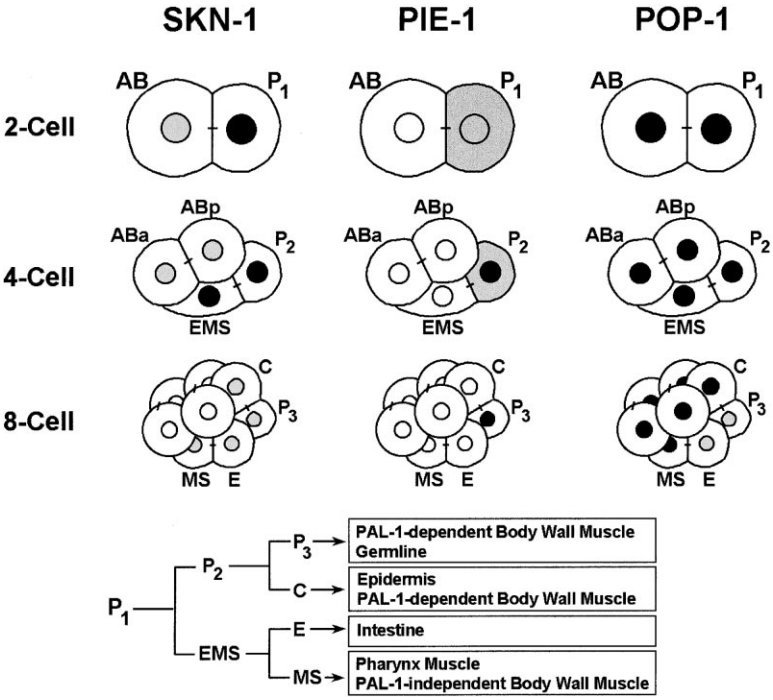

Figure 1. Early Cleavages in *C. elegans* Embryos and Cell Types Arising from the P_1 Blastomere

The immunolocalization of maternal SKN-1 (Bowerman et al., 1992), PIE-1 (Mello et al., 1996), and POP-1 (Lin et al., 1995) is shown as follows: gray, low levels; black, high levels. Maternal PAL-1 shows similar localization to SKN-1 but is not detected in AB descendants at the two- and four-cell stage (Hunter and Kenyon 1996). This figure is patterned after Schnabel and Priess (1997).

eage. In embryos depleted of *med* function, MS and E generate epidermis and body wall muscle, similar to a normal C cell, instead of their normal mesendodermal derivatives. While the activity of several maternal genes limits *med* expression to the EMS lineage, misexpression of *med-1* is sufficient to convert non-EMS blastomeres into mesendodermal precursors. SKN-1 binds directly to the *med-1* promoter and is sufficient to activate the *med* genes wherever it is expressed in early somatic blastomeres, with one notable exception: SKN-1-dependent activation of the MEDs, and acquisition of EMS fate in the C cell are blocked by SGG-1, a GSK-3β kinase homolog. These results demonstrate a dual role for SGG-1 in repression and activation of the mesendoderm; while SGG-1 is required positively for the Wnt-mediated activation of endoderm in the EMS lineage, it is an inhibitor of mesendoderm in the C lineage, explaining how C development can occur in a cell containing SKN-1 and lacking PIE-1.

Results

med-1 and *med-2* Encode Nearly Identical GATA Factors

Of the five somatic "founder cells" (AB, MS, E, C, and D) born during the early asymmetric cleavages in *C. elegans*, only one, the E cell, is known to be completely specified by a zygotic gene or genes. Two neighboring genes, *end-1* and *end-3*, encode GATA-type transcription factors redundantly required for specification of the relatively simple E lineage (Zhu et al., 1997; our unpublished observations). The apparent neomorphic activity of an unusual *end-3* mutation that inappropriately activates MS development in the E lineage (our unpublished observations) led us to propose that there may be a GATA factor normally required to specify the MS founder cell fate. Two nearly identical GATA factors, encoded by the *med-1 X* and *med-2 III* genes (*med*, *m*esend*o*derm specification) fulfilled this prediction (see below). Though on different chromosomes, these genes are nearly identical over a ~1.3 kbp region, suggesting they arose by a recent duplication (Figure 2A). Their structures predict intronless transcription units that are 98% identical. The upstream and downstream regions are slightly more divergent, with about 90% identity. Each gene encodes a 174 amino acid predicted protein containing a single CXNC-X_{18}-CNXC zinc finger and basic domain characteristic of GATA-type transcription factors (Figures 2B and 2C). The high degree of similarity of their coding and flanking sequences suggests that *med-1* and *med-2* may be functionally redundant.

Loss of *med* Gene Function Results in Transformations in MS and E Fates Resembling Loss of *skn-1*

We assessed the role of *med-1* and *med-2* by RNA-mediated interference (RNAi; Fire et al., 1998). The similarity between *med-1* and *med-2* enabled us to target both genes with a single double-stranded RNA species (see Experimental Procedures). For simplicity, we will hereafter refer to arrested *med-1(RNAi)*; *med-2(RNAi)* embryos as *med-1,2(RNAi)* or *med* mutant embryos. *med* mutants arrest as well-developed partially elongated embryos containing a truncated pharynx (Figure 3B): only the anterior pharynx is detected in *med* mutants, based on morphology and expression of molecular markers (Figures 3C and 3D). Moreover, *glp-1(RNAi)*; *med-1,2(RNAi)* embryos lack pharynx entirely (Table 1 and data not shown), confirming that the pharynx in *med* mutant embryos is solely AB derived and consistent with a complete absence of MS-derived pharynx.

med embryos lack not only MS-derived pharynx but

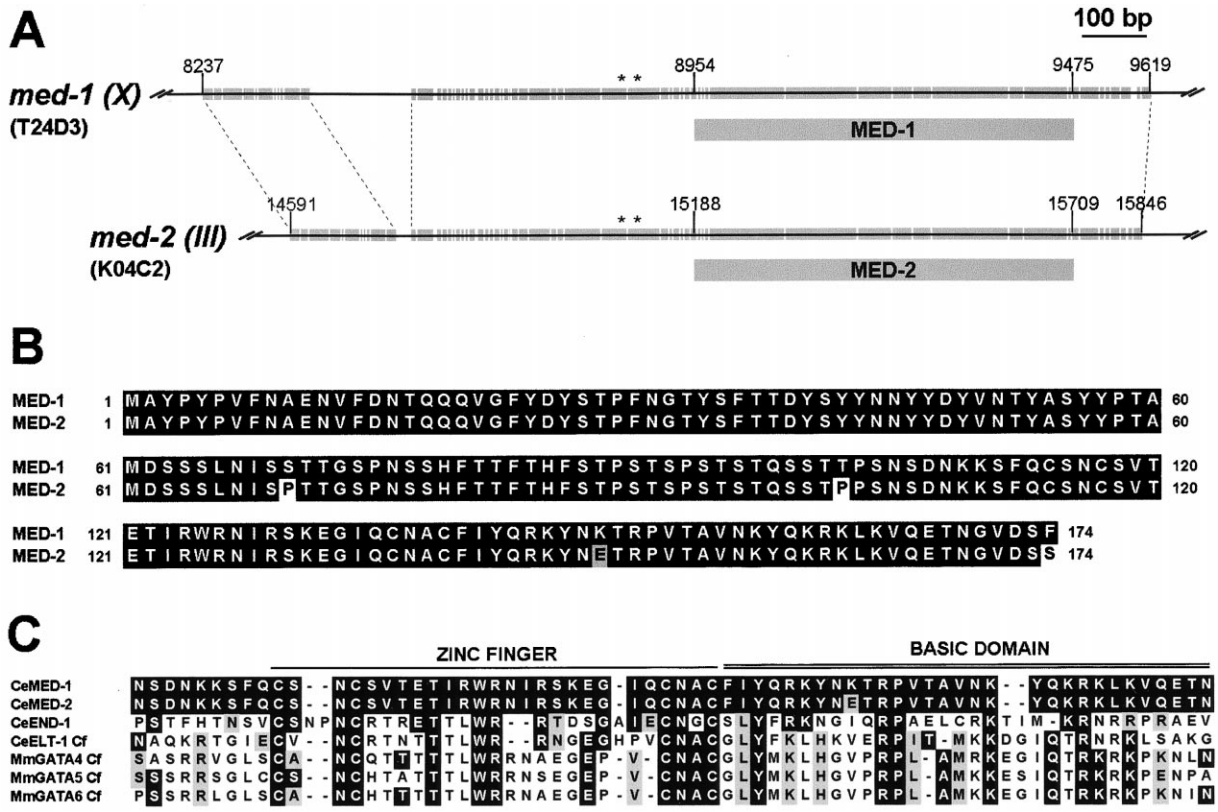

Figure 2. *med-1* and *med-2* Loci and Their Predicted Gene Products, MED-1 and MED-2

(A) *med-1* and *med-2* loci on cosmids T24D3 and K04C2, with base pair positions indicated. Gray rectangles indicate blocks of nucleic acid identity, with white lines indicating mismatches. Two SKN-1 site clusters are denoted by asterisks. The intronless open reading frames (ORFs) are shown below each gene.

(B) Alignment of MED-1 and MED-2 ORFs. Identities with MED-1 are indicated by a black background, while gray indicates a conservative change.

(C) Alignment of MED-1 and MED-2 zinc finger and basic domains with other GATA factors. END-1 and ELT-1 are from *C. elegans* (Spieth et al., 1991; Zhu et al., 1997), while GATA-4,5,6 are from mouse. For ELT-1 and GATA-4,5,6, only the carboxyl zinc finger (Cf) is shown. Block colors are as in (B). Accession numbers: *med-1*, AF302237; *med-2*, AF302238.

probably all cell types normally produced by this founder cell. Though MS and P_2 both generate body muscles (Sulston et al. 1983), the muscle arising from each can be distinguished based on their mechanism of specification. P_2-derived muscle requires maternal expression of the CAUDAL-like PAL-1 transcription factor (Hunter and Kenyon, 1996), whereas that from MS does not. In *pal-1* mutant embryos, body muscles are specifically missing from the posterior of the embryo, reflecting the loss of P_2-derived, and continued production of MS-derived, muscle (Hunter and Kenyon, 1996). We found that *pal-1(RNAi); med-1,2(RNAi)* mutants lack muscle almost entirely, implying that the MS-type body wall muscle is not made in *med* mutants (data not shown). The defects in MS specification are manifested early in the MS lineage of *med* embryos: expression of *lag-2*, which encodes a ligand for a Notch-like receptor (Henderson et al., 1994), is specifically absent from the early MS lineage, yet continues to be expressed in the early AB lineage in mutant embryos (Figures 3E and 3F). Together these results imply that most or all cell types arising from the MS lineage are incorrectly specified in *med* mutant embryos.

While all *med* mutant embryos lack MS-derived tissues, ~50% of arrested embryos also lack a differentiated intestine (Table 1; Figures 3G and 3H), which derives exclusively from the E founder cell (Sulston et al., 1983; Figure 1). Embryos lacking maternal SKN-1 show a similar defect: although nearly all *skn-1(zu67)* mutants fail to make MS-derived tissues, 80% do not make intestine (Bowerman et al., 1992). We examined whether two other properties of the E lineage that are abnormal in *skn-1* mutants are also affected in *med* mutants: gastrulation and timing of cell division (Bowerman et al., 1992). Wild-type gastrulation begins at the 28-cell stage, when the two E daughter cells move from the surface to the interior of the embryo. The E daughters divide only after their ingression is complete. We observed that the E daughters in *med* embryos divided, on average, 10 min earlier than in wild type and usually remained on the ventral surface of the embryo during division (Figures 3I–3L). Finally, we found that expression of *end-1* and *end-3*, which are first detectable in the E cell and are the earliest known markers of E-specific fate (Zhu et al., 1997; M. F. M. and J. H. R., unpublished data), is significantly diminished in *med* embryos (data not

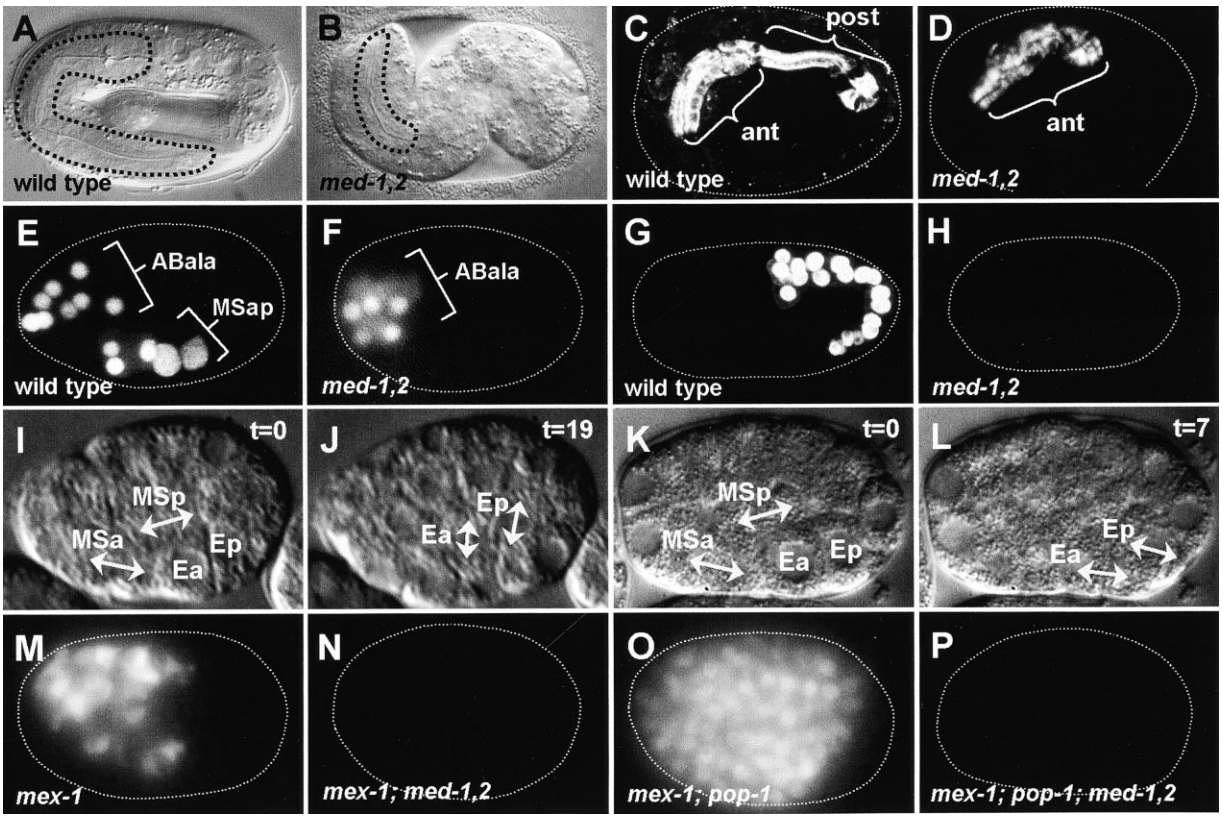

Figure 3. *med* Mutant Embryos Lack MS- and E-Derived Tissues

(A) Wild-type embryo elongated to ~3× egg length, with pharynx outlined.

(B) *med-1,2(RNAi)* mutant embryo showing 1.5-fold elongation and abnormally small pharynx.

(C) Pharynx muscle detected by mAb9.2.1 showing anterior (ant) and posterior (post) halves of wild-type pharynx.

(D) *med* mutant showing absence of posterior pharynx.

(E) Expression of *lag-2*::GFP in ABala and MSap descendants (bracketed) of an ~200-cell embryo.

(F) Specific loss of MSap-derived expression in a *med* mutant.

(G) Wild-type gut-specific *elt-2*::GFP expression (Fukushige et al., 1998) in a 2-fold embryo.

(H) Lack of gut cells in a terminal *med* embryo.

(I–L) Video stills from time-lapse recordings. (I and J) Wild-type E daughters migrated into the interior of the embryo and divided an average of 18.4 min ($n = 7$) after the MS daughters. (K and L) In *med* mutants, the E cells divided an average of only 8.7 min after ($n = 7$; $p < 0.001$). In 6/7 embryos the E cells divided on the ventral surface, rather than internally. Time (t) is indicated in minutes after MS daughter division.

(M–P) *med-1,2* are required for ectopic MS- and E-like tissues (also see Table 1). (M) Ectopic pharynx in a *mex-1(RNAi)* embryo shown by expression of *ceh-22*::GFP (Okkema and Fire, 1994). (N) Absence of pharynx cells in a *mex-1(RNAi); med-1,2(RNAi)* mutant embryo. (O) Ectopic gut nuclei (*elt-2*::GFP) in a *mex-1(RNAi)*; *pop-1(RNAi)* double mutant. In such mutants, endoderm is derepressed in all MS-like cells such that the four AB granddaughters and MS adopt E-like fates (Lin et al., 1995). (P) Absence of intestinal cells in a *mex-1(RNAi); pop-1(RNAi); med-1,2(RNAi)* embryo.

In fluorescence micrographs, the boundary of the eggshell has been indicated with a dotted line. A *C. elegans* embryo is about 50 μm in length. Anterior is to the left, and dorsal is up.

shown), confirming the defects in endoderm development.

In addition to the defects in differentiation of MS- and E-derived tissues, *med* embryos often contain internal cuticle-lined cavities similar to those observed in *skn-1* mutants, suggesting that MS and E have adopted the fate of the C cell, which generates cuticle-secreting epidermis (Bowerman et al., 1992). To test directly whether MS adopts a C-like fate in *med* mutant embryos, we analyzed the differentiated cell types produced when MS is isolated by laser ablation of all other cells. As shown in Table 2, while an isolated wild-type C blastomere always produced epidermis, an isolated MS cell never did. In contrast, approximately half (5/9) of the isolated *med* mutant MS blastomeres generated epidermal cells, consistent with the proportion of unoperated embryos that showed a *med* phenotype. Moreover, production of muscles from MS in *med* mutant embryos, like those from a wild-type C cell, is PAL-1 dependent (data not shown). In similar experiments, we observed epidermal and muscle cells arising from a small number of isolated *med* mutant E cells (data not shown). We conclude that, like *skn-1* mutants (Bowerman et al., 1992), MS and E can both adopt a C-like fate in *med* mutant embryos, with the only notable difference being that MS (and presumably E; Lin et al., 1995), retain the ability to induce secondary mesoderm, including anterior pharynx, in AB descendants (see Discussion).

Table 1. Tissue Types Produced in *med* Mutant and Heat Shock *med-1* Embryos

Genotype[a]	Pharynx[b] (%)	Intestine[c] (%)
Wild type	100% (n > 500)[d]	100% (n > 500)
glp-1(RNAi)	100% (197)	100% (197)
med-1,2(RNAi)	100% (143)	48% (143)[e]
glp-1(RNAi); med-1,2(RNAi)	53% (72)	59% (34)[f]
mex-1(RNAi)	100% (93)	ND[g]
mex-1(RNAi); med-1,2(RNAi)	89% (115)	ND
glp-1(RNAi); pie-1(RNAi)	100% (31)	100% (31)
glp-1(RNAi); pie-1(RNAi); med-1,2(RNAi)	40% (77)	59% (46)[f]
pop-1(RNAi)	ND	100% (>500)
pop-1(RNAi); med-1,2(RNAi)	ND	78% (92)
mex-1(RNAi); pop-1(RNAi)	ND	98% (124)
mex-1(RNAi); pop-1(RNAi); med-1,2(RNAi)	ND	69% (117)
glp-1(RNAi); wrm-1(RNAi)	100% (96)	0% (96)
glp-1(RNAi); wrm-1(RNAi); med-1,2(RNAi)	75% (75)	0% (75)
glp-1(RNAi); mom-2(RNAi)	100% (78)	89% (78)
glp-1(RNAi); mom-2(RNAi); med-1,2(RNAi)	43% (51)	0% (29)[f]
glp-1(RNAi); lit-1(RNAi)	97% (113)	0% (113)
glp-1(RNAi); lit-1(RNAi); med-1,2(RNAi)	78% (119)	0% (119)
Wild type (EMS ablated)	0% (14)	0% (14)
hs-*med-1* (EMS ablated)	56% (9)	56% (9)

[a] In embryos depleted for the WRM-1/β-catenin or MOM-2/Wnt ligand components of the Wnt pathway (Rocheleau et al., 1997; Thorpe et al., 1997) or for the LIT-1 kinase component of the MAPK pathway (Kaletta et al., 1997; Rocheleau et al., 1999), E adopts an MS-like fate. Other phenotypes are described in the text and in the legends to Figures 4 and 5. In some experiments, a *glp-1(RNAi)* background was used to eliminate AB-derived pharynx cells (Priess et al., 1987). For wild type, embryos were scored just prior to hatching; for all other genotypes, only arrested embryos were scored.
[b] Pharynx muscle was scored by *ceh-22*::GFP expression (Okkema and Fire, 1994).
[c] Intestinal cells were scored by polarized light birefringence or expression of *elt-2*::GFP (Fukushige et al., 1998).
[d] Total number of embryos scored is shown in brackets.
[e] Underlined figures indicate statistical significance ($p < 0.002$) compared with the results shown in the line immediately above.
[f] Only embryos with no pharynx were scored.
[g] ND, not determined.

Table 2. Differentiated Cell Types Produced by Isolated Blastomeres

		Cell Types Produces			
Genotype	Blastomere Isolated[a]	Pharynx Cells[b]	Intestinal Cells[c]	Body Wall Muscle[d]	Hypodermal Cells[e]
Wild type	MS	ND[f]	0/12	8/8	0/10
	C	ND	0/9	8/8	9/9
med-1,2(RNAi)[g]	MS	ND	0/15	6/6	5/9
sgg-1(RNAi)	P_2	0/8	7/8	ND	ND
sgg-1(RNAi); skn-1(zu67)	Intact	ND	12/117	ND	ND
sgg-1(RNAi); pal-1(RNAi)	Intact	ND	158/164	ND	ND
	P_2	18/24	21/24	ND	ND
	Ca	11/12	0/12	ND	ND
	Cp	0/8	7/8	ND	ND
sgg-1(RNAi); pal-1(RNAi); skn-1(zu67)	Intact	ND	4/114	ND	ND
sgg-1(RNAi); pal-1(RNAi); skn-1(RNAi)	P_2	0/8	0/8	ND	ND
sgg-1(RNAi); pal-1(RNAi); med-1,2(RNAi)	P_2	1/7	2/7	ND	ND

[a] Ablation of early blastomeres was performed as previously described (Moskowitz and Rothman, 1996; Zhu et al., 1997). To isolate P_2, ABa, ABp, and EMS were ablated in four-cell embryos. To isolate MS, ABa, ABp, and P_2 were ablated in four-cell stage embryos, then E was ablated following division of EMS. To isolate C, ABa, ABp, and EMS were ablated, followed by P_3 after P_2 divided. To isolate Ca or Cp, Cp or Ca was ablated following division of an isolated C cell. In some experiments, no cells were ablated ("intact").
[b] Pharynx cells were scored by expression of *ceh-22*::GFP (Okkema and Fire, 1994).
[c] Intestinal cells were scored by polarized light birefringence and nuclear morphology.
[d] Body wall muscle cells were scored by *hlh-1*::GFP expression (Krause et al., 1990).
[e] Hypodermal cells were scored by *lin-26*::GFP expression (Labouesse et al., 1996).
[f] ND, not determined.
[g] *med-1,2(RNAi)* embryos were obtained from hermaphrodite parents in a time window known to produce between 50%–70% mutant progeny (see Experimental Procedures).

med-1 Expression Is Sufficient to Convert Non-EMS Blastomeres into Mesendodermal Precursors

To assess whether *med-1* can reprogram non-EMS lineages into mesendoderm, we expressed it ubiquitously throughout early embryos under the control of a heat shock promoter (Stringham et al., 1992). We found that such embryos arrest with large numbers of pharynx muscles occupying up to ~30%, and gut cells occu-

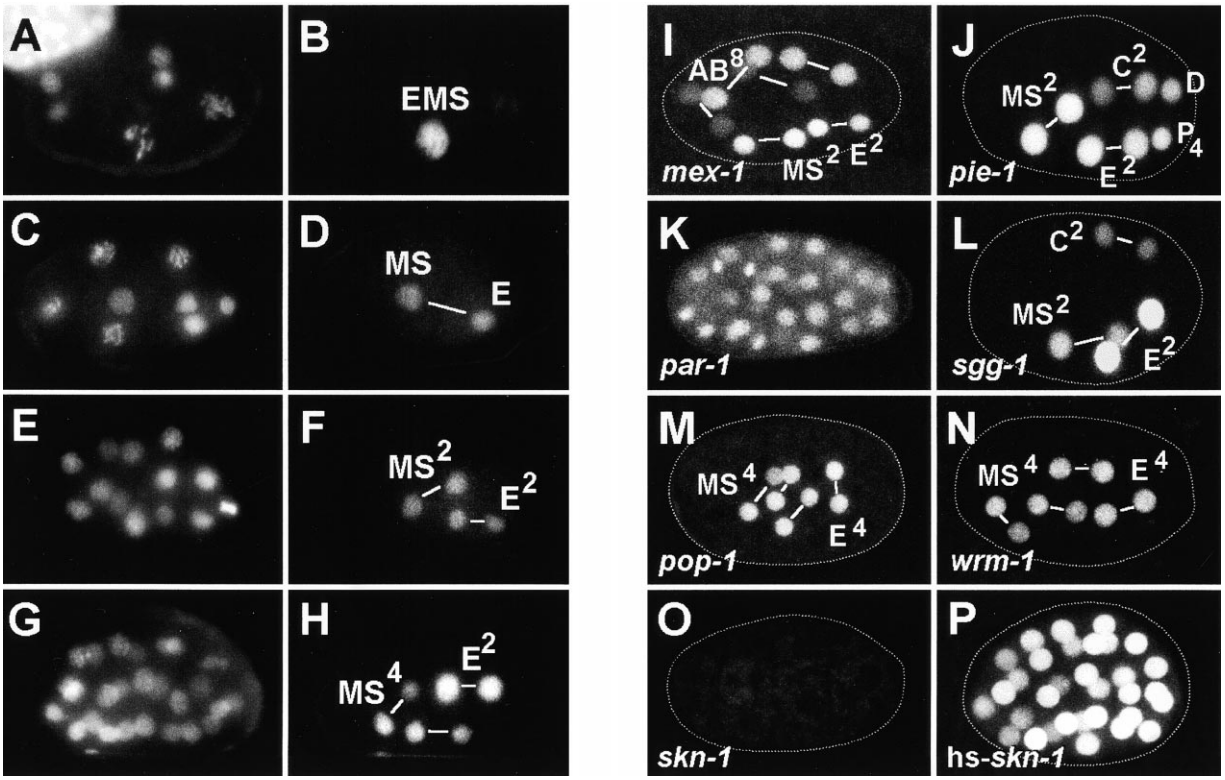

Figure 4. *med-1* Transgene Expression Correlates with SKN-1 Activity

(A–H) Nuclei stained with 4′,6-diamidino-2-phenylindole (DAPI, left column) and anti-cmyc staining (B) or GFP fluorescence (D, F, and H). (A and B) Onset of *med-1*::cmyc::MED-1 expression in a six-cell stage embryo. The bright fluorescence in the upper left of (A) is from an adjacent embryo. The faint signal in P_2 in (B) is nonspecific, as similar staining occurred in nontransgenic embryos (data not shown). (C–H) Expression of *med-1*::GFP::MED-1. (C and D) 8-cell stage; (E and F) 16-cell stage; (G and H) 28-cell stage.

(I–O) Expression of a chromosomally integrated *med-1*::GFP::MED-1 transgene in mutant backgrounds generated by RNAi. (I) Additional *med-1* expression in the AB lineage in a *mex-1(RNAi)* embryo. In *mex-1* mutants, SKN-1 levels become abnormally elevated in AB descendants, causing the granddaughters of AB to adopt MS-like fates and to produce GLP-1-independent pharynx inappropriately (Mello et al., 1992; Bowerman et al., 1993). (J) Expression in all P_1 descendants in a *pie-1(RNAi)* embryo. SKN-1 is able to activate MS-like and E-like fates in the daughters of P_2 when transcription is derepressed in a *pie-1* mutant (Mello et al., 1992). (K) Widespread expression of *med-1* in a *par-1(RNAi)* embryo. In *par-1* mutants, misexpression of SKN-1 frequently causes all four-cell stage blastomeres to produce GLP-1-independent pharynx (Bowerman et al., 1993). (L) Expression in descendants of EMS, and at slightly reduced levels in C descendants, in a *sgg-1(RNAi)* embryo. (M) and (N) *med-1* expression persists in EMS descendants in mutants lacking either the β-catenin homolog WRM-1 or the maternal Lef-1-like protein POP-1 (Lin et al., 1995; Rocheleau et al., 1997; Thorpe et al., 1997). (O) Absence of expression in *skn-1(RNAi)*, a result similar to that seen in *skn-1(zu67)* and *pos-1(RNAi)* (data not shown).

(P) Overexpression of SKN-1 from an independent hs-*skn-1* extrachromosomal array causes widespread *med-1* expression 90 min after heat shock.

Anterior is to the left, and dorsal is up.

pying up to ~70%, of the embryonic volume. Moreover, while EMS-ablated wild-type embryos always lack gut and pharynx (Priess and Thomson, 1986; Table 1), similar embryos expressing *med-1* ubiquitously generally differentiated large amounts of these tissues (Table 1). Similar effects were observed even in embryos lacking maternal contribution of either SKN-1 or another factor, POS-1 (data not shown), both of which are normally essential to specify the fate of EMS (Bowerman et al., 1992; Tabara et al., 1999). We conclude that *med-1* is a robust activator of mesendoderm development that can function independently of SKN-1 or POS-1 activity throughout the embryo.

med-1,2 Are Zygotically Expressed Downstream of SKN-1 in Mesendodermal Precursors

Based on translational fusion reporter constructs, *med-1* expression is first detectable in EMS at the six-cell stage (Figures 4A and 4B). Thereafter, expression becomes weaker and persists in all EMS daughters until there are 8 E and ~20 MS descendants (Figures 4C–4H; data not shown). A *med-2* reporter gives a similar expression pattern (data not shown). As transgenes are generally not expressed in the germline (Kelly et al., 1997), these patterns are likely to reflect zygotic expression of the *med*s. Consistent with early zygotic expression, *med-1* transcripts are reproducibly detected in 4-cell (EMS stage) embryos by RT–PCR and become largely undetectable by the 12-cell stage, after E and MS are born (S. Robertson and R. Lin, personal communication). Expression of maternal SKN-1 appears to anticipate expression of *med-1,2*: while SKN-1 is first detectable at the two-cell stage, its expression peaks at the four-cell stage, where it is at high levels in EMS and P_2 (Figure 1; Bowerman et al., 1993). SKN-1 is not active in P_2, because the nucleus of P_2 is kept transcriptionally

silent by maternal PIE-1 (Seydoux et al., 1996); thus, at the four-cell stage, SKN-1 activity is present specifically in EMS, where *med* expression is first detected.

We found that *med-1* expression and function correlate with SKN-1 activity when SKN-1 is inappropriately activated in non-EMS lineages. In several maternal mutants that show ectopic SKN-1 activity, we observed a corresponding ectopic expression of *med-1* (Figures 4I–4L). Moreover, the *med*s are required for production of ectopic MS- and E-like cells in these mutants (Table 1; Figures 3M–3P). We also found that ubiquitous zygotic expression of SKN-1 from a heat shock–driven hs-*skn-1* transgene causes widespread expression of both *med* reporters (Figure 4P; data not shown), suggesting that high SKN-1 levels are sufficient to activate the *med*s. Conversely, expression of both *med* reporters is blocked in *skn-1* and *pos-1* mutant embryos (Figure 4O; data not shown), consistent with an essential function for both in activation of zygotic *med* expression. We further explored the possibility that the *med*s are intimately required for specification of E and MS fates in any context by examining the requirement for the *med*s when E and MS fates are inappropriately apportioned within the EMS lineage. As expected, the *med*s are essential to specify the fates of the EMS daughters when both are transformed to E-like cells or both to MS-like cells, as the result of mutations in Wnt/MAPK pathway components (Table 1); *med* expression is unaffected under these conditions (Figures 4M and 4N). We conclude that the *med*s are activated by, and function downstream of, SKN-1 in the EMS lineage and are essential to specify E and MS fates in any context.

med-1 Is Directly Activated by Maternal SKN-1

The dependence of *med-1,2* expression on SKN-1 activity prompted us to look for evidence of a direct interaction between SKN-1 and the *med-1* promoter. A group of six SKN-1 consensus binding sites (Blackwell et al., 1994) is present in two overlapping clusters in the promoters of both *med-1* and *med-2* (Figures 2A and 5B). We tested the in vivo and in vitro function of these sites in *med-1*. First, we found that point mutations that simultaneously alter all of the SKN-1 sites abolish reporter expression, while a smaller 180 bp fragment containing the wild-type sites is sufficient for normal expression (Figures 5A and 5B). Further, we found that a polypeptide containing the SKN-1 DNA binding domain (Blackwell et al., 1994; Rupert et al., 1998) results in an electrophoretic mobility shift of this fragment, while point mutation of the sites abolishes this shift (Figures 5B and 5C). Taken with the reporter expression, heat shock overexpression, and genetic data, these results indicate that SKN-1 directly activates transcription of *med-1* in vivo, marking a direct link between a maternal regulator and a zygotic gene in *C. elegans*.

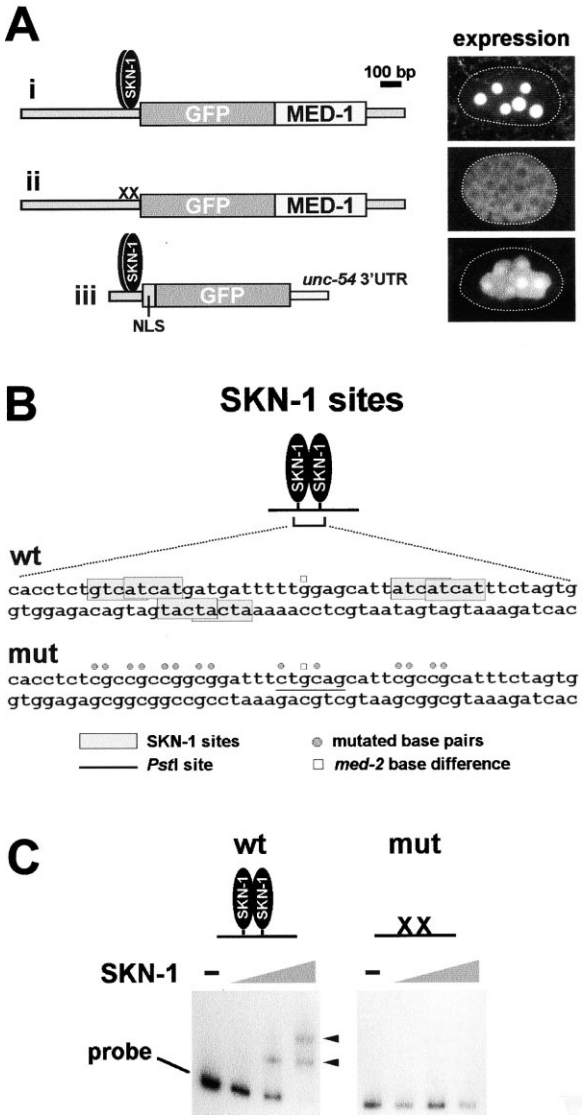

Figure 5. SKN-1 Interacts Directly with the *med-1* Promoter

(A) Reporter expression requires SKN-1 sites. (i) Unmodified *med-1*::GFP::MED-1 reporter. Putative SKN-1 site clusters are shown. (ii) Mutation of SKN-1 sites as in (B) abolishes expression and response to hs-*skn-1* (data not shown). (iii) A 180 bp fragment of the *med-1* promoter is sufficient to direct EMS lineage expression and response to hs-*skn-1* (data not shown). The reporter nuclear localization signal (NLS) permits some cytoplasmic GFP signal. Anterior is to the left, and dorsal is up.

(B) Nucleotide sequences of the SKN-1 binding region in wild-type (wt) and mutated (mut) forms of probe/promoters. Substitutions generated by PCR, and the single bp difference with the corresponding region of the *med-2* promoter, are also indicated.

(C) Electrophoretic mobility shift assay shows that recombinant SKN-1 shifts a wild-type 180 bp probe, but not a mutated probe. Assays were performed using a recombinant SKN-1 DNA binding fragment (a gift from P. Rupert) at 0, 150, 300, and 600 nM as described (Blackwell et al., 1994).

SGG-1/GSK-3β Kinase Inhibits SKN-1-Mediated Activation of *med-1,2* in the C Lineage

While SKN-1 protein is expressed outside the EMS lineage (Figure 1; Bowerman et al., 1993), at least two activities restrict SKN-1-dependent *med* activation to EMS: PIE-1 represses transcription in the P lineage (Seydoux and Fire, 1994), and POS-1, which is required for transcriptional activation of the *med*s by SKN-1 in EMS, is absent from the AB lineage (Tabara et al., 1999). However, although the C cell, like EMS, contains SKN-1 and POS-1, and lacks PIE-1 (Seydoux and Fire, 1994; Tabara et al., 1999), the *med*s are not expressed, and mesendoderm is not made, in the C lineage. This difference between C and EMS cannot be explained by the C-pro-

moting activity of PAL-1 per se, since PAL-1 is present in both EMS and C, and removal of PAL-1 does not cause C to adopt an EMS-like fate (Hunter and Kenyon, 1996). Why then does PAL-1 override SKN-1 in C, allowing development of muscle and epidermis instead of mesendoderm?

A *C. elegans* GSK-3β homolog, SGG-1, has been shown to act positively in the *C. elegans* Wnt pathway by transducing the Wnt signal that both reorients the EMS mitotic spindle and induces endoderm from EMS (Schlesinger et al., 1999). Paradoxically, however, while E frequently fails to make endoderm, the C cell often produces endoderm in *sgg-1* mutant embryos. We asked whether this transformation results from aberrant expression of the *med*s in the C lineage and found, indeed, that *med-1* is expressed in both EMS and C descendants in *sgg-1* mutants (Figure 4L). This ectopic expression is SKN-1 dependent, as expression of *med-1*::GFP was not detected in a *skn-1(zu67); sgg-1(RNAi)* background (n = 21). Moreover, the ectopic gut produced in *sgg-1* mutants requires *skn-1(+)* activity (Table 2).

Because the cascade of SKN-1 and MED activities in EMS results in specification of the fates of both its daughters, E and MS, we postulated that C might adopt an EMS-like, rather than an E-like fate in *sgg-1(RNAi)* embryos. The nucleus of Cp, the posterior daughter of C, contains lower POP-1 levels than its anterior sister, Ca, analogous to the lower levels in E vs. MS (Lin et al., 1998). We therefore predicted that in *sgg-1* mutants, only Cp might adopt an E-like fate. Indeed, expression of an E-lineage marker, *end-1*::GFP, is seen only in Cp descendants in *sgg-1(RNAi)* (data not shown). However, Ca does not appear to adopt an MS-like fate in these mutants, since pharynx was not produced when P_2 (the mother of C) was isolated in *sgg-1(−)* embryos (Table 2). We hypothesized that in *sgg-1(−)* embryos, PAL-1 competes with the MEDs in Ca, thereby blocking *med*-dependent activation of MS fate. Indeed, we found that simultaneous removal of both SGG-1 and PAL-1 not only results in transformation of Cp to an E-like cell, but also Ca to an MS-like cell (Table 2). Both the MS-like and E-like fates adopted by the C daughters require SKN-1 and MED-1,2 (Table 2).

We conclude that SGG-1 prevents SKN-1-dependent activation of the *med*s in the C lineage, revealing the mechanism that prevents the C lineage from generating mesendoderm.

Discussion

We have identified two zygotic regulators, MED-1 and -2, that are necessary and sufficient to program *C. elegans* mesendoderm development. We find that EMS-restricted *med* expression, and mesendoderm development, require the combined action of a number of maternal factors that control embryonic polarity or transcriptional regulation, explaining how mesendoderm development is limited to a single blastomere (Figure 6). These findings reveal that, although mesendoderm arises from a broad territory of many thousands of cells in vertebrates and only a single cell in *C. elegans*, the gene regulatory network underlying mesendoderm is similar in these widely divergent organisms (discussed below).

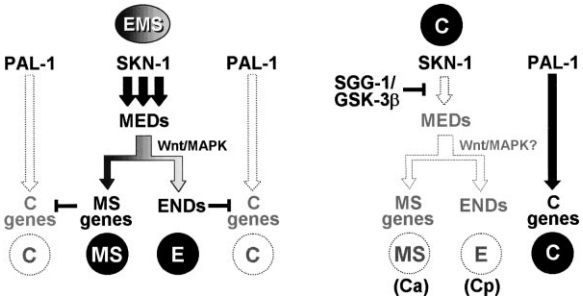

Figure 6. A Model for SKN-1-Dependent Mesendoderm Specification

Maternal SKN-1 activates *med* expression in the mesendodermal precursor EMS. In the anterior daughter MS, MED-1 and MED-2 (MEDs) promote MS fate in conjunction with unmodified POP-1. In E, Wnt/MAPK signaling results in modification of POP-1, permitting the MEDs to promote endoderm fate by activating *end-1* and *end-3* (ENDs). Although PAL-1 protein is present in EMS, C fate is blocked by the targets of the MEDs. In the C cell, SGG-1/GSK-3β blocks the ability of SKN-1 to activate the *med*s. The reduced amounts of SKN-1 in wild-type C as compared to EMS may result in lower activity, shown by only one arrow between SKN-1 and the MEDs. For simplicity, POP-1 levels, the GLP-1/Notch ligand activated by SKN-1, and the requirement for POS-1 in *med* expression are not shown.

med-1,2 Act at the Convergence of SKN-1 and POS-1 Activities

skn-1, pos-1, and *med-1,2* mutant embryos show many similarities with regard to EMS defects: MS and E fail to produce their normal cell types and instead produce cells normally made by the C blastomere (Bowerman et al., 1992; Tabara et al., 1999). As neither *skn-1* nor *pos-1* mutant embryos express *med-1,2*, which are direct targets of SKN-1, the EMS defects in *skn-1* and *pos-1* mutants are attributable to the failure to activate *med-1,2*. As SKN-1 protein is expressed normally in *pos-1* mutants, and POS-1 itself is cytoplasmic (Tabara et al., 1999), *pos-1* may function indirectly to allow SKN-1 to activate *med-1,2* in EMS. The *med* genes cannot be the sole targets of SKN-1, however; while pharynx is completely absent in *skn-1* mutants (Bowerman et al., 1992), *med-1,2* and *pos-1* mutants lack only MS-derived pharynx (Tabara et al., 1999). Thus, the transformed MS cell in *med-1,2* and *pos-1* mutants is nonetheless capable of inducing secondary mesoderm, including pharynx, in AB by the GLP-1/Notch pathway. There must therefore be at least one other zygotic SKN-1 target, a Delta-like ligand for the GLP-1 receptor (perhaps encoded by redundant genes), in the EMS lineage.

Wnt/MAPK Signaling and Modulation of MED-1,2 Specificity

The MEDs are able to activate two very different developmental programs, resulting in specification of the MS and E lineages respectively. The apportionment of these two fates is determined by the Wnt/MAPK-signaled status of each EMS daughter. While E fate is specified by Wnt/MAPK signaling, MS fate is specified in the absence of signaling (Rocheleau et al., 1997; Thorpe et al., 1997). How might the MEDs respond to a cell's Wnt/MAPK status? One possibility is that the MEDs promote MS

fate by default; in a signaled cell (E), MED target specificity is altered, allowing activation of the E-specifying *end* genes. Alternatively, the MEDs by themselves might promote E fate; POP-1 in its nonsignaled form would block *end-1,3* activation in MS, allowing the MEDs to activate MS genes. Indeed, when *med-1* is expressed throughout early embryos, endoderm is the predominant fate adopted by non-EMS blastomeres; such embryos also show widespread expression of *end-1* and *end-3* (M. F. M. and J. H. R., unpublished observations). We propose, therefore, that the MEDs activate the *end* genes in E, while in MS, POP-1 repression of the *end*s allows the MEDs to activate MS-specific target genes.

SGG-1/GSK-3β and Mesendoderm Development in *C. elegans*

GSK-3β is generally found to act as a negative regulator in the Wnt signaling pathway (Cadigan and Nusse, 1997). This versatile kinase phosphorylates β-catenin, a coactivator of the transcription factor Lef-1, resulting in its degradation. However, SGG-1, a GSK-3β homolog in *C. elegans*, appears to act positively in the Wnt pathway, being required for Wnt-mediated polarization of both endoderm potential and mitotic spindle alignment within EMS (Schlesinger et al., 1999). In the absence of SGG-1 and certain other Wnt pathway components, both endoderm induction and spindle orientation in EMS are defective, suggesting that these gene products act in a common process (Rocheleau et al., 1997; Thorpe et al., 1997; Schlesinger et al., 1999). Nevertheless, no biochemical activities have been demonstrated for SGG-1/GSK-3β, and its precise role in regulating either aspect of EMS polarity remains undefined.

Our results show that in addition to its roles in polarizing EMS, SGG-1/GSK-3β performs a novel function in the C cell. In the C lineage, SGG-1/GSK-3β is required to block SKN-1-dependent activation of the *med* genes, thus preventing the ectopic generation of EMS-like fates. We found, however, that while SKN-1 activity in *sgg-1(−)* results in the conversion of Cp to an E-like cell, PAL-1 must also be depleted in *sgg-1(−)* to reveal a Ca to MS transformation. In wild-type embryos, SKN-1 is present at lower levels in C than in EMS, while PAL-1 protein persists in the C daughters, where SKN-1 is not detectable (Bowerman et al., 1993; Hunter and Kenyon, 1996). We propose that the higher PAL-1:SKN-1 ratio in C compared to EMS prevents the MEDs from activating MS fates in the Ca lineage when *sgg-1(−)* is absent.

How does SGG-1/GSK-3β influence the activity of SKN-1 in C? SGG-1 could prevent *med* activation in C by directly inactivating SKN-1 through phosphorylation. Alternatively, depletion of SGG-1 might influence C fate indirectly by altering the transcriptional status of chromatin, thereby allowing SKN-1 to gain access to the *med* genes. Regardless of the mechanism of SGG-1/GSK-3β function, there is no evidence that Wnt signaling influences its activity in C. In addition to its classically described role in glucagon-regulated glycogen metabolism, there are precedents for regulation and function of GSK-3 in a developmental context by non-Wnt mechanisms (e.g., Dominguez and Green, 2000; Hoeflich et al., 2000) and it is reasonable to suppose that SGG-1 acts through a Wnt-independent mechanism in the C lineage. Other mutants that result in production of endoderm from the C lineage have been identified (J. Priess, personal communication; M. Soto and C. Mello, personal communication); these may reveal a new pathway in which SGG-1 acts and might unveil how its SKN-1-inhibitory action is restricted to the C lineage.

Similar Mechanisms Regulate Mesendoderm Development in Vertebrates and *C. elegans*

In vertebrates, a broad set of cell types is generated from a single tissue territory, mesendoderm. The mesendoderm becomes subdivided into endoderm and a subset of the mesoderm ("splanchnopleural") that generates heart and blood (Warga and Nüsslein-Volhard, 1999). Similarly, in *C. elegans*, a broad set of cell types is generated from a single cell, EMS. The EMS lineage becomes subdivided into endoderm (E cell) and a subset of mesoderm (MS cell) that generates part of the heart-like pharynx (Haun et al., 1998) and coelomocytes (putative primitive blood cells). Both zebrafish and *C. elegans* first express a GATA factor (Faust/GATA5 in zebrafish; Reiter et al., 1999; MED-1,2 in *C. elegans;* this work) throughout the mesendoderm prior to gastrulation that is sufficient to direct mesendoderm development in non-mesendodermal cells. In both *C. elegans* and vertebrates, a set of conserved regulators acts after each germ layer type has been segregated from the mesendoderm. These include HNF-3-like and HNF-4-like factors in the endoderm (Duncan et al., 1994; Azzaria et al., 1996; Odenthal and Nüsslein-Volhard, 1998; K. Koh and J. H. R., unpublished results) and the cardiac/pharynx-promoting Nkx2.5/CEH-22 factors in the mesoderm (Okkema and Fire, 1994; Evans, 1999). These observations suggest that a conserved gene regulatory network may underly mesendoderm specification in all triploblastic metazoans. The generation of a common progenitor of endoderm and a subset of the mesoderm may reflect a decisive event in metazoan evolution that has been preserved in both a large group of cells in vertebrates and in a single cell in *C. elegans*.

Experimental Procedures

Plasmids and Cloning

DNA manipulations and PCR were performed according to standard protocols. The *med-1*::GFP::MED-1 reporter pMM280 was constructed by introducing a BamHI site after the predicted start codon by ligation-mediated PCR and inserting an S65C GFP coding region amplified from plasmid pPD95.67 (a gift from A. Fire). A fragment encoding the 35 carboxy-terminal amino acids of c-myc was similarly inserted to make the *med-1*::cmyc::MED-1 plasmid pMM410. Both plasmids retain the MED-1 ORF and the 5′ and 3′ flanking sequences conserved with the *med-2* locus. The *med-2*::GFP reporter pMM266 was made by cloning a 1.0 kbp PCR product (containing 700 bp of 5′ flanking sequence and 106 codons of the *med-2* ORF) into pPD95.67. Reporter fusions of *med-1* and *med-2* with longer 5′ sequences (up to 2.2 kbp) also show EMS lineage expression, at reduced intensity. Heat shock constructs were made by cloning the *med-1* or *skn-1* ORFs into both pPD49.78 and pPD49.83 (from A. Fire). A *pop-1* cDNA clone was a gift from R. Lin; a *pal-1* cDNA was a gift from B. Tsung and C. Hunter; cosmids T24D3 and K04C2 were from A. Coulson. Oligonucleotide sequences are available upon request.

Reverse Genetics

Southern hybridization confirmed that *med-1* and *med-2* are distinct (data not shown). The *med-1 X* ORF is located on cosmid T24D3,

bases 8954–9475 and partially overlaps the predicted gene T24D3.1. The *med-2 III* ORF is on K04C2, bases 15188–15709. RNA synthesis was performed using T7 MEGAscript (Ambion) on PCR-generated templates tagged with the T7 promoter sequence. RNA was purified by organic extraction and alcohol precipitation as described in the manual and injected as described (Fire et al.,1998). dsRNA with the entire *med-1* or *med-2* ORF, or the *med-1* ORF lacking the DNA binding domain, gave similar interference results. Injection of *med-1* dsRNA eliminated expression of *med-1* and *med-2* GFP reporters in a fraction of progeny embryos. Although RNAi targeted to some genes can produce 100% mutant progeny (Fire et al., 1998), those fertilized 5–7 hr postinjection at 20°C show maximal *med-1,2* interference (50%–70% mutant). As a similar window occurs with *end-1* and *end-3*, which are required zygotically for E fate (M. F. M. and J. H. R., unpublished data), this may be a general feature of RNAi targeted to early zygotic genes. Some *med* mutant embryos were produced by heat shock overexpression of sense and antisense cDNAs from an array, after Tavernarakis et al. (2000). Control injections of dsRNAs targeted to maternal genes reproduced their known null phenotypes. For *glp-1(RNAi)*, although all embryos lacked anterior pharynx, 10% elongated, resembling *glp-1(e2072)* mutants (Priess et al., 1987). Multiply phenocopied embryos were produced by simultaneous dsRNA injection, except for *sgg-1; pal-1; med-1,2* mutants, for which *sgg-1* and *pal-1* dsRNAs were coinjected, followed by *med-1* dsRNA after 24 hr.

C. elegans Strains and Manipulation

culs2 V (*ceh-22*::GFP) was a gift from Peter Okkema; *ccls7963 V* (*hlh-1*::GFP) was a gift from J. Hsieh and A. Fire; a *lag-2*::GFP strain (*wls42*) was made by M. Fukuyama from pJK597 (a gift from D. Gao and S. Henderson); an *elt-2*::GFP strain (*wls84*) was made by D. Braun using pJM66 (a gift from T. Fukushige and J. McGhee); an *end-1*::GFP strain (*wls28*) was made by J. Zhu; *unc-119(ed3) III* was made in the laboratory of D. Pilgrim (Maduro and Pilgrim, 1995); N2, EU1 (a balanced *skn-1(zu67)* strain) and *him-8(e1489) V* were obtained from the *C. elegans* Genetics Center (St. Paul, MN). DNA injections were performed as described (Mello et al., 1991) using either *rol-6*^{D} (pRF4; Mello et al., 1991) or *unc-119* rescue (pDP#MM016B; Maduro and Pilgrim, 1995) as transformation markers. At least two independent lines were analyzed for each reporter. Integrants were induced by γ-rays (3000 rad) or UV (400 J/m^2) and identified by screening F_2 progeny for 100% transmission. To construct the hs-*skn-1*; *med-1*::GFP strain, *unc-119(ed3)* males rescued by a [hs-*skn-1* + *unc-119*(+)] array were mated with *unc-119(ed3)* hermaphrodites carrying an independent [*med-1*::GFP + *rol-6*^{D}] array; Rol non-Unc progeny were used to establish double-transgenic lines. A similar strategy was used for other hs + reporter combinations. For heat shock experiments, embryos were incubated for 30 min at 33°C. In terminal hs-*med-1* embryos, pharynx was scored by mAb9.2.1 staining or expression of *ceh-22*::GFP (Okkema and Fire, 1994), and gut was scored by mAb1CB4 staining or gut granule birefringence.

Microscopy and Immunofluorescence

Fixation and antibody staining were performed as described (Zhu et al., 1997). The cmyc epitope was detected with mAb9E10 (Boehringer Mannheim; 1:200). DIC and fluorescence images were acquired with an Optronics VI-470 camera. Confocal micrographs were made using a BioRad 1024 microscope. Fluorescence images from multiple focal planes were combined and enhanced for contrast using Photoshop 4.0 (Adobe) and Confocal Assistant 4.02 (T.C. Brelje).

Acknowledgments

The authors wish to thank Roger Patient for insightful discussions; Scott M. Robertson and Rueyling Lin for communicating unpublished results; D. Braun, S. Gendreau, M. Fukuyama, P. Okkema, A. Fire, J. Hsieh, T. Fukushige, J. McGhee, A. Fire, R. Lin, B. Tsung, C. Hunter, and A. Coulson for strains and plasmids; P. Rupert for providing the purified SKN-1 binding domain polypeptide; and A. Labrousse and A. van der Bliek for providing access to a gamma irradiator. M.F.M. is a postdoctoral fellow of the Natural Sciences and Engineering Research Council of Canada. Some of the strains used in this work were provided by the Caenorhabditis Genetics Center, which is funded by the National Center for Research Resources of the National Institutes of Health (NIH). This work was supported by grants from the NIH (GM49869) to B. B., and from the March of Dimes (FY2000-659) and the NIH (HD37487) to J. H. R.

Received August 29, 2000; revised January 25, 2001.

References

Angerer, L.M., and Angerer, R.C. (2000). Animal-vegetal axis patterning mechanisms in the early sea urchin embryo. Dev. Biol. *218*, 1–12.

Azzaria, M., Goszczynski, B., Chung, M.A., Kalb, J.M., and McGhee, J.D. (1996). A fork head/HNF-3 homolog expressed in the pharynx and intestine of the *Caenorhabditis elegans* embryo. Dev. Biol. *178*, 289–303.

Batchelder, C., Dunn, M.A., Choy, B., Suh, Y., Cassie, C., Shim, E.Y., Shin, T.H., Mello, C., Seydoux, G., and Blackwell, T.K. (1999). Transcriptional repression by the *Caenorhabditis elegans* germ-line protein PIE-1. Genes Dev. *13*, 202–212.

Blackwell, T.K., Bowerman, B., Priess, J., and Weintraub, H. (1994). Formation of a monomeric DNA binding domain by Skn-1 bZIP and homeodomain elements. Science *266*, 621–628.

Bowerman, B., Eaton, B.A., and Priess, J.R. (1992). *skn-1*, a maternally expressed gene required to specify the fate of ventral blastomeres in the early *C. elegans* embryo. Cell *68*, 1061–1075.

Bowerman, B., Draper, B.W., Mello, C.C., and Priess, J.R. (1993). The maternal gene *skn-1* encodes a protein that is distributed unequally in early *C. elegans* embryos. Cell *74*, 443–452.

Bowerman, B., Ingram, M.K., and Hunter, C.P. (1997). The maternal *par* genes and the segregation of cell fate specification activities in early *Caenorhabditis elegans* embryos. Development *124*, 3815–3826.

Cadigan, K.M., and Nusse, R. (1997). Wnt signaling: a common theme in animal development. Genes Dev. *11*, 3286–3305.

Dominguez, I., and Green, J.B. (2000). Dorsal downregulation of GSK3beta by a non-Wnt-like mechanism is an early molecular consequence of cortical rotation in early *Xenopus* embryos. Development *127*, 861–868.

Duncan, S.A., Manova, K., Chen, W.S., Hoodless, P., Weinstein, D.C., Bachvarova, R.F., and Darnell, J.E. (1994). Expression of transcription factor HNF-4 in extraembryonic endoderm, gut, and nephrogenic tissue of the developing mouse embryo: HNF-4 is a marker for primary endoderm in the implanting blastocyst. PNAS *91*, 7598–7602.

Evans, S.M. (1999). Vertebrate tinman homologues and cardiac differentiation. Semin. Cell. Dev. Biol. *10*, 73–83.

Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., and Mello, C.C. (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature *391*, 806–811.

Fukushige, T., Hawkins, M.G., and McGhee, J.D. (1998). The GATA-factor *elt-2* is essential for formation of the *Caenorhabditis elegans* intestine. Dev. Biol. *198*, 286–302.

Goldstein, B. (1992). Induction of gut in *Caenorhabditis elegans* embryos. Nature *357*, 255–257.

Goldstein, B. (1993). Establishment of gut fate in the E lineage of *C. elegans*: the roles of lineage-dependent mechanisms and cell interactions. Development *118*, 1267–1277.

Goldstein, B. (1995). An analysis of the response to gut induction in the *C. elegans* embryo. Development *121*, 1227–1236.

Haun, C., Alexander, J., Stainier, D.Y., and Okkema, P.G. (1998). Rescue of *Caenorhabditis elegans* pharyngeal development by a vertebrate heart specification gene. PNAS *95*, 5072–5075.

Henderson, S.T., Gao, D., Lambie, E.J., and Kimble, J. (1994). *lag-2* may encode a signaling ligand for the GLP-1 and LIN-12 receptors of *C. elegans*. Development *120*, 2913–2924.

Hoeflich, K.P., Luo, J., Rubie, E.A., Tsao, M.S., Jin, O., and Woodgett,

J.R. (2000). Requirement for glycogen synthase kinase-3beta in cell survival and NF-kappaB activation. Nature *406*, 86–90.

Hunter, C., and Kenyon, C. (1996). Spatial and temporal control target *pal-1* blastomere-specification activity to a single blastomere lineage in *C. elegans* embryos. Cell *87*, 217–226.

Kaletta, T., Schnabel, H., and Schabel, R. (1997). Binary specification of the embryonic lineage in *Caenorhabditis elegans*. Nature *390*, 294–298.

Kelly, W.G., Xu, S.Q., Montgomery, M.K., and Fire, A. (1997). Distinct requirements for somatic and germline expression of a generally expressed *Caenorhabditis elegans* gene. Genetics *146*, 227–238.

Kimelman, D., and Griffin, K.J. (2000). Vertebrate mesendoderm induction and patterning. Curr. Opin. Genet. Dev. *10*, 350–356.

Krause, M., Fire, A., Harrison, S.W., Priess, J., and Weintraub, H. (1990). CeMyoD accumulation defines the body wall muscle cell fate during *C. elegans* embryogenesis. Cell *63*, 907–919.

Labouesse, M., Hartwieg, E., and Horvitz, H.R. (1996). The *Caenorhabditis elegans* LIN-26 protein is required to specify and/or maintain all non-neuornal ectodermal cell fates. Development *122*, 2579–2588.

Lin, R., Thompson, S., and Priess, J.R. (1995). *pop-1* encodes an HMG box protein required for the specification of a mesoderm precursor in early *C. elegans* embryos. Cell *83*, 599–609.

Lin, R., Hill, R.J., and Priess, J.R. (1998). POP-1 and anterior-posterior fate decisions in *C. elegans* embryos. Cell *92*, 229–239.

Maduro, M., and Pilgrim, D. (1995). Identification and cloning of *unc-119*, a gene expressed in the *Caenorhabditis elegans* nervous system. Genetics *141*, 977–988.

Mello, C.C., Kramer, J.M., Stinchcomb, D., and Ambros, V. (1991). Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. EMBO J. *10*, 3959–3970.

Mello, C.C., Draper, B.W., Krause, M., Weintraub, H., and Priess, J.R. (1992). The *pie-1* and *mex-1* genes and maternal control of blastomere identity in early *C. elegans* embryos. Cell *70*, 163–178.

Mello, C.C., Schubert, C., Draper, B., Zhang, W., Lobel, R., and Priess, J.R. (1996). The PIE-1 protein and germline specification in *C. elegans* embryos. Nature *382*, 710–712.

Meneghini, M.D., Ishitani, T., Carter, J.C., Hisamoto, N., Ninomiya-Tsuji, J., Thorpe, C.J., Hamill, D.R., Matsumoto, K., and Bowerman, B. (1999). MAP kinase and Wnt pathways converge to downregulate an HMG-domain repressor in *Caenorhabditis elegans*. Nature *399*, 793–797.

Moskowitz, I.P.G., and Rothman, J. (1996). *lin-12* and *glp-1* are required zygotically for early embryonic cellular interactions and are regulated by maternal GLP-1 signaling in *Caenorhabditis elegans*. Development *122*, 4105–4117.

Odenthal, J., and Nüsslein-Volhard, C. (1998). Fork head domain genes in zebrafish. Dev. Genes Evol. *208*, 245–258.

Okkema, P., and Fire, A. (1994). The *Caenorhabditis elegans* NK-2 class homeoprotein CEH-22 is involved in combinatorial activation of gene expression in pharyngeal muscle. Development *120*, 2175–2186.

Osada, S.I., and Wright, C.V. (1999). *Xenopus* nodal-related signaling is essential for mesendodermal patterning during early embryogenesis. Development *126*, 3229–3240.

Priess, J.R., and Thomson, J.N. (1986). Cellular interactions in early *C. elegans* embryos. Cell *48*, 241–250.

Priess, J.R., Schnabel, H., and Schnabel, R. (1987). The *glp-1* locus and cellular interactions in the early *C. elegans* embryo. Cell *51*, 601–611.

Reiter, J.F., Alexander, J., Rodaway, A., Yelon, D., Patient, R., Holder, N., and Stainier, D.Y. (1999). Gata5 is required for the development of the heart and endoderm in zebrafish. Genes Dev. *13*, 2983–2995.

Rocheleau, C.E., Downs, W.D., Lin, R., Wittmann, C., Bei, Y., Cha, Y.-H., Ali, M., Priess, J.R., and Mello, C.C. (1997). Wnt signaling and an APC-related gene specify endoderm in early *C. elegans* embryos. Cell *90*, 707–716.

Rocheleau, C.E., Yasuda, J., Shin, T.H., Lin, R., Sawa, H., Okano, H., Priess, J.R., Davis, R.J., and Mello, C.C. (1999). WRM-1 activates the LIT-1 protein kinase to transduce anterior/posterior polarity signals in *C. elegans*. Cell *97*, 717–726.

Rupert, P.B., Daughdrill, G.W., Bowerman, B., and Matthews, B.W. (1998). A new DNA-binding motif in the Skn-1 binding domain-DNA complex. Nat. Struct. Biol. *5*, 484–491.

Schierenberg, E. (1987). Reversal of cellular polarity and early cell-cell interaction in the embryos of *Caenorhabditis elegans*. Dev. Biol. *122*, 452–463.

Schlesinger, A., Shelton, C.A., Maloof, J.N., Meneghini, M., and Bowerman, B. (1999). Wnt pathway components orient a mitotic spindle in the early *Caenorhabditis elegans* embryo without requiring gene transcription in the responding cell. Genes Dev. *13*, 2028–2038.

Schnabel, R., and Priess, J.R. (1997). Specification of cell fates in the early embryo. In C. elegans II, D.L. Riddle, T. Blumenthal, B.J. Meyer, and J.R. Priess, eds. (Cold Spring Harbor, NY: CSHL Press), pp. 361–382.

Seydoux, G., and Fire, A. (1994). Soma-germline asymmetry in the distributions of embryonic RNAs in *Caenorhabditis elegans*. Development *120*, 2823–2834.

Seydoux, G., Mello, C.C., Pettitt, J., Wood, W.B., Priess, J.R., and Fire, A. (1996). Repression of gene expression in the embryonic germ lineage of *C. elegans*. Nature *382*, 713–716.

Spieth, J., Shim, Y.H., Lea, K., Conrad, R., and Blumenthal, T. (1991). *elt-1*, an embryonically expressed *Caenorhabditis elegans* gene homologous to the GATA transcription factor family. Mol. Cell. Biol. *11*, 4651–4659.

Stringham, E.G., Dixon, D.K., Jones, D., and Candido, E.P.M. (1992). Temporal and spatial expression patterns of the small heat shock (hsp-16) genes in transgenic *Caenorhabditis elegans*. Mol. Biol. Cell *3*, 221–233.

Sulston, J.E., Schierenberg, E., White, J.G., and Thomson, J.N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev. Biol. *100*, 64–119.

Tabara, H., Hill, R.J., Mello, C.C., Priess, J.R., and Kohara, Y. (1999). *pos-1* encodes a cytoplasmic zinc-finger protein essential for germline specification in *C. elegans*. Development *126*, 1–11.

Tavernarakis, N., Wang, S.L., Dorovkov, M., Ryazanov, A., and Driscoll, M. (2000). Heritable and inducible genetic interference by double-stranded DNA encoded by transgenes. Nat. Genet. *24*, 180–183.

Tenenhaus, C., Schubert, C., and Seydoux, G. (1998). Genetic requirements for PIE-1 localization and inhibition of gene expression in the embryonic germ lineage of *Caenorhabditis elegans*. Dev. Biol. *200*, 212–224.

Thorpe, C.H., Schlesinger, A., Carter, J.C., and Bowerman, B. (1997). Wnt signaling polarizes an early *C. elegans* blastomere to distinguish endoderm from mesoderm. Cell *90*, 795–705.

Warga, R.M., and Nüsslein-Volhard, C. (1999). Origin and development of the zebrafish endoderm. Development *128*, 827–838.

Zhu, J., Hill, R.J., Heid, P.J., Fukuyama, M., Sugimoto, A., Priess, J.R., and Rothman, J.H. (1997). *end-1* encodes an apparent GATA factor that specifies the endoderm precursor in *Caenorhabditis elegans* embryos. Genes Dev. *11*, 2883–2896.