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Developmental patterning in the *Caenorhabditis elegans* hindgut

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Abstract

Developmental pattern formation allows cells within a tissue or organ to coordinate their development and establish cell types in relationship to one another. To better characterize the developmental patterning events within one organ, the *C. elegans* hindgut, we have analyzed the expression pattern of several genes using green fluorescent protein-based reporter transgenes. In wild-type animals, these genes are expressed in subsets of hindgut cells rather than in individual cell types. In mutant animals, we find that some, but not all, genes expressed in cells with altered development exhibit a corresponding alteration of gene expression. The results are consistent with a model where a combination of factors contribute to each cell's fate, and address how developmental information converges to specify cell types.

Keywords: Cell lineage; EGL-38; Organogenesis

Introduction

During animal development, different cell types result from events that establish and refine developmental patterns. The development of the *C. elegans* hindgut provides an example where developmental patterning occurs among cells of divergent developmental backgrounds. Within the hindgut, 11 cells arrange into five tiers (Fig. 1; Sulston et al., 1983). These 11 cells include eight distinct cell types that arise in the embryonic cell lineage from diverse points (Fig. 2). Genetic analysis has provided insight into some features of the patterning events that contribute to the distinct cell types. For example, mutations in *mab-9* result in the dorsal posterior hindgut cells F and B developing like their ventral neighbors U and Y (Chisholm and Hodgkin, 1989), whereas mutations in *egl-38* result in the F and U cells developing with some features of their posterior neighbors B and Y (Chamberlin et al., 1997). These mutants indicate that regional patterning plays a role in the development of this organ.

Identification and analysis of existing mutants with defective hindgut development have utilized the fact that cells of the posterior hindgut (U, F, B, and Y) are male-specific blast cells (Sulston et al., 1980). To complement and extend the cell lineage analysis of mutants, we have collected and characterized a set of genes that serve as molecular markers of hindgut cells. These genes are expressed in subsets of hindgut cells and together allow the different types to be distinguished. The gene expression patterns reflect subdivisions within the organ that may correspond to developmental patterning events and functionally relevant patterns of gene activation. We have tested this idea by using molecular and genetic analyses.

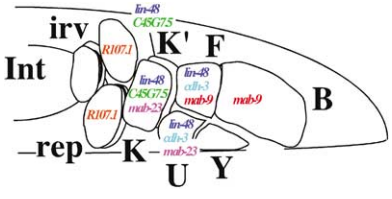

Fig. 1. Diagram of hindgut cells in early L1 stage, including genes reported to express within the cells (after Chamberlin et al., 1999). Anterior left, dorsal up in all figures.

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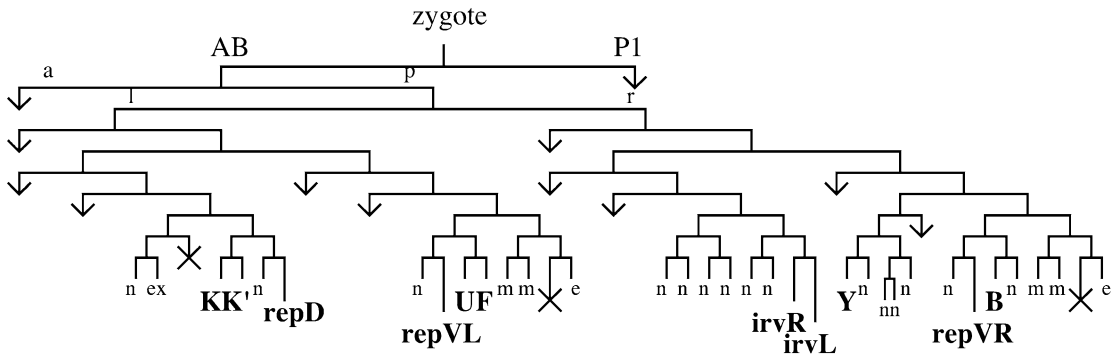

Fig. 2. Embryonic lineage relationship among cells of the hindgut (after Sulston et al., 1983). A vertical line represents a cell and a horizontal line represents a cell division. An arrow indicates the cell divides further, but does not produce any cells of the hindgut. n, neuron; m, muscle; e, epidermis; ex, excretory cell; ×, cell death.

Table 1
Summary of hindgut gene expression patterns

Gene	Cell								Reference
	irv	rep	K	K′	U	F	B	Y	
2CB7	+	−	−	−	−	−	−	−	Bowerman et al., 1992
R107.1	−	+	−	−	−	−	−	−	This work; Lynch et al., 1995
ceh-6	−	−	+	−	+	+	+	+	Burglin and Ruvkun, 2001
egl-5	−	−	+	−	+	+	+	+	Wang et al., 1993
lin-48	−	−	+	+	+	+	−	−	Johnson et al., 2001
C45G7.5	−	−	+	+	−	−	−	−	This work
cdh-3	−	−	−	−	+	+	−	−	Pettitt et al., 1996
mab-23	−	−	+	−	+	−	−	−	Lints and Emmons, 2002
mab-9	−	−	−	−	−	+	+	−	Woollard and Hodgkin, 2000

Table 2
Expression of hindgut genes in *egl-38* mutants[a]

Transgene	Expression	*egl-38* genotype	% of animals with expression in *x* cells[d]					*N*
			4	3	2	1	0	
R107.1::gfp	RepD, RepVL, RepVR	+		**100**	0	0	0	25
		sy294		**96**	4	0	0	25
		s1775		**100**	0	0	0	20
lin-48::gfp[b]	K, K′, U, F	+	**57**	26	16	1	0	69
		sy294	0	0	0	0	**100**	30
		s1775	0	0	0	0	**100**	20
cdh-3::gfp[c]	U, F	+			**72**	15	13	109
		sy294			47	43	10	108
		s1775			50	30	20	20
mab-23::gfp	K, U	+			**74**	26	0	62
		sy294			0	0	**100**	30
C45G7.5::gfp	K, K′	+			**62**	16	22	87
		sy294			12	4	**84**	25
		s1775			0	9	**91**	23

[a] The category with the majority of animals is indicated in boldface.
[b] Data summarized from Johnson et al. (2001)
[c] Wild type and *egl-38(sy294)* data summarized from Chamberlin et al. (1999).
[d] Percentage of animals observed with expression in the indicated (*x*) number of cells: 4 = 4 cells expressing, 3 = 3 cells expressing, etc.

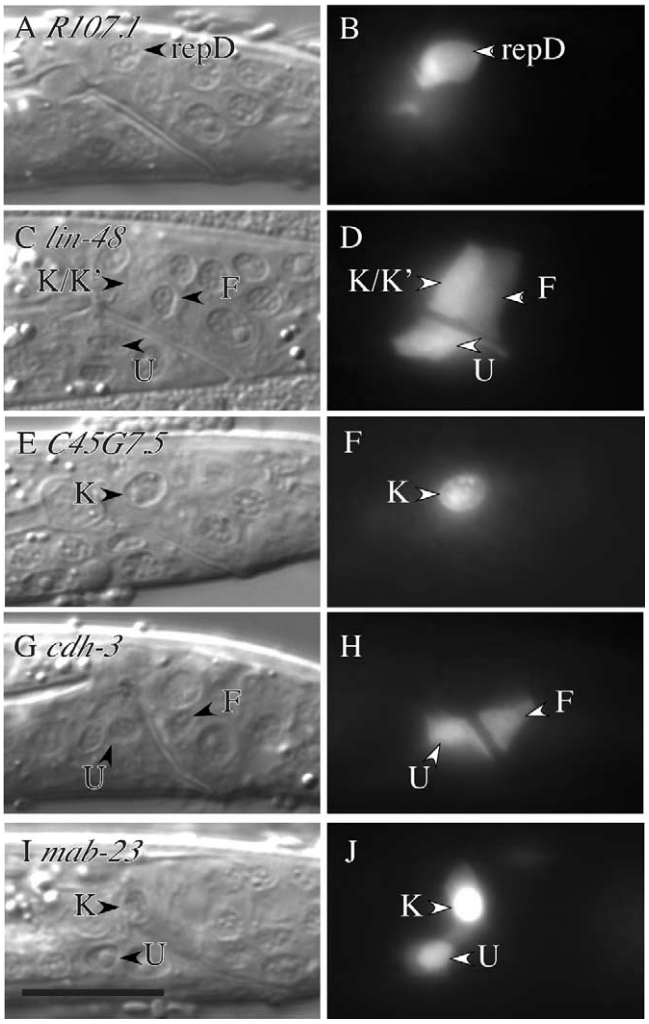

Fig. 3. Gene expression patterns reveal hindgut subdomains. A, C, E, G, and I are DIC (differential interference contrast) images of L1 or L2 animals, and B, D, F, H, and J are epifluorescence images to visualize GFP (green fluorescent protein). For *lin-48*, K and K′ are out of the plane of focus, but the fluorescence from these cells is detected (K/K′). Additional expressing cells are out of the plane of focus for *R107.1* and *C45G7.5*. Bar is 10 μm.

Materials and methods

Strains

The following strains were cultured according to standard techniques, described by Hodgkin (1997): Linkage group (LG) II: *mab-9(e1245)*; LG III: *lin-48(sa469)*, *cdh-3(pk87)*, *unc-119(e2498)*, and *pha-1(e2123)*; LG IV: *egl-38(sy294)*, *egl-38(s1775)*, *dpy-20(e1282)*, and *dpy-20(e1362)*; LG V: *mab-23(bx118)*, and *him-5(e1490)*.

Transgenes were as follows: *pkEx246* (*cdh-3::gfp*, Pettitt et al., 1996), *saEx459* (*lin-48::gfp*, Johnson et al., 2001), *bxEx83* (*mab-23::gfp*, Lints and Emmons, 2002), *guEx127* (*C45G7.5::gfp*), *guIs1* (*R107.1::gfp*), and *saIs14* (*lin-48::gfp*).

Table 3
Production of ectopic spicule cuticle in mutants

Genotype[a]	% with spicules	*N*
+[b]	0	10
mab-23	0	12
lin-48[b]	67	21
lin-48; *mab-23*	76	25
lin-48 cdh-3; *mab-23*	64	28

[a] All strains include *him-5(e1490)*.
[b] Data summarized from Chamberlin et al., (1999).

Table 4
Expression of hindgut genes in *lin-48* mutants

Transgene	Cells	Genotype	% of animals with expression in *x* cells					*N*
			4	3	2	1	0	
lin-48::gfp	K, K′, U, F	+	**57**	26	16	1	0	69
		lin-48	**65**	22	12	1	0	65
cdh-3::gfp[a]	U, F	+			**72**	15	13	109
		lin-48			**70**	19	11	109
mab-23::gfp	K, U	+			**74**	26	0	62
		lin-48			39	37	24	54
C45G7.5::gfp	K, K′	+			**62**	16	22	87
		lin-48			38	8	**54**	26

[a] Data summarized from Chamberlin et al. (1999).

Construction and analysis of transgenes

Green fluorescent protein (GFP) reporter transgenes were constructed by using upstream sequences for each gene cloned into pPD vectors provided by Andy Fire. Transgenes were injected into animals with appropriate marker DNA. The activity of each transgene was assessed in animals from heritable transgenic lines as described (Johnson et al., 2001). Cells were scored positive for expression if any GFP was detected above background.

cdh-3

A 1-kb region of upstream DNA was amplified with polymerase chain reaction (PCR) from cosmid ZK112 and cloned into pPD95.67. Deletions were generated by using forward PCR primers corresponding to different positions in *cdh-3*. Point mutations were generated by using the QuikChange

Table 5
mab-23 expression in hindgut mutants

Genotype	% of animals with expression in each cell				*N*
	U	F	K	K′	
+	89	2	82	8	62
lin-48	62	0	55	45	55
mab-9	84	70	88	23	57
plin-48::mab-9	33	5	64	10	58

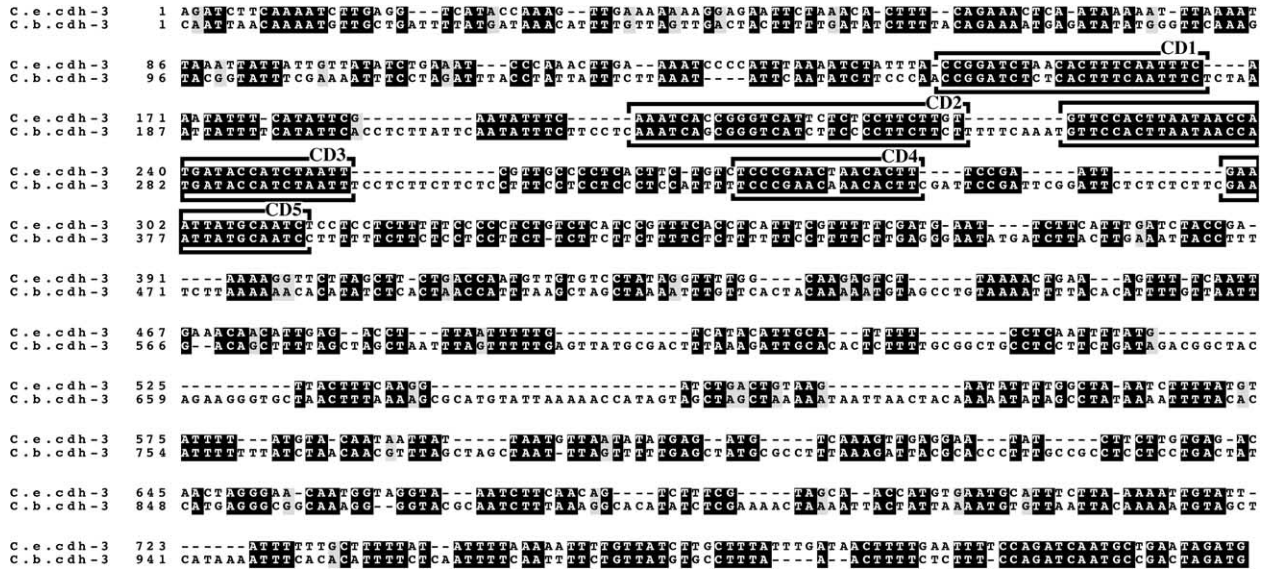

Fig. 4. Alignment of *cdh-3* upstream region from *C. elegans* and *C. briggsae* identifies conserved sequence blocks. The sequence ends with the predicted start codon for each gene. Conserved domains (CD) are boxed. Sequences from http://genome.wustl.edu/.

site-directed mutagenesis protocol (Stratagene). The changes in italics were used: CD3 GTTCCACTTAAT-AAC*gccagctg*CATCTAATT, CD4 *cgagctc*CTAA*t*ACTT.

plin-48::mab-9

Reverse transcription (RT)-PCR was used to isolate *mab-9* cDNA from *C. elegans* total RNA and cloned into MCSII of pPD49.26. A 3-kb *Hin*dIII fragment upstream of *lin-48* was cloned into MCSI. The resulting clone was injected together with a *mab-23::gfp* clone (Lints and Emmons, 2002).

Cell kill experiments

The B or B.a cell was killed in male larvae, and spicule socket cell differentiation scored in adults as described (Chamberlin et al., 1999).

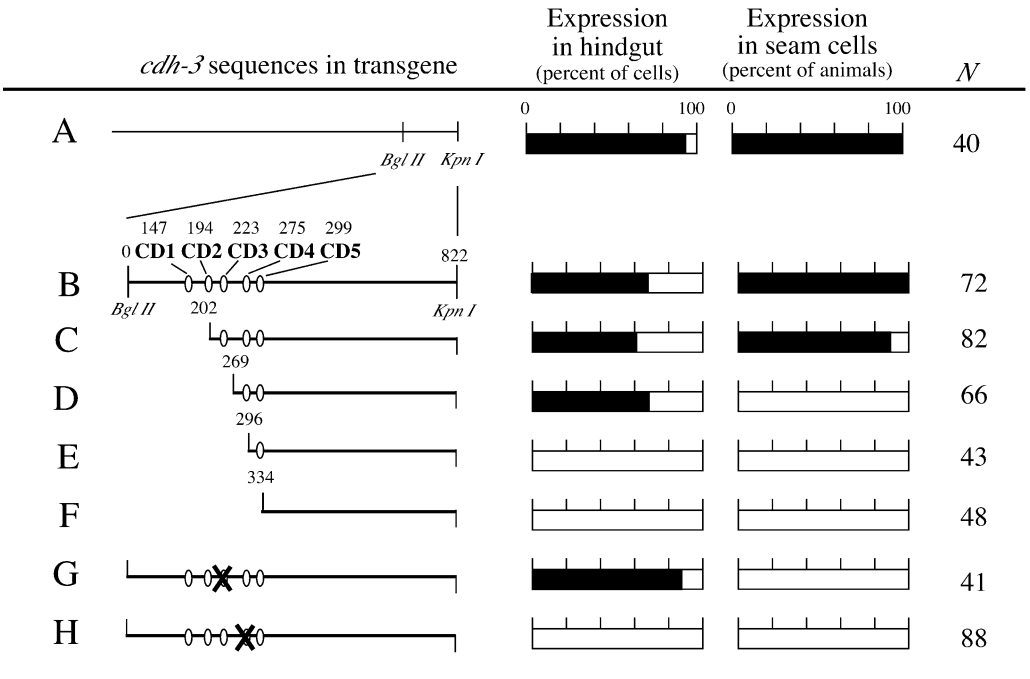

Fig. 5. Two conserved domains in *cdh-3* mediate gene expression in hindgut and seam cells. The conserved domains shown in Fig. 4 are indicated with ovals. Transgenes containing mutant sequences are indicated with an X. Black bar, percentages of cells expressing green fluorescent protein (GFP); white bar, not expressing GFP; N, number of animals scored.

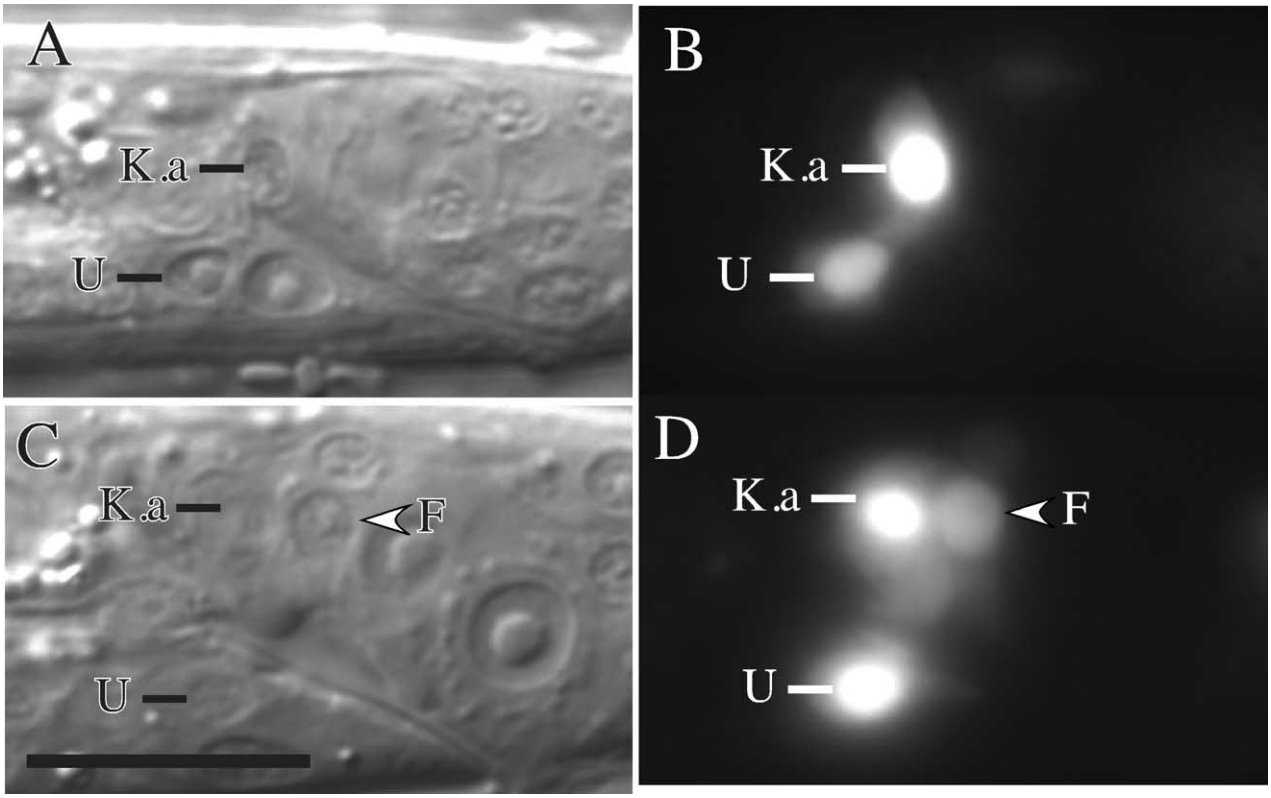

Fig. 6. Ectopic expression of *mab-23* in the presumptive F cell of *mab-9* mutants. In wild-type animals (A and B), *mab-23* is expressed in U and K. In *mab-9* mutants (C and D), *mab-23* expression is also detected in the F cell (arrowhead).

Results and discussion

Gene expression patterns define hindgut subdomains

Table 1 summarizes the hindgut expression pattern of genes reported to be expressed in hindgut cells of *C. elegans* larvae. Representative photographs of genes analyzed in this study are in Fig. 3. Each cell type has the potential to be uniquely identified based on the combination of expressed genes. For example, within the four cells that express *lin-48*, *C45G7.5* defines the anterior cells K and K′, and *cdh-3* defines the posterior cells U and F. Both of these pairs of cells are embryonic siblings (Fig. 2). Expression of *mab-23* distinguishes the cells within each sibling pair, with expression in K and U. Our analysis of the regulatory inputs that establish these gene expression patterns indicates both combinatorial and coordinate regulation play a role.

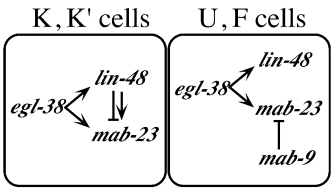

Fig. 7. Model for genetic relationship among hindgut genes in the mid-hindgut cells. *egl-38* is essential for the expression of *lin-48* and *mab-23* in all cells of the mid-hindgut. The roles for *lin-48* and *mab-9* in affecting *mab-23* expression differ between the more anterior cells (K, K′) and the more posterior cells (U, F).

Coordinate regulation of gene expression

The Pax transcription factor EGL-38 is important for the development of hindgut cell types (Chamberlin et al., 1997). Expression of *lin-48* requires EGL-38, and expression in all four hindgut cell types is mediated through the same regulatory elements (Johnson et al., 2001). To further investigate the function of *egl-38* in regulating hindgut gene expression, we observed *R107.1*, *cdh-3*, *C45G7.5*, and *mab-23* expression in *egl-38* mutants (Table 2). We found that expression of *mab-23* was eliminated in a manner similar to that of *lin-48*, whereas *cdh-3* and *C45G7.5* exhibited a reduction of expression. From these results we conclude that, even within the same cells, *egl-38* affects the expression of different hindgut genes to different extents.

Since *cdh-3* expression exhibits limited dependence on *egl-38*, we inferred that another factor or factors mediates its expression in the U and F cells. To understand *cdh-3* regulation in these cells, we carried out an analysis of its regulatory region. We identified five blocks of sequence upstream of the predicted start codon that are conserved between *C. elegans* and *C. briggsae* (Fig. 4). Reporter

Table 6
Deduced K and K′ lineage based on *lin-48::gfp* expressing cells in L2 larvae[a]

Genotype	Percentage of animals									Total % of wild type	*N*
	K K′	K K′	K K′	K K′	K K′ ?	K K′ ? ?	K K′ ?	K K′ ?	K K′ ? ?		
+	0	0	57	4	11	0	14	0	14	96	28
mab-23	0	0	68	14	9	0	9	0	0	86	22
lin-48	28	16	24	16	4	4	4	4	0	32	25

[a] Boxed columns indicate patterns expected in wild type. Expression from *sals14* is not always at detectable levels, and sometimes division from only one, or no, cells can be scored. Filled circle indicates green fluorescent protein (GFP) detected in the cell (or its progeny). Empty circle indicates GFP not detected and the cell was not scored.

transgenes containing these sequences express in hindgut cells U and F and the seam cells of the epidermis. A series of deletion and point mutation clones allowed us to identify regions important for seam cell expression and hindgut expression (Fig. 5). One element, CD4, is critical for hindgut expression, but does not bear similarity to sequences bound by EGL-38. Thus, for two genes tested [*lin-48* (Johnson et al., 2001) and *cdh-3* (this work)], expression in all the hindgut cells is coordinately regulated by single regulatory elements, consistent with single factors or factor complexes mediating the expression in all hindgut cell types.

Combinatorial regulation of gene expression

To further explore the regulatory relationship among hindgut genes, we constructed double and triple mutants among *lin-48*, *cdh-3*, and *mab-23* (Table 3), and examined gene expression in *lin-48* mutants (Table 4). These experiments indicate independence among the genes, as *cdh-3* and *mab-23* do not enhance a *lin-48* spicule phenotype, and only *mab-23* and *C45G7.5* expression exhibit partial sensitivity to *lin-48* genotype. *mab-23* expression was analyzed in more detail in *lin-48* and *mab-9* mutants (Table 5). In *lin-48* mutants, *mab-23* expression is random and approximately equal in both K and K′. In *mab-9* mutants, expression of *mab-23* is increased in the presumptive F cell (Fig. 6). In addition, ectopic expression of *mab-9* under control of *lin-48* promoter sequences results in reduction of *mab-23* expression in U. Taken together, the results indicate different genes influence *mab-23* expression in different cells (Fig. 7). Finally, although *mab-23* expression reflects cell type, *mab-23* is not necessary for either U or K cell type (Lints and Emmons, 2002; Table 6, data not shown). In contrast, although *lin-48* expression is symmetric, it affects either establishment or maintenance of asymmetry between K and K′ (Tables 5 and 6).

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