Expression Pattern, Regulation, and Biological Role of Runt Domain Transcription Factor, *run*, in *Caenorhabditis elegans*

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The *Caenorhabditis elegans run* gene encodes a Runt domain factor. *Runx1*, *Runx2*, and *Runx3* are the three known mammalian homologs of *run*. *Runx1*, which plays an essential role in hematopoiesis, has been identified at the breakpoint of chromosome translocations that are responsible for human leukemia. *Runx2* plays an essential role in osteogenesis, and inactivation of one allele of *Runx2* is responsible for the human disease cleidocranial dysplasia. To understand the role of *run* in *C. elegans*, we used transgenic *run::GFP* reporter constructs and a double-stranded RNA-mediated interference method. The expression of *run* was detected as early as the bean stage exclusively in the nuclei of seam hypodermal cells and lasted until the L3 stage. At the larval stage, expression of *run* was additionally detected in intestinal cells. The regulatory elements responsible for the postembryonic hypodermal seam cells and intestinal cells were separately located within a 7.2-kb-long intron region. This is the first report demonstrating that an intron region is essential for stage-specific and cell type-specific expression of a *C. elegans* gene. RNA interference analysis targeting the *run* gene resulted in an early larva-lethal phenotype, with apparent malformation of the hypodermis and intestine. These results suggest that *run* is involved in the development of a functional hypodermis and gut in *C. elegans*. The highly conserved role of the Runt domain transcription factor in gut development during evolution from nematodes to mammals is discussed.

The PEBP2/CBF (polyomavirus enhancer-core binding protein 2/core binding factor) family of transcription factors is composed of two subunits, α and β (3, 5, 33, 34, 44). The α subunit contains an evolutionarily conserved 128-amino-acid region that harbors two different activities, the ability to bind DNA and the ability to interact with the β subunit. This conserved region has been named the Runt domain (20) because *Drosophila runt* (21) is the first identified gene which contains the conserved region. The function of *Runt* was initially characterized as one of the pair-rule genes involved in segmentation (15). Further studies have demonstrated that *runt* has roles in two other developmental processes in *Drosophila*, sex determination and neurogenesis (11, 12). Another *Drosophila* Runt domain-encoding gene, *Lozenge*, is involved in prepat- terning photoreceptor precursor cells in the developing eye and is required in hematopoiesis for the specification of blood cell lineage (9, 10).

In mammals there are three PEBP2 α genes, *Runx1*/PEBP2 α B, *CBFA2/AML1* (2, 30), *Runx2*/PEBP2 α A/*CBFA1* (34), and *Runx3*/PEBP2 α C/*CBFA3* (4, 27). Recent work has identified important roles for these genes in hematopoiesis and osteogenesis in mouse and human. *Runx1* has been found at

the breakpoint of chromosome translocations associated with human leukemia (16, 31, 38). Targeted disruption of the *Runx1* gene in mice resulted in a lack of definitive hematopoiesis in the fetal liver (35, 43). More recently, it has been demonstrated that *Runx2*, another Runt domain-encoding gene, plays an essential role in osteogenesis. Mice with a homozygous mutation in *Runx2* died just after birth because of breathing difficulties caused by a complete lack of ossification (23, 36). Mutations in the *Runx2* gene have also been demonstrated to be responsible for the human disease cleidocranial dysplasia, an autosomal dominant disorder (25, 32).

The full genomic sequencing of *C. elegans* is essentially complete and has recently been published (1). More than 30% of the predicted genes from the genomic sequence of *C. elegans* have a human homolog, many of which have been implicated in human disease (13, 17). This observation, along with increasing evidence that biological processes and molecular pathways are highly conserved, has made the nematode *C. elegans* a model organism for the study of developmental regulation. The *C. elegans* homolog of *Runx*, *run*, was recently identified in the *C. elegans* genome database (6).

In this paper, we used *run::GFP* reporter constructs to generate transgenic animals and a double-stranded RNA (dsRNA)-mediated interference (RNAi) method to determine the role of *run* in *C. elegans*. Our results show that the *run* gene is expressed exclusively in the nuclei of hypodermal seam cells and intestinal cells. Expression was first detected at the bean stage and the late embryonic stage for hypodermal and intestinal cells, respectively, and lasted until the L3 stage of development. The cell type specificity and expression at specific

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FIG. 1. Schematic representation of the genomic DNA structure of *run* and *run::GFP* fusion constructs. The open boxes represent non-coding exons, and the solid boxes represent coding exons (6). Restriction enzyme sites used for the deletion constructs are indicated. An asterisk indicates the deletion point at the 5' end of the PCR-based deletion. Numbers in parentheses were obtained from the nucleotide sequence of the B0414 cosmid.

stages of development are controlled by an unusually long intron located between exons 3 and 4. Targeted inactivation of *run* by RNAi resulted in an early larva-lethal phenotype, with malformation of the hypodermis and intestine.

MATERIALS AND METHODS

Materials. Reagents were purchased from the following vendors: restriction enzymes from Takara (Tokyo, Japan); T4 DNA ligase from New England Biolabs (Beverly, Mass.); and T3 and T7 RNA polymerases from Promega (Madison, Wis.). All other chemicals of the purest grade available were obtained from commercial sources.

Worm strain and culture. Wild-type worms were the *C. elegans* Bristol strain N2. Worms were grown on NGM plates as described previously (8).

Construction of *run::GFP* reporter genes. A cosmid clone, B0414, containing the entire *run* gene was obtained from the *C. elegans* genome project. To construct pPD-run-FL, a genomic DNA fragment of approximately 13 kb (nucleotides [nt] 3870 to 16791 of B0414) containing *run* was subcloned into the *Sma*I site of the reporter gene expression vector pPD95.79 (a gift from A. Fire, Carnegie Institute of Washington, Baltimore, Md.). The genomic DNA fragment included all the exons and introns of *run* and a 5' region containing the adjacent 3' noncoding region of a gene homologous to *histone H1*. A *Stu*I site located six nucleotides upstream of the translation termination codon of *run* was used for in-frame fusion with *GFP* (Fig. 1).

pPD-run-ΔMB was constructed by deleting an *Mlu*I/*Bgl*II fragment (nt 6830 to 12116) from pPD-FL. pPD-run-Δ*M was constructed by deleting nt 5140 to 6830 of B0414 from pPD-run-FL using a PCR-based deletion method (39). pPD-Δ*S and pPD-run-Δ*B were constructed by deleting *Mlu*I/*Stu*I and *Mlu*I/*Bgl*II fragments, respectively, from pPD-run-Δ*M. The pSNAP25 plasmid contains the genomic region of *C. elegans* *SNAP-25* from the TATA box to 13 amino acids in exon 1 inserted into the pPD95.77 GFP reporter vector. An *Hpa*I/*Mlu*I fragment of B0414 cosmid DNA (corresponding to nt 4849 to 6830) was inserted into the *Sac*I site of pSNAP25 to generate sense-oriented pSR-HM-p(4849–6830) and reverse-oriented pSR-HM-m(6830–4849) plasmids. Similarly, pSR-MB-p and pSR-MB-m plasmids were constructed by inserting an *Mlu*I/*Bgl*II fragment into the *Sac*I site of pSNAP25.

pSR-HM(5102–6378), pSR-HM(5102–5826), and pSR-HM(5102–5438) were constructed by inserting the indicated region of B0414 amplified by PCR into

pSNAP25 that had been double-digested with *Hind*III and *Xba*I. The PCR primers used for these plasmids are as follows: HM-5102 (forward primer), 5'-TTTATGGAAGCTTTTGTGGAAGGTT-3'; HM-6378 (reverse primer), 5'-AGAATCTAGACAATTACAGCGATT-3'; HM-5826 (reverse primer), 5'-TCGATCTAGAGTGAAGCCCTTGCC-3'; and HM-5438 (reverse primer), 5'-CATTTGTCTAGACAATTGTGTCA-3'. The *Hind*III and *Xba*I restriction enzyme sites in these primers used for subcloning are in italics.

Germ line transformation. Germ line transformations were carried out by standard protocols (29). All *GFP* constructs were injected at a concentration of 100 ng/μl. Dominant *rol-6* pRF4 DNA was coinjected at 100 ng/μl. Transgenic lines inheriting the gene of interest were selected by their rolling behaviors, and the animals were observed for *GFP* expression.

Fixation and microscopy. Worms were collected from an unsaturated plate into a 1.7-ml microcentrifuge tube in M9 buffer. The worms were then centrifuged, washed with M9 buffer, and fixed overnight at 4°C by adding 20% paraformaldehyde to a final concentration of 2%. The fixed worms were then washed three times with phosphate-buffered saline (PBS) buffer and mounted on a slide glass. A Bio-Rad confocal microscope was used for observing the expression patterns and taking photos. Nomarski photos were taken with a Carl Zeiss Axoplan microscope.

Run immunostaining. Embryos and young larvae were permeabilized by freeze-cracking and fixed in methanol (95%) and then in acetone for 5 min each at –20°C. Specimens were rehydrated in a 75, 50, and 25% methanol series (3 min at each step), then washed in PBS three times for 10 min each. The blocking reaction was carried out in 5% goat serum for 3 to 5 h at room temperature. Monoclonal antibody raised against the mammalian Runt domain (10B7G8) (19) was added (1:20), and the specimens were incubated overnight at 4°C. After washing in PBS three times for 10 min each, secondary antibody conjugated with fluorescein isothiocyanate (FITC) (Sigma) was added according to the manufacturer's instructions.

RNAi assay. Sense and antisense RNA of the *run* gene was generated by in vitro transcription of a pBluescript SK(+) vector containing *run* cDNA. The *run* cDNA fragment contained 380 bp of the 5'-UTR and 130 bp of the 3'-UTR. PCRs were carried out using T3 and T7 RNA polymerases. To make double-stranded RNA, RNA generated from both forward and reverse reactions was mixed in a 1:1 ratio in TE (Tris-EDTA) buffer at 20 or 50 ng/μl. dsRNA was injected into the syncytial gonad of young wild-type animals (pPD-run-FL) (Fig. 1). Every 12 h the P₀ individuals were moved to new plates, and the number of F₁ progeny eggs was counted. Each progeny was inspected for any defects and for embryonic lethality by Nomarski and fluorescent microscopy.

RESULTS

Run is expressed in the lateral hypodermis and intestine of *C. elegans*. A 12,920-bp genomic DNA fragment containing the *C. elegans* *run* gene was obtained from cosmid clone B0414 and fused to a green fluorescent protein (GFP) reporter construct (pPD-run-FL) (Fig. 1). As illustrated in Fig. 2A, reporter gene expression in embryos from all transformed lines was first detected exclusively in hypodermal seam cells at the bean stage of development. Reporter gene expression was observed in the nuclei of these cells, and expression persisted throughout embryogenesis. Expression of *run* in seam cell nuclei was detected up until the L3 stage. Between the late embryonic stage and L3 stage, expression was also apparent in gut cell nuclei, with the anterior ring of four cells (int-1) showing the highest level of expression. Expression of *run* in binucleated gut cells with large nuclei became evident at the L2 stage. At the L3 stage, expression of *run* in gut cells was significantly decreased except in those cells in the most anterior part of the intestine. Expression in seam cells remained virtually unchanged. By the L4 stage, expression of *run* was undetectable (data not shown). Endogenous *run* expression was confirmed by immunohistochemical staining with antibody raised against the Runt domain. As shown in Fig. 2B, the pattern of endogenous *run* expression was essentially identical to that of *run::GFP* in the embryonic

FIG. 2. Expression pattern of *run* at various stages. (A) *run::GFP* reporter transgene. (a) Bean stage, (b) comma stage, (c) twofold stage, (d) L1 stage, (e) L2 (upper) and L3 (lower) stage. Arrows indicate the anterior ring of four intestinal cells (int-1). Note that *run* is expressed only in lateral hypodermal cells in the embryonic stage and is additionally expressed in intestine cells in the larval stage. Bar, 50 μ m. (B) Endogenous expression pattern of *run*. (a and c) Nomarski images and (b and d) antibody staining of *run* visualized with FITC. (a and b) An embryo of the bean stage. Hypodermal cells express *run*. (c and d) An animal at the L2 stage. Intestine and hypodermal cells express *run*. Intestine nuclei are indicated by arrows, and hypodermal nuclei are indicated by arrowheads.

and larval stages, suggesting that the *run::GFP* reporter gene could represent the expression of endogenous *run*.

Stage- and tissue-specific expression is controlled by intronic sequence. The *run* gene contains an exceptionally long

intron (7.2 kb) between exons 3 and 4. Because of the length of this intron, we examined whether it plays roles in modulating gene expression at various stages of development and in determining expression in various cell types. For this purpose we constructed four deletion constructs from pPD-*run*-FL, as shown in Fig. 1. Deletion of 1.7 kb in the 5' region of intron 3 (pPD-*run*- Δ^* M) resulted in dramatic reduction of expression in the hypodermal seam cells at larval stage (Fig. 3A). However, the reporter activity of the construct remained unchanged in the intestinal cells and embryonic seam cells (Fig. 3B). Further deletion of intron 3 up to the *StuI* site (pPD-*run*- Δ^* S) showed a similar expression pattern, but the expression level was much reduced (data not shown). In contrast, deletion of 5.3 kb in the 3' region (pPD-*run*- Δ MB) resulted in a complete loss of intestinal cell expression and little change in seam cell expression at the larval stage (Fig. 3A). When the majority of the intron was deleted (pPD-*run*- Δ^* B), reporter activity was difficult to detect at any stage. Cell type-specific expression of various deletion constructs is summarized in Fig. 3C.

FIG. 3. Expression pattern of *run::GFP* reporter transgenes with deletions within intron 3 at the L1 stage. (A) GFP activity of pPD-run- Δ^*M (a and b) and pPD-run- ΔMB (c and d). Deletion of a 1.7-kb fragment in the 5' region and a 5.3-kb fragment in the 3' region of intron 3 resulted in the loss of hypodermal seam cell and intestinal cell expression of the reporter gene, respectively. (B) GFP activity of pPD-run- Δ^*M . Deletion of the 1.7-kb fragment abolished postembryonic seam cell expression, but did not change the embryonic seam cell and intestinal cell expression. (a) GFP activity. (b) Nomarski photomicrograph. (C) Summary of the expression of *run* in deletion constructs within intron 3.

These results suggest that intron 3 is essential for stage-specific and cell type-specific expression of *run*. The intron can be separated into two functionally distinct regions, a 5', 1.7-kb region and a 3', 5.3-kb region, which are required for seam cell-specific and intestinal cell-specific expression, respectively, at the larval stage.

To examine whether the intron regions are sufficient for specifying cell type-specific expression, we examined the ability of the intronic regions to act as enhancers in a heterologous expression system. We generated pSR-HM-p(4849–6830), pSR-HM-m(6830–4849), pSR-MB-p, and pSR-MB-m by inserting the 2-kb (including the 1.7-kb region) or the 5.3-kb intron fragment into the pPD-SNAP25-GFP reporter plasmid. This plasmid contains the basal promoter of SNAP25, a neuron-specific gene (S. Hwang and J. Lee, unpublished results) (Fig. 4A). The pSNAP25 plasmid alone did not produce any expression of GFP. As shown in Fig. 4B, insertion of the 2-kb [pSR-HM-p(4849–6830)] and 5.3-kb [pSR-MB-p(6830–12116)] fragments resulted in seam cell-specific and intestinal cell-specific expression, respectively, at the larval stage. Plasmids containing the intron fragment in the reverse orientation (pSR-HM-m and pSR-MB-m) showed essentially the same results, but the activities were much lower (data not shown).

The intron region required for hypodermal seam cell expression at the larval stage was further mapped using plasmid constructs pSR-HM-p(5102–6378), pSR-HM-p(5102–5826) and pSR-HM-p(5102–5438). As summarized in Fig. 4C, the minimum region for hypodermal seam cell expression lies between nt 5102 and 5826. It was also demonstrated that the region between nt 5827 and 6378 is required for full activity. Mapping of the minimum region required for intestinal specific expression within the 5.3-kb region of the intron is the subject of a separate study.

These results strongly suggest that 0.7- and 5.3-kb fragments of intron 3 contain enhancer elements that are sufficient for seam cell and intestinal cell expression, respectively, at the larval stage.

Phenotypes of worms with RNA interference of *run*. Double-stranded RNAi analysis of the *run* gene revealed an early larva-lethal phenotype with apparent malformation of the hypodermis and the intestine (Fig. 5). Using RNA at a concentration of 20 ng/ μ l, 186 animals out of 4,226 eggs (4.4%) treated with RNAi did not hatch, and 836 animals (19.8%) hatched but showed visibly abnormal phenotypes at early larval stage. The remaining animals did not have any defects. At 50 ng/ μ l, 98 animals out of 1,468 eggs treated with RNAi (6.7%) failed to hatch, and 292 animals (19.9%) displayed visible abnormal phenotypes at the larval stage (Table 1).

At both concentrations of dsRNAi, typical phenotypes were an abnormal morphology of the hypodermal tissue and distortion of the morphology of the intestine. This phenotype was specific to the *run* dsRNA interference, since we did not observe such a phenotype in the control experiment in which TE alone was microinjected (Table 1). Other organs such as the pharynx appeared to be normal. Eggs that failed to hatch apparently finished development, because fully developed organs, such as the pharynx, could be observed (Fig. 5a). We did not observe massive cell death in the hypodermis or the intestine. However, in some cases int-1 cells died at the L1 stage (Fig. 5c). In conclusion, inactivation of *run* by RNAi appears to

C

	Lateral hypodermis	Intestine
pSR-HM-m	++	-
pSR-HM-p	++++	-
pSR-HM-(5102-6378)	++++	-
pSR-HM-(5102-5826)	++	-
pSR-HM-(5102-5438)	-	-
pSR-MB-m	+/-	++
pSR-MB-p	+/-	++++

cause a malformation in the development of the hypodermis and intestine. No significant effects on cell degeneration or on the formation of other organs by the embryo were observed.

DISCUSSION

The *C. elegans run* gene, encoding the runt domain transcription factor, is expressed in hypodermal seam cells and gut cells and is essential for the development of the corresponding tissues in early larvae. Expression of *run* can first be detected in hypodermal seam cells at the bean stage. Expression was observed in the nuclei of all hypodermal seam cells and remained evident up until the L3 stage. The expression pattern of *run* in hypodermal cells partly overlaps that of *Elt-1*, which has been demonstrated to play an early role in specifying epidermal cell fate. *Elt-1* is expressed in hypodermal cells which appear ≈ 260 min postfertilization (about the 365-cell stage) along with hypodermal seam cells (37). *Run* expression can also be first detected at 260 min postfertilization; however, its expression is restricted to seam cells, whereas *Elt-1* is expressed in all major hypodermal cells.

On each side of the newly hatched animal, there exists a single longitudinal row of specialized hypodermal cells, called seam cells. These cells are the only cells that divide after hatching. Seam cells divide following a stem cell pattern, resulting in an anterior daughter cell that fuses with the hypodermal syncytium, *hyp7*, and a posterior daughter cell that remains in the seam, where it is capable of further cell division (42). The majority of seam cells are blast cells that provide hypodermal nuclei and a postdeirid neuroblast (42). The male sensory rays are also derived from the seam cells. Targeted inactivation of *run* by RNAi resulted in abnormal morphology of hypodermal seam cells, suggesting that *run* is essential for the formation of normal hypodermis. Screening and characterization of *run* mutant *C. elegans* will be required to understand whether *run* also plays a role in the formation of postdeirid neuroblast cells and sensory rays.

The *C. elegans* gut is a major organ, involved not only in digestion but also in storage and macromolecule synthesis (7, 22). The intestine is derived exclusively from the founder cell E, which gives rise to no other tissue. At hatching, these cells are mononucleate, but most of them subsequently become binucleate through nuclear division (41). Furthermore, at the end of each larval stage, all the intestinal nuclei undergo endoreduplication of their DNA without accompanying nuclear

FIG. 4. Cell type-specific enhancer activity of intron 3. (A) Heterologous reporter constructs of the *run-intron3::GFP* fusion gene. Constructs are driven by parts of intron 3 of *run*, which is joined to the basal promoter of SNAP25 fused to *GFP*. Intron sequences essential for hypodermal seam cell expression and intestinal cell expression are indicated by hatched and shaded boxes, respectively. (B) Enhancer activity of intron 3 of *run*. (a) pSR-HM-p(4849–6830) and (b) pSR-MB-p. GFP activity is detected in the cytoplasm because the GFP used in this experiment does not contain a nuclear localization signal. Bar, 50 μ m. (C) Summary of enhancer activities located within intron 3 of *run*. Note that the 0.7-kb fragment in the 5' region and the 5.3-kb DNA fragment in the 3' region of intron 3 are sufficient for hypodermal seam cell- and intestinal cell-specific expression, respectively, at the larval stage.

FIG. 5. Phenotypes resulting from RNAi of *run*. (a) A typical egg phenotype. Although very much degraded, the egg contained a visible pharynx structure, indicating that most embryonic development had occurred. (b and c) A typical L1 phenotype. The outline of the animal is severely distorted. Arrow indicates lack of int-1 cells.

division. Endoreduplication within intestinal cells results in large cell nuclei.

At the larval stage, *run* expression was detected in the intestine as well as in the lateral hypodermis. Expression of *run* in the anterior ring of four cells (int-1) and in binucleated intes-

tinal cells can be clearly demonstrated at the L2 stage (Fig. 2). The mechanism by which the E blastomere, the clonal progenitor of the gut, becomes the functional gut is poorly understood. This is probably due to the paucity of information on transcription factors involved in the process. *End-1* and *elt-2* are two transcription factors that are known to play essential roles in the formation of the intestine. Both are GATA-like transcription factors that are expressed at the beginning of embryogenesis (2E cell stage) (14, 46). In contrast to the early embryonic expression of these transcription factors, *run* expression was only detected from the late embryonic stage and lasted until the L3 stage. This result suggests that *run* is involved in the establishment of a functional gut rather than in the determination of gut cell fate during embryogenesis.

This interpretation is supported by results from the RNAi experiments targeting *run*. Targeted disruption of *run* resulted in degeneration or malformation of hypodermal cells and intestinal cells. It is worth noting that targeted inactivation of *run* did not result in the lack of an intestine but rather in intestinal malformation. In comparison, loss-of-function phenotypes of either *end-1* or *elt-2* genes result in a complete lack of intestinal cells (14, 46). *Runx3/PEBP2αC*, a mammalian homolog of *run*, is expressed mainly in epithelial cells of the gastrointestinal tract. Most *Runx3* mutant mice die within 24 h after birth due to a malfunctioning gastrointestinal tract that fails to absorb nutrients (Li et al., unpublished data). The major phenotype of mice with a targeted deletion of *Runx3* is therefore quite similar to that of *C. elegans* with targeted inactivation of *run*. These results strongly suggest that *run* is required for the formation of a functional gut and that its role in gut development is evolutionarily conserved from nematodes to mammals. Although *end-1* and *elt-2* are also known to be gut-specific transcription factors in *C. elegans*, to date there has been no evidence to show that GATA family genes are involved in mammalian gut development. Therefore, *run* is the first gene that has been shown to be essential for both nematode and mammalian gut development.

An intimate relationship between runt domain transcription

TABLE 1. Effects of RNAi of *run*^a

Amt of dsRNA injected (ng/μl)	Time after injection (h)	Total no. of eggs collected	No. (%) of dead embryos (%)	No. (%) with larval defect	RNAi effect rate (%)
0 (TE control)	0–12	121	7 (5.8)	6 (5.0)	13 (10.8)
	12–24	342	4 (1.2)	5 (1.5)	9 (2.6)
	24–36	331	1 (0.3)	0 (0.0)	1 (0.3)
	36–48	292	1 (0.3)	2 (0.7)	3 (1.0)
	48–60	25	0 (0.0)	0 (0.0)	0 (0.0)
20	0–12	301	40 (13.3)	98 (32.6)	138 (45.8)
	12–24	973	57 (5.9)	226 (23.2)	283 (29.1)
	24–36	1,204	32 (2.7)	180 (15.0)	212 (17.6)
	36–48	1,209	56 (4.6)	247 (20.4)	303 (25.1)
	48–60	539	1 (0.2)	85 (15.7)	86 (16.0)
50	0–12	142	15 (10.6)	40 (28.2)	55 (38.7)
	12–24	313	13 (4.2)	60 (19.2)	73 (23.3)
	24–36	371	24 (6.5)	79 (21.3)	103 (27.7)
	36–48	401	29 (7.2)	75 (18.7)	104 (25.9)
	48–60	241	17 (7.1)	38 (15.8)	55 (22.8)

^a The numbers of dead embryos and abnormal larvae were compared to the total numbers of embryos laid at each time interval. Negative control was performed by microinjecting TE alone.

factor and transforming growth factor beta (TGF- β) superfamily signaling has been reported. Receptors for the TGF- β superfamily are composed of type I and II receptors. Following ligand binding, type I receptors associate with type II receptors and become activated through phosphorylation. The activated receptor-associated kinase phosphorylates Smads, which move into the nucleus to stimulate the transcription of a set of target genes (28). *Runx2* and *Runx3* are important targets of TGF- β signaling and are induced at the transcriptional level (26, 40). Furthermore, Runx proteins interact physically with activated Smads and function together (18, 26, 45). The *C. elegans* TGF- β -like type I receptor (*sma-6*), which can activate Smads of *C. elegans* (*sma-2*, *sma-3*, and *sma-4*), is expressed mainly in the intestine, and its expression overlaps that of *run* (24). It would therefore be of interest to study whether the functional relationship between TGF- β signaling and Runt domain transcription factor in mammals is conserved in the nematode.

An unusual characteristic of the genome of *C. elegans* is the short intron length. Introns in *C. elegans* are typically 45 to 55 nucleotides long, and more than half are shorter than 60 nucleotides. However, the third intron of the *C. elegans run* gene is 7.2 kb in length. All other introns in the *run* gene are shorter than 1 kb. Because tissue-specific enhancer elements have been identified within intron regions of fruit fly and mammalian genes, we examined whether the long intron of *run* plays a role in cell type-specific and stage-specific expression of the gene. Our results demonstrate that the long intron region contains enhancer elements required for stage-specific and cell type-specific expression.

The intron region could be separated into two functionally distinct fragments (a 0.7-kb fragment in the 5' region and a 5.3-kb fragment in the 3' region). The 0.7-kb fragment in the 5' region is sufficient for seam cell-specific expression at the larval stage. On the other hand, the 5.3-kb fragment in the 3' region was sufficient for intestinal cell-specific expression at the larval stage. For embryonic seam cell expression, intron 3 is required but not sufficient. This is the first report demonstrating that an intron region is essential for stage-specific and cell type-specific expression of a *C. elegans* gene.

In this paper, we show that *run* is expressed in the lateral hypodermis and intestine and that cell type-specific and stage-specific expression is regulated by enhancer elements located in an intron. Our results also demonstrate that *run* is essential for the development of a functional hypodermis and intestine and that the role of *run* in *C. elegans* gut development has been evolutionarily conserved from nematodes to mammals.

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