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GENE EXPRESSION

In situ analysis of C. elegans vitellogenin fusion gene expression in integrated transgenic strains: effect of promoter mutations on RNA localization

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Expression of the Caenorhabditis elegans vitellogenin (*vit*) genes is initiated at the larva-to-adult molt in all of the 30 to 34 nuclei of the hermaphrodite intestine. A series of strains in which DNA carrying a *vit* fusion gene was integrated at low copy number was analyzed by in situ hybridization to determine whether the transgene showed the same tissue-specific expression. Strains with only 247 bp of 5′-flanking DNA accumulated the mRNA product of the introduced vitellogenin gene only in the adult hermaphrodite intestine, and uniformly in all of the intestinal cells. When similar strains carrying *vit* fusion genes with promoter modifications were tested, no loss of tissue specificity was observed. Surprisingly, however, strains with modified promoters that resulted in reduced levels of expression displayed a novel pattern of transgene RNA localization within their intestines. Strains with severe promoter defects accumulated the transgene mRNA in the central part of the intestine but lacked the mRNA at both ends. Those with less severe promoter mutations lacked the transgene mRNA only in the most anterior intestinal cells. We hypothesize that genes with altered promoters require higher activator concentrations to express the reporter gene, thus revealing an inherent asymmetry in activator levels, lowest in the anterior cells and highest in the central cells of the intestine.

Vitellogenins are precursors of the yolk proteins stored in developing oocytes in many species. In a variety of organisms, the site of vitellogenin synthesis is separate from the site of yolk accumulation (reviewed by Wahli, 1988). The vertebrates produce vitellogenin in liver cells, from which it is secreted into the circulatory system (Wallace and Dumont, 1968). In Drosophila, the yolk precursors are synthesized in the fat body (Warren et al., 1979) and in ovarian follicle cells (Brennan et al., 1982). The major endodermal tissue in the nematode Caenorhabditis elegans is the intestine, which synthesizes vitellogenin (Kimble and Sharrock, 1983) and secretes it into the coelomic fluid for uptake by the developing oocytes.

In C. elegans the vitellogenins are encoded by a gene family of five closely related genes, *vit-1* to *vit-5*, and a more divergent member, *vit-6* (Blumenthal et al., 1984; Spieth and Blumenthal, 1985). All of the nematode vitellogenins and the mRNAs that encode them are produced in the same stage-, sex-, and tissue-specific manner, suggesting their coordinate control by a common regulatory mechanism. No tissue other than the adult hermaphrodite intestine synthesizes vitellogenin or its mRNA (Kimble and Sharrock, 1983; Blumenthal et al., 1984; Schedin et

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al., 1991). The nematode and vertebrate vitellogenin genes are distant members of a single gene family (Spieth et al., 1985; Nardelli et al., 1987; and Spieth et al., 1991). In vertebrates, vitellogenin synthesis is under hormonal control. Estrogen response elements (EREs) have been identified in the 5′ regions and shown to act as inducible enhancers able to confer estrogen responsiveness to a heterologous promoter (Klein-Hitpass et al., 1986). In the nematode system, no hormone that might act as an estrogen homologue has been identified. Despite the conservation of sequence between nematode and vertebrate vitellogenin coding sequences, EREs have not been found in the nematode genes. Comparisons of the 5′ regions of the six genes in C. elegans and five genes in C. briggsae have revealed conserved, repeated heptamers, the Vit Promoter Element 1 (VPE1) and VPE2 (Spieth et al., 1985; Zucker-Aprison and Blumenthal, 1989). In all of the putative promoter regions, these sequences are found in approximately the same relative positions. Their conserved sequences and positions argue for their functional importance as cis elements in nematode vitellogenin gene regulation.

We have tested the functional importance of VPE1 and VPE2 in the *vit-2* promoter in vivo, using the transgenic system developed by Fire (1986). Germ-line transformation was achieved by injection of constructs containing a copy of the *sup-7* gene, which was used for selection. The selected strains had low-copy-number tandem arrays of the injected DNA integrated into chromosomal DNA. Initially, we established that a novel vitellogenin gene fusion (*vit-2/vit-6*) introduced into the germ line with 3.9 kb of upstream sequence gave correctly regulated tissue-, stage-, and sex-specific expression (Spieth et al., 1988). More recently, we constructed a series of strains carrying modified promoters with deletions and point mutations in the VPEs, and demonstrated that both VPE1 and VPE2 represent activation sites. Furthermore, we detected no changes in sex- or stage-specificity of *vit* expression due to the modified promoters (MacMorris et al., 1992).

While the initial analysis of tissue-specific transgene expression was accomplished by in vitro protein synthesis with dissected intestines, we sought a method that would allow us to examine the products of all tissues at the RNA level. For that purpose we adapted the in situ hybridization methods developed by Edwards and Wood (1983) for the specific detection of the *vit-2/vit-6* fusion gene product. We report here that none of the modified promoters changed the tissue-specific pattern of expression. However, mutations that lowered expression levels also resulted in spatially altered patterns of expression within the intestines. We hypothesize that these in situ hybridization results reveal an underlying asymmetry in the distribution of activators within the intestine.

Materials and methods

Growth and synchronization of populations of C. elegans

C. elegans strains were grown on NGM plates, as described by Brenner (1974). Synchronous populations of adults were obtained by isolating embryos from gravid adults with an alkaline hypochlorite solution and allowing them to hatch without food to produce an arrested population of larvae, as described previously in Sulston and Hodgkin (1988).

Strain construction

The transgenic strains were derived by germ-line transformation of *tra-3(e1107)* mutant worms. The *vit* promoter constructs were cloned into a pUC-derived plasmid containing the *sup-7* gene (Fig. 1), and transformants were selected on the basis of suppression of the amber-suppressible *tra-3* mutation (Fire, 1986). The promoter modifications diagrammed in Figure 1 were made by site-directed mutagenesis, and terminal deletions by BAL 31 digestion or restriction endonuclease digestions to remove deleted fragments. Most of them are described in detail in MacMorris et al. (1992).

In situ hybridization

The methods for in situ hybridization were modified from those of Schedin et al. (1991).

Sample preparation. Adult hermaphrodites 1–2 days past the L4–adult transition were cut just posterior to the pharynx in M9 buffer containing 10 mM tetramisole on prechilled slides precoated with 0.2% gelatin, 0.02% chromium potassium sulfate. After the worms were lightly squashed with a 22 mm coverslip, they were quick-frozen in liquid nitrogen. Coverslips were cracked off the frozen slides, and slides were immediately

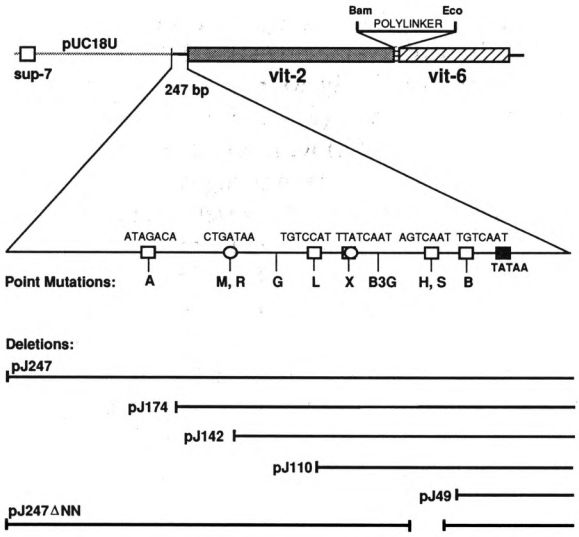


Figure 1. The *vit-2/6* fusion gene and promoter mutants. The fusion gene was cloned into a pUC-derived plasmid containing *sup-7* (MacMorris et al., 1992). The polylinker region connecting the *vit-2* and *vit-6* partial genes is 15 nucleotides long from the BamH I to the EcoR I sites. Only 247 bp of *vit-2* 5′-flanking DNA are included in this construct. This DNA contains 5 VPE1 (□) and 2 VPE2 (○) elements, allowing a one-nucleotide mismatch to the consensus sequences. The sequences of the elements are shown. Modifications in the region are shown below the promoter diagram: each vertical line marks the region changed. Deletion constructs are named according to the length of promoter remaining. The 247ΔNN promoter is an 18 bp internal deletion that removes the second most proximal VPE1. The sequences of the elements in each of the mutants are as follows: A, GtCTaGa; M, cGgatCC; R, ctgataTC; G, TCT AGATCT; L, tgGccat; X, ttCtAGat; B3G, AGATCTaGA; H, CTCGaGt; S, TGTCGGTAC; B, tgGCcaat. Uppercase letters indicate the changed nucleotides.

immersed in 2% paraformaldehyde in 1X M9 buffer, 0.1% Triton X-100. Fixation for 10 minutes was followed by dehydration through a graded series of ethanol. Slides with fixed samples were air-dried, baked at 80°C for 15 minutes, and then stored at 4°C.

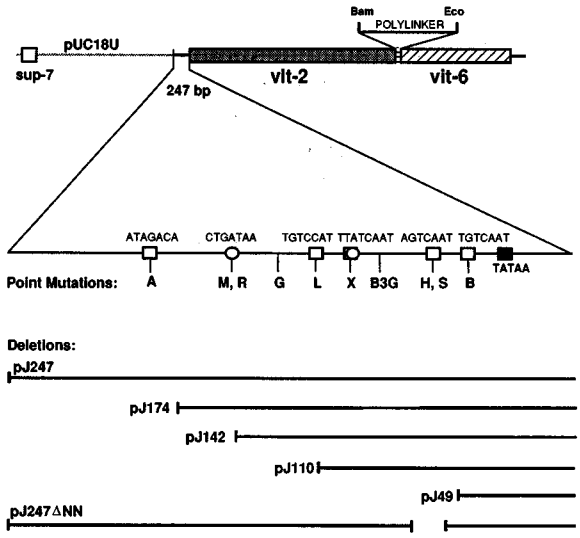
Probe preparation. RNA probes were prepared by T7 RNA polymerase transcription (Tabor and Richardson, 1985) using ^{35}S-UTP (>1000 Ci/mmol: Amersham). The *vit-2* probe was prepared by T7 transcription of DJ1, pTZ18U with a 2 kb fragment of *vit-2* cloned into the Sph I site in 3′–5′ (reverse) orientation, and digested with Dde I. The resulting transcript has 90 nucleotides complementary to *vit-2* that may also cross-hybridize to other *vit* transcripts. The template for transcription for the polylinker probe (PL) was pTZ18U (Mead et al., 1986) linearized with Hind III to produce a 54 nucleotide RNA complementary to the polylinker. Twenty-seven nucleotides of this RNA are complementary to the polylinker region and the flanking restriction sites within the *vit-2/6* fusion gene RNA.

Hybridization. The hybridization mixture contained a standard amount of transcript determined in separate experiments to be effectively in local excess, in 70% deionized formamide, 5X Denhardts, 2X SSPE, 10 mM DTT, 1 μg/ml E. coli rRNA. (1X SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, 1 mM EDTA, pH 7.4.) Ten to 15 μl of hybridization mixture were placed on each sample and overlaid with a siliconized, baked cover slip and hybridized at 37°C overnight. Slides were washed extensively, beginning with 32°C, 70% formamide, 1X SSPE, 0.05% SDS, 1 mM DTT; ending with aqueous washes (0.1X SSPE, 0.05% SDS, 1 mM DTT) at 53°C. Similar stringency was maintained in washes by compensating for the decrease in formamide concentration with increased temperature and lowered salt concentration. Washed slides were dehydrated through a graded series of ethanol and oven-dried.

Autoradiography. Slides were dipped in Kodak NTB2 emulsion diluted 1:1 with water, dried in a moist chamber (Angerer and Angerer, 1981), and then stored in black boxes with dessicant at 4°C. Typical exposure times were 2–7 days. The autoradiographic image was developed for 4 minutes in half-strength D19 at 15–16°C, followed by a 30 second treatment in 0.1% acetic acid and 5 minute fixation in Kodak Fixer. After photographic development, the slides were stained in 1 μg/ml DAPI (4′,6-Diamidine-2-phenylindole dihydrochloride, Boehringer Mannheim) and mounted in Gelutol (Monsanto).

Microscopy and photography. Processed slides were analyzed with a Zeiss Axioskop microscope using simultaneous epifluorescence (at 365 nm wavelength) and darkfield optics. Tri-X film was used for microphotography.

Strains tested by in situ hybridization. The following strains were hybridized with the *vit-2* internal probe: BL203 (pJ247), BL341 and BL342 (pJ247R), BL300 (pJ174-homologous integrant), BL330 and BL332 (pJ247ΔNN), BL310 and BL311 (pJ142het), BL360 and BL361 (pJ247B),



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N2 (wild-type), CB3168 (*him-1; mab-3*) males. Hybridization was confined to the intestines in each case. The transgene-specific PL probe detected no signal in the following strains: N2, *him-5* males, BL203 L4s, BL315 (pJ142); BL320, BL322 (pJ49); BL360, BL361, BL364het (pJ247B); BL390, BL394 (pJ247X); BL423, BL424 (pJ247M), and BL440 (pJ110). The following strains hybridized the PL probe to intestinal cells only: pJ247 strains: BL203, BL207(het), BL209(het); pJ142 strains: BL300 (homologous integrant), BL301 (het), BL303(het), BL304(het); pJ142 strains: BL310(het), BL311(het), BL422 (homologous integrant); pJ247ΔNN strains: BL330, BL332; pJ247R strains: BL341, BL342; pJ247L strains: BL385(het), BL386; BL360 (pJ247B3G); BL391 (pJ247X); BL410 (pJ247S); BL425 (pJ247H); BL430 (pJ247G); BL470 (pJ247A). A single strain, BL350 (pJ247M), displayed ectopic hybridization of the PL probe in a region of the gonad (MacMorris et al., 1992). The strains that were maintained as obligate heterozygotes are designated by "het" after the name.

Results

In vivo function of *vit-2* promoter tested in transgenic worms

The *vit-2/6* fusion gene used as a reporter in our transgenic study of promoter function is shown in Figure 1. The 5′ portion of *vit-2*, as well as 247 bp of its 5′-flanking region, was fused to the 3′ end of *vit-6* including its 3′ untranslated region. Within the *vit-2* 5′-flanking 247 bp are 5 VPE1s and 2 VPE2s that have been modified by point mutations and deletions as shown. Analysis of promoter function in the mutated strains illustrated that several of the VPEs function as activators; modifications can result in loss or modulation of expression levels. We detected no changes in stage- or sex-specific expression with these promoter mutations (MacMorris et al., 1992).

RNA–RNA hybridization specifically detects mRNA of the *vit-2/6* fusion gene

We showed previously by protein synthesis with isolated intestines that the *vit-2/6* fusion gene mRNA accumulated in the intestine and not in other isolated body parts in transgenic lines with an unmodified 3.9 kb promoter (Spieth et al., 1988) and in transgenic worms with 247 bp unmodified promoter (MacMorris et al., 1992). In order to assay the RNA products throughout the body, we performed in situ hybridization analysis (Edwards and Wood, 1983; Schedin et al., 1991) with an RNA probe complementary to an internal region of *vit-2* that recognizes *vit-2/6* mRNA, as well as the endogenous *vit-2* product. In the 11 transgenic lines containing wild-type and altered promoters that we examined, label was confined to the intestines of adult hermaphrodites, suggesting that ectopic expression from the fusion genes controlled by the modified promoters does not occur.

To detect solely the *vit-2/6* mRNA, we synthesized a 54 nucleotide transcript of pTZ18U polylinker that is complementary to the only region of the *vit-2/6* fusion gene not shared with *vit-2* and *vit-6*: 27 nucleotides of vector sequence between the *vit*-gene fragments. With this probe (called PL), the hybridization signal was confined to the intestines of adult hermaphrodites (Fig. 2) and was detected only in strains that accumulated the novel 155 kDa polypeptide product of the fusion gene (MacMorris et al., 1992). A control probe of similar length transcribed from the opposite strand of the polylinker region gave no intestinal hybridization.

Non-uniform intestinal localization of PL probe

Hybridization of the PL probe to semi-squashed, cut worm samples of BL203, a strain containing a single copy of the fusion gene under the control of the unmodified 247 bp promoter, resulted in silver grains distributed uniformly over the intestine (Fig. 2A and B). Control strains lacking an integrated fusion gene (not shown) or those in which the promoter was inactivated by mutation or deletion (Fig. 2I and J) exhibited no hybridization signal with the PL probe. Many strains that were examined had reduced levels of fusion protein expression as a result of promoter modifications (MacMorris et al., 1992). Some of these strains had reduced levels of hybridization signal, while others displayed some high-level but non-uniform patterns of hybridization. The data accumulated from 25 strains representing 15 promoter variants (Table 1) with a wide range of expression levels defined five general classes of hybridization patterns (Figs. 2 and 3). In the class that resembled wild-type (Class I), intestines were uniformly labeled (Fig. 2A and B). Class II in-

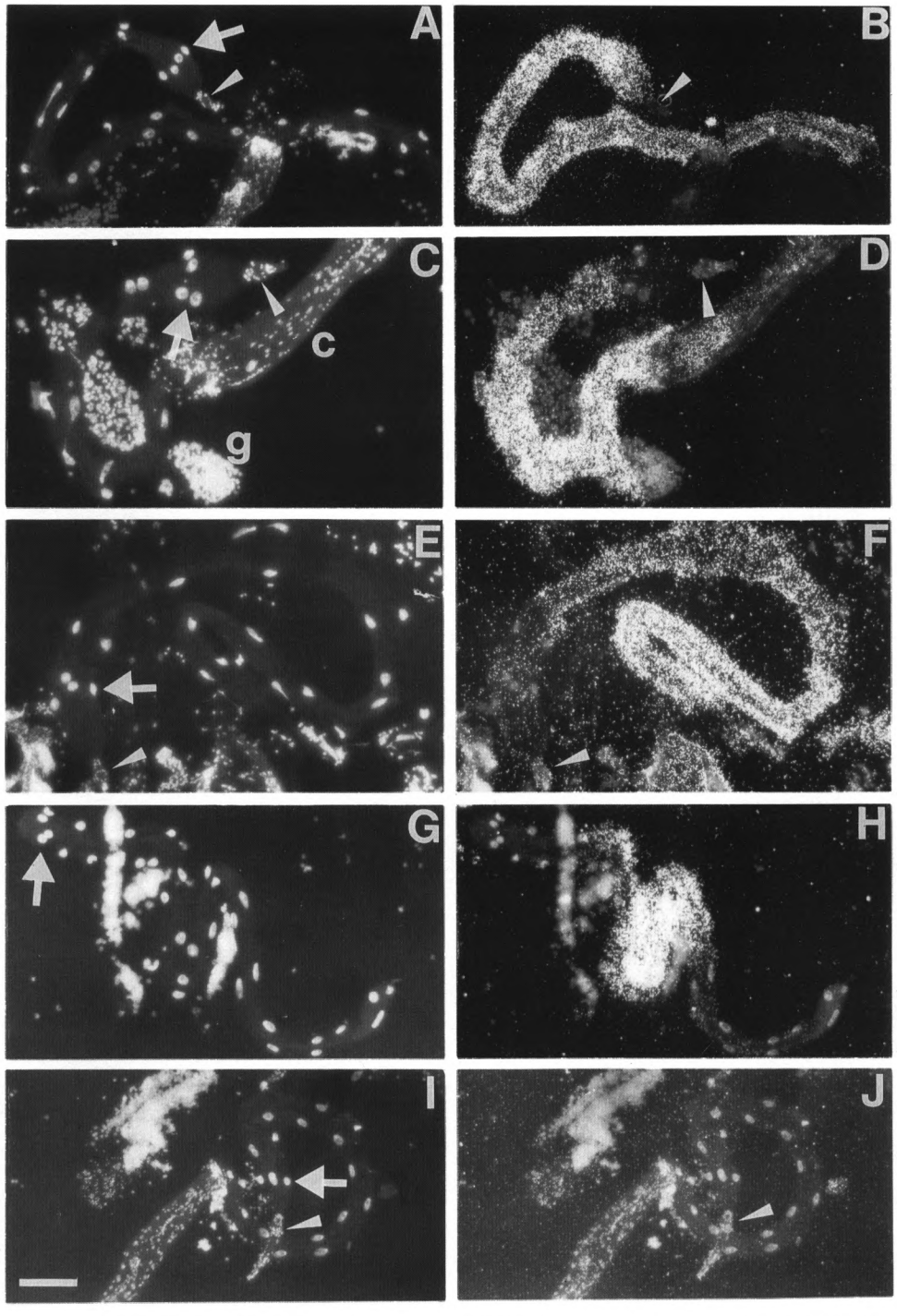

Figure 2. In situ hybridization of PL probe defines five classes of labeling. Worms were observed with simultaneous dark-field and epifluorescence to visualize RNA localization and DAPI-stained nuclei. Punctate bright spots in the right panels are silver grains indicating sites of RNA hybridization. Panels on the left show the corresponding DAPI-stained nuclei alone. Intestines are the predominant tissue in each panel, characterized by large DAPI-stained nuclei. An arrow points to the first set of intestinal nuclei at the anterior end in each. A smaller arrowhead indicates pharyngeal structures when present. **A** and **B.** Class I: BL203 (pJ247); **C** and **D.** Class II: BL330 (pJ247ΔNN); **E** and **F.** Class III: BL304 (pJ174het); **G** and **H.** Class IV: BL391 (pJ247X); **I** and **J.** Class V: BL424 (pJ247M). c: a portion of worm carcass from which most of the intestine has been extruded. g: gonadal tissue containing many small nuclei. Magnification bar = 100 μm.

Table 1. Intestinal localization of fusion gene mRNA.

Classes of intestinal labeling as shown in Figures 2 and 3. I: Uniform labeling of all intestinal cells. II: Anterior set of intestinal cells lack label. III: Anterior two sets of intestinal cells lack label. IV: Mid-intestinal region labeled, with negative anterior and posterior ends of intestine. V: Intestine uniformly lacking label. ND: not determinable. n = number of intestinal pieces scored. Fusion protein (fp155) level was estimated from quantitative Western blots as reported by MacMorris et al. (1992) for all strains except BL300, BL422, BL410, and BL430, which were measured by the same method.

Strain	Construct	Protein level	n	Percentage of intestines in class				
				I	II	III	IV	V
Deletion strains								
BL203	pJ247	++++	58	97	3	0	0	0
BL207	pJ247het	++++	40	95	5	0	0	ND
BL300	pJ174hom*	++++	12	100	0	0	0	0
BL422	pJ142hom*	++++	14	100	0	0	0	0
BL303	pJ174het	++++	24	59	35	6	0	ND
BL301	pJ174het	+++	17	17	71	4	8	ND
BL305	pJ174het	+++	8	62	13	0	25	ND
BL304	pJ174het	++	43	0	40	60	0	ND
BL310	pJ142het	+	24	0	33	17	50	ND
BL315	pJ142	+	21	0	0	0	0	100
BL311	pJ142het	+	2	0	0	100	0	ND
BL440	pJ110	−	10	0	0	0	0	100
BL322	pJ49	−	12	0	0	0	0	100
Point mutation strains								
BL460	pJ247B3G	++++	13	69	31	0	0	0
BL385	pJ247Lhet	+++	17	0	70	29	0	ND
BL386	pJ247L	+++	21	0	33	52	5	10
BL330	pJ247ΔNN	+++	146	0	82	9	8	1
BL332	pJ247ΔNN	+++	160	0	91	5	3	1
BL341	pJ247R	+++	5	0	100	0	0	0
BL425	pJ247H	+++	37	19	75	3	0	5
BL410	pJ247S	++	33	0	6	67	15	12
BL430	pJ247G	++	39	0	8	18	26	49
BL391	pJ247X	+	66	0	2	18	52	27
BL394	pJ247Xhet	−	17	0	0	0	0	100
BL423	pJ247Mhet	−	18	0	0	0	0	100

* BL300 and BL422 are homologous integrants of pJ174 and pJ142, respectively. In each case expression of fp155 is controlled by the endogenous *vit-2* promoter (Broverman et al., 1993).

testines lacked label only over the first set of four intestinal nuclei. Frequently the limits of the labeled region were very distinct, defining a sharp boundary (Fig. 2C and D). Samples in which the unlabeled zone extended posteriorly to include the second set of two intestinal nuclei were designated Class III. Within this classification were those samples in which there was a distinct boundary after the second set of intestinal cells (not shown), as well as those in which the signal increased gradually toward the posterior (Fig. 2E and F). Class IV mosaic intestines showed a region of high-density signal bounded both anteriorly and posteriorly by regions lacking label (Fig. 2G and H). They ranged from those in which the positive region extended for more than half of the intestine to those in which there was a small labeled region of only 2–3 sets of intestinal nuclei bounded by negative stretches. In many intestines the transitions were gradual, with intermediate zones between the positive and negative regions. Finally, in Class V intestines none of the cells was labeled (Fig. 2I and J).

For each of the 25 strains examined, we scored intestinal samples according to the above classification scheme (Table 1, Fig. 3). The terminal bulb of the pharynx remained attached to the intestine in many samples and provided a landmark for orientation of the intestine. The most anterior set of intestinal cells is also distinctive in having four nuclei at approximately the same anterior–posterior level. Thus the anterior end of the intestine could be identified even in cases in which the pharyngeal bulb was no longer attached to the intestine to provide an anterior marker (e.g., Fig. 2G). Only those intestines with clear landmarks for orientation were included in the analysis, although hundreds of unoriented intestinal fragments were recorded and confirmed that the patterns were not excision artifacts.

Some of the strains were not viable or fertile as homozygotes and thus were maintained as

PROTEIN LEVELS	HYBRIDIZATION CLASS I	II	III	IV	V
++++	83	15	1	1	0
+++	5	80	10	5	2
++	0	19	48	13	20
+	0	8	17	41	34
-	0	0	0	0	100

Figure 3. Hybridization patterns correlate with levels of expression. In situ results arranged according to hybridization patterns (Class I–V; see Figs. 2 and 4B) and the level of transgenic protein expression measured by Western analysis (MacMorris et al., 1992). Numbers given are percentages of the total for each set of strains. Protein levels: + + + + (100–150% of BL203 control) (number of samples, n = 161) Strains BL203 (pJ247), BL207 (pJ247het), BL300 (homologous pJ174), BL422 (homologous pJ142), BL303 (pJ174-het), BL460 (pJ247B3G); + + + (32–88%) (n = 406) Strains BL301 (pJ174het), BL305 (pJ174het), BL330 (pJ247ΔNN), BL332 (pJ247ΔNN), BL341 (pJ247R), BL385 (pJ247Lhet), BL386 (pJ247L), BL425 (pJ247H); + + (14–20%) (n = 115) Strains BL304 (pJ174het), BL410 (pJ247S), BL430 (pJ247G); + (1–6%) (n = 113) Strains BL310 (pJ142het), BL311 (pJ142het), BL315 (pJ142), BL391 (pJ247X); -(0%) (n = 47) Strains BL322 (pJ49), BL394 (pJ247X), BL423 (pJ247M), BL440 (pJ110).

stocks containing a mixture of the fertile heterozygotes and sterile non-transgenic *tra-3* siblings. In those cases, varying proportions of the individuals examined on any slide displayed an absence of hybridization. Those individuals were presumed to be the segregants that did not contain the fusion gene and were therefore not included in the counts. As a result, the Class V non-expressors are underrepresented in the data.

We found a good correlation between the measured levels of fusion protein as detected on Western blots (MacMorris et al., 1992) and the hybridization patterns (Fig. 3). Strains with high levels of the fusion protein exhibited uniform hybridization throughout the intestine. In the strains with lower protein levels, the first set of intestinal cells more often lacked signal. As the level of protein decreased, we observed a higher percentage of intestines with the areas of signal bounded both anteriorly and posteriorly by negative areas.

Discussion

Pattern of RNA localization reflects level of promoter function

We developed a reliable technique for detecting transgenic *vit-2/6* fusion gene expression using in situ hybridization of RNA probes to squashed and cut worms. Our in situ hybridization results indicated that tissue-specific repressor elements were not disabled by any of the changes in promoter sequence, since ectopic expression of the *vit-2/6* fusion gene was never seen. We found instead that when expression was detectable, label was confined to intestines of adult hermaphrodites. Interestingly, the intestines were not uniformly labeled but exhibited a predictable correspondence between protein expression levels and the pattern seen with in situ hybridization. Strains with fully functional promoters had uniformly labeled intestines. A progression in the dysfunction of the *vit* promoter was accompanied first by a loss of label in the anteriormost cells. As expression was more severely affected by the promoter modifications, a greater proportion of the anterior end was unlabeled. Eventually a negative region was seen at the posterior end as well, with the mid-region containing higher levels.

These patterns were not specific to the polylinker probe, which detects fusion gene mRNA. We have demonstrated that in a strain in which homologous integration placed the endogenous *vit-2* gene under the control of a mutant promoter, drastic reductions in expression of the endogenous gene product and elevated levels of the transgenic fusion protein resulted (Broverman et al., 1993). In those strains the *vit-2/6* transgene integrated within the homologous region of *vit-2*, creating tandem *vit-2/6* and *vit-2* genes, each under the control of the other's promoter. Using the homologous integrant strain and a probe to specifically detect the *vit-2* product, we found patterns of expression predicted for the mutant promoter (Broverman et al.,

1993). The same homologous integrant strain examined with the PL probe, specific for the *vit-2/6* product under the control of the endogenous *vit-2* promoter, had uniformly labeled intestines—the wild-type pattern in each case (Table 1). These results strongly support the contention that the pattern of labeling by in situ hybridization reflects the level of promoter function.

Intestinal labeling patterns do not follow the cell lineage

The zones of cells that typically lacked label in the mutant strains do not represent clonal groups. The four cells composing the first anterior set are not a clone. As shown in Figure 4A, the smallest clonal unit containing those four cells also contains most of the anterior half of the intestine (Sulston et al., 1983). If our integrated transgene had been inactivated in some cells at an early stage, we would have expected that the pattern of hybridization reflected these clonal relationships. Because of apparent cell rearrangements within this lineage, clonal patches would not have produced large contiguous regions encompassing the middle, nor would they have left successive sets of anterior cells as distinct unlabeled regions, as we found (Fig. 4B). The patterns of RNA accumulation must reflect factors other than cell lineage.

The lineage of the intestine does indicate some differences between regions within the intestine. The embryonic intestine contains twenty mononucleate cells, which form a hollow tube. Near the end of the first larval stage, most of the nuclei divide, creating binucleated cells. The four nuclei of the first anterior set (Int1) and the pair of Int2 nuclei do not divide and remain mononucleate. Interestingly, these nuclei are the most sensitive to partial inactivation of the promoter. These cells are apparently different in several ways. Recently, Beh et al. (1991) reported an intestinal acid phosphatase in C. elegans that is not expressed in the same two anterior sets of cells. The nuclei in the four posteriormost cells variably fail to divide as well, giving rise to a twenty-cell intestine with 30 to 34 nuclei (Sulston and Horvitz, 1977). Just before each larval molt, the DNA within these nuclei endoreduplicates, producing adult intestinal nuclei, each with the same total DNA content of 32C (Hedgecock and White, 1985). Therefore, it is not likely that differences between intestinal cells in ploidy or transgene copy number would account for the patterns we have found.

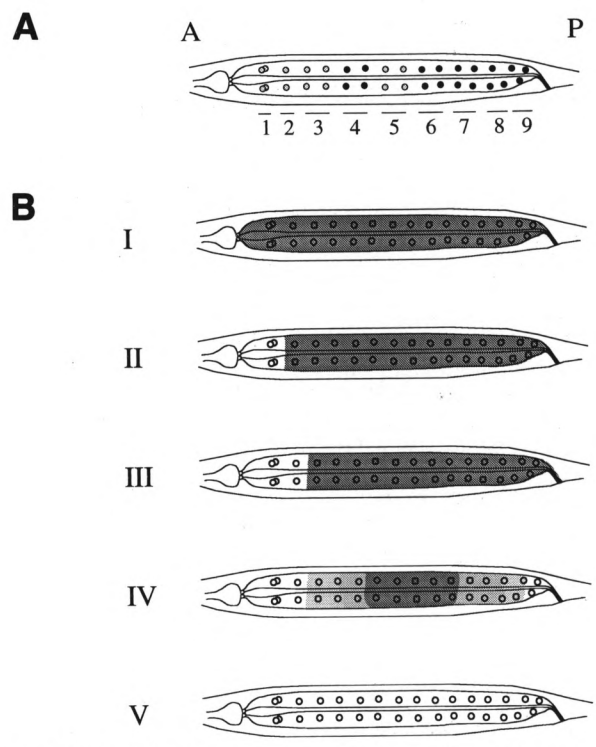

Figure 4. Hybridization patterns do not follow intestinal cell lineage. **A.** Schematic diagram of an intestine attached to the terminal bulb of the pharynx showing clonally derived regions. Thirty-two nuclei are shown, grouped in nine sets, labeled 1–9. The lineal descendants of the E_p cell are dark, and the descendants of E_a are light (data from Sulston and Horvitz, 1977). **B.** Intestinal diagram showing hybridization classes I–V, as illustrated in Figure 2 and described in the text. Dark stippling indicates regions containing intense hybridizing RNA within the intestine. Light stippling indicates lower hybridization level. A, anterior; P, posterior.

Intestinal patterns could reflect an underlying distribution of activators

One possible explanation for the uneven distribution of fusion gene expression in strains carrying mutant promoters would be an inherently uneven distribution of activator(s). According to this model, the activator localization pattern is present but not seen with wild-type promoters, because they are less sensitive to activator concentration. We suggest that the mutant promoters have a lower binding affinity for activating factors, which raises the threshold of activator required for expression. In this model, the anteriormost cells of a strain with

a weak mutant promoter have a sub-threshold level of activator(s) and therefore no fusion gene expression. In a strain with a severely impaired promoter, the threshold is significantly higher, so that only mid-intestinal cells have sufficient activator to promote expression. The data are consistent with models in which an activating substance peaks in the mid-region of the intestine or in which there are oppositely-oriented gradients of two required factors, with a critical level of each achieved only in the mid-region in mutant strains.

Schedin et al. (1991) reported nonuniform intestinal localization of *vit-5* and *vit-6* mRNA in intestines of mosaic intersexes with labeling confined to the anterior ends. In contrast, they also found preferential labeling of posterior intestinal cells in studies of late L4 to very young adult wild-type worms. At later time points, the labeled region extended further to the anterior. Schedin et al. (1991) postulated that they had detected two gradients that represented different signals acting to promote vitellogenesis. One signal originated at the anterior end to establish sexual identity of intestinal cells, and the other originated at the posterior end and acted as a temporal signal responsible for initiating *vit* transcription in hermaphrodite-committed intestinal cells. While the nonuniform intestinal sex-specification may occur at a stage prior to vitellogenesis to result in a hermaphrodite-specific intestinal program, it may also initiate a cascade of sex-specific activators, which themselves are more concentrated in the anterior. If such "anterior activators" act first to ready the intestinal cells for the postulated "posterior temporal activator," then Schedin et al. would have seen posterior expression only at early times. In our studies, the lack of labeling of both ends of the intestine implies that if two activators are involved, then the mutant promoters have reduced affinity for both factors. While some of the patterns we see suggest the mutants are merely delayed in expression, we did not find wild-type patterns of hybridization when older worms were assayed.

Another possibility is that activator levels may be highest in the intestinal regions that lie adjacent to the vitellogenic portions of the gonad, suggesting inductive tissue interactions between the gonad and intestine. The influences would have to originate in the somatic gonad if required for vitellogenin synthesis, since the sterile mutant strain *glp-4(bn2)I* lacks germ cells (Beanan and Strome, 1992) and yet expresses the *vit* genes (M. Beanan and S. Strome, personal communication). In addition, mab-3 males synthesize vitellogenins despite the presence of a relatively normal male gonad, suggesting that inductive influences do not derive specifically from hermaphrodite-specific gonadal structures. We have not, however, examined the patterns of *vit* RNA localization in either of these strains.

A cell-specific on/off switch

Our observations of differences in expression levels between cells within an individual highlight an important implication of this study. In individuals with promoters acting with lowered efficiency, transcription levels were not simply lowered in all cells. Rather, some cells and some individuals lacked expression, while others even within the same intestine had high levels. We interpret our results in terms of graded distributions of activator(s), with a response limited to the cells in which activator levels exceed a threshold. This suggests that a gradual, linear increase in activator concentration can act as an effective off/on switch for gene expression. In Drosophila there is precedent for such a mechanism. The *bicoid* gene product is distributed in embryos in an anterior–posterior gradient that has been shown to effect a switch with relatively sharp spatial boundaries for target genes with different binding affinities (Driever et al., 1989; Struhl et al., 1989). We might expect such an activation switch if multiple activators bind either cooperatively (Ptashne, 1986) or independently (Fiering et al., 1990).

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