Regulation of Organogenesis by the *Caenorhabditis elegans* FoxA Protein PHA-4

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Regulation of Organogenesis by the *Caenorhabditis elegans* FoxA Protein PHA-4

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The *pha-4* locus encodes a forkhead box A (FoxA/HNF3) transcription factor homolog that specifies organ identity for *Caenorhabditis elegans* pharyngeal cells. We used microarrays to identify pharyngeal genes and analyzed those genes to determine which were direct PHA-4 targets. Our data suggest that PHA-4 directly activates most or all pharyngeal genes. Furthermore, the relative affinity of PHA-4 for different TRTTKRY (R = A/G, K = T/G, Y = T/C) elements modulates the onset of gene expression, providing a mechanism to activate pharyngeal genes at different developmental stages. We suggest that direct transcriptional regulation of entire gene networks may be a common feature of organ identity genes.

Cells acquire distinct fates during development in response to a host of regulators, many of which encode transcription factors. A long-standing issue has been the nature of the target genes controlled by these transcriptional regulators: Do they activate a few genes at the top of a hierarchy, or do they activate many genes that function at multiple stages throughout development? This question has been difficult to resolve because identification of downstream genes has relied largely on genetic approaches, which demand easily observed phenotypes. We used a genomic approach to investigate how the predicted transcription factor PHA-4 promotes development of an organ, the *Caenorhabditis elegans* pharynx.

The *C. elegans* pharynx is a neuromuscular organ that pumps food in from the environment and initiates digestion. The precursors of the pharynx are generated by distinct genetic programs and cell lineages and are assembled into a 92-cell primordium that undergoes complex patterning and morphogenesis (*1*–*3*). Ultimately, the precursors acquire different fates within the pharynx, including muscles, neurons, glands, structural cells, valves, and epithelia (*4*, *5*).

The *pha-4* loss-of-function phenotype and the expression pattern of PHA-4 reveal that all cells of the pharynx, regardless of their distinct ancestry or ultimate cell fate, share a common pharyngeal identity that is specified by *pha-4* (*6*–*8*). *pha-4* encodes a protein that most closely resembles forkhead box A (FoxA) transcription factors (*6*, *8*), which have been implicated in gut development in other organisms (e.g., *Drosophila* Forkhead and mammalian FoxA2) (*9*). Few target genes are known for FoxA members. For example, the only identified direct target of PHA-4 is the pharyngeal myosin gene *myo-2*, indicating that PHA-4 can function late in development (*8*). We identified and analyzed PHA-4 targets to study how FoxA proteins function during organogenesis.

Identification of pharyngeal genes. To identify pharyngeally expressed genes, we probed *C. elegans* microarrays (*10*) with labeled complementary DNA from embryos that produced either excess pharyngeal cells (*par-1* mutants) (*11*) or no pharyngeal cells (*skn-1* mutants) (*12*). We performed three independent experiments with microarrays that contained 62% of the *C. elegans* genome (11,917 out of 19,099 predicted genes) and identified 240 candidate pharyngeal genes with an average *par-1*/*skn-1* ratio ≥2.0 (*13*).

Three lines of evidence indicate that our microarray experiments selectively identified pharyngeally expressed genes. First, the microarrays contained 18 genes previously shown to be expressed in the embryonic pharynx. We identified 15 of these genes (*13*), including genes expressed at low levels (e.g., *ceh-22*) (*14*). Second, a search of messenger RNA (mRNA) in situ hybridization data demonstrated that 59 out of 70 of our positives were pharyngeally expressed (*15*). Third, we constructed green fluorescent protein (GFP) reporters for 10 randomly chosen microarray positives and found that 8 out of 10 were expressed predominantly or exclusively in the pharynx (*13*). In total, the expression patterns of 96 of our microarray positives were characterized here or elsewhere, and 82% (79 out of 96) were selectively expressed in the pharynx (*13*).

Pharyngeal expression depends on predicted PHA-4 binding sites. To determine whether pharyngeally expressed genes were direct PHA-4 targets, we searched for predicted PHA-4 binding sites within our microarray positive reporters. The consensus binding site for PHA-4 orthologs is TRTTKRY (R = A/G, K = T/G, Y = T/C) (*16*), and PHA-4 binds a TRTTKRY element in the promoter of *myo-2*, the only known direct target of PHA-4 (*8*). All eight of our pharyngeally expressed reporters carried three or more TRTTKRY elements within their likely regulatory elements, and two of our nonpharyngeal reporters had one or no element (*13*).

Pharynx-specific expression of eight out of eight reporter constructs depended on TRTTKRY elements (Fig. 1A). For every promoter analyzed, elimination of one or more TRTTKRY elements resulted in either a complete loss of pharyngeal GFP (Fig. 1A, i through vi) or a substantial decrease in expression and a delay in the observed onset of expression (Fig. 1A, vii and viii). Moreover, pharyngeal expression of each of three additional genes [*avr-15* (*17*), H30A04.1/*eat-20* (*18*), and *peb-1* (*19*)] was dependent on an intron containing two or more TRTTKRY elements (*13*). We also constructed GFP reporters for one additional microarray positive and one gene with a borderline *par-1*/*skn-1* ratio (=1.9), neither of which contained any TRTTKRY elements in its upstream region. Neither reporter was expressed in the pharynx (*13*).

Our findings suggest that expression in the pharynx is critically dependent on TRTTKRY elements for genes expressed either early (e.g., M05B5.2) or later (e.g., ZK816.4) in pharynx development. Because our reporter constructs relied on randomly selected genes, our findings suggest that many, perhaps all, genes selectively expressed in the pharynx depend on TRTTKRY elements.

PHA-4 functions during early and late pharyngeal development. Based on the known expression patterns of *C. elegans* Fox genes, PHA-4 is the best candidate to recognize the pharyngeal TRTTKRY elements. Previous studies showed that *pha-4* functions early in embryogenesis (*7*). If PHA-4 directly activates most or all pharyngeal genes, then PHA-4 should also be required during later pharynx development. To test this idea, we engineered a temperature-sensitive configuration of *pha-4* [*pha-4*(*ts*)] and shifted these animals from permissive to restrictive temperatures at different developmental time points (*13*).

We found that *pha-4* activity was required throughout development for viability (Fig. 2A). Shifting *pha-4*(*ts*) to a restrictive temperature any time after the formation of pharyngeal precursors, but before the pharynx was fully developed (early and late embryos), resulted in 100% lethality. Late embryos shifted to restrictive temperatures arrested as first stage (L1) larvae

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with a "stuffed" pharynx phenotype (*20*) that resembled that of animals with defects in pharyngeal pumping (*21*). We also observed substantial lethality among *pha-4*(*ts*) animals shifted as L1's, after pharynx development was complete (Fig. 2A). Adults that developed from these L1's were small and unhealthy, consistent with a feeding defect (*20*). These data demonstrate that *pha-4* activity is required during both early and late times in embryonic development.

To characterize the temperature-sensitive phenotype further, we analyzed expression of three of our reporters. For D2096.6 and C44H4.1 reporters, a reduction in PHA-4 activity in the mature pharynx resulted in a reduction of pharyngeal GFP. Conversely, restoration of PHA-4 activity was sufficient to reactivate pharyngeal expression (Fig. 2, B through H). This effect was specific because a reporter expressed in nonpharyngeal cells (coelomocytes) showed no change in expression when animals were shifted to a restrictive temperature.

The third pharynx reporter, *myo-2*::GFP, showed no appreciable decrease in expression. *myo-2* expression is probably maintained by the combined actions of other transcription factors, including CEH-22 and PEB-1 (*14*, *19*). This hypothesis is consistent with our finding that the PHA-4 site of *myo-2* is important for initial expression of a *myo-2* reporter but not for later expression (Fig. 1A).

PHA-4 binds TRTTKRY elements in vitro. A second line of evidence that indicates that TRTTKRY elements are recognized by PHA-4 is that PHA-4 binds this consensus in vitro. We tested the ability of four different TRTTKRY sequences to compete against a labeled TGTTTGC-bearing probe for binding to PHA-4 in electrophoretic mobility shift assays (EMSAs) (*8*, *13*). The TGTTTGC-bearing sequence represented a characterized PHA-4 site from the *myo-2* promoter (myo2-WT) (*8*). We tested three more sites identified in our reporter assays: TGTTGAC from M05B5.2 (myo2-M), TGTTTGT from D2096.6 (myo2-D), and TATTTGT from *ceh-22* (myo2-ceh). These sequences were placed within the context of myo2–wild-type (myo2-WT) flanking sequences to minimize any effects of sequences outside the TRTTKRY core. Each of these TRTTKRY sequences competed specifically against myo2-WT compared to a nonspecific competitor (myo2-non, TGAACAG) (*8*) (Fig. 3A).

Examination of in vitro binding curves revealed that PHA-4 bound different TRTTKRY sequences with different affinities. Quantitative analyses demonstrated that myo2-ceh had four times less affinity for PHA-4 than did myo2-WT (paired *t* test of the log of the median inhibitory concentration [IC_{50}], $P = 0.023$) (Fig. 3C), whereas the myo2-WT, myo2-M, and myo2-D sites had comparable affinities. We observed differences between the myo2-WT site (TGTTTGC) and the D site (TGTTTGT) when we used endogenous flanking sequence from D2096.6 rather than *myo-2*. We performed EMSA with labeled myo2-WT, as above, and two cold competitors: D2096-WT (wild-type D2096.6 sequence, which includes TGTTTGT) and D2096-mut (D2096.6 sequence mutated to include TGTTTGC) (Fig. 3B). In the context of endogenous D2096.6 promoter sequence, we observed that D2096-mut had a twofold higher affinity than D2096-WT (Fig. 3D) (paired *t* test of log [IC_{50}], $P = 0.015$). Our ability to detect this difference in the context of D2096.6, but not in the *myo-2* sequence, suggests that sequences flanking TRTTKRY influence the binding affinity of PHA-4.

Onset of expression is modulated by altering the affinity of TRTTKRY elements for PHA-4. The affinity of TRTTKRY elements for PHA-4 influenced the onset of expression of six out of six GFP reporters in a manner consistent with the in vitro binding data (Fig. 4A). We generated "up" mutations (from a low- to high-affinity site) and "down" mutations in TRTTKRY elements of our GFP reporters. Predicted down mutations in M05B5.2 or T05E11.3 promoters (from TGTTGAC or TGTTTGC to TGTTTGT, respectively) resulted in a delay of 2 to 3 hours in the onset of pharyngeal expression as compared to unaltered reporters (Fig. 4, A through E). Conversely, when predicted weaker elements were mutated to TGTTTGC, we noticed a shift of 1 to 3 hours in the onset of expression to earlier stages (Fig. 4A and F through H) (*22*). Despite differences in the onset of expression, alterations in the sequence of TRTTKRY elements did not affect the strength of GFP fluorescence in later stages.

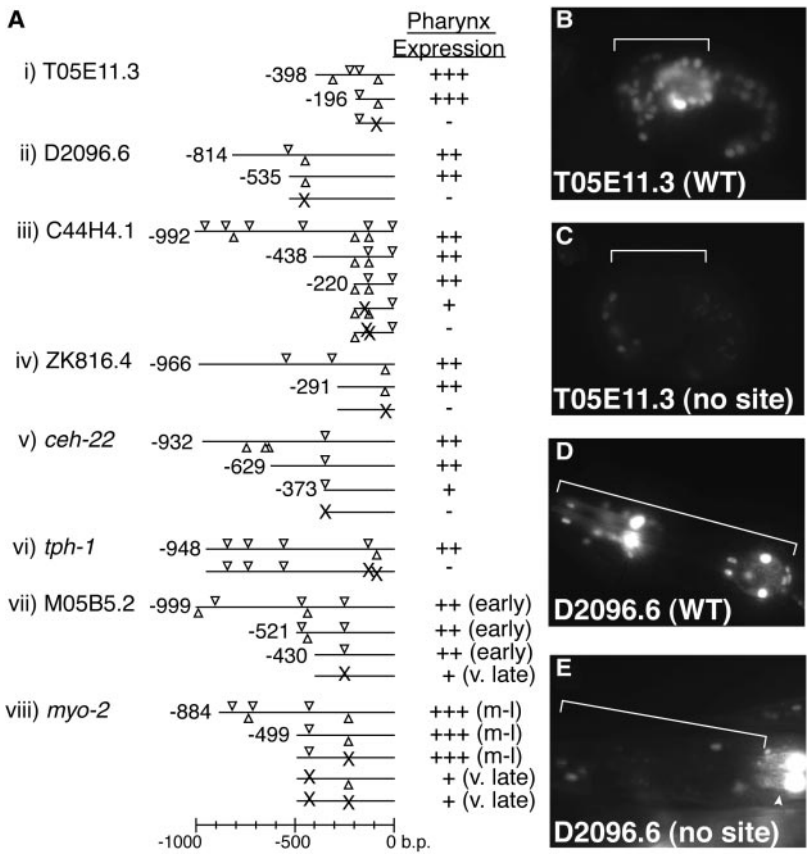


Fig. 1. Pharyngeal expression requires TRTTKRY elements. (**A**) Diagrams of promoter fragments used to drive expression of a GFP::HIS2B reporter (*13*). Triangles represent TRTTKRY elements on the top or bottom strand, and X's indicate eliminated TRTTKRY elements. Numbers indicate the 5′ end relative to the predicted start codon of each gene. "Pharynx expression" is the relative strength of pharyngeal GFP at its peak in embryogenesis, assessed visually. For M05B5.2 and *myo-2*, notes in parentheses indicate the relative onset of GFP expression (*22*). *ceh-22*, *myo-2*, and *tph-1* are known to be expressed in the pharynx (*14*, *32*–*34*). (**B** through **E**) Embryos and larvae expressing GFP. Anterior is left, and dorsal is up. Brackets indicate pharyngeal cells. (B) Pharyngeal expression of the –196 T05E11.3::GFP reporter with a wild-type predicted PHA-4 site [TGTTTGC; shown in (A)i]. (C) GFP expression of the –196 T05E11.3::GFP reporter with a disrupted site (TGGATCC) is faintly visible in cells outside the pharynx [shown in (A)i]. (D) Pharyngeal expression of the –535 D2096.6::GFP reporter containing the wild-type predicted PHA-4 site [TGTTTGT; shown in (A)ii]. (E) GFP expression of the –535 D2096.6::GFP reporter with a disrupted site [TCCGCGG; shown in (A)ii]. Faint GFP fluorescence is detected in epidermal and gut (arrowhead) cells.

If the affinity of PHA-4 for TRTTKRY elements regulates onset of expression, then a decrease in PHA-4 levels should mimic a down mutation of a PHA-4 binding site. We tested this prediction with the early-expressed, panpharyngeal M05B5.2 reporter in our *pha-4*(*ts*) strain (*13*). In animals grown at an intermediate temperature (20°C), the pharynx was formed, but was often disorganized, suggesting that PHA-4 was present at insufficient levels to promote proper pharynx development (*20*). Under these conditions, expression of the M05B5.2 reporter was delayed as compared to the same strain grown at a permissive temperature (Fig. 5, A through D). Eventually, expression of this reporter reached a level equivalent to that of unshifted controls. This result is consistent with our model and suggests that PHA-4 is critical for controlling the onset of pharyngeal gene expression.

The effect of affinity on the onset of pharyngeal expression may be generally true. An analysis of the promoters of our microarray positives revealed a striking correlation between the rank of positive genes (according to their differential expression in microarray experiments) and the probability of a gene having a high-affinity PHA-4 binding site in its promoter (Fig. 5E). Genes that were highly ranked were more likely to contain a moderate- to high-affinity site than were lower ranked genes (X^2 goodness-of-fit, $P = 0.07$). Conversely, lower ranked genes were slightly more likely to contain only a low-affinity PHA-4 site. We suggest that this correlation was revealed because we isolated RNA from embryos at mid-stages of pharyngeal development, when the abundance of a given mRNA reflected the onset of its expression.

Affinity model for pharyngeal expression. We propose that (i) PHA-4 directly regulates a wide array of genes expressed at multiple stages of pharyngeal development, and (ii) the affinity of TRTTKRY elements for PHA-4 regulates the relative onset of the expression of target genes. Two observations suggest that temporal regulation by PHA-4 is important in vivo. First, mutations that alter the affinity of TRTTKRY elements shift the onset of GFP expression by 1 to 3 hours, which is a substantial effect given the rapid development of *C. elegans* embryos (<14 hours). Second, using our *pha-4*(*ts*) strain, we observed a concordance between the temperature at which we first observed lethality and the temperature at which we first detected delayed GFP expression (Fig. 5) (*20*).

We propose that high-affinity sites bind PHA-4 in early embryos whereas low-affinity sites may not bind until later, after PHA-4 levels increase. Consistent with this idea, PHA-4 is expressed at low levels at the ~50-cell stage of embryogenesis and increases as development progresses (*6*). Moreover, 15 out of 23 genes expressed during early pharyngeal development [as determined by mRNA in situ hybridization (*15*) or our reporters] carry high-affinity sites, and the remaining 8 genes carry sites whose affinity has not been tested. This affinity model is reminiscent of the threshold response of genes to a gradient of *bicoid* along the anteroposterior axis or *dorsal* activity along the dorsoventral axis of the *Drosophila* embryo (*23*, *24*). PHA-4 target genes may demonstrate temporally, rather than spatially, restricted threshold responses so that genes required for early aspects of pharynx development (e.g., regulators) are expressed before those required for later aspects of development (e.g., differentiation markers).

The affinity of a PHA-4 binding site does not correlate with the absolute onset of pharyngeal gene expression. The most striking example of this discrepancy is the different behavior of T05E11.3 and *myo-2* reporters. Both genes depend on a TGTTTGC site for expression in the pharynx, but T05E11.3 expression is first visible in early embryos, and *myo-2* expression is not visible until ~3 to 4 hours later (Fig. 4). These data suggest that a high-affinity PHA-4 site may be necessary but not sufficient for early activation of transcription. In some cases, the availability of other activators may determine the onset of expression. For example,

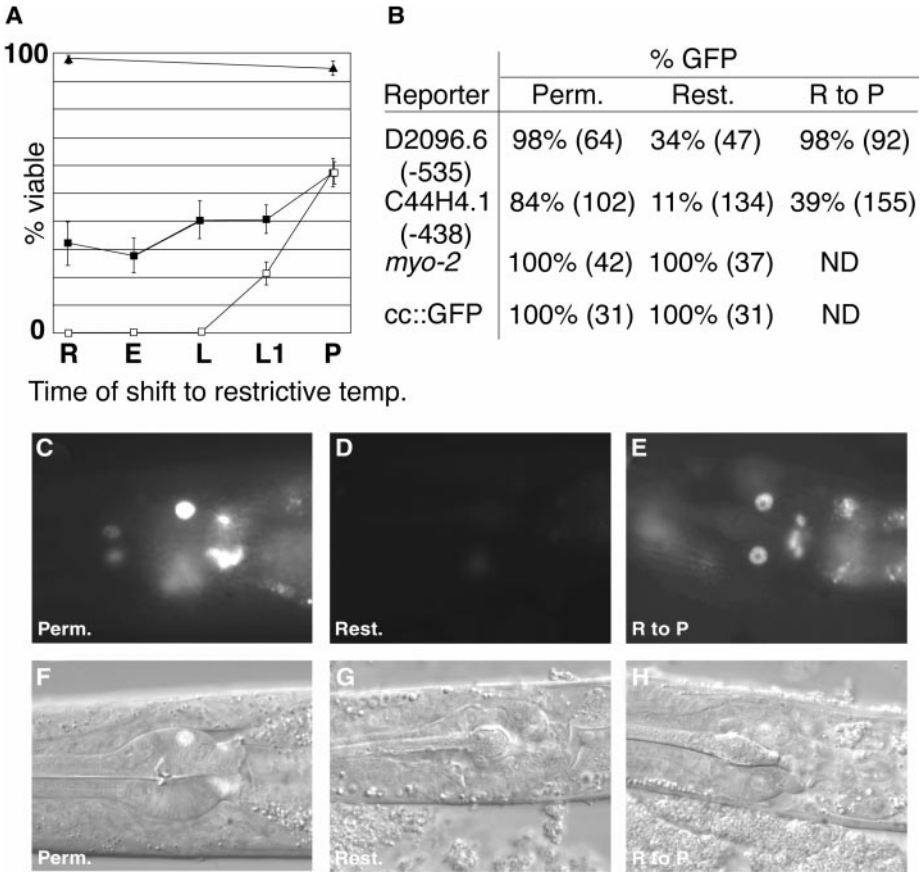
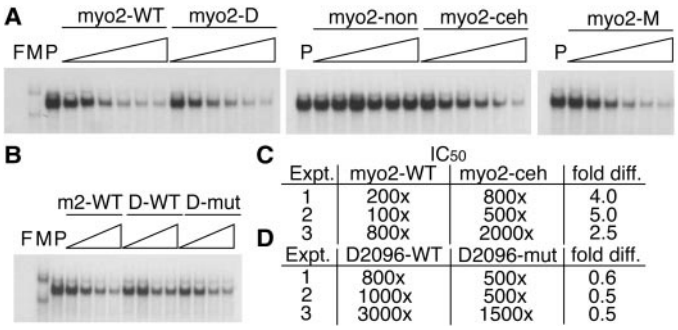


Fig. 2. *pha-4* is required early and late in pharynx development. (**A**) Viability of a *pha-4*(*ts*) strain when shifted to a restrictive temperature at different times in development. Triangles represent data for the *smg-1*(*cc546ts*) control strain; open boxes represent data for the *pha-4*(*ts*) strain, *smg-1*(*cc546ts*);*pha-4*(*zu225*); and solid boxes represent data for the temperature-insensitive strain, *smg-1*(*r861*);*pha-4*(*zu225*) (*13*). "% viable" is measured as the percentage of embryos that developed into adults. The horizontal axis shows the time of shift to a restrictive temperature (*13*): "R" embryos were grown completely at restrictive temperatures, "E" were shifted as early embryos, "L" were shifted as late embryos, "L1" were shifted after hatching, and "P" were grown continuously at permissive temperatures. Data represent the 95% confidence interval for "% viable," using a normal approximation to the binomial distribution. (**B**) *pha-4* activity is required early and late for expression of pharyngeal genes. Values are the percentage of transgenic animals with detectable pharyngeal GFP (lines 1 to 3) or coelomocyte GFP (line 4). (B) through (H) "Perm." animals were grown at permissive temperatures; "Rest." animals were grown at permissive temperatures and later shifted to restrictive temperatures; and "R to P" animals were grown at permissive temperatures, shifted to restrictive temperatures, and later returned to permissive temperatures (*13*). (**C** through **H**) GFP expression (C through E) and merged Nomarski/differential interference contrast–GFP images (F through H) of *pha-4*(*ts*) animals carrying a D2096.6::GFP::HIS2B reporter. Posterior bulbs of adult pharynges are shown.

other transcription factors that activate *myo-2* expression are expressed later in development than PHA-4 (*14*).

Implications for other developmental regulators. FoxA proteins in many species may directly regulate most or all genes within an organ. Both mammalian and *Drosophila* orthologs of PHA-4 are required for early aspects of digestive tract formation and also directly activate late-acting genes (*16*, *25*–*27*). Moreover, heterozygous Foxa/+ mice have numerous abnormalities, demonstrating that vertebrates are sensitive to the dosage of FoxA proteins, consistent with the model presented here (*28*, *29*). More generally, direct regulation of an entire genetic network may be a hallmark of organ identity factors. The selector gene *SCALLOPED* directly activates genes that function at two distinct steps of the regulatory hierarchy leading to wing formation in *Drosophila* (*30*). Similarly, the paired-type homeodomain factor Pax6/*eyeless* is both necessary and sufficient for specification of eye formation in *Drosophila* imaginal discs, where it directly activates the expression of both early- and late-acting genes (*31*). These observations suggest that organ identity factors may use a strategy similar to that of PHA-4 to establish gene expression during organogenesis.



C

Expt.	myo2-WT	myo2-ceh	fold diff.
1	200x	800x	4.0
2	100x	500x	5.0
3	800x	2000x	2.5

D

Expt.	D2096-WT	D2096-mut	fold diff.
1	800x	500x	0.6
2	1000x	500x	0.5
3	3000x	1500x	0.5

Fig. 3. PHA-4 binds to different TRTTKRY elements with different affinities. (**A** and **B**) EMSA with radiolabeled duplex (myo2-WT) and varying amounts of cold competitor duplex. F, free probe; M, probe + reticulocyte lysate; and P, probe + PHA-4. (A) Amounts of cold competitor were, from left to right: 10×, 100×, 300×, 600×, 1000×, and 3000× molar excess relative to the probe. Cold competitor duplexes differ from myo2-WT only in the PHA-4 site, as indicated. Experiments shown were performed using the same reagents to allow a comparison of relative binding affinities. (B) Amounts of cold competitor were, from left to right: 100×, 300×, 1000×, and 3000× molar excess relative to the probe. (**C** and **D**) Quantitation of competition reactions provided estimates of the IC_{50}. IC_{50} values are expressed as fold excess of competitor and are inversely proportional to binding affinity (*35*). (C and D) IC_{50} values from three independent replicates of the experiments in (A and B), respectively.

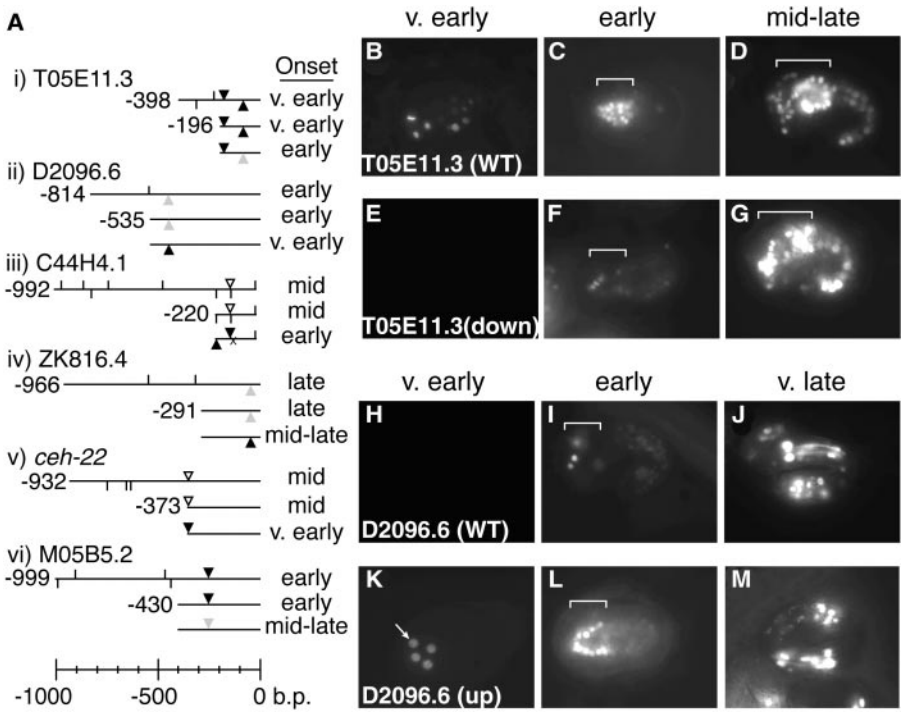


Fig. 4. The onset of expression in the pharynx can be altered by changes in the affinity of TRTTKRY elements for PHA-4. (**A**) Diagrams of promoter fragments used to drive expression of a GFP::HIS2B reporter. Vertical lines represent TRTTKRY elements that were not mutated in these experiments or whose relative affinity for PHA-4 has not been determined in vitro. Triangles represent TRTTKRY elements mutated in these experiments: black, gray, and open triangles represent high- (TGTTTGC or TGTTGAC), medium- (TGTTTGT), and low- (TATTTGT) affinity PHA-4 binding sequences, respectively. "Onset" refers to the developmental stage at which expression of GFP in the pharynx is first detected (*22*). (**B** through **M**) Embryos expressing GFP; brackets indicate the developing pharynx. (B through D) Pharyngeal expression of the –196 T05E11.3::GFP reporter containing the wild-type predicted PHA-4 site [TGTTTGC; shown in (A)iii]. (E through G) Pharyngeal expression of the –196 T05E11.3::GFP reporter containing a mutated site [TGTTTGT; shown in (A)iii]. (H through J) Pharyngeal expression of the –535 D2096.6::GFP reporter containing the wild-type predicted PHA-4 site [TGTTTGT; shown in (A)ii]. (K through M) Expression of the –535 D2096.6::GFP reporter containing a mutated site [TGTTTGC; shown in (A)ii].

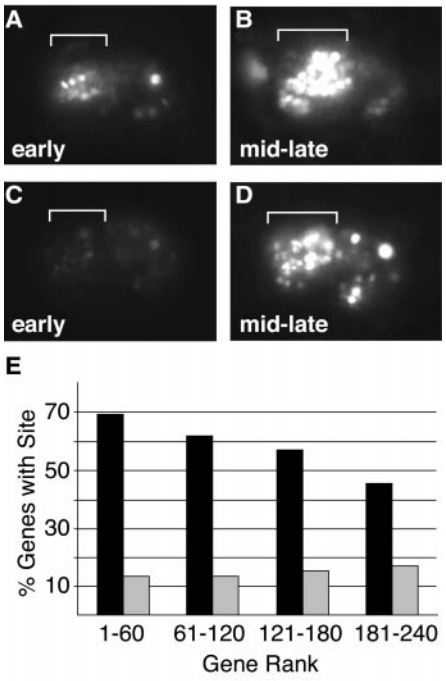


Fig. 5. Reduction of *pha-4* activity reduces early pharyngeal expression. (**A** through **D**) Expression of the –521 M05B5.2::GFP::HIS2B reporter in *pha-4*(*ts*) embryos incubated at permissive [24° (A) and (B)] and intermediate temperatures [20° (C) and (D)]. Brackets indicate the location of the developing pharynx. (**E**) Frequency of TRTTKRY elements in the upstream regions of microarray positives. Genes are ranked according to their differential expression in our microarray experiments; 1 is the highest rank (*par-1/skn-1* = 25.0), and 240 is the lowest (*par-1/skn-1* = 2.0). Bars show the percentage of genes in each rank group with one or more TRTTKRY elements. Black bars represent the frequency of genes with high- and moderate-affinity sites (defined by in vivo and in vitro experiments as TGTTTGC, TGTTTGT, and TGTTGAC). Shaded bars represent the frequency of genes containing only a low-affinity PHA-4 site (defined by in vivo and in vitro experiments as TATTTGT).

Evolution of organogenesis. Our findings suggest that a complex gene network regulated by PHA-4 could have evolved by serial recruitment of target genes. If the digestive tract of primitive organisms was a simple epithelial sac, then direct targets of ancestral PHA-4 may have been those that encoded digestive enzymes and those required to form epithelia. As additional genes, including transcription factors, fell under PHA-4 control, patterns of gene expression within the resulting organ structures may have increased in complexity. This model provides a possible explanation for the fact that FoxA proteins play a conserved role in foregut development in animals whose foreguts are morphologically and mechanistically different from one another. FoxA proteins may have acquired different target genes in different organisms, enabling new cell types or morphologies to emerge.

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Tunneling Spectroscopy of the Elementary Excitations in a One-Dimensional Wire

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The collective excitation spectrum of interacting electrons in one dimension has been measured by controlling the energy and momentum of electrons tunneling between two closely spaced, parallel quantum wires in a GaAs/AlGaAs heterostructure while measuring the resulting conductance. The excitation spectrum deviates from the noninteracting spectrum, attesting to the importance of Coulomb interactions. An observed 30% enhancement of the excitation velocity relative to noninteracting electrons with the same density, a parameter determined experimentally, is consistent with theories on interacting electrons in one dimension. In short wires, 6 and 2 micrometers long, finite size effects, resulting from the breaking of translational invariance, are observed.

Electronic systems such as metals contain a vast number of mobile electrons. Surprisingly, despite the Coulomb repulsion between them, many electronic properties of metals can be well described in terms of independent particles with a finite lifetime, each carrying charge e and spin half (*1*, *2*). Most importantly, their excitation spectrum, which for noninteracting electrons is simply determined by the underlying band mass m, is only slightly modified by the Coulomb interactions.

However, such a simple description of electronic systems, known as Landau Fermi-liquid theory (*1*), is valid only in two and three spatial dimensions. In one-dimensional (1D) metals, where electrons are forced to move on a line, the single-particle description completely breaks down by even the slightest Coulomb repulsion. Instead, the excitations of an interacting 1D system, being fluctuations of the charge and spin densities, are well described by collective modes that involve the entire electron population (*3*). These collective modes are decoupled into two separate kinds: collective spin modes and collective charge modes. Coulomb interactions couple primarily to the latter, thereby strongly influencing their dispersion. Conversely, the excitation spectrum of the spin modes, typically unaffected by interactions, remains similar to the noninteracting case. For example, low-energy charge excitations propagate with velocity $v_p = v_F/g$, where $v_F = \hbar k_F/m$ is the Fermi velocity ($\hbar = h/2\pi - h$ is Planck's constant, and k_F is the Fermi wave vector). The parameter g, which characterizes the interactions, is given by $g \approx \{1+[U/(2E_F)]\}^{-1/2}$, where U is the Coulomb interaction energy between particles in the wire and E_F is the Fermi energy (*4*). Thus, the stronger the Coulomb repulsion is, the larger the propagation velocity is.

Although interactions fundamentally alter the excitation spectrum of a 1D wire, they do not affect its conductance (*5*–*10*). In fact, a clean 1D wire has universal conductance irrespective of its length, density, and dispersion (*11*), ren-

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