IDENTIFICATION AND CHARACTERIZATION OF EFL-3,

A *C. ELEGANS* E2F TRANSCRIPTION FACTOR

APPROVED BY SUPERVISORY COMMITTEE

Leon Avery, Ph.D. (Mentor)

Michael Buszczak, Ph.D. (Chair)

Melanie Cobb, Ph.D.

Rene Galindo, Ph.D.

IDENTIFICATION AND CHARACTERIZATION OF EFL-3, A *C. ELEGANS* E2F TRANSCRIPTION FACTOR

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JENNIFER ANN WINN

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JENNIFER ANN WINN, M.S.

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Mentor: LEON AVERY, Ph.D.

The development of an organism depends on cells receiving and executing their specific fates, though how this process is regulated remains largely unknown. Here, we identify a mechanism by which a specific cell fate, apoptosis, is determined through the cooperative efforts of Hox and E2F proteins. E2F transcription factors are critical, conserved regulators of the cell cycle and apoptosis. However, little is known about the two most recently discovered mammalian E2Fs—E2F7 and E2F8. In

the nematode *Caenorhabditis elegans*, we identify a novel E2F7/8 homolog, EFL-3, and show that EFL-3 functions cooperatively with LIN-39, providing the first example in which these two major developmental pathways—E2F and Hox—are able to directly regulate the same target gene. Our studies demonstrate that LIN-39 and EFL-3 function in a cell type-specific context to regulate transcription of the *egl-1* BH3-only cell death gene and determine cell fate during development.

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PRIOR PUBLICATIONS

Winn JA, Mason RE, Robbins AL, Rooney WL, Hays DB. QTL mapping of a high protein digestibility trait in *Sorghum bicolor*. *International Journal of Plant Genomics*. 2009 Jul 7;2009:471853.

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BCL-2- B-cell lymphoma 2

BH3- Bcl-2 homology domain 3

CED- cell death defective

CEP- *C. elegans* p53

DPL- dimerization partner-like

E2F- E2 promoter binding factor

EFL- E2F-like

EGL- egg-laying defective

HOX- homeobox

HSN- hermaphrodite-specific neuron

IP- immunoprecipitation

LIN- lineage defective

MAB- male abnormal

Muv- multivulva

RB- retinoblastoma

UNC- uncoordinated

Vul- vulvaless

CHAPTER ONE

Introduction

CELL DEATH

Genetic studies of the programmed cell death cascade

During the development of many organisms, a subset of cells undergoes programmed cell death. The process by which cell death is executed has now been extensively studied (CHINNAIYAN *et al.* 1997a; CHINNAIYAN *et al.* 1997b; CONRADT and HORVITZ 1998; ELLIS and HORVITZ 1986; HENGARTNER *et al.* 1992; SESHAGIRI and MILLER 1997; WU *et al.* 1997a; WU *et al.* 1997b; YUAN *et al.* 1993; ZOU *et al.* 1997). However, how the apoptotic pathway is controlled in individual cells is not well understood, though it is of great importance to human disease, as the pathway is frequently abnormally regulated in cancer (see review by CHONGHAILE and LETAI 2008). The research presented here examines how a cell death gene, *egl-1*, is regulated within the context of the nematode *Caenorhabditis elegans*'s ventral nerve cord. *C. elegans* is often used as a model to study cell death, as the core apoptotic pathway was first defined in *C. elegans* (CHINNAIYAN *et al.* 1997a; CHINNAIYAN *et al.* 1997b; CONRADT and HORVITZ 1998; HENGARTNER *et al.* 1992; SESHAGIRI and MILLER 1997;

WU *et al.* 1997a; WU *et al.* 1997b; YUAN *et al.* 1993; ZOU *et al.* 1997) and homologs of the pathway were later confirmed to be highly evolutionarily conserved in other species, including mammals and *Drosophila* (Fig. 1-1).

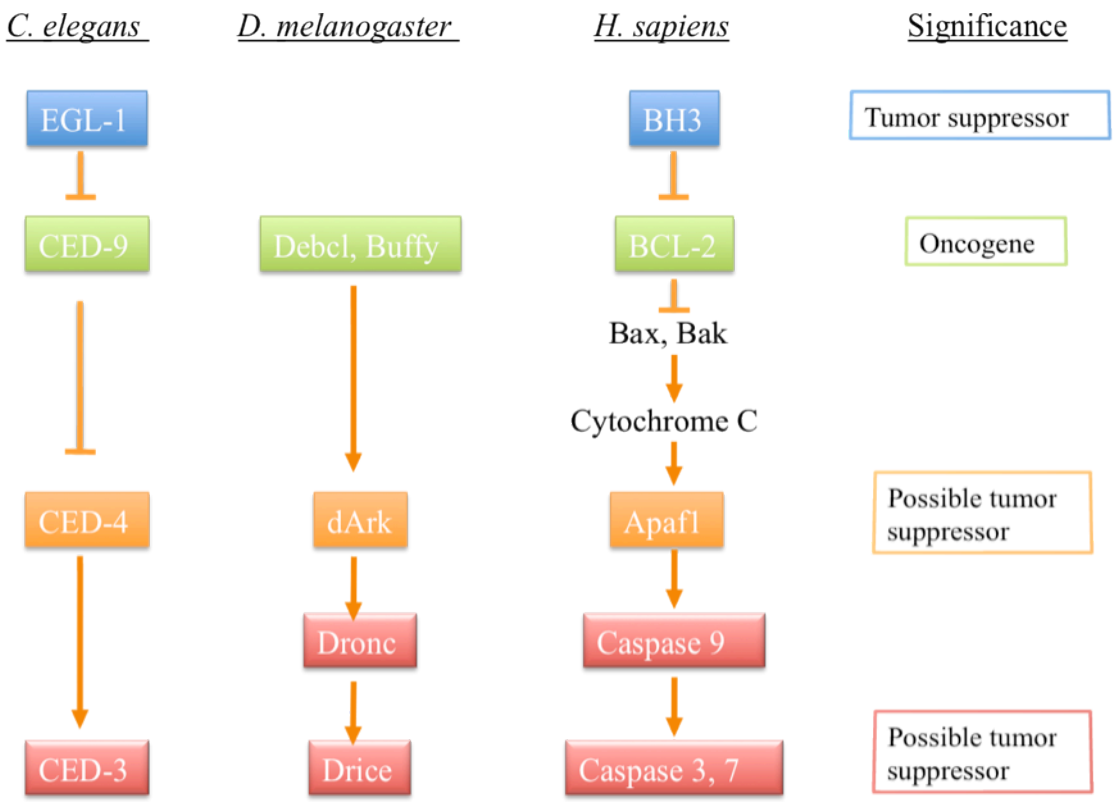


FIGURE 1-1 The core cell death pathway is conserved across species [modified from (HAY and GUO 2006; POTTS and CAMERON 2011)].

Identifying *C. elegans* as a model to study apoptosis

C. elegans was first chosen as a candidate model organism to study cell death and other developmental processes by Sydney Brenner in the 1960's. By that time, significant advances had been made in the field of genetics, and approaches were being introduced to take the known molecular fundamentals of genetics and apply them to a more complex biological setting. To Brenner, the ideal characteristics of a model organism included easily grown, small and with few cells, and genetically tractable (WOOD 1988). Nematodes of the genus *Caenorhabditis* met these requirements, and he ultimately chose *C. elegans* as an ideal, simple genetic model organism. To begin this work, Brenner and colleagues identified the pattern of cell divisions and fates within individual *C. elegans* nematodes.

***C. elegans* undergo an essentially invariant pattern of development**

Work by Robert Horvitz and others in Brenner's lab identified a characteristic of *C. elegans* that made the worm particularly amenable to genetic studies of cell death: wildtype *C. elegans* follow an essentially invariant pattern of development. That is, grown under identical conditions two N2 worms, from hereon referred to as the "wildtype," will develop cells of the same number and fate (KIMBLE and HIRSH 1979; SULSTON and

HORVITZ 1977; SULSTON *et al.* 1983). Thus, by knowing the numbers and locations of cells typically destined to die, it is possible to study how various mutations may affect the pattern of cell death.

Apoptosis within the ventral nerve cord

Each wildtype hermaphrodite nematode gives rise to 1090 somatic nuclei, with 131 of those cells undergoing programmed cell death (SULSTON *et al.* 1983). 113 cells apoptose during the worm's embryonic development. The remaining eighteen deaths occur during the postembryonic stages of L1-L4, before the worms reach adulthood (SULSTON and HORVITZ 1977). Of these, ten occur across the length of the ventral nerve cord (Fig. 1-2).

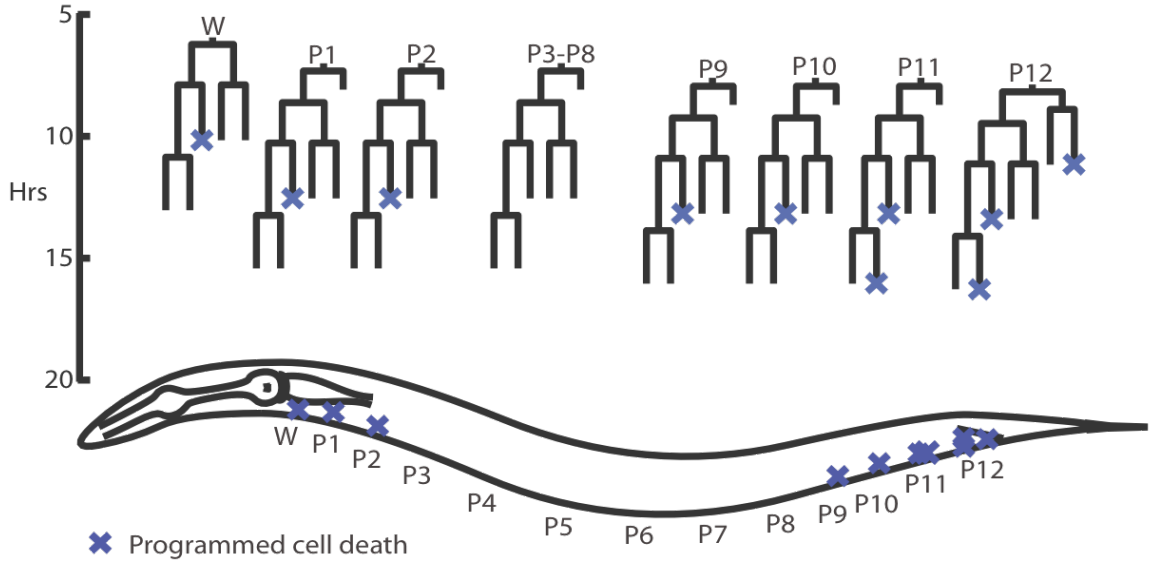


FIGURE 1-2 Cell death within the ventral nerve cord. Hours post-hatching are shown [derived from (SULSTON 1976)].

Identifying the *egl-1* cell death gene

Early *C. elegans* cell death research used the ventral nerve cord to identify genes that caused an aberrant pattern of apoptosis. Mutagenesis screens identified a mutation, *n487*, in the gene termed "*egl-1*" that causes the death of a pair of hermaphrodite-specific neurons (HSN's), leading to an Egl ("egl-laying defective") phenotype (CONRADT and HORVITZ 1998). The Egl phenotype is quite obvious and easy to score, as hermaphrodites with an *egl-1(n487)* mutation become bloated with eggs. Using the Egl phenotype as an indicator of *egl-1* function, it was found that *egl-1(n487)*

animals, heterozygous with a deficiency that eliminates the *egl-1* locus, were not Egl but appeared nearly wildtype (CONRADT and HORVITZ 1998). Thus, they reasoned, *egl-1(n487)* is not a loss-of-function (*lf*) mutation but, rather, may be a gain-of-function (*gf*) mutation. Similarly, another allele identified, *n1084*, is also a *gf*, but functions in a semi-dominant manner; *n1084/deletion* animals show a partial Egl phenotype. This partial *gf* background allowed for the screening of *egl-1 lf* mutations, and the *lf* allele *n3082* was identified. 0% of *egl-1(n1084 n3082)* animals exhibited an Egl phenotype, and 99% of the animals had surviving HSN's, similar to wildtype (CONRADT and HORVITZ 1998).

Conradt and Horvitz also found that loss-of-function *egl-1* had an effect not only in HSN survival, but also in survival of cells within the pharynx. Compared with wildtype, *egl-1(n1084 n3082)* animals displayed an average of 11.1 extra cells surviving in the pharynx, indicating that *egl-1* functions to repress cell death other contexts besides the egg-laying muscles.

EGL-1 is a BH3-only domain protein

A BLAST search of proteins homologous to EGL-1 yielded no clear hits but did display a small region of nine residues that are conserved with

mammalian BH3-only domain proteins. EGL-1 appeared to be similar in protein structure to mammalian Bik, Bid, Harakiri, Bad, Bak, and Bax in the BH3 domain (CONRADT and HORVITZ 1998). Furthermore, like mammalian BH3 proteins, EGL-1 is capable of binding the *C. elegans* Bcl-2 protein, CED-9, and a deletion of EGL-1's BH3 domain disrupts EGL-1 binding to CED-9, providing further support that EGL-1 is a BH3-only domain protein (CONRADT and HORVITZ 1998; DEL PESO *et al.* 1998).

Placing *egl-1* in the apoptosis pathway

Previous screens for genes that affect cell death had yielded *ced-9*, *ced-3*, and *ced-4* (CONRADT and HORVITZ 1998; ELLIS and HORVITZ 1986; HENGARTNER and HORVITZ 1994; YUAN and HORVITZ 1992; YUAN *et al.* 1993). *egl-1(gf)* was originally placed upstream of *ced-9*, *ced-4*, and *ced-3* in the cell death pathway (HENGARTNER *et al.* 1992), and upon identification of an *egl-1(lf)* allele, *egl-1* was confirmed to be upstream of the three other cell death genes (CONRADT and HORVITZ 1998).

In most cells programmed to die, apoptosis is initiated by transcriptional regulation of *egl-1* (CONRADT and HORVITZ 1998). The EGL-1 protein binds the BCL-2 family member CED-9, releasing the APAF-1 homolog CED-4

to activate the caspase CED-3, killing the cell (CHINNAIYAN *et al.* 1997a; CHINNAIYAN *et al.* 1997b; CONRADT and HORVITZ 1998; HENGARTNER *et al.* 1992; SESHAGIRI and MILLER 1997; WU *et al.* 1997a; WU *et al.* 1997b; YUAN *et al.* 1993; ZOU *et al.* 1997).

Observing cell death in *C. elegans*

To observe cell deaths in *C. elegans*, there are two typical methods: Nomarski microscopy and fluorescent reporters. Using Nomarski microscopy, cell deaths were recorded by an observer watching individual cells for a specific change in morphology. A dying cell, within thirty minutes, will become “button-like” and then be engulfed by surrounding cells (SULSTON and HORVITZ 1977).

To facilitate this process, a mutation in a cell-corpse engulfment gene, *ced-1*, may be used. In a *ced-1* background, the cells that are programmed to die continue the apoptotic pathway until the cell is dead, but without corpse engulfment from neighboring cells, the “button-like” cell corpses remain present, thus facilitating the counting of cell deaths.

A second method that researchers use to study cell death is through fluorescent reporters. Of the four core cell death genes—*egl-1*, *ced-9*,

ced-3, and *ced-4*—all except *ced-9* function to promote cell death, making them putative reporters of cell death. However, it is the activation, not expression, of CED-4 and CED-3 that promote their action in the cell death cascade. *egl-1* expression is the most viable cell death transcriptional reporter of the core genes, and indeed, a P_{egl-1}*gfp* reporter faithfully mimics the pattern of cell death observed in wildtype worms.

The P_{egl-1}*gfp* reporter also correctly responds to mutations that are known to affect cell death. For instance, mutations in a Hox transcription factor, *lin-39*, leads to an increase in the number of cells undergoing cell death, as observed by DIC microscopy and P_{egl-1}*gfp* expression, as described in Chapter III. It should be noted that although *egl-1* expression is not synonymous with cell death, essentially all cells that express *egl-1* go on to die.

HOX GENES REGULATE DEVELOPMENT

The Hox family of transcription factors affects spatial development

In the development of multicellular organisms, specifications need to be made to differentiate the cells of one body region from another. One way in which these specifications are made is through transcription factors that

are differentially expressed or differentially regulated according to spatial restrictions.

One important family of transcription factors that performs the task of spatial identification is Hox genes. Hox genes function as transcriptional regulators and are characterized by their ~60 amino acid homeodomain motif. Hox genes and their ability to regulate spatial cell fate determination was first identified in *Drosophila*, in which eight Hox genes each function within a limited context to define specific cells within a body region (see review by McGinnis and Krumlauf 1992). Later, Hox genes were identified in *C. elegans* and mammals and were shown to perform conserved functions—defining spatial patterning (McGinnis and Krumlauf 1992).

Hox genes are often located in clusters within the genome (McGinnis and Krumlauf 1992). In *C. elegans*, three well-studied Hox genes are found within one cluster that affects anterior-posterior development within the ventral nerve cord. These genes include *lin-39*, *mab-5*, and *egl-5* (Chisholm 1991; Clark *et al.* 1993; Costa *et al.* 1988; Wang *et al.* 1993). *lin-39* functions to specify cell fate in the midbody (Clark *et al.* 1993),

mab-5 functions in the posterior (COSTA *et al.* 1988), and *egl-5* functions in the tail (CHISHOLM 1991).

The *lin-39* Hox gene regulates cell fate

The Hox gene *lin-39* has been studied in the ventral nerve cord primarily for its function in vulval development. *lin-39(null)* mutants exhibit a vulvaless ("Vul") phenotype, in which the animals are not capable of properly releasing eggs. This phenotype is induced by loss of *lin-39* in the vulval precursor cells (MALOOF and KENYON 1998). In this subset of cells, Ras signaling allows for the derepression of *lin-39*, which leads to the upregulation of vulval fate-specific genes (CHEN and HAN 2001; MALOOF and KENYON 1998). Aside from vulval fate, *lin-39* also functions to regulate cell death within the ventral nerve cord. Specifically, *lin-39* represses the *egl-1* cell death gene in a subset of midbody neurons (POTTS *et al.* 2009). This finding will be further discussed in Chapter III.

CHAPTER TWO

Materials and Methods

Worm strains

Wildtype refers to the Bristol N2 strain. All worms were maintained under standard conditions (BRENNER 1974), at 20°C, unless otherwise noted. The RNAi screen background was *ced-1(e1735) I; lin-39(n709ts) III; ced-3(n717) IV; mxIs14 X. mxIs14* is an integrated P_{egl-1}*histone:gfp* transgene (LIU *et al.* 2006). The strains used to identify VA, VB, and VC neurons included: *wdIs4[*P_{unc-4}*gfp] II* (PFLUGRAD *et al.* 1997), *wdIs6[*P_{del-1}*gfp] II* (WINNIER *et al.* 1999), *nIs106[*P_{lin-11}*gfp] X* (REDDIEN *et al.* 2001), *lin-39(n709ts)*, and *ced-3(n717)*. *wdIs4* and *wdIs6* were generously provided by David Miller. Strains used to study how cell death is affected by E2Fs include *efl-1(se1) V*, which was raised at 26°C, *dpl-1(n2994) II*, and *efl-3(gk835) / mIn1(mIs14 dpy-10(e128)) II*.

RNAi analyses and imaging

dsRNA-expressing bacteria from the Ahringer RNAi library were fed to worms according to the procedure described by Kamath *et al.* (2001). L4 animals were fed on RNAi bacteria and their L2 progeny scored 2-3 days later. For RNAi clones used after the initial screen, the validity of gene

targets was confirmed by sequencing. *dpl-1* RNAi was constructed by PCR, amplifying a 1.1 kb segment of *dpl-1* genomic DNA. The *dpl-1* segment was then dropped into the L4440 RNAi backbone, using the following primers to introduce *Nco*I and *Pst*I sites for cloning: 5'-TAGCCATGGACAAACTACGATCCCCGTATC-3' and 5'-CTACTGCAGCTTACTGGCAATGATTTCGTC-3'. For RNAi screening and for all images, we used a Zeiss Axiophot microscope.

Conservation across species

To identify putative E2F binding sites in P_{egl-1}, the 7.67 kb *Bam*HI-*Stu*I promoter of *egl-1* (LIU *et al.* 2006) was first searched using MatInspector (www.genomatix.de) for General Core Promoter binding sites, with a matrix similarity of 5, indicating similarity across many species. All sequences identified as possible E2F or E2F/DP binding sites were then examined for conservation with *C. briggsae* and *C. remanei* using Family Relations II and Cartwheel (Caltech, http://cartwheel.caltech.edu/).

To search for domains conserved between human and *C. elegans* E2Fs, human E2F proteins were examined in Uniprot (www.uniprot.org) for their respective domains. Each E2F domain was aligned with *C. elegans* E2F

protein sequences (Wormbase www.wormbase.org) using MacVector Pustell Protein Matrix software.

Reporter constructs

P_{egl-1}gfp is *P_{egl-1}histone:gfp* (LIU *et al.* 2006). To create *$P_{egl-1(mut)}$gfp*, the *P_{egl-1}gfp* construct was first split into two parts. A 3.5 kb *Pst*I fragment was dropped out, creating pJW001, and the 3.5 kb *Pst*I fragment was cloned into pBluescript II KS, creating pMP024. Mutations were made using Phusion (Finnzymes) site-directed mutagenesis. In pJW001, two mutations were made, changing 5'-TTTCCCGCATGAA-3' to 5'-TTTAGGCCTTGAA-3' and 5'-AGTTCCCGCGTTT-3' to 5'-AGTACTAGTGTTT-3'. In pMP024, three mutations were made, changing 5'-TTTCGCGCATT-3' to 5'-TTTCGATCATT-3', 5'-TTTCGCGCATTTC-3' to 5'-TTTAGGCCTTTTC-3', and 5'-ATTGCGCGAGACC-3' to 5'-ATTACTAGTGACC-3'. Mutations were confirmed by sequencing. The pMP024 PstI fragment was then excised and recombined with pJW001 to create the final *$P_{egl-1(mut)}$gfp* construct. *$P_{egl-1(mut)}$gfp* (5ng/μL) was injected into *ced-1(e1735) I; ced-3(n717) IV; lin-15(n765) X* with the *lin-15* rescuing construct pL15EK (50ng/μL). Non-Muv transgenic progeny were maintained. Three stable transgenic lines gave similar *gfp* expression. Mutations of single sites induced no

change in *egl-1* expression, indicating that a combination of multiple binding sites is required for E2F regulation of *egl-1*. One line was crossed with *lin-39(n1760) III* to give *lin-39;* $P_{egl\text{-}1(mut)}$*gfp.*

$P_{efl\text{-}3}$*mCherry* was created by first amplifying a 3.2 kb fragment upstream of *efl-3*'s ATG start. To this fragment, *Sph*I and *Xma*I sites were added by PCR using the following primers: 5'-TAGTAGGCATGCCCAGCAGTGTGACTGTACATGTTC-3' and 5'-CTACTACCCGGGATTTGTTGAGCTCAATTACCAGATG-3'. $P_{efl\text{-}3}$ was cloned into a pPD95.70 construct in which the *gfp* coding sequence was replaced with *mCherry*. The final $P_{efl\text{-}3}$*mCherry* construct was confirmed by sequencing. $P_{efl\text{-}3}$*mCherry* (50ng/μL) was injected with a $P_{myo\text{-}2}$*gfp* (30ng/μL) co-injection marker into N2 worms. Three stable transgenic lines gave similar *mCherry* expression.

CHAPTER THREE

Results

A Hox gene, *lin-39*, specifies cell fate and apoptosis according to spatial positioning

Hox genes provide cells with spatial cues to determine their location within an organism. *C. elegans* has been used to study the Hox family of transcription factors, particularly in the context of the ventral nerve cord. The ventral nerve cord is a model to study how cells differentiate and determine cell death based on varying developmental cues. The ventral nerve cord is generated from 12 P and 1W blast cell lineages (Fig. 3-1A,B), each of which produces up to five types of motor neurons—VA, VB, VC, VD, and AS—and one hypodermal cell (SULSTON 1976). All ventral nerve cord lineages are similar in terms of the number of progeny they create and what types of neurons are generated, but because the lineages are spread across the length of the body, each is under the control of different spatial cues, leading to variations in cell fate based on developmental context. In the midbody, the survival of one neuron type, the VC neuron, allows the innervation of egg-laying muscles, but in the anterior and posterior lineages, where the VC-lineal equivalents are unnecessary, the neurons undergo apoptosis (CLARK *et al.* 1993). The

Hox gene *lin-39* provides the spatial information to determine VC neuron survival vs. death; without *lin-39* to tell the midbody VC neurons where within the animal they are located, the neurons undertake the fate (death) of their lineal equivalents in the anterior or posterior of the animal (CLARK *et al.* 1993; POTTS *et al.* 2009) (Fig. 3-1).

EFL-3 is identified in a screen for repressors of cell death redundant with LIN-39

Although the Hox transcription factor LIN-39 is expressed in all five neuron types generated by each midbody P3-8 lineage, only the VC neurons express *egl-1* and subsequently undergo apoptosis in a *lin-39* mutant (MALOOF and KENYON 1998) (Fig. 3-1). With loss of LIN-39, what causes the VC neurons to die and the other four neuron types to remain alive? One possibility is that there is a repressor of *egl-1* that functions redundantly with *lin-39* in the VA, VB, VD, and/or AS neurons.

Using an RNAi screen of 387 transcription factors and 263 chromatin remodeling factors (KAMATH and AHRINGER 2003), we sought repressors of *egl-1* that are redundant with Hox function in the non-VC motor neurons. *egl-1* expression was determined by a $P_{egl\text{-}1}$*gfp* reporter in a cell death-defective (*ced-3*) background so cells that initiate the cell death

cascade would express P_{egl-1}*gfp* but the execution of death would be blocked by a downstream mutation in the caspase *ced-3,* allowing the cells to remain alive for scoring by fluorescence microscopy. In the screening background *lin-39*; *ced-3;* P_{egl-1}*gfp*, we discovered that the gene *F49E12.6*, which we refer to as *efl-3,* represses *egl-1* expression in a subset of ventral nerve cord neurons and in a manner that is at least partially redundant with *lin-39* (Fig. 3-1).

EFL-3 and LIN-39 repress *egl-1* in the ventral nerve cord in a partially redundant manner

Additional neurons expressed *egl-1* on *efl-3(RNAi)* in a *lin-39* background (Fig. 3-2A). In the midbody, loss of LIN-39 was sufficient to derepress *egl-1* in the VC neurons. Additional loss of EFL-3 led to derepression of *egl-1* in the VA and VB neurons. Loss of EFL-3 alone was not sufficient to induce any change in *egl-1* expression in the midbody. In the posterior, where LIN-39 is not expressed, loss of EFL-3 alone was sufficient to result in ectopic *egl-1* expression. These findings suggest that in the midbody, EFL-3 and LIN-39 act partially redundantly to repress *egl-1* transcription in the VA and VB motor neurons.

EFL-3 is the *C. elegans* homolog of mammalian E2F7 and E2F8

The predicted protein structure of EFL-3 includes two DNA binding domains characteristic of the E2F family of transcription factors (Fig. 3-3). Like E2F7 and E2F8, EFL-3 lacks a transactivation domain and binding domains for Rb and cyclin A. EFL-3 is the only predicted protein in the completely sequenced *C. elegans* genome that shares all these characteristics with E2F7 and E2F8.

EFL-3 induces cell death without altering VA and VB neuron differentiation

To examine whether a differentiation defect might explain the additional *egl-1*-expressing neurons we observed in the screen, we examined the expression of cell type-specific *gfp* markers after treatment with *efl-3(RNAi)* (Fig. 3-4). We found that the VA, VB, and VC cell-type differentiations were apparently unaltered with *efl-3(RNAi)*, supporting the hypothesis that *efl-3* represses *egl-1* expression without altering the identity of the VA, VB, and VC neurons.

EFL-3 directly represses *egl-1* in the VA and VB motor neurons

In the sole murine E2F7/8 study (Li *et al.* 2008), widespread apoptosis is observed in embryos with loss of E2F7/8, but it is unclear whether the

apoptosis occurred indiscriminately or in a cell type-specific manner. Furthermore, whether E2F7/8 regulation of cell death is direct (E2Fs binding to the promoters of cell death genes to repress transcription) or indirect (possibly as the result of cell cycle deregulation) is unclear. In *C. elegans*, we sought to investigate these two questions. Our hypothesis was that EFL-3 might function directly at the site of a cell death gene, *egl-1*, to repress its transcription in the VA and VB neurons.

To determine whether EFL-3 is able to directly bind P_{egl-1} *in vivo*, we mutagenized 2-5 nucleotides at each of the five consensus E2F binding sites across the 7.6 kb *egl-1* promoter, to give $P_{egl-1(mut)}$*gfp*. In *ced-3;* $P_{egl-1(mut)}$*gfp* transgenic animals, we observed an increase in the number of ventral nerve cord neurons expressing *egl-1* (Fig. 3-5) when compared with *ced-3;* P_{egl-1}*gfp* animals (Fig. 3-1, 3-2A). Thus, an E2F likely binds *egl-1* to directly regulate its transcription.

It is thought that all E2Fs are capable of binding a similar E2F consensus sequence (DE BRUIN *et al.* 2003; DI STEFANO *et al.* 2003; LOGAN *et al.* 2005; ZHENG *et al.* 1999), so it is possible that *efl-1* or *efl-2* is capable of repressing *egl-1*. However, loss of *efl-1* and/or *efl-2*, or their putative obligate dimerization partner *dpl-1* did not affect cell death in the ventral

nerve cord, though *efl-3* did (Fig. 3-2B). Furthermore, *ced-3; $P_{egl-1(mut)}$gfp* animals expressed GFP in two neurons per midbody lineage (Fig. 3-5), similar to the two additional neurons per midbody lineage that expressed *egl-1* in *lin-39; ced-3; P_{egl-1}gfp; efl-3(RNAi)* animals as compared to control RNAi (Fig. 3-1). Additionally, though *efl-3(RNAi)* induced additional neurons to express *egl-1* in *ced-3; P_{egl-1}gfp* animals, *efl-3(RNAi)* induced no change in expression in *ced-3; $P_{egl-1(mut)}$gfp* animals, suggesting that *efl-3* repression had been lost by removing P_{egl-1}'s E2F binding sites (Fig. 3-2A).

EFL-3 is a stronger repressor of *egl-1* in the midbody VA and VB neurons than LIN-39

In the midbody of *P_{egl-1}gfp* transgenic animals, no neurons express *egl-1*, but in the midbody of *$P_{egl-1(mut)}$gfp* animals, there were two neurons per lineage that expressed *egl-1* (Fig. 3-5). We knew these to be VA and VB neurons, based on differential interference contrast analysis. Furthermore, in *lin-39; ced-3; $P_{egl-1(mut)}$gfp* animals, we observed three cells per midbody lineage expressing *egl-1*—the VA, VB, and VC neurons (Fig. 3-5). The pattern of *egl-1* expression in *lin-39; ced-3; $P_{egl-1(mut)}$gfp* animals was similar to the pattern observed in *lin-39; ced-3; P_{egl-1}gfp; efl-3(RNAi)* animals, but more consistent (see ranges of *gfp*-expressing neurons,

Table 3-1). One likely reason for the inconsistency of *egl-1* expression in *lin-39; ced-3; P_{egl-1}gfp; efl-3(RNAi)* animals is that *efl-3(RNAi)* provides incomplete knockdown of *efl-3*, as supported by the observation that *efl-3(RNAi)* worms are viable, but deletion alleles of *efl-3*, *gk835* and *gk896*, are larval lethal.

ced-3; $P_{egl-1(mut)}$gfp animals (compared with *ced-3; P_{egl-1}gfp* animals) exhibited an increase of midbody neurons expressing *egl-1*, independent of *lin-39*. Previously, we had shown that *lin-39* loss was required for *efl-3(RNAi)* to have an effect on *egl-1* expression in the midbody (Fig. 3-2A). Now, however, with E2F binding sites removed, we have created an essentially null condition in terms of EFL-3 binding P_{egl-1}*gfp*, indicating that in the midbody VA and VB neurons, LIN-39 functions as a weak repressor and EFL-3 functions as a strong repressor of *egl-1*. That is, complete loss of LIN-39 (via a null allele) and partial loss of EFL-3 (via RNAi) is sufficient to derepress *egl-1*, while complete loss of EFL-3 alone (via P_{egl-1} mutagenesis) is sufficient to derepress *egl-1* (Fig. 3-2A).

One possible mechanism to explain the partial redundancy of LIN-39 and EFL-3 in the midbody VA and VB neurons is that by abolishing E2F sites, we inadvertently disrupted one or more Hox sites as well, leading to

derepression of *egl-1*. However, this hypothesis seems unlikely, as a previous study has shown that of the 116 putative P_{egl-1} Hox/Pbx binding sites, LIN-39 is predicted to repress *egl-1* at multiple sites or indirectly, by binding one or more P_{egl-1}-bound transcription factors (POTTS *et al.* 2009).

A second hypothesis is that LIN-39 represses *egl-1* by forming a complex with EFL-3 bound to P_{egl-1}. Partial knockdown of EFL-3 (via RNAi) would lead to variable expression *egl-1* due to a minimal amount of EFL-3 being present to allow for inconsistent LIN-39 and EFL-3 repression of *egl-1*, and elimination of E2F binding sites would lead to a more predictable pattern of *egl-1* derepression, which is what we observed (Fig. 3-2, Table 3-1).

To test directly whether complete loss of *efl-3* function is sufficient to cause ectopic expression of *egl-1*, we attempted to look at *efl-3(gk835); ced-3;* P_{egl-1}*gfp* animals. This experiment failed because the majority of these worms die as larvae before completing cell deaths in the ventral nerve cord. We found, however, that a rare few *ced-1*; *efl-3*; P_{egl-1}*gfp* animals are able to survive long enough to display a GFP pattern in the ventral nerve cord matching that of *ced-3;* $P_{egl-1(mut)}$*gfp* animals (Fig. 3-5). (*ced-1* is necessary for the normal engulfment of cell corpses.) In *ced-1;*

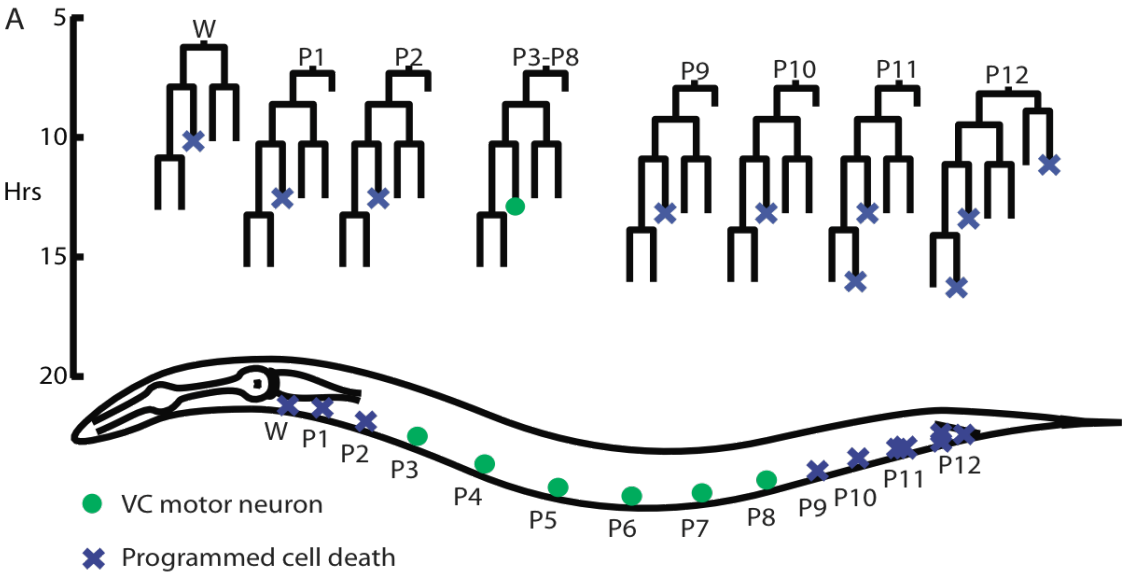
efl-3; P_{egl-1}gfp animals, similar to *ced-3; $P_{egl-1(mut)}$gfp* animals, two neurons per midbody lineage expressed *egl-1*, supporting the hypothesis that *efl-3* is a repressor of *egl-1* in the VA and VB neurons.

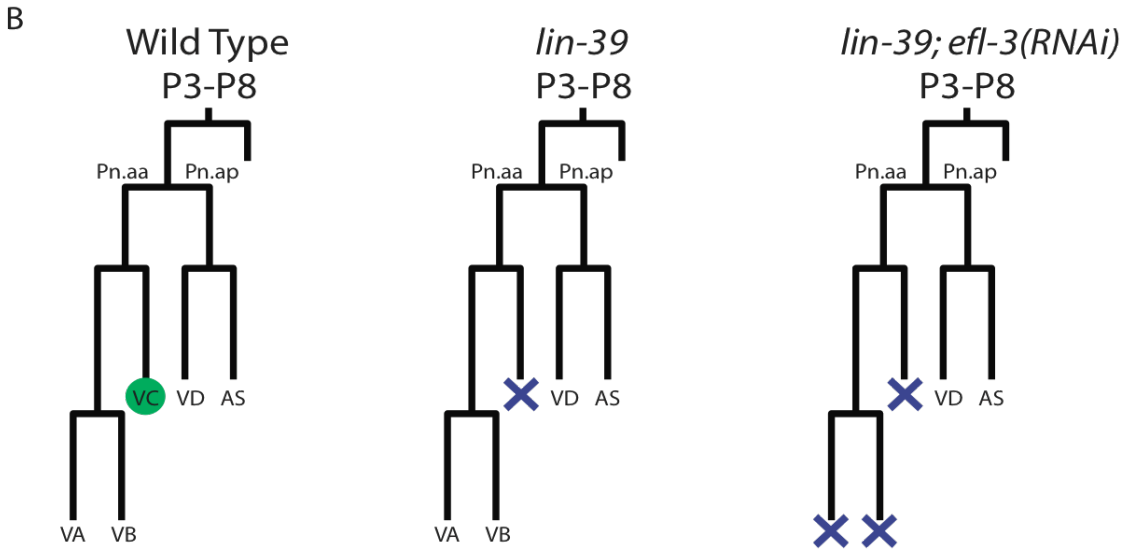
EFL-3 is expressed in the VA and VB motor neurons

EFL-3 is required in the VA and VB neurons to repress *egl-1* in a manner partially redundant with LIN-39, but loss of LIN-39 alone is sufficient to de-repress *egl-1* in the VC neurons. Why doesn't EFL-3 affect *egl-1* expression in the VC neurons? One possibility is that EFL-3 is not present in the VC neurons. To determine the pattern of *efl-3* expression, we created a *P_{efl-3}mCherry* reporter. *P_{efl-3}mCherry* transgenic worms displayed expression beginning in multiple cells in embryos (Fig. 3-6A). The expression continued throughout the lifespan of the worm, with maximal expression appearing in the L1-L2 larval stages. Additionally, EFL-3 expression was observed consistently in head neurons from L1 to adult (Fig. 3-6A).

In the ventral nerve cord, EFL-3 was most clearly expressed during the L2 stage. By crossing *P_{efl-3}mCherry* transgenic animals with integrated lines of VA- (*P_{unc-4}*), VB- (*P_{del-1}*), and VC- (*P_{lin-11}*) specific GFP markers, we determined that EFL-3 is expressed in the VA and VB neurons but not the

VC neurons (Fig. 3-6B). This finding provides insight as to why EFL-3 represses *egl-1* in the VA and VB but not the VC neurons.


A
5
10
Hrs
15
20
W
P1
P2
P3-P8
P9
P10
P11
P12
W
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
P11
P12
VC motor neuron
Programmed cell death


B
Wild Type
P3-P8
Pn.aa
Pn.ap
VC
VD
AS
VA
VB
lin-39
P3-P8
Pn.aa
Pn.ap
VD
AS
VA
VB
lin-39; efl-3(RNAi)
P3-P8
Pn.aa
Pn.ap
VD
AS

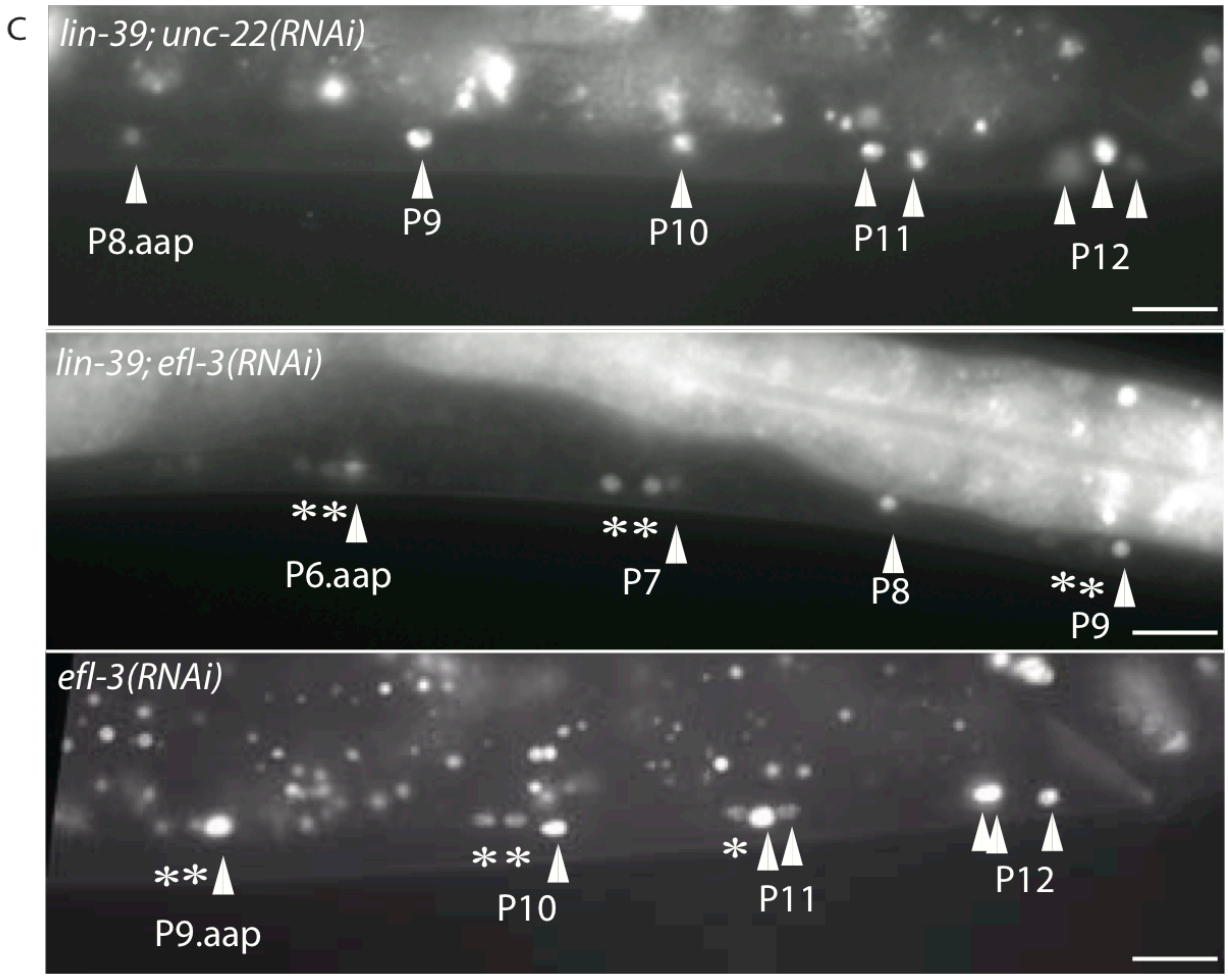


FIGURE 3-1 EFL-3 and LIN-39 function in a context-specific manner to repress *egl-1* transcription in the ventral nerve cord. (A) The ventral nerve cord is derived from 13 lineages (W and P1-12) along the length of the animal. In the anterior (W, P1-P2) and posterior (P9-P12) lineages, ten neurons undergo programmed cell death. (B) Each midbody (P3-P8) lineage produces a hypodermal cell and five neuron types, which survive

in wild type animals. Loss of LIN-39 induces one neuron type, the VC, to express the cell death gene *egl-1* and die in each midbody lineage; the pattern of cell deaths in the posterior is unchanged (CLARK *et al.* 1993). (C) On *unc-22* control RNAi, *lin-39* mutants express $P_{egl\text{-}1}$*gfp* in cells that undergo programmed cell death. In *lin-39; efl-3(RNAi)* animals, additional neurons in the midbody and posterior express $P_{egl\text{-}1}$*gfp*. In *efl-3(RNAi)* animals, there is an increase in posterior neurons expressing $P_{egl\text{-}1}$*gfp*. Arrowheads indicate neurons expressing $P_{egl\text{-}1}$*gfp* in a *lin-39* mutant background, and asterisks indicate additional neurons expressing $P_{egl\text{-}1}$*gfp*. All animals shown are *ced-1; ced-3;* $P_{egl\text{-}1}$*gfp*. Scale bars represent 10μm.

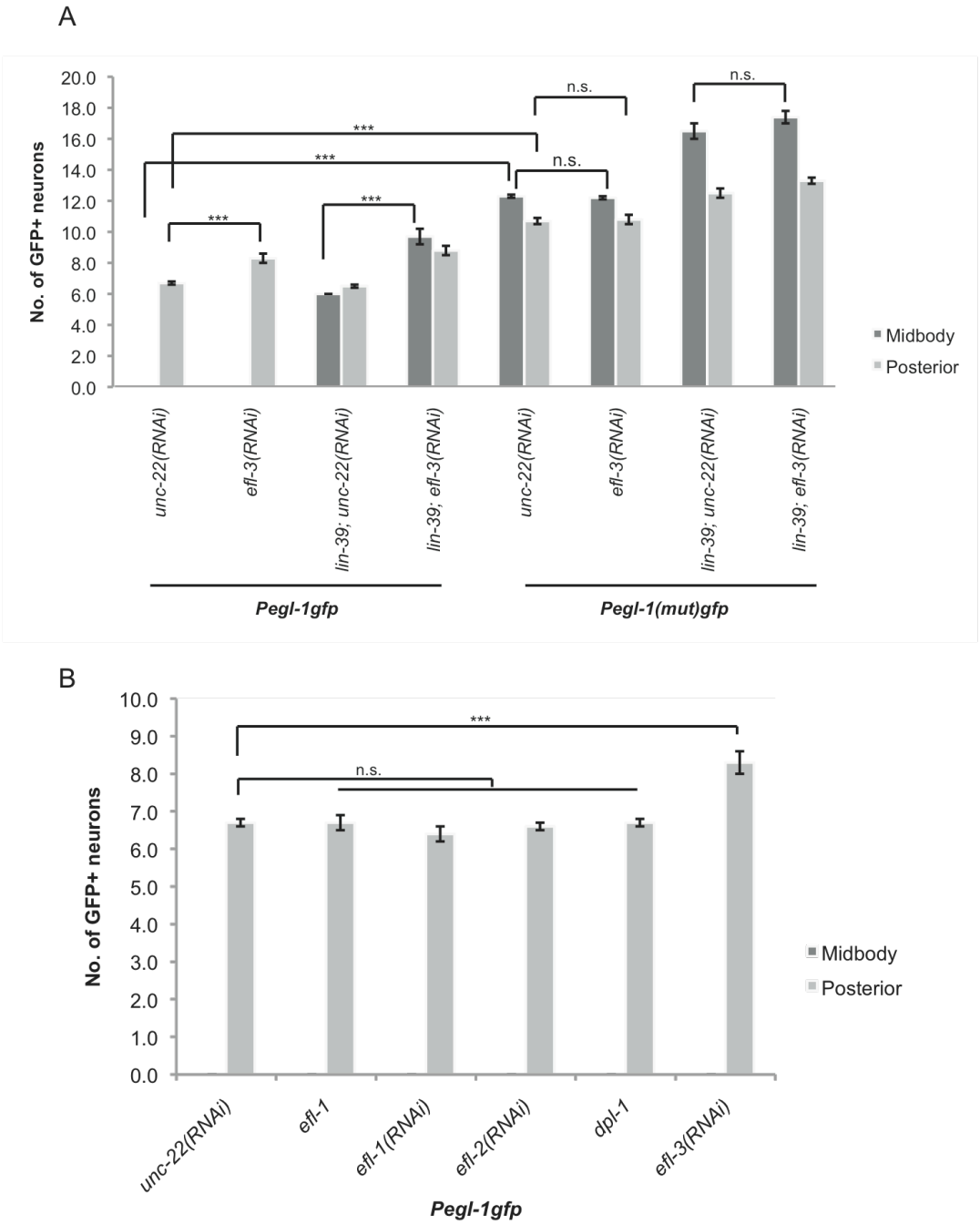


FIGURE 3-2 EFL-3 directly represses *egl-1*. (A) *egl-1* is repressed by *efl-3* and *lin-39* in a context-specific manner. A repressive E2F, likely EFL-3,

directly binds P_{egl-1}. (B) Loss of *efl-3* induces an increase in the number of neurons expressing *egl-1*. All strains are homozygous for *ced-3*. Standard errors are shown. "***" represents $p<0.0001$, "n.s." represents $p>0.05$.

Genotype	Midbody Average			Midbody Range	Posterior Average			Posterior Range
ced-3; P_{egl-1}gfp; unc-22(RNAi)	0.0	±	0.0	0	6.7	±	0.1	4-7
ced-3; P_{egl-1}gfp; efl-3(RNAi)	0.0	±	0.0	0	8.3	±	0.3	7-12
lin-39; ced-3; P_{egl-1}gfp; unc-22(RNAi)	6.0	±	0.0	6	6.5	±	0.1	5-7
lin-39; ced-3; P_{egl-1}gfp; efl-3(RNAi)	9.7	±	0.5	6-15	8.8	±	0.3	7-13
ced-3; $P_{egl-1(mut)}$gfp; unc-22(RNAi)	12.3	±	0.1	12-14	10.7	±	0.2	9-13
ced-3; $P_{egl-1(mut)}$gfp; efl-3(RNAi)	12.2	±	0.1	10-13	10.8	±	0.3	8-14
lin-39; ced-3; $P_{egl-1(mut)}$gfp; unc-22(RNAi)	16.5	±	0.5	13-19	12.5	±	0.3	11-15
lin-39; ced-3; $P_{egl-1(mut)}$gfp; efl-3(RNAi)	17.4	±	0.4	13-19	13.3	±	0.2	12-15
ced-3; P_{egl-1}gfp; efl-1	0.0	±	0.0	0	6.7	±	0.2	6-7
ced-3; P_{egl-1}gfp; efl-1(RNAi)	0.0	±	0.0	0	6.4	±	0.2	5-7
ced-3; P_{egl-1}gfp; efl-2(RNAi)	0.0	±	0.0	0	6.6	±	0.1	6-7
dpl-1; ced-3; P_{egl-1}gfp	0.0	±	0.0	0	6.7	±	0.1	6-7

Table 3-1 EFL-3 directly represses *egl-1* in a context-specific manner.

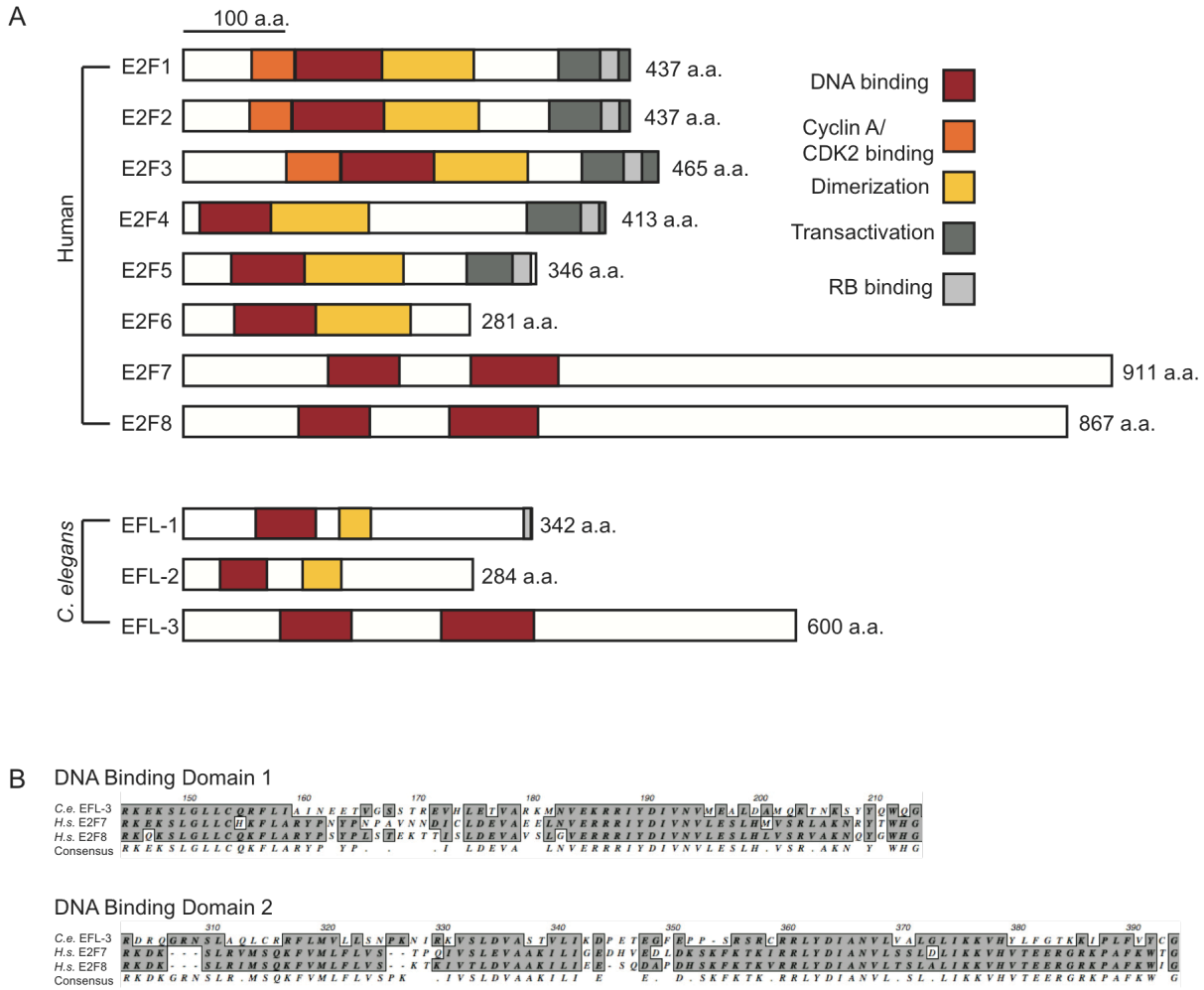


FIGURE 3-3 EFL-3 is a homolog of mammalian E2F7 and E2F8. (A) The mammalian and *C. elegans* E2F families of transcription factors. (B) Alignment of the DNA binding domains of *C. elegans* EFL-3 and *H. sapiens* E2F7 and E2F8.

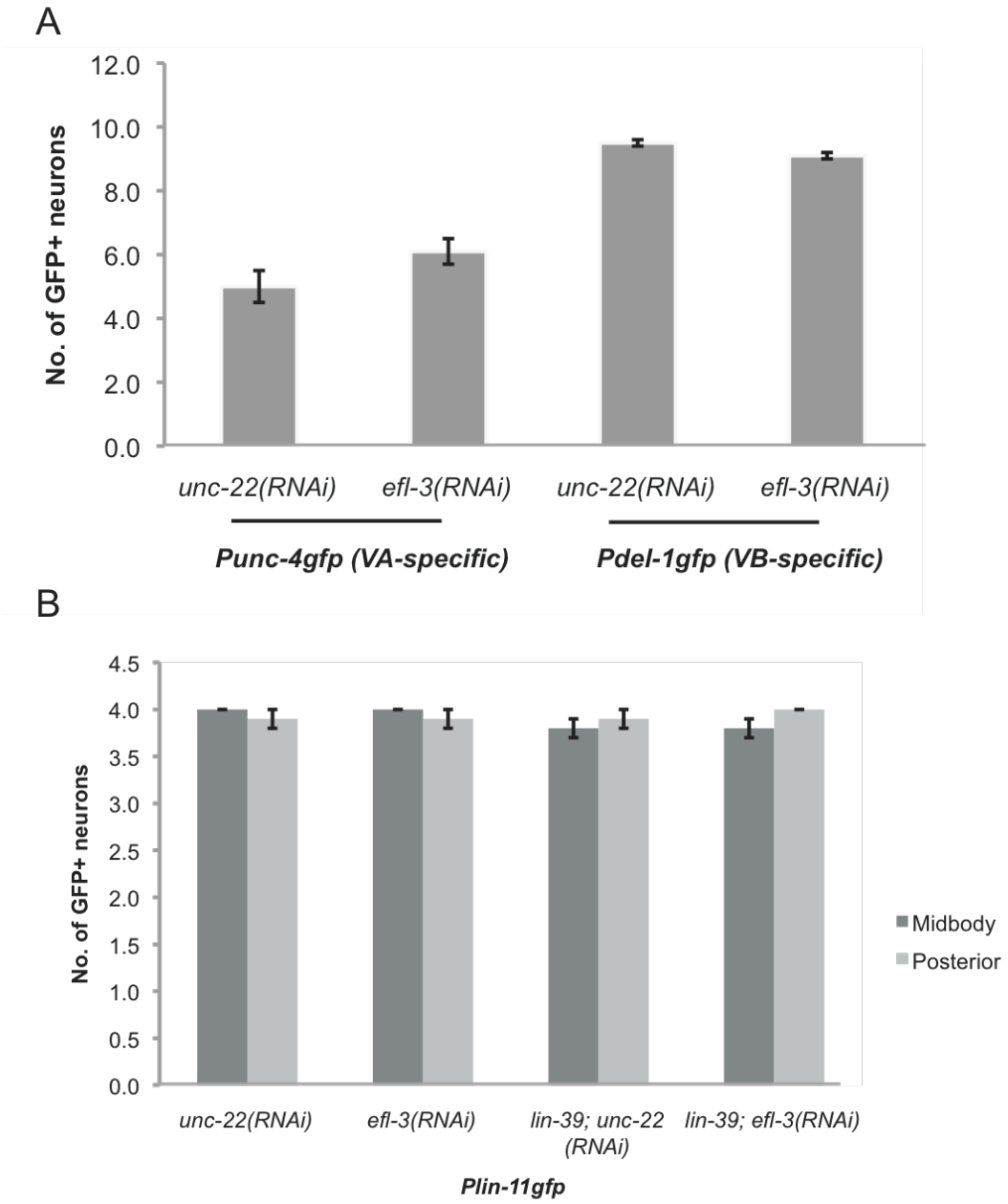


FIGURE 3-4 Loss of *efl-3* does not affect VA, VB, and VC identity. (A) Differentiation of the VA and VB neurons is unaffected by *efl-3(RNAi)*. (B)

Differentiation of the VC neurons is unaffected with *efl-3(RNAi)*, as observed with the VC-specific $P_{lin\text{-}11}$*gfp* reporter. The $P_{lin\text{-}11}$*gfp* strain is homozygous for *ced-3.* Standard errors are shown.

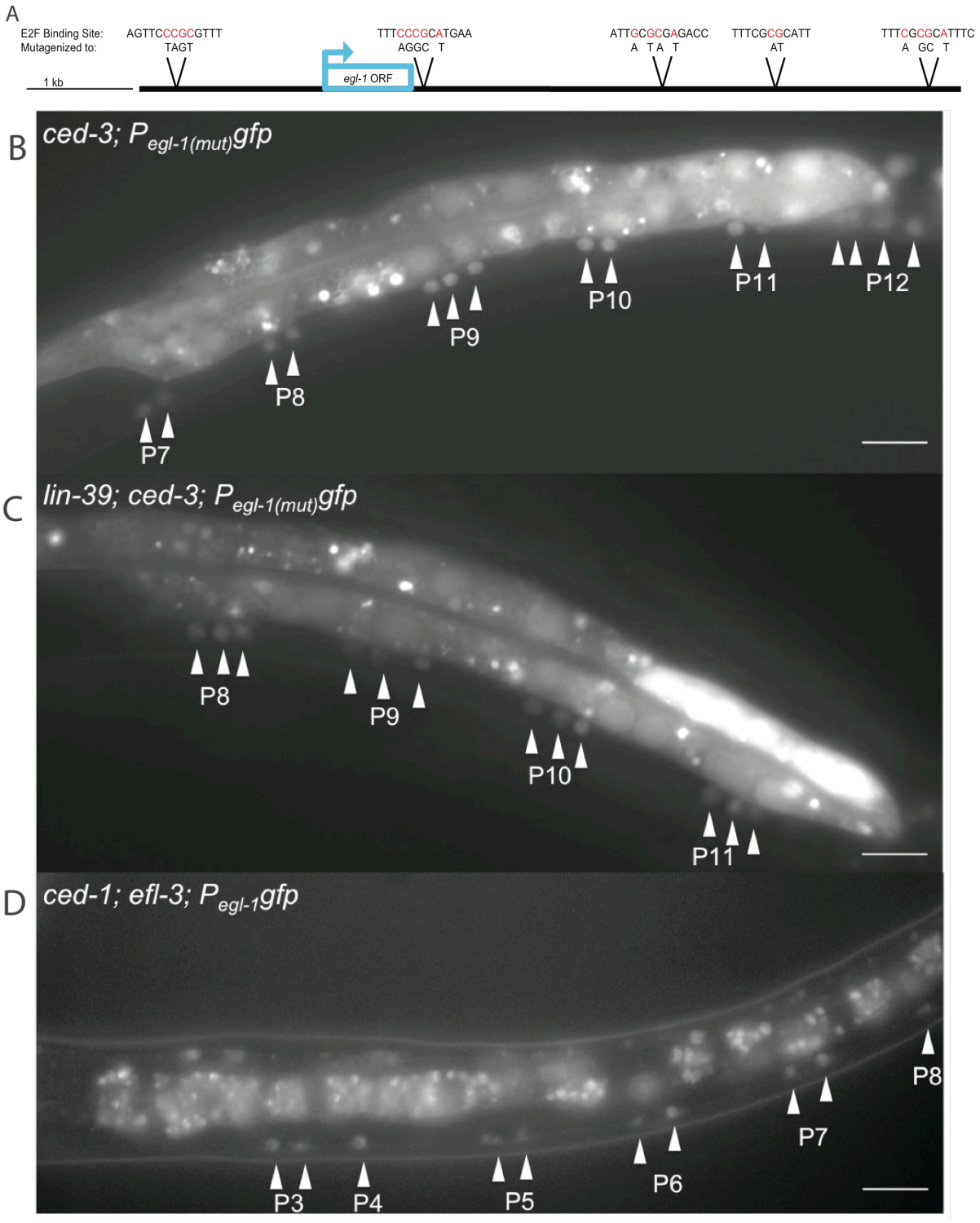
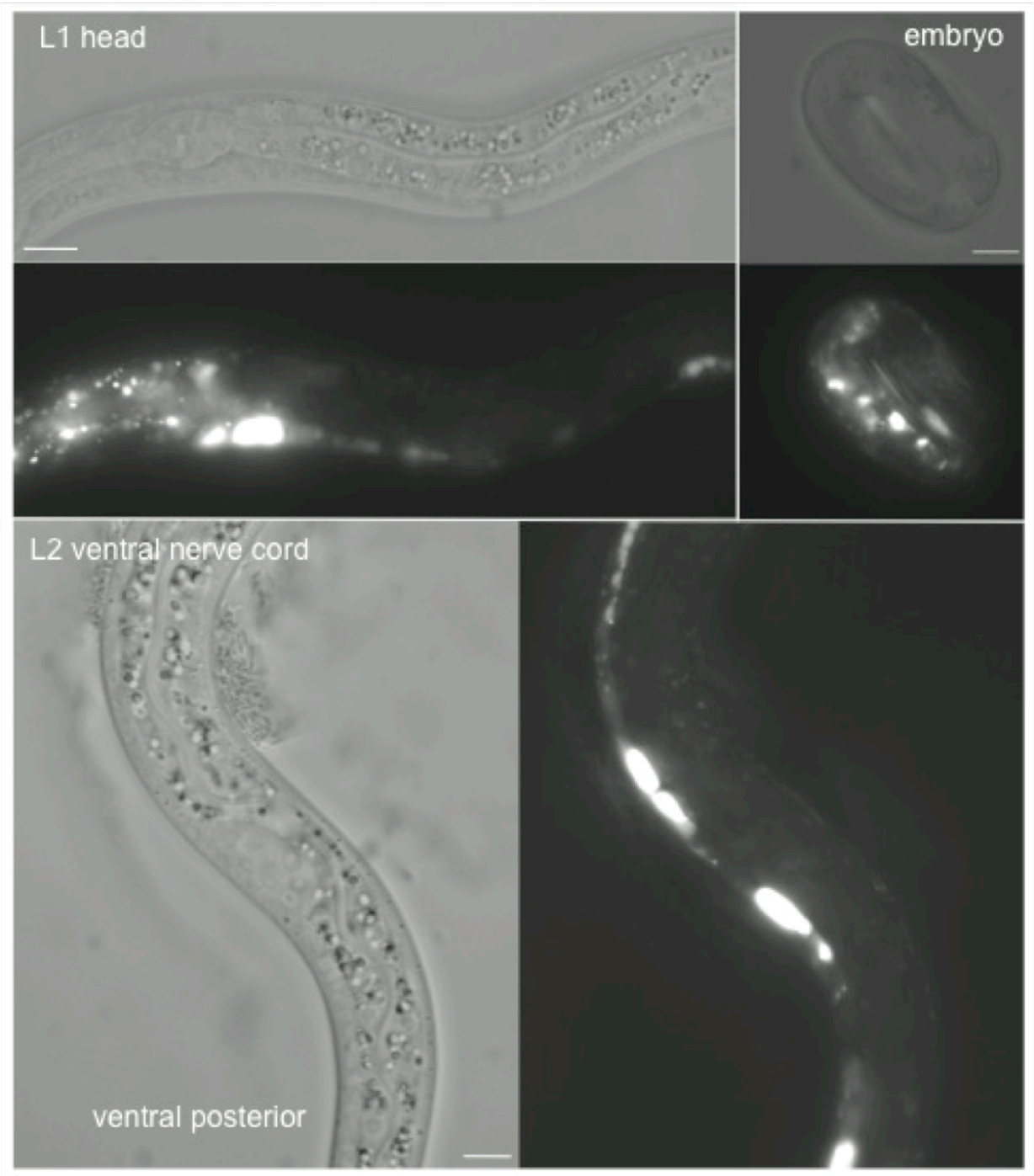

A
E2F Binding Site:
Mutagenized to:
AGTTCCCGCGTTT
TAGT
TTTCCCGCATGAA
AGGC T
ATTGCGCGAGACC
A TA T
TTTCGCGCATT
AT
TTTCGCGCATTTC
A GC T
1 kb
egl-1 ORF
B
ced-3; P egl-1(mut) gfp
P7
P8
P9
P10
P11
P12
C
lin-39; ced-3; P egl-1(mut) gfp
P8
P9
P10
P11
D
ced-1; efl-3; P egl-1 gfp
P3
P4
P5
P6
P7
P8

FIGURE 3-5. (A) Five E2F consensus sites were identified in P_{egl-1}. At each site, the nucleotides shown in red were mutated to abolish E2F binding. All five mutated sites were combined into one construct to give $P_{egl-1(mut)}$. (B) Loss of E2F binding sites in P_{egl-1} results in ectopic *egl-1* expression in midbody P3-P8 and posterior P9-P12 lineages. Mutations in P_{egl-1}*gfp* induce a doublet pattern of neurons in the midbody P3-P8 lineages and an increase in neurons in the posterior expressing *egl-1*. (C) In *lin-39;* $P_{egl-1(mut)}$*gfp* animals, GFP is expressed in a triplet pattern in the midbody lineages. (D) *efl-3(null);* P_{egl-1}*gfp* animals exhibit ectopic *egl-1* expression. Scale bars represent 10 μm. Animals in (B) and (C) are homozygous for *ced-3*, and the animal in (D) is homozygous for *ced-1*.

A



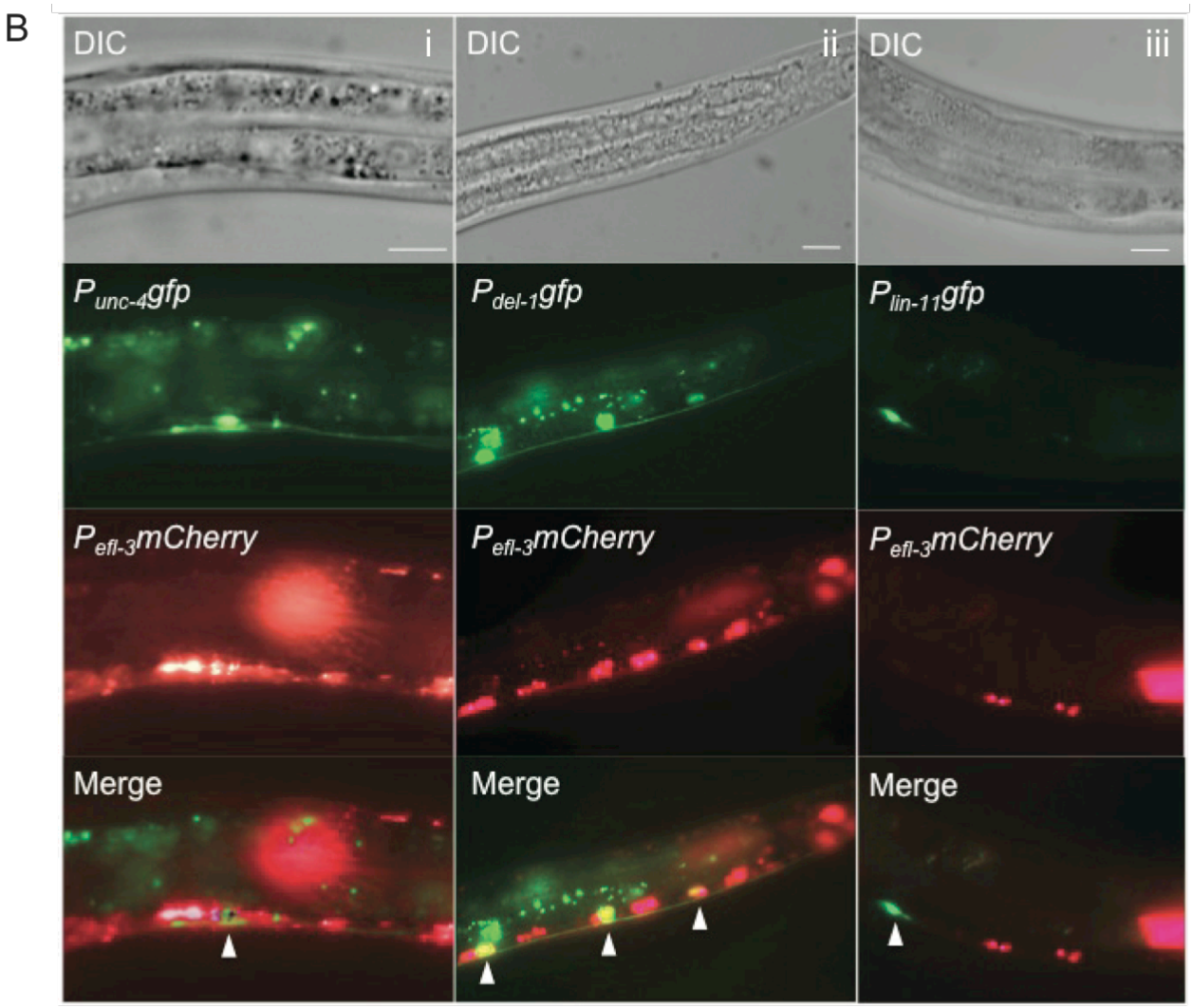


FIGURE 3-6 Expression of *efl-3*. (A) $P_{efl\text{-}3}$*mCherry* is expressed throughout development in embryos, L1 animals (with highest expression in the head), and L2 animals (with highest expression in the head and ventral nerve cord). Three independent transgenic lines exhibit the same expression pattern. (B) $P_{efl\text{-}3}$*mCherry* is expressed in the (i) VA neurons

and (ii) VB neurons. (iii) EFL-3 is not expressed in the VC neurons. Images are false-colored epifluorescence of L2-L3 animals. Scale bars represent 10 μm.

CHAPTER FOUR

Conclusions and Recommendations

Here, we examined how a newly-identified E2F, *efl-3*, is able to regulate transcription of the *egl-1* cell death gene. We found that *efl-3* functions in the ventral nerve cord VA and VB cells to repress *egl-1* in a manner partially redundant with a Hox, *lin-39*, but *efl-3* function is not required to repress *egl-1* in a third cell type, the VC neurons (Fig. 4-1). In the posterior, where *lin-39* is not expressed, *efl-3* alone is sufficient to repress *egl-1*. Across the ventral nerve cord, *lin-39* provides information to developing lineages to tell them where they are located, and within each lineage, *efl-3* provides information to the developing VA and VB neurons to tell them how their fate should be different than that of the VC neurons. Thus, spatial information from *lin-39* Hox is integrated with lineage-specific information from *efl-3* at the site of the *egl-1* cell death gene to determine cell fate (Fig. 4-1).

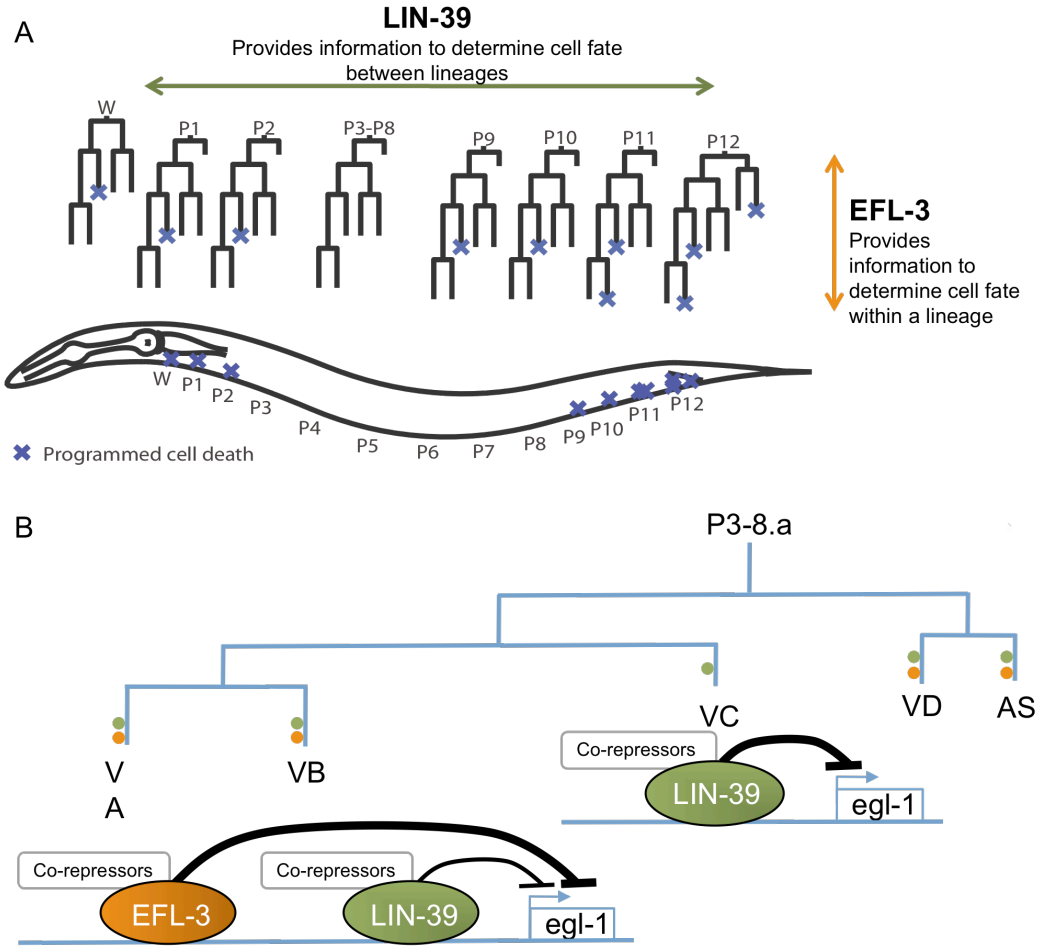


FIGURE 4-1 EFL-3 and LIN-39 interact to specify ventral nerve cord development. (A) LIN-39 provides spatial-specific information to developing lineages. EFL-3 provides information within each lineage to specify cell fate between the different cell types. (B) In the midbody P3-8 lineages, EFL-3 and LIN-39 integrate their lineage- and spatial-specific cues on the *egl-1* promoter. EFL-3 and LIN-39 co-repress *egl-1* in the VA

and VB neurons, with LIN-39 functioning as a weaker repressor. In the VC neurons, LIN-39 is necessary to repress *egl-1*. EFL-3 is not expressed in the VC neurons and has no apparent affect on *egl-1* expression in the VCs. Green dots represent neurons that express *lin-39*, orange dots represent neurons that express *efl-3*.

Our findings demonstrate a context in which two major developmental pathways—Hox and E2F—cooperatively regulate a target gene, *egl-1*. This interaction is dependent on context, with the transcription of *egl-1* being determined in a spatial- and cell type-specific manner. Although our studies examined how Hox and E2F interact within the limited setting of the *C. elegans* ventral nerve cord, it is possible that the mechanism of cell fate regulation by Hox and E2F is relevant across species. Hox proteins have previously demonstrated broad functional conservation, with *Drosophila* Hox proteins able to function in *C. elegans* (HUNTER and KENYON 1995), and vertebrate Hox proteins able to function in *Drosophila* (LUTZ *et al.* 1996; MALICKI *et al.* 1990; MCGINNIS *et al.* 1990; ZHAO *et al.* 1993). In the E2F family, mechanistic similarities can also be drawn between species, particularly between mouse, *Drosophila*, and *C. elegans*, though precise functional comparisons are more difficult given the variety and redundancy of E2F family proteins and their extensive range of functions (VAN DEN HEUVEL and DYSON 2008).

Do *lin-39* and *efl-3* interact in other contexts?

For future studies, it would be interesting to determine whether the two families' cooperative action is limited to cell death regulation, or if their interaction affects other developmental processes, as well. For instance,

lin-39, *efl-1*, and *dpl-1* have each been shown to regulate vulval development. *lin-39* represses a vulvaless ("Vul") phenotype (MALOOF and KENYON 1998), while *efl-1* and *dpl-1* function with other members of the SynMuv family of genes to repress a multi-vulval phenotype (CEOL and HORVITZ 2001). Because Hox and E2F proteins have been shown to regulate vulval development, and *lin-39* and *efl-3* co-regulate cell fate within the ventral nerve cord, it is possible that *lin-39* may interact with *efl-3* to regulate vulval formation. To test this, I would examine vulval fate with loss of *lin-39* and *efl-3*. *lin-39; efl-3(RNAi)* animals showed no obvious defects in vulval fate, so I would begin by increasing the efficacy of *efl-3(RNAi)* by adding an *rrf-3* (a putative RNA-directed RNA polymerase) mutation into the *lin-39; efl-3(RNAi)* background. Loss of *rrf-3* leads to an increase in RNAi sensitivity, including within neurons, without causing obvious morphological defects (SIMMER *et al.* 2002). Thus, it is possible that *efl-3(RNAi)*-induced defects, whether in the vulva or elsewhere in the worm, would be more obvious in an *rrf-3* background.

Furthermore, I would test the effects of *efl-3* loss on other developmental processes, including cell death in the pharynx. *efl-1* and *dpl-1* repress cell death in the anterior pharynx (REDDIEN *et al.* 2007), so I hypothesize that *efl-3* may work antagonistically to the "activator" E2Fs in the pharynx to

repress cell death. I would test the effects of *efl-3* loss (with and without *lin-39*) by counting the number of cell corpses in a *ced-1* background. (*rrf-3* could also be used in this experiment to promote *efl-3(RNAi)* sensitivity.)

In the developmental processes in which I find *efl-3* is critical, it would be interesting to examine whether human E2f7 and/or E2f8 are able to compensate for loss of *efl-3*. I propose to create transgenes of $P_{efl\text{-}3}$*E2f7*, $P_{efl\text{-}3}$*E2f8*, and $P_{efl\text{-}3}$*E2f7* and $P_{efl\text{-}3}$*E2f8* (co-injected) to observe rescue of *efl-3(null)*'s lethality, rescue of cell death in *efl-3(RNAi)* animals, and rescue of any vulval defects. If human E2f7/8 can compensate for *efl-3*, it would provide support for the claim that the mechanisms of *efl-3* function identified in *C. elegans* are likely conserved in mammals.

In which pathways does EFL-3 function?

In the research presented here, I have shown that *efl-3* is able to repress the *egl-1* cell death gene in a manner partially redundant with *lin-39*. I am curious, now, as to how *efl-3* functions in other contexts and through what mechanisms.

In the sole mouse study of E2F7/8, the widespread apoptosis observed in E2F7/8 double knockout mice was suppressed by a mutation in p53. To observe whether this same effect is observable in *C. elegans*, I created *cep-1; efl-3* double mutant animals. These double mutants were occasionally able to survive longer than *efl-3(null)* animals. Furthermore, *cep-1; efl-3* worms tended to be able to give rise to several progeny, a feat which was extremely rare for *efl-3* animals. Approximately 1 in 100 *cep-1; efl-3* animals could produce progeny (between 1-30 offspring), and these progeny could occasionally survive for >6 generations. Approximately 1 in 1000 *efl-3* animals could produce progeny (between 1-5), and these progeny would not survive past 2 generations. It is possible that *cep-1*'s suppression of *efl-3*'s lethality could be the result of an increased rate of background mutations, but it is tempting to speculate that loss of *cep-1* directly leads to increased viability in *efl-3* animals. I would hypothesize that loss of *efl-3* may lead to a fatal increase in *cep-1* levels (possibly through cell cycle distress and DNA damage), and that additional loss of *cep-1* rescues the lethality. Further experiments would include quantifying the rescue of *efl-3* lethality in a *cep-1* background and searching for other suppressors of *efl-3*. These suppressors may include other genes critical in the DNA damage pathway. Furthermore, I propose performing an unbiased screen to search for *efl-3* suppressors. Results from this screen

would be particularly interesting because they may lead to the placement of *efl-3* in an unexpected pathway.

Another method to determine putative pathways through which *efl-3* functions would be to identify its binding partners. Using a $P_{efl\text{-}3}$*efl-3::FLAG* transgenic line, I would perform a Co-IP followed by mass spectrometry to identify proteins bound to EFL-3. This could be performed using worms at specific developmental stages to determine how EFL-3's function may change throughout development. Furthermore, I would be interested in determining whether EFL-3 is able to bind LIN-39 directly. This finding could help explain why loss of E2F binding sites in $P_{egl\text{-}1}$ leads to loss of *efl-3* and *lin-39* repression.

Where is EFL-3 required?

Using $P_{efl\text{-}3}$*mCherry* as a reporter, I found that *efl-3* is highly expressed during development, particularly in the head. This finding has led me to question: In which cells is *efl-3* expressed? To answer this, I propose using cell type-specific promoters to drive *efl-3* expression in *efl-3(null)* animals. The aim of this experiment is to identify promoters that are able to rescue *efl-3(null)* lethality, and by doing so, gain insight into the locations and processes in which *efl-3* functions.

Concluding remarks

efl-3 and its mammalian homologs, E2f7/8, are critical regulators of development. This paper presents the mechanism by which *efl-3* is able to determine cell fate, specifically by repressing apoptosis. In the ventral nerve cord, we have identified a context in which Hox and E2F co-regulate the *egl-1* cell death gene. It is likely that this mechanism is conserved across species, as E2F, Hox, and the cell death pathway are highly conserved. Though our work focused on the ventral nerve cord of *C. elegans*, the research presented here will hopefully provide insight into how cell fate is determined in other contexts.

APPENDIX I

Acknowledgments

We would like to thank the Caenorhabditis Genetics Center at the University of Minnesota for providing some of the *C. elegans* strains and the NIH (Grant #HL46154) for funding.

REFERENCES

BRENNER, S., 1974 The genetics of Caenorhabditis elegans. Genetics **77:** 71-94.

CEOL, C. J., and H. R. HORVITZ, 2001 dpl-1 DP and efl-1 E2F act with lin-35 Rb to antagonize Ras signaling in C. elegans vulval development. Mol Cell **7:** 461-473.

CHEN, Z., and M. HAN, 2001 C. elegans Rb, NuRD, and Ras regulate lin-39-mediated cell fusion during vulval fate specification. Curr Biol **11:** 1874-1879.

CHINNAIYAN, A. M., D. CHAUDHARY, K. O'ROURKE, E. V. KOONIN and V. M. DIXIT, 1997a Role of CED-4 in the activation of CED-3. Nature **388:** 728-729.

CHINNAIYAN, A. M., K. O'ROURKE, B. R. LANE and V. M. DIXIT, 1997b Interaction of CED-4 with CED-3 and CED-9: a molecular framework for cell death. Science **275:** 1122-1126.

CHISHOLM, A., 1991 Control of cell fate in the tail region of C. elegans by the gene egl-5. Development **111:** 921-932.

CHONGHAILE, T. N., and A. LETAI, 2008 Mimicking the BH3 domain to kill cancer cells. Oncogene **27 Suppl 1:** S149-157.

CLARK, S. G., A. D. CHISHOLM and H. R. HORVITZ, 1993 Control of cell fates in the central body region of C. elegans by the homeobox gene lin-39. Cell **74:** 43-55.

CONRADT, B., and H. R. HORVITZ, 1998 The C. elegans protein EGL-1 is required for programmed cell death and interacts with the Bcl-2-like protein CED-9. Cell **93:** 519-529.

COSTA, M., M. WEIR, A. COULSON, J. SULSTON and C. KENYON, 1988 Posterior pattern formation in C. elegans involves position-specific expression of a gene containing a homeobox. Cell **55:** 747-756.

DE BRUIN, A., B. MAITI, L. JAKOI, C. TIMMERS, R. BUERKI *et al*., 2003 Identification and characterization of E2F7, a novel mammalian E2F family member capable of blocking cellular proliferation. J Biol Chem **278:** 42041-42049.

DEL PESO, L., V. M. GONZALEZ and G. NUNEZ, 1998 Caenorhabditis elegans EGL-1 disrupts the interaction of CED-9 with CED-4 and promotes CED-3 activation. J Biol Chem **273:** 33495-33500.

DI STEFANO, L., M. R. JENSEN and K. HELIN, 2003 E2F7, a novel E2F featuring DP-independent repression of a subset of E2F-regulated genes. EMBO J **22:** 6289-6298.

ELLIS, H. M., and H. R. HORVITZ, 1986 Genetic control of programmed cell death in the nematode C. elegans. Cell **44:** 817-829.

HAY, B. A., and M. GUO, 2006 Caspase-dependent cell death in Drosophila. Annu Rev Cell Dev Biol **22:** 623-650.

HENGARTNER, M. O., R. E. ELLIS and H. R. HORVITZ, 1992 Caenorhabditis elegans gene ced-9 protects cells from programmed cell death. Nature **356:** 494-499.

HENGARTNER, M. O., and H. R. HORVITZ, 1994 C. elegans cell survival gene ced-9 encodes a functional homolog of the mammalian proto-oncogene bcl-2. Cell **76:** 665-676.

HUNTER, C. P., and C. KENYON, 1995 Specification of anteroposterior cell fates in Caenorhabditis elegans by Drosophila Hox proteins. Nature **377:** 229-232.

KAMATH, R. S., and J. AHRINGER, 2003 Genome-wide RNAi screening in Caenorhabditis elegans. Methods **30:** 313-321.

KAMATH, R. S., M. MARTINEZ-CAMPOS, P. ZIPPERLEN, A. G. FRASER and J. AHRINGER, 2001 Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in Caenorhabditis elegans. Genome Biol **2:** RESEARCH0002.

KIMBLE, J., and D. HIRSH, 1979 The postembryonic cell lineages of the hermaphrodite and male gonads in Caenorhabditis elegans. Dev Biol **70:** 396-417.

LI, J., C. RAN, E. LI, F. GORDON, G. COMSTOCK *et al*., 2008 Synergistic function of E2F7 and E2F8 is essential for cell survival and embryonic development. Dev Cell **14:** 62-75.

LIU, H., T. J. STRAUSS, M. B. POTTS and S. CAMERON, 2006 Direct regulation of egl-1 and of programmed cell death by the Hox protein MAB-5 and by CEH-20, a C. elegans homolog of Pbx1. Development **133:** 641-650.

LOGAN, N., A. GRAHAM, X. ZHAO, R. FISHER, B. MAITI *et al*., 2005 E2F-8: an E2F family member with a similar organization of DNA-binding domains to E2F-7. Oncogene **24:** 5000-5004.

LUTZ, B., H. C. LU, G. EICHELE, D. MILLER and T. C. KAUFMAN, 1996 Rescue of Drosophila labial null mutant by the chicken ortholog Hoxb-1 demonstrates that the function of Hox genes is phylogenetically conserved. Genes Dev **10:** 176-184.

MALICKI, J., K. SCHUGHART and W. MCGINNIS, 1990 Mouse Hox-2.2 specifies thoracic segmental identity in Drosophila embryos and larvae. Cell **63:** 961-967.

MALOOF, J. N., and C. KENYON, 1998 The Hox gene lin-39 is required during C. elegans vulval induction to select the outcome of Ras signaling. Development **125:** 181-190.

MCGINNIS, N., M. A. KUZIORA and W. MCGINNIS, 1990 Human Hox-4.2 and Drosophila deformed encode similar regulatory specificities in Drosophila embryos and larvae. Cell **63:** 969-976.

MCGINNIS, W., and R. KRUMLAUF, 1992 Homeobox genes and axial patterning. Cell **68:** 283-302.

PFLUGRAD, A., J. Y. MEIR, T. M. BARNES and D. M. MILLER, 3RD, 1997 The Groucho-like transcription factor UNC-37 functions with the neural specificity gene unc-4 to govern motor neuron identity in C. elegans. Development **124:** 1699-1709.

POTTS, M. B., and S. CAMERON, 2011 Cell lineage and cell death: Caenorhabditis elegans and cancer research. Nat Rev Cancer **11:** 50-58.

POTTS, M. B., D. P. WANG and S. CAMERON, 2009 Trithorax, Hox, and TALE-class homeodomain proteins ensure cell survival through repression of the BH3-only gene egl-1. Dev Biol **329:** 374-385.

REDDIEN, P. W., E. C. ANDERSEN, M. C. HUANG and H. R. HORVITZ, 2007 DPL-1 DP, LIN-35 Rb and EFL-1 E2F act with the MCD-1 zinc-finger protein to promote programmed cell death in Caenorhabditis elegans. Genetics **175:** 1719-1733.

REDDIEN, P. W., S. CAMERON and H. R. HORVITZ, 2001 Phagocytosis promotes programmed cell death in C. elegans. Nature **412:** 198-202.

SESHAGIRI, S., and L. K. MILLER, 1997 Caenorhabditis elegans CED-4 stimulates CED-3 processing and CED-3-induced apoptosis. Curr Biol **7:** 455-460.

SIMMER, F., M. TIJSTERMAN, S. PARRISH, S. P. KOUSHIKA, M. L. NONET *et al.*, 2002 Loss of the putative RNA-directed RNA polymerase RRF-3 makes C. elegans hypersensitive to RNAi. Curr Biol **12:** 1317-1319.

SULSTON, J. E., 1976 Post-embryonic development in the ventral cord of Caenorhabditis elegans. Philos Trans R Soc Lond B Biol Sci **275:** 287-297.

SULSTON, J. E., and H. R. HORVITZ, 1977 Post-embryonic cell lineages of the nematode, Caenorhabditis elegans. Dev Biol **56:** 110-156.

SULSTON, J. E., E. SCHIERENBERG, J. G. WHITE and J. N. THOMSON, 1983 The embryonic cell lineage of the nematode Caenorhabditis elegans. Dev Biol **100:** 64-119.

VAN DEN HEUVEL, S., and N. J. DYSON, 2008 Conserved functions of the pRB and E2F families. Nat Rev Mol Cell Biol **9:** 713-724.

WANG, B. B., M. M. MULLER-IMMERGLUCK, J. AUSTIN, N. T. ROBINSON, A. CHISHOLM *et al*., 1993 A homeotic gene cluster patterns the anteroposterior body axis of C. elegans. Cell **74:** 29-42.

WINNIER, A. R., J. Y. MEIR, J. M. ROSS, N. TAVERNARAKIS, M. DRISCOLL *et al*., 1999 UNC-4/UNC-37-dependent repression of motor neuron-specific genes controls synaptic choice in Caenorhabditis elegans. Genes Dev **13:** 2774-2786.

WOOD, 1988 *The Nematode Caenorhabditis elegans*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

WU, D., H. D. WALLEN, N. INOHARA and G. NUNEZ, 1997a Interaction and regulation of the Caenorhabditis elegans death protease CED-3 by CED-4 and CED-9. J Biol Chem **272:** 21449-21454.

WU, D., H. D. WALLEN and G. NUNEZ, 1997b Interaction and regulation of subcellular localization of CED-4 by CED-9. Science **275:** 1126-1129.

YUAN, J., and H. R. HORVITZ, 1992 The Caenorhabditis elegans cell death gene ced-4 encodes a novel protein and is expressed during the period of extensive programmed cell death. Development **116:** 309-320.

YUAN, J., S. SHAHAM, S. LEDOUX, H. M. ELLIS and H. R. HORVITZ, 1993 The C. elegans cell death gene ced-3 encodes a protein similar to mammalian interleukin-1 beta-converting enzyme. Cell **75:** 641-652.

ZHAO, J. J., R. A. LAZZARINI and L. PICK, 1993 The mouse Hox-1.3 gene is functionally equivalent to the Drosophila Sex combs reduced gene. Genes Dev **7:** 343-354.

ZHENG, N., E. FRAENKEL, C. O. PABO and N. P. PAVLETICH, 1999 Structural basis of DNA recognition by the heterodimeric cell cycle transcription factor E2F-DP. Genes Dev **13:** 666-674.

ZOU, H., W. J. HENZEL, X. LIU, A. LUTSCHG and X. WANG, 1997 Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3. Cell **90:** 405-413.