

A complex regulatory network coordinating cell cycles during *Caenorhabditis elegans* development is revealed by a genome-wide RNAi screen

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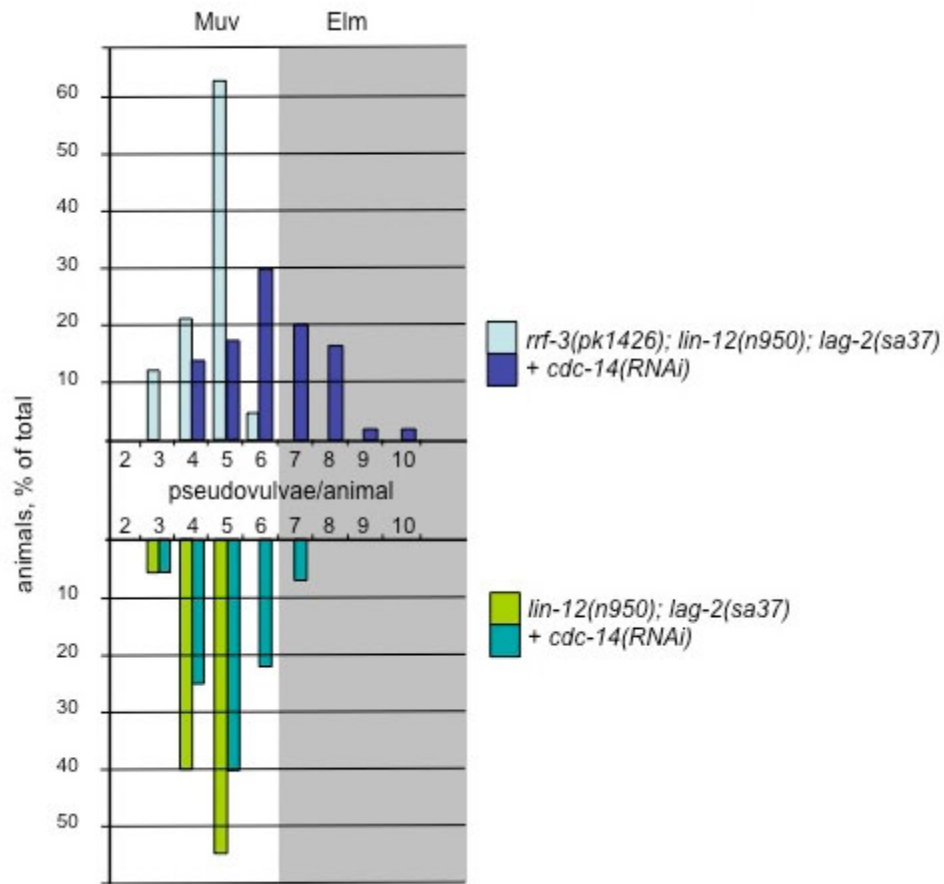


Figure S1 The VW22 strain incorporates several favorable characteristics. The *rrf-3(pk1426)* mutation enhances the Elm phenotype of *cdc-14(RNAi)*. Comparison of pseudovulva number produced by *lin-12(n950); lag-2(sa37)* double mutant (lower graph) and *rrf-3(pk1426); lin-12(n950); lag-2(sa37)* triple mutant (upper graph) animals. Animals displaying the Elm phenotype are indicated by grey shading.

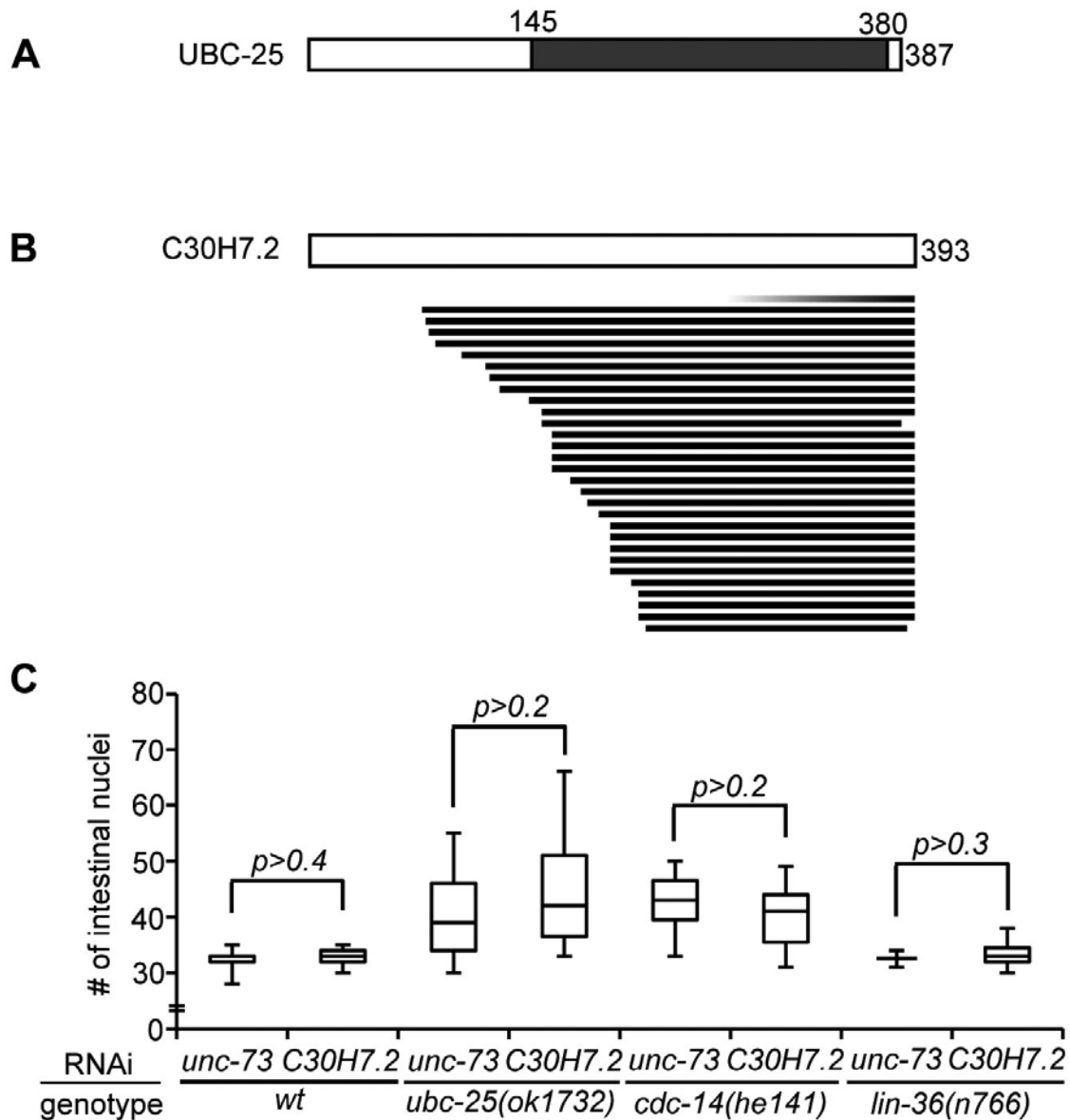


Figure S2 UBC-25 yeast two-hybrid screen identifies C30H7.2. (A) Diagram of UBC-25 open reading frame used in Y2H screen. Ubiquitin conjugating domain is shaded dark grey. The UBC-25 cDNA was cloned in-frame with the LexA DNA binding domain. Approximately 7.8×10^7 potential interactions within a high complexity *C. elegans* cDNA library were screened. (B) Schematic diagram illustrating the open reading frames of the thirty clones (black lines) representing C30H6.2 that were isolated in the UBC-25 Y2H screen. The cDNA inserts were sequenced from both the 5' and 3' directions. The 5' sequence of a single clone was not determined and is shown as a fading black line. (C) RNAi-mediated inhibition of C30H7.2 did not significantly alter the number of intestinal nuclei in *wt*, *unc-25(ok1732)*, *cdc-14(he141)*, or *lin-36(n766)* mutant animals. Intestinal nuclei were examined during the L4 stage.

Table S1 Genes identified in the Elm phenotype RNAi screen

Available for download as an Excel file at <http://www.g3journal.org/lookup/suppl/doi:10.1534/g3.114.010546/-/DC1>

Table S2 *ubc-25(ok1732)* causes temperature-sensitive viability defect

Temp.	wild type		<i>ubc-25(ok1732)</i>	
	Brood size	% Emb	Brood size	% Emb
15°C	189.4±19.7 (n=8)	0.8 (n=1510)	181.9±34.5 (n=11)	2.6 (n=2001)
20°C	273.0±34.6 (n=10)	2.7 (n=2734)	236.4±53.8 (n=11)	6.7 (n=2600)
25°C	190.6±32.5 (n=7)	1.6 (n=1576)	64.9±15.5 (n=9)	69.8 (n=584)

Table S3 The *ubc-25(ok1732)* mutation does not disturb the cell-cycle quiescence of the M, V, and Z cell lineages

lineage	age	reporter	wild type	<i>ubc-25(ok1732)</i>
M	L2	<i>hlh-8::GFP</i>	16.5±0.7 (n=24)	17.7±0.6 (n=20)
V	L4	<i>scm::GFP</i>	16.1±0.2 (n=20)*	16.1±0.6 (n=20)*
Z	L4	<i>lag-2::GFP</i>	2.0±0.0 (n=39)	2.0±0.0 (n=35)

*one side of GFP expressing V cells were counted per animal

Table S4 Comparison of wild type and *ubc-25(ok1732)* E lineage cell cycle lengths

cell	average cell division length*	
	wild type n=2	<i>ubc-25(ok1732)</i> (% of wt) n=5
E	36.5±0.7	32.4±2.4 (89)
Ea	41.0±1.4	35.4±4.4 (86)
Ear	67.0±9.9	47.2±2.9 (70)
Eara	125±21.2	56.6±6.3 (45)

*time (minutes) from mitosis producing named cell to division of cell

Table S5 Several *ubc* genes act redundant to *ubc-25*.

RNAi target*	intestinal nuclei, avg \pm std dev	
	wild type	<i>ubc-25(ok1732)</i>
<i>unc-73</i>	33.1 \pm 1.4	38.3 \pm 6.5
<i>cdc-14</i>	35.3 \pm 2.8	48.5 \pm 10.2
<i>ubc-1</i>	33.0 \pm 2.6	54.5 \pm 9.2
<i>ubc-2</i>	Let	Let
<i>ubc-3</i>	31.9 \pm 1.3	38.9 \pm 5.7
<i>ubc-6</i>	32.6 \pm 1.2	44.5 \pm 11.3
<i>ubc-7</i>	32.5 \pm 1.1	36.3 \pm 7.9
<i>ubc-8</i>	N/A	N/A
<i>ubc-9</i>	34.5 \pm 3.4	Let
<i>ubc-12</i>	31.7 \pm 1.8	Let
<i>ubc-13</i>	N/A	N/A
<i>ubc-14</i>	N/A	N/A
<i>ubc-15</i>	32.5 \pm 1.1	38.8 \pm 5.0
<i>ubc-16</i>	32 \pm 2.0	40.6 \pm 8.3
<i>ubc-17</i>	32.9 \pm 1.6	50.4 \pm 12.2
<i>ubc-18</i>	33.2 \pm 2.6	38.2 \pm 5.6
<i>ubc-19</i>	N/A	N/A
<i>ubc-20</i>	33.3 \pm 2.4	53.9 \pm 9.3
<i>ubc-21</i>	31.7 \pm 0.9	47.8 \pm 13.8
<i>ubc-22</i>	32.6 \pm 2.1	42.7 \pm 8.9
<i>ubc-23</i>	32.9 \pm 1.1	45.6 \pm 7.6
<i>ubc-24</i>	32.6 \pm 2.0	39.1 \pm 7.7
<i>ubc-25</i>	32.3 \pm 2.0	44.2 \pm 10.3
<i>ubc-26</i>	N/A	N/A

*no *ubcs* numbered 4, 5, 10 and 11 in *C. elegans*

N/A=not available in RNAi Library

unc-73 and *cdc-14* are negative and positive controls, respectively.

n \geq 15 for all experiments.

Table S6 Examination of putative *elm* genes for enhancement of extra intestinal nuclei phenotypes

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