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Woo Chi and Valerie Reinke *Development*, **133**, 3147-3157.

There was an error in the title of the ePress version of this article published online on 19 July 2006. E2F was mistakenly written as EZF. The final online version and the print version are both correct.

We apologise to authors and readers for this mistake.

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Promotion of oogenesis and embryogenesis in the *C. elegans* gonad by EFL-1/DPL-1 (E2F) does not require LIN-35 (pRB)

Woo Chi and Valerie Reinke*

In *Caenorhabditis elegans*, EFL-1 (E2F), DPL-1 (DP) and LIN-35 (pRb) act coordinately in somatic tissues to inhibit ectopic cell division, probably by repressing the expression of target genes. EFL-1, DPL-1 and LIN-35 are also present in the germline, but do not always act together. Strong loss-of-function mutations in either *efl-1* or *dpl-1* cause defects in oogenesis that result in sterility, while *lin-35* mutants are fertile with reduced broods. Microarray-based expression profiling of dissected gonads from *efl-1*, *dpl-1* and *lin-35* mutants reveals that EFL-1 and DPL-1 promote expression of an extensively overlapping set of target genes, consistent with the expectation that these two proteins function as a heterodimer. Regulatory regions upstream of many of these target genes have a canonical E2F-binding site, suggesting that their regulation by EFL-1/DPL-1 is direct. Many EFL-1/DPL-1 responsive genes encode proteins required for oogenesis and early embryogenesis, rather than cell cycle components. By contrast, LIN-35 appears to function primarily as a repressor of gene expression in the germline, and the genes that it acts on are for the most part distinct from those regulated by EFL-1 and/or DPL-1. Thus, in vivo, *C. elegans* E2F directly promotes oogenesis and embryogenesis through the activation of a tissue-specific transcriptional program that does not require LIN-35.

KEY WORDS: *C. elegans*, Germline, E2F, Microarrays, Gene expression, pRB, DP

INTRODUCTION

In mammals, the retinoblastoma tumor suppressor (pRB) plays a crucial role in controlling cellular proliferation and differentiation, in large part by regulating the activity of a transcription factor called E2F. E2F is typically a heterodimer composed of one E2F and one DP subunit. In the canonical model for Rb pathway function, pRB binds to E2F in the G_0 or early G_1 phase of the cell cycle, preventing activation and/or promoting repression of target genes (reviewed by Trimarchi and Lees, 2002). Phosphorylation of pRB by cyclinD/cdk4 releases E2F, which is then free to activate genes important for DNA synthesis, promoting progression from G_1 into S phase. However, the roles of pRB and E2F are more diverse than this model indicates, as these proteins modulate apoptosis, senescence and differentiation, as well as cell cycle progression (reviewed by Dimova and Dyson, 2005; Cobrinik, 2005).

Greatly complicating the understanding of this crucial pathway is the fact that each of these proteins is a member of a gene family. The mouse and human genomes each encode three pRB-like 'pocket' proteins, eight E2F-like proteins and two DP-like proteins (reviewed by Dimova and Dyson, 2005). Family members can exhibit both redundant and independent functions. For instance, loss of pRB is compensated by the activity of another pocket protein, p107, in mouse embryo fibroblasts (Sage et al., 2003). p107 and the third pocket protein p130 have independent functions as well, as they typically bind to a different set of E2Fs than does pRB (reviewed by Trimarchi and Lees, 2002). E2F proteins are functionally divided into those that activate transcription (E2F1-3) and those that repress transcription (E2F4-8). Activator E2Fs are primarily bound by pRB, repressors E2F4 and E2F5 are bound by p107 and p130, while E2F6-8 lack any pocket protein-binding domain. Moreover, deletion mutations in any of six endogenous E2F genes in mice do not display the same spectrum of defects, suggesting that each protein acts at distinct times and places in development and organ homeostasis (reviewed by Dimova and Dyson, 2005). In vivo, different E2Fs appear to be able to both promote and hinder tumor formation, and the mechanisms underlying these diverse outcomes are largely unknown.

Recent microarray and chromatin immunoprecipitation experiments in tissue culture cells have identified E2F target genes that expand the role of E2F/DP beyond the regulation of G1/S phase transition, including genes that act in mitosis, DNA repair and recombination (Ishida et al., 2001; Muller et al., 2001; Ren et al., 2002). In Drosophila S2 cells, the sole activator E2F, dE2F1, primarily activates cell cycle-regulated genes, while the repressor dE2F2 targets developmentally regulated genes independent of the cell cycle (Dimova et al., 2003). Overall, these and other analyses have led to the concept that E2Fs activate genes that promote the cell cycle and repress genes required for differentiation. However, most of these analyses have been performed in cell culture, and how tissue-specificity and developmental context impact the regulation of E2F target genes is just beginning to be addressed.

The hermaphrodite nematode *Caenorhabditis elegans* provides both a streamlined pathway and the opportunity for in-vivo analysis. Its genome encodes a single pRB-like protein (LIN-35), a single DP-like protein (DPL-1) and three E2F-like proteins (EFL-1, EFL-2 and F49E12.6). Of the three E2F-like proteins in *C. elegans*, a phenotype has been attributed only to *efl-1*, which is most closely related to the mammalian repressor E2F4 (Ceol and Horvitz, 2001; Page et al., 2001). RNA-mediated interference studies of *efl-2* and F49E12.6 have not identified any apparent phenotype (Boxem and van den Heuvel, 2002; Ceol and Horvitz, 2001; Kamath et al., 2003; Rual et al., 2004; Sonnischen et al., 2005).

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The *C. elegans* pRB/E2F pathway is required for multiple aspects of somatic development. *lin-35*, *efl-1* and *dpl-1* are all components of the SynMuv B pathway, which inhibits ectopic vulval development redundantly with a second pathway, the SynMuv A pathway (Lu and Horvitz, 1998; Ceol and Horvitz, 2001). In addition to Rb and E2F orthologs, the SynMuv B pathway includes components of a histone deacetylase complex that probably acts to repress transcription (Lu and Horvitz, 1998; Korenjak et al., 2004). LIN-35, EFL-1 and DPL-1 proteins have been demonstrated to physically associate in vitro (Ceol and Horvitz, 2001). Together, the common phenotype, physical association and similarity to mammalian proteins support the argument that LIN-35, EFL-1 and DPL-1 act in concert to repress gene expression. Additionally, *lin-35*, *efl-1* and *dpl-1* function together in G1 cell cycle control in other tissues that exhibit postembryonic cell division, including the intestine and ventral cord (Boxem and van der Heuvel, 2002). In all of the above cases, *lin-35*, *efl-1* and *dpl-1* act together to negatively regulate cell division. Notably, *lin-35* acts redundantly with other genes such as *ubc-18* and *xnp-1* during pharynx and somatic gonad development, respectively, without regulating cell proliferation (Fay et al., 2004; Bender et al., 2004).

Here we investigate the function of *lin-35*, *efl-1* and *dpl-1* in the germline. Null phenotypes of *efl-1*, *dpl-1* and *lin-35* reveal distinct requirements for these proteins in regulating germline development. While EFL-1 and DPL-1 are essential for fertility, LIN-35 is dispensable. Global gene expression profiling of dissected gonads from *lin-35*, *efl-1* and *dpl-1* mutants reveals an extensively overlapping gene expression program for EFL-1 and DPL-1. Loss of *efl-1* and *dpl-1* decreased expression of a common set of genes that promote specific aspects of a developmental program – oogenesis and early embryogenesis – rather than cell cycle. We identified an over-represented sequence in the 5′ regions of these genes that closely resembles the mammalian E2F-binding site. LIN-35 plays only a minor role in the regulation of these genes and instead appears chiefly to repress a distinct set of genes with diverse functions in the germline. Our results demonstrate that, in vivo, E2F can directly initiate a developmental program by activating genes that promote differentiation with only minor effects on the cell cycle.

MATERIALS AND METHODS

Strains and maintenance

Nematode strain maintenance was as described (Sulston and Hodgkin, 1988). All strains were grown at 20°C. The following variants were used: N2 (wild type), *lin-35(n745)* I (Lu and Horvitz, 1998), *unc-4(e120)*; *dpl-1(n3316)* II (Ceol and Horvitz, 2001), *efl-1(n3639)* V (Coel and Horvitz, 2001), *rme-2(b1005)* IV (Grant and Hirsh, 1999) and AZ212 (H2B:GFP) (Praitis et al., 2001). We examined two different isolates of *lin-35(n745)* and found that both isolates exhibited sterility at 25°C in our hands, a phenotype not previously reported.

Gonad dissection and microarray analysis

Wild-type and mutant worms were staged by bleaching gravid adults to collect eggs, which were then hatched in S-basal solution in the absence of food. Starved L1 larvae were cultured with food (bacterial strain OP50) and harvested 70-72 hours later, the first point at which *efl-1(n3639)* mutants could be distinguished from siblings. Adult worms were placed in dissection buffer (M9 with 0.1% levamisole and 0.001% Tween 20) on a coverslip. For each strain, a pair of 30 1/2-gauge needles was used to extrude approximately 50 gonad arms, excising each just proximal to the spermatheca. Dissected gonads were carefully isolated from carcasses and transferred into an Eppendorf tube. Total RNA from each sample (~100 ng) was isolated using Trizol (Invitrogen) and amplified with T7 RNA polymerase using one round of linear amplification (Baugh et al., 2001). Three independent *dpl-1* and *lin-35* mutant samples and four independent *efl-1* mutant samples were collected.

Fluorescence-labeled cDNA probe for DNA microarray hybridization was prepared from 3 μg of amplified RNA as described (DeRisi et al., 1997). *lin-35* and *efl-1* mutant cDNA was labeled with Cy3 and compared to wild-type (N2) cDNA labeled with Cy5. Cy3-labeled *unc-4*;*dpl-1* mutant cDNA was compared to *unc-4* cy5-labeled cDNA. *Caenorhabditis elegans* whole genome microarrays were used for hybridization as described (Jiang et al., 2001). Each slide was scanned using an Axon scanner (Molecular Devices, Sunnyvale, CA), and the expression levels for each gene in each channel were collected using GenePix 3.0 software. Cy5/Cy3 ratios were calculated and normalized by setting the overall median of ratios to one. All data have been deposited in GEO under Accession Number GSE5071.

For each set of mutant data, the repeats were averaged, and a Z test [Z=(observed–expected)/SE] was performed in Excel. A moderate correction for multiple testing (~17,600 genes) was performed by multiplying the calculated *P*-value by 10,000. After this correction, all genes with up- or downregulation greater than twofold, $P<0.05$ in any given mutant were selected. The hypergeometric probability test (http://elegans.uky.edu/MA/progs/overlap_stats.html) was used to calculate the significance of overlap of gene groups. We determined whether transcripts of Group I-IV genes are bound by GLD-1, based on a minimum criteria of >1.5× enrichment in GLD-1 immunoprecipitated samples compared to control immunoprecipitations ($P<0.01$). Genes in Groups III and IV had very little overlap with GLD-1-bound transcripts (1/42 and 1/84, respectively), while genes in Groups I and II had a significantly enriched overlap with candidate GLD-1 targets (26/74 and 5/43, respectively).

Regulatory motif analysis

To identify candidate regulatory sequences in the 5′ noncoding regions of target genes, the online program MEME (Mutiple Em for Motif Elicitation) http://meme.sdsc.edu/meme/intro.html) was applied to sequences upstream taken from the start codon of each target gene to the neighboring gene, up to 1 kb. Each group of genes (Groups I-IV) was examined separately, as well as two control sets of genes, one with oogenic germline-enriched expression not regulated by EFL-1/DPL-1, and one that does **not** show germline-enriched expression. Manual examination was performed by taking all variations of the MEME-derived consensus motif [e.g. TTC(G/C)CGC(C/G)] and searching through each 5′ regulatory sequence for an exact match. Manual examination of upstream regions of those Group I genes in which MEME failed to find the E2F consensus motif did not uncover any additional instances of the motif. The motif sequence logo was created using the online program (http://weblogo.berkeley.edu/logo.cgi).

Immunofluorescence

RME-2 and MEX-5 localization was performed as described (Kelly et al., 2002). Briefly, gonads were dissected as described above, fixed in 3.7% paraformaldehyde, and mounted on a slide. The slides were frozen on a dry ice block and the coverslip cracked off before storing the slides in –20°C methanol. The slides were washed three times in phosphate-buffered saline (PBS), blocked in PBS containing 0.1% Tween 20 and 0.5 mg/ml BSA, and then incubated at 4°C overnight with α-RME-2 (1:100; gift from B. Grant), α-MEX-5 (undiluted; gift from J. Priess) and α-LIN-3 (1:50; Santa Cruz Biotechnology, Santa Cruz, CA). The samples were then stained with DAPI, washed and incubated at room temperature for 3 hours with a fluorescent secondary antibody (Molecular Probes, Carlsbad, CA). After further washing in PBS, the slides were mounted with anti-fade solution and viewed using a Zeiss Axioplan 2 imaging epifluorescence microscope.

RNAi

Group I genes with oogenic germline-enriched expression that were reported to have embryonic lethal RNAi phenotypes (25) were tested for PIE-1:GFP mis-localization. Of these, ten gave embryonic lethality in our hands: T22F3.3, T05G5.7, *spn-4*, *rnr-2*, *rme-2*, R05H5.3, *puf-3*, *pos-1*, C28C12.2 and *cyb-3*. *mex-5* and *mex-6* were used as controls. PCR using gene-specific primers containing T7 sequences was followed by in-vitro transcription using T7 RNA polymerase. The resulting dsRNA was ethanol precipitated with Pellet Paint (Novagen) and resuspended to a concentration of ~2 mg/ml. *pie-1*::GFP worms (Reese et al., 2000) were synchronized and grown until the L4 stage. Worms were washed in M9 and soaking buffer (0.25× Mg^{++}-free M9, 3 mmol/l spermidine, 0.05% gelatin) twice before soaking.

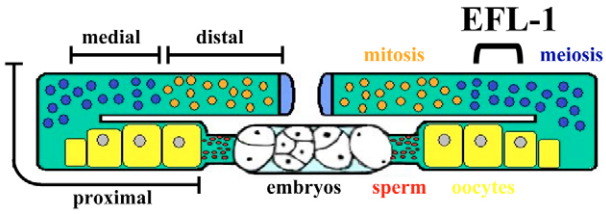

Fig. 1. *C. elegans* germline. The right arm of the gonad shows germ cell development. The left arm of the gonad delineates the distal, medial and proximal regions, as referred to in the text. Fertilized embryos are present in the uterus. Orange, proliferation; blue, meiotic prophase I; yellow, maturing oocytes; red, sperm; brackets, region in which EFL-1 protein is detectably expressed.

Approximately 30 worms in 2 μl soaking buffer were added to 2 μl dsRNA for each gene, incubated for 24 hours at 20°C, and then plated to NGM seeded with OP50 bacteria. For each gene giving embryonic lethality, embryos released from the uterus of ~10 mothers were examined using a Zeiss Axioplan 2 microscope. Cross-RNAi between *mex-5* and *mex-6* transcripts probably occurred, resulting in PIE-1 mis-localization (Schubert et al., 2000).

RESULTS

The *C. elegans* germline

Each lobe of the bi-lobed hermaphrodite germline is arranged along a distal-to-proximal axis, with germ cells progressing from an undifferentiated mitotic state to fully differentiated gametes along this axis [Fig. 1; reviewed by Hubbard and Greenstein (Hubbard and Greenstein, 2005)]. During the fourth larval stage, spermatogenesis occurs in the proximal germline. Upon the onset of adulthood, oogenesis then initiates in the proximal germline. Early stage oocytes enlarge substantially by incorporating cytoplasm from the central core of the germline and taking up yolk proteins that are synthesized by the intestine. Oocyte cytoplasm contains many maternally provided mRNAs and proteins that are necessary for proper embryonic development. The most proximal oocyte undergoes maturation and ovulation, becoming fertilized when it comes into contact with sperm in the spermatheca. Fertilization triggers rapid eggshell formation and completion of meiosis. The newly fertilized embryos pass from the spermatheca into the uterus, where they are held briefly before being laid.

dpl-1 and *efl-1*, but not *lin-35*, are required for fertility

Immunolocalization studies show that DPL-1 protein is present in all germline nuclei except sperm (Ceol and Horvitz, 2001). EFL-1 protein is also present in germline nuclei, but its expression is detected only in the medial region, when nuclei are in the pachytene stage of meiosis I (Page et al., 2001) (Fig. 1). Partial loss-of-function point mutations in either *dpl-1* or *efl-1* result in temperature-sensitive maternal-effect embryonic lethality due to defects in embryonic asymmetry (Page et al., 2001). Additionally, Page et al. (Page et al., 2001) demonstrated using genetic mosaic analysis that loss of *efl-1* activity in the germline, but not the soma, was sufficient to cause this embryonic lethality. To identify the primary defect upon complete loss of *dpl-1* and *efl-1* activity, we examined animals bearing probable null alleles in *dpl-1* or *efl-1*. The *dpl-1(n3316)* allele contains a deletion that removes the first half of the protein, including the EFL-1-binding domain. The *efl-1(n3639)* allele harbors an early stop mutation, truncating the protein upstream of the predicted DP- and pRB-binding domains (Ceol and Horvitz, 2001).

The *dpl-1(n3316)* and *efl-1(n3639)* mutant phenotypes are very similar: germ cells of all stages are present and the gonad has essentially normal morphology, but adults display nearly 100% penetrant sterility (i.e. do not lay fertilized embryos) (Coel and Horvitz, 2001) (Fig. 2C,D). Most oocytes either do not enter or do not exit the spermatheca normally, and over time the proximal gonad

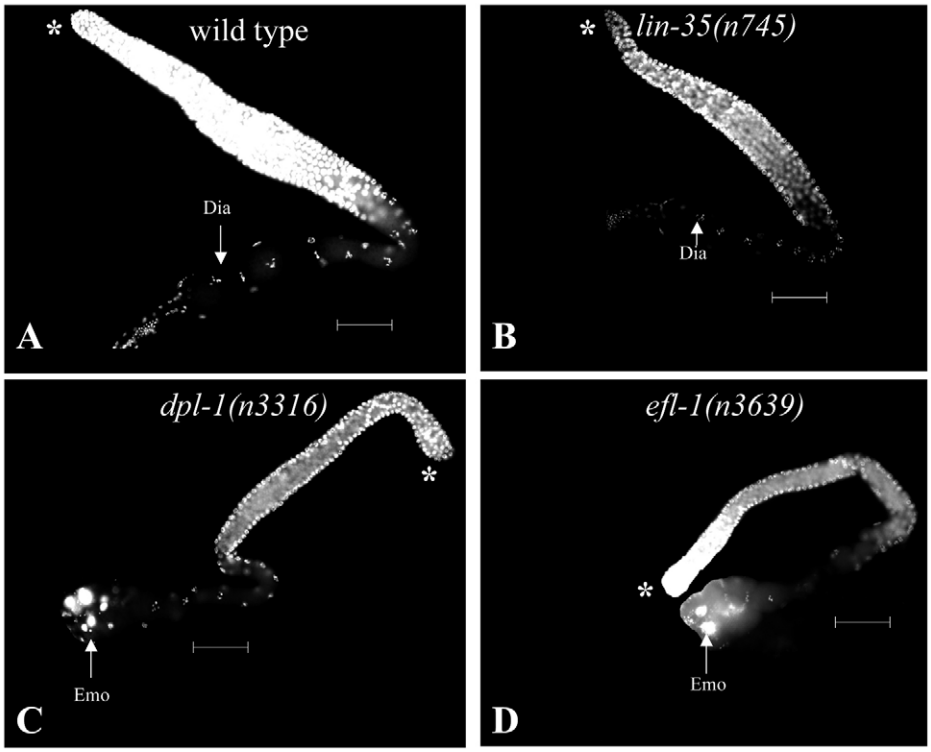

Fig. 2. Dissected gonads from *efl-1*, *dpl-1* and *lin-35* mutants. Hermaphrodite gonads from (**A**) wild type (N2); (**B**) *lin-35(n745)*; (**C**) *dpl-1(n3316);* and (**D**) *efl-1(n3639)*. Distal end of gonad marked with asterisk. Scale bars: 50 μm. Dia, oocyte in diakinesis of meiosis I; Emo, endomitotic.

in *efl-1* and *dpl-1* mutants fills up with degenerating oocytes that become endomitotic. The occasional oocyte that does pass through the spermatheca frequently fails to develop a complete eggshell, and does not undergo normal cell division. The *efl-1(n3639)* and *dpl-1(n3316)* phenotypes persist even when exogenous sperm is provided from wild-type males, indicating that the phenotypes are not due solely to defective spermatogenesis.

Aberrant ovulation and the endomitotic (Emo) phenotype can be caused by defects in either the oocyte or the somatic gonad (McCarter et al., 1997; McCarter et al., 1999). To test the site of action of *dpl-1* and *efl-1*, we injected dsRNA corresponding to *dpl-1* or *efl-1* into wild-type and *rrf-1* mutants, which can carry out RNAi in the germline but not the soma (Sijen et al., 2001). Relative to wild-type controls, *rrf-1*;*efl-1(RNAi)* and *rrf-1*;*dpl-1(RNAi)* animals displayed a decreased incidence of the Emo phenotype. This observation suggests that *efl-1* and *dpl-1* do play a role in the soma, possibly in the somatic gonad, to regulate ovulation (data not shown). However, *rrf-1*;*efl-1(RNAi)* and *rrf-1*;*dpl-1(RNAi)* animals were still completely sterile and collected partial, torn oocytes in the uterus, indicating that defects in ovulation, though more subtle, still occurred. This observation suggests a role for *efl-1* and *dpl-1* in the germline to promote fertility. In sum, the null phenotype for both *efl-1* and *dpl-1* in the germline is more severe than in previously studied point mutants (Page et al., 2001).

The *lin-35(n745)* mutation truncates the 961-amino acid LIN-35 protein after the first 150 amino acids, removing the A and B pocket domains, and probably acting as a null (Lu and Horvitz, 1998). The germline of *lin-35(n745)* mutants generally appears morphologically normal (Fig. 2B). Unlike *dpl-1(n3316)* and *efl-1(n3639)* mutants, *lin-35(n745)* animals are fertile and do not display obvious defects in oogenesis or ovulation. However, the brood size of *lin-35(n745)* mutants is 41% of wild type (132±13 versus 320±42; Student's *t* test: $P<<0.001$).

EFL-1 and DPL-1 regulate a common set of genes and LIN-35 regulates a distinct set of genes

Because EFL-1, DPL-1 and LIN-35 are predicted to act as transcriptional regulators, we used whole-genome *C. elegans* DNA microarrays to identify candidate target genes. To focus on the role of EFL-1, DPL-1 and LIN-35 in the germline, we isolated gonads away from the rest of the animal. For each mutant or control sample, we collected ~50 dissected adult gonads, carefully discarding the spermatheca and uterus as well as the rest of the carcass. The only somatic cells retained were the distal tip cell and gonadal sheath cells, ensuring that the vast majority of the isolated RNA derived from germ cells. Because the germline morphology of *efl-1(n3639)*, *dpl-1(n3316)* and *lin-35(n745)* mutants are visually similar to wild type, non-specific effects on gene expression should be minimal.

We used linear amplification to increase the amount of RNA for all samples, and reverse transcribed the RNA to labeled cDNA. Using microarrays containing ~90% of predicted *C. elegans* genes (Jiang et al., 2001), we performed at least three hybridizations directly comparing each mutant to a control. The resulting data were analyzed using both an average fold-difference and a *Z*-test with correction for multiple testing (see Materials and methods). We have focused on 272 genes whose expression is either up- or downregulated in *efl-1*, *dpl-1* or *lin-35* mutants relative to controls, exceeding a twofold difference at $P<0.05$ (see Table S1 in the supplementary material). Throughout this paper, we will use the term 'downregulated' to refer to target genes with lower expression in *dpl-1*, *efl-1* and/or *lin-35* mutants relative to controls, and 'upregulated' to refer to genes with higher expression in any mutant relative to controls.

We first examined the overlap and independence between the different mutant datasets (Fig. 3). Our results show that DPL-1 and EFL-1 both function primarily to promote gene expression. At our twofold cutoff, 116 genes were downregulated and 30 genes were upregulated in *dpl-1* mutants. Similarly, in *efl-1* mutants, 114 genes were downregulated and 20 genes were upregulated. Remarkably, the two mutants showed downregulation of 70% of the same target genes (95/135; $P<<0.001$) (Fig. 3A). This extensive overlap is consistent with these two proteins acting as a heterodimer to regulate transcription. Only a subset (36) of the 95 genes downregulated in *efl-1* and *dpl-1* mutants were also downregulated in *lin-35* mutants. Instead, distinct genes were primarily upregulated in *lin-35* mutants; strikingly few of these were also regulated in either *efl-1* or *dpl-1* mutants (Fig. 3B). The above observations suggest that EFL-1/DPL-1 can function without LIN-35 to regulate expression of many target genes and, conversely, that LIN-35 often acts independently of EFL-1/DPL-1. When LIN-35 does act on genes regulated by EFL-1/DPL-1, it cooperates with, rather than antagonizes, EFL-1/DPL-1 (Fig. 3A).

Examining the overlap among preselected gene groups can exclude genes that come close but fail to meet selection criteria, partially disguising general trends. We therefore used hierarchical clustering to place the 272 genes into groups with similar regulation (Eisen et al., 1998) (Fig. 4). From this analysis, we defined four expression groups that encompass 248 of the 272 genes; the remaining 24 genes showed variable expression and were not included in a group (Fig. 4; see Table S1 in the supplementary material). Group I comprised 75 genes with decreased levels in *dpl-1(n3316)* and *efl-1(n3639)* mutants compared with controls and were termed 'downregulated in *efl-1* and *dpl-1*'. Sixty-five out of 75 genes in this group were regulated more than twofold ($P<0.05$) by both EFL-1 and DPL-1. Seven of the remaining ten genes just missed the twofold cutoff in one mutant and the remaining three lacked data for one mutant. Thus these genes are almost uniformly under the control of both EFL-1 and DPL-1, suggesting that they are targets of an EFL-1/DPL-1 heterodimer. Only 16 out of the 75 genes in Group I were significantly downregulated in *lin-35* mutants, although many more were moderately affected.

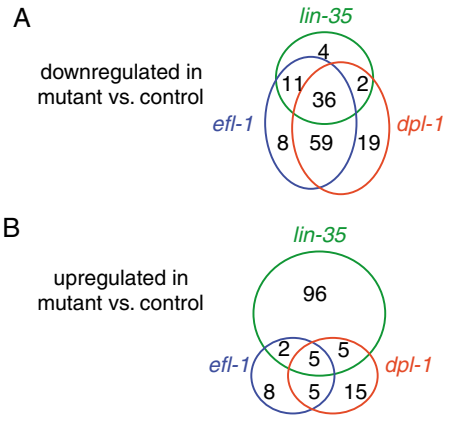

Fig. 3. Target genes of *lin-35, efl-1* and *dpl-1*. The overlap between genes regulated at least twofold, $P<0.05$ in at least one mutant are displayed by Venn diagram. Genes are considered overlapping if they are regulated >1.5×, $P<0.05$ in the second mutant. Regulated genes are divided into downregulated in mutants (**A**) and upregulated in mutants (**B**). Three genes (*col-178*, *asp-1* and F11A3.2) are represented twice, as they show opposite regulation in two mutants (see Table S1 in the supplementary material).

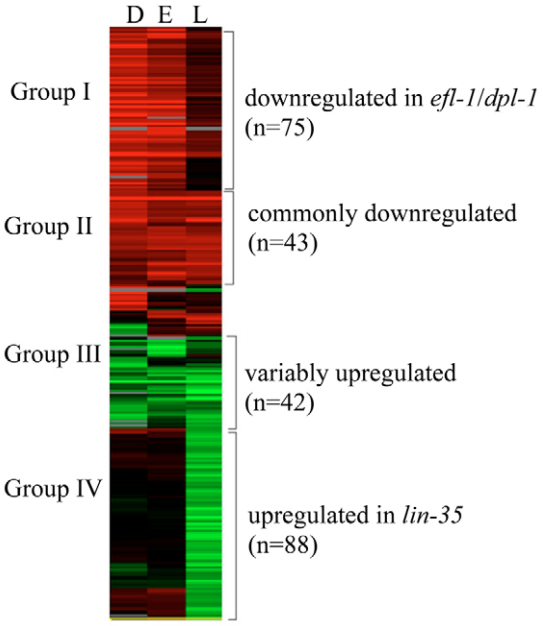

Fig. 4. Hierarchical cluster analysis of *lin-35*-, *efl-1*- and *dpl-1*-regulated genes. The 272 genes are displayed in rows; columns represent the average of repeats for each mutant (D, *dpl-1*; E, *efl-1*; L, *lin-35*). Red, lower expression in mutant samples relative to controls; black, equivalent expression in mutant relative to control; green, higher expression in mutant relative to control samples.

Group II genes (43 genes) generally had lower expression in *efl-1*, *dpl-1* and *lin-35* mutants than in control gonads. Twenty-three genes were significantly regulated in all three mutants, and 36 were regulated in at least two mutants, so we call Group II genes 'commonly downregulated'. Conversely, genes in Group III (42 genes) had higher expression in one or more mutants relative to controls. However, only five were significantly upregulated in all three mutants and only 17 were upregulated in at least two mutants, so we termed this group 'variably upregulated'. Group IV contains 88 genes with significantly increased levels only in the *lin-35* mutant relative to controls; we referred to Group IV genes as 'upregulated in *lin-35*'. From these data, we conclude that *efl-1* and *dpl-1* mutants show strong similarity in gene regulation over all four groups, even if a given gene did not surpass the selection criteria in one mutant, reinforcing the notion that EFL-1 and DPL-1 are likely to act as a heterodimer and regulate common targets. *lin-35* shared common targets with *efl-1* and *dpl-1* for one of the four groups (Group II), but showed distinct regulation in the other three groups. Most notably, *lin-35* had minimal effect on Group I genes regulated by *efl-1* and *dpl-1*, and instead regulated expression of a large set of genes not affected by loss of *efl-1* or *dpl-1* (Group IV).

A consensus E2F-binding site is present upstream of EFL-1/DPL-1-activated genes

To identify candidate regulatory sequence motifs for each group, we examined the sequence upstream of each open reading frame (maximum 1 kb) using the MEME program, which identifies over-represented sequences de novo (Bailey and Elkan, 1994). The most significant hit among any of the groups was the sequence TTC(G/C)CGC(C/G) (P<<0.001), found upstream of 58 out of 75 Group I (downregulated in *efl-1* and *dpl-1*) genes (Fig. 5A; see Table S1 in the supplementary material). This sequence bears striking similarity to the known mammalian E2F consensus site TTTCGCGC (Chittenden et al., 1991), suggesting that most Group I genes are likely to be direct targets of the EFL-1/DPL-1 transcription factor. The TTCGCGCC motif was present within the first 500 base pairs upstream of the translation start in 48 genes. Multiple copies of the motif were present in 28 genes, and the duplicate sites were often juxtaposed (Fig. 5B). MEME did not identify the E2F consensus motif in Groups II, III or IV, in a control set of oogenic germline-enriched genes not responsive to EFL-1, DPL-1 or LIN-35 (n=38), or in a control set of genes that did not show germline-enriched expression (n=51).

Our ability to find an E2F consensus site in Group I genes but not the other three groups suggests that most genes in Groups II-IV are not regulated by direct binding of an E2F heterodimer, but are either indirect or downstream. To determine whether at least some genes in Groups II, III and IV might be direct targets, we examined their 5′ regulatory sequence manually and identified 13 Group II, 8 Group III and 30 Group IV genes with a *C. elegans* consensus E2F site (see Table S1 in the supplementary material). Thus, it is possible that the regulatory regions upstream of certain genes in these groups are directly bound by EFL-1/DPL-1.

MEME detected a different motif, TTTTCCAG, in the regulatory regions of Group IV 'upregulated in *lin-35*' genes (P<<0.001; Fig. 5C). This consensus sequence was present in 58/88 regulatory sequences, but its location was not biased toward the translation start site (Fig. 5D). We could find no clear match to any known transcription factor consensus sequence for this motif in any database. No significant motifs were identified in Group II or Group III genes.

The germline expression pattern of downregulated genes differs from upregulated genes

We examined the spatial expression patterns of genes in groups I-IV using data from an online in-situ hybridization database (Y. Kohara, personal communication; http://nematode.lab.nig.ac.jp), which has images of expression patterns available for 151 of the 248 genes (see Table S1 in the supplementary material). Images are available for 54 Group I 'downregulated in *efl-1* and *dpl-1*' genes, and 47 of these show staining in the germline. Notably, 37 genes have undetectable levels in the distal germline with an abrupt increase in the medial germline that persists proximally, often into embryos (Fig. 6A-D). Onset of expression coincides with the appearance of EFL-1, which is detectable only in the mid-pachytene region of the medial germline (Page et al., 2001) (see Fig. 1). Fewer Group II 'commonly downregulated' genes show detectable expression in the germline based on in-situ data (12/26); however, 10 of 12 were expressed in a medial/proximal restricted expression pattern, similar to Group I genes. Thus, germline expression of most genes downregulated in the mutants (Groups I and II) is generally restricted to the medial and proximal gonad, coincident with peak EFL-1 protein levels.

Conversely, most Group III and IV genes are broadly expressed in the wild-type germline. Out of 46 Group IV 'upregulated in *lin-35*' genes with in-situ images, 35 have detectable germline expression, with 32 visible in both the distal and proximal germline and three restricted to the medial/proximal germline (Fig. 6E-H). Only 6/16 Group III genes show detectable germline expression, but four of these have broad expression and two show medial/proximal restricted expression. Given that our dissected gonad microarray data indicates that these genes are normally at lower levels in the wild-type germline than in the *lin-35* mutant germline, this broad germline expression was unexpected. One possibility is that LIN-35 acts broadly to decrease, but not abrogate, expression of these genes,

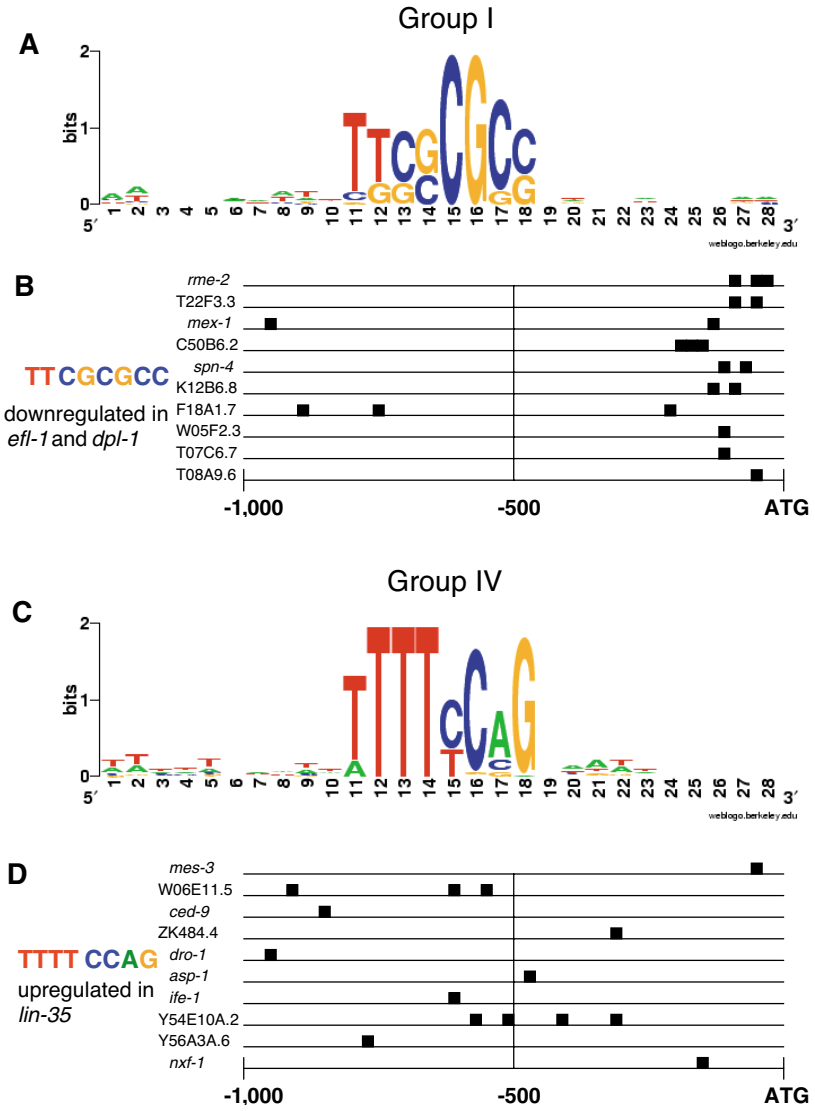

Fig. 5. Identification of candidate regulatory motifs upstream of target genes. (**A**,**C**) Graphic representation of motifs identified through MEME. The height of a letter represents the relative frequency of occurrence. (**B**,**D**) Location of putative motifs within a 1 kb region upstream of selected candidates. Small boxes indicate the location of the motif. Scale at bottom in nucleotides.

because elevated levels are slightly detrimental to germline function. Another possibility is that LIN-35 acts to repress expression of these genes in a specific region of the germline, but that this effect is not substantial enough to be visible by in-situ hybridization methods, which are difficult to quantify. Finally, we could be detecting LIN-35-mediated repression of these germline-expressed genes in the few somatic cells that are included in our analysis (distal tip and sheath cells) rather than in germ cells, which would be difficult to distinguish by in situ. This last possibility is consistent with the observation that *lin-35* prevents ectopic germline gene expression in the soma (Wang et al., 2005).

EFL-1 and DPL-1 are required for normal expression of RME-2 and MEX-5

We independently tested whether two Group I genes, *rme-2* and *mex-5*, were regulated in a manner consistent with the microarray results. *rme-2*, which encodes the yolk receptor (Grant and Hirsh, 1999), and *mex-5*, which encodes a CCCH zinc finger protein required for correct embryonic polarity (Schubert et al., 2000), both require *efl-1* and *dpl-1* activity for their expression, based on our microarray experiments. *lin-35* has a moderate effect on *mex-5* but not *rme-2* expression (see Table S1 in the supplementary material). We performed semi-quantitative RT-PCR of both genes in wild type, *efl-1*, *dpl-1* and *lin-35* mutants and saw decreased expression of both *rme-2* and *mex-5* in *efl-1* and *dpl-1* mutants relative to wild type, with minimal effects in *lin-35* mutants, consistent with our microarray data (see Fig. S1 in the supplementary material). We also performed immunohistochemistry to determine whether localization and expression level of RME-2 and MEX-5 differs in *efl-1*, *dpl-1* and *lin-35* mutants compared to wild type (Fig. 7). In wild-type gonads, RME-2 localizes to the oocyte membrane. We saw similar levels and localization in *lin-35* mutants, but *efl-1* or *dpl-1* mutants displayed severely reduced RME-2 levels, even at tenfold higher exposure times, consistent with a role for EFL-1 and DPL-1 in promoting expression of *rme-2*.

MEX-5 showed a slightly different response from RME-2 to loss of *efl-1* and *dpl-1*. In wild type, MEX-5 began to accumulate in the cytoplasm of late pachytene germ cells, increasing markedly in oocytes (Fig. 7B). In *lin-35* mutants, MEX-5 expression appeared very similar to wild type. In *efl-1* and *dpl-1* mutants, no MEX-5 expression was evident in the cytoplasm of pachytene nuclei or immature oocytes, but was still present in the most proximal oocytes (Fig. 7H,K). This result suggests that EFL-1 and DPL-1 are important for increasing levels of *mex-5* expression, but are not solely responsible for its activation. This finding is consistent with a previous observation that MEX-5 was still present in *efl-1(se1ts)* or

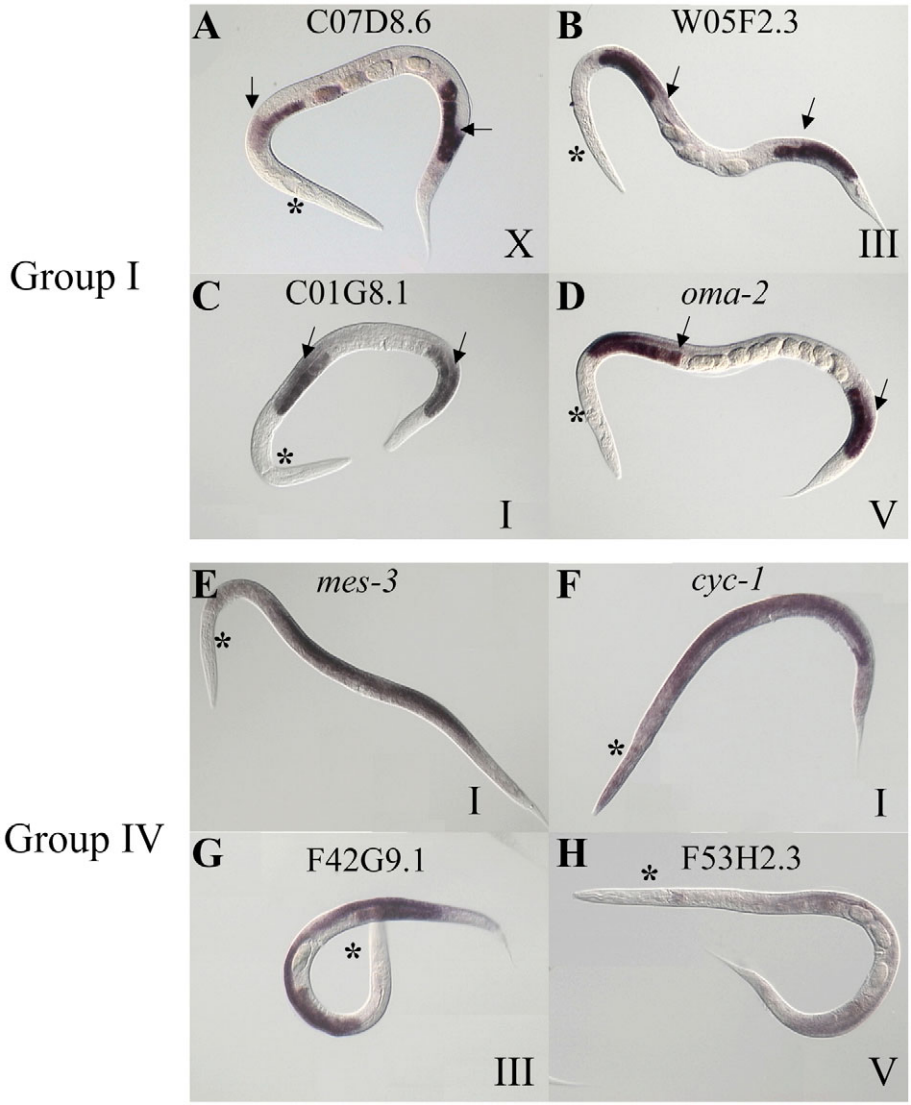

Fig. 6. In-situ hybridization patterns of example Group I and Group IV genes. The head of the animal is marked by an asterisk. (**A-D**) Autosomal Group I genes initiate detectable expression in the medial germline, while X-linked genes are more proximal. Arrows mark medial germline and onset of expression. (**E-H**) Group IV genes generally show broad expression, including distal and proximal germline. In-situ data courtesy of Y. Kohara (http://nematode.lab.nig.ac.jp).

dpl-1(zu355) mutant embryos (Page et al., 2001). Thus, for both Group I genes we tested, we saw decreased expression in both *efl-1* and *dpl-1* mutants, but not in *lin-35* mutants, relative to wild type, consistent with the microarray results.

To test whether the reduced expression of RME-2 and MEX-5 seen in *efl-1* and *dpl-1* mutants was a downstream, non-specific effect of decreased oogenesis or oocyte maturation, we examined LIN-3. *lin-3* is expressed during oogenesis, but is not differentially regulated in *lin-35*, *dpl-1* or *efl-1* mutants in our microarray experiments. In wild type, LIN-3 is localized to the oocyte membrane (Fig. 7C), while in *lin-3(RNAi)* animals this staining is absent (see Fig. S2 in the supplementary material). LIN-3 localization was not altered in *lin-35*, *dpl-1* or *efl-1* mutants relative to wild type, suggesting that oogenesis genes not identified in our microarray study are probably still expressed normally in *dpl-1* and *efl-1* mutants.

Group I genes promote oogenesis and early embryogenesis

Studies in mammalian cells have focused on the role of E2F in regulating genes important for promotion of the cell cycle, particularly those acting in DNA synthesis (reviewed by Trimarchi and Lees, 2002). However, recent global expression studies in mammalian and *Drosophila* cell culture have found that various E2Fs can activate genes that act at other phases of the cell cycle, as well as repressing genes that act to promote differentiation (Ren et al., 2002; Dimova et al., 2003). The complexity of the Rb/E2F network has made it difficult to perform and interpret in-vivo studies, leaving a gap in our understanding of how accurately the transcriptional properties of E2F in culture reflect its role in vivo.

In our experiments, we found that EFL-1/DPL-1 primarily promotes expression of pro-differentiation genes in the wild-type germline (Table 1). Group I 'downregulated in *efl-1* and *dpl-1*' genes include only a few likely to have direct roles in the meiotic and mitotic cell cycle, such as a ribonucleotide reductase subunit *rnr-2*, and two cyclin B orthologs, *cyb-3* and *cyb-2.1* (Sonneville and Gonczy, 2004) (www.wormbase.org).

Table 1. Known genes in Group I

Oocyte development	Metabolism/ structural	Cell division	Embryonic asymmetry
rme-2	*gpd-1-4*	*rnr-2*	*mex-1*
oma-2	*gln-2, -6*	*cyb-3*	*mex-5*
egg-2	*tbb-2, -4*	*cyb-2.1*	*mex-6*
cej-1	*sqv-4*		*spn-4*
			pos-1

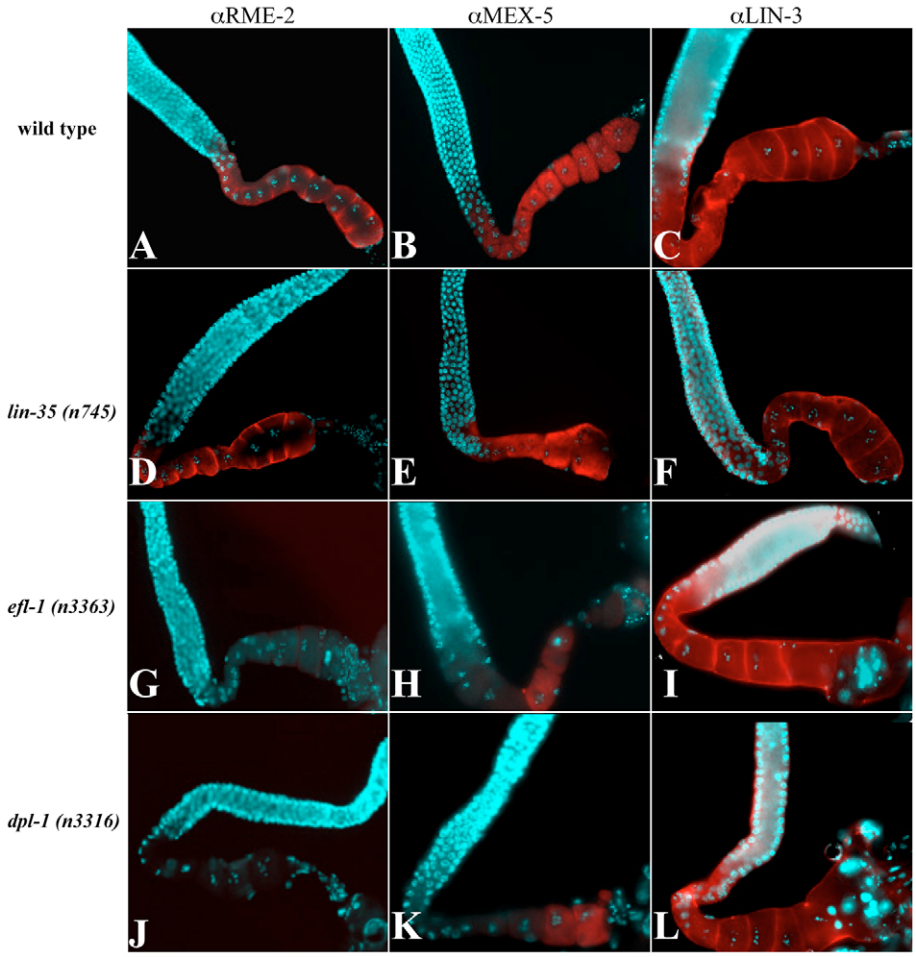

Fig. 7. Reduction of RME-2 and MEX-5 levels in *efl-1(n3363)* and *dpl-1(n3316)* mutants. (**A-L**) Adult germlines from each genotype were dissected, fixed and stained with an antibody to the corresponding protein as labeled. Blue, DNA; red, antibody. Panels G and J are at 10× exposure relative to A. For each antibody and genotype, *n*>20. The reduced RME-2 and MEX-5 staining seen in *efl-1* and *dpl-1* mutants occurred in all gonads examined. LIN-3 staining on the oocyte surface disappears upon RNAi of *lin-3*, whereas the diffuse staining does not (see Fig. S2 in the supplementary material).

Four previously studied targets encode proteins with decreased expression that is likely to contribute to the defects in fertilization and eggshell formation seen in *dpl-1(n3316)* and *efl-1(n3639)* mutants. These include the yolk receptor RME-2, the Tis11-like oocyte maturation protein OMA-2, the predicted chitin-binding protein CEJ-1, and an EGF-related protein required for fertilization, EGG-2 (Grant and Hirsh, 1999; Detwiler et al., 2001; Lee and Schedl, 2001; Kadandale et al., 2005). In particular, *rme-2* mutants display ovulation defects and have a reduced brood size as a consequence (Grant and Hirsh, 1999). Thus, decreased *rme-2* expression is probably a significant component of the *dpl-1* and *efl-1* phenotypes.

Other Group I 'downregulated in *efl-1*and *dpl-1*' genes act to establish polarity and cell fate determination in the early embryo, such as *spn-4*, *mex-5*, *mex-6* and *mex-1*. *pos-1* is also regulated by EFL-1/DPL-1, although it is not in Group I. SPN-4, POS-1 and MEX-1 are localized to posterior blastomeres in the early embryo and are important for establishing asymmetry in the early cell divisions. Additionally, SPN-4 and POS-1 act together in the posterior blastomeres to negatively regulate translation of the *glp-1* mRNA (Ogura et al., 2003). MEX-5 and MEX-6 are localized to anterior blastomeres and also establish embryonic asymmetry (Schubert et al., 2000). Loss of *pos-1*, *mex-5/6* or *mex-1* activity results in ectopic localization of the germline-specific PIE-1 protein in somatic cells, either through mis-specification of the germ lineage or through a failure to degrade PIE-1 in somatic blastomeres, and results in embryonic lethality (Schubert et al., 2000; Tabara et al., 1999; Guedes and Priess, 1997).

Because four genes downregulated in *efl-1* and *dpl-1* mutants are known to affect PIE-1 localization, we tested several other target genes for their requirement in correctly localizing a PIE-1:GFP fusion protein (Reese et al., 2000). We examined a subset of genes with reported RNAi phenotypes that were embryonic lethal in large-scale screens. Out of ten genes tested, we found one, T22F3.3, with inactivation by RNAi that results in persistence of PIE-1 in somatic blastomeres at a frequency similar to the rate of embryonic lethality (9%) (see Fig. S3 in the supplementary material). T22F3.3 encodes the only obvious glycogen phosphorylase in the *C. elegans* genome and presumably plays a crucial role in modulating glucose availability. Mutations in human glycogen phosphorylase cause McArdle disease, which impairs muscle function (Dimaur et al., 2002). Alterations in available energy levels could conceivably impact some of the processes required for timely degradation of PIE-1 in the soma.

Although Group II genes share some common expression characteristics with Group I genes as described above, they generally encode different types of proteins. For instance, Group II genes include seven that encode histones H2A, H2B or H4, but none encoding H3 or H1. In human cells, the promoters of several histone H2 genes are also bound by E2F4 (Ren et al., 2002). RNAi of several Group II genes produces embryonic lethal phenotypes based on large-scale studies, including those encoding histones, the translation elongation factor EFT-2, and chaperone proteins such as HSP-3 and PDI-2.

The predicted protein functions of the genes in Groups III and IV are heterogeneous, and obvious trends are not readily apparent. Five Group III genes show significant upregulation in all three mutants,

including *akt-2*, a kinase that acts downstream of insulin signaling, *toh-2*, a metalloprotease, and *cdr-4*, a cadmium-responsive glutathione-S-transferase. Group IV 'upregulated in *lin-35*' genes include several that encode transcriptional regulatory proteins, such as *mes-3*, *dro-1* and *ntl-1*, as well as four uncharacterized proteins with histone acetyltransferase domains. Genes encoding the DNA synthesis licensing factor CDT-1, the anti-apoptotic factor CED-9 and the Frizzled receptor MOM-5 are also repressed by LIN-35 in the gonad. Thus, the upregulated genes encode proteins of a wider variety of functions than genes downregulated in *efl-1* and *dpl-1* mutants.

DISCUSSION

The connection between the transcriptional activity of E2F and its in-vivo role in differentiation is one of the most poorly understood aspects of E2F function. Using the germline of *C. elegans* as a model system, we investigated the function of the core pRb/E2F pathway and uncovered an unexpected role for the EFL-1/DPL-1 (E2F) heterodimeric transcription factor in vivo. Identification of genes regulated in *efl-1*, *dpl-1* and *lin-35* mutant gonads indicates that EFL-1/DPL-1 probably functions as a transcriptional activator, while LIN-35 has a repressive role. Importantly, genes upregulated by EFL-1 and DPL-1 (Group I) have canonical E2F-binding sites in their 5′ regulatory sequences, and their expression is first detectable in the same region of the germline at which EFL-1 reaches peak levels, indicating that they are probably direct targets of EFL-1/DPL-1. They encode proteins that promote oogenesis, fertilization and early embryogenesis, rather than cell cycle regulators. Thus, even though EFL-1 most closely resembles mammalian repressor E2F4, our results demonstrate that E2F directly promotes development through activation of pro-differentiation genes, suggesting a new mechanism by which E2Fs can function as tumor suppressors in vivo.

Limited compensation by Rb/E2F family members

The streamlined nature of the pRb/E2F pathway in *C. elegans* reduces the possibility of compensation by other family members. The *dpl-1* locus encodes the only DP-related protein in the *C. elegans* genome; thus, loss of *dpl-1* should remove all E2F activity, as most E2Fs require a DP subunit to regulate gene expression. Our results show that *dpl-1* does not regulate many genes in addition to those regulated by *efl-1*. This extensive overlap of *dpl-1* and *efl-1* in regulating germline gene expression strongly suggests that EFL-2 or F49E12.6 do not compensate for loss of EFL-1 and that EFL-1 is the major component of E2F activity in the germline.

lin-35 is the only pocket protein encoded in the worm genome and clearly has a distinct role in regulating a distinct set of genes (Group IV). However, additional processes in which LIN-35 participates could be buffered by the action of other proteins. In somatic tissues, genes of diverse functions have exhibited redundancy with *lin-35*, including components of the SynMuv A or C pathways, the cell cycle inhibitor *cki-1*, and a regulator of APC activity, *fzr-1* (Ferguson and Horvitz, 1989; Ceol and Horvitz, 2004; Boxem and van den Heuvel, 2002; Fay et al., 2002). Additionally, a mutation of *lin-35* enhances the meiotic recombination defect of *him-17* mutants in the germline (Reddy and Villeneuve, 2004). Thus LIN-35 could have a role in germ cell division or differentiation that is not apparent in *lin-35* mutants because of redundancy with other factors.

Relationship between EFL-1, DPL-1 and LIN-35 in the germline

Based on both genetic and biochemical evidence, components of the *C. elegans* Rb pathway act together to repress gene expression in somatic tissues (reviewed by Kipreos, 2005). In both *C. elegans* and *Drosophila*, recent work has demonstrated that genes normally expressed in the germline are repressed in the soma by pRB and E2F (Dimova et al., 2003; Wang et al., 2005). Our study is the first in-vivo demonstration of distinct roles for E2F and pRB in regulating gene expression in the germline. We have shown that E2F mainly functions to activate, rather than repress, genes important for oocyte and embryo differentiation (Group I genes), and that the role of LIN-35(pRB) in this process is minimal or dispensable. Possibly, LIN-35 dissociates from the E2F complex in the germline, freeing E2F to activate target gene expression. E2F activity could also be controlled by accumulation of EFL-1 protein at the mid-pachytene stage of meiosis I rather than by association of LIN-35. Notably, in *Drosophila*, chromatin immunoprecipitation studies demonstrate that both pRB and E2F can be found at the promoters of genes whose expression is not dependent on pRB (Dimova et al., 2003; Stevaux et al., 2005). By analogy, in the *C. elegans* germline, LIN-35 could be present at the promoters of Group I genes but not be rate-limiting for their expression.

LIN-35 instead acts to downregulate a distinct set of genes (Group IV) that do not require EFL-1 or DPL-1 for their expression. The absence of canonical E2F binding sites from the 5′ regulatory regions of most Group IV genes suggests that LIN-35 is targeted to these sites through the activity of a different DNA-binding factor, one that potentially binds the TTTTCCAG site that we found highly represented among Group IV genes. Several instances of an E2F-independent function of pRB have been described (Sellers et al., 1998; Thomas et al., 2001; Gagrica et al., 2004). Alternatively, LIN-35 could be acting with either EFL-2 or F49E12.6 in a DPL-1-independent manner that does not require an E2F consensus site. Finally, the increased expression of these genes upon loss of LIN-35 activity could be indirect or occurring in the few somatic cells present in our samples. Additional experiments will be necessary to distinguish among these possibilities.

Tissue-specific EFL-1/DPL-1 transcriptional program

Our results demonstrate that EFL-1/DPL-1 can display tissue specificity in both target gene selection and in the manner of gene regulation (activation or repression). In somatic tissues, EFL-1/DPL-1 represses genes such as *fkh-6*, *mat-3* or *cye-1* in various cell types to control the timing and nature of cell division (Chang et al., 2004; Garbe et al., 2004; Tilmann and Kimble, 2005; Grishok and Sharp, 2005). However, in the germline, EFL-1/DPL-1 does not regulate these target genes, nor is it crucial for proliferation of the germline stem cell population. Instead, EFL-1/DPL-1 activates the expression of a distinct set of genes whose protein products participate in oocyte differentiation and embryogenesis. Indeed, many EFL-1/DPL-1 target genes such as *rme-2* and *mex-1* have known roles only in oogenesis and embryogenesis and are likely to be expressed specifically in the maternal germline. The presence of canonical E2F-binding sites in these promoters strongly indicates that their regulation by EFL-1/DPL-1 is direct, although the mechanisms establishing the EFL-1/DPL-1 germline-specific program are unknown. Possibly, unknown germline-specific transcriptional co-activators or chromatin conformation might influence the activity of EFL-1/DPL-1.

Coordination of gene expression with developmental events

Several transcripts expressed in the medial germline are translationally repressed by the mRNA-binding protein GLD-1, including two that we identified as EFL-1/DPL-1 targets (*rme-2* and

cej-1) (Jones and Schedl, 1995; Lee and Schedl, 2001). We have generated an expanded list of candidate GLD-1 targets by identifying mRNAs that co-immunoprecipitate with GLD-1 and probing microarrays (M.-H. Lee, V.R. and T. Schedl, unpublished). Candidate GLD-1 target transcripts had significantly enriched overlap with Group I and II genes (26/74 and 5/43, respectively; see Materials and methods). Group I transcripts bound by GLD-1 include several with roles in oogenesis and embryogenesis, such as *rme-2*, *cej-1*, *oma-2* and *egg-2*, as well as *spn-4*, *cyb-2.1* and *cyb-3*. Thus, the germline has established a multi-tier mechanism for ensuring that the expression of these target genes is properly coordinated with oocyte development. First, detectable EFL-1 protein is restricted to the medial germline, which limits the significant accumulation of the target transcripts before the pachytene stage of meiosis I. Second, once expressed, many of these transcripts are probably translationally suppressed by GLD-1 until oogenesis. This dual regulation ensures that crucial components of oogenesis are available but held inactive until germ cells are at the appropriate stage of development.

Conclusion

Our results underline the importance of examining gene expression profiles in vivo in order to gain an accurate understanding of the natural function of transcription regulators. The primary role of EFL-1 in the *C. elegans* germline is to coordinately activate a cohort of genes required for the final stages of oocyte development and particular aspects of early embryonic development, suggesting that E2F activity in vivo adapts to tissue-specific environments. Future functional studies of target genes will help us to better understand how a transcription factor coordinates the transition from gamete to embryo.

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Supplementary material

Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/133/16/3147/DC1

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Supplemental Table 1
EFL-1/DPL-1/LIN-35 regulated genes

Group I Downregulated in *efl-1 /dpl-1* mutants

			Regulation (ctl/mut, log2)					Expression Pattern		Binding Site Analysis	
Wormbase ID	**Name**	**Chro.**	***dpl-1***	***efl-1***	***lin-35***	**Function**	**RNAi**	**In situ pattern**	**EST**	**TTCGCGCC**	**TTTTCCAG**
AH6.5	mex-6	II	**1.067**	**0.994**	0.171	RNA metabolism	Emb Bmd D	medial germline	yk38b2	yes	no
B0272.1	tbb-4	X	**1.033**	**0.607**	0.332	Structural	Emb Lvl Pna	embryos	yk313f12	no	no
B0416.4		X	**1.074**	0.786	0.113	Proteolysis	WT	no image	no ESTs	no	no
C01G8.1		I	**2.161**	**1.423**	0.498	Unknown	WT	medial germline	yk542h7	yes	no
C03C10.3	rnr-2	III	**1.987**	**1.177**	0.5	Replication/repair	Emb Ste Pvl	broad gonad	yk78c11	yes	yes
C05C10.5		II	**1.123**	**0.854**	0.336	Unknown	Emb Him Br	medial germline	yk91b4	yes	no
C07D8.6		X	**2.243**	**2.06**	0.395	Metabolism	WT	proximal germline	yk609e5	yes	no
C07G2.1	cej-1	III	**1.5**	**1.33**	**0.643**	Cell surface	WT	medial germline	yk32f3	yes	no
C17E7.9a		V	**1.339**	**0.956**	0.137	Unknown	WT	embryo	yk613c1	no	no
C17F4.5		II	**1.072**	**0.816**	0.556	Proteolysis	Sma	faint germline	yk345h11	yes	yes
C27C12.3		X	**1.303**	**1.37**	0.166	Unknown	WT	proximal germline	yk238b3	yes	no
C28C12.2		IV	**1.512**	**1.048**	0.251	Unknown	Emb Him Lv	medial germline	yk595h12	yes	no
C28D4.3	gln-6	IV	**1.189**	**1.871**	0.191	Metabolism	Emb	medial germline	yk82c9	yes	no
C36E8.5	tbb-2	III	**1.022**	**0.772**	0.002	Structural	Emb Lva Pn	broad gonad/soma	yk120c4	yes	no
C45H4.14		V	**1.088**	**0.884**	0.391	Unknown	WT	medial germline	yk108c3	yes	no
C50B6.2		V	**1.83**	**1.526**	0.584	Transcription	Emb Unc	medial germline	yk717g3	yes	no
EEED8.3		II	**1.296**	**0.9**	0.08	Metabolism	Emb	no image	no ESTs	no	no
F01F1.12		III	**1.053**	**0.827**	0.367	Metabolism	Emb Stp Egl	embryos/broad soma	yk100f9	yes	no
F16H11.3		X	**1.609**	**1.967**	**0.8**	Cell surface	WT	no image	no ESTs	yes	no
F18A1.7		II	**1.144**	**1.143**	0.187	Unknown	Emb	medial germline	yk595d11	yes	no
F26G5.1		V	**1.109**	**1**	**0.637**	Unknown	Emb	medial germline	yk166d9	yes	no
F27C8.6		IV	**1.317**	**0.985**	0.499	Metabolism	Emb	medial germline	yk585h8	yes	yes
F29F11.1	sqv-4	V	**1.067**	**0.616**	0.062	Metabolism	Emb	proximal germline	yk709d5	yes	no
F29G9.7		V	**1.348**	**1.356**	0.536	Proteolysis	WT	no image	no ESTs	yes	no
F31F6.1		X	**1.811**	**1.629**	0.374	Unknown	WT	no image	no ESTs	yes	no
F31F6.2		X	**1.938**	**1.052**	0.219	Unknown	WT	no image	no ESTs	yes	no
F31F6.3		X	**1.365**	**0.931**	0.373	Unknown	WT	no image	no ESTs	yes	no
F33H1.2	gpd-4	II	**1.306**	**0.786**	0.306	Metabolism	Emb Gon Lv	medial germline	yk83e12	no	no
F35C8.7		X	**1.197**	**0.826**	0.307	Cell surface	WT	proximal germline	yk90d4	yes	no
F35E12.3		V	**1.006**	ND	0.229	Proteolysis	WT	no image	no ESTs	no	no
F40G12.11		V	ND	**1.681**	ND	Unknown	WT	proximal germline	yk741b2	yes	no
F54D11.3		V	**1.172**	**1.72**	**0.68**	Unknown	WT	no image	no ESTs	yes	no
H02I12.5		IV	**1**	**0.624**	0.19	Unknown	Emb	no image	no ESTs	yes	no
K03H1.1	gln-2	III	**0.853**	**1.11**	0.236	Metabolism	Emb	medial germline	yk66d10	no	no
K04C1.5		X	**1.241**	**0.796**	0.301	Signaling	Emb	no image	no ESTs	yes	no
K10B2.3		II	**1.656**	**1.302**	**0.587**	Cell surface	WT	no image	yk440f3	yes	no
K10B3.7	gpd-3	X	**1.171**	**0.653**	0.133	Metabolism	Emb Gon Sr	broad soma	yk256g8	no	no
K10B3.8	gpd-2	X	**1.09**	0.52	0.054	Metabolism	Emb Gon Sr	broad soma	yk256g8	yes	no
K12B6.8		V	**1.086**	0.933	0.384	Proteolysis	WT	no image	no ESTs	yes	no

Supplemental Table 1
EFL-1/DPL-1/LIN-35 regulated genes

R01H2.3	egg-2	III	**1.118**	**0.922**	0.704	Cell surface	WT	no image	no ESTs	yes	no
R05H5.3		II	**1.126**	**1.07**	0.324	Metabolism	Emb	medial germline	yk115e11	yes	no
R06C7.4		I	**1.804**	**1.629**	**0.819**	Unknown	WT	medial germline	yk411b9	no	no
R11A5.4		I	**0.817**	**1.029**	0.533	Metabolism	WT	embryos/broad soma	yk293g1	yes	no
R12C12.5		II	0.91	**1.014**	**0.614**	Cell trafficking	WT	faint germline	yk108b10	yes	no
T05F1.2		I	1.783	**1.589**	**0.98**	Unknown	WT	medial germline	yk567e3	yes	no
T05G5.7		III	**1.716**	**1.072**	0.4	Unknown	Emb	medial germline	yk164c11	yes	no
T06E6.2a	cyb-3	V	**1.972**	**1.37**	0.521	Replication/repair	Emb Ste Pvl	medial germline	yk609h2	no	no
T08A9.6		X	1.296	**1.065**	**0.658**	Proteolysis	WT	no image	no ESTs	yes	yes
T09F3.3	gpd-1	II	**1.504**	**0.799**	0.401	Metabolism	Emb Gon Lv	medial germline	yk83e12	yes	no
T10C6.7		V	**2.148**	**2.077**	**0.759**	Proteolysis	WT	no image	no ESTs	yes	yes
T10C6.8		V	**2.014**	**2.044**	**1.129**	Proteolysis	WT	no image	no ESTs	no	no
T11F8.1		IV	**1.48**	**1.661**	0.577	Unknown	WT	no image	no ESTs	yes	no
T11F8.3	rme-2	IV	**2.898**	**2.271**	0.467	Cell surface	Emb Ste Gr	medial germline	yk46e4	yes	no
T12G3.6		IV	**1.784**	**1.399**	0.118	Unknown	WT	broad soma	yk185d7	no	no
T19H12.2		V	**1.084**	**0.677**	0.142	Unknown	Emb	distal germline	yk670b10	yes	yes
T21C9.13		V	**1.87**	**1.294**	0.377	Unknown	WT	medial germline	yk508b5	yes	no
T21E3.1		I	**1.121**	**0.722**	0.293	Signaling	Emb Ste Lv	proximal germline	yk122d7	yes	yes
T22F3.3		V	**1.616**	**1.746**	**0.663**	Metabolism	Emb	distal germline	yk41h6	yes	no
T25E12.5		V	**1.947**	**1.248**	**0.909**	Metabolism	WT	medial germline	yk558b4	yes	no
VF13D12L.1		II	**1.191**	**0.93**	0.5	Signaling	Emb Lvl Unc	faint germline	yk114b5	yes	no
W01A8.1	mdt-28	I	**1.3**	**0.958**	0.392	Transcription	WT	proximal germline	yk540b10	yes	no
W02A2.7	mex-5	IV	**1.354**	**1.404**	**0.613**	RNA metabolism	Emb Dpy Ex	germline	yk585e12	no	no
W02F12.3		V	**1.879**	**1.475**	0.58	Unknown	WT	medial germline	yk610h12	yes	no
W03C9.7	mex-1	II	**1.341**	**1.148**	**0.631**	RNA metabolism	Emb Stp Ex	medial germline	yk568d5	yes	no
W05F2.3		I	**1.678**	**1.076**	**0.717**	Unknown	WT	medial germline	yk462h7	yes	no
W06D11.3		X	**1.44**	0.858	0.111	Unknown	Emb	no image	no ESTs	yes	no
Y102A5C.18	efl-1	V	ND	**1.248**	-0.084	Transcription	WT	broad gonad	yk617e4	no	no
Y43E12A.1	cyb-2.1	IV	**1.146**	**0.773**	0.423	Replication/repair	Emb Stp Lv	medial germline	yk341e8	yes	no
Y45F10A.2	puf-3	IV	**1.435**	**1.05**	0.053	RNA metabolism	Emb	medial germline	yk602c3	yes	no
Y55D5A.2		III	**1.399**	**1.134**	0.44	Unknown	WT	no image	no ESTs	yes	no
ZC308.4		I	**1.634**	**1.132**	-0.066	Unknown	WT	no image	no ESTs	yes	no
ZC404.8	spn-4	V	**1.555**	**1.027**	0.073	RNA metabolism	Emb	medial germline	yk568b11	yes	no
ZC513.6	oma-2	V	**1.346**	**1.24**	0.351	RNA metabolism	WT	medial germline	yk585f5	yes	no
ZK637.13		III	**1.327**	**1.371**	0.484	Unknown	WT	distal germline	yk66b9	yes	no
ZK858.3		I	**1.347**	**0.973**	0.456	Cell surface	WT	medial germline	yk490d4	yes	no

Group II Commonly downregulated in mutants

			Regulation (ctl/mut, log2)					Expression Pattern		Binding Site Analysis	
Wormbase ID	**Name**	**Chro.**	***dpl-1***	***efl-1***	***lin-35***	**Function**	**RNAi**	**In situ pattern**	**EST**	**TTCGCGCC**	**TTTTCCAG**
B0035.8	his-48	IV	**0.863**	**1.108**	**1.104**	Replication/repair	Emb Ste Lv	broad gonad	yk515f3	yes	no
B0403.4	tag-320	X	**1.465**	**1.244**	**1.515**	Metabolism	Lva Unc	embryos/intestine	yk104c5	no	no

Supplemental Table 1
EFL-1/DPL-1/LIN-35 regulated genes

C02E11.1		V	**1.04**	**1.096**	**0.823**	Proteolysis	WT	proximal germline	yk523b6	yes	yes
C03E10.5		V	**1.401**	**0.904**	1.073	Cell surface	WT	spermatheca	yk539d3	no	no
C07A12.4	pdi-2	X	**0.8**	**0.884**	**1.229**	Chaperone	Emb Lvl Clr	broad soma/germline?	yk123d1	no	no
C09B8.4		X	**1.257**	1.426	**1.273**	Unknown	WT	embryos	yk736c2	no	no
C15H9.6	hsp-3	X	**0.789**	**0.865**	**1.095**	Chaperone	Emb Ste Dp	broad soma/germline?	yk37d11	no	no
C17E7.4		V	**1.121**	**0.728**	**0.706**	Unknown	Emb	medial germline	yk502d2	yes	no
C27C12.4		X	**0.782**	**0.632**	**1.031**	Unknown	WT	no image	no ESTs	no	no
C39D10.7		X	**1.119**	**1.523**	**1.403**	Cell surface	WT	proximal germline	yk43c10	no	no
F08F3.6		V	**1.017**	0.456	0.551	Proteolysis	WT	medial germline	yk267c7	yes	no
F11G11.2	gst-7	II	**0.61**	0.457	**1.001**	Metabolism	WT	proximal germline	yk106e10	no	yes
F14D7.2		V	**1.358**	0.929	1.062	RNA metabolism	Exp	medial germline	yk502c10	yes	no
F17E9.12	his-31	IV	**0.94**	**1.207**	0.938	Replication/repair	Emb Ste Pvl	no image	no ESTs	no	no
F20H11.5		III	**0.965**	**1.794**	**1.186**	Metabolism	WT	no image	no ESTs	no	no
F25H5.4	eft-2	I	**1.167**	**1.208**	**1.37**	Translation	Emb Ste Pvl	broad soma/germline?	yk451d11	yes	no
F27B3.2	acr-21	III	**0.977**	**1.283**	0.822	Cell surface	WT	no image	no ESTs	no	no
F35H10.1	his-30	IV	**1.001**	**1.208**	**1.282**	Replication/repair	Emb Ste Egl	no image	no ESTs	no	no
F41C3.5		II	**1.215**	**0.974**	**1.033**	Proteolysis	WT	somatic gonad	yk557d5	no	no
F42G8.3a	pmk-2	IV	0.536	**0.894**	**1.012**	Signaling	WT	no staining	yk524d4	no	no
F45F2.2	his-39	V	**1.127**	**1.286**	**1.28**	Replication/repair	Emb Ste Pvl	no image	no ESTs	no	yes
F45F2.4	his-7	V	0.566	**0.981**	**1.032**	Replication/repair	Emb Ste Lva	no image	no ESTs	no	no
F48E3.3		X	0.672	**0.763**	**1.124**	Metabolism	WT	broad soma/germline?	yk112d12	no	no
F48E3.4		X	0.4	**1.064**	**0.821**	Proteolysis	WT	embryo image only	yk728d11	no	no
F54C9.8	puf-5	II	**1.205**	0.568	0.593	RNA metabolism	WT	medial germline	yk134g3	yes	no
F54F7.2		X	**1.734**	**0.939**	**1.82**	Unknown	WT	no image	no ESTs	yes	no
F55G1.11	his-60	IV	0.568	**1.133**	**1.111**	Replication/repair	Emb Ste Gr	no image	yk599g11	no	no
H02I12.6	his-66	IV	**1.075**	**1.19**	**1.064**	Replication/repair	Emb Ste Pvl	no image	no ESTs	yes	no
H13N06.6	tbh-1	X	0.887	**1.641**	1.621		WT	somatic gonad	yk543f3	no	no
K08H10.1	lea-1	V	**1.259**	**0.923**	0.998	Unknown	WT	broad gonad/soma	yk43h9	no	no
M116.2		IV	**1.344**	**0.881**	**0.881**	Metabolism	WT	nonspecific staining	yk1016d0	no	no
R74.3	xbp-1	III	0.452	**0.611**	**1.066**	Chaperone	WT	intestine	yk110e5	no	no
T03E6.7	cpl-1	V	**2.726**	**2.135**	**2.303**	Proteolysis	Emb Gro Un	intestine	yk136h7	no	yes
T03G11.6		X	**1.242**	**0.915**	**0.858**	Unknown	WT	no image	yk1520c0	yes	no
T04D3.5		I	0.607	0.292	**1.032**	Unknown	Lva	proximal germline	yk602f5	no	no
T10C6.10		V	**1.344**	**0.818**	**0.755**	Proteolysis	WT	no image	no ESTs	yes	no
T10C6.9		V	**0.833**	**1.184**	0.799	Proteolysis	WT	no image	no ESTs	yes	no
Y4C6A.3		IV	**1.361**	**0.974**	**0.969**	Proteolysis	WT	no image	no ESTs	no	no
Y62H9A.5		X	0.408	**1.147**	0.605	Unknown	WT	spermatheca/embryos	yk506a10	no	no
Y71H10A.1		X	**0.724**	**1.02**	**1.158**	Metabolism	Emb	proximal germline	yk7f1	no	yes
ZC373.2		X	0.655	**1.483**	1.216	Unknown	WT	no image	yk730a12	no	no
ZK546.15	try-1	II	**1.075**	**0.797**	**0.895**	Proteolysis	WT	proximal germline	yk241h9	yes	no
ZK813.3		X	**1.018**	**0.809**	**0.887**	Cell surface	WT	spermatheca	yk124d10	no	no

Supplemental Table 1
EFL-1/DPL-1/LIN-35 regulated genes

Group III Variably upregulated in mutants

			Regulation (ctl/mut, log2)					Expression Pattern		Binding Site Analysis	
Wormbase ID	**Name**	**Chro.**	***dpl-1***	***efl-1***	***lin-35***	**Function**	**RNAi**	**In situ pattern**	**EST**	**TTCGCGCC**	**TTTTCCAG**
B0280.4	odd-1	III	**-0.759**	**-1.183**	-1.141	Transcription	WT	no image	no ESTs	yes	no
C01B4.9		V	ND	-0.243	**-1.319**	Cell surface	WT	no image	no ESTs	yes	no
C04F6.3	cht-1	X	**-1.727**	-0.108	-0.784	Proteolysis	Emb	broad gonad	yk109d2	no	no
C08D8.1		V	-0.208	ND	**-1.061**	Unknown	WT	no image	no ESTs	no	no
C09B8.6	hsp-25	X	-0.473	**-1.323**	-1.732	Chaperone	WT	somatic gonad	yk163h9	no	no
C09G5.7		II	**-1.005**	**-1.083**	**-2.601**	Unknown	WT	no image	no ESTs	no	no
C27F2.2	nca-2	III	**-1.105**	**-0.754**	-1.712	Cell surface	WT	broad soma/embryo	yk24d9	no	yes
C31H1.6a		IV	**-1.448**	-1.636	0.102	Unknown	WT	no image	yk1304f03	no	yes
C50B6.8		V	**-1.305**	-0.641	**-0.67**	Transcription	WT	no image	no ESTs	no	no
C54D10.10		V	**-2.479**	-2.297	-4.653	Proteolysis	WT	no image	no ESTs	no	no
F08F3.9		V	**-0.615**	**-1.337**	-0.703	RNA metabolism	WT	broad gonad	yk215a11	no	no
F17C8.6		III	**-1.096**	-0.835	-1.677	Cell surface	WT	no image	no ESTs	yes	yes
F28H6.1a	akt-2	X	**-1.071**	**-0.858**	**-1.1**	Signaling	WT	pharynx/intestine	yk232g7	no	no
F35E12.10		V	ND	**-1.856**	-0.451	Unknown	WT	no staining	yk744h9	no	yes
F36D3.4		V	ND	-0.332	**-1.464**	Structural	WT	spermatheca	yk125h12	no	no
F36H12.7	msp-19	IV	-0.543	**-1.029**	-1.532	Structural	WT	no image	yk57h4	no	no
F38E1.10		V	-0.566	**-1.617**	-0.735	Unknown	WT	no image	no ESTs	no	no
F40E10.1	hch-1	X	**-1.402**	-0.066	-0.116	Proteolysis	WT	embryo	yk376d4	no	no
F42A9.1a	tag-137	IV	-0.752	-0.159	**-1.158**	Signaling	WT	broad gonad	yk270f12	yes	no
F42E11.4	tni-1	X	-0.476	**-1.085**	-0.931	Structural	Emb Egl Dpy	embryo/posterior soma	yk103h4	no	no
F46E10.2		V	**-1.048**	-0.233	-0.074	Unknown	WT	embyro	yk572b7	no	no
F55C5.9	srh-16	V	ND	**-1.362**	**-3.191**	Cell surface	WT	no image	no ESTs	no	no
F56D1.6	cex-1	II	-0.588	**-1.717**	**-1.489**	Signaling	WT	no image	no ESTs	no	no
F56G4.2	pes-2	I	**-1.274**	-0.514	-0.997	Proteolysis	WT	no image	no ESTs	no	no
F56G4.3		I	**-1.369**	**-0.717**	-0.907	Proteolysis	WT	no image	no ESTs	no	no
F57A10.2		V	**-1.536**	-0.523	-1.597	Signaling	WT	proximal germline	yk58e1	no	no
JC8.8		IV	**-0.604**	-0.541	**-1.068**	Unknown	WT	spermatheca	yk702c5	no	no
K01D12.11	cdr-4	V	**-1.316**	**-2.877**	**-0.736**	Chaperone	WT	intestine	yk736g5	no	no
K08C7.3	epi-1	IV	**-0.931**	-0.23	**-1.184**	Cell surface	Ste Muv Dpy	broad soma/vulva/emb	yk531d10	no	yes
R13H9.2	msp-57	IV	-0.428	-0.752	**-2.039**	Structural	WT	no image	yk94d8	yes	no
R151.5	toh-2	III	**-1.32**	**-1.531**	**-1.909**	Proteolysis	WT	embryo/intestine	yk101h3	no	no
T26F2.2		V	**-1.523**	-0.665	-0.454	Unknown	WT	no image	no ESTs	no	no
T27E4.8	hsp-16.1	V	-0.41	**-2.059**	-0.409	Chaperone	WT	no image	no ESTs	no	no
T27E4.9	hsp-16.49	V	-0.428	**-1.621**	-0.379	Chaperone	WT	no image	no ESTs	no	no
T27E9.2		III	-0.3	-0.428	**-1.014**	Metabolism	Gro	no image	yk112d7	no	no
Y19D10A.11		V	**-1.355**	-0.311	**-1.225**	Cell surface	WT	no image	no ESTs	yes	yes
Y46H3A.3	hsp-16.2	V	0.006	**-1.176**	0.393	Chaperone	WT	no image	yk1108c09	no	no
Y55B1AR.1	lec-6	III	**-0.826**	**-1.017**	-0.738	Metabolism	WT	intestine	yk566a11	no	no
Y55F3BR.4		IV	**-1.788**	-1.15	**-1.553**	Cell surface	WT	no image	yk280e4	no	no

Supplemental Table 1
EFL-1/DPL-1/LIN-35 regulated genes

Y59E9AR.7		IV	-0.659	-1.53	**-2.902**	Structural	WT	proximal germline	yk412e1	no	no
Y67D8A.3		IV	**-1.135**	-0.163	-0.262	Transcription	WT	no image	no ESTs	yes	no
Y82E9BR.16		III	**-1.029**	**-0.894**	**-0.641**	Cell trafficking	Emb Lva	faint germline/embryo	yk599b8	yes	yes

Group IV Upregulated in *lin-35* mutants

			Regulation (ctl/mut, log2)					Expression Pattern		Binding Site Analysis	
Wormbase ID	**Name**	**Chro.**	***dpl-1***	***efl-1***	***lin-35***	**Function**	**RNAi**	**In situ pattern**	**EST**	**TTCGCGCC**	**TTTTCCAG**
B0336.3		III	0.119	0.126	**-1.036**	RNA metabolism	Bmd Gro	broad gonad	yk87e1	no	yes
B0511.9a		I	0.378	0.41	**-1.239**	Unknown	Emb Ocs	faint germline	yk340e12	no	yes
C06G3.7	trxr-1	IV	-0.149	-0.087	**-1.481**	Metabolism	WT	broad staining	yk457d4	yes	no
C08F11.7		IV	-0.128	-0.016	**-1.41**	Unknown	WT	no image	no ESTs	no	yes
C09H10.5		II	-0.034	0.072	**-1.315**	Unknown	WT	no image	no ESTs	no	yes
C10H11.8		I	0.156	0.146	**-1.531**	Signaling	Lva Gro	no image	no ESTs	no	no
C15H11.3	nxf-1	V	ND	0.341	**-1.073**	RNA metabolism	Emb	no image	no ESTs	no	yes
C15H9.4		X	0.338	0.344	**-1.105**	Cell surface	Gro,Sma	no image	no ESTs	yes	yes
C17D12.7		I	-0.041	0.059	**-1.636**	Unknown	WT	faint germline	yk627a2	no	no
C17H12.2		IV	0.081	0.199	**-1.475**	Unknown	WT	faint broad gonad	yk386a5	no	yes
C26E6.3		III	-0.114	-0.149	**-1.09**	Unknown	Emb	broad gonad	yk863e11	no	no
C34B7.4		I	0.238	0.343	**-1.518**	Transcription	WT	no image	no ESTs	no	no
C34F6.2	col-178	X	0.314	**0.689**	**-1.108**	Structural	WT	broad soma	yk92a12	yes	yes
C44B7.10		II	0.215	0.205	**-1.197**	Metabolism	WT	broad soma/germline?	yk20f6	no	yes
C48B6.3		I	0.049	0.237	**-1.87**	Unknown	WT	faint germline	yk487e9	no	yes
C49A1.1		I	-0.118	-0.032	**-1.21**	Cell surface	WT	no image	no ESTs	yes	yes
C54G4.8	cyc-1	I	-0.204	-0.066	**-1.352**	Metabolism	Emb Ste Pvl	distal germline/soma	yk609b2	no	yes
C55C2.2	ssp-19	I	0.145	-0.086	**-1.347**	Unknown	WT	spermatheca	yk263a8	yes	no
C56G2.15		III	-0.128	0.094	**-1.376**	Transcription	WT	no staining	yk425c9	no	no
C56G2.1a		III	0.089	0.089	**-1.409**	RNA metabolism	Emb	broad gonad/soma	yk14c7	no	yes
D2092.5		I	0.179	0.132	**-1.16**	Unknown	WT	embryo image only	yk1234g0	yes	yes
DC2.5		V	-0.026	0.047	**-1.451**	Metabolism	WT	no image	no ESTs	yes	yes
F02E9.2a	lin-28	I	0.061	0.107	**-1.749**	RNA metabolism	Egl	faint staining	yk401f5	yes	yes
F08G5.4	col-130	IV	-0.022	0.181	**-1.175**	Structural	WT	intestine/vulva	yk204e8	no	no
F11A3.2		V	**0.792**	**0.748**	**-1.157**	Translation	Emb Ste Stp	no image	yk1359c0	yes	yes
F14F8.4	srz-103	V	-0.06	0.134	**-1.182**	Cell surface	WT	no image	no ESTs	no	yes
F22B5.2	eif-3.G	II	0.536	0.402	**-1.161**	Translation	Emb Stp Dp	germline	yk499d6	no	yes
F26A1.13		III	0.056	0.188	**-1.369**	Replication/repair	WT	no image	no ESTs	no	no
F26A1.14		III	0.057	0.038	**-1.644**	Replication/repair	WT	no image	no ESTs	no	yes
F26F2.7		V	0.171	-0.017	**-1.111**	Unknown	Emb	no image	yk395f6	no	yes
F31D4.2		V	-0.01	-0.047	**-1.49**	Unknown	WT	broad soma	yk676h2	yes	yes
F33H1.4		II	-0.318	-0.256	**-1.884**	Transcription	Emb Lva Un	broad gonad	yk367g3	no	no
F36F12.2		V	0.105	0.527	**-1.129**	Unknown	WT	no image	no ESTs	no	no
F42G9.1		III	0.14	-0.066	**-1.626**	Signaling	WT	broad gonad	yk110g9	no	no
F43C1.2b	mpk-1	III	0.14	-0.034	**-1.244**	Signaling	Emb Ste Pvl	broad gonad	yk531h7	yes	yes

Supplemental Table 1
EFL-1/DPL-1/LIN-35 regulated genes

F45F2.11		V	0.193	-0.021	**-1.848**	Unknown	WT	no image	no ESTs	no	yes
F48E8.2		III	0.17	0.184	**-1.061**	Unknown	Emb	no image	yk1570f02	no	no
F53A2.5	dro-1	III	0.317	0.334	**-2.286**	Transcription	WT	no image	no ESTs	no	no
F53H1.4		IV	0.259	0.228	**-1.466**	Transcription	WT	broad gonad	yk547a6	yes	no
F53H2.3		V	-0.011	-0.095	**-1.594**	Unknown	WT	broad gonad/intestine	yk569e3	yes	yes
F54C1.3	mes-3	I	0.138	0.108	**-1.421**	Transcription	Stp	distal germline	yk270a10	no	yes
F54H12.1a	aco-2	III	-0.515	-0.033	**-1.85**	Metabolism	Emb Ste Pvl	broad soma	yk60d11	yes	no
F55H2.5		III	0.063	0.151	**-1.48**	Metabolism	WT	no image	no ESTs	no	yes
F56C9.1	gsp-2	III	0.198	0.348	**-1.635**	Signaling	Emb Egl Me	broad gonad	yk150g8	yes	yes
F56D1.2		II	0.443	0.557	**-1.081**	Unknown	WT	body wall muscle	yk130c8	no	no
F57B9.2	ntl-1	III	0.397	0.845	**-1.316**	Transcription	Emb Ste Exp	broad gonad	yk19a2	yes	yes
F57F5.5	pkc-1	V	-0.183	-0.224	**-1.241**	Signaling	WT	no staining	yk587d2	yes	no
H05L14.2		I	0.108	0.147	**-1.314**	Unknown	WT	broad gonad	yk542d3	no	yes
K03D10.3		I	-0.061	-0.004	**-1.144**	Transcription	WT	faint germline	yk499f7	no	yes
K03H1.11		III	0.006	-0.067	**-2.252**	Unknown	Emb	no image	no ESTs	no	no
K08C9.2		I	0.015	-0.051	**-1.153**	Unknown	Ste	spermatheca	yk380d10	no	yes
K11D2.3	unc-101	I	0.205	0.091	**-1.442**	Cell trafficking	Unc	faint germline	yk456b2	yes	no
M03C11.4		III	0.49	0.371	**-1.762**	Transcription	Emb	proximal germline	yk293d10	no	yes
M110.4	ifg-1	II	-0.253	-0.264	**-1.544**	Translation	Emb Ste Pcl	broad gonad	yk150f8	no	yes
R06C1.2		I	-0.384	-0.062	**-1.025**	Metabolism	Emb Lva	distal germline	yk573d7	no	yes
R06F6.2		II	0.052	0.02	**-1.213**	Cell trafficking	Emb Mlt Sm	broad gonad	yk15d5	no	no
R08H2.3	srh-170	V	0.086	0.078	**-1.466**	Cell surface	WT	no image	no ESTs	yes	yes
R144.7	larp-1	III	-0.261	0.161	**-1.607**	RNA metabolism	Emb Gro Un	no image	yk1239f07	no	yes
T07A5.2		III	0.107	0.483	**-1.491**	Unknown	WT	broad gonad	yk125e8	no	no
T07C4.8	ced-9	III	0.11	0.418	**-1.306**	Cell death	Emb	broad gonad	yk113b7	no	no
T23D8.1	mom-5	I	-0.026	-0.116	**-2.442**	Cell surface	Emb Egl Bm	broad gonad	yk471e5	no	yes
W05H9.3		X	-0.573	-0.143	**-1.544**	Unknown	WT	embryo image only	yk1316f10	yes	yes
W06E11.5		III	0.212	-0.003	**-1.63**	Unknown	WT	no image	no ESTs	no	no
W08G11.3		V	0.348	0.224	**-1.634**	Unknown	WT	no image	yk1370b1(	no	yes
Y102A5A.1		V	-0.008	-0.074	**-1.961**	Transcription	WT	no image	yk430g8	yes	yes
Y104H12D.3		IV	-0.135	-0.068	**-1.05**	Metabolism	WT	embryo image only	yk1190e1(	no	no
Y116A8C.36	itsn-1	IV	0.239	0.254	**-1.522**	Cell trafficking	WT	no image	yk1635c02	no	yes
Y19D10A.10		V	-0.252	-0.25	**-1.312**	Cell surface	WT	no image	no ESTs	no	yes
Y34D9A.11	spp-23	I	-0.049	0.014	**-1.057**	Signaling	WT	no image	yk1534d1:	yes	yes
Y38E10A.22		II	-0.067	-0.08	**-1.296**	Unknown	WT	no image	no ESTs	no	yes
Y39B6A.20	asp-1	V	**1.113**	0.273	**-1.321**	Proteolysis	Ced	intestine	yk101b5	no	yes
Y45G12C.7	srd-73	V	-0.017	0.358	**-1.37**	Cell surface	WT	no image	no ESTs	yes	yes
Y47G6A.10	spg-7	I	-0.067	0.013	**-1.169**	Proteolysis	Emb Ste Lva	distal germline	yk282e3	no	yes
Y49G5B.1		V	-0.094	-0.144	**-1.095**	Unknown	Emb	broad gonad	yk252b8	no	yes
Y53C10A.3		I	0.132	-0.017	**-1.439**	Transcription	Emb Sma Lv	no image	no ESTs	no	yes
Y53G8AR.6		III	0.29	0.148	**-1.5**	Transcription	Emb	no staining	yk571d3	yes	no
Y54E10A.15	cdt-1	I	0.428	0.43	**-1.205**	Replication/repair	Emb Ste Pvl	proximal germline	yk593e9	yes	yes

Supplemental Table 1
EFL-1/DPL-1/LIN-35 regulated genes

Y54E10A.2	I	-0.067	-0.125	**-1.506**	Cell surface	Emb Pvl Exp	germline	yk31b12	no	no
Y55F3AR.3	IV	0.111	-0.02	**-1.92**	Chaperone	Emb Ste Pvl	no image	yk1601c0	yes	no
Y56A3A.31	III	0.078	0.092	**-1.144**	Unknown	WT	embryo image only	yk1509e0	no	yes
Y56A3A.6	III	-0.291	-0.149	**-1.027**	Unknown	Ste Pvl Gro	faint germline	yk531e8	yes	yes
Y61A9LA.8	V	0.267	0.39	**-1.322**	RNA metabolism	WT	distal germline	yk654a12	yes	yes
Y65A5A.3	IV	-0.139	-0.07	**-1.945**	Unknown	WT	no image	no ESTs	yes	yes
Y71H2AM.9	III	0.058	0.036	**-1.853**	Cell surface	WT	no image	yk1524d0	no	yes
Y87G2A.13	I	0.001	0.171	**-1.065**	Cell surface	WT	no image	yk1666f11	no	no
ZK484.4	I	0.134	0.127	**-1.171**	Unknown	Emb Stp	broad gonad	yk455c4	no	yes
ZK795.2	IV	-0.314	-0.119	**-1.036**	Unknown	WT	proximal germline	yk206f7	no	no
ZK896.9	IV	0.229	0.382	**-1.075**	Cell surface	WT	no image	no ESTs	yes	yes

Regulated genes not in group

			Regulation (ctl/mut, log2)					Expression Pattern		Binding Site Analysis	
Wormbase ID	**Name**	**Chro.**	***dpl-1***	***efl-1***	***lin-35***	**Function**	**RNAi**	**In situ pattern**	**EST**	**TTCGCGCC**	**TTTTCCAG**
B0513.4		IV	0.268	**1.079**	0.928	Unknown	WT	no image	yk309c11	ND	ND
C02G6.1		V	**1.607**	0.169	0.352	Proteolysis	WT	no image	yk1381a0	ND	ND
C02G6.2		V	**1.416**	0.326	0.314	Proteolysis	WT	no image	no ESTs	ND	ND
C03B1.12	lmp-1	X	**1.166**	0.422	0.126	Metabolism	Clr	medial germline	yk117e4	ND	ND
C10H11.10	kca-1	I	**1.01**	0.395	-0.24	Cell trafficking	Lon	broad gonad	yk560c6	ND	ND
C28F5.4		II	**1.882**	0.551	0.233	Proteolysis	WT	no image	no ESTs	ND	ND
C34C6.4		II	0.021	**1.207**	0.339	Metabolism	Ste	broad gonad	yk380g5	ND	ND
C36E6.5	mlc-2	X	ND	ND	**-1.148**	Structural	Egl Lva Slu	body wall muscle	yk232e10	ND	ND
C43E11.11		I	-0.091	0.136	**1.97**	Cell trafficking	Emb Gro	broad gonad	yk349a3	ND	ND
D2045.1	atx-2	III	**-1.042**	0.439	0.641	RNA metabolism	Emb Ste	broad gonad	yk201d3	ND	ND
F44E7.4		V	**2.354**	0.474	-0.145	Proteolysis	WT	broad gonad	yk133a3	ND	ND
F45B8.1	rgs-11	X	**1.175**	0.073	0.233	Signaling	WT	no staining	yk81b9	ND	ND
F52E1.1	pos-1	V	**2.03**	**1.807**	-0.088	RNA metabolism	Emb	medial germline	yk602f11	ND	ND
F53G12.1	rab-11.1	I	**-1.255**	0.08	0.206	Cell trafficking	Emb Ste Lvl	broad gonad	yk604d10	ND	ND
M05B5.4		I	-0.126	0.306	**1.103**	Metabolism	WT	embryo	yk712f2	ND	ND
R09E12.3		V	0.199	**1.423**	**1.381**	Chaperone	WT	broad gonad	yk117d2	ND	ND
T01C3.3		V	**1.804**	-0.351	0.364	Proteolysis	WT	medial germline	yk104f4	ND	ND
T03D3.5		V	-0.363	0.576	**1.4**	Unknown	WT	embryo	yk526g7	ND	ND
T04H1.2		V	**-1.771**	0.424	1.186	Translation	WT	broad gonad	yk484e10	ND	ND
T05G11.1		V	**1.08**	0.115	0.557	Transcription	WT	no image	no ESTs	ND	ND
T23G7.1	dpl-1	II	1.608	**0.193**	0.305	Transcription	Emb Stp Pvl	broad gonad	yk473g5	ND	ND
Y18D10A.17	car-1	I	**-1.152**	0.308	0.36	RNA metabolism	Emb Stp Slu	broad gonad	yk575h5	ND	ND
Y75B12B.1		V	ND	**1.066**	-0.214	Unknown	WT	no image	no ESTs	ND	ND
Y79H2A.11	zyg-8	III	**-1.026**	0.27	0.412	Structural	Bmd Dpy Ur	broad gonad	yk54f7	ND	ND

ND - not determined
bold - statistically significant p<0.05, adjusted Z test