Published in final edited form as:
Cell Cycle. 2008 October ; 7(19): 3083–3090.

The *let-7* microRNA interfaces extensively with the translation machinery to regulate cell differentiation

Xavier C. Ding[1], **Frank J. Slack**[2,*], and **Helge Großhans**[1,*]

[1]Friedrich Miescher Institute for Biomedical Research (FMI); Basel, Switzerland [2]Department of Molecular; Cellular and Developmental Biology; Yale University; New Haven, Connecticut USA

Abstract

MicroRNAs (miRNAs) are noncoding RNAs that regulate numerous target genes through a posttranscriptional mechanism and thus control major developmental pathways. The phylogenetically conserved *let-7* miRNA regulates cell proliferation and differentiation, thus functioning as a key regulator of developmental timing in *C. elegans* and a tumor suppressor gene in humans. Using a reverse genetic screen, we have identified genetic interaction partners of *C. elegans let-7*, including known and novel potential target genes. Initial identification of several translation initiation factors as suppressors of a *let-7* mutation led us to systematically examine genetic interaction between *let-7* and the translational machinery, which we found to be widespread. In the presence of wild-type *let-7*, depletion of the translation initiation factor eIF3 resulted in precocious cell differentiation, suggesting that developmental timing is translationally regulated, possibly by *let-7*. As overexpression of eIF3 in humans promotes translation of mRNAs that are also targets of *let-7*-mediated repression, we suggest that eIF3 may directly or indirectly oppose *let-7* activity. This might provide an explanation for the opposite functions of *let-7* and eIF3 in regulating tumorigenesis.

Keywords

miRNA; let-7; translation factor; heterochronic; *C. elegans*; RNAi; eIF3; eIF6

Introduction

MicroRNAs (miRNAs) are small, untranslated RNAs involved in numerous developmental pathways (reviewed in ref. 1). They function through an antisense mechanism where binding of an miRNA to complementary sequences in its target mRNAs (‘cognate mRNAs’) causes cognate mRNA repression, but the mechanisms of target mRNA repression are less clear. Many different, and some-times contradictory, miRNA modes of action have been proposed (reviewed in refs. 2 and 3). These include inhibition of target mRNA translation either at the initiation or elongation step, target mRNA degradation in a non-endonucleolytic fashion, which may or may not result from deadenylation, and co-translational protein degradation. MicroRNAs may thus act through multiple mechanisms. These mechanisms may either

*Correspondence to: Frank J. Slack; Department of Molecular; Cellular and Developmental Biology; Yale University; New Haven, Connecticut USA; frank.slack@yale.edu/ Helge Großhans; Friedrich Miescher Institute for Biomedical Research (FMI); Basel, Switzerland; helge.grosshans@fmi.ch.

function redundantly or as alternate pathways that affect only individual subsets of miRNAs and/or cognate mRNAs.2,3

The *C. elegans let-7* miRNA was originally identified as a component of the heterochronic pathway,4 which controls the temporal fate of cells during postembryonic development (reviewed in ref. 5). Postembryonic development proceeds through the four larval stages, L1 through L4, followed by the sexually mature, adult stage. During normal development, cells adopt fates that are characteristic of the developmental stage of the animal, e.g., certain cells divide while others may exit the cell cycle and differentiate. Mutations in heterochronic genes may cause cells to prematurely adopt fates that are normally observed at a later developmental stage, i.e., cause precocious phenotypes. Alternatively, the mutant cells may display retarded phenotypes, i.e., characteristics typical of cells in earlier developmental stages. Partial loss of *let-7* activity causes retarded phenotypes, i.e., repetition of fourth larval stage (L4) cell fates, while more complete loss of activity causes animals to die by bursting through the vulva at the larval-to-adult transition.[4] These phenotypes are due to overexpression of *let-7* target genes and can be partially suppressed by knock-down of individual *let-7* target genes.[6–10]

let-7 is conserved in higher eukaryotes, with a striking 100% sequence identity in the case of the mature *let-7* of *C. elegans* and humans.[11,12] This, and the observation that *let-7* expression is temporally regulated in invertebrates as well as vertebrates,[11] suggests that *let-7* function may also be conserved. This view is supported by our recent finding that *C. elegans let-7* regulates *let-60/ras* expression, while human *let-7* regulates the *let-60* orthologue *RAS*.[7,13] Human *let-7* also regulates the chromatin-binding factor HMGA2, and failure of *let-7*-mediated HMGA2 repression promotes oncogenic transformation.[14–18] Consistent with overproliferation of cells with reduced *let-7* expression, *let-7* also represses the expression of the cell cycle regulator CDC25A (refs. 19 and 20). Reduced *let-7* expression in lung cancer[13,21] may contribute to tumorigenic transformation through upregulation of these oncogenes,[22,23] and reduced *let-7* expression levels are prognostic for poor patient survival.[21,24] *let-7* has also been shown to function as a tumor suppressor in breast cancer, where it controls proliferation and differentiation of tumor initiating cells.[25] The converging results from these different experimental systems have supported a model of *let-7* functioning as an important regulator of stem cell fates in both normal and tumor cells.[26] To achieve this function, *let-7* expression is highly regulated not only at the transcriptional level, but, as recent data suggest, also posttranscriptionally (reviewed in ref. 26).

To identify interaction partners of the *let-7* miRNA, which might include novel *let-7* targets, regulators of *let-7* expression, mediators of *let-7* activity, or heterochronic genes, we devised a high-throughput, functional genomics screen based on RNA interference (RNAi). Through this screen, we identified 41 known and novel interaction partners of *let-7*. As several genes directly or indirectly involved in translation were found among the novel *let-7* suppressors, we systematically examined genetic interactions between *let-7* and the core translational machinery and found them to be widespread. Consistent with translational control of the heterochronic pathway, we found that depletion of several of these genes, in particular subunits of the tumor promoting translation initation factor eIF3, caused abnormal timing of cell differentiation in the presence of wild-type *let-7*.

Results

A reverse genetics screen reveals translation factors as suppressors of *let-7*

The temperature sensitive *let-7*(*n2853*) allele contains a point mutation in the mature *let-7* sequence that impairs target binding.[4,27] In addition, reduced accumulation of the mutant *let-7* RNA28 further impairs target repression and as a result, mutant animals die by bursting through the vulva at the larval-to-adult transition when grown at or above 20°C (reviewed in

ref. 4). RNAi-mediated knockdown of individual *let-7* targets can partially suppress this lethality.[6–10] To identify novel interaction partners of the *let-7* miRNA, we carried out a feeding RNAi screen to uncover additional suppressors of the *let-7*(*n2853*) bursting phenotype. We performed this screen by RNAi on synchronized L1 stage larvae to avoid missing factors whose efficient depletion would cause sterility and/or embryonic lethality. Using a previously described feeding library of bacteria producing double-strand RNA,[29] we individually tested the suppressing effect of RNAi on almost 90% of the genes on chromosome I, i.e., ca. 2,400 genes (Fig. 1). We found that 41 genes could efficiently suppress the conditional lethality of the *let-7* mutation when knocked down through RNAi by feeding (Table 1). Some but not all of the suppressor genes contained *let-7* complementary sites, as defined previously,[7] in their 3′ untranslated regions (UTR) suggesting that these genes may be targets of the *let-7* RNA (Table 1).

Our screening procedure was validated by two observations. First, we blindly identified *lin-41*, the only known heterochronic gene in the chromosome I library, as a potent suppressor of *let-7*(*n2853*) when depleted. *lin-41* is a known downstream target of the *let-7* miRNA whose depletion had previously been shown to suppress *let-7*(*n2853*) (refs. 4 and 10). No RNAi construct targeting *lin-28*, another heterochronic gene and known suppressor of *let-7* encoded on chromosome I, was included in the RNAi library.[29] A second gene, *lss-4* was identified independently by us through a computational approach and also subsequently validated as a *let-7* target.[7] Second, seven genes in the library are targeted by two independent RNAi constructs,[29] and we identified both clones for four of these genes, *rpl-24.2, Y65B4BR.5, imb-5/xpo-2* and *spg-7*. In the remaining three cases differences in the RNAi phenotypes elicited by each pair of constructs were already previously noted.[29]

The largest class of suppressors identified in our screen is comprised of genes with a predicted function in the metabolism of RNA or protein, which account for nearly half (20/41) of the suppressors (Fig. 2). Genes from this category showed a 50% increase over the frequency found by Fraser et al.,[29] who queried the library for genes eliciting any phenotype when depleted in wild-type animals (Fig. 2). This observation may suggest that genes of this functional class are particularly important as targets and/or mediators of *let-7* function or are heterochronic genes. Given the tight posttranscriptional regulation of *let-7* expression,[26] it will also be of considerable interest to test in future work whether any of these novel *let-7* interactors control *let-7* maturation.

Our list of suppressors contained several translation initiation factors: two putative subunits of the eukaryotic translation initiation factor (eIF) 3, *eif-3.H* and *eif-3.E* (*C41D11.2* and *B0511.10*, respectively) and the delta subunit of eIF2B (*F11A3.2*). We also identified *eif-6* (*C47B2.5*) as a *let-7* suppressor, an unexpected result given previous data showing that depletion of eIF6 abrogated miRNA target repression.[30] However, recent data from Drosophila S2 cells suggest that eIF6 may not be widely required for miRNA activity.[31,32]

As our initial screen did not cover the whole genome, we tested additional translation factors for a genetic interaction with *let-7*. Many translation factors have identifiable homologues in *C. elegans*[33] and we performed a systematic RNAi screen of these factors for suppression of the *let-7*(*n2853*) phenotype (Table 2). In most cases, knock-down of translation factors induced a slow growth or developmental arrest phenotype. We could frequently avoid developmental arrest by mixing bacteria that carried the dsRNA producing plasmid with those carrying a plasmid without insert. We found that additional translation factors could suppress the *let-7* mutation, in fact, most of the translation factors tested, including initiation, elongation, as well as termination factors, showed partial suppression. Many, but not all suppressors caused slow growth (Table 2), ruling out for at least a subset of translation factors that delayed development is the cause for *let-7* suppression. Moreover, an approximately wild-type rate of development

also suggests that at least this subset of suppressors affects general translation only weakly. As a corollary, *let-7* function and/or the heterochronic pathway appear to be highly sensitive to altered translation levels.

Depletion of translation factors affects the heterochronic pathway

To ascertain that the suppression of the *let-7* vulval bursting phenotype through impaired translation was specific, i.e., mediated through a modulation of the heterochronic pathway rather than an indirect consequence of, for instance, aberrant vulval development, we investigated whether depletion of individual translation factors caused heterochronic phenotypes in the seam cells. Seam cells are a subset of hypodermal cells that display a stem-cell-like division pattern during larval stages. At the larval to adult transition, seam cells exit the cell cycle and fuse to form a syncytium, which subsequently secretes collagenous structures termed alae. *let-7* is expressed in the seam cells[34] and in its absence, seam cells fail to terminally differentiate at this time and instead divide again.[4] By contrast, overexpression of *let-7* causes the opposite, precocious phenotype and seam cells fuse after the L3-to-L4 molt.[4] We examined the effect of RNAi against *eif-3.B, eif-3.C, eif-3.E, eif-3.H* and *eif-6* in animals carrying a wild-type allele of *let-7*. We selected these factors because they were amongst the first suppressors we had identified. While seam cell fusion is not observed in mock-treated animals at early L4 stage, significant numbers of *eif-3*(*RNAi*) and *eif-6*(*RNAi*) animals displayed precocious seam cell fusion, as did the *lin-41*(*RNAi*) positive control animals (Fig. 3). In the case of *eif-3.B* (*RNAi*) and *eif-3.C*(*RNAi*) we again had to dilute the RNAi-inducing bacteria with inert bacteria to avoid developmental arrest of the affected animals. These findings directly demonstrate that knock-down of this subset of the suppressing translation factors causes heterochronic defects.

To obtain further evidence for a role of translation factors in the heterochronic pathway we analyzed the genetic interaction between *eif-3.E* or *eif-3.H* and *lin-41*. *lin-41* codes for a protein that prevents premature execution of adult fates by repressing production of the transcription factor LIN-29 until the L4 stage.[10] LIN-41 protein levels themselves are regulated through the interaction of *let-7* miRNA with the *let-7* complementary sites in the 3′ UTR of *lin-41* mRNA.[4,10,27] *lin-41* loss-of-function mutations lead to partially penetrant precocious phenotypes in the seam cells and we previously showed that the penetrance of this phenotype can be enhanced when a second *let-7* interactor, the *let-7* target *daf-12*, is also knocked down.[7] Similarly, while only 53.1% of the *lin-41*(*ma104*) animals display precocious alae (±2.4% SEM), the penetrance of this phenotype was significantly ($p < 0.05$, student's t-test) increased to 65.0% ± 1.2% for *lin-41*(*ma104*); *eif-3.E*(*RNAi*) animals and 80.1% ± 0.4% for *lin-41*(*ma104*); *eif-3.H*(*RNAi*) animals.

Analysis of seam cell fusion and alae formation thus indicate that the translation factors investigated modulate the heterochronic pathway. This shows that depletion of these factors does not simply superficially rescue the *let-7*(*n2853*) mutant phenotype but impacts on a pathway known to be regulated by *let-7*. These data would suggest that translation of one or several heterochronic genes, possibly *let-7* target genes, is inefficient and therefore particularly susceptible to further decreases in translation activity. Interestingly, overexpression of eIF3 subunits has been linked to various cancers (reviewed in ref. 35), and our findings indicate that the opposing effects of eIF3 and *let-7* on cell differentiation might be a contributing factor (see Discussion).

Discussion

We have identified several novel genetic interaction partners of the *let-7* miRNA. Some of these contain predicted *let-7* binding sites, and future work may establish them as bona fide *let-7* targets. Here, we have focused on the observation that our screen identified several translation initiation factors whose depletion allowed survival of *let-7*(*n2853*) worms (Table

1). Systematic depletion of individual translation factors subsequently allowed us to identify suppressors at each step of translation:[33] initiation, elongation and termination (Table 2). These suppressors include a subunit of eIF2, which is part of the eIF2/GTP/Met-tRNA$_i^{Met}$ ternary complex and all but one subunit of the eIF2B factor, which catalyzes guanine nucleotide exchange on eIF2 bound to GDP. Among the factors required to recruit the 40S ribosomal subunit to the ternary complex to form the 43S pre-initiation complex (PIC), we observed that eIF1A and all but one of the eIF3 subunits that permitted larval growth results are suppressors. The eIF4F complex, which comprises the cap-binding eIF4E, the scaffolding eIF4G, and the ATP-dependent RNA helicase eIF4A, recruits the 43S PIC to mRNA via an interaction between eIF3 and eIF4G. Among these factors, none of the eIF4E homologues (*ife-1* to *ife-5*) individually tested showed suppression, eIF4G and one homologue of eIF4A (*F57B9.3*) showed developmental arrest, whereas the eIF4A homologue encoded by the *F57B9.6* ORF showed potent suppression. Additionally eIF5, eIF5A and eIF5B were also identified as suppressors. Finally, all translation elongation and termination factors tested showed either a developmental block or suppression of the bursting phenotype.

Based on this result, it seems that suppression of the *let-7*(*n2853*) bursting phenotype can be rescued by decreasing the activity of virtually any step of the translation initiation process as well as the elongation and termination steps. Indeed, some of the other suppressors found in our screen are predicted, by homology, to be part of the ribosome (*rpl-24.2*) or function in its biogenesis (the putative snoRNP proteins W01B11.3 and Y48G1A.4 and the putative nucleolar GTPases homologous to yeast Lsg1p and Nog2p) and we also found two poly(A)-tail binding proteins, *pab-1* and *pabp-2*. However, not all translation factors could suppress *let-7* lethality. This may be due to redundancy (e.g., *ife-1* to *ife-5*), inefficient depletion by RNAi or a genuine lack of interaction between these two genes.

Unexpectedly, we also observed that depleting eIF6 rescues *let-7*(*n2853*) animals and causes precocious heterochronic phenotypes in the presence of wild-type *let-7*, although this factor was reported to be required for miRNA mediated repression.[30] If eIF6 were similarly required for *let-7* function, we would have expected to see the opposite phenotypes, i.e., enhancement of weak *let-7* alleles and or retarded heterochronic phenotypes. Our data would thus argue against an involvement of eIF6 in *let-7* function, consistent with earlier reports from *D. melanogaster* cells that eIF6 does not seem to be generally involved in promoting miRNA function.[31,32]

It is also surprising to see that *eif-3.D*, along with almost all other eIF3 subunits, is found as an efficient suppressor. This observation is in contrast with a recent report indicating that *eif-3.D*(*RNAi*) in an RNAi sensitized strain enhanced the weak *let-7*(*mg279*) loss-of-function allele, as determined by increased vulval bursting.[36] However, we found that *eif-3.D*(*RNAi*) can induce vulval bursting even in wild-type animals where ca. 20% of animals die by bursting despite having functional *let-7*. It is possible that this bursting phenotype may dominate over a weak *let-7* allele, particularly when RNAi is performed in an RNAi sensitized strain, as done in the earlier report.[36]

We are particularly intrigued to see that depletion of eIF3 subunits causes precocious seam cell differentiation in the presence of wild-type *let-7*. This is because several of the thirteen subunits of human eIF3 have altered expression levels in cancers including lung, breast, cervical, esophageal, prostate and testicular cancers, and this aberrant expression is likely to contribute to oncogenesis.[35] For instance, INT6/eIF3e was originally identified as a common integration site of mouse mammary tumor virus,[37] and expression of the truncated INT6/eIF3e gene product, but not of the wild-type eIF3e gene, is sufficient to transform a number of cell lines.[38,39] Conversely, INT6/eIF3e loss-of-heterozygosity and decreased expression appear to be associated with breast and non-small cell lung cancers,[37] suggesting that INT6/eIF3e activity

is particularly dosage dependent. Recently, eIF3h overexpression was shown to increase tumorigenic phenotypes in various cell lines,[40] and eIF3h was also found in a genome-wide association screen for loci conferring increased risk for colorectal cancer.[41] Finally, eIF3a, the largest eIF3 subunit, is overexpressed in human lung, breast, cervical and esophageal cancers,[42–45] and reduction of eIF3a levels in two human lung and breast cancer cell lines, respectively, is sufficient to suppress the malignant phenotypes in vitro.[46] eIF3a expression is also higher in fetal than in more differentiated tissues,[47] and thus reciprocal to *let-7* expression.[26]

As expression of eIF3 subunits in human cells appears to be highly coordinated[48] such that forced overexpression of individual subunits leads to increased accumulation of other subunits and incorporation into functional eIF3 complexes, it appears likely that additional eIF3 subunits are deregulated in tumors.

When viewed together with the fact that *let-7* functions as a tumor suppressor gene,[26] these findings suggest that the opposing roles of eIF3 and *let-7* on cell differentiation might be conserved beyond *C. elegans*. Indeed, increased amounts of eIF3 specifically stimulate translation of mRNAs involved in cell proliferation, in particular MYC and cyclin D1 (reviewed in ref. 48)—mRNAs that are repressed by *let-7*.[26] We might thus speculate that the opposing activities of eIF3 and *let-7* on a subset of cellular mRNAs contribute to the oncogenic functions of eIF3.

Taken together, we find widespread suppression of *let-7* loss-of-function through decreased cellular translation activity, suggesting that *let-7* targets or other heterochronic genes may be translationally regulated to allow proper timing of cell differentiation.

Materials and Methods

let-7(*n2853*) suppressor screen and RNAi

Wild-type (N2) and *let-7*(*n2853*) (MT7626) strains used in this work were provided by the Caenorhabditis Genetics Center (CGC), which is founded by the NIH National Center for Research Resources. The screen was performed using RNAi by feeding with a published RNAi library[29] covering ca. 90% of the genes on *C. elegans* chromosome I. Additional RNAi clones were obtained from RNAi libraries,[49,50] or were created in the laboratory as follows by PCR on genomic DNA using the primers listed below. PCR fragments were digested with *Xba*I and *Kpn*I (pXD10, pXD11 and pXD12), *Nde*I/*Xho*I (pHG8) or *BamH*I/*Xho*I (pHG9) and ligated into L4440 (reviewed in ref. 51). The resulting constructs were transformed in *E. coli* HT115 for feeding RNAi experiments.

The screen was performed as illustrated in Figure 1, with every step done in duplicate. Supplements were used at the following concentrations: ampicillin: 100 μg/ml, tetracycline: 12.5 μg/ml, IPTG: 1 mM. Retesting and testing of additional translation factors was done at 20°C and 25°C on 6-cm diameter plates as described.[7] In some experiments, carbenicillin was used instead of ampicillin. Suppressor identity was confirmed through plasmid DNA recovery followed by sequencing. In some cases we were unable to stage worms reliably because RNAi caused gonad migration defects in the absence of oocytes. These candidates were discarded.

Enhancement of *lin-41*(*ma104*) precocious phenotypes was scored in at least two independent experiments with ≥19 animals per strain and a total of ≥100 animals scored per strain.

Oligonucleotide sequences

Synthetic sequences are in lowercase, restriction sites underlined.

Name	5′ to 3′ sequence	Plasmid
eIF1_F1	gctctagagcTGAAGTTGCTCACCATCTCG	pXD10
eIF1_R1	ggggtacccACTTCCTCGCCACTTCTTCA	(L4440-eIF1)
cif-1_F1	gctctagagcGATGATGTCAAGCAGCTCCA	pXD11
cif-1_R1	ggggtacccCATTGTTCCGTTCCGAATCT	(L4440-cif-1)
eIF5B_F1	gctctagagcGAAGAGGATTCGGATGGTGA	pXD12
eIF5B_R1	ggggtacccACCTCCTTCCTCTTGGCAAT	(L4440-eIF5B)
eif-3.B_F1	caccatATGGTCGAAATTGACTTTAAT	pHG8
eif-3.B_R1	tttctcgagTTAGTCTCTCATCTCCTCC	(L4440-eif-3.B)
eif-3.C_F1	tttggatccTGTCTCGCTTCTTCCATGC	pHG9
eif-3.C_R1	tttctcgagTTAGAAGGCTCGTGGCTTT	(L4440-eif-3.C)

Precocious seam cells fusion

Precocious seam cell fusion was analyzed using strain *wIs79*[*ajm-1::gfp; scm::gfp*] (ref. 9). Microscopy images were acquired using a Zeiss Axioplan microscope equipped with a Zeisscam CCD camera and Axiovision software. Images were cropped and levels adjusted using Adobe Photoshop software.

Acknowledgments

X.D. was supported by a PhD student fellowship from the Boehringer Ingelheim Foundation, F.J.S by NIH R01 grant (GM62594). Part of H.G.'s work on the project was supported by a Human Frontier Science Program Postdoctoral Fellowship. Research in H.G.'s laboratory is supported by the FMI, part of the Novartis Research Foundation.

References

1. Bushati N, Cohen SM. microRNA Functions. Annu Rev Cell Dev Biol 2007;23:175–205. [PubMed: 17506695]
2. Eulalio A, Huntzinger E, Izaurralde E. Getting to the Root of miRNA-Mediated Gene Silencing. Cell 2008;132:9–14. [PubMed: 18191211]
3. Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat Rev Genet 2008;9:102–14. [PubMed: 18197166]
4. Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G. The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. Nature 2000;403:901–6. [PubMed: 10706289]
5. Moss EG. Heterochronic genes and the nature of developmental time. Curr Biol 2007;17:425–34. [PubMed: 17320389]
6. Abrahante JE, Daul AL, Li M, Volk ML, Tennessen JM, Miller EA, Rougvie AE. The *Caenorhabditis elegans* hunchback-like gene lin-57/hbl-1 controls developmental time and is regulated by microRNAs. Dev Cell 2003;4:625–37. [PubMed: 12737799]
7. Grosshans H, Johnson T, Reinert KL, Gerstein M, Slack FJ. The temporal patterning microRNA let-7 regulates several transcription factors at the larval to adult transition in *C. elegans*. Dev Cell 2005;8:321–30. [PubMed: 15737928]
8. Lall S, Grün D, Krek A, Chen K, Wang YL, Dewey CN, Sood P, Colombo T, Bray N, Macmenamin P, Kao HL, Gunsalus KC, Pachter L, Piano F, Rajewsky N. A genome-wide map of conserved microRNA targets in *C. elegans*. Curr Biol 2006;16:460–71. [PubMed: 16458514]
9. Lin SY, Johnson SM, Abraham M, Vella MC, Pasquinelli A, Gamberi C, Gottlieb E, Slack FJ. The *C. elegans* hunchback homolog, hbl-1, controls temporal patterning and is a probable microRNA target. Dev Cell 2003;4:639–50. [PubMed: 12737800]

10. Slack FJ, Basson M, Liu Z, Ambros V, Horvitz HR, Ruvkun G. The lin-41 RBCC gene acts in the *C. elegans* heterochronic pathway between the let-7 regulatory RNA and the LIN-29 transcription factor. Mol Cell 2000;5:659–69. [PubMed: 10882102]
11. Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kuroda MI, Maller B, Hayward DC, Ball EE, Degnan B, Muller P, Spring J, Srinivasan A, Fishman M, Finnerty J, Corbo J, Levine M, Leahy P, Davidson E, Ruvkun G. Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. Nature 2000;408:86–9. [PubMed: 11081512]
12. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. Science 2001;294:853–8. [PubMed: 11679670]
13. Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, Labourier E, Reinert KL, Brown D, Slack FJ. RAS is regulated by the let-7 microRNA family. Cell 2005;120:635–47. [PubMed: 15766527]
14. Lee YS, Dutta A. The tumor suppressor microRNA let-7 represses the HMGA2 oncogene. Genes Dev 2007;21:1025–30. [PubMed: 17437991]
15. Mayr C, Hemann MT, Bartel DP. Disrupting the Pairing Between let-7 and Hmga2 Enhances Oncogenic Transformation. Science 2007;315:1576–9. [PubMed: 17322030]
16. Wang T, Zhang X, Obijuru L, Laser J, Aris V, Lee P, Mittal K, Soteropoulos P, Wei JJ. A micro-RNA signature associated with race, tumor size, and target gene activity in human uterine leiomyomas. Genes Chromosomes Cancer 2007;46:336–47. [PubMed: 17243163]
17. Hebert C, Norris K, Scheper MA, Nikitakis N, Sauk JJ. High mobility group A2 is a target for miRNA-98 in head and neck squamous cell carcinoma. Mol Cancer 2007;6:5. [PubMed: 17222355]
18. Shell S, Park SM, Radjabi AR, Schickel R, Kistner EO, Jewell DA, Feig C, Lengyel E, Peter ME. Let-7 expression defines two differentiation stages of cancer. Proc Natl Acad Sci USA 2007;104:11400–5. [PubMed: 17600087]
19. Huang JC, Babak T, Corson TW, Chua G, Khan S, Gallie BL, Hughes TR, Blencowe BJ, Frey BJ, Morris QD. Using expression profiling data to identify human microRNA targets. Nat Methods 2007;4:1045–9. [PubMed: 18026111]
20. Johnson CD, Esquela-Kerscher A, Stefani G, Byrom M, Kelnar K, Ovcharenko D, Wilson M, Wang X, Shelton J, Shingara J, Chin L, Brown D, Slack FJ. The let-7 MicroRNA Represses Cell Proliferation Pathways in Human Cells. Cancer Res 2007;67:7713–22. [PubMed: 17699775]
21. Takamizawa J, Konishi H, Yanagisawa K, Tomida S, Osada H, Endoh H, Harano T, Yatabe Y, Nagino M, Nimura Y, Mitsudomi T, Takahashi T. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. Cancer Res 2004;64:3753–6. [PubMed: 15172979]
22. Esquela-Kerscher A, Trang P, Wiggins JF, Patrawala L, Cheng A, Ford L, Weidhaas JB, Brown D, Bader AG, Slack FJ. The let-7 microRNA reduces tumor growth in mouse models of lung cancer. Cell Cycle 2008;7
23. Kumar MS, Erkeland SJ, Pester RE, Chen CY, Ebert MS, Sharp PA, Jacks T. Suppression of non-small cell lung tumor development by the let-7 microRNA family. Proc Natl Acad Sci USA 2008;105:3903–8. [PubMed: 18308936]
24. Yu SL, Chen HY, Chang GC, Chen CY, Chen HW, Singh S, Cheng CL, Yu CJ, Lee YC, Chen HS, Su TJ, Chiang CC, Li HN, Hong QS, Su HY, Chen CC, Chen WJ, Liu CC, Chan WK, Li KC, Chen JJ, Yang PC. MicroRNA Signature Predicts Survival and Relapse in Lung Cancer. Cancer Cell 2008;13:48–57. [PubMed: 18167339]
25. Yu F, Yao H, Zhu P, Zhang X, Pan Q, Gong C, Huang Y, Hu X, Su F, Lieberman J, Song E. let-7 Regulates Self Renewal and Tumorigenicity of Breast Cancer Cells. Cell 2007;131:1109–23. [PubMed: 18083101]
26. Büssing I, Slack FJ, Grosshans H. let-7 microRNAs in development, stem cells and cancer. Trends Mol Med. 2008 doi: 10.1016/j.molmed.2008.07.001.
27. Vella MC, Choi EY, Lin SY, Reinert K, Slack FJ. The *C. elegans* microRNA let-7 binds to imperfect let-7 complementary sites from the lin-41 3′UTR. Genes Dev 2004;18:132–7. [PubMed: 14729570]
28. Bagga S, Bracht J, Hunter S, Massirer K, Holtz J, Eachus R, Pasquinelli AE. Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation. Cell 2005;122:553–63. [PubMed: 16122423]

29. Fraser AG, Kamath RS, Zipperlen P, Martinez-Campos M, Sohrmann M, Ahringer J. Functional genomic analysis of *C. elegans* chromosome I by systematic RNA interference. Nature 2000;408:325–30. [PubMed: 11099033]
30. Chendrimada TP, Finn KJ, Ji X, Baillat D, Gregory RI, Liebhaber SA, Pasquinelli AE, Shiekhattar R. MicroRNA silencing through RISC recruitment of eIF6. Nature 2007;447:823–8. [PubMed: 17507929]
31. Eulalio A, Rehwinkel J, Stricker M, Huntzinger E, Yang SF, Doerks T, Dorner S, Bork P, Boutros M, Izaurralde E. Target-specific requirements for enhancers of decapping in miRNA-mediated gene silencing. Genes Dev 2007;21:2558–70. [PubMed: 17901217]
32. Eulalio A, Huntzinger E, Izaurralde E. GW182 interaction with Argonaute is essential for miRNA-mediated translational repression and mRNA decay. Nat Struct Mol Biol 2008;15:346–53. [PubMed: 18345015]
33. Rhoads, RE.; Dinkova, TD.; Korneeva, NL. Mechanism and regulation of translation in *C. elegans*. In: The *C. elegans* Research Community. , editor. WormBook. Wormbook; 2006. p. 1-18.
34. Johnson SM, Lin SY, Slack FJ. The time of appearance of the *C. elegans* let-7 microRNA is transcriptionally controlled utilizing a temporal regulatory element in its promoter. Dev Biol 2003;259:364–79. [PubMed: 12871707]
35. Dong Z, Zhang JT. Initiation factor eIF3 and regulation of mRNA translation, cell growth and cancer. Crit Rev Oncol Hematol 2006;59:169–80. [PubMed: 16829125]
36. Parry DH, Xu J, Ruvkun G. A Whole-Genome RNAi Screen for *C. elegans* miRNA Pathway Genes. Curr Biol 2007;17:2013–22. [PubMed: 18023351]
37. Marchetti A, Buttitta F, Pellegrini S, Bertacca G, Callahan R. Reduced expression of INT-6/eIF3-p48 in human tumors. Int J Oncol 2001;18:175–9. [PubMed: 11115556]
38. Rasmussen SB, Kordon E, Callahan R, Smith GH. Evidence for the transforming activity of a truncated Int6 gene, in vitro. Oncogene 2001;20:5291–301. [PubMed: 11536042]
39. Mayeur GL, Hershey JW. Malignant transformation by the eukaryotic translation initiation factor 3 subunit p48 (eIF3e). FEBS Lett 2002;514:49–54. [PubMed: 11904180]
40. Zhang L, Smit-McBride Z, Pan X, Rheinhardt J, Hershey JW. An oncogenic role for the phosphorylated h-subunit of human translation initiation factor eIF3. J Biol Chem. 2008
41. Tomlinson IP, Webb E, Carvajal-Carmona L, Broderick P, Howarth K, Pittman AM, Spain S, Lubbe S, Walther A, Sullivan K, Jaeger E, Fielding S, Rowan A, Vijayakrishnan J, Domingo E, Chandler I, Kemp Z, Qureshi M, Farrington SM, Tenesa A, Prendergast JG, Barnetson RA, Penegar S, Barclay E, Wood W, Martin L, Gorman M, Thomas H, Peto J, Bishop DT, Gray R, Maher ER, Lucassen A, Kerr D, Evans DG, CORGI Consortium, Schafmayer C, Buch S, Völzke H, Hampe J, Schreiber S, John U, Koessler T, Pharoah P, van Wezel T, Morreau H, Wijnen JT, Hopper JL, Southey MC, Giles GG, Severi G, Castellví-Bel S, Ruiz-Ponte C, Carracedo A, Castells A, EPICOLON Consortium, Försti A, Hemminki K, Vodicka P, Naccarati A, Lipton L, Ho JW, Cheng KK, Sham PC, Luk J, Agúndez JA, Ladero JM, de la Hoya M, Caldés T, Niittymäki I, Tuupanen S, Karhu A, Aaltonen L, Cazier JB, Campbell H, Dunlop MG, Houlston RS. A genome-wide association study identifies colorectal cancer susceptibility loci on chromosomes 10p14 and 8q23.3. Nat Genet 2008;40:623–30. [PubMed: 18372905]
42. Bachmann F, Banziger R, Burger MM. Cloning of a novel protein overexpressed in human mammary carcinoma. Cancer Res 1997;57:988–94. [PubMed: 9041205]
43. Dellas A, Torhorst J, Bachmann F, Banziger R, Schultheiss E, Burger MM. Expression of p150 in cervical neoplasia and its potential value in predicting survival. Cancer 1998;83:1376–83. [PubMed: 9762939]
44. Chen G, Burger MM. p150 expression and its prognostic value in squamous-cell carcinoma of the esophagus. Int J Cancer 1999;84:95–100. [PubMed: 10096238]
45. Pincheira R, Chen Q, Zhang JT. Identification of a 170-kDa protein overexpressed in lung cancers. Br J Cancer 2001;84:1520–7. [PubMed: 11384103]
46. Dong Z, Liu LH, Han B, Pincheira R, Zhang JT. Role of eIF3 p170 in controlling synthesis of ribonucleotide reductase M2 and cell growth. Oncogene 2004;23:3790–801. [PubMed: 15094776]

47. Liu Z, Dong Z, Yang Z, Chen Q, Pan Y, Yang Y, Cui P, Zhang X, Zhang JT. Role of eIF3a (eIF3 p170) in intestinal cell differentiation and its association with early development. Differentiation 2007;75:652–61. [PubMed: 17381544]

48. Zhang L, Pan X, Hershey JW. Individual overexpression of five subunits of human translation initiation factor eIF3 promotes malignant transformation of immortal fibroblast cells. J Biol Chem 2006;282:5790–800. [PubMed: 17170115]

49. Rual JF, Ceron J, Koreth J, Hao T, Nicot AS, Hirozane-Kishikawa T, Vandenhaute J, Orkin SH, Hill DE, van den Heuvel S, Vidal M. Toward improving *Caenorhabditis elegans* phenome mapping with an ORFeome-based RNAi library. Genome Res 2004;14:2162–8. [PubMed: 15489339]

50. Kamath RS, Fraser AG, Dong Y, Poulin G, Durbin R, Gotta M, Kanapin A, Le Bot N, Moreno S, Sohrmann M, Welchman DP, Zipperlen P, Ahringer J. Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. Nature 2003;421:231–7. [PubMed: 12529635]

51. Timmons L, Fire A. Specific interference by ingested dsRNA. Nature 1998:395–85.

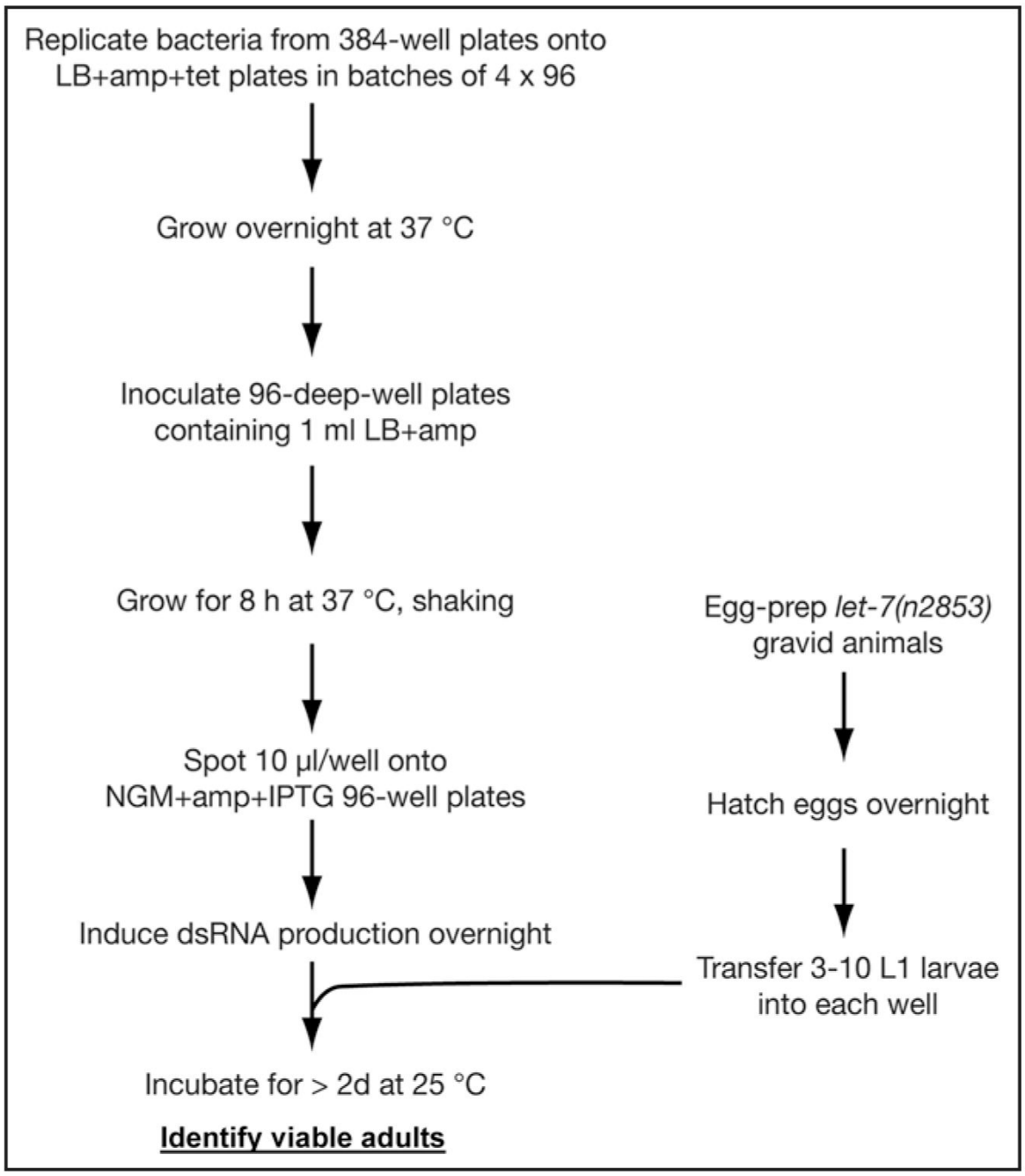

Figure 1.
A high-throughput reverse genetics screen to identify suppressors of *let-7*(*n2853*) lethality. See main text and Materials and Methods for details.

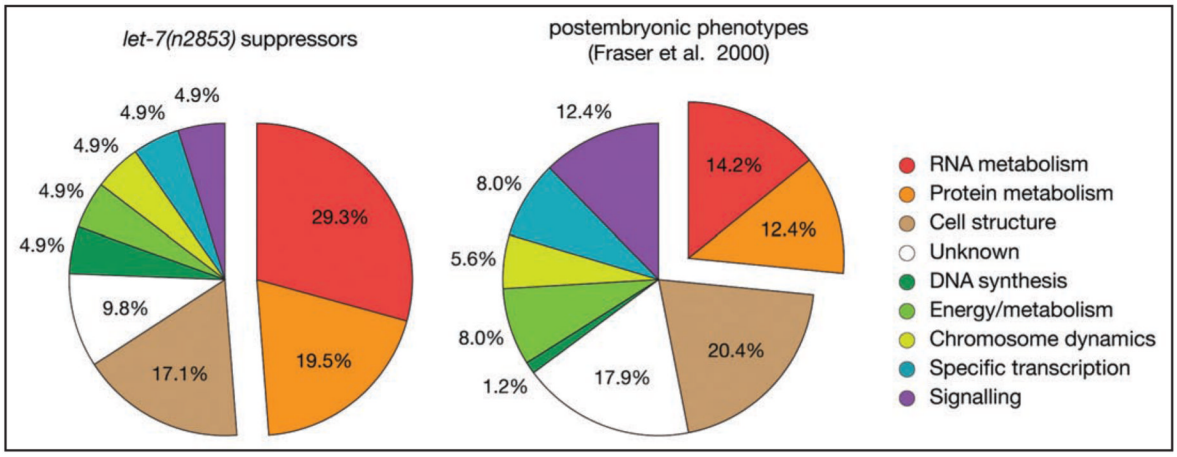

Figure 2.

let-7 suppressors are enriched for factors involved in RNA and protein metabolism. Genes involved in RNA and protein metabolism are enriched among the suppressors of the *let-7* (*n2853*) mutation. Indicated are the distributions across functional classes of genes causing suppression of the *let-7*(*n2853*)-associated lethality (left chart) or visible phenotypes in otherwise wild-type animals (right chart; assembled from data in ref. 29). Assignments to functional classes are from Wormbase Release WS188. Where gene assignments had changed from those used by Fraser et al., their data were adjusted accordingly. Note that both studies used an identical RNAi library.

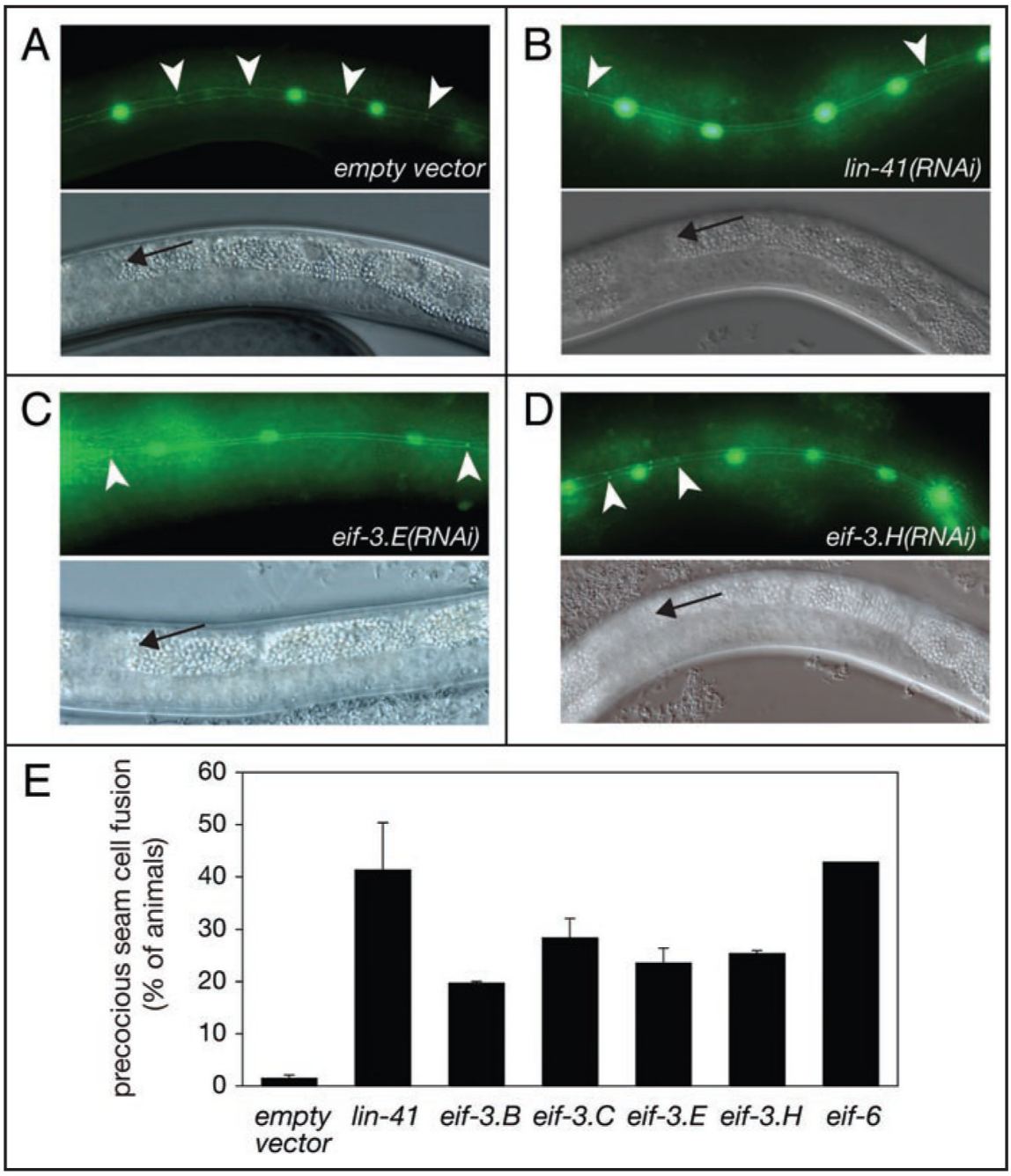

Figure 3.

Reduced levels of eIF3-subunits cause precocious seam cell fusion. Synchronized *N2*; *wIs79* L1-stage larvae were grown on bacteria producing the indicated dsRNA and examined for presence of precocious seam cell fusion upon reaching early-L4 stage. (A–D) Photomicrographs of animals grown on the indicated bacteria. *Arrowheads* point to AJM-1/GFP signal between seam cells observed in the absence of cell fusion. *Arrows* in lower panels indicate the distal tips of the gonads, visualized through Nomarski optics, which demonstrate the appropriate early L4 developmental stage. Anterior of each animal is left, ventral down. Note that GFP and Nomarski micrographs show different parts of the same animal. (E) Percentages of animals with precocious seam cell fusion were averaged from at least two

independent experiments. To avoid developmental arrest in the case of *eif-3.B* and *eif-3.C* subunits, and gonadal migration defects in the case of the *eif-3.E*, animals were fed bacteria expressing the appropriate dsRNA, diluted appropriately (1:2 to 1:6) with bacteria producing mock dsRNA. $n \geq 82$ for each. Error bars correspond to SEM.

Table 1

Suppressors of *let-7*(*n2853*) lethality identified in a screen

ORF[a]	Locusb	Function/Homologies[b,c]	LCS[d]	Suppression[e]
DNA synthesis				
W02D9.1	*pri-2*	DNA primase	−	+++
Y54E10A.15	*cdt-1*	*Hs* CDT1, *Dm* dup	−	+++
RNA metabolism				
B0511.6		DEAD box helicase, *Dm* pit	−	++
C17E4.5	*pabp-2*	Poly(A)-binding protein, *Hs* PABN1	−	++
C36B1.3	*rbp-3*	RNA polymerase II subunit	−	++
C53H9.2		Nucleolar GTPase, *Sc* Lsg1p	1	+
F14B4.3		RNA Polymerase I, second largest subunit	1	++
T19A6.2	*ngp-1*	Nucleolar GTPase, *Sc* Nog2p	−	+++
W01B11.3	*nol-5*	snoRNP associated, *Sc* Nop5p	1	++
W04A4.5		*Hs* Integrator isoform 1 (U1, U2 RNA processing)	−	+++
Y48G1A.4		snoRNP associated, *Sc* Nop14p	nd	++
Y54E10BR.6	*rbp-7*	RNA polymerase II subunit	−	++
Y71F9B.4	*snr-7*	*Hs* SNRPG (spliceosome subunit)	−	++
Y106G6H.2	*pab-1*	Poly(A)-binding protein, *Sc* Pab1p	−	++
Protein metabolism				
B0511.10	*eif-3.E*	Translational initiation factor eIF3 subunit	−	++
C03D6.8	*rpl-24.2*	Ribosomal protein L24-family, *Sc* Rlp24p	−	++
C12C8.3	*lin-41*	NHL domains	2	+++
C41D11.2	*eif-3.H*	Translational initiation factor eIF3 subunit	−	++
C47B2.5	*eif-6*	Translational initiation factor eIF6	−	++
Y47G6A.10	*spg-7*	AAA-ATPase, protease	−	++
F11A3.2[f]		Translational initiation factor, eIF2Bδ	−	+++
Y65B4BR.5		Nascent polypeptide associated complex α-chain	−	++
Energy/metabolism				
T09B4.9		Mitochondrial inner membrane translocase, *Hs* TIM44	−	++
W09C5.8		Subunit IV of cytochrome c oxidase	−	+
Chromosome dynamics				
C45G3.1	*aspm-1*	*Dm* Asp	−	+
T03F1.9	*hcp-4*	CENP-C homologue	−	++
Cell structure				
F56A3.3	*npp-6*	Nuclear pore protein	−	+++
T19B4.2	*npp-7*	Nuclear pore protein	2	+++
T21E12.4	*dhc-1*	Dynein heavy chain	−	++
Y48G1A.5	*imb-5*	*Sc* Cse1p, *Hs* CAS/CSE1	1	++
Y71F9AM.5	*nxt-1*	NTF2-family	−	+
Y105E8A.9	*apg-1*	γ-adaptin AP-1	1	++
H15N14.2	*nsf-1*	Vesicle fusion	−	+

ORF[a]	Locusb	Function/Homologies[b,c]	LCS[d]	Suppression[e]
Specific transcription				
C01G8.9	*lss-4*	*Dm* osa/eld	3	+++
F57B10.1		CREB/ATF family transcription factor	–	++
Signalling				
K12C11.2	*smo-1*	*Hs* SUMO-1	–	+
ZC581.1	*nekl-2*	NEK kinase family	–	++
Unknown				
F20G4.1	*smgl-1*	*Hs* Neuroblastoma amplified gene protein	–	++
F56A3.4	*spd-5*	Coiled coils	–	++
T23D8.3		*Hs* LTV1; in operon with eif-3C	–	++
Y63D3A.5	*tfg-1*	*Hs* TFG1 (TrkA-fused gene)	–	++

[a] Some ORFs were targeted by more than one dsRNA construct and/or construct names might differ from those of the target ORF indicated here.

[b] Gene loci names according to Wormbase, Release 188. Gene names were not considered when assigning functional classes in cases where no published information or sequence homologies were available to support the gene designation.

[c] *Sc*: *Saccharomyces cerevisiae*, *Dm*: *Drosophila melanogaster*, *Hs*: *Homo sapiens*. In most cases functions are predicted from homologies.

[d] LCS: *let-7* complementary site as identified in[7]. *nd*: 3′ UTR was not included in dataset used for LCS prediction.

[e] +, >30%, ++, >50%, +++, >80% survival.

[f] Sequencing revealed plasmid contained other than predicted insert, targeting the indicated ORF (chromosome V).

Table 2

Genetic interactions between *let-7* and the translation machinery

Translation factor	locus	ORF	*let-7*(*n2853*) suppression
Initiation factors			
eIF1		T27F7.3b	−
eIF1A[a]		H06H21.3	++
eIF2β[b]	*iftb-1*	K04G2.1	++
eIF2γ[d]		Y39G10AR.8	×
eIF2A		E04D5.1 (a, b)	−
eIF2Bα		ZK1098.4	−
eIF2Bβ		Y47H9C.7	++
eIF2Bγ	*ppp-1*	C15F1.4	++
eIF2B∂		F11A3.2	+++
eIF2Bεd		D2085.3	+++
eIF3a[c,d]	*egl-45*	C27D11.1	×
eIF3b[c,d]	*eif-3.B*	Y54E2A.11a	+++
eIF3c[c,d]	*eif-3.C*	T23D8.4	+++
eIF3d[d]	*eif-3.D*	R08D7.3	++
eIF3e[d]	*eif-3.E*	B0511.10	+++
eIF3f[d]	*eif-3.F*	D2013.7	+++
eIF3g[d]	*eif-3.G*	F22B5.2	×
eIF3h	*eif-3.H*	C41D11.2	++
eIF3i[b]	*eif-3.I*	Y74C10AR.1	×
eIF3k	*eif-3.K*	T16G1.11	−
eIF3m[a]	*cif-1*	K08F11.3	+++
eIF4A[b,d]	*inf-1*	F57B9.6	×
eIF4A[a,d]		*F57B9.3*	+++
eIF4E-1+5	*ife-1*	F53A2.6	−
	ife-5	Y57A10A.30 (a, b)	
eIF4E-2	*ife-2*	R04A9.4	−
eIF4E-3	*ife-3*	B0348.6 (a, b, c)	−
eIF4E-4	*ife-4*	C05D9.5	−
eIF4G[b,d]	*ifg-1*	M110.4 (a, b)	×
eIF4H	*drr-2*	T12D8.2	−
eIF5[b]		C37C3.2 (a, b, c)	++
eIF5A[d]	*iff-2*	F54C9.1	+++
eIF5B	*iffb-1*	Y54F10BM.2	+++
eIF6	*eif-6*	C47B2.5	++
Elongation factors			
eEF1A[b,d,e]	*eft-3*	F31E3.5	++

Translation factor	locus	ORF	*let-7*(*n2853*) suppression
	eft-4	R03G5.1 (a, b, c, d)	
eEF1B[e]		Y41E3.10	++
		F54H12.6	
eEF2[b,d]	*eft-2*	F25H5.4	+++
eEF2[c,d]	*eft-1*	ZK328.2	++
Release factors			
eRF1[c,d]		T05H4.6 (a, b)	×
eRF3[b,d]		H19N07.1	++

In some cases, RNAi titration was performed to overcome developmental block by mixing bacteria that carried the dsRNA producing plasmid with those carrying a plasmid without insert.

[a] 1:1 dilution

[b] 1:5 dilution

[c] 1:10 dilution

[d] slow growth

[e] multiple RNAi targets. Suppression: −,<20%, +, >20%, ++, >40%, +++, >80%, x, developmental block, n ≥ 60 worms for each. Control animals fed with bacteria carrying empty L4440 vector showed never more than 10% survival, whereas *daf-12*(*RNAi*), our positive control, showed never less than 90% survival.