Transcriptional adaptation in *Caenorhabditis elegans*

Vahan Serobyan[1]*, Zacharias Kontarakis[1†], Mohamed A El-Brolosy[1], Jordan M Welker[1], Oleg Tolstenkov[2,3‡], Amr M Saadeldein[1], Nicholas Retzer[1], Alexander Gottschalk[2,3,4], Ann M Wehman[5], Didier YR Stainier[1]*

[1]Department of Developmental Genetics, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany; [2]Institute for Biophysical Chemistry, Goethe University, Frankfurt Am Main, Germany; [3]Cluster of Excellence Frankfurt - Macromolecular Complexes (CEF-MC), Goethe University, Frankfurt Am Main, Germany; [4]Buchmann Institute for Molecular Life Sciences (BMLS), Goethe University, Frankfurt Am Main, Germany; [5]Rudolf Virchow Center, University of Würzburg, Würzburg, Germany

*For correspondence:
Vahan.Serobyan@mpi-bn.mpg.de (VS);
Didier.Stainier@mpi-bn.mpg.de (DYRS)

Present address: †Genome Engineering and Measurement Lab, ETH Zurich, Functional Genomics Center Zurich of ETH Zurich, University of Zurich, Zurich, Switzerland; ‡Sars International Centre for Marine Molecular Biology, University of Bergen, Bergen, Norway

Abstract Transcriptional adaptation is a recently described phenomenon by which a mutation in one gene leads to the transcriptional modulation of related genes, termed adapting genes. At the molecular level, it has been proposed that the mutant mRNA, rather than the loss of protein function, activates this response. While several examples of transcriptional adaptation have been reported in zebrafish embryos and in mouse cell lines, it is not known whether this phenomenon is observed across metazoans. Here we report transcriptional adaptation in *C. elegans*, and find that this process requires factors involved in mutant mRNA decay, as in zebrafish and mouse. We further uncover a requirement for Argonaute proteins and Dicer, factors involved in small RNA maturation and transport into the nucleus. Altogether, these results provide evidence for transcriptional adaptation in *C. elegans*, a powerful model to further investigate underlying molecular mechanisms.

Introduction

Transcriptional adaptation is the ability of certain mutations in a gene to modulate the expression of related genes, referred to as adapting genes (*El-Brolosy and Stainier, 2017*; *El-Brolosy et al., 2019*; *Ma et al., 2019*). At the molecular level, the mutant mRNA, rather than the loss of protein function, is responsible for this transcriptional modulation (*Rossi et al., 2015*; *El-Brolosy and Stainier, 2017*; *Sztal et al., 2018*; *El-Brolosy et al., 2019*; *Ma et al., 2019*). According to one model (*El-Brolosy et al., 2019*), the mutant mRNA, via its degradation products, modulates the expression of adapting genes via transcriptional regulators including antisense RNAs and histone modifiers. According to another model (*Ma et al., 2019*), the premature termination codon (PTC) containing mutant mRNA interacts with a histone modifier complex leading to transcriptional upregulation of the adapting gene(s). Sequence similarity with the mutant mRNA determines which genes get upregulated during transcriptional adaptation (*El-Brolosy et al., 2019*). In some cases, the upregulated genes share functionality with the mutated gene leading to functional compensation. However, while transcriptional adaptation is often discussed in the context of genetic robustness (*Rossi et al., 2015*; *El-Brolosy et al., 2019*; *Ma et al., 2019*), it does not always lead to functional compensation (*Rossi et al., 2015*). In addition, transcriptome analyses suggest that even genes with limited sequence similarity with the mutant mRNA can be upregulated during transcriptional adaptation (*El-Brolosy et al., 2019*), although clearly more work is required to determine whether the upregulation of these genes is a direct or indirect effect of transcriptional adaptation.

Understanding the mechanisms of transcriptional adaptation will help us better comprehend why for a given gene some mutations cause disease while others do not (*Castel et al., 2018*). However, despite the importance and growing interest in many aspects of genetic compensation, transcriptional adaptation has currently only been investigated in vertebrates. Thus, it remains unclear whether this phenomenon is observed across metazoans. The evolutionary importance of related genes that have compensatory effects has also been discussed in non-vertebrate eukaryotes (*Conant and Wagner, 2004*; *Plata and Vitkup, 2014*). However, it is not known whether these examples of compensation are due to protein feedback loops or transcriptional adaptation.

Only a few factors are known to be involved in the transcriptional adaptation response thus far, and others, including some involved in RNA processing and transport, are likely required. In addition, it is not clear whether the mechanisms of transcriptional adaptation are common or whether each particular case occurs in a different manner, especially at the step leading to transcriptional modulation. Also, as different paralogs or related genes are expressed in distinct tissues and/or at different times (*Laisney et al., 2010*; *Kryuchkova-Mostacci and Robinson-Rechavi, 2015*; *Radomska et al., 2016*; *Pasquier et al., 2017*; *Jojic et al., 2018*), it is currently unclear whether the expression of adapting genes can appear in tissues where, and/or at times when, they are not normally expressed.

In this study, we provide examples of transcriptional adaptation in *Caenorhabditis elegans* and show the ectopic expression of an extrachromosomal reporter in a tissue where it is not normally expressed. In addition, we analyze these transcriptional adaptation models after RNAi-mediated knockdown of different genes involved in RNA metabolism and find that the upregulation of the adapting genes requires factors involved in the maturation and transport into the nucleus of small RNAs (sRNAs).

Results

Examples of transcriptional adaptation in *C. elegans*

Actins are essential structural components of eukaryotic cells as they mediate a wide range of cellular processes (*Pollard and Cooper, 1986*). Actin genes are often present in multiple copies in higher eukaryotic genomes and hints of transcriptional adaptation modulating their expression have been reported. For example, mouse embryonic fibroblasts (MEFs) mutant for β-Actin (*Actb*) display increased expression of other Actins including α- and γ-Actin (ACTA and ACTG1/2) as measured by Western blots (*Tondeleir et al., 2012*). Similarly, *Actg1* knockout, but not knockdown, in MEFs leads to an increase in *Acta* mRNA levels (*Patrinostro et al., 2017*), and zebrafish *actc1b* mutants exhibit mild muscle defects because of the transcriptional upregulation of *actc1a* (*Sztal et al., 2018*). Furthermore, *Actg1* mutant MEFs and *Actb* mutant mouse embryonic stem cells (mESCs) display increased mRNA levels of *Actg2* and *Actg1*, respectively, and this upregulation is triggered not by the loss of protein function but by mutant mRNA decay (*El-Brolosy et al., 2019*). Thus, we decided to investigate actin genes in *C. elegans* to test for transcriptional adaptation. The *C. elegans* genome contains five actin genes which display high similarity in their DNA and protein sequences (*MacQueen et al., 2005*). We started by analyzing several mutant alleles for *act-1*, *act-2*, *act-3* and *act-5*, and determined mutant transcript levels. We found significantly reduced *act-5* mRNA levels in *act-5(dt2019)* mutants compared to wild type (*Figure 1A*), likely caused by nonsense-mediated decay (NMD) due to a premature termination codon (ptc) in the first exon (*Figure 1A*, *Figure 1—figure supplement 1*). Most mutant alleles of *act-5* cause severe phenotypes including lethality (*Estes et al., 2011*; *MacQueen et al., 2005*), sterility (*Cui et al., 2004*), and paralysis (*Etheridge et al., 2015*). However, the *act-5(dt2019)* allele, hereafter referred to as *act-5(ptc)*, does not exhibit any obvious phenotype (*MacQueen et al., 2005*), an observation we confirmed. We analyzed the mRNA levels of all actin genes in *act-5(ptc)* mutants (*Figure 1A*, *Figure 1—figure supplement 2*), and observed the upregulation of *act-3* mRNA and pre-mRNA (*Figure 1B*, *Figure 1—figure supplement 3*), consistent with a transcriptional adaptation response.

We also examined the *act-5(dt2017)* partial deletion allele, hereafter referred to as *act-5(Δ1)*, (*Figure 1—figure supplement 1*) and found no significant change in *act-5* mRNA levels in homozygous mutants compared to wild type (*Figure 1A*). Notably, *act-3* mRNA levels in *act-5(Δ1)* mutants were not changed compared to wild type (*Figure 1B*). To further test whether *act-3* upregulation in *act-5*
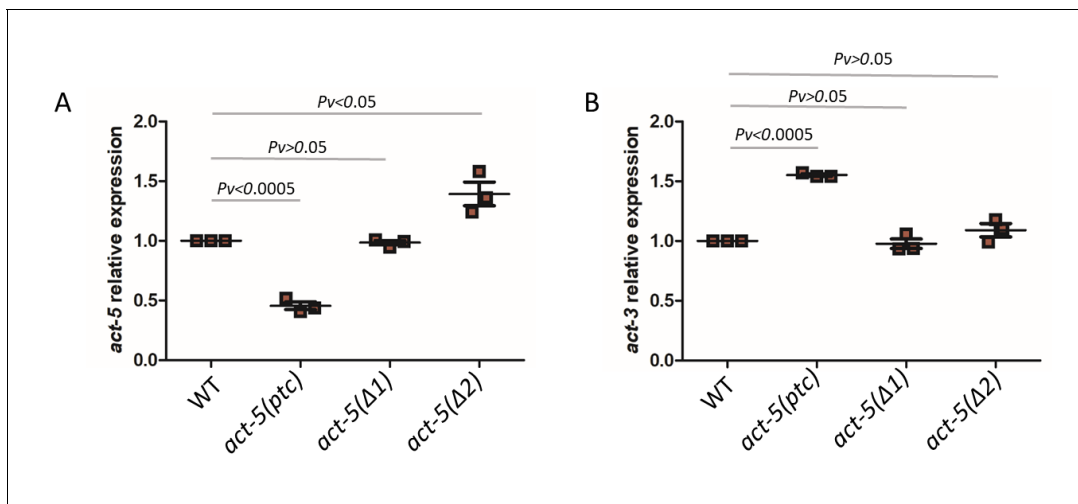
Figure 1. mRNA levels of *act-5* and *act-3* in WT and mutant alleles. qPCR analysis of *act-5* (**A**) and *act-3* (**B**) mRNA levels in WT and *act-5(ptc)*, *act-5(Δ1)*, and *act-5(Δ2)* mutants. *act-3* mRNA levels are upregulated when *act-5* mutant mRNA levels are reduced (i.e., only in the *act-5(ptc)* allele). WT expression levels are set at 1. Data are mean ± S.E.M.; average dCt values are shown in *Figure 1—source data 1*. Two-tailed Student's t-test was used to calculate *P* values.

The online version of this article includes the following source data and figure supplement(s) for figure 1:

Source data 1. Average dCt values from qPCR analysis of *act-5* and *act-3* mRNA levels in WT and *act-5* mutants.
Source data 2. Average dCt values from qPCR analysis of *act-1*, *act-2* and *act-4* mRNA levels in WT and *act-5(ptc)* mutants.
**Figure supplement 1.** Organization of *act-5* locus.
Figure supplement 2. mRNA levels of *act-1* (**A**), *act-2* (**B**) and *act-4* (**C**) in WT and *act-5(ptc)* mutants.
Figure supplement 3. Pre-mRNA levels of *act-3* in WT and *act-5(ptc)* mutants.

mutants represents a model of transcriptional adaptation, we analyzed another *act-5* deletion allele (*ok1397*) (*Estes et al., 2011*), hereafter referred to as *act-5(Δ2)*. The *ok1397* deletion removes part of the promoter region and the first two exons (*Figure 1—figure supplement 1*). We examined this allele for the presence of any transcripts and identified a new isoform which is present in mutants but not in wild type (see Materials and methods) and consists of only 3′ sequence (*Figure 1—figure supplement 1*). As with the *act-5(Δ1)* deletion allele, we found no changes in *act-5* or *act-3* mRNA levels in *act-5(Δ2)* mutants compared to wild type (*Figure 1B*).

In multicellular organisms, paralogous genes are often expressed in distinct spatiotemporal patterns, an indication of subfunctionalization (*Guschanski et al., 2017*). For example, in *C. elegans*, *act-3* is expressed in the pharynx (*Hunt-Newbury et al., 2007*) while *act-5* is expressed in intestinal cells (*MacQueen et al., 2005*). The models of transcriptional adaptation suggest a cell-autonomous mechanism, that is the mutant mRNA can cell-autonomously trigger transcriptional adaptation. In order to test this hypothesis, we generated transcriptional reporter constructs with the *act-3* or *act-5* promoter region driving the expression of a red florescent protein gene (*Merzlyak et al., 2007*). As expected, we observed expression of the extrachromosomal *act-5p::rfp* transgene in the intestine in wild-type animals (*Figure 2A*) as well as in *act-5(ptc)* mutants (*Figure 2—figure supplement 1*). Likewise, expression of the extrachromosomal *act-3p::rfp* transgene was only observed in the pharynx in wild-type animals (*Figure 2B*), consistent with previous studies (*Hunt-Newbury et al., 2007*). However, extrachromosomal *act-3p::rfp* expression was also observed in the intestine in *act-5(ptc)* mutants (*Figure 2C*), consistent with transcriptional adaptation. In summary, we saw upregulation of expression from a synthetic and extrachromosomal *act-3* promoter in tissues where *act-5* is expressed, supporting the model that the mutant mRNA cell-autonomously triggers transcriptional adaptation.

To identify an additional example of transcriptional adaptation in *C. elegans*, we turned to the *titin* gene family (*Figure 3—source data 1*). Due to their size, *titin* genes are frequent targets of random mutagenesis, and several PTC alleles have been identified (*Jorgensen and Mango, 2002*; *Lipinski et al., 2011*). We focused on *unc-89* which has many nonsense alleles that do not exhibit an obvious phenotype, potentially indicating functional compensation. We identified three different
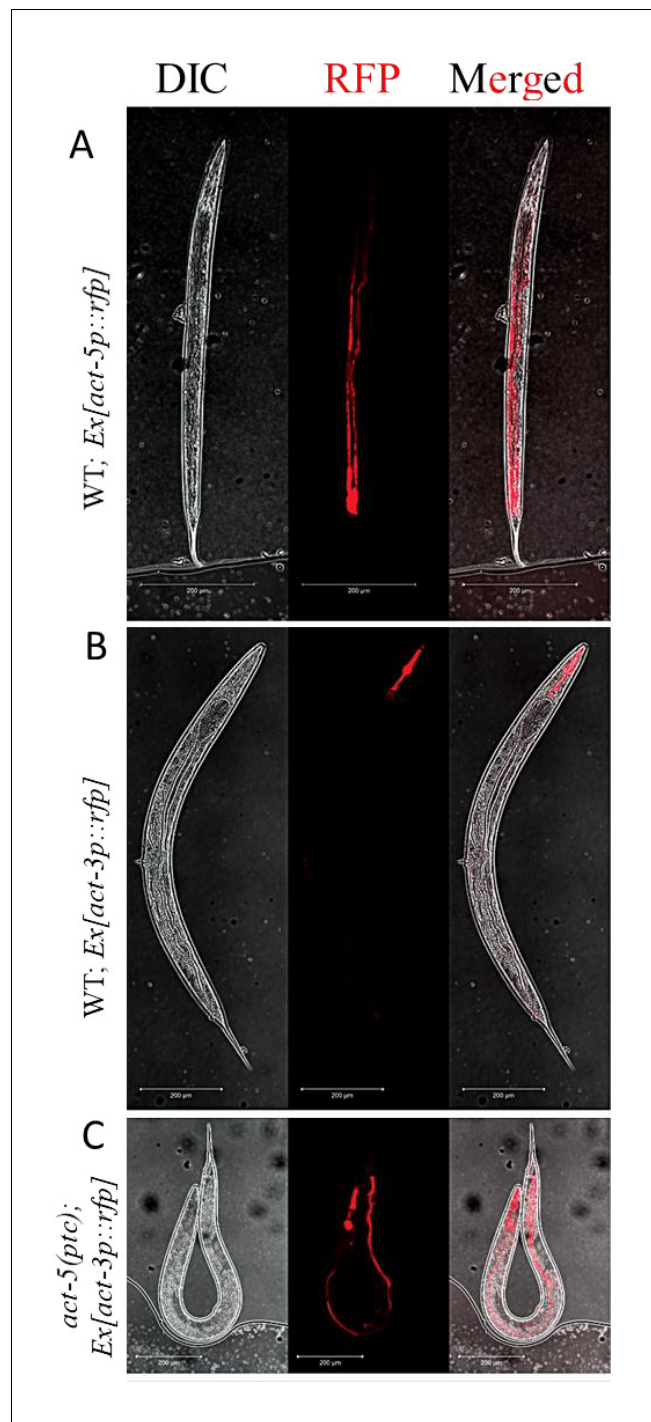
Figure 2. Extrachromosomal reporter expression in WT and mutant alleles. (**A**) *act-5p::rfp* extrachromosomal reporter expression was observed in the intestine in 153 of 300 WT animals. (**B**) *act-3p::rfp* extrachromosomal reporter expression was observed in the pharynx in 182 of 400 WT animals. (**C**) *act-3p::rfp* extrachromosomal reporter expression was observed in the pharynx and intestine in 138 of 320 *act-5(ptc)* mutants.
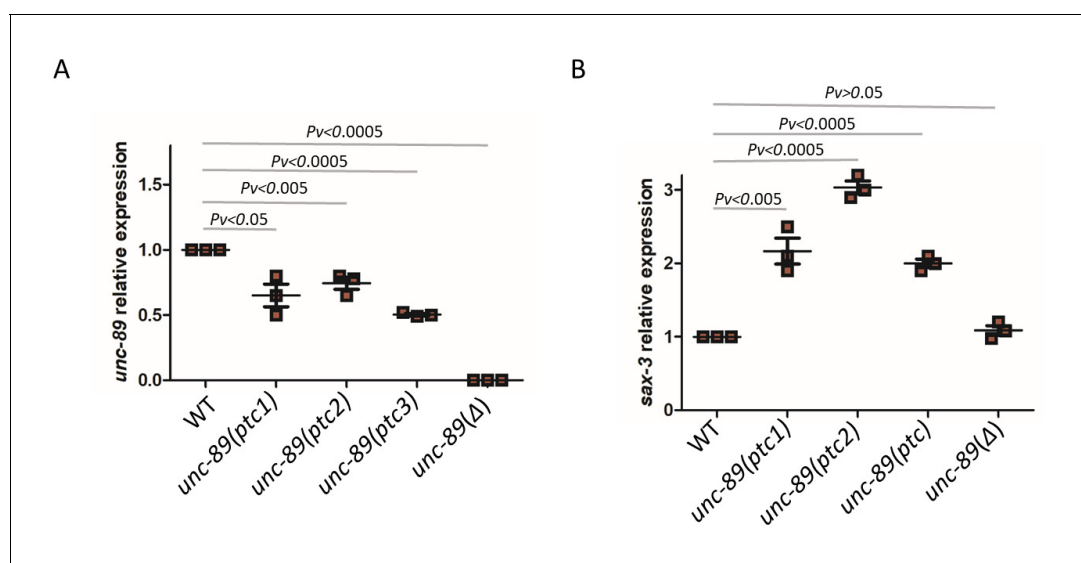The online version of this article includes the following figure supplement(s) for figure 2:

Figure supplement 1. *act-5p::rfp* extrachromosomal reporter expression.

unc-89 alleles (*gk469156*, *gk509355*, *gk506355*) which exhibit lower levels of mutant mRNA compared to wild type (*Figure 3A*) and lack an obvious phenotype. Analyzing the mRNA levels of 10 *titin* related genes (*him-4*, *ttn-1*, *ketn-1*, *sax-3*, *unc-22*, *unc-52*, *sax-7*, *rig-6*, *unc-40*, and *unc-73*), we found that *sax-3* was upregulated in all three *unc-89*(*ptc*) alleles (*Figure 3—figure supplement 1*), both at the mRNA (*Figure 3B*) and pre-mRNA (*Figure 3—figure supplement 2*) levels. To test whether this upregulation of *sax-3* was due to transcriptional adaptation and not to the loss of UNC-89 function, we generated a 16 kb deletion (*bns7000*) in *unc-89*, hereafter referred to as *unc-89*(Δ), using CRISPR/Cas9 genome editing. This deletion removes part of the promoter region and the first several exons (*Figure 3—figure supplement 1*). Hence, most *unc-89* isoforms are not observed in *unc-89*(Δ) mutants (*Figure 3A*). Homozygous *unc-89*(Δ) worms are maternal-effect sterile and exhibit growth defects, phenotypes not observed in the *unc-89*(*ptc*) alleles. In the RNA-less *unc-89*(Δ) allele, *sax-3* was not upregulated (*Figure 3B*), indicating that *sax-3* upregulation is not due to the loss of UNC-89 function and that the mutant mRNA needs to be present for the transcriptional adaptation response. Thus, *sax-3* upregulation in the *unc-89*(*ptc*) alleles is a second example of transcriptional adaptation in *C. elegans*.

To test whether the observed changes in gene expression in *act-5*(*ptc*) and *unc-89*(*ptc*) mutants were specific, we measured *unc-89* and *sax-3* expression in *act-5*(*ptc*) mutants as well as *act-5* and *act-3* expression in *unc-89*(*ptc*) mutants. We observed no significant differences (*Figure 3—figure supplement 3*), suggesting that there is specificity to the gene expression changes.

Identifying additional regulators of transcriptional adaptation

The mutant mRNA has been reported to activate transcriptional adaptation in zebrafish embryos and mouse cell lines (*El-Brolosy et al., 2019*; *Ma et al., 2019*). In order to identify additional factors involved in transcriptional adaptation, we performed a candidate RNA interference (RNAi) screen,

Figure 3. mRNA levels of *unc-89* and *sax-3* in WT and mutant alleles. qPCR analysis of *unc-89* (C) and *sax-3* (D) mRNA levels in WT and *unc-89*(*ptc1*), *unc-89*(*ptc2*), *unc-89*(*ptc3*), and *unc-89*(Δ) mutants. *sax-3* mRNA levels in *unc-89* alleles are upregulated when *unc-89* mutant mRNA levels are reduced, except in the deletion allele. WT expression levels are set at 1. Data are mean ± S.E.M.; average dCt values are shown in *Figure 3—source data 2*. Two-tailed Student's t-test was used to calculate *P* values.

The online version of this article includes the following source data and figure supplement(s) for figure 3:

Source data 1. List of *ttn-1* paralogous genes based on WormBase release WS266.
Source data 2. Average dCt values from qPCR analysis of *unc-89* and *sax-3* mRNA levels in WT and *unc-89* mutants.
Source data 3. Distance, in nucleotides, from each PTC to the next exon-intron junction and to the stop codon in each *unc-89* isoform in the *unc-89*(*ptc1*), *unc-89*(*ptc2*), and *unc-89*(*ptc3*) alleles.
Figure supplement 1. Organization of *unc-89* locus.
Figure supplement 2. Pre-mRNA levels of *sax-3* in WT and *unc-89*(*ptc1*), *unc-89*(*ptc2*), *unc-89*(*ptc3*) mutants.
Figure supplement 3. mRNA levels in WT and mutant alleles.

focusing on genes involved in RNA metabolism (*Figure 4—source data 1*). We knocked down genes involved in mRNA processing including splicing and nonsense-mediated decay, as well as other genes involved in small RNA synthesis and maturation. We measured the mRNA levels of the mutant and adapting genes in order to position RNAi candidates upstream or downstream of mRNA decay. If the gene targeted by RNAi is required for mutant mRNA decay, we expect to see the mRNA levels of the mutant and adapting genes to be similar to wild-type levels. If the gene targeted by RNAi is involved in transcriptional adaptation downstream of mutant mRNA decay, we expect to see the levels of mutant mRNA remaining lower than in wild type, but the levels of the adapting gene's mRNA to be similar to wild-type levels. Finally, if the gene targeted by RNAi is not involved in transcriptional adaptation, we expect to see the levels of mutant mRNA remain lower than in wild type and the expression levels of the adapting gene to remain higher than in wild type. For example, when we knocked down *drsh-1*, a gene involved in miRNA biogenesis (*Denli et al., 2004*), we saw no significant changes in the mRNA levels of the mutant or adapting genes compared to control (*Figure 4—figure supplement 1A,B,C,D*), suggesting that *drsh-1* is not involved in regulating transcriptional adaptation.

Transcriptional adaptation requires the activity of decay factors (*El-Brolosy et al., 2019*; *Ma et al., 2019*), and UPF1, SMG6, and XRN1 were reported to be differentially required in various zebrafish embryo and mouse cell line models of transcriptional adaptation (*El-Brolosy et al., 2019*). In order to test whether NMD factors are involved in regulating transcriptional adaptation in *C. elegans*, we knocked down several NMD genes including *smg-2* (the *C. elegans* orthologue of *Upf1*), *smg-4* (*Upf3*) and *smg-6* (*Smg6*). Knockdown of *smg-2* and *smg-4* blocked the transcriptional adaptation response in all three *unc-89(ptc)* alleles but not in the *act-5(ptc)* allele (*Figure 4*). Conversely, knockdown of *smg-6* blocked the transcriptional adaptation response in the *act-5(ptc)* allele but not in the three *unc-89(ptc)* alleles (*Figure 4*). A differential requirement for Upf1 and Smg6 between gene models was also observed in mouse cells (*El-Brolosy et al., 2019*).

As RNAi efficiency can vary in different tissues (*Ratliff et al., 2006*; *Zhuang and Hunter, 2011*), we generated double mutant strains with *smg-2*, *smg-4*, or *smg-6* mutant alleles and the *act-5(ptc)* and *unc-89(ptc)* alleles to exclude the possibility of tissue-specific knockdown. Analysis of the double mutant strains confirmed the observations made in the RNAi experiments (*Figure 5*). For example, we found that the levels of *act-5* mRNA were lower in *smg-2; act-5(ptc)* and *smg-4; act-5(ptc)* double mutants than in *smg-2* and *smg-4* single mutants, and that the levels of the adapting gene's mRNA were higher (*Figure 5—source data 1*), further indicating that *smg-2* and *smg-4* are not required for transcriptional adaptation in the *act-5* model. However, in *smg-4; unc-89(ptc)* animals, the mRNA levels of the mutant (*unc-89*) and adapting (*sax-3*) genes were similar to those in *smg-4* single mutants. Furthermore, these animals exhibited a mild uncoordinated phenotype and grew slowly, suggesting a lack of functional compensation. These data further indicate that *smg-4* is required for transcriptional adaptation in the *unc-89* model. We could not obtain *smg-6; act-5(ptc)* viable mutants due to severe larval lethality, possibly as a consequence of blocking the transcriptional adaptation response, that is *act-3* upregulation. *smg-6; unc-89(ptc)* mutants exhibited lower levels of *unc-89* mRNA and higher levels of adapting gene mRNA in comparison to single *smg-6* mutants (*Figure 5—source data 1*) similar to the observations in the RNAi experiments. Thus, there are differential requirements for decay factors in different models of transcriptional adaptation.

Previous data indicate that the exonuclease Xrn1 is involved in regulating the transcriptional adaptation response in mouse cells (*El-Brolosy et al., 2019*). Therefore, we tested the role of exonucleases in transcriptional adaptation in *C. elegans*, specifically the exonuclease gene *xrn-1* (*Jones et al., 2012*) and the XRN-2 partner gene *paxt-1* (*Miki et al., 2014*) (*Figure 4*). We found that knocking down *xrn-1* or *paxt-1* led to mutant (*act-5* and *unc-89*) mRNA levels similar to wild-type levels. Furthermore, the transcriptional adaptation response was blocked, suggesting that the degradation and processing of mutant transcripts is important to trigger transcriptional adaptation.

We next looked for additional factors required for transcriptional adaptation. Pre-mRNA splicing and NMD are closely related processes via the positioning and use of the exon junction complex (EJC) (*Lejeune and Maquat, 2005*; *Kashima et al., 2010*; *Fukumura et al., 2016*). SR-protein kinases (SRPK) and their substrates, serine/arginine-rich (SR) splicing factors, are key components of the splicing machinery and are well conserved across phyla (*Kuroyanagi et al., 2000*; *Black, 2003*; *Galvin et al., 2011*). Multiple SR proteins are components of the EJC (*Singh et al., 2012*), consistent with a previously suggested role of SR proteins in mRNA surveillance (*Zhang and Krainer, 2004*).

process	gene	act-5/act-3		unc-89/sax-3	
		mutant mRNA reduction	upregulation of adapting gene	mutant mRNA reduction	upregulation of adapting gene
	control	Yes	Yes	Yes	Yes
mRNA splicing	spk-1	No	No	No	No
	rsp-6	No	No	Yes	Yes
mRNA decay and processing	smg-2	Yes	Yes	No	No
	smg-4	Yes	Yes	No	No
	smg-6	No	No	Yes	Yes
	xrn-1	No	No	No	No
	paxt-1	No	No	No	No
small RNA biogenesis	ergo-1	Yes	No	Yes	No
	nrde-3	Yes	No	Yes	No
	rrf-3	Yes	No	Yes	No
	dcr-1	Yes	No	Yes	No

Figure 4. Factors regulating transcriptional adaptation identified in RNAi-mediated knockdown screen.

The online version of this article includes the following source data and figure supplement(s) for figure 4:

Source data 1. List of genes and RNAi clones tested in the screen; average dCt values of qPCR analyses of act-5 and act-3 mRNA levels in WT and act-5 mutants as well as of unc-89 and sax-3 mRNA levels in WT and unc-89 mutants.

Figure supplement 1. qPCR analysis of act-5 (A) and act-3 (B) mRNA levels in WT and act-5(ptc) mutants as well as of unc-89 (C) and sax-3 (D) mRNA levels in WT and unc-89(ptc) mutants upon drsh-1 RNAi-mediated knockdown by two independent clones.

Figure supplement 2. qPCR analysis of act-5 (A) and act-3 (B) mRNA levels in WT and act-5(ptc) mutants as well as of unc-89 (C) and sax-3 (D) mRNA levels in WT and unc-89(ptc) mutants upon spk-1 RNAi-mediated knockdown by two independent clones.

Figure 4 continued on next page

Figure 4 continued

Figure supplement 3. qPCR analysis of *act-5* (**A**) and *act-3* (**B**) mRNA levels in WT and *act-5(ptc)* mutants as well as of *unc-89* (**C**) and *sax-3* (**D**) mRNA levels in WT and *unc-89(ptc)* mutants upon *nrde-3* RNAi-mediated knockdown by two independent clones.

Knocking down the SRPK gene *spk-1* resulted in mutant mRNA levels similar to wild-type levels, and blocked transcriptional adaptation in all *act-5* and *unc-89 ptc* alleles (***Figure 4—figure supplement 2A,B,C,D***). We also identified the SR family gene *rsp-6* as a regulator of transcriptional adaptation in the *act-5* model, but were unable to identify a single SR protein whose knockdown influenced the transcriptional adaptation response in the *unc-89* model (***Figure 4—source data 1***), possibly due in part to the complexity of the *unc-89* gene structure including the large number of isoforms (***Tourasse et al., 2017***).

The next group of genes we targeted encode factors involved in small RNA (sRNA) biogenesis, maturation and transport into the nucleus. We tested several pathways (***Figure 4—source data 1***) and observed that the argonaute proteins ERGO-1 and NRDE-3, the RNA-dependent RNA polymerase RRF-3, as well as the ribonuclease DCR-1 regulate the transcriptional adaptation response downstream of mRNA decay (*i.e.*, the mutant mRNA was still degraded but the adapting gene was not upregulated) (***Figure 4***, ***Figure 4—figure supplement 3A,B,C,D***, ***Figure 4—source data 1***). These RNAi data were confirmed by analyzing double mutants of the *act-5(ptc)* or *unc-89(ptc)* alleles with *ergo-1*, *nrde-3*, and *rrf-3* (***Figure 5***), and all these animals exhibited phenotypes comparable to the

process	gene (allele)	act-5/act-3		unc-89/sax-3	
		mutant mRNA reduction	upregulation of adapting gene	mutant mRNA reduction	upregulation of adapting gene
mRNA decay	*smg-2(e2008)*	Yes	Yes	not tested	not tested
	smg-4(ma116)	Yes	Yes	No	No
	smg-6(r896)	not tested	not tested	Yes	Yes
small RNA biogenesis	*ergo-1(gg100)*	Yes	No	Yes	No
	nrde-3(gg66)	Yes	Yes	Yes	No
	rrf-3(mgm373)	Yes	No	Yes	No

Figure 5. Factors regulating transcriptional adaptation analyzed in double mutants.
The online version of this article includes the following source data and figure supplement(s) for figure 5:

Source data 1. List of genes and alleles for each gene tested in the double mutant analysis; average dCt values from qPCR analyses of *act-5* and *act-3* mRNA levels in WT and *act-5* mutants as well as of *unc-89* and *sax-3* mRNA levels in WT and *unc-89* mutants.
Figure supplement 1. Partial data from double mutant analysis.

act-5 or *unc-89* deletion alleles analyzed in this study including larval lethality, slow growth and unco-ordinated movements, indicating lack of functional compensation. Notably, ERGO-1, NRDE-3, RRF-3, and DCR-1 are involved in 26G RNA biogenesis (*Pavelec et al., 2009*; *Vasale et al., 2010*; *Fischer, 2010*; *Grishok, 2013*; *Yvert, 2014*), suggesting that 26G RNAs could play a role in transcriptional adaptation.

Together, these results indicate that mRNA decay as well as small RNA biogenesis and transport are critical in triggering transcriptional adaptation.

Discussion

Recent advances in reverse genetic tools have significantly expanded our ability to generate genetic modifications in a wide range of organisms (*Housden et al., 2017*). However, some engineered mutants exhibit no apparent phenotype, renewing interest in the concept of genetic robustness. Genetic compensation, and in particular transcriptional adaptation, have been proposed as a means to achieve genetic robustness upstream of protein feedback loops. Despite the potential importance of transcriptional adaptation, its underlying molecular mechanisms remain relatively unexplored. Here, we report two cases of transcriptional adaptation in *C. elegans*. By carrying out a small RNAi screen and a follow up analysis using double mutants, we identified several new factors that regulate transcriptional adaptation and further validated previously identified ones.

In the *C. elegans act-5* model, the mutant gene and related adapting gene (*act-5* and *act-3*, respectively) are primarily expressed in distinct tissues. However, using an extrachromosomal transcriptional reporter, we observed that in *act-5(ptc)* mutants, the *act-3* promoter adapts to drive transcription in the primary site of *act-5* expression, the intestine. As *act-5(ptc)* mutants do not exhibit any obvious phenotype when the transcriptional adaptation response is intact, we predict that ACT-3 and/or other proteins are able to compensate for the loss of ACT-5. Indeed, when we disrupted transcriptional adaptation, *act-5(ptc)* mutants did not survive. Thus, transcriptional adaptation can in some cases entail the change in the pattern of expression of related gene(s) and suppress phenotypes that would alter the animal's fitness.

Based on the factors identified in this study, we hypothesize that the transcriptional adaptation response consists of at least three critical processes: mutant mRNA decay, sRNA maturation and sRNA transport. In terms of mutant mRNA decay, we found that the machinery can be gene-specific. In our experiments, SMG-6 is involved in *act-5(ptc)* mRNA decay, while SMG-2 (UPF1) and SMG-4 (UPF3) impact *unc-89(ptc)* mRNA decay. Similar observations were made in mouse *Actb* and *Rela* mutant cells in which siRNA-mediated knock down of SMG6 blocked the transcriptional adaptation response in *Actb* mutant mESCs but had little influence on *Rela* mutant MEFs. Conversely, siRNA-mediated knockdown of UPF1 blocked the transcriptional adaptation response in *Rela* mutant MEFs but not in *Actb* mutant mESCs (*El-Brolosy et al., 2019*). Consistently, mutant mRNA decay can involve different factors in the same organism (*Nickless et al., 2017*), possibly due to differential expression of the decay factors. However, we cannot exclude the possibility that SMG6 could function as a decay factor independent of NMD, especially since it has been reported to have NMD-independent cleavage activity (*Gehring et al., 2005*; *Glavan et al., 2006*; *Huntzinger et al., 2008*; *Chakrabarti et al., 2014*).

Transcriptional adaptation can be triggered by the degradation products of the mutant mRNA (*El-Brolosy et al., 2019*), which could seed the generation of sRNAs (*Mattick and Makunin, 2005*; *Boivin et al., 2018*). We found that factors involved in sRNA maturation and transport, including RRF-3, DCR-1, ERGO-1 and NRDE-3, also regulate transcriptional adaptation. Transcriptional modulation of genes by sRNAs of approximately 20–30 nucleotides in length is a widespread and diverse feature of prokaryotes (*Melamed et al., 2019*) and eukaryotes (*Ambros et al., 2003*; *Yigit et al., 2006*; *Hutvagner and Simard, 2008*; *Portnoy et al., 2011*; *Castel and Martienssen, 2013*; *Rechavi and Lev, 2017*; *Billmyre et al., 2019*). Notably, the factors we identified are known to be involved in somatic gene regulation by sRNAs, described as the RRF-3 pathway (*Gent et al., 2010*). RRF-3 is an RNA-dependent RNA polymerase involved along with the DICER complex in the biogenesis of 26-nucleotide RNAs with 5′ bias for guanosine monophosphate (26G-RNAs) (*Han et al., 2009*; *Gent et al., 2010*; *Vasale et al., 2010*). 26G sRNAs associate with the Argonaute protein ERGO-1, which is involved in the further maturation of sRNAs and is required to separate the sRNA duplex (*Han et al., 2009*; *Gent et al., 2010*; *Fischer et al., 2011*). Mature sRNAs interacting with

Argonaute proteins can direct post-transcriptional gene silencing (*Vasale et al., 2010*; *Phillips et al., 2012*), or be transported into the nucleus (*Guang et al., 2008*; *Buckley et al., 2012*; *Shirayama et al., 2012*). NRDE-3 is another Argonaute protein involved in transporting sRNAs into the nucleus (*Guang et al., 2008*), and we found that knocking it down, and knocking it out, blocked transcriptional adaptation while not affecting mutant mRNA levels. While sRNAs are best known as repressors of gene expression, they can also function as activators (*Li et al., 2006*; *Janowski et al., 2006*; *Turunen et al., 2009*; *Portnoy et al., 2011*; *Wedeles et al., 2013*; *Li, 2017*), although the underlying mechanisms remain poorly understood (*Portnoy et al., 2011*). Some of these activating sRNAs interact with Argonaute proteins (*Seth et al., 2013*), and they can target gene regulatory sequences including promoters. Whether they can also interfere with antisense RNAs, which usually function to repress gene expression (*Faghihi and Wahlestedt, 2009*; *Modarresi et al., 2012*), is a hypothesis worth testing given our observations in zebrafish embryos and mouse cell lines (*El-Brolosy et al., 2019*) as well as the previously suggested role of Argonaute proteins in such a process (*Ghanbarian et al., 2017*).

The transcriptional adaptation factors identified here came from a candidate screen where we specifically targeted pathways involved in RNA metabolism. With this study, we have established *C. elegans* as a genetic model system to perform unbiased screens to help reveal further mechanisms of transcriptional adaptation, a newly uncovered phenomenon contributing to genetic robustness.

Materials and methods

Key resources table

Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Gene (*Caenorhabditis elegans*)	*act-1*		CELE_T04C12.6	WormBase ID: WBGene00000063
Gene (*Caenorhabditis elegans*)	*act-2*		CELE_T04C12.5	WBGene00000064
Gene (*Caenorhabditis elegans*)	*act-3*		CELE_T04C12.4	WBGene00000065
Gene (*Caenorhabditis elegans*)	*act-4*		CELE_M03F4.2	WBGene00000066
Gene (*Caenorhabditis elegans*)	*act-5*		CELE_T25C8.2	WBGene00000067
Gene (*Caenorhabditis elegans*)	*unc-89*		CELE_C09D1.1	WBGene00006820
Gene (*Caenorhabditis elegans*)	*sax-3*		CELE_ZK377.2	WBGene00004729
Strain, strain background (*C. elegans*)	N2	CGC, Bristol strain		wild type
Strain, strain background (*C. elegans*)	IN2049	*MacQueen et al., 2005*		*act-5(ptc); dtIs 419[act-5+ rol-6(d)]*
Strain, strain background (*C. elegans*)	IN2051	*MacQueen et al., 2005*		*act-5(Δ1); dtIs 419[act-5+ rol-6(d)]*
Strain, strain background (*C. elegans*)	VC971	CGC, *Estes et al., 2011*		*+/mT1; act-5(Δ2)/ mT1 [dpy-10(e128)].*
Strain, strain background (*C. elegans*)	CB4043	CGC, *Hodgkin et al., 1989*		*smg-2(e2008);him-5(e1490)*
Strain, strain background (*C. elegans*)	CB4355	CGC, *Pulak and Anderson, 1993*		*smg-4(ma116);him-8(e1490)*
Strain, strain background (*C. elegans*)	TR1396	CGC, *Pulak and Anderson, 1993*		*smg-6(r896)*
Strain, strain background (*C. elegans*)	YY168	CGC, *Pavelec et al., 2009*		*ergo-1(gg100)*

Continued on next page

Continued

Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Strain, strain background (*C. elegans*)	YY158	CGC, *Guang et al., 2008*		*nrde-3(gg66)*
Strain, strain background (*C. elegans*)	YY13	CGC, *Pavelec et al., 2009*		*rrf-3(mg373)*
Strain, strain background (*C. elegans*)	DYS0005	This study, crossed IN2049 to N2		*act-5(ptc)*
Strain, strain background (*C. elegans*)	DYS0004	This study, crossed IN2049 to N2		*+/act-5(Δ1)*
Strain, strain background (*C. elegans*)	DYS0012	This study, injected in N2		*Ex[act-5p::RFP]*
Strain, strain background (*C. elegans*)	DYS0014	This study, injected in N2		*Ex[act-3p::RFP]*
Strain, strain background (*C. elegans*)	DYS0015	This study, crossed DYS0014 to DYS0004		*act-5(ptc);Ex[act-3p::RFP]*
Strain, strain background (*C. elegans*)	DYS0042	This study, crossed DYS0012 to DYS0005		*act-5(ptc);Ex[act-5p::RFP]*
Strain, strain background (*C. elegans*)	VC40114	CGC, Million Mutation Project		*unc-89(ptc1)*
Strain, strain background (*C. elegans*)	VC40193	CGC, Million Mutation Project		*unc-89(ptc2)*
Strain, strain background (*C. elegans*)	VC40199	CGC, Million Mutation Project		*unc-89(ptc3)*
Strain, strain background (*C. elegans*)	DYS0028	This study, crossed VC40114 to N2		*unc-89(ptc1)*
Strain, strain background (*C. elegans*)	DYS0030	This study, crossed VC40193 to N2		*unc-89(ptc2)*
Strain, strain background (*C. elegans*)	DYS0031	This study, crossed VC40199 to N2		*unc-89(ptc3)*
Strain, strain background (*C. elegans*)	DYS0037	This study, induced by CRISPR/Cas9		*unc-89(Δ)*
Strain, strain background (*C. elegans*)	DYS0008	This study, crossed DYS0005 to CB4043		*smg-2(e2008); act-5(ptc)*
Strain, strain background (*C. elegans*)	DYS0057	This study, crossed DYS0005 to CB4355		*act-5(ptc); smg-4(ma116)*
Strain, strain background (*C. elegans*)	DYS0047	This study, crossed DYS0028 to CB4355		*unc-89(ptc1); smg-4(ma116)*
Strain, strain background (*C. elegans*)	DYS0048	This study, crossed DYS0030 to CB4355		*unc-89(ptc2); smg-4(ma116)*
Strain, strain background (*C. elegans*)	DYS0050	This study, crossed DYS0031 to CB4355		*unc-89(ptc3); smg-4(ma116)*
Strain, strain background (*C. elegans*)	DYS0053	This study, crossed DYS0028 to TR1396		*unc-89(ptc1); smg-6(r896)*
Strain, strain background (*C. elegans*)	DYS0055	This study, crossed DYS0030 to TR1396		*unc-89(ptc2); smg-6(r896)*
Strain, strain background (*C. elegans*)	DYS0056	This study, crossed DYS0031 to TR1396		*unc-89(ptc3); smg-6(r896)*
Strain, strain background (*C. elegans*)	DYS0010	This study, crossed DYS0005 to YY168		*act-5(ptc); ergo-1(gg100)*
Strain, strain background (*C. elegans*)	DYS0054	This study, crossed DYS0028 to YY168		*unc-89(ptc1); ergo-1(gg100)*
Strain, strain background (*C. elegans*)	DYS0051	This study, crossed DYS0030 to YY168		*unc-89(ptc2); ergo-1(gg100)*

Continued on next page

Continued

Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Strain, strain background (*C. elegans*)	DYS0052	This study, crossed DYS0031 to YY168		*unc-89(ptc3); ergo-1(gg100)*
Strain, strain background (*C. elegans*)	DYS0045	This study, crossed DYS0005 to YY158		*act-5(ptc); nrde-3(gg66)*
Strain, strain background (*C. elegans*)	DYS0065	This study, crossed DYS0028 to YY158		*unc-89(ptc1); nrde-3(gg66)*
Strain, strain background (*C. elegans*)	DYS0072	This study, crossed DYS0030 to YY158		*unc-89(ptc2); nrde-3(gg66)*
Strain, strain background (*C. elegans*)	DYS0066	This study, crossed DYS0031 to YY158		*unc-89(ptc3); nrde-3(gg66)*
Strain, strain background (*C. elegans*)	DYS0046	This study, crossed DYS0005 to YY13		*rrf-3(mg373); act-5(ptc)*
Strain, strain background (*C. elegans*)	DYS0070	This study, crossed DYS0028 to YY13		*unc-89(ptc1); rrf-3(mg373)*
Strain, strain background (*C. elegans*)	DYS0062	This study, crossed DYS0030 to YY13		*unc-89(ptc2); rrf-3(mg373)*
Strain, strain background (*C. elegans*)	DYS0063	This study, crossed DYS0031 to YY13		*unc-89(ptc3); rrf-3(mg373)*
Commercial assay or kit	In-Fusion HD Cloning	Clontech	Clontech:639647	
Commercial assay or kit	Superscript III reverse transcriptase	Takara	Cat. No: 18080–044	
Commercial assay or kit	SMARTer RACE cDNA Amplification Kit	Takara	Cat. N. 634860	
Commercial assay or kit	Advantage 2 PCR kit	Takara	Cat. N. 639207	
RNAi construct	mv_C18D11.4	BioScience		*rsp-8*
RNAi construct	sjj2_C18D11.4	BioScience		*rsp-8*
RNAi constructs	mv_C33H5.12	BioScience		*rsp-6*
RNAi constructs	sjj2_C33H5.12	BioScience		*rsp-6*
RNAi constructs	mv_W02B12.3	BioScience		*rsp-1*
RNAi constructs	sjj2_W02B12.3	BioScience		*rsp-1*
RNAi constructs	mv_D2089.1	BioScience		*rsp-7*
RNAi constructs	sjj2_D2089.1	BioScience		*rsp-7*
RNAi constructs	mv_B0464.5	BioScience		*spk-1*
RNAi constructs	sjj2_B0464.5	BioScience		*spk-1*
RNAi constructs	mv_R05D11.6	BioScience		*paxt-1*
RNAi constructs	sjj2_R05D11.6	BioScience		*paxt-1*
RNAi constructs	mv_F43E2.8	BioScience		*hsp-4*
RNAi constructs	sjj2_F43E2.8	BioScience		*hsp-4*
RNAi constructs	sjj2_Y39G8C.1	BioScience		*xrn-1*
RNAi constructs	mv_Y48G8AL.6	BioScience		*smg-2*
RNAi constructs	sjj2_Y48G8AL.6	BioScience		*smg-2*
RNAi constructs	sjj2_F46B6.3	BioScience		*smg-4*
RNAi constructs	mv_Y54F10AL.2	BioScience		*smg-6*
RNAi constructs	sjj2_Y54F10AL.2	BioScience		*smg-6*
RNAi constructs	mv_F26B1.2	BioScience		*hrpk-1*
RNAi constructs	sjj2_F26B1.2	BioScience		*hrpk-1*

Continued

Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
RNAi constructs	mv_F26E4.10	BioScience		*drsh-1*
RNAi constructs	sjj2_F26E4.10	BioScience		*drsh-1*
RNAi constructs	mv_T22A3.5	BioScience		*pash-1*
RNAi constructs	sjj2_T22A3.5	BioScience		*pash-1*
RNAi constructs	sjj2_F26A3.8	BioScience		*rrf-1*
RNAi constructs	mv_ R06C7.1	BioScience		*wago-1*
RNAi constructs	sjj2_ R06C7.1	BioScience		*wago-1*
RNAi constructs	mv_F58G1.1	BioScience		*wago-4*
RNAi constructs	sjj2_F58G1.1	BioScience		*wago-4*
RNAi constructs	sjj2_F10B5.7	BioScience		*rrf-3*
RNAi constructs	mv_M88.5	BioScience		*zbp-1*
RNAi constructs	sjj2_M88.5	BioScience		*zbp-1*
RNAi constructs	sjj2_K12H4.8	BioScience		*dcr-1*
RNAi constructs	mv_T20G5.11	BioScience		*rde-4*
RNAi constructs	sjj2_T20G5.11	BioScience		*rde-4*
RNAi constructs	mv_F36H1.2	BioScience		*kdin-1*
RNAi constructs	mv_K12B6.1	BioScience		*sago-1*
RNAi constructs	sjj2_K12B6.1	BioScience		*sago-1*
RNAi constructs	mv_K08H10.7	BioScience		*rde-1*
RNAi constructs	sjj2_K08H10.7	BioScience		*rde-1*
RNAi constructs	sjj2_R09A1.1	BioScience		*ergo-1*
RNAi constructs	mv_R04A9.2	BioScience		*nrde-3*
RNAi constructs	sjj2_R04A9.2	BioScience		*nrde-3*

Culture conditions and strains

All wild-type worms were the N2 reference strain. All *C. elegans* strains were kept on 6 cm plates with nematode growth medium agar and fed with a lawn of *E. coli* OP50 grown in 500 µl Luria broth, except for the RNAi mediated knockdown experiments where the worms were fed with *E. coli* expressing the respective double-stranded RNA. Cultures were maintained at 20°C. Also, to minimize the potential for laboratory evolution of the trait, a new culture of the strains was revived annually from frozen stocks. All plates with fungal or bacterial contamination were excluded from the experiments.

Synchronization of cultures for RNA isolation

Worms from healthy cultures were washed off of plates using M9 buffer and passed through a 41 µm filter (Millipore Cat. No SCNY00040) with vacuum; antibiotics (Ampicillin, Chloramphenicol) were added (50 µg/ml final concentration) to eliminate remaining food bacteria, and the worms were then incubated on a shaker at room temperature for 15 min. Worms were centrifuged at 3000 rpm for 5 min to pellet early larval stage animals. The buffer was aspirated and 1 ml of fresh buffer was added to resuspend the pellet. Samples were confirmed to be primarily L1 and L2 stage larvae by observing two 5 µl samples on a 6 cm nematode growth medium plate. Starving cultures or cultures that had more than one male were excluded from the experiments.

qPCR analysis

Total RNA from synchronized cultures or manually picked young adults was isolated using TRIzol (ambion by Takara). For reverse transcription (RT), Superscript III reverse transcriptase (Invitrogen, Cat. No: 18080–044) was used following manufacturer's instructions. We used 1–2 µg total RNA for

each RT reaction. The qPCR experiments were performed on a CFX Connect Real-Time System (Bio-rad-Roche Diagnostics) as described previously (*El-Brolosy et al., 2019*). *cdc-42* and *Y45F10D.4* (*iscu-1*) were used as reference genes as described previously (*Hoogewijs et al., 2008*), and the Ct values ranged from 12.3 to 28.4 for *cdc-42* and 11.8 to 26 for *Y45F10D.4*. The Ct values for all other genes were aimed to be below 30.

The following primers were used to amplify the cDNA of target genes: *Y45F10D.4* (forward 5'-CGAGAACCCGCGAAATGTCGGA-3' and reverse 5'- CGGTTGCCAGGGAAGATGAGGC-3'), *cdc-42* (forward 5'-AGCCATTCTGGCCGCTCTCG-3' and reverse 5'- GCAACCGCTTCTCGTTTGGC-3'), *act-1* (forward 5'-ACGACGAGTCCGGCCCATCC-3' and reverse 5'-GAAAGCTGGTGGTGACGATGGTT-3'), *act-2* (forward 5'-GCGCAAGTACTCCGTCTGGATCG-3' and reverse 5'- GGGTGTGAAAATCCGTAAGGCAGA-3'), *act-3* (forward 5'-AAGCTCTTCGCCTTACCATTTTCTC-3' and reverse 5'-ACAGAGCAAATTGTAGTGGGGTCTTC-3'), *act-4* (forward 5'-AGAGGCTCTCTTCCAGCCATCCTTC-3' and reverse 5'-TGATCTTGATCTTCATGGTGGATGG-3'), *act-5* (forward 5'- AAGTGCGATGTCGACATCAGAAAG-3' and reverse 5'- TAATCTTGATCTTCATTGTGCTTGG-3'), *act-5d* (forward 5'- AAGTGCGATGTCGACATCAGAAAG-3' and reverse 5'- TAATCTTGATCTTCATTGTGCTCCGG-3'), *unc-89* (forward 5'-AAGGCTGAACTTGTCATCGAAGGAG-3' and reverse 5'-TCATCTCCACAACATTACCCTCGTG-3'), *sax-3* (forward 5'-TGCCGTTTGTCCCGTAACAACTATG-3' and reverse 5'-ATCTTCTGAAGCTGACGGGGAGAAC-3'), *act-3* pre-mRNA (forward 5'-TTTTTCAGAACCATGAAGATCA-3' and reverse 5'-GAAAATGGTAAGGCGAAGAGC-3'), *sax-3* pre-mRNA (forward 5'-TGTAAAACCGCACTGCACAAT-3' and reverse 5'-TCCACCAAGAGCCTGAAAAC-3'). PCR efficiency was determined using external standards on plasmid mini-preparation of cloned PCR products. Expression levels were analyzed by basic relative quantification. qPCR data are based on three biological replicates and three technical replicates for each biological replicate.

Rapid amplification of cDNA ends (RACE)

Total RNA from manually picked young wild-type and *act-5(Δ2)* mutant adults was isolated using TRIzol (ambion by Takara). 5' and 3' RACE ready cDNA was synthesized by reverse transcription PCR using a SMARTer RACE cDNA Amplification Kit following manufacturer's protocol (Cat. N. 634860, Takara). PCRs were performed using an Advantage 2 PCR kit (Cat. N. 639207, Takara). The following gene-specific primers and nested gene-specific primers were used to amplify 3' and 5' cDNA ends: act5GSP2 (5'-ACCACCGGAATCGTTTTGGACACCGGAG-3'), act5NGSP2 (5'-GAAGGATATGCCCTCCCACATGCCATCC-3'), act5GSP1 (5'-AAAAATCAGCTTAGAAGCACTTTCGGTG-3'), act5NGSP1 (5'-TCGATGGGCCGGACTCGTCGTACTCCTG-3'), unc89GSP2 (5'-TTTGGTACCATTTGTATAGAGGCGAGTG-3'), unc89NGSP2 (5'-TTCTGAACTGGACAAATCTTGCTTTTCG-3), unc89N1GSP2 (5'- ACTTTCCAGTATCTCCTGGATGTTGCTTC-3'), and unc89N2GSP2 (5'- TTTGAATACTTTTTGATGAACCGTGTGC-3'). RACE experiment revealed an isoform with an alternative start which is present only in *act-5(Δ2)* mutants (*Figure 1—figure supplement 1A*). This new isoform is not affected by the large deletion, and thus the corresponding mRNA is not degraded (*Figure 1A*).

Plasmid construction and genetic transformation

To study the expression of *act-5*, we generated a reporter construct with an *act-5* promoter region (2.5 kb from III:13606066 to 13608569) fused to turboRFP in a pUC19 vector. Similarly, a pUC19 vector containing turboRFP was fused with an *act-3* promoter region (4.5 kb from V:11073234 to 11077791). The germ line of wild-type animals was injected with the generated plasmids (10 ng ul^{-1}). The transgenic lines were subsequently crossed with *act-5(ptc)* mutants to transfer the extrachromosomal array to the mutant background.

Confocal microscopy

A Zeiss LSM 700 confocal microscope was used to image adult worms.

RNA interference mediated knockdown

RNAi was performed by feeding double-stranded RNA-expressing bacteria at 25°C from the early larval stage through adulthood (60–75 hr) as previously described (*Fraser et al., 2000*). For the genes whose knockdown from an early larval stage caused lethality or sterility, we started the RNAi treatment at later stages (L4, adult). Also, for some clones (mv_R05D11.6, sjj2_R05D11.6,

sjj2_Y39G8C.1), we diluted the double-stranded RNA-expressing bacteria with empty vector (L4440)-containing bacteria, in order to obtain milder effects. RNAi constructs were obtained from available libraries (Source BioScience) and verified by sequencing. RNAi clones used in this study are listed in the key resources table.

CRISPR/Cas9 induced mutations

To generate the CRISPR/Cas9-induced *unc-89* deletion allele (*bns7000*), two sgRNAs (final concentration 4 uM each) were injected with Cas9 protein (0.35 ug/ul), and a *dpy-10* sgRNA (2.5 uM) was used as a co-injection marker along with a repair oligo (PSdpy-10-PS; 0.73 uM) (*Dickinson and Goldstein, 2016*).

sgRNA1: 5′-GGTAGTTAGCGACCCCATGAGGG-3′.
sgRNA2: 5′-ACAGACTGGTAAACAAACGAGGG-3′

The following primers were used for genotyping: dunc-89–1 forward (5′-ATACCACCACATGTC TCTTC-3′), dunc-89–2 forward (5′-GCTAAAAGTCAGAGTTCCAC-3′), dunc-89–3 reverse (5′- GGA TGGGTTTACATAAAAT-3′), dunc-89–4 reverse (5′-TGAAAAAGAAACAACAAAA-3′), dunc-89–5 forward (5′-TAACAAAAAGCTCAAAATG-3′), dunc-89–6 reverse (5′-GGATAGATTTCTGTTGGAGA-3′). The external primers flank a 19612 bp region in wild types and amplify a 3601 bp fragment in *bns7000* mutants. The internal primers with different combinations amplify 500-2600 bp products in wild types.

Double mutant analysis

All the double mutants exhibited gene expression levels as in the RNAi treated animals with one exception. *act-5(ptc); nrde-3* double mutants exhibited *act-5* mRNA levels as in the RNAi experiments but also some upregulation of the adapting gene, unlike what was observed in the RNAi experiments (*Figures 4* and *5*, *Figure 5—figure supplement 1A and B*). One possible explanation is related to an alternative start site of *nrde-3* (*Tourasse et al., 2017*) which might be used only in some tissues and thus could lead to some protein function in the allele used in our study.

Statistical evaluation

To calculate the significance of the differences for the expression data, we performed two-tailed Student's t-test. Mean ± SEM is indicated in graphs. All statistical analyses were implemented in the program Statistica v. 9. Graphs were generated in Prism5.

Gene structure visualization

The *act-5* and *unc-89* loci were visualized using the GSDS gene structure visualization tool (*Guo et al., 2007*).

Additional information

Competing interests

Didier YR Stainier: Senior editor, *eLife*. The other authors declare that no competing interests exist.

Author contributions

Vahan Serobyan, Conceptualization, Data curation, Formal analysis, Validation, Visualization, Methodology, Writing - original draft, Writing - review and editing; Zacharias Kontarakis, Conceptualization, Data curation, Formal analysis, Validation, Visualization, Methodology, Writing - review and editing; Mohamed A El-Brolosy, Data curation, Formal analysis, Methodology, Writing - review and editing; Jordan M Welker, Ann M Wehman, Validation, Writing - review and editing; Oleg Tolstenkov, Data curation, Methodology; Amr M Saadeldein, Data curation; Nicholas Retzer, Data curation, Validation; Alexander Gottschalk, Supervision; Didier YR Stainier, Conceptualization, Resources, Supervision, Funding acquisition, Investigation, Writing - original draft, Project administration, Writing - review and editing

Author ORCIDs

Vahan Serobyan (iD) https://orcid.org/0000-0001-5055-8422
Oleg Tolstenkov (iD) https://orcid.org/0000-0002-6484-9965
Amr M Saadeldein (iD) https://orcid.org/0000-0002-5971-2083
Alexander Gottschalk (iD) http://orcid.org/0000-0002-1197-6119
Ann M Wehman (iD) http://orcid.org/0000-0001-9826-4132
Didier YR Stainier (iD) https://orcid.org/0000-0002-0382-0026

Decision letter and Author response

Decision letter https://doi.org/10.7554/eLife.50014.sa1
Author response https://doi.org/10.7554/eLife.50014.sa2

Additional files

Data availability

All data generated or analyzed during this study are included in the manuscript and supporting files.

References

Ambros V, Lee RC, Lavanway A, Williams PT, Jewell D. 2003. MicroRNAs and other tiny endogenous RNAs in *C. elegans*. *Current Biology* **13**:807–818. DOI: https://doi.org/10.1016/S0960-9822(03)00287-2, PMID: 12747828

Billmyre KK, Doebley AL, Spichal M, Heestand B, Belicard T, Sato-Carlton A, Flibotte S, Simon M, Gnazzo M, Skop A, Moerman D, Carlton PM, Sarkies P, Ahmed S. 2019. The meiotic phosphatase GSP-2/PP1 promotes germline immortality and small RNA-mediated genome silencing. *PLOS Genetics* **15**:e1008004. DOI: https://doi.org/10.1371/journal.pgen.1008004, PMID: 30921322

Black DL. 2003. Mechanisms of alternative pre-messenger RNA splicing. *Annual Review of Biochemistry* **72**:291–336. DOI: https://doi.org/10.1146/annurev.biochem.72.121801.161720, PMID: 12626338

Boivin V, Deschamps-Francoeur G, Scott MS. 2018. Protein coding genes as hosts for noncoding RNA expression. *Seminars in Cell & Developmental Biology* **75**:3–12. DOI: https://doi.org/10.1016/j.semcdb.2017.08.016, PMID: 28811264

Buckley BA, Burkhart KB, Gu SG, Spracklin G, Kershner A, Fritz H, Kimble J, Fire A, Kennedy S. 2012. A nuclear Argonaute promotes multigenerational epigenetic inheritance and germline immortality. *Nature* **489**:447–451. DOI: https://doi.org/10.1038/nature11352

Castel SE, Cervera A, Mohammadi P, Aguet F, Reverter F, Wolman A, Guigo R, Iossifov I, Vasileva A, Lappalainen T. 2018. Modified penetrance of coding variants by cis-regulatory variation contributes to disease risk. *Nature Genetics* **50**:1327–1334. DOI: https://doi.org/10.1038/s41588-018-0192-y

Castel SE, Martienssen RA. 2013. RNA interference in the nucleus: roles for small RNAs in transcription, epigenetics and beyond. *Nature Reviews Genetics* **14**:100–112. DOI: https://doi.org/10.1038/nrg3355, PMID: 23329111

Chakrabarti S, Bonneau F, Schüssler S, Eppinger E, Conti E. 2014. Phospho-dependent and phospho-independent interactions of the helicase UPF1 with the NMD factors SMG5-SMG7 and SMG6. *Nucleic Acids Research* **42**:9447–9460. DOI: https://doi.org/10.1093/nar/gku578, PMID: 25013172

Conant GC, Wagner A. 2004. Duplicate genes and robustness to transient gene knock-downs in *Caenorhabditis elegans* . *Proceedings of the Royal Society of London. Series B: Biological Sciences* **271**:89–96. DOI: https://doi.org/10.1098/rspb.2003.2560

Cui M, Fay DS, Han M. 2004. *lin-35/Rb* cooperates with the SWI/SNF complex to control *Caenorhabditis elegans* larval development. *Genetics* **167**:1177–1185. DOI: https://doi.org/10.1534/genetics.103.024554, PMID: 152 80233

Denli AM, Tops BB, Plasterk RH, Ketting RF, Hannon GJ. 2004. Processing of primary microRNAs by the microprocessor complex. *Nature* **432**:231–235. DOI: https://doi.org/10.1038/nature03049, PMID: 15531879

Dickinson DJ, Goldstein B. 2016. CRISPR-Based methods for *Caenorhabditis elegans* Genome Engineering. *Genetics* **202**:885–901. DOI: https://doi.org/10.1534/genetics.115.182162, PMID: 26953268

El-Brolosy MA, Kontarakis Z, Rossi A, Kuenne C, Günther S, Fukuda N, Kikhi K, Boezio GLM, Takacs CM, Lai S-L, Fukuda R, Gerri C, Giraldez AJ, Stainier DYR. 2019. Genetic compensation triggered by mutant mRNA degradation. *Nature* **568**:193–197. DOI: https://doi.org/10.1038/s41586-019-1064-z

El-Brolosy MA, Stainier DYR. 2017. Genetic compensation: A phenomenon in search of mechanisms. *PLOS Genetics* **13**:e1006780. DOI: https://doi.org/10.1371/journal.pgen.1006780

Estes KA, Szumowski SC, Troemel ER. 2011. Non-Lytic, Actin-Based Exit of Intracellular Parasites from *C. elegans* Intestinal Cells. *PLOS Pathogens* **7**:e1002227. DOI: https://doi.org/10.1371/journal.ppat.1002227

Etheridge T, Rahman M, Gaffney CJ, Shaw D, Shephard F, Magudia J, Solomon DE, Milne T, Blawzdziewicz J, Constantin-Teodosiu D, Greenhaff PL, Vanapalli SA, Szewczyk NJ. 2015. The integrin-adhesome is required to maintain muscle structure, mitochondrial ATP production, and movement forces in *Caenorhabditis elegans*. *The FASEB Journal* **29**:1235–1246. DOI: https://doi.org/10.1096/fj.14-259119, PMID: 25491313

Faghihi MA, Wahlestedt C. 2009. Regulatory roles of natural antisense transcripts. *Nature Reviews Molecular Cell Biology* **10**:637–643. DOI: https://doi.org/10.1038/nrm2738, PMID: 19638999

Fischer SE. 2010. Small RNA-mediated gene silencing pathways in *C. elegans*. *The International Journal of Biochemistry & Cell Biology* **42**:1306–1315. DOI: https://doi.org/10.1016/j.biocel.2010.03.006, PMID: 20227516

Fischer S, Benz J, Späth B, Jellen-Ritter A, Heyer R, Dörr M, Maier LK, Menzel-Hobeck C, Lehr M, Jantzer K, Babski J, Soppa J, Marchfelder A. 2011. Regulatory RNAs in *Haloferax volcanii*. *Biochemical Society Transactions* **39**:159–162. DOI: https://doi.org/10.1042/BST0390159, PMID: 21265765

Fraser AG, Kamath RS, Zipperlen P, Martinez-Campos M, Sohrmann M, Ahringer J. 2000. Functional genomic analysis of *C. elegans* chromosome I by systematic RNA interference. *Nature* **408**:325–330. DOI: https://doi.org/10.1038/35042517, PMID: 11099033

Fukumura K, Wakabayashi S, Kataoka N, Sakamoto H, Suzuki Y, Nakai K, Mayeda A, Inoue K. 2016. The exon junction complex controls the efficient and faithful splicing of a subset of transcripts involved in mitotic Cell-Cycle progression. *International Journal of Molecular Sciences* **17**:1153. DOI: https://doi.org/10.3390/ijms17081153

Galvin BD, Denning DP, Horvitz HR. 2011. SPK-1, an SR protein kinase, inhibits programmed cell death in *Caenorhabditis elegans* . *PNAS* **108**:1998–2003. DOI: https://doi.org/10.1073/pnas.1018805108

Gehring NH, Kunz JB, Neu-Yilik G, Breit S, Viegas MH, Hentze MW, Kulozik AE. 2005. Exon-junction complex components specify distinct routes of nonsense-mediated mRNA decay with differential cofactor requirements. *Molecular Cell* **20**:65–75. DOI: https://doi.org/10.1016/j.molcel.2005.08.012, PMID: 16209946

Gent JI, Lamm AT, Pavelec DM, Maniar JM, Parameswaran P, Tao L, Kennedy S, Fire AZ. 2010. Distinct phases of siRNA synthesis in an endogenous RNAi pathway in *C. elegans* soma. *Molecular Cell* **37**:679–689. DOI: https://doi.org/10.1016/j.molcel.2010.01.012, PMID: 20116306

Ghanbarian H, Wagner N, Michiels JF, Cuzin F, Wagner KD, Rassoulzadegan M. 2017. Small RNA-directed epigenetic programming of embryonic stem cell cardiac differentiation. *Scientific Reports* **7**:41799. DOI: https://doi.org/10.1038/srep41799, PMID: 28165496

Glavan F, Behm-Ansmant I, Izaurralde E, Conti E. 2006. Structures of the PIN domains of SMG6 and SMG5 reveal a nuclease within the mRNA surveillance complex. *The EMBO Journal* **25**:5117–5125. DOI: https://doi.org/10.1038/sj.emboj.7601377, PMID: 17053788

Grishok A. 2013. Biology and mechanisms of short RNAs in *Caenorhabditis elegans*. *Advances in Genetics* **83**:1–69. DOI: https://doi.org/10.1016/B978-0-12-407675-4.00001-8, PMID: 23890211

Guang S, Bochner AF, Pavelec DM, Burkhart KB, Harding S, Lachowiec J, Kennedy S. 2008. An argonaute transports siRNAs from the cytoplasm to the nucleus. *Science* **321**:537–541. DOI: https://doi.org/10.1126/science.1157647, PMID: 18653886

Guo AY, Zhu QH, Chen X, Luo JC. 2007. [GSDS: a gene structure display server]. *Hereditas* **29**:1023–1026. DOI: https://doi.org/10.1360/yc-007-1023, PMID: 17681935

Guschanski K, Warnefors M, Kaessmann H. 2017. The evolution of duplicate gene expression in mammalian organs. *Genome Research* **27**:1461–1474. DOI: https://doi.org/10.1101/gr.215566.116, PMID: 28743766

Han T, Manoharan AP, Harkins TT, Bouffard P, Fitzpatrick C, Chu DS, Thierry-Mieg D, Thierry-Mieg J, Kim JK. 2009. 26g endo-siRNAs regulate spermatogenic and zygotic gene expression in *Caenorhabditis elegans*. *PNAS* **106**:18674–18679. DOI: https://doi.org/10.1073/pnas.0906378106, PMID: 19846761

Hodgkin J, Papp A, Pulak R, Ambros V, Anderson P. 1989. A new kind of informational suppression in the nematode *Caenorhabditis elegans*. *Trends in Genetics* **123**:301.

Hoogewijs D, Houthoofd K, Matthijssens F, Vandesompele J, Vanfleteren JR. 2008. Selection and validation of a set of reliable reference genes for quantitative sod gene expression analysis in *C. elegans*. *BMC Molecular Biology* **9**:9. DOI: https://doi.org/10.1186/1471-2199-9-9, PMID: 18211699

Housden BE, Muhar M, Gemberling M, Gersbach CA, Stainier DY, Seydoux G, Mohr SE, Zuber J, Perrimon N. 2017. Loss-of-function genetic tools for animal models: cross-species and cross-platform differences. *Nature Reviews Genetics* **18**:24–40. DOI: https://doi.org/10.1038/nrg.2016.118, PMID: 27795562

Hunt-Newbury R, Viveiros R, Johnsen R, Mah A, Anastas D, Fang L, Halfnight E, Lee D, Lin J, Lorch A, McKay S, Okada HM, Pan J, Schulz AK, Tu D, Wong K, Zhao Z, Alexeyenko A, Burglin T, Sonnhammer E, et al. 2007. High-throughput in vivo analysis of gene expression in *Caenorhabditis elegans*. *PLOS Biology* **5**:e237. DOI: https://doi.org/10.1371/journal.pbio.0050237, PMID: 17850180

Huntzinger E, Kashima I, Fauser M, Saulière J, Izaurralde E. 2008. SMG6 is the catalytic endonuclease that cleaves mRNAs containing nonsense codons in metazoan. *RNA* **14**:2609–2617. DOI: https://doi.org/10.1261/rna.1386208, PMID: 18974281

Hutvagner G, Simard MJ. 2008. Argonaute proteins: key players in RNA silencing. *Nature Reviews Molecular Cell Biology* **9**:22–32. DOI: https://doi.org/10.1038/nrm2321, PMID: 18073770

Janowski BA, Huffman KE, Schwartz JC, Ram R, Nordsell R, Shames DS, Minna JD, Corey DR. 2006. Involvement of AGO1 and AGO2 in mammalian transcriptional silencing. *Nature Structural & Molecular Biology* **13**:787–792. DOI: https://doi.org/10.1038/nsmb1140, PMID: 16936728

Jojic B, Amodeo S, Bregy I, Ochsenreiter T. 2018. Distinct 3' UTRs regulate the life-cycle-specific expression of two *TCTP* paralogs in *Trypanosoma brucei*. *Journal of Cell Science* **131**:jcs206417. DOI: https://doi.org/10.1242/jcs.206417, PMID: 29661850

Jones CI, Zabolotskaya MV, Newbury SF. 2012. The 5' → 3' exoribonuclease XRN1/Pacman and its functions in cellular processes and development. *Wiley Interdisciplinary Reviews: RNA* **3**:455–468. DOI: https://doi.org/10.1002/wrna.1109, PMID: 22383165

Jorgensen EM, Mango SE. 2002. The art and design of genetic screens: *Caenorhabditis elegans*. *Nature Reviews Genetics* **3**:356–369. DOI: https://doi.org/10.1038/nrg794, PMID: 11988761

Kashima I, Jonas S, Jayachandran U, Buchwald G, Conti E, Lupas AN, Izaurralde E. 2010. SMG6 interacts with the exon junction complex via two conserved EJC-binding motifs (EBMs) required for nonsense-mediated mRNA decay. *Genes & Development* **24**:2440–2450. DOI: https://doi.org/10.1101/gad.604610, PMID: 20 930030

Kryuchkova-Mostacci N, Robinson-Rechavi M. 2015. Tissue-Specific evolution of protein coding genes in human and mouse. *PLOS ONE* **10**:e0131673. DOI: https://doi.org/10.1371/journal.pone.0131673, PMID: 26121354

Kuroyanagi H, Kimura T, Wada K, Hisamoto N, Matsumoto K, Hagiwara M. 2000. SPK-1, a *C. elegans* SR protein kinase homologue, is essential for embryogenesis and required for germline development. *Mechanisms of Development* **99**:51–64. DOI: https://doi.org/10.1016/S0925-4773(00)00477-9, PMID: 11091073

Laisney JA, Braasch I, Walter RB, Meierjohann S, Schartl M. 2010. Lineage-specific co-evolution of the egf receptor/ligand signaling system. *BMC Evolutionary Biology* **10**:27. DOI: https://doi.org/10.1186/1471-2148-10-27, PMID: 20105326

Lejeune F, Maquat LE. 2005. Mechanistic links between nonsense-mediated mRNA decay and pre-mRNA splicing in mammalian cells. *Current Opinion in Cell Biology* **17**:309–315. DOI: https://doi.org/10.1016/j.ceb.2005.03.002, PMID: 15901502

Li LC, Okino ST, Zhao H, Pookot D, Place RF, Urakami S, Enokida H, Dahiya R. 2006. Small dsRNAs induce transcriptional activation in human cells. *PNAS* **103**:17337–17342. DOI: https://doi.org/10.1073/pnas.0607015103, PMID: 17085592

Li LC. 2017. Small RNA-guided transcriptional gene activation (RNAa) in mammalian cells. In: *RNA Activation*. Singapore: Springer. p. 1–20.

Lindeboom RG, Supek F, Lehner B. 2016. The rules and impact of nonsense-mediated mRNA decay in human cancers. *Nature Genetics* **48**:1112–1118. DOI: https://doi.org/10.1038/ng.3664, PMID: 27618451

Lipinski KJ, Farslow JC, Fitzpatrick KA, Lynch M, Katju V, Bergthorsson U. 2011. High spontaneous rate of gene duplication in *Caenorhabditis elegans*. *Current Biology* **21**:306–310. DOI: https://doi.org/10.1016/j.cub.2011.01.026, PMID: 21295484

Ma Z, Zhu P, Shi H, Guo L, Zhang Q, Chen Y, Chen S, Zhang Z, Peng J, Chen J. 2019. PTC-bearing mRNA elicits a genetic compensation response via Upf3a and COMPASS components. *Nature* **568**:259–263. DOI: https://doi.org/10.1038/s41586-019-1057-y, PMID: 30944473

MacQueen AJ, Baggett JJ, Perumov N, Bauer RA, Januszewski T, Schriefer L, Waddle JA. 2005. ACT-5 is an essential *Caenorhabditis elegans* actin required for intestinal microvilli formation. *Molecular Biology of the Cell* **16**:3247–3259. DOI: https://doi.org/10.1091/mbc.e04-12-1061, PMID: 15872090

Mattick JS, Makunin IV. 2005. Small regulatory RNAs in mammals. *Human Molecular Genetics* **14**:R121–R132. DOI: https://doi.org/10.1093/hmg/ddi101, PMID: 15809264

Melamed S, Adams PP, Zhang A, Zhang H, Storz G. 2019. RNA-RNA interactomes of ProQ and hfq reveal overlapping and competing roles. *Molecular Cell*. DOI: https://doi.org/10.1016/j.molcel.2019.10.022, PMID: 31761494

Merzlyak EM, Goedhart J, Shcherbo D, Bulina ME, Shcheglov AS, Fradkov AF, Gaintzeva A, Lukyanov KA, Lukyanov S, Gadella TW, Chudakov DM. 2007. Bright monomeric red fluorescent protein with an extended fluorescence lifetime. *Nature Methods* **4**:555–557. DOI: https://doi.org/10.1038/nmeth1062, PMID: 17572680

Miki TS, Richter H, Rüegger S, Großhans H. 2014. PAXT-1 promotes XRN2 activity by stabilizing it through a conserved domain. *Molecular Cell* **53**:351–360. DOI: https://doi.org/10.1016/j.molcel.2014.01.001, PMID: 24462208

Modarresi F, Faghihi MA, Lopez-Toledano MA, Fatemi RP, Magistri M, Brothers SP, van der Brug MP, Wahlestedt C. 2012. Inhibition of natural antisense transcripts in vivo results in gene-specific transcriptional upregulation. *Nature Biotechnology* **30**:453–459. DOI: https://doi.org/10.1038/nbt.2158, PMID: 22446693

Nickless A, Bailis JM, You Z. 2017. Control of gene expression through the nonsense-mediated RNA decay pathway. *Cell & Bioscience* **7**:26. DOI: https://doi.org/10.1186/s13578-017-0153-7, PMID: 28533900

Pasquier J, Braasch I, Batzel P, Cabau C, Montfort J, Nguyen T, Jouanno E, Berthelot C, Klopp C, Journot L, Postlethwait JH, Guiguen Y, Bobe J. 2017. Evolution of gene expression after whole-genome duplication: new insights from the spotted gar genome. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* **328**:709–721. DOI: https://doi.org/10.1002/jez.b.22770

Patrinostro X, O'Rourke AR, Chamberlain CM, Moriarity BS, Perrin BJ, Ervasti JM. 2017. Relative importance of β_{cyto}- and γ_{cyto}-actin in primary mouse embryonic fibroblasts. *Molecular Biology of the Cell* **28**:771–782. DOI: https://doi.org/10.1091/mbc.e16-07-0503, PMID: 28077619

Pavelec DM, Lachowiec J, Duchaine TF, Smith HE, Kennedy S. 2009. Requirement for the ERI/DICER complex in endogenous RNA interference and sperm development in *Caenorhabditis elegans*. *Genetics* **183**:1283–1295. DOI: https://doi.org/10.1534/genetics.109.108134, PMID: 19797044

Phillips CM, Montgomery TA, Breen PC, Ruvkun G. 2012. MUT-16 promotes formation of perinuclear mutator foci required for RNA silencing in the *C. elegans* germline. *Genes & Development* **26**:1433–1444. DOI: https://doi.org/10.1101/gad.193904.112, PMID: 22713602

Plata G, Vitkup D. 2014. Genetic robustness and functional evolution of gene duplicates. *Nucleic Acids Research* **42**:2405–2414. DOI: https://doi.org/10.1093/nar/gkt1200, PMID: 24288370

Pollard TD, Cooper JA. 1986. Actin and actin-binding proteins A critical evaluation of mechanisms and functions. *Annual Review of Biochemistry* **55**:987–1035. DOI: https://doi.org/10.1146/annurev.bi.55.070186.005011, PMID: 3527055

Portnoy V, Huang V, Place RF, Li LC. 2011. Small RNA and transcriptional upregulation. *Wiley Interdisciplinary Reviews: RNA* **2**:748–760. DOI: https://doi.org/10.1002/wrna.90, PMID: 21823233

Pulak R, Anderson P. 1993. mRNA surveillance by the *Caenorhabditis elegans smg* genes. *Genes & Development* **7**:1885–1897. DOI: https://doi.org/10.1101/gad.7.10.1885, PMID: 8104846

Radomska KJ, Sager J, Farnsworth B, Tellgren-Roth Å, Tuveri G, Peuckert C, Kettunen P, Jazin E, Emilsson LS. 2016. Characterization and expression of the zebrafish qki paralogs. *PLOS ONE* **11**:e0146155. DOI: https://doi.org/10.1371/journal.pone.0146155, PMID: 26727370

Ratliff EP, Gutierrez A, Davis RA. 2006. Transgenic expression of CYP7A1 in LDL receptor-deficient mice blocks diet-induced hypercholesterolemia. *Journal of Lipid Research* **47**:1513–1520. DOI: https://doi.org/10.1194/jlr.M600120-JLR200, PMID: 16609145

Rechavi O, Lev I. 2017. Principles of transgenerational small RNA inheritance in *Caenorhabditis elegans*. *Current Biology* **27**:R720–R730. DOI: https://doi.org/10.1016/j.cub.2017.05.043, PMID: 28743023

Rossi A, Kontarakis Z, Gerri C, Nolte H, Hölper S, Krüger M, Stainier DY. 2015. Genetic compensation induced by deleterious mutations but not gene knockdowns. *Nature* **524**:230–233. DOI: https://doi.org/10.1038/nature14580, PMID: 26168398

Seth M, Shirayama M, Gu W, Ishidate T, Conte D, Mello CC. 2013. The *C. elegans* CSR-1 argonaute pathway counteracts epigenetic silencing to promote germline gene expression. *Developmental Cell* **27**:656–663. DOI: https://doi.org/10.1016/j.devcel.2013.11.014, PMID: 24360782

Shirayama M, Seth M, Lee HC, Gu W, Ishidate T, Conte D, Mello CC. 2012. piRNAs initiate an epigenetic memory of nonself RNA in the *C. elegans* germline. *Cell* **150**:65–77. DOI: https://doi.org/10.1016/j.cell.2012.06.015, PMID: 22738726

Singh G, Kucukural A, Cenik C, Leszyk JD, Shaffer SA, Weng Z, Moore MJ. 2012. The cellular EJC interactome reveals higher-order mRNP structure and an EJC-SR protein nexus. *Cell* **151**:750–764. DOI: https://doi.org/10.1016/j.cell.2012.10.007, PMID: 23084401

Sztal TE, McKaige EA, Williams C, Ruparelia AA, Bryson-Richardson RJ. 2018. Genetic compensation triggered by actin mutation prevents the muscle damage caused by loss of actin protein. *PLOS Genetics* **14**:e1007212. DOI: https://doi.org/10.1371/journal.pgen.1007212, PMID: 29420541

Tondeleir D, Lambrechts A, Müller M, Jonckheere V, Doll T, Vandamme D, Bakkali K, Waterschoot D, Lemaistre M, Debeir O, Decaestecker C, Hinz B, Staes A, Timmerman E, Colaert N, Gevaert K, Vandekerckhove J, Ampe C. 2012. Cells lacking β-actin are genetically reprogrammed and maintain conditional migratory capacity. *Molecular & Cellular Proteomics* **11**:255–271. DOI: https://doi.org/10.1074/mcp.M111.015099, PMID: 2244 8045

Tourasse NJ, Millet JRM, Dupuy D. 2017. Quantitative RNA-seq meta-analysis of alternative exon usage in *C. elegans*. *Genome Research* **27**:2120–2128. DOI: https://doi.org/10.1101/gr.224626.117, PMID: 29089372

Turunen MP, Lehtola T, Heinonen SE, Assefa GS, Korpisalo P, Girnary R, Glass CK, Väisänen S, Ylä-Herttuala S. 2009. Efficient regulation of VEGF expression by promoter-targeted lentiviral shRNAs based on epigenetic mechanism: a novel example of epigenetherapy. *Circulation Research* **105**:604–609. DOI: https://doi.org/10.1161/CIRCRESAHA.109.200774, PMID: 19696410

Vasale JJ, Gu W, Thivierge C, Batista PJ, Claycomb JM, Youngman EM, Duchaine TF, Mello CC, Conte D. 2010. Sequential rounds of RNA-dependent RNA transcription drive endogenous small-RNA biogenesis in the ERGO-1/Argonaute pathway. *PNAS* **107**:3582–3587. DOI: https://doi.org/10.1073/pnas.0911908107, PMID: 20133583

Wedeles CJ, Wu MZ, Claycomb JM. 2013. Protection of germline gene expression by the *C. elegans* argonaute CSR-1. *Developmental Cell* **27**:664–671. DOI: https://doi.org/10.1016/j.devcel.2013.11.016, PMID: 24360783

Yigit E, Batista PJ, Bei Y, Pang KM, Chen CC, Tolia NH, Joshua-Tor L, Mitani S, Simard MJ, Mello CC. 2006. Analysis of the *C. elegans* argonaute family reveals that distinct argonautes act sequentially during RNAi. *Cell* **127**:747–757. DOI: https://doi.org/10.1016/j.cell.2006.09.033, PMID: 17110334

Yvert G. 2014. 'Particle genetics': treating every cell as unique. *Trends in Genetics* **30**:49–56. DOI: https://doi.org/10.1016/j.tig.2013.11.002, PMID: 24315431

Zhang Z, Krainer AR. 2004. Involvement of SR proteins in mRNA surveillance. *Molecular Cell* **16**:597–607. DOI: https://doi.org/10.1016/j.molcel.2004.10.031, PMID: 15546619

Zhuang JJ, Hunter CP. 2011. Tissue specificity of *Caenorhabditis elegans* enhanced RNA interference mutants. *Genetics* **188**:235–237. DOI: https://doi.org/10.1534/genetics.111.127209, PMID: 21385728