RESEARCH ARTICLE

Exon-dependent transcriptional adaptation by exon-junction complex proteins Y14/RNP-4 and MAGOH/MAG-1 in Caenorhabditis elegans

Jesus Fernandez-Abascal, Lei Wang, Bianca Graziano, Christina K. Johnson, Laura Bianchi

Department Physiology and Biophysics, University of Miami Miller School of Medicine, Miami, Florida

Abstract

Transcriptional adaptation is a powerful gene regulation mechanism that can increase genetic robustness. Transcriptional adaptation occurs when a gene is mutated and is mediated by the mutant RNA, rather than by protein feedback loops. We show here that transcriptional adaptation occurs in the C. elegans clh family of Cl− channels and that it requires exon-junction complex (EJC) proteins RNP-4, MAG-1, and eiF4AIII. Depending on which exons are deleted in distinct clh-1 alleles, different clh genes are regulated in an EJC-dependent manner. Our results support the idea that different transcriptional adaptation outcomes may be directed by the differential interaction of the EJC with its target mutant RNAs.

Author summary

The expansion of molecular tools designed to introduce mutations in genes across different models has revealed that sometimes mutations do not cause any apparent phenotype. This phenomenon is called genetic robustness and it can be mediated by the mechanism of transcriptional adaptation. In transcriptional adaptation, the degradation of the mutant RNA causes the up or downregulation of genes that functionally compensate for the mutant gene. Using the genetically amenable nematode C. elegans, we show here that transcriptional adaptation depends on proteins of the Exon Junction Complex, a protein complex important for RNA stability and localization, and protein translation. Further, we show that different mutations of the same gene lead to different transcriptional adaptation outcomes and variable functional compensation. Our results bring new insights into the still poorly understood phenomenon of transcriptional adaptation.
Introduction

Transcriptional adaptation is a form of genetic compensation in which the mutation in a gene leads to the change in the expression level and/or pattern of related genes. This phenomenon has been confirmed in zebrafish, mouse cell cultures, and more recently in *Caenorhabditis elegans* [1–4]. It has also been suggested in other model organisms, including yeast, *Arabidopsis*, *Drosophila*, and mouse [5–9]. Transcriptional adaptation does not depend on the loss of protein function but rather on mutant mRNA. At least two models of transcriptional adaptation have been proposed in which the common denominator is the presence of a premature termination codons (PTC) in the mutant mRNA. One model proposed by Ma and colleagues, is based on studies in zebrafish and it involves the interaction of the PTC containing mutant mRNA with the histone modifier COMPASS complex, leading to enhancement of histone H3 Lys4 trimethylation at the transcription start site regions of the adapting genes, which causes their upregulation [4]. This type of mechanism may co-regulate genes in operons. Another model involves the degradation of the PTC containing mRNA via a process termed nonsense-mediated decay (NMD) and the formation of small RNA species that then interact with RNA binding proteins, are transferred to the nucleus, and may regulate gene expression via interaction with histone modifiers [1,2,10].

Serobyan and colleagues recently showed for the first time that transcriptional adaptation occurs also in *C. elegans*. The authors showed that in this organism, transcriptional adaptation of actin and titin genes requires the function of factors involved in mRNA decay, as well as of Argonaute proteins and Dicer, which are involved in small RNA maturation and transport into the nucleus, thus supporting a model involving PTC mediated NMD [3]. Furthermore, SPK-1 and RSP-6, two serine/arginine proteins involved in mRNA binding and splicing [11,12], and homologous to components of the exon-junction complex, were recently found to also participate in transcriptional adaptation [3]. The EJC has been known to enhance NMD, via recruitment of UPF1 (an RNA decay factor called SMG-2 in *C. elegans*), when deposited on a mRNA containing a PTC [13]. However, other EJC proteins, including core proteins Y14 (RNP-4 in *C. elegans*), MAGOH1 (MAG-1 in *C. elegans*), and eIF4AIII (F33D11.10 in *C. elegans*) have not been tested for their involvement in transcriptional adaptation [14–19]. Moreover, the involvement of EJC proteins in transcriptional adaptation raises the possibility of different transcriptional adaptation outcomes based on different exon/exon junctions present in distinct mutations of the same gene [9]. The conservation of basic molecular mechanisms from *C. elegans* to higher organisms, urges the exploitation of this pioneering organism to better understand transcriptional adaptation, a potential modifier of disease severity that could be harnessed for treatment.

The *clh* family of Cl- channels in *C. elegans* consists of six genes, named *clh-1* through *clh-6*, located on chromosome II (*clh-1-3* and *clh-5*), chromosome V (*clh-6*), and chromosome X (*clh-4*). *clh* genes are homologous to the mammalian CLCN genes, are expressed in various tissues, and participate in several important biological processes [20–24]. For example, CLH-1 regulates pH in the amphid sensory organ of *C. elegans*, mediates Cl- efflux from amphid glia for GABA regulation of the mechanosensory neuron ASH, and participates in regulating the activity of sensory neurons that modulate the navigation in response to food [25–27]. Furthermore, the knock-out of *clh-1* causes wider body and abnormal alae structure, underscoring the function of this Cl- channel in the hypodermal seam cells [20]. Among the six *clh* genes, *clh-1* is the only one for which three different mutants have been isolated [20,25,28], and thus, it represents a useful tool to study transcriptional adaptation.

In this study, using real time PCR, RNA interference, phenotypic measurements, quantification of the brood size, and nose touch behavioral assays, we sought to determine whether
transcriptional adaptation occurs in the clh family and what factors might be involved. Using the three clh-1 mutants, we report here the following findings for the clh family that may apply to other gene families: 1) transcriptional adaptation is allele-specific, 2) transcriptional adaptation involves the upregulation of some genes and the downregulation of others, 3) the EJC proteins RNP-4, MAG-1, and F33D11.10 (from here on referred to as eIF4AIII) are required for transcriptional adaptation, and 4) functional compensation correlates with downregulation of genes of the same family. Our study shows that different transcriptional adaptation outcomes with variable functional compensations are directed in different mutants of the same gene, adding to our understanding of this important genetic compensation mechanism.

Results

Transcriptional adaptation leads to different adapting gene profiles in clh mutant alleles

We first acquired three clh-1 knock-out strains (ok658, qa900, and qa901) [20,25,28] and determined whether the mRNA levels for the six clh genes was altered in these mutants (Fig 1). The clh-1(ok658) mutation consists of the deletion of 1029 bp containing exons 3–5 and a thymidine insertion at position 2911_2912 [28] (Fig 1A). In this mutant, a premature stop codon (PTC) is introduced at position 313–315 of the mRNA sequence (the wild type RNA length is 2613 bp) (S1A Fig). The clh-1(qa900) has an in-frame deletion of 1857 bp containing exons 6–9 and part of exon 10 [20], and the clh-1(qa901) has a deletion of 2071 bp containing exons 4–9 and part of exon 10 [20] (Fig 1A). In qa901 a PTC is introduced at position 511–513 of the mRNA sequence (S1A Fig).

We found that in these three mutants, the levels of clh-1 mRNA are different (Fig 1B). While in alleles ok658 and qa901 the clh-1 mRNA is reduced as compared to wild type (ratio of each clh-1 mRNA in mutant versus wild type: 0.45 ± 0.05 and 0.15 ± 0.04, respectively), in qa900 mutant it is on average at the same level (1.57 ± 0.33, not statistically different than wild type). These results are consistent with the NMD phenomenon, by which PTCs are recognized as signals to target RNA for degradation [29]. Indeed, the only mutant in which clh-1 mRNA level is like wild type is qa900, which consists of an in-frame deletion. Consistent with the idea that transcriptional adaptation is not activated in mutants lacking a PTC, the mRNA levels of all the other clh genes are unaltered in qa900 (Fig 1C–1G). On the other hand, we found that in both ok658 and qa901 mutants, other clh genes had different mRNA levels as compared to wild type animals, suggesting that transcriptional adaptation is operative in these mutants. Interestingly though, there are differences between the two mutants (Fig 1B–1G and Table 1). clh-2 mRNA level is smaller in qa901 but unaltered in the ok658 mutant (Fig 1C, 0.31 ± 0.04 and 1.04 ± 0.15 versus wild type, respectively), whereas clh-4 mRNA levels are higher in ok658 and lower in qa901 (Fig 1E, 2.10 ± 0.29 and 0.47 ± 0.09 versus wild type, respectively). In both mutant alleles, the expression levels of the other genes, clh3, clh-5, and clh-6, are like in wild type (Fig 1D, 1F–1G). To confirm these data, we performed additional qRT-PCRs using probes spanning other exon boundaries in clh-1, clh-2, and clh-4 mRNAs and we obtained similar results (S1B–S1D Fig). These data show different transcriptional adaptation profiles based on the mutant clh-1 transcript.

The three clh-1 mutants exhibit different body and brood sizes

Transcriptional adaptation is thought to provide functional compensation via change in expression of related genes (reviewed in [30]). Thus, we wondered whether qa900, the mutant allele in which there is no change in expression of the other clh genes, displayed a phenotype
Moreover, we asked whether ok658 and qa901, which display different mRNA profiles for clh-2 and clh-4, differed in their phenotypes. clh-1(ok658) mutants are nose touch insensitive, and clh-1(qa900) and clh-1(qa901) have wider bodies [20,27]. We thus compared the nose touch phenotype and body size across all three mutants (Fig 2A and ...)
2B). In addition, we compared brood size since we noticed that clh-1(qa900) produced significantly fewer progenies (Fig 2C). When we compared nose touch avoidance across the three mutants, we found no statistical differences (avoidance index was 0.32 ± 0.045, 0.44 ± 0.052, and 0.44 ± 0.05 for ok658, qa900, and qa901, respectively) (Fig 2A) [27]. Thus, the nose touch insensitive phenotype is shared by the three clh-1 mutants, suggesting that this phenotype is not functionally compensated.

On the other hand, we found differences in the other two phenotypes. For body size, we found that while ok658 mutants are similar to wild type in width and length (width, WT = 57.88 ± 0.66 μm, ok658 = 59.31 ± 0.67 μm and length, WT = 1050 ± 10.16 μm, ok658 = 1036 ± 11.09 μm, respectively), qa900 and qa901 mutants are wider and shorter (width, qa900 = 68.31 ± 1.43 μm, qa901 = 65.74 ± 1.36 μm and length, qa900 = 869.9 ± 11.41 μm, qa901 = 952.4 ± 12.95 μm, respectively), as it was previously reported (S2A Fig) [20]. The difference in body proportions is particularly evident in Fig 2B, where we plotted the ratio between width and length. These data show that qa900 mutant has the most severe phenotype having widest and shortest body. Thus, these results suggest that changes in expression of other clh genes in

Table 1. Mutant clh-1 and unc-89 that cause transcriptional adaptation and their adapting genes.

| Genotype       | Adapte d genes | Change     |
|----------------|----------------|------------|
| clh-1 (ok658)  | clh-4          | Upregulated|
| clh-1 (qa901)  | clh-2          | Downregula ted |
|                | clh-4          | Downregula ted |
| unc-89         | sax-3          | Upregulated |

https://doi.org/10.1371/journal.pgen.1010488.t001

Fig 2. Variable phenotypic compensation in the three clh-1 mutants. (A) Nose touch responses in the three clh-1 mutants; wild type and trpa-1 worms were used as positive and negative controls respectively [27,31]. Data are expressed as individual animals (open circles) and as mean ± SEM, n = 20 except qa901 that was 10. (B) Body size ratios (width/length) of WT, clh-1 mutants, and clh-1 RNAi. The length of the worms was determined by measuring the distance between the tip of the nose and the end of the tail in anaesthetized animals laying straight on agarose pads. The width was determined by measuring the distance between the vulva opening and the back of the worm. Data are expressed as individual data points (open circles) and as violin plots (n = 44, 45, 27, 25, and 20 respectively). (C) Brood size of WT, clh-1 mutants, and clh-1 RNAi. The columns represent the average brood size of each genotype, and the open circles represent individual worms (n = 6, 6, 5, 6, and 11 respectively). Data represent mean ± SEM and were obtained by counting the number of adults found in plates where individual worms were grown for 24 hours over 5 consecutive days. Statistical analysis was by one-way ANOVA followed by Tukey's. Statistical differences are shown in the graphs as p values. Data used for this figure are reported in the S1 Table.
*clh-1*(ok658) and *clh-1*(qa901) mutants may result in compensation of the wider and shorter body phenotype.

When we analyzed the brood size in the three *clh-1* mutants, we once again found that *qa900* was the mutant with the most severe phenotype having the smallest brood size among the three mutants (brood size in WT, *ok658*, *qa900*, and *qa901* was $249.8 \pm 12.39$, $204.8 \pm 18.21$, $106.2 \pm 2.7$, and $261 \pm 14.9$, respectively) (Fig 2C). Taken together, these data suggest that the body size and the brood size may be phenotypes that are transcriptionally compensated in both *ok658* and *qa901* mutants.

**The specificity of transcriptional adaptation in the *clh-1* mutants**

Transcriptional adaptation is induced by mutations in the genome but not by the knockdown of a gene [1]. To gather further support that the changes in *clh-2* and *clh-4* mRNA levels seen in *ok658* and *qa901* mutants might be due to transcriptional adaptation, we performed *clh-1* knockdown experiments. While we confirmed knockdown of *clh-1* (Fig 3A), we found no differences in the levels of mRNA of the other *clh* genes (Fig 3A–3F). These data lend further support to the idea that the different mRNA levels for *clh-2* and *clh-4* observed in *ok658* and *qa901* are due to transcriptional adaptation. Next, we analyzed body and brood size in *clh-1* RNAi worms and found that these phenotypes were significantly different than wild type (Fig 2B and 2C). Parenthetically, *clh-1* RNAi also causes nose touch insensitivity [27]. These data further support the idea that the wider and short body, and the smaller brood size are due to uncompensated loss of *clh-1* function in *C. elegans*.

To test whether changes in *clh-2* and *clh-4* gene expression in *clh-1* mutants were specific, we analyzed the transcript of titin-related gene *sax-3* in the three mutants (Fig 3G). *sax-3* mRNA undergoes transcriptional adaptation in mutants of the titin gene *unc-89*, so we added *unc-89* mutant as positive control [3]. As previously reported by Serobyan and colleagues, in *unc-89* mutants the adapting gene *sax-3* is upregulated (Fig 3G and Table 1), while the mRNA level of *unc-89* transcript itself is lower than in WT, consistent with the NMD process (Fig 3H) [3]. We found that neither *sax-3* or *unc-89* mRNA levels were altered in *ok658* and *qa901* mutant alleles (Fig 3G and 3H). Interestingly, we found slight downregulation of *sax-3* and slight upregulation of *unc-89* in *qa900* mutant, that might be related to the severely altered body size and proportions in this mutant, given that *unc-89* and *sax-3* encode for titin-related genes. Taken together, these data support that changes in the expression of *clh-2* and *clh-4* seen in *ok658* and *qa901* mutants are not stochastic.

**The transcriptional adaptation of *clh-2* requires RNA biogenesis factor ERGO-1**

To gather further support for transcriptional adaptation, we looked at the similarity between *clh-1* and the other *clh* genes. Indeed, transcriptional adaptation has been reported to involve more frequently similar genes, even though non-similar genes have been also shown to undergo up or down regulation in PTC-bearing mutants [2]. Using blastn and a word size of 20, we found significant similarity between *clh-1* and *clh-2*, *clh-3*, *clh-4*, and *clh-5*. More specifically, we found that *clh-2* contains a continuous stretch of 20 bp that is 100% identical to a stretch of nucleotides in *clh-1* exon 13. In addition, *clh-2* shares 68% to 85% homology with *clh-1* in stretches of nucleotides varying in length between 32 and 228 bp across 785 bp total. We also found significant homology with *clh-3* (70% identity across 320 bp), *clh-4* (66% identity across 113 bp), and *clh-5* (88% identity across 49 bp). These similarities further support the idea that the differences seen in *clh-2* and *clh-4* mRNA levels in *clh-1*(ok658) and *clh-1*(qa901) might be due to transcriptional adaptation. To gather experimental evidence for this
Fig 3. Transcriptional adaptation is dependent on mutant RNA and is gene-family specific. (A–F) mRNA levels of the six clh genes in wild type animals treated with clh-1 RNAi compared to control growth conditions. (G) mRNA levels of sax-3 in clh-1 and unc-89 mutants. (H) mRNA levels of unc-89 in clh-1 and unc-89 mutants. Data are expressed as mean ± SEM and normalized to WT levels, that were taken as 1. The pmp-3 mRNA levels were used as internal control. Three independent experiments with three technical replicates were performed. The horizontal dotted line corresponds to the level in control conditions (A–F) and in WT (G and H). All statistical differences are reported in the graphs (unpaired two-tailed t-test). Data used for this figure are reported in the S1 Table.

https://doi.org/10.1371/journal.pgen.1010488.g003

conclusion, we quantified the pre-mRNA levels of clh-2 and clh-4 and of clh-4 in the qa901 and ok658 mutants, respectively. We found that the pre-mRNA levels of clh-2 and clh-4 are smaller than in WT in the qa901 mutant, and that the pre-mRNA level of clh-4 is higher than in WT in the ok658 mutant (S1E–S1F Fig). Thus, the changes in steady state RNA levels of clh-2 and clh-4 seen in qa901 and ok658 (Figs 1C and 1E and S1C–S1E) correspond to changes in transcription of these genes, supporting the idea that they are the result of transcriptional adaptation.

Next we asked whether ERGO-1, a protein involved in small RNA biogenesis [32–35], and required for transcriptional adaptation in C. elegans titin and actin families [3], was required for changes in clh-2 and clh-4 mRNA levels in clh-1 mutants. First, consistent with the idea that ERGO-1 regulates transcriptional adaptation downstream of mRNA decay, we found that the mutant clh-1 mRNA levels were still downregulated in ergo-1 RNAi (S1G Fig), whose effectiveness was confirmed by qRT-PCR (S1J Fig) [3]. Second, we found different outcomes for clh-2 and clh-4 mRNAs in ergo-1 RNAi. While the mRNA levels of clh-2, which were downregulated in qa901 (Fig 1G), were now upregulated (S1H Fig), upregulation of clh-2 in ergo-1 RNAi was also seen with clh-2 probe spanning 11–12 exons: 2.247 ± 0.29 relative to WT), the mRNA levels of clh-4 for ok658 and qa901 were unchanged as compared to control conditions (S1I Fig). These results support the requirement for small RNA biogenesis for clh-2 downregulation in qa901, but not for the changes in clh-4 mRNA levels in ok658 and qa901. Intriguingly, clh-2 is the only clh gene in which a 20 bp sequence sharing 100% identity with clh-1 is found. Taken together, these results suggest that different transcriptional adaptation mechanisms may be operative in the same mutant to target different adapting genes.

Transcriptional adaptation of clh genes requires the Exon Junction Complex

Serobyan and colleagues reported a list of proteins involved in transcriptional adaptation, including splicing factors spk-1 and rsp-6 [3]. However, the core components of the EJC Y14/RNP-4, MAGOH/MAG-1, and elf4AIII were not included in their study [14,15,17–19,36]. The EJC is involved in transcriptional adaptation via its role in NMD. EJCs are deposited on the mRNA during splicing in the nucleus, remain on mRNAs even after transport to the cytosol, and are then removed from the mRNA by the ribosome during the pioneer round of translation. If a PTC is present upstream of an EJC, then this is not dislodged from the mRNA during translation leading to the recruitment of RNA decay factor UPF1 (smg-2 in C. elegans) that degrades the RNA [13].

To experimentally determine the potential role of EJC proteins in transcriptional adaptation in clh-1 mutants, we performed knockdown by RNAi feeding in wild type and clh-1 mutants, as well as in the unc-89 mutant as a control (Figs 4, S3 and S4 and Table 2). When we analyzed the mRNA levels of the adapting genes clh-2 and clh-4 in clh-1 mutants, and of sax-3 in unc-89 mutant treated with rnp-4 RNAi, we found significant reduction of transcriptional adaptation (Figs 4A–4E, S3G and S3H). Thus, the clh-2 mRNA levels were no longer downregulated in qa901 worms but were like the levels seen in wild type (Fig 4A) and, therefore,
Fig 4. Exon-junction proteins and the non-sense mediated decay protein SMG-2 mediate transcripational adaptation of clh and titin genes. (A-E) mRNA levels of clh-2 and clh-4 in clh-1 mutant alleles upon knockdown of rnp-4. (F-J) mRNA levels of clh-2 and clh-4 in clh-1 mutant alleles upon knockdown of mag-1. (K-O) mRNA levels of clh-2 and clh-4 in clh-1 mutant alleles upon knockdown of eif4AIII (P-T) mRNA levels of clh-2 and clh-4 in clh-1 mutant alleles upon knockdown of smg-2. Data are expressed as mean ± SEM and normalized to WT levels or NGM condition, that were taken as 1 (black dotted line). Three independent experiments with three technical replicates were...
higher than the levels seen in qa901 grown under control conditions (Fig 4B). The clh-4 mRNA levels were no longer up- and downregulated in ok658 and qa901 worms, respectively (Fig 4C), thus they were lower and higher than the levels observed in these mutants grown in control conditions (Fig 4D and 4E, respectively), and the sax-3 mRNA levels were no longer upregulated in unc-89 worms (S3G Fig). We also found that the clh genes that do not undergo transcriptional adaptation in both clh-1 mutants remained unaltered when rnp-4 was knocked down (S3A–S3C Fig). Interestingly, the mRNA levels of the mutant clh-1 were still lower than the wild type (S3D Fig). However, comparison with the control conditions revealed that the mutant clh-1 RNA is not as low in qa901, suggesting reduced degradation in this mutant (S3E Fig), a phenomenon not observed in ok658, despite similar rnp-4 knockdown efficiency in the two mutants (S3F Fig).

To determine whether the effects of rnp-4 RNAi were specific, we knocked down the gene ZC155.4 which encodes an ortholog of human glycerophosphodiester phosphodiesterase 1 (GDE1). The gene product of ZC155.4 is predicted to participate in lipid metabolic processes and is not expected to be involved in transcriptional adaptation or RNA processing. We found that knock down of ZC155.4 had no effect on the mRNA levels of clh-1, clh-2, and clh-4 in clh-

Table 2. Summary of the effects of knocking down control gene ZC155.4, rnp-4, mag-1, eiF4AIII, and smg-2 on transcriptional adaptation. "Yes" indicates that the gene is either up or down-regulated, "no" indicates no change in transcript level as compared to wild type, and "reduced" indicates that the indicated genes were up or down-regulated but not to the same extent as in control conditions.

| RNAi gene | Function | Genotype / Adapted Gene | Transcriptional adaptation |
|-----------|----------|-------------------------|---------------------------|
| -         | -        | clh-1 (ok658) / clh-4    | yes                       |
|           |          | clh-1 (qa901) / clh-2    | yes                       |
|           |          | clh-1 (qa901) / clh-4    | yes                       |
|           |          | unc-89 / sax-3           | yes                       |
| ZC155.4 (control) | glycerophosphodiester phosphodiesterase 1 | clh-1 (ok658) / clh-4    | yes                       |
|           |          | clh-1 (qa901) / clh-2    | yes                       |
|           |          | clh-1 (qa901) / clh-4    | yes                       |
| rnp-4     | mRNA splicing (EJC) | clh-1 (ok658) / clh-4    | no                        |
|           |          | clh-1 (qa901) / clh-2    | no                        |
|           |          | clh-1 (qa901) / clh-4    | reduced                   |
|           |          | unc-89 / sax-3           | no                        |
| mag-1     | mRNA splicing (EJC) | clh-1 (ok658) / clh-4    | no                        |
|           |          | clh-1 (qa901) / clh-2    | reduced                   |
|           |          | clh-1 (qa901) / clh-4    | reduced                   |
|           |          | unc-89 / sax-3           | no                        |
| eiF4AIII  | mRNA splicing (EJC) | clh-1 (ok658) / clh-4    | reduced                   |
|           |          | clh-1 (qa901) / clh-2    | reduced                   |
|           |          | clh-1 (qa901) / clh-4    | reduced                   |
|           |          | unc-89 / sax-3           | Not tested                |
| smg-2     | mRNA decay and processing (NMD) | clh-1 (ok658) / clh-4    | no                        |
|           |          | clh-1 (qa901) / clh-2    | yes                       |
|           |          | clh-1 (qa901) / clh-4    | no                        |
|           |          | unc-89 / sax-3           | no                        |
and clh-1(qa901) mutants (S3I–S3K Fig), supporting the specificity of the effects seen in rnp-4 RNAi.

The RNAi of the other EJC component, MAG-1 caused also substantial decrease of transcriptional adaptation in both the clh and titin families (Figs 4F–4J and S4D). Thus, clh-2 and clh-4 mRNA levels were no longer downregulated in qa901 (Fig 4F, 4G, 4H and 4J), the upregulation of clh-4 in ok658 worms was significantly reduced (Fig 4H and 4I), and the upregulation of sax-3 in unc-89 mutants was absent (S4D Fig). As seen for rnp-4 RNAi, mag-1 RNAi did not affect the downregulation of clh-1 transcript in ok658 mutant but reduced the downregulation of clh-1 in qa901, suggesting reduced degradation of the clh-1 transcript in this mutant (S4A and S4B Fig). In the case of mag-1 RNAi too, knockdown shows the same effectiveness in the two clh-1 mutants (S4B Fig). Finally, we tested the involvement of the core component of the EJC eIF4AIII and obtained results similar to the ones obtained with RNAi of rnp-4 and mag-1 (Figs 4K–4O and S4E–S4G). Taken together, these findings are consistent with the idea that the process of NMD is EJC-dependent in C. elegans. Although they also reveal that, at least in the case of ok658 mutant, knockdown of components of the EJC does not lead to changes in degradation of the mutant RNA, suggesting an EJC-independent NMD mechanism in this clh-1 mutant [37,38]. Thus in C. elegans EJC-dependent and EJC-independent NMD appear to exist side by side [39].

The UPF1 homolog SMG-2 and transcriptional adaptation of clh genes

Serobyan and colleagues reported that smg-2 and smg-4 genes are required for transcriptional adaptation of unc-89 alleles, whereas smg-6 is required for the transcriptional adaptation in act-5 [3]. SMG-2, SMG-4, and SMG-6 are all RNA decay factors that have been implicated in transcriptional adaptation in zebrafish embryos and mouse cell lines, in addition to C. elegans [2,4]. We thus wondered whether transcriptional adaptation in clh-1 mutants required SMG-2, the C. elegans ortholog of the ATP-dependent RNA helicase upstream frameshift 1 (UPF1) [40]. We found that the knockdown of smg-2, partially blocked the transcriptional adaptation of the adapting gene clh-2 in qa901 worms (Fig 4P and 4Q) and fully blocked changes in the clh-4 transcript in ok658 and qa901 worms (Fig 4R–4T). As reported by Serobyan and colleagues, smg-2 knockdown blocked the transcriptional adaptation of sax-3 (S4K Fig) and the decay of unc-89 mRNA in unc-89 worms (S4L Fig) [3]. Furthermore, a block of decay of the clh-1 transcript was observed in smg-2 RNAi (S4H and S4I Fig), and the efficiency of the RNAi treatment was confirmed by qRT-PCR (S4J Fig), thus confirming that mutant clh-1 transcripts are degraded via the NMD pathway. To conclude, the RNA decay factor smg-2 is required for degradation of the mutant clh-1 transcripts and for the changes in clh-2 and clh-4 mRNA levels seen in clh-1(ok658) and clh-1(qa901) mutants.

RNP-4 and MAG-1 are not required in development for transcriptional adaptation

In the knockdown experiments we performed, we grew worms from egg to adult on RNAi plates [41]. To determine whether RNP-4 and MAG-1 are required in development for transcriptional adaptation, we repeated knockdown experiments by growing worms on RNAi plates from the last larval stage L4 to adulthood (24 hours) (S5A–S5L Fig). Under these conditions, we obtained results that were overall similar to the results obtained from animals that were reared from egg to adult on the RNAi plates, albeit the block of clh-4 transcriptional adaptation appeared weaker (compare S5D–S5F and S5J–S5L Fig with Fig 4C–4E and 4H–4J). Similarly, smg-2 RNAi in late larvae/young adults reduced transcriptional adaptation, though not to the same extent as in experiments in which animals were reared on RNAi plates from...
These results support the idea that RNP-4, MAG-1, and SMG-2 are not essential during development for the transcriptional adaptation observed in the clh family.

**Effects of loss of transcriptional adaptation on the phenotypes of clh-1 mutants**

The three clh-1 mutants we analyzed here have similar nose touch avoidance phenotype, but different brood size and body's width/length phenotypes (Fig 2). Interestingly, clh-1(qa900) which does not contain a PTC and in which we did not observe any change in the levels of the other clh genes’ transcripts, displays the most severe phenotypes having the largest width/length ratio and the smallest brood size. We thus wondered whether knockdown of the factors we found to be involved in transcriptional adaptation of the clh family in mutants clh-1(ok658) and clh-1(qa901) would exacerbate the phenotypes in these mutants and render them more similar to clh-1(qa900). Thus, we analyzed body’s size in rnp-4, mag-1, and smg-2 knockdown animals and brood size in rnp-4 and smg-2 knockdown. The extremely low number of progenies in all strains treated with eiF4III RNAi prevented meaningful analysis of brood size under these conditions (S2I Fig).

We found that knockdown of rnp-4 and mag-1 exacerbated the width/length ratio in qa901 worms, while it did not have any effect in ok658 (Figs 5A, 5B and S2C–S2F). Interestingly, smg-2 knockdown, which reduced transcriptional adaptation in ok658 and qa901 mutants, but not as effectively as rnp-4 and mag-1 knockdown, did not have any effect on the body size of any of the mutants (Figs 5C, S5G and S5H). The brood size was on average decreased in all the strains in rnp-4 knockdown, as previously reported [19]. However, the effect on the brood size of rnp-4 knockdown was most evident in qa901 mutant which produced a number of progenies as low as qa900 mutant (Fig 5D). Knockdown of smg-2 did not have any effect on the brood size either despite leading to at least partial block of transcriptional adaptation as mentioned above (Fig 5E). The results with smg-2 RNAi suggest that the level of transcriptional adaption under these conditions is still sufficient to induce functional compensation. These results support the idea that in qa901 mutant, knockdown of rnp-4 and mag-1 causes the worsening of the phenotypes, suggesting that in this mutant transcriptional adaptation leads to functional compensation. It is interesting to note that in qa901, both clh-2 and clh-4 genes are downregulated. On the contrary, even though we observe block of upregulation of clh-4 in ok658 mutant treated with rnp-4 and mag-1 RNAi, the phenotypes remain unaffected, suggesting that other genes compensate the phenotypes in ok658, perhaps in an rnp-4 and mag-1 independent manner.

**Discussion**

With the expansion of genetic tools that engineer mutations in genes, there is a growing interest in understanding mechanisms of genetic compensation. Indeed, across species, genetic mutations often do not result in any apparent phenotype. C. elegans is a genetically amenable organism that can be used for rapidly advancing our understanding of the mechanisms of genetic compensation, including transcriptional adaptation.

The work that we present here adds to our understanding of transcriptional adaptation in C. elegans, so far described only in another manuscript [3]. We report here that the EJC proteins RNP-4, MAG-1, and eiF4AIII are needed for transcriptional adaptation in the clh and titin families and show that the transcriptional adaptation outcome of the adapting genes depends on the specific PTC-bearing mutant alleles. More specifically, transcriptional adaptation in the clh family can result in the downregulation or the upregulation of the adapting
genes, it is, at least in part, dependent on the RNA biogenesis factor ERGO-1 [3], while the RNA decay factor SMG-2 appears commonly required, at least for transcriptional adaptation in the 2 clh-1 mutants we analyzed [13,33–35].

Our data support that the introduction of a premature stop codon (PTC) is a key factor in promoting transcriptional adaptation in C. elegans. Indeed, we show that qa900 mutant, consisting in an in-frame deletion (Figs 1A and S1), does not lead to change in the expression of other clh genes. On the contrary, ok658 and qa901 alleles introduce PTC and lead to changes
in expression level of clh-2 and clh-4 (Fig 1), in line with what was shown by Serobyan and colleagues for the actin and titin families in C. elegans [3]. Our smg-2 RNAi data support the idea that the PTC-bearing mRNAs undergo degradation via the NMD mechanism [42] and thus, that the lower levels of mutant clh-1 detected in these mutants are not likely due to reduced transcription of clh-1. Interestingly though, this is as far as the commonality of transcription adaptation mechanism between the two PTC-bearing clh-1 mutants goes.

Our analysis of the requirement for EJC proteins and for RNA biogenesis factor ERGO-1 for transcriptional adaptation in ok658 and qa901 reveals differences between the two mutants. While changes in clh-2 and clh-4 expression levels in both ok658 and qa901 mutants are blocked when EJC proteins are knocked down (Table 2), the outcome of this treatment on the PTC-bearing clh-1 mRNA level is different. While in ok658 it is still degraded, in qa901 it is not (S3E, S4B and S4F Figs). This result suggests that in ok658 the EJC complex is required for transcriptional adaptation (perhaps indirectly via other genes) [2], but it is not required for NMD of the mutant clh-1 mRNA, consistent with an EJC-independent NMD in this mutant as previously described in C. elegans and in other systems [37,39,43,44]. This model would still be consistent with the fact that smg-2 RNAi reduces transcriptional adaptation in both ok658 and qa901 mutants, given that at least in human cell lines UPF1, the mammalian homolog of SMG-2, is needed for both EJC-dependent and EJC-independent NMD [39]. Future studies in which the entire transcriptome is compared between ok658 and qa901 mutants may be needed to shed more light on the difference between mechanisms of transcriptional adaptation in these mutants.

Importantly, in qa901, where we find requirement for ERGO-1, we also observe worsening of the phenotype when EJC proteins are knocked down, consistent with the idea that in this mutant transcriptional adaptation leads to at least partial functional compensation. Indeed, under rnp-4 and mag-1 RNAi conditions, qa901 body and brood size are similar to qa900 mutant. Interestingly though, in this mutant both clh-2 and clh-4 mRNA are downregulated. While the study of transcriptional adaptation has been focusing primarily on understanding how adapted genes become upregulated, the downregulation of genes has been observed before [2,3]. For example, in act-5(dt2019) mutants, while act-3 becomes upregulated, act-4 becomes downregulated [3]. On a larger scale, El-Brolosy and colleagues reported the downregulation, in addition to the upregulation, of hundreds of genes in three mouse knock-out cell lines [2]. One attractive model is that small RNAs produced by the degradation of mutant clh-1 directly silence clh-2 and clh-4 mRNA [45]. Alternatively, small RNA, once bound to RNA binding proteins, may act on the promoter regions of these genes (reviewed in [46]). Intriguingly, clh-1 shares some identity with clh-4, and even more so, with clh-2 (100% identity over a stretch of 20 nucleotides). Our data also show that the downregulation of clh-2 and/or of clh-4 lead to functional compensation. Although the involvement of other genes cannot be excluded, how might the downregulation of a gene lead to functional compensation? This is not clear, but effects mediated by antisense transcripts or the proteins themselves, especially if the proteins have opposite effects on cellular physiology, can be envisioned [2].

In neither ok658 nor qa901 the nose touch avoidance phenotype is compensated. Similarly, mutations in the C. elegans Na+/K+-ATPase α-subunits eat-6 and catp-1 cause upregulation of the homologous gene catp-2. However, the upregulation of catp-2 does not compensate for the nose touch insensitive phenotype of eat-6 and catp-1 mutants [47]. Other examples of failed functional compensation can be found in zebrafish. For example, vegfaa (Vascular endothelial growth factor A) mutants, there is an upregulation of the related gene vegfab, but these animals still show vascular hypoplasia [48]. Failed functional compensation in transcriptional adaptation may be associated with phenotypes, such as nose touch avoidance or vascularization, that are still compatible with survival and reproduction.
Finally, we must caution on the fact that in our work we have not analyzed a full locus deletion of clh-1; instead, we have compared our results obtained in PTC-bearing ok658 and qa901 mutants with the in-frame deletion qa900 where clh-1 RNA is still present. On the contrary, Serobyan and colleagues using an RNA-less unc-89 mutant were able to show that transcriptional adaptation indeed requires the presence of the mutant mRNA [3]. Thus, we cannot exclude the possibility that other mechanisms such as loss of CLH-1 function are at play here.

In summary, we have shown here that the EJC plays a key role in transcriptional adaptation in C. elegans. Furthermore, we report that transcriptional adaptation can lead to either the up or down regulation of related genes, depending on the mutant allele, and that functional compensation is variable not only depending on the mutation but also depending on the phenotype. Finally, we have confirmed that transcriptional adaptation requires the presence of PTC-bearing mRNA. The data presented here urge consideration of this genetic mechanism of compensation whenever working with C. elegans knock-out strains. The good practice of the worm community of analyzing knock-out, knockdown, and rescue strains must be maintained to safeguard from misinterpretation of phenotypes. Furthermore, the genetic amenability of C. elegans makes it an excellent model to advance the study the molecular underpinnings of this important, yet still poorly understood, phenomenon.

Materials and methods

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Laura Bianchi (lbianchi@med.miami.edu).

C. elegans growth and maintenance

Experiments were performed using healthy 1 day old adult hermaphrodites. Nematodes were grown on standard grow medium (NGM) seeded with Escherichia coli (OP50 strain) and kept at 20°C. N2 Bristol was the wild type strain.

C. elegans strains

N2 strain from CGC was used as wild type [49]. The clh-1 mutants, strain RB1052 trpa-1 (ok999), and strain VC40193 unc-89(gk509355) were also purchased from CGC [20,28,50]. Strains XA900 and XA901 were originally outcrossed at least 4 times [20]. Strain BLC588 clh-1 (ok658) was obtained after outcrossing 3 times the RB833 strain. The unc-89 strain used in this study (BLC532) was obtained after outcrossing 3 times the VC40193 strain. The full list of the strains used in this study is reported in Table 3.

Table 3. Nematode strains used in this study.

| Genotype | Strain Name | Reference |
|----------|-------------|-----------|
| Wild type | N2          | CGC [49]  |
| trpa-1 (ok999) | RB1052 | CGC [28]  |
| clh-1 (ok658)  | RB833      | CGC [28]  |
| clh-1 (ok658)  | BLC588     | This study|
| clh-1 (qa900)  | XA900      | [20]      |
| clh-1 (qa901)  | XA901      | [20]      |
| unc-89 (gk509355) | VC40193 | [50]      |
| unc-89 (gk509355) | BLC532 | This study|

https://doi.org/10.1371/journal.pgen.1010488.t003
**C. elegans** synchronization

Gravid adults were rinsed off plates using 2 ml of M9 buffer (22.1 mM KH$_2$PO$_4$, 42.3 mM Na$_2$PO$_4$, 85.6 mM NaCl) and then transferred to tubes for centrifugation at 4300 rpm for 5 minutes. Pelleted worms were resuspended in 400 μl of bleach solution (22.7% bleach, 0.1 M NaOH). When ~ 90% of the eggs were released, the reaction was stopped with 10 ml of M9 buffer. Eggs were centrifuged at 4300 rpm for 5 minutes and washed with M9 buffer twice. The pelleted eggs were then resuspended in 100 μl M9 buffer and inoculated onto seeded NGM plates.

**Quantitative real-time PCR**

One day old adult hermaphrodites were rinsed off plates using 2 ml of M9 buffer and transferred into tubes containing 12 ml M9 buffer. Tubes were centrifuged for 3 minutes at 2400 rpm prior to the pellet being washed 4 times with M9. The pellet was then resuspended in 1 ml TRIzol reagent (ThermoFisher) and exposed to 6 cycles of liquid N$_2$ for 30 seconds and bath of 37˚C for 2 minutes. The solution was then transferred into fresh tubes and mixed with 200 μl chloroform. After 5 minutes incubation on ice, the tubes were centrifuged for 15 minutes at 15000 rpm at 4˚C. The top transparent layer was then transferred to a fresh tube containing 800 μl of isopropanol and centrifuged for 10 minutes at 15000 rpm at 4˚C. The pellet was then resuspended in 75% ethanol solution and centrifuged again for 5 minutes at 15000 rpm at 4˚C. Finally, the pellet was resuspended in water and heated at 62˚C for 10 minutes. The RNA concentration was measured using a spectrophotometer and only samples with OD$_{260/280}$ between 1.8 and 2 were used for further analysis. One μg of RNA per sample was used for reverse transcription with the High-Capacity RNA-to-cDNA kit (Applied Biosystems) according to manufacturer’s instructions. For PCR amplification, 25 ng of cDNA were used with FAM dye labeled probes (Table 4, ThermoFisher) and TaqMan Universal Master Mix II in a CXF Connect Real-Time PCR detection system (Bio-Rad) following manufacturer’s instructions. For the rnp-4 gene, the SYBR Green PCR method was used due to lack of commercial TaqMan rnp-4 FAM dye labeled specific probe. Briefly, SYBR Green qPCR experiments were performed using PowerUp SYBR Green PCR Master Mix (Applied Biosystems, USA), following the manufacturer’s instructions. cDNA (25 ng) and 50 nM of the paired-primer mix were used for each reaction. The melting curve was performed and analyzed to make sure there were no nonspecific PCR products. To measure pre-mRNA levels with SYBR chemistry, extracted RNA samples were processed to remove genomic DNA before reverse transcription using a DNase kit (Qiagen, Netherlands) and following manufacturer’s instructions. The pre-RNA clh-2 and clh-4, the rnp-4 and pmp-3 primers’ sequences used for the SYBR Green method are shown in Table 4. The gene pmp-3 was used as an endogenous calibrator for both methods of qPCR. For the SYBR Green method, pmp-3 primers were designed at the same location as pmp-3 FAM dye labeled probe. The pmp-3 primers are also in Table 4. The relative mRNA levels were calculated using the 2$^{-ΔΔCt}$ method [51,52]. Wild type was used as the reference sample, taken as 1-fold expression level, is indicated on each figure legend.

**Body measurements**

To measure body length and width, we immobilized synchronized 1 day old adults in 2% agarose (in M9 buffer) pads using 100 mM sodium azide. We used a Evos FL Auto 2 Imaging System (Invitrogen) microscope to acquire images with an 40x objective (Olympus). Acquisition was done with the Evos FL Auto software. To determine the length, the distance from the tip of the nose to the end of the tail was measured in animals that were laying straight on the agar pad. The width was determined by measuring the distance from vulva opening to back of the
worm. Fiji (ImageJ) was used in both cases for data analysis [53]. Measures and ratio width/length were plotted on Prism 8 for Windows (Version 8.4.2).

### Nose touch

Nose touch assays were performed as previously described [27]. In brief, healthy 1 day old adults were placed in a NGM plate containing a thin layer of OP50 and allowed to crawl for 30 minutes. An eyelash was placed perpendicular to a forward moving animal so the worm would touch it with the nose while crawling forward. A response was recorded as positive if the worm showed an aversive response (reversal or head withdraw) or as negative if the worm kept moving forward over, under or along the eyelash. Each worm was tested 5 times with an interval of at least 30 seconds between touches. The average response of each worm was calculated and used for data curation (see S1 Table). The experiments were performed blind to genotype.

### Table 4. Probes and primers used for qRT-PCR amplification for TaqMan and SYBR chemistries. We note that all probes selected span exon-exon junctions that are shared between different splice variants of the same clh gene, so they do not distinguish between splice variants.

| Gene | Assay ID | Probe spans exon boundary |
|------|----------|--------------------------|
| clh-1 | Ce02420887_g1 | 1–2 |
| clh-1 | Ce02420885_g1 | 15–16 |
| clh-2 | Ce02439134_g1 | 11–12 |
| clh-2 | Ce02439147_g1 | 9–10 |
| clh-3 | Ce02434744_g1 | 14–15 |
| clh-4 | Ce02494081_g1 | 14–15 |
| clh-4 | Ce02494095_g1 | 9–10 |
| clh-5 | Ce02436786_g1 | 8–9 |
| clh-6 | Ce02482293_g1 | 1–2 |
| unc-89 | Ce02416187_g1 | 27–28 |
| sax-3 | Ce02419838_g1 | 11–12 |
| ergo-1 | Ce02470935_m1 | 4–5 |
| eIF4AIII | Ce02418330_g1 | 1–2 |
| smg-2 | Ce02414128_m1 | 3–4 |
| pmp-3 | Ce02485188_m1 | 4–5 |

**Primers for rnp-4 used for SYBR chemistry**

| Sequence | Probe spans exon boundary |
|----------|--------------------------|
| Forward  | 5'–AACGCAGAGGGAAGCCAACG–3' | 2–3 |
| Reverse  | 5'–TCAGCGCTTTCCAGAAGTCT–3' |

**Primers for clh-2 pre-mRNA used for SYBR chemistry**

| Sequence | Probe spans exon boundary |
|----------|--------------------------|
| Forward  | 5'–CCGATCTTCGTTTGGTGAGT–3' | 2–3 |
| Reverse  | 5'–TGAGATGAGTGCGAGTTGAGAAT–3' |

**Primers for clh-4 pre-mRNA used for SYBR chemistry**

| Sequence | Probe spans exon boundary |
|----------|--------------------------|
| Forward  | 5'–TTGCCGGATCTTCGATTTCCGGAAGG–3' | 2–3 |
| Reverse  | 5'–ATTCCGTGAAACATATTCCAT–3' |

**Primers for pmp-3 used for SYBR chemistry**

| Sequence | Probe spans exon boundary |
|----------|--------------------------|
| Forward  | 5'–GGAATCTTTGCGATTTCTAT–3' | 4–5 |
| Reverse  | 5'–ATTCGGTGAAACATATTCCAT–3' |

[https://doi.org/10.1371/journal.pgen.1010488.t004](https://doi.org/10.1371/journal.pgen.1010488.t004)
**Brood size**

The quantification of the brood size was performed as previously described [54]. Individual worms were picked at L1 stage into separate plates containing empty OP50 or HT115 E. coli transformed with the target RNAi construct. For L4 to young adult assays, worms were picked at L4 stage. After reaching day one adulthood, worms were transferred into fresh plates for 5 consecutive days. Progenies from each plate were counted at late larva to adult stage.

**Knockdown by dsRNA feeding**

A 770 bp exon-rich sequence from the genomic *rnp-4* gene was amplified by PCR using the following primers: forward (5’CTTAAGCTTAGAGATGGAGGATGTGGTGCC) and reverse (5’GTAGCTAGCTCAGCGCTTTCCAGAAGTG). A 1128 bp exon-rich sequence from the genomic *clh-1* gene was amplified by PCR using the following primers: forward (5’GACTCAGGCTTAGGCTTAG) and reverse (5’CTCCAACCACGGCATAAAGTCC). A 995 bp exon-rich sequence from the genomic *eiF4AIII* gene was amplified by PCR using the following primers: forward (5’CGTCGTAATCTTGCACCCGAG) and reverse (5’CTCCGTGGTAGTTTGGGTCTTAG). The PCR products were then separately cloned into a L4440 vector containing T7 polymerase promoters to read the sequence in both sense and antisense. The vectors were transformed into HT115 E. coli that were then used to inoculate NGM plates containing IPTG. The HT115 E. coli strains expressing the L4440 vector containing a 584 bp exon-rich sequence from the genomic *mag-1* gene, a 651 bp exon-rich sequence from the genomic *smg-2* gene, a 1155 bp exon-rich sequence from the genomic *ZC144.5* gene, or a ≥ 1 kb exon-rich sequence from the genomic *ergo-1* gene were part of the Ahringer library [55] and were a gift from Kevin Collins. *C. elegans* eggs were seeded on the RNAi plates and allowed to grow for 2.5 days to adulthood prior to RNA extraction [45]. We observed reduced brood size in *rnp-4* RNAi plates, as previously reported, supporting RNAi efficiency in our hands [19], as well as almost complete sterility associated with *eiF4AIII* RNAi treatment.

**Statistics**

For qRT-PCR, the values obtained with the 2^ΔΔCt_\text{method}, which avoid a false depiction of the variation, were used for statistical analysis between the target samples and their own reference sample (wild type or control) using unpaired t-test [56,57]. For phenotypic comparisons, ANOVA followed by Tukey’s was used. The statistics used for each graph are reported in the figure legends. The software Prism 8 for windows, version 8.4.2. was used.

**Supporting information**

S1 Fig. Schematic representation of the *clh-1* mRNA alleles, qRT-PCR with different primers, and *ergo-1* RNAi. Related to Fig 1. Drawings represent the spliced *clh-1* WT and mutant alleles. Exons are numbered. The position of the first introduced premature STOP codon is indicated. (B-D) mRNA levels of *clh-1*, *clh-2* and *clh-4* genes in WT and mutant alleles by qPCR using probes different from the ones used for Fig 1. Thus, a probe spanning exons 15–16 was used for *clh-1*, one spanning exons 9–10 was used for *clh-2*, and one spanning exons 9–10 was used for *clh-4*. (E-F) Pre-mRNA levels of *clh-2* and *clh-4* in WT, *ok658* and *qa901* mutants (G-) Knockdown of *ergo-1* by RNAi feeding in WT, *ok658* and *qa901* worms. The *clh-1* mRNA levels are smaller for both mutants as compared to WT (G). The RNAi of *ergo-1* causes upregulation of *clh-2* mRNA levels (H). The *clh-4* mRNA levels are not modified under *ergo-1* RNAi treatment in both mutants (I). The *ergo-1* mRNA levels are smaller in RNAi as compared to NGM conditions (J). (B-J) pmp-3 was used as internal control. Data are expressed...
as mean ± SEM and normalized to WT levels or NGM condition, that were taken as 1. Three independent experiments with three technical replicates were performed. Dashed lines indicate values from Fig 1 and are shown here for comparison: blue (panel I), corresponding to clh-4 mRNA levels of ok658 in NGM (2.1, from Fig 1E); red (panels H and I), corresponding to clh-2 and clh-4 mRNA levels, respectively, of qa901 in NGM (0.31 from Fig 1C, and 0.47 from Fig 1E, respectively). The horizontal black dotted line corresponds to WT or control conditions, as indicated. All statistical differences are reported in the graphs (unpaired two-tailed t-test). Data used for this figure are reported in the S1 Table.

S2 Fig. Body width and length in control and knockdown conditions, and brood size in eIF4AIII RNAi. Related to Figs 2 and 5. Body width and length of WT and clh-1 mutants in control conditions and clh-1 RNAi, (A-B), rnp-4 RNAi (C-D), mag-1 RNAi (E-F), and smg-2 RNAi (G-H). Data are expressed as individual data points (open circles) and as violin plots (A-B: n = 44, 45, 27, 25, and 20, respectively; C-D: n = 19, 26, 14, and 20, respectively; E-F: n = 19, 17, 21, and 20, respectively; and G-H: n = 26, 21, 24, and 28, respectively). Statistical analysis was by one-way ANOVA followed by Tukey’s post test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). The horizontal dashed lines represent the values obtained in control conditions (from Fig 2C, WT: gray, ok658: dark blue, qa900: blue, qa901: light blue). Data used for this figure are reported in the S1 Table.

S3 Fig. RNP-4 is needed for transcriptional adaptation. Related to Fig 4. (A-C) clh-3, clh-5, and clh-6 mRNA levels, respectively, in ok658 and qa901 mutants treated with rnp-4 RNAi. (D) clh-1 mRNA levels in ok658 and qa901 mutants in which rnp-4 was knocked down, showing that clh-1 transcripts are reduced. (E) clh-1 mRNA levels in ok658 and qa901 worms in which rnp-4 was knocked down compared to their levels in control conditions, showing that mRNA degradation is reduced in qa901 worms. (F) rnp-4 mRNA in control conditions and rnp-4 knockdown showing efficacy of the knockdown. (G) mRNA levels of sax-3 in unc-89 mutants upon knockdown of rnp-4, showing rescue of transcriptional adaptation. (H) unc-89 mRNA levels in unc-89 worms in which rnp-4 was knocked down, showing that unc-89 transcript is still reduced when rnp-4 is knocked down. (I-K) clh-1, clh-2, and clh-4 mRNA levels, respectively, in ok658, qa900, and qa901 mutants in which control gene ZC155.4 was knocked down. Data are expressed as mean ± SEM and normalized to WT levels or control conditions (NGM), that were taken as 1. The horizontal black dotted line corresponds to WT or control conditions, as indicated. The pmp-3 mRNA levels were used as internal control. Three independent experiments with three technical replicates were performed. The dashed green line corresponds to mRNA levels of sax-3 in unc-89 worms in control conditions (from Fig 3G). All statistical differences are reported in the graphs (unpaired two-tailed t-test). See S1 Table for the data.

S4 Fig. MAG-1 and eIF4AIII are needed for transcriptional adaptation. Related to Fig 4. (A) clh-1 mRNA levels in ok658 and qa901 worms in which mag-1 was knocked down, showing that clh-1 transcripts are reduced. (B) clh-1 mRNA levels in ok658 and qa901 worms in which mag-1 was knocked down compared to their levels in control conditions, showing that mRNA degradation is reduced in qa901 worms. (C) mag-1 mRNA in control conditions and mag-1 knockdown showing efficacy of the knockdown. (D) mRNA levels of sax-3 in unc-89 worms in mag-1 RNAi, showing rescue of transcriptional adaptation. (E) clh-1 mRNA levels in ok658 and qa901 worms in which eIF4AIII was knocked down, showing that clh-1 transcripts
are reduced. (F) clh-1 mRNA levels in ok658 and qa901 worms in which eif4AIIII was knocked down compared to their levels in control conditions, showing that mRNA degradation is reduced in qa901 worms. (G) eif4AIIII mRNA in control conditions and eif4AIIII knockdown showing efficacy of the knockdown. (H) clh-1 mRNA levels in ok658 and qa901 worms in which smg-2 has been knocked down. (I) clh-1 mRNA levels in ok658 and qa901 worms in which smg-2 was knocked down compared to their levels in control conditions, showing that mRNA degradation is reduced in both strains. (J) smg-2 mRNA levels in ok658 and qa901 mutants in control and smg-2 RNAi, showing the efficacy of RNAi. (K) mRNA levels of sax-3 in unc-89 mutants upon knockdown of smg-2, showing rescue of transcriptional adaptation. (L) unc-89 mRNA levels in unc-89 worms in which smg-2 has been knocked down. Data are expressed as mean ± SEM and normalized to WT levels or NGM conditions, that were taken as 1. The horizontal black dotted line corresponds to WT or control conditions, as indicated. The pmp-3 mRNA levels were used as internal control. Three independent experiments with three technical replicates were performed. Dashed lines correspond to mRNA levels of sax-3 in unc-89 worms in control conditions (green, from Fig 3G). All statistical differences are reported in the graphs (unpaired two-tailed t-test). See S1 Table for the data.

S5 Fig. RNP-4 and MAG-1 are not required during development for transcriptional adaptation. Related to Fig 5. (A-F) mRNA levels of clh-1, clh-2, and clh-4 in clh-1 mutants upon knockdown of rnp-4 from L4 to Young Adult (L4toYA). (G-L) and (M-S) Same as in A-F for mag-1 and smg-2 RNAi, respectively. Data are expressed as mean ± SEM and normalized to WT levels or NGM condition, that were taken as 1 (black dotted line). Three independent experiments with three technical replicates were performed, and pmp-3 mRNA levels were used as internal control. Dashed lines correspond to mRNA levels of qa901 in control (red, from Fig 1C and 1E) and mRNA levels of ok658 in control (blue, from Fig 1E). The horizontal black dotted line corresponds to WT or control conditions, as indicated. All statistical differences are reported in the graphs (unpaired two-tailed t-test). Data used for this figure are reported in the S1 Table.

S1 Table. Data and statistics. File containing all the data points used for the main and supplementary figures, as well as the statistic calculations.

Acknowledgments
We thank Rong Grace Zhai and Robert W. Keane for sharing of equipment vital to collection of data presented in this paper, Kevin Collins for the L4440 vector and the HT115 Escherichia coli strain, as well as for the E. coli mag-1 and smg-2 RNAi clones. We thank Braulio Cervantes for help with the quantification of the brood sizes.

Author Contributions

Conceptualization: Jesus Fernandez-Abascal, Laura Bianchi.

Data curation: Jesus Fernandez-Abascal, Lei Wang, Bianca Graziano, Christina K. Johnson.

Formal analysis: Jesus Fernandez-Abascal, Christina K. Johnson.

Funding acquisition: Laura Bianchi.

Investigation: Jesus Fernandez-Abascal, Lei Wang, Bianca Graziano, Christina K. Johnson.
Methodology: Jesus Fernandez-Abascal, Laura Bianchi.

Project administration: Laura Bianchi.

Resources: Laura Bianchi.

Supervision: Laura Bianchi.

Validation: Jesus Fernandez-Abascal, Laura Bianchi.

Visualization: Jesus Fernandez-Abascal.

Writing – original draft: Jesus Fernandez-Abascal.

Writing – review & editing: Jesus Fernandez-Abascal, Lei Wang, Bianca Graziano, Christina K. Johnson, Laura Bianchi.

References

1. Rossi A, Kontarakis Z, Gerri C, Nolte H, Holper S, Kruger M, et al. Genetic compensation induced by deleterious mutations but not gene knockdowns. Nature. 2015; 524(7564):230–3. Epub 2015/07/15. https://doi.org/10.1038/nature14580 PMID: 26168398.

2. El-Brolosy MA, Kontarakis Z, Rossi A, Kuenne C, Gunther S, Fukuda N, et al. Genetic compensation triggered by mutant mRNA degradation. Nature. 2019; 568(751):193–7. Epub 2019/04/05. https://doi.org/10.1038/s41586-019-1064-z PMID: 30944477; PubMed Central PMCID: PMC6707827.

3. Serobyan V, Kontarakis Z, El-Brolosy MA, Welker JM, Tolstenkov O, Saadeldin AM, et al. Transcriptional adaptation in Caenorhabditis elegans. Elife. 2020;9. Epub 2020/01/18. https://doi.org/10.7554/eLife.50014 PMID: 31951195; PubMed Central PMCID: PMC6968918.

4. Ma Z, Zhu P, Shi H, Guo L, Zhang Q, Chen Y, et al. PTC-bearing mRNA elicits a genetic compensation response via Upf3a and COMPASS components. Nature. 2019; 568(751):259–63. Epub 2019/04/05. https://doi.org/10.1038/s41586-019-1057-y PMID: 30944473.

5. Jost AP, Weiner OD. Probing Yeast Polarity with Acute, Reversible, Optogenetic Inhibition of Protein Function. ACS Synth Biol. 2015; 4(10):1077–85. Epub 2015/06/03. https://doi.org/10.1021/acssynbio.5b00053 PMID: 26035630; PubMed Central PMCID: PMC4609243.

6. Braun N, Wyzykowski J, Muller P, David K, Couch D, Perrot-Rechenmann C, et al. Conditional repression of AUXIN BINDING PROTEIN1 reveals that it coordinates cell division and cell expansion during postembryonic shoot development in Arabidopsis and tobacco. Plant Cell. 2008; 20(10):2746–62. Epub 2008/10/28. https://doi.org/10.1105/tpc.108.059048 PMID: 18952781; PubMed Central PMCID: PMC2590743.

7. Yamamoto S, Jaiswal M, Charrl WL, Gambin T, Karaca E, Mirzaa G, et al. A drosophila genetic resource of mutants to study mechanisms underlying human genetic diseases. Cell. 2014; 159(1):200–14. Epub 2014/09/27. https://doi.org/10.1016/j.cell.2014.09.002 PMID: 25259927; PubMed Central PMCID: PMC4298142.

8. De Souza AT, Dai X, Spencer AG, Reppen T, Menzie A, Roesch PL, et al. Transcriptional and phenotypic comparisons of Ppara knockout and siRNA knockdown mice. Nucleic Acids Res. 2006; 34(16):4486–94. Epub 2006/09/02. https://doi.org/10.1093/nar/gkl609 PMID: 16945951; PubMed Central PMCID: PMC1636368.

9. Szilai TE, Stainier DYR. Transcriptional adaptation: a mechanism underlying genetic robustness. Development. 2020;147(15). Epub 2020/08/21. https://doi.org/10.1242/dev.188452 PMID: 32816303.

10. Haimovich G, Medina DA, Causse SZ, Garber M, Millan-Zambrano G, Barkai O, et al. Gene expression is circular: factors for mRNA degradation also foster mRNA synthesis. Cell. 2013; 153(5):1000–11. Epub 2013/05/28. https://doi.org/10.1016/j.cell.2013.05.012 PMID: 23706738.

11. Kuroyanagi H, Kimura T, Wada K, Hisamoto N, Matsumoto K, Hagiwara M. SPK-1, a C. elegans SR protein kinase homologue, is essential for embryogenesis and required for germline development. Mech Dev. 2000; 99(1–2):51–64. Epub 2000/11/25. https://doi.org/10.1016/s0925-4773(00)00477-9 PMID: 11091073.

12. Longman D, Johnstone IL, Caceres JF. Functional characterization of SR and SR-related genes in Caenorhabditis elegans. EMBO J. 2000; 19(7):1625–37. Epub 2000/04/04. https://doi.org/10.1093/emboj/19.7.1625 PMID: 10747030; PubMed Central PMCID: PMC310231.

13. Maquat LE. Nonsense-mediated mRNA decay: splicing, translation and mRNP dynamics. Nat Rev Mol Cell Biol. 2004; 5(2):89–99. Epub 2004/03/26. https://doi.org/10.1038/nrm1310 PMID: 15040442.
14. Kataoka N, Diem MD, Kim VN, Yong J, Dreyfuss G. Magoh, a human homolog of Drosophila mago nashi protein, is a component of the splicing-dependent exon-exon junction complex. EMBO J. 2001; 20(22):6424–33. Epub 2001/11/15. https://doi.org/10.1093/emboj/20.22.6424 PMID: 11707413; PubMed Central PMCID: PMC125744.

15. Kataoka N, Yong J, Kim VN, Velazquez F, Perkinson RA, Wang F, et al. Pre-mRNA splicing imprints mRNA in the nucleus with a novel RNA-binding protein that persists in the cytoplasm. Mol Cell. 2000; 6(3):673–82. Epub 2000/10/13. https://doi.org/10.1016/s1097-2765(00)00065-4 PMID: 11030346.

16. Le Hir H, Gatfield D, Braun IC, Forler D, Izaurralde E. The protein Mago provides a link between splicing and mRNA localization. EMBO Rep. 2001; 2(12):1119–24. Epub 2001/12/18. https://doi.org/10.1093/embo-reports/kve245 PMID: 11743026; PubMed Central PMCID: PMC1084163.

17. Li Q, Imataka H, Morino S, Rogers GW Jr., Merrick WC, et al. Eukaryotic translation initiation factor 4AII (eIF4AII) is functionally distinct from eIF4AI and eIF4AII. Mol Cell Biol. 1999; 19(11):7336–46. Epub 1999/10/13. https://doi.org/10.1128/MCB.19.11.7336 PMID: 11030346; PubMed Central PMCID: PMC847272.

18. Rechert VL, Le Hir H, Jurica MS, Moore MJ. 5' exon interactions within the human spliceosome establish a framework for exon junction complex structure and assembly. Genes Dev. 2002; 16(21):2778–91. Epub 2002/11/05. https://doi.org/10.1101/gad.1030602 PMID: 12414731; PubMed Central PMCID: PMC187475.

19. Kawano T, Kataoka N, Dreyfuss G, Sakamoto H. Ce-Y14 and MAG-1, components of the exon-exon junction complex, are required for embryogenesis and germline sexual switching in Caenorhabditis elegans. Mech Dev. 2004; 121(1):27–35. Epub 2004/01/07. https://doi.org/10.1016/j.mod.2003.11.003 PMID: 14706697.

20. Petalcorin MI, Oka T, Koga M, Ogura K, Wada Y, Ohshima Y, et al. Disruption of clh-1, a chloride channel gene, results in a wider body of Caenorhabditis elegans. J Mol Biol. 1999; 294(2):347–55. Epub 1999/12/28. https://doi.org/10.1006/jmbi.1999.3241 PMID: 10610763.

21. Schreier AM, Friedrich T, Pusch M, Jentsch TJ. CLC chloride channels in Caenorhabditis elegans. J Biol Chem. 1999; 274(48):34238–44. Epub 1999/11/24. https://doi.org/10.1074/jbc.274.48.34238 PMID: 10567397.

22. Nehrke K, Begenisich T, Pilato J, Melvin JE. Into ion channel and transporter function. Caenorhabditis elegans CIC-type chloride channels: novel variants and functional expression. Am J Physiol Cell Physiol. 2000; 279(6):C2052–66. Epub 2000/11/18. https://doi.org/10.1152/ajpcell.2000.279.6.C2052 PMID: 11078724.

23. Rutledge E, Bianchi L, Christensen M, Boehler C, Morrison R, Brosiat A, et al. CLH-3, a CIC-2 anion channel ortholog activated during meiotic maturation in C. elegans oocytes. Curr Biol. 2001; 11(3):161–70. Epub 2001/03/07. https://doi.org/10.1016/s0960-9822(01)00051-3 PMID: 11231150.

24. Branicky R, Miyazaki H, Strange K, Schafer WR. The voltage-gated anion channels encoded by clh-3 regulate egg laying in C. elegans by modulating motor neuron excitability. J Neurosci. 2014; 34(3):764–75. Epub 2014/01/17. https://doi.org/10.1523/JNEUROSCI.3112-13.2014 PMID: 24431435; PubMed Central PMCID: PMC3891957.

25. Grant J, Matthewman C, Bianchi L. A Novel Mechanism of pH Buffering in C. elegans Glia: Bicarbonate Transport via the Voltage-Gated CIC Cl- Channel CLH-1. J Neurosci. 2015; 35(50):16377–97. Epub 2015/12/18. https://doi.org/10.1523/JNEUROSCI.3237-15.2015 PMID: 26674864; PubMed Central PMCID: PMC4679820.

26. Park C, Sakurai Y, Sato H, Kanda S, Iino Y, Kunitomo H. Roles of the ClC chloride channel CLH-1 in food-associated salt chemotaxis behavior of C. elegans. Elife. 2021; 10. Epub 2021/08/07. https://doi.org/10.7554/eLife.55701 PMID: 34392228; PubMed Central PMCID: PMC7834019.

27. Fernandez-Abascal J, Bianchi L. The ClC Cl(-) channel CLH-1 mediates HCO3 (-) efflux from the amphid sheath glia in C. elegans. MicroPubl Biol. 2022;2022. Epub 2022/01/21. https://doi.org/10.1791/micropub.biology.000510 PMID: 35047763; PubMed Central PMCID: PMC8758995.

28. Consortium CeDM. large-scale screening for targeted knockouts in the Caenorhabditis elegans genome. G3 (Bethesda). 2012; 2(11):1415–25. Epub 2012/11/23. https://doi.org/10.1534/g3.112.003830 PMID: 23173093; PubMed Central PMCID: PMC3484672.

29. Peltz SW, Brown AH, Jacobson A. mRNA destabilization triggered by premature translational termination depends on at least three cis-acting sequence elements and one trans-acting factor. Genes Dev. 1993; 7(9):1737–54. Epub 1993/09/01. https://doi.org/10.1101/gad.7.9.1737 PMID: 8370523.

30. El-Brolosy MA, Stainier DYR. Genetic compensation: A phenomenon in search of mechanisms. PLoS Genet. 2017; 13(7):e1006780. Epub 2017/07/14. https://doi.org/10.1371/journal.pgen.1006780 PMID: 28704371; PubMed Central PMCID: PMC5509088.
Exon-dependent transcriptional adaptation by exon-junction complex proteins

31. Kindt KS, Viswanath V, Macpherson L, Quast K, Hu H, Patapoutian A, et al. Caenorhabditis elegans TRPA-1 functions in mechanosensation. Nat Neurosci. 2007; 10(5):568–77. Epub 2007/04/24. https://doi.org/10.1038/nn1866 PMID: 17450139.

32. Vasale JJ, Gu W, Thivierge C, Batista PJ, Claycomb JM, Youngman EM, et al. Sequential rounds of RNA-dependent RNA transcription drive endogenous small-RNA biogenesis in the ERGO-1/Argonaute pathway. Proc Natl Acad Sci U S A. 2010; 107(8):3582–7. Epub 2010/02/06. https://doi.org/10.1073/pnas.0911908107 PMID: 20133583; PubMed Central PMCID: PMC2840456.

33. Han T, Manoharan AP, Harksins TT, Bouffard P, Fitzpatrick C, Chu DS, et al. 26G endo-siRNAs regulate spermatogenic and zygotic gene expression in Caenorhabditis elegans. Proc Natl Acad Sci U S A. 2009; 106(44):18674–9. Epub 2009/10/23. https://doi.org/10.1073/pnas.0906378106 PMID: 19846761; PubMed Central PMCID: PMC2765456.

34. Gent JI, Lamm AT, Pavelec DM, Maniar JM, Parameswaran P, Tao L, et al. Distinct phases of siRNA synthesis in an endogenous RNAi pathway in C. elegans soma. Mol Cell. 2010; 37(5):679–89. Epub 2010/02/02. https://doi.org/10.1016/j.molcel.2010.01.012 PMID: 20116306; PubMed Central PMCID: PMC2838994.

35. Fischer SE, Montgomery TA, Zhang C, Fahlgren N, Breen PC, Hwang A, et al. The ERI-6/7 helicase acts at the first stage of an siRNA amplification pathway that targets recent gene duplications. PLoS Genet. 2011; 7(11):e1002369. Epub 2011/11/22. https://doi.org/10.1371/journal.pgen.1002369 PMID: 22102826; PubMed Central PMCID: PMC3213143.

36. Le Hir L, Gatfield D, Izaurralde E, Moore MJ. The exon-exon junction complex provides a binding platform for factors involved in mRNA export and nonsense-mediated mRNA decay. EMBO J. 2001; 20(17):4987–97. Epub 2001/09/05. https://doi.org/10.1093/emboj/20.17.4987 PMID: 11532962; PubMed Central PMCID: PMC1256161.

37. Buhler M, Steiner S, Mohn F, Paillusson A, Muhlemann O. EJC-independent degradation of nonsense immunoglobulin-mu mRNA depends on 3' UTR length. Nat Struct Mol Biol. 2006; 13(5):462–4. Epub 2006/04/20. https://doi.org/10.1038/nsmb1081 PMID: 16622410.

38. Longman D, Plasterk RH, Johnstone IL, Caceres JF. Mechanistic insights and identification of two novel factors in the C. elegans NMD pathway. Genes Dev. 2007; 21(9):1075–85. Epub 2007/04/18. https://doi.org/10.1101/gad.177707 PMID: 17437990; PubMed Central PMCID: PMC1855233.

39. Metze S, Herzog VA, Ruepp MD, Muhlemann O. Comparison of EJC-enhanced and EJC-independent NMD in human cells reveals two partially redundant degradation pathways. RNA. 2013; 19(10):1432–48. Epub 2013/08/22. https://doi.org/10.1261/rna.038893.113 PMID: 23962664; PubMed Central PMCID: PMC3854533.

40. Leeks P, Wood JM, Lee BS, Culbertson MR. Gene products that promote mRNA turnover in Saccharomyces cerevisiae. Mol Cell Biol. 1992; 12(5):2165–77. Epub 1992/05/01. https://doi.org/10.1128/mcb.12.5.2165-2177.1992 PMID: 1569496; PubMed Central PMCID: PMC364388.

41. Timmons L, Fire A. Specific interference by ingested dsRNA. Nature. 1998; 395(6705):855. Epub 1998/03/05. https://doi.org/10.1038/27579 PMID: 9804418.

42. Conti E, Izaurralde E. Nonsense-mediated mRNA decay: molecular insights and mechanistic variations across species. Curr Opin Cell Biol. 2005; 17(3):316–25. Epub 2005/04/20. https://doi.org/10.1016/j.ceb.2005.04.005 PMID: 15901503.

43. Muhirad D, Parker R. Aberrant mRNAs with extended 3' UTRs are substrates for rapid degradation by mRNA surveillance. RNA. 1999; 5(10):1299–307. Epub 1999/11/27. https://doi.org/10.1038/s135583.8299990829 PMID: 10573121; PubMed Central PMCID: PMC1369852.

44. Puliaf R, Anderson P. mRNA surveillance by the Caenorhabditis elegans smg genes. Genes Dev. 1993; 7(10):1885–97. Epub 1993/10/01. https://doi.org/10.1101/gad.7.10.1885 PMID: 8104846.

45. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature. 1998; 391(6659):806–11. Epub 1998/03/05. https://doi.org/10.1038/35888 PMID: 9486653.

46. Chen X, Rechavi O. Plant and animal small RNA communications between cells and organisms. Nat Rev Mol Cell Biol. 2022; 23(3):185–203. Epub 2021/10/29. https://doi.org/10.1038/s41580-021-00425-y PMID: 34707241; PubMed Central PMCID: PMC9208737.

47. Johnson CK, Fernandez-Abascal J, Wang Y, Wang L, Bianchi L. The Na(+)-K(+)-ATPase is needed in glia of touch receptors for responses to touch in C. elegans. J Neurophysiol. 2020; 123(5):2064–74. Epub 2020/04/16. https://doi.org/10.1152/jn.00636.2019 PMID: 32292107; PubMed Central PMCID: PMC7444924.

48. Kontarakis Z, Stainier DYS. Genetics in Light of Transcriptional Adaptation. Trends Genet. 2020; 36(12):926–35. Epub 2020/09/16. https://doi.org/10.1016/j.tig.2020.08.008 PMID: 32928563.

49. Brenner S. The genetics of Caenorhabditis elegans. Genetics. 1974; 77(1):71–94. Epub 1974/05/01. https://doi.org/10.1093/genetics/77.1.71 PMID: 4366476; PubMed Central PMCID: PMC1213120.
50. Thompson O, Edgley M, Strasbourger P, Filbotte S, Ewing B, Adair R, et al. The million mutation project: a new approach to genetics in Caenorhabditis elegans. Genome Res. 2013; 23(10):1749–62. Epub 2013/06/27. https://doi.org/10.1101/gr.157651.113 PMID: 23800452; PubMed Central PMCID: PMC3787271.

51. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the ΔΔC(T) Method. Methods. 2001; 25(4):402–8. https://doi.org/10.1006/meth.2001.1262 PMID: 11846609.

52. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 2001; 29(9):e45. https://doi.org/10.1093/nar/29.9.e45 PMID: 11328886; PubMed Central PMCID: PMC55695.

53. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, et al. Fiji: an open-source platform for biological-image analysis. Nat Methods. 2012; 9(7):676–82. Epub 2012/06/30. https://doi.org/10.1038/nmeth.2019 PMID: 22743772; PubMed Central PMCID: PMC3855844.

54. Hodgkin J, Barnes TM. More is not better: brood size and population growth in a self-fertilizing nematode. Proc Biol Sci. 1991; 246(1315):19–24. Epub 1991/10/22. https://doi.org/10.1098/rspb.1991.0119 PMID: 1684664.

55. Kamath RS, Ahringer J. Genome-wide RNAi screening in Caenorhabditis elegans. Methods. 2003; 30(4):313–21. Epub 2003/06/28. https://doi.org/10.1016/s1046-2023(03)00050-1 PMID: 12828945.

56. Yuan JS, Reed A, Chen F, Stewart CN Jr. Statistical analysis of real-time PCR data. BMC Bioinformatics. 2006; 7:85. Epub 2006/03/01. https://doi.org/10.1186/1471-2105-7-85 PMID: 16504059; PubMed Central PMCID: PMC1395339.

57. Pipelers P, Clement L, Vynck M, Hellemans J, Vandesompele J, Thas O. A unified censored normal regression model for qPCR differential gene expression analysis. PLoS One. 2017; 12(8):e0182832. Epub 2017/08/18. https://doi.org/10.1371/journal.pone.0182832 PMID: 28817597; PubMed Central PMCID: PMC5560691.