Regulation of Caenorhabditis elegans RNA Interference by the daf-2 Insulin Stress and Longevity Signaling Pathway

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A conserved insulin-like signaling pathway regulates metabolism, development, stress resistance, and life span in Caenorhabditis elegans (Lee et al. 2003). This pathway involves an insulin-like receptor DAF-2, a phosphatidylinositol-3-OH kinase (PI3K) AGE-1, and the kinase AKT-1, AKT-2 (Paradis and Ruvkun 1998), and PKD-1 (Paradis et al. 1999), as well as the fork head transcription factor DAF-16 (Lee et al. 2003). Loss-of-function mutations in daf-2 or age-1 cause the worm to arrest at the dauer diapause stage rather than proceeding to reproductive development (Ogg et al. 1997). In addition, the daf-2 and age-1 mutants have increased life span and stress resistance, compared with wild type (Ogg et al. 1997; Honda and Honda 2002; Lee et al. 2003). Both the dauer-constitutive and life span phenotypes of the daf-2 or age-1 mutants are suppressed by loss-of-function mutation in the downstream daf-16 gene (Ogg et al. 1997).

RNA interference, or RNAi, is well conserved across phylogeny and protects the genome from invasive genetic elements such as transposons and viruses (Drai and Hannon 2003). Exogenous double-stranded RNA (dsRNA) is cleaved by the RNAi III Dicer into 22-nucleotide short interfering RNA (siRNA) (Bernstein et al. 2001). siRNA is incorporated into a protein complex called RNA-induced silencing complex (RISC), which recognizes and destroys the target mRNA based on the sequence homology between the target mRNA and the trigger dsRNA. siRNA is cleaved by the RNase III Dicer into 22-nucleotide short interfering RNA (siRNA) (Bernstein et al. 2001). siRNA is incorporated into a protein complex called RNA-induced silencing complex (RISC), which recognizes and destroys the target mRNA based on the sequence homology between the target mRNA and the trigger dsRNA.

RNAi Mediates RNAi Response via mRNA Abundance

The phenotypic difference between age-1 and wild type in response to dsRNA correlates with the different change of the target mRNA level. RNAi of the histone gene his-44 causes 100% early larval arrest in age-1(mg305), but does not cause this phenotype in wild type. Similarly, other RNAs of a wide range of histone genes is enhanced in age-1(mg305). Consistent with the enhanced RNAi phenotype, northern analysis shows that after feeding his-44 dsRNA, his-44 mRNA level is significantly decreased in age-1(mg305), whereas no change is observed in wild type (Fig. 1). This decrease in his-44 mRNA level was similar to that induced by the previously known RNAi enhancer mutant rrf-3(pk1426) (Simmer et al. 2002), which also enhances the lethality induced by his-44 RNAi. Therefore, the insuline-like pathway enhances RNAi by facilitating the degradation of target mRNA.

The Dauer-Regulatory Pathways Overlap with the RNAi Pathway

Three parallel signaling cascades regulate dauer arrest in C. elegans: the daf-2/insulin-like, daf-7/TGF-β-like, and daf-11/cyclic GMP pathways (Li et al. 2003). Mutants lacking activity of either pathway arrest at the dauer stage; however, only mutations in the daf-2 insulin-like pathway...
increase longevity and stress resistance of reproductively growing animals (Tissenbaum and Ruvkun 1998; Wolkow et al. 2000). To decide whether the RNAs-enhanced phenotype is caused by some general dauer-inducing signal shared by all three pathways, we tested the RNAi response of dauer-constitutive mutants in the other two pathways, daf-17/TGF-β and daf-11/guanosine cyclase. daf-13(mg29ts) is no more sensitive to RNAi than wild type. daf-17(mg1372) is slightly more sensitive than wild type to lin-1 (22% Muv vs. 0% in wild type) and hour-1 (41% embryonic lethal vs. 28% in wild type), but no different than wild type in response to his-44 dsRNA (7% embryonic lethal vs. 12% in wild type) or col-183 dsRNA (not dumpy) (Table 1). The weak RNAi enhancement of a daf-7 mutant may reflect the known cross-talk between the insulin and TGF-β pathways (Lee et al. 2001; Li et al. 2003). We conclude that RNAi, like stress resistance and longevity, is most affected by mutations in the insulin-like pathway.

CONCLUSIONS

Mutations in the insulin-like pathway enhance RNAi response and this enhancement is dependent on the DAF-16 fork head transcription factor (Lee et al. 2001). Model for how insulin-like signaling affects RNAi is that components that positively regulate RNAi, like dcr-1 and rde-1, are positively regulated by DAF-16, or components that negatively regulate RNAi, like erv-1 (Kennedy et al. 2004) and rde-1 (Simmer et al. 2002), are negatively regulated by DAF-16. The DAF-16 binding site has been determined and 947 C. elegans genes were identified to bear at least one DAF-16 binding site within 1 kilobase (kb) upstream of their predicted transcriptional start sites. Among these genes, 17 genes are orthologous between Drosophila and C. elegans, highlighting the response pathways that may be conserved (Lee et al. 2003). None of those conserved genes with consensus DAF-16 binding sites correspond to known RNAi factors, but many regulatory steps in RNAi have yet to be identified.

Recent study shows that a conserved and pancreatic islet-specific microRNA, miR375, regulates the insulin signaling pathway in mammals (Poy et al. 2004). Over-expression of miR375 suppresses glucose-induced insulin secretion and, conversely, inhibition of endogenous miR-375 function enhances insulin secretion (Poy et al. 2004). MicroRNA processing shares the same machinery with RNAi, which includes the RNAse III Bicr and the Argonaute proteins ALG-1 and ALG-2 (Grishok et al. 1997). The fact that mutation in the insulin-like pathway enhances RNAi suggests a possible negative feedback between insulin signaling and miRNA/siRNA processing.

The nuclear localization of DAF-16 is regulated by insulin-like signaling (Lee et al. 2001). Model for how insulin-like signaling affects RNAi is that components positively regulate RNAi, like dcr-1 and rde-1, are positively regulated by DAF-16, or components that negatively regulate RNAi, like erv-1 (Kennedy et al. 2004) and rde-1 (Simmer et al. 2002), are negatively regulated by DAF-16. The DAF-16 binding site has been determined and 947 C. elegans genes were identified to bear at least one DAF-16 binding site within 1 kilobase (kb) upstream of their predicted transcriptional start sites. Among these genes, 17 genes are orthologous between Drosophila and C. elegans, highlighting the response pathways that may be conserved (Lee et al. 2003). None of those conserved genes with consensus DAF-16 binding sites correspond to known RNAi factors, but many regulatory steps in RNAi have yet to be identified.

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### Table 1. The Dauer-Constitutive Mutants Show Different Sensitivity to RNAi

<table>
<thead>
<tr>
<th>Strains</th>
<th>Feeding RNAa (phenotype)</th>
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<tbody>
<tr>
<td></td>
<td>% of L1/L2 arrest</td>
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<tr>
<td>age-1(mg1375ts)</td>
<td>73 ± 2</td>
</tr>
<tr>
<td>daf-17(mg1372ts)</td>
<td>91 ± 6</td>
</tr>
<tr>
<td>daf-17(mg29ts)</td>
<td>7 ± 3</td>
</tr>
<tr>
<td>daf-17(mg1372ts)</td>
<td>9 ± 6</td>
</tr>
<tr>
<td>ref-1(pk1426)</td>
<td>86 ± 11</td>
</tr>
<tr>
<td>Wild type (N2)</td>
<td>12 ± 9</td>
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</tbody>
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Animals were fed bacteria expressing dsRNA targeting to lin-1 or daf-13 from the L1 stage at 25°C. For the lin-1 and col-183 experiment, dauers from 25°C were recovered at 20°C and allowed to proceed to adulthood when the multiple vulval (Muv) and dumpy body shape phenotypes were scored. For the col-183 experiment, –, +, and ++ indicate no (–), dumpy (+), and extremely dumpy (++) body shape. For the hour-1 experiment, animals at young adult stage were fed lin-1 dsRNA and the percentage of embryonic lethality among their progeny was scored.

### Table 2. The age-1 Mutant Has Enhanced Response to lin-1 dsRNA

<table>
<thead>
<tr>
<th>Strains</th>
<th>% of Muv animals</th>
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</thead>
<tbody>
<tr>
<td>age-1(mg1375ts)</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>daf-17(mg29ts)</td>
<td>1 ± 3</td>
</tr>
<tr>
<td>ref-1(pk1426)</td>
<td>64 ± 6</td>
</tr>
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Animals of the indicated genotypes were fed bacteria expressing lin-1 dsRNA at 20°C since the L1 larval stage. The percentage of animals with multiple vulval structures (Muv) was scored. For this experiment with age-1(mg1375ts) at 25°C, animals arrested as dauers and were then recovered at 20°C to proceed to adulthood when the Muv phenotype was scored.

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Grishok A., Pasquinelli A.E., Conte D., Li N., Parrish S., Ha I., Arabidopsis Plasterk 2003) as well as resistance to virus infection also depend in part on RNAi pathways. It is intriguing to contemplate that the aging regulation by the enhanced. It is possible that the change in chromatin structure in the daf-2 mutants re- leases RNA components from heterochromatin to now allow a more robust silencing of mRNAs.

Mutants lacking the insulin-like signaling, such as daf-2 and age-1, are more resistant than wild type to environmental stresses (Honda and Honda 2002) as well as pathogens (Garza et al. 2003). RNA components also mediate silencing of transposons in C. elegans (Sijen and Plasterk 2003) as well as resistance to virus infection in Arabidopsis (Mourrain et al. 2000). Thus the coupling of RNA responses to the stress resistance pathway of daf-2 insulin-like signaling makes biological sense—as part of a stress resistance pathway, RNA responses may be enhanced. It is intriguing to contemplate that the aging regulation by the daf-2 insulin signaling pathway could also depend in part on RNA pathways.

ACKNOWLEDGMENTS

We thank W. Li, S. Kennedy, C. Wolko, H.A. Tissenbaum, A. Hart, and J. Kaplan for insightful discussion.

REFERENCES


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Access the most recent version at doi:10.1101/sqb.2004.69.429

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