Cell

EXTENDED EXPERIMENTAL PROCEDURES

Computational Procedures

FastqC Reports

We first visualized the fastq files using "fastqC" (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and validated their quality, based on length, nucleotide distribution and sequencing quality. All samples were of high quality, and only contained highly represented sequences due to the presence of adaptor sequences.

Adaptor Clipping

Adapters were removed from the small RNA libraries using "CutAdapt" (Martin, 2011). We clipped the adaptor sequences "TGGAATTCTCGGGTGCCAAGGC" and filtered out any reads which were shorter than 15 nucleotides after the adaptor was removed. In average ~90% of the reads were successfully clipped and ~2%–10% of the reads were filtered out since they were too short.

Bowtie Mapping

We mapped the clipped reads to version ce10 (WS220) of the *C. elegans* genome using bowtie2 (v. 2.1.0) (Langmead and Salzberg, 2012) with local sensitive approach, which aligns with restriction to seed sequence of at least 20 nucleotides.

Reads Counting

We counted the reads using the python script "htseq-count" (Anders et al., 2014) and "ensembl" annotation of the genome (V.66). Small RNA reads that were in the antisense orientation to annotated genes were counted. In an additional analysis we counted the small RNA reads against the whole genome of the worm, using genomic index of 2,500 bases as a "bin."

Differential Expression Analysis; Small RNAs

The counted reads were then submitted to examination of differential expression of small RNAs which aligned against specific genes. We used the DESeq2 method (Anders and Huber, 2010), which is based on a negative binomial distribution, and compared the STGs levels between the different conditions. We considered an STG to be differentially expressed if its adjusted p value (Benjamini-Hochberg correction; FDR) was smaller than 0.1.

Differential Expression Analysis; mRNA

We first normalized the reads for "reads per million" (RPM) and then compared the different conditions as described earlier (see Differential Expression Analysis; small RNAs) based on their fold-change. Transcripts which exhibited more than 1.5 fold-changes between the compared conditions and in addition had more than 5n RPM in total (where "n" is the number of the compared conditions) were considered as changed.

PCA and MA-Plot

We used the DESeq2 package and generated PCA and MA-plots of the different conditions. Log-transformation of the reads and the "plotPCA" function were used to generate the PCA graphs.

Data Clustering

We clustered the data based on the small RNA levels of the 1,175 STGs which were differentially expressed following starvation of the parents. The R package "pvclust" (Suzuki and Shimodaira, 2006) was used in order to determine the significance of the different nodes of the dendrogram. The clustered small RNA levels were normalized for RPM before clustering.

UCSC Visualization

We used the WS220 UCSC genome browser to visualize the small RNA libraries that align to different interesting loci. We used "samtools" (Li et al., 2009) to convert the aligned SAM files into BAM format and for indexing them.

Enrichment Analysis

We used a hypergeometric distribution testing in order to determine enrichment of specific groups of genes.

GO Analysis

We used the GOrilla web-server to determine GO enrichments of the differentially expressed and inherited STGs. We used the option of comparing two unranked lists, with a background list of all the genes of *C. elegans*, generated from ensembl version 66. Additionally, we used the option of comparing one ranked list to generate Figure S3.

SUPPLEMENTAL REFERENCES

Anders, S., and Huber, W. (2010). Differential expression analysis for sequence count data. Genome Biol. *11*, R106.

Anders, S., Pyl, P.T., and Huber, W. (2014). HTSeq A Python framework to work with high-throughput sequencing data (Cold Spring Harbor: Cold Spring Harbor Labs Journals).

Langmead, B., and Salzberg, S.L. (2012). Fast gapped-read alignment with Bowtie 2. Nat. Methods *9*, 357–359.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., and Durbin, R.; 1000 Genome Project Data Processing Subgroup (2009). The Sequence Alignment/Map format and SAMtools. Bioinformatics *25*, 2078–2079.

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal *17*, 10.

Suzuki, R., and Shimodaira, H. (2006). Pvclust: an R package for assessing the uncertainty in hierarchical clustering. Bioinformatics *22*, 1540–1542.