The 5'–3' exoribonuclease *xrn-1* is essential for ventral epithelial enclosure during *C. elegans* embryogenesis

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ABSTRACT

Ribonucleases have been studied in yeast and bacteria, but their biological significance to multicellular organisms is virtually unknown. However, there is increasing evidence that specific, timed transcript degradation is critical for regulation of many cellular processes, including early development and RNA interference. In this report we have investigated the effects of the 5'–3' exoribonuclease *xrn-1* on the development of the nematode worm *Caenorhabditis elegans*. Silencing of *xrn-1* expression using RNA interference results in embryos that fail to complete ventral enclosure, where the outer layer of cells normally closes over the mesoderm in a purse-string movement. Our data suggest that *xrn-1* is involved in a critical aspect of epithelial movement and reveal an unexpected link between RNA stability and morphogenesis. Because *xrn-1* is highly conserved in all eukaryotes, it is possible that it plays a role in similar morphological processes such as dorsal or thorax closure in *Drosophila* and wound healing in humans. In contrast to work in human tissue culture cells, where the 3'–5' pathway has been shown to be the most important for degradation of mRNAs, our work shows that the 5'–3' degradation pathway is crucially important at a critical stage of development in *C. elegans*. We have also investigated whether *xrn-1* can influence the response of *C. elegans* to RNA interference. Our data indicate that *xrn-1* plays a facilitating, but not crucial role in this process.

Keywords: RNA stability; RNA degradation; ribonuclease; RNA interference; embryonic development

INTRODUCTION

Ribonucleases are key factors in the control of mRNA degradation, which is one of the least understood aspects of the control of gene regulation. However, in multicellular organisms, it is increasingly evident that differential regulation of mRNA stability is crucial for normal embryonic development (Cooperstock and Lipshitz 1997; Fontes et al. 1999). In *Caenorhabditis elegans*, a number of genes that are post-transcriptionally regulated have been identified, which include the *xol-1* RNA that is responsible for primary sex determination, the maternal RNAs *glp-1*, *apx-1*, and *pal-1* that control embryonic polarity, and the germ-line sex determination *fem-3* and *tra-2* RNAs. This post-transcriptional regulation is characterized by binding of specific proteins to the 3' UTR of the transcript, which results in repression of translation coupled with RNA degradation (Puoti et al. 2001; Goodwin and Ellis 2002). The

ribonucleases that are responsible for this degradation are unknown.

In the yeast *Saccharomyces cerevisiae*, where mRNA degradation pathways have been extensively analyzed, degradation of mRNA in the 3'–5' direction after de-adenylation occurs through a multicomponent complex of ribonucleases known as the exosome, assisted by the helicase Ski2p (Jacobs Anderson and Parker 1998). The predominant degradation pathway for most RNAs in yeast, however, is decapping followed by degradation in a 5'–3' direction by the processive exoribonuclease Xrn1p (Camponigro and Parker 1996; Mitchell and Tollervey 2000). In *S. cerevisiae*, null mutations in *XRN1* result in slow growth, sporulation defects, and an increase in cell size (Larimer and Stevens 1990; Tishkoff et al. 1991; Larimer et al. 1992; Tishkoff et al. 1995). Mutations in genes encoding proteins in both the 5' and 3' degradation pathways are synthetically lethal, showing that these pathways are essential for viability (Jacobs Anderson and Parker 1998).

In this paper, we have analyzed the effect of the 5'–3' exoribonuclease *xrn-1* on developmental processes in the nematode worm *C. elegans*. We show that this exoribonuclease is crucial at a specific stage of development, where epithelial sheets move together and seal along the ventral

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side of the embryo. We also demonstrate that *xrn-1* is involved in the mechanism of RNA interference, but is not crucial in the RNA interference process.

RESULTS AND DISCUSSION

To study the effect of a 5'–3' exoribonuclease on the development of *C. elegans* we have used RNA-mediated interference to silence the expression of *xrn-1*. The 5'–3' exoribonuclease XRN1 is extremely well conserved in all eukaryotes with its ortholog in *C. elegans* being 61% identical to yeast Xrn1p in the amino-terminal region, which contains the essential magnesium binding site (Fig. 1A). This conservation extends throughout the entire 180-kD protein, with the two other amino-terminal domains being 47% and

52% identical to yeast Xrn1p and the remaining carboxy-terminal half of the protein showing 23% identity to Xrn1p. The *C. elegans* XRN-1 is 63% identical in the amino-terminal region to *Drosophila* PACMAN, which has previously been shown to be differentially expressed throughout development and also degrade nucleic acids in a 5'–3' direction (Till et al. 1998; Chernukhin et al. 2001). The *xrn-1* gene is located on chromosome II (Y39G8C.1) at map position 19.36. The length of the *xrn-1* cDNA is predicted to be 5406 bp encoding a protein of 1801 amino acids, which is similar in length to *Drosophila* PACMAN and yeast XRN1p. The similarity of *C. elegans* Xrn1 to *Drosophila* PACMAN and mouse Xrn1 is emphasized in the evolutionary comparison shown in Figure 1B. These three proteins are more closely related to each other than to yeast Xrn1p, with the nuclear versions of all four proteins (orthologous

FIGURE 1. (A) Alignment of the amino-terminal 181 amino acids of the 205-kD *C. elegans* *xrn-1* protein with orthologs from *Drosophila* (accession no. Q9VW11), mouse (accession no. Q35651), and *S. cerevisiae* (accession no. P22147) using Bioedit software (<http://www.mbio.ncsu.edu/Bioedit/bioedit.html>). (B) Phylogenetic tree of the above amino acid sequences together with the *Schizosaccharomyces pombe* XRN1 homolog ExoII (P40383). The related *S. cerevisiae* nuclear 5'–3' exoribonuclease RAT1 (accession number Q02792) and its orthologs from *Drosophila* (accession number Q95RS5, CG10354), mouse (accession number Q61489), *C. elegans* (accession number Q9U299, Y48B6A.3), *S. pombe* (accession number P40848), and *Arabidopsis thaliana* (accession numbers Q9FQ02, Q9FQ03, Q9FQ04) are also given. These sequences were aligned using CLUSTALW and a tree constructed using weighted neighbor-joining (PHYMLIP). The phylogenetic tree was drawn using the Phylo dendron software (<http://iubio.bio.indiana.edu/treeapp/treeprint-form.html>).

to *S. cerevisiae* Rat1p), being grouped together on a distant part of the evolutionary tree (Fig. 1B).

To silence *xrn-1* expression, a region at the 3' end of the coding region, which had no significant matches to other sequences in the database, was chosen for targeting. Injection of double-stranded RNA corresponding to this region of *xrn-1* into adult hermaphrodites results in embryos that arrest at the twofold stage of development, 48 h after injection. These embryos fail to complete ventral enclosure, where the epithelial cells normally stretch over both sides of the embryo and then seal together on the ventral side. Subsequent attempted elongation movements cause the internal cells to ooze through the hole in the epidermis, resulting in "bulged out" embryos (Fig. 2A, panels A, B) that subse-

quently die. We also introduced double-stranded *xrn-1* RNA into hermaphrodites by feeding (Kamath et al. 2001). This results in embryos with similar ventral enclosure defects and short arrested L1 larvae that fail to elongate properly (data not shown). These data suggest that *xrn-1* is involved in events during ventral enclosure such as the control of changes in cell shape, cell movement, or cell adhesion.

The specific effects of *xrn-1* on development were surprising as mutations in *XRN1*, the ortholog in *S. cerevisiae*, have pleiotropic effects. To confirm that the injected double-stranded *xrn-1* RNA was down-regulating *xrn-1* protein levels we used Western blotting on embryos from uninjected and injected hermaphrodites (150 of each). These results showed that *xrn-1* protein in embryos from hermaphrodites injected with double-stranded *xrn-1* RNA was reduced to undetectable levels compared to uninjected controls (Fig. 2B), confirming the efficacy of RNA interference in *xrn1* (*RNAi*) embryos.

To examine the effect of *xrn-1* on morphogenesis in more detail we used embryos where the gene encoding the junction adhesion molecule *ajm-1* (*jam-1*) is fused in-frame to GFP (strain SU93). In this strain, all cells of the embryos are outlined during the twofold stage and changes in cell shape that take place during elongation can be readily visualised (Mohler et al. 1998; Raich et al. 1999). The hypodermis of *C. elegans* originates as six rows of cells positioned on the dorsal surface of the embryo. Shortly after they are born, the two rows of dorsal-most cells interdigitate and then fuse to each other in a process known as dorsal intercalation to form the dorsal syncytium (Fig. 2C, panel A). After dorsal intercalation begins, the anterior leading ventral hypodermal (epidermal) cells on both sides of the embryo elongate toward the ventral midline before fusing or forming stable adherens junctions. The hypodermal cells posterior to the leading edge cells, known as the ventral pocket cells, then become wedge-shaped and stretch as they approach the ventral midline (Fig. 2C, panel C). The ventral pocket is then closed by an actomyosin purse-string mechanism that pulls together the edges of the hypodermal sheet at the ventral midline to seal the embryo (Fig. 2C, panel D). Finally the embryo elongates

FIGURE 2. (A) *xrn-1*(*RNAi*) *C. elegans* embryos (panels A, B) compared to wild-type embryos (panel C). The wild-type embryo at the top right of panel C has completed ventral enclosure and is at the twofold stage. The remaining two wild-type embryos are at the threefold stage, have completed elongation, and are actively moving. The embryos in panels A and B are the progeny of hermaphrodites injected with double-stranded *xrn-1* RNA. These embryos have failed to complete ventral enclosure, with consequent oozing of internal cells through the hole in the epidermis and subsequent elongation has not occurred. The scale bar represents 10 μ m and applies to all panels. (B) Western blot showing that XRN-1 is down-regulated in embryos from hermaphrodites injected with double-stranded *xrn-1* RNA (lane 2) compared to uninjected controls (lane 1). The expression of the loading control (actin) is unaffected. (C) Ventral enclosure in wild-type and *xrn-1*(*RNAi*) embryos carrying the adherens junction marker *ajm-1::GFP* (*jam-1::GFP*) viewed using fluorescence superimposed over the DIC image. Embryos are positioned with the anterior to the left. Panel A: Dorsal view of a wild-type embryo at completion of dorsal intercalation (340 min after fertilization). Lateral seam cells on the right of the embryos are marked with an arrow. Panel B: Lateral view of a wild-type embryo (430 min after fertilization) showing the prominent lateral seam cells (arrow). Panel C: Ventral view of a wild-type embryo beginning normal ventral enclosure (310 min postfertilization). Anterior leading cells are indicated with arrows and ventral pocket cells with arrowheads. Panel D: Ventrolateral view of an embryo on completion of ventral enclosure (360 min postfertilization). Panels E and F: Dorsal view of *xrn-1*(*RNAi*) embryos showing retraction of the lateral seam cells (white arrow) onto the dorsal surface of the embryo and contraction of the dorsal syncytium (blue arrow) into a narrow band after failure of ventral enclosure. Panel G: Ventral view of a *xrn-1*(*RNAi*) embryo. Note the disorganization of the anterior leading cells and pocket cells. Panel H: Ventrolateral view of a *xrn-1*(*RNAi*) embryo showing failure of the ventral pocket cells (arrow) to reach the ventral midline. Scale bar, 10 μ m.

into a worm shape by contraction of circumferentially organized actin filaments and microtubules with consequent elongation of the hypodermal cells along the anterior-posterior axis (Fig. 2A, panel C; Chin-Sang and Chisholm 2000; Simske and Hardin 2001).

The *xrn-1(RNAi)* embryos expressing *ajm-1::GFP* display a dorsal retraction phenotype, with the hypodermal cells retracted back onto the dorsal surface of the embryo (Fig. 2C, panels E, F) as a result of failure of ventral enclosure. The lateral seam cells are arranged correctly in relation to each other but are on the dorsal, rather than the lateral side of the embryo and the dorsal syncytium has contracted into a narrow band (cf. Fig. 2C, panels E, F and Fig. 2C, panel A). On the ventral side of affected embryos, the anterior hypodermal cells are disorganised (Fig. 2C, panel G) or undergo partial migration and fusion together with attempted elongation of the anterior portion of the embryo (Fig. 2C, panel H). We saw no evidence of correct elongation and adhesion of the ventral pocket cells in affected embryos, although we cannot rule out that the ventral pocket cells reach the midline and then retract after failure to adhere to each other. The failure of enclosure on the ventral side of the embryo and retraction of the hypodermis dorsally results in the protrusion of gut and other cells from the inside of the embryo (Fig. 2A, panels A and B) and subsequent death of the embryo.

To determine the expression pattern of *xrn-1* in *C. elegans* we constructed a GFP fusion to genomic *xrn-1*. The *xrn-1* gene spans 20 kb of sequence, has 17 introns, and an upstream intergenic region of 3.1 kb that presumably includes the promoter sequences. Because of the large size of genomic *xrn-1* we fused the GFP reporter, in-frame, to the second exon of *xrn-1* and included the entire intergenic upstream region. This fusion construct was used to generate three transgenic lines. Analysis of the GFP expression in adults shows that it is expressed in the hypodermis and circumferentially around the rectum (Fig. 3A). No other specific expression was detected. In embryos, the expression of the GFP reporter is extremely faint or undetectable. However, this experiment is limited in scope as we are using a fusion to GFP that only includes part of the gene; therefore XRN-1 could be expressed elsewhere or localized in manners that we cannot detect with this construct. The expression of *xrn-1* in the hypodermis that we observed is consistent with a role in controlling cell adhesion or cell shape.

We also explored the possibility that *xrn-1*, as an exoribonuclease, might be involved in the mechanism of RNA interference (Bosher and Labouesse 2000; Hutvagner and Zamore 2002). Normally, in RNAi experiments, affected embryos may be observed 12–24 h after injection of the hermaphrodites. We observed that embryos showing the *xrn-1(RNAi)* phenotype were produced 48–72 h after injection of the hermaphrodites, a delay that suggested to us that the *xrn-1* double-stranded RNA may partially repress the

FIGURE 3. (A) (Panels A, B) Expression pattern of *xrn-1::GFP*. GFP fluorescence pattern of animals carrying a *rol-6* marker and a GFP reporter fused to the second exon of *xrn-1*, expressed as a nonintegrated extrachromosomal array. (B) Effect of *xrn-1* on RNA interference. Panels A and B: Expression of an integrated *histone::GFP* marker in the nuclei of oocytes (A) and embryos (B) in the germline of wild-type hermaphrodites. Panel C: *histone::GFP* hermaphrodites injected with double-stranded GFP RNA showing complete silencing of GFP expression, 48 h postinjection. Panel D: Co-injection of *dcr-1* and GFP double-stranded RNA into hermaphrodites showing faint fluorescence in oocytes. Panel E: Silencing of GFP expression in adult worms co-injected with double-stranded GFP RNA and double-stranded *unc-22* RNA 48 h postinjection. Panel F: *histone::GFP* hermaphrodites co-injected with *xrn-1* and GFP double-stranded RNA showing faint fluorescence in the oocyte nuclei of the germline 48 h after gonad injection. Panel G: Triple injection of double-stranded *xrn-1*, *dcr-1*, and GFP RNA into hermaphrodites results in faint fluorescence in oocytes in most worms. Panel H: Control triple injection of *unc-22*, *mab-9*, and GFP double-stranded RNA showing no fluorescence except for autofluorescence in the gut. Scale bar, 50 μ m.

RNA interference process. To test this possibility we used a strain carrying a integrated copy of *histoneH2B::GFP* (Strome et al. 2001), which is expressed clearly in germ-line nuclei of hermaphrodites and all nuclei of early embryos

(Fig. 3B, panels A, B). Injection of double-stranded *GFP* RNA into these embryos completely silences the *histone::GFP* expression after 48 h (Fig. 3B, panel C).

To validate the above system for investigating the effects of particular genes on RNA interference we used double-stranded *dcr-1* RNA. The gene *dcr-1* encodes the homolog of DICER, which cleaves double-stranded RNA to give the crucial first step in the RNAi process (Zamore et al. 2000; Bernstein et al. 2001). Co-injection of double-stranded *dcr-1* RNA with double-stranded *GFP* RNA resulted in weak fluorescence in most animals (68%, $n = 22$; Fig. 3B, panel D) indicating that, as expected, *dcr-1* reduces the efficiency of RNA interference. No fluorescence was detected in control hermaphrodites injected with double-stranded *unc-22* plus double-stranded *GFP* RNA used at comparable concentrations showing that there is no dilution effect (Fig. 3B, panel E). In each case, the double-stranded RNAs were injected individually into another group of worms to ensure that they produced the expected phenotype (data not shown). This result is similar to previously published data showing that animals mutant for the *dcr-1* gene have defects in RNA interference in some, but not all conditions (Zamore et al. 2000; Bernstein et al. 2001).

Because the *histone::GFP* system proved to be a reliable indicator of efficiency of RNA interference for a gene known to be involved in this process, we co-injected double-stranded *xrn-1* RNA with double-stranded *GFP* RNA into *histone::GFP* hermaphrodites. In 64% ($n = 25$) of the hermaphrodites injected we could see weak fluorescence in the oocyte nuclei or the syncytial gonads, 48 h after injection (Fig. 3B, panel F) indicating that *xrn-1* reduced the efficiency of RNA interference in most animals. The residual RNA interference activity may be due to redundancy or alternative pathways.

To determine whether *dcr-1* enhances the effect of *xrn-1* on RNA interference, we injected double-stranded *dcr-1*, *xrn-1*, and *GFP* RNA into hermaphrodites. These triple injections resulted in most hermaphrodites showing faint fluorescence of *histone::GFP* in oocytes and early embryos (79%, $n = 33$; Fig. 3B, panel G). Control triple injections using ds *unc-22*, *mab-9*, and *GFP* RNA at comparable concentrations resulted in no fluorescence (Fig. 3B, panel H). These RNAs were used because *unc-22* and *mab-9* are thought not to be involved in RNA degradation pathways. Control injections using double-stranded *xrn-1* RNA, double-stranded *unc-22* RNA, and double-stranded *GFP* RNA gave faint fluorescence, as did injections of double-stranded *dcr-1* RNA, double-stranded *unc-22* RNA, and double-stranded *GFP* RNA (data not shown). In each case, these double-stranded RNAs were injected individually into a control group of animals to confirm that they gave the expected phenotype (data not shown). If *xrn-1* and *dcr-1* are in different but parallel pathways leading to RNA interference, then it would be expected that *xrn-1* would enhance

the effect of *dcr-1* on RNA interference and brighter staining by the reporter would occur. Our results for the triple injections, where we observe faint staining, suggest that *xrn-1* may be in the same pathway as *dcr-1*. The residual RNA interference activity, therefore, may be the result of a redundant or alternative pathway that is distinct from *dcr-1* and *xrn-1*.

Our experiments show that *xrn-1* may play a facilitating role in the mechanism of RNA interference. Because the RISC complex (RNA-induced silencing complex) is known to have ribonuclease activity (Hammond et al. 2000), is very large, and some of its components have not yet been identified, it is possible that XRN-1 may be part of this complex. The role of *xrn-1* in RNA interference is, however, not crucial, suggesting redundancy within this complex or that RNA interference occurs by multiple pathways. The evidence for multiple pathways or redundancy is supported by recent experiments showing that animals mutant for the *dcr-1* gene have defects in RNA interference in some, but not all conditions (Zamore et al. 2000; Bernstein et al. 2001; Knight and Bass 2001). In addition, depletion of dFXR (the *Drosophila* homolog of the fragile X mental retardation protein), which is known to be associated with the RISC complex, results in only partial loss of RNAi efficiency in *Drosophila* S2 cells (Caudy et al. 2002).

Our results show, for the first time, that the 5'–3' degradation pathway plays a biologically significant role in organisms other than the yeast *S. cerevisiae*. The relative importance of the 5'–3' and 3'–5' degradation pathways in the cells of metazoa have only recently been experimentally analyzed. In human tissue culture cells, it has been shown that degradation of unstable RNAs such as *c-myc* occurs in a 3'–5' direction through the exosome, which has led to the suggestion that the 5'–3' pathway is of minor importance in multicellular organisms (Wang & Kiledjian 2001; Mukherjee et al. 2002; van Hoof and Parker 2002). However, our results, using whole organisms, rather than individual tissue culture cells, show that the 5'–3' degradation pathway is crucial at critical stages of development. These results suggest a regulatory role for *xrn-1* in developmental processes. In *C. elegans xrn-1(RNAi)* embryos, it is likely that lack of exoribonuclease activity leads to up-regulation of a particular RNA or RNAs, which in turn lead to the observed phenotype.

This is the first time that a ribonuclease has been shown to have a specific effect on a developmental process. Because development until the twofold stage proceeds normally, *xrn-1* is presumably not essential during the initial cell divisions, but does become critical during ventral enclosure, which requires significant changes in cell movement and cell adhesion. Similar morphogenetic movements, where epithelial sheets move together and then seal are common during development of all multicellular organisms. For example, dorsal and thorax closure in *Drosophila*, wound healing in humans, and closure of the hind-brain during

neural tube formation in vertebrates (Jacinto et al. 2001) are known to be similar morphogenetic processes. Because *xrn-1* is highly conserved in all eukaryotes, it is possible that it plays a role in these processes in other organisms. Our recent work in *Drosophila* shows that mutations in *pacman* (the homolog of *xrn-1*) leads to defects in thorax closure (D.P. Grima, K.C. Wan, Y. Okada, and S.F. Newburg, in prep.).

Complete failure of enclosure with a similar retraction of the hypodermis onto the dorsal side of the *C. elegans* embryo has also been observed in embryos mutant for various adhesion and signalling molecules. Maternal and zygotic loss of HMR-1/cadherin results in a similar phenotype to that of *xrn-1(RNAi)* embryos, and this is thought to be due to the failure of formation of adherens junctions at the ventral midline (Costa et al. 1998; Raich et al. 1999). Inactivation of the *C. elegans* APC-related gene *apc-1* also leads to failure of enclosure (Hoier et al. 2000), but also results in dorsal intercalation defects, which are not seen in *xrn-1(RNAi)* embryos. Ventral enclosure defects are also seen in embryos mutant for the *vab-1* ephrin receptor tyrosine kinase and its ephrin ligand *vab-2* (Chin-Sang et al. 1999). It is thought that ephrin signaling may set up signalling events between the underlying substrate where they are expressed and overlying, migrating hypodermal cells (Chin-Sang et al. 1999; Chin-Sang and Chisholm 2000). It is possible that the 5'–3' exoribonuclease XRN-1 modulates the expression of one or more of these molecules.

CONCLUSIONS

We have shown that the 5'–3' exoribonuclease *xrn-1* is essential for ventral enclosure in *C. elegans*. This morphogenetic process, where two epithelial sheets move together and seal, also occurs during dorsal closure in *Drosophila* and wound healing in humans, therefore *xrn-1*, which is highly conserved, may be involved in similar processes in other organisms. Our results show an unexpected link between RNA stability and morphogenesis in *C. elegans* demonstrating the crucial importance of regulated RNA stability in multicellular development. In contrast to work on tissue culture cells, our results show that the 5'–3' mRNA degradation pathway is biologically significant. Our data also demonstrate that *xrn-1* plays a facilitating but not crucial role in the RNA interference process and that *xrn-1* is likely to be in the same pathway as *dcr-1*.

MATERIALS AND METHODS

Strains and growth conditions

All *C. elegans* strains were derived from the wild-type Bristol strain N2 and all experiments were performed at 20°C.

RNA interference

For RNA interference, T7 and T3 RNA polymerase promoter sequences were added to the PCR primer sequences to facilitate antisense and sense in vitro transcription. RNA was then synthesized directly from gel-purified product essentially as described (Fire et al. 1998) and injected into the gonad of young N2 adults at a concentration of 1.5 mg/mL. The primers used were as follows: *xrn-1* forward primer: 5'-ATTAACCCTCACTAAAGAACAA GCCGAAGGATACG-3'; *xrn-1* reverse primer: 5'-AATACGACTC ACTATAGTTC AACACCATCGACTCC-3'; *dcr-1* forward primer: 5'-AATACGACTCACTATAGCATTCATCCTATCTCTGC-3'; *dcr-1* reverse primer: 5'-ATTAACCCTCACTAAAGCGACATCAGCCA TCAGTG-3'; *mab-9* forward primer: 5'-ATTAACCCTCACTAA AGCTAATCCTAAACTCAATGCAC-3'; *mab-9* reverse primer: 5'-AATACGACTCACTATAGCTTTATTGAAATTTCTGCAGG-3'; *GFP* forward primer: 5'-ATTAACCCTCACTAAAGGAGAGGGT GAAGGTGATG-3'; *GFP* reverse primer: 5'-AATACGACTCACT ATAGGGTCTGCTAGTTGAACGC-3'.

All of the above reactions used genomic DNA as template except for *GFP* double-stranded RNA, where the PCR product was amplified from the plasmid pPD95.75. *unc-22* double-stranded RNA was prepared from a plasmid LT61 (from the Fire lab vector kit) using T7 promoter primers. RNAs were co-injected at equal concentrations into the germline of young adult worms expressing histone::GFP (F54E12.4::GFP = H2B::GFP; Strome et al. 2001) and the fluorescence of nuclei within the gonad monitored 48 h after injection.

To construct a plasmid expressing double-stranded *xrn-1* RNA, a PCR fragment generated using the above *xrn-1* primers was cloned into the "feeding vector" L4440. *Escherichia coli* (strain HT115) expressing double-stranded RNA was fed to young adult N2 worms as described (Kamath et al. 2001).

Construction of transgenic worms

To generate transgenic worms expressing a *xrn-1::GFP* fusion, the entire intergenic region upstream of *xrn-1*, plus genomic DNA that included the first intron and part of the second exon (4.6 kb) was amplified by PCR and cloned, in-frame, into the GFP reporter plasmid pPD95.75 (kindly supplied in the Fire Lab. vector kit, Carnegie Institute of Washington, Baltimore). This GFP fusion was then injected into worms along with the pRF4 *rol-6* marker and transgenic Rol lines examined for their GFP expression using a Zeiss Axiophot microscope.

Western blotting

One-hundred-fifty hermaphrodites were injected with *xrn-1* double-stranded RNA, and after 48 h, the embryos were collected by treating the hermaphrodites with bleach (Hope 1999). Embryos were then boiled in 2 × loading buffer (250 mM Tris at pH 6.8, 4% SDS, 10% glycerol, 0.006% bromophenol blue, 2% mercaptoethanol, plus protease cocktail inhibitors [Roche]) and loaded onto a 7% NuPAGE Tris-Acetate gel (Invitrogen) and electrophoresed at 150 V for 1 h. Embryos from 150 noninjected hermaphrodites were used as a control. Proteins were transferred onto Immobilon membrane at 90 mA for 16 h using a BioRad transblot system. Proteins were detected using a rabbit polyclonal antibody raised

against the *Drosophila* Xrn-1 homolog (1:2000) and a peroxidase conjugated anti-rabbit secondary antibody (1:80,000; Sigma). The actin loading control was detected using a monoclonal mouse antiactin clone C4 antibody (1:10,000; ICN) and a peroxidase conjugated antimouse secondary antibody (1:80,000; Sigma).

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REFERENCES

- Bernstein, E., Caudy, A.A., Hammond, S.M., and Hannon, G.J. 2001. Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* **409**: 363–366.
- Bosher, J.M. and Labouesse, M.L. 2000. RNA interference: Genetic wand and genetic watchdog. *Nat. Cell Biol.* **2**: E31–E36.
- Camponigro, G. and Parker, R. 1996. Mechanisms and control of mRNA turnover in *Saccharomyces cerevisiae*. *Microbiol. Rev.* **60**: 233–249.
- Caudy, A.A., Myers, M., Hannon, G.J., and Hammond, S.M. 2002. Fragile X-related protein and VIG associate with the RNA interference machinery. *Genes & Dev.* **16**: 2491–2496.
- Chernukhin, I.V., Seago, J.E., and Newbury, S.F. 2001. The *Drosophila* 5'–3' exoribonuclease *pacman*. *Methods Enzymol.* **342**: 293–302.
- Chin-Sang, I.D. and Chisholm, A.D. 2000. Form of the worm: Genetics of epidermal morphogenesis in *C. elegans*. *Trends Genet.* **16**: 544–551.
- Chin-Sang, I.D., George, S.E., Ding, M., Moseley, S.L., Lynch, A.S., and Chisholm, A.D. 1999. The ephrin VAB-2/EFN-1 functions in neuronal signaling to regulate epidermal Morphogenesis in *C. elegans*. *Cell* **99**: 781–790.
- Cooperstock, R.L. and Lipshitz, H.D. 1997. Control of mRNA stability and translation during *Drosophila* development. *Sem. Cell. Dev. Biol.* **8**: 541–549.
- Costa, M., Raich, W., Agbunag, C., Leung, B., Hardin, J., and Priess, J.R. 1998. A putative catenin-cadherin system mediates morphogenesis of the *Caenorhabditis elegans* embryo. *J. Cell Biol.* **141**: 297–308.
- Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., and Mello, C.C. 1998. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **391**: 806–811.
- Fontes, A.M., Ito, J., and Jacobs-Lorena, M. 1999. Control of messenger RNA stability during development. *Curr. Top. Dev. Biol.* **44**: 171–202.
- Goodwin, E.B. and Ellis, R.E. 2002. Turning clustering loops: Sex determination in *Caenorhabditis elegans*. *Curr. Biol.* **12**: R111–R120.
- Hammond, S.M., Bernstein, E., Beach, D., and Hannon, G.J. 2000. An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells. *Nature* **404**: 293–296.
- Hoier, E.F., Mohler, W.A., Kim, S.K., and Hajnal, A. 2000. The *Caenorhabditis elegans* APC-related gene *apr-1* is required for epithelial cell migration and Hox gene expression. *Genes & Dev.* **14**: 874–886.
- Hope, I.A., ed. 1999. *C. elegans, a practical approach*. Oxford University Press, New York.
- Hutvagner, G. and Zamore, P.D. 2002. RNAi: Nature abhors a double-strand. *Curr. Opin. Genet. Dev.* **12**: 225–232.
- Jacinto, A., Martinez-Arias, A., and Martin, P. 2001. Mechanisms of epithelial fusion and repair. *Nat. Cell Biol.* **3**: E117–E123.
- Jacobs Anderson, J.S. and Parker, R. 1998. The 3' to 5' degradation of yeast mRNAs is a general mechanism for mRNA turnover that requires the SKI2 DEVH box protein and 3' to 5' exonucleases of the exosome complex. *EMBO J.* **17**: 1497–1506.
- Kamath, R.S., Martinez-Campos, M., Zipperlen, P., Fraser, A.G., and Ahringer, J. 2001. Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in *Caenorhabditis elegans*. *Genome Biol.* **2**: 1–10.
- Knight, S.W. and Bass, B.L. 2001. A role for the RNase III enzyme DCR-1 in RNA interference and germ line development in *Caenorhabditis elegans*. *Science* **293**: 2269–2271.
- Larimer, F.W. and Stevens, A. 1990. Disruption of the gene XRN1, coding for a 5' → 3' exoribonuclease, restricts yeast cell growth. *Gene* **95**: 85–90.
- Larimer, F.W., Hsu, C.L., Maupin, M.K., and Stevens, A. 1992. Characterization of the XRN1 gene encoding a 5' → 3' exoribonuclease: Sequence data and analysis of disparate protein and mRNA levels of gene-disrupted yeast cells. *Gene* **120**: 51–57.
- Mitchell, P. and Tollervey, D. 2000. mRNA stability in eukaryotes. *Curr. Opin. Genet. Dev.* **10**: 193–198.
- Mohler, W.A., Simske, J.S., Williams-Masson, E.M., Hardin, J.D., and White, J.G. 1998. Dynamics and ultrastructure of developmental cell fusions in the *Caenorhabditis elegans* hypodermis. *Curr. Biol.* **8**: 1087–1090.
- Mukherjee, D., Gao, M., O'Connor, J.P., Raijmakers, R., Pruijn, G., Lutz, C.S., and Wilusz, J. 2002. The mammalian exosome mediates the efficient degradation of mRNAs that contain AU-rich elements. *EMBO J.* **21**: 165–174.
- Puoti, A., Pugnale, P., Belfiore, M., Schlappi, A.C., and Saudan, Z. 2001. RNA and sex determination in *Caenorhabditis elegans*. Post-transcriptional regulation of the sex-determining *tra-2* and *fem-3* mRNAs in the *Caenorhabditis elegans* hermaphrodite. *EMBO Rep.* **2**: 899–904.
- Raich, W.B., Agbunag, C., and Hardin, J. 1999. Rapid epithelial-sheet sealing in the *Caenorhabditis elegans* embryo requires cadherin-dependent filopodial priming. *Curr. Biol.* **9**: 1139–1146.
- Simske, J.S. and Hardin, J. 2001. Getting into shape: Epidermal morphogenesis in *Caenorhabditis elegans* embryos. *Bioessays* **23**: 12–23.
- Strome, S., Powers, J., Dunn, M., Reese, K., Malone, C.J., White, J., Seydoux, G., and Saxton, W. 2001. Spindle dynamics and the role of γ -tubulin in early *Caenorhabditis elegans* embryos. *Mol. Biol. Cell* **12**: 1751–1764.
- Till, D.D., Linz, B., Seago, J.E., Elgar, S.J., Marujo, P.E., Elias, M., McClellan, J.A., Arraiano, C.M., McCarthy, J.E.G., and Newbury, S.F. 1998. Identification and developmental expression of a 5'–3' exoribonuclease from *Drosophila melanogaster*. *Mech. Dev.* **79**: 51–55.
- Tishkoff, D.X., Johnson, A.W., and Kolodner, R.D. 1991. Molecular and genetic analysis of the gene encoding the *Saccharomyces cerevisiae* strand exchange protein Sep1. *Mol. Cell. Biol.* **11**: 2593–2608.
- Tishkoff, D.X., Rockmill, B., Roeder, G.S., and Kolodner, R.D. 1995. The SEP1 mutant of *Saccharomyces cerevisiae* arrests in pachytene and is deficient in meiotic recombination. *Genetics* **139**: 495–509.
- van Hoof, A. and Parker, R. 2002. Messenger RNA degradation: Beginning at the end. *Curr. Biol.* **12**: R285–R287.
- Wang, Z. and Kiledjian, M. 2001. Functional link between the mammalian exosome and mRNA decapping. *Cell* **107**: 751–762.
- Zamore, P.D., Tuschl, T., Sharp, P.A., and Bartel, D.P. 2000. RNAi: Double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. *Cell* **101**: 25–33.

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