Protection of germline immortality by the soma via a secreted endoribonuclease

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Abstract
In sexually reproducing organisms maintenance of germ stem cell immortality is fundamental for transmitting genetic material to future generations. While previous research has mainly considered intrinsic regulatory mechanisms in the germline, our recent study has found a direct contribution of somatic cells in preserving germline immortality via the somatically expressed endoribonuclease ENDU-2 in Caenorhabditis elegans. We have identified ENDU-2 as a secreted protein that can be taken up by the germline. Here, we discuss how ENDU-2 might uncouple its RNA-binding and RNA-cleavage activities to control gene expression via either an endoribonuclease dependent or an independent way. We also speculate on a possible functional conservation of its mammalian homologs in mediating cell-cell communication as well as its potential significance in understanding human pathogenesis such as cancer development.

KEYWORDS
ENDOU, germline, immortality, RNA-binding protein, RNA, Soma, stress

INTRODUCTION
Even though germline is physically set apart from the soma early in embryogenesis, the communication between the soma and germline is insensitively studied in multicellular model organisms to understand how reproduction and somatic maintenance are coordinated during development and upon alteration in environmental conditions. Up to date, we know that the germline is not only anatomically connected to the surrounded somatic tissues, but also relies on somatic supply of small molecules, such as nutrients, ligands, and hormones, for normal development as well in response to stress. Gene expression in the germline can be additionally directly regulated by miRNAs that are enclosed in multivesicular bodies and released by somatic cells. In contrast to somatic cells, the germline is characterized by its competence of maintaining immortality that allows reproduction for unlimited generations. Previous studies describing how germline immortality is established have almost exclusively focused on intrinsic regulatory mechanisms in the germline, such as telomere maintenance, germline specific histone modification as well as contribution of certain small RNA pathways. It was poorly known whether the soma is also involved in maintenance of germline immortality, especially under stress conditions, even though the differentiated somatic cells are supposed to be more prone to sense environmental alterations than totipotent germ stem cells. Here we outline our recent findings about protection and maintenance of germline immortality by the soma via secretion of the endoribonuclease ENDU-2 in C. elegans. We found that ENDU-2 binds to miRNA and affects abundance of transcripts in both soma and germline. Based on these
findings we propose that ENDU-2 confers a non-cell-autonomous mechanism to control gene expression across tissue boundaries, thus mediating soma-to-germline communication.

**ENDU-2 IS A MEMBER OF THE CONSERVED POLY-U SPECIFIC ENDORIBONUCLEASE FAMILY**

*C. elegans* ENDU-2, together with ENDU-1, are members of a highly conserved poly-U specific endoribonuclease (ENDOU) family that exists from virus to human. The most conserved regions, XenomoD domains, share a putative active site that may be involved in diverse RNA processing pathways. Studies ranging from viral to human orthologs of these members reveal that the XenomoD domain can bind to and cleave RNA in a Mn$^{2+}$ dependent manner, leading to production of molecules with 2',3'-cyclic phosphate ends in which the 2'- and 3'-positions of ribose is bridged by the phosphate. XenomoD/ENDOU in Xenopus is involved in biogenesis of U16 and U68 small nuclear RNAs through the intron cleavage of pre-mRNAs. Recently, both Xenopus XenomoD and human ENDOU have been shown to promote tubular endoplasmic reticulum (ER) formation via triggering local mRNA decay on the ER membrane upon Ca$^{2+}$ release. Additionally, human ENDOU has been found to cleave the upstream open reading frame of C/EBP homologous protein mRNA to promote its translation. The overall physiological roles of ENDOU were poorly described until the viral ENDOU orthologue Nsp15 has drawn great attention due to its presence in all corona virus. Nsp15 ensures virus replication and the viral ENDOU orthologue Nsp15 has drawn great attention due to implicating that ENDU-2 probably acts independent of pRNA pathway to preserve germline immortality. The finding that the impaired reproduction in ENDU-2(lf) animals is reversible at 15°C additionally indicates that an alternative repair mechanism at low temperature is able to suspend the germline malfunction caused by lack of ENDU-2.

By analyzing the expression pattern of ENDU-2, we observed that an EGFP tagged ENDU-2 protein localized in the extracellular space, suggesting that ENDU-2 is secreted. Using computational prediction tools we found that 19 amino acids at the N-terminus of the ENDU-2 protein compose a putative ER targeting peptide. Four observations support the notion that the identified ER targeting peptide allows secretion of ENDU-2 via the ER-Golgi secretory pathway for its subsequent uptake into the germline. First, immunofluorescence staining reveals the presence of the ENDU-2 protein in the germline even though ENDU-2 mRNA is not detectable in the gonad. Second, elimination of the ER targeting sequence abolishes both germline localization of ENDU-2 and its function in preserving germline immortality. Third, adding the ENDU-2 ER targeting sequence to the N-terminus of EGFP is sufficient to trigger secretion of EGFP. Fourth, replacement of the predicted ER targeting sequence of ENDU-2 with the ER targeting sequence from another secreted protein, SEL-1, suffices its secretion and germline function. These data collectively suggest that guiding ENDU-2 into the secretory pathway is necessary and sufficient for its uptake as well as its protective role in the germline. In addition, expression of transgenic ENDU-2 in the neurons or intestine is sufficient to ensure germline immortality, while muscular or somatic gonadal expressed ENDU-2 fails to do this. This is a striking result, as ENDU-2 as a secreted protein is supposed to have the same activity independent of its tissue origin. Our current hypothesis is that tissue specific interaction partners of ENDU-2 might also contribute to its germline activity.

**SOMATIC CELLS ENSURE GERMLINE IMMORTALITY VIA SECRETED ENDU-2**

During maintenance of endu-2 loss-of-function (lf) mutant animals, we noticed that their brood size gradually decreased over generations at 20°C, the standard culture temperature of worms. This results in a complete sterility after 10–20 generations. Such mortal germline (Mrt) phenotype is caused by loss-of-germline immortality that impedes the indefinite dividing capacity of germ stem cells. In addition, the Mrt phenotype in endu-2(If) mutant animals is temperature dependent: at 25°C, it requires a lower number of generations to reach 100% sterility, while growth at 15°C is able to reverse the already existing partial sterile phenotype caused by growth of the previous generations at higher temperature. These observations suggest that decreased telomerase activity is less likely the cause of the Mrt phenotype in endu-2(If) animals, as the role of telomerase to preserve germline immortality is independent of temperature. Mutants in the nuclear RNAi pathway that is involved in transgenerational RNAi inheritance in the germline also have a temperature dependent Mrt phenotype. Loss of endu-2 function, however, does not impede transgenerational RNAi inheritance, suggesting that ENDU-2 acts independently of the nuclear RNAi pathway to ensure germline immortality. Moreover, mutants defective in pRNA function became gradually sterile due to abnormal accumulation of ribosomal siRNAs that lead to RNAi mediated gene silencing of rRNAs. Therefore, the Mrt phenotype in pRNA mutants could be suppressed by inactivation of nuclear RNAi factor hrde-1. hrde-1 mutation, however, did not suppress the Mrt phenotype in endu-2(If) mutant, implicating that ENDU-2 probably acts independent of pRNA pathway to preserve germline immortality. The finding that the impaired reproduction in ENDU-2(lf) animals is reversible at 15°C additionally indicates that an alternative repair mechanism at low temperature is able to suspend the germline malfunction caused by lack of ENDU-2.

**RNA-BINDING AND RNA-CLEAVAGE ACTIVITIES OF ENDU-2 ARE SEPARABLE FOR REGULATING GENE EXPRESSION IN THE SOMA AND THE GERMLINE**

Our RIP-seq analysis reveals that ENDU-2 binds to about 25% of total protein-coding mRNAs. In addition, adding Ca$^{2+}$ or Mn$^{2+}$ can trigger ENDU-2 dependent RNA decay in the lysate, corresponding to previous studies on ENDU, XenomoD and Nsp15 which demonstrate that their RNA cleavage activities are relatively nonspecific. We
propose that ENDU-2 likely affects mRNA stability to control transcript levels. To identify the transcripts regulated by ENDU-2, we performed transcriptome analyses of whole animals as well as isolated gonads. Combined with the data from the RIP-seq, about 60 mRNAs are considered as ENDU-2 direct targets in the soma as they are directly bound and negatively regulated by ENDU-2 in the whole animal’s transcriptomic study but not in the gonadal transcriptome analysis. In addition, ENDU-2 binds and negatively regulates about 200 transcripts in the gonad. The finding that less than 10% of the ENDU-2 bound mRNAs are also regulated by ENDU-2 implicates that ENDU-2 is able to distinguish bound-only and bound-cleavage targets. It is not clear yet how ENDU-2 could selectively hydrolyze certain associated transcripts and which additional regulatory inputs contribute to discriminate RNA-cleavage from RNA-binding. Binding of ENDU-2 to a large number of transcripts might also provide a platform for recruitment of additional RNA-binding proteins required for the functional activity of ENDU-2.

The germline transcripts negatively regulated by ENDU-2 are primarily actively expressed in the soma but not in the germline of wild type animals, indicating a crucial role of ENDU-2 to safeguard a germline specific gene expression program. Furthermore, using specific ENDU-2 mutant variants that differ in their RNA-binding and RNA-cleavage activities, we find that the RNA-binding but not the cleavage activity plays an essential role in preventing misexpression of soma-specific genes in the germline and maintenance of germline immortality. In contrast, the RNA-cleavage activity of ENDU-2 is required for the down-regulation of specific mRNA targets in the soma. These results suggest that ENDU-2 can uncouple its RNA-binding and -cleavage capabilities to control gene expression via distinct mechanisms.

OPEN QUESTIONS AND OUTLOOK

The detailed mechanism used by ENDU-2 to prevent misexpression of soma-specific genes in the germline remains enigmatic. Several key questions still need to be addressed. (1) How does ENDU-2 recognize which germline targets should be repressed? Only ENDU-2 from the neurons and the intestine, but not from the other tissues, is able to preserve germline immortality. Coincidently, our transcriptomic data reveal that ENDU-2 in the germline preferentially reduces transcript levels that have enriched expression in neurons or intestine. Are these neuronal or intestinal specific transcripts repressed by neuronal ENDU-2 or intestinal derived ENDU-2, respectively? (2) Does ENDU-2 repress misexpression of soma-specific genes in the germline at the transcriptional level or post-transcriptional level? The RNA-cleavage independent function of ENDU-2 suggests that ENDU-2 might belong to a surveillance machinery to recognize leaking transcription of soma-specific genes in the germline for subsequent decay by additional RNases. Alternatively, ENDU-2 could repress transcription of these soma specific genes by a yet unidentified mechanism in addition to its known role as an endoribonuclease. (3) Does ENDU-2 act in the P granules to fulfill its function? Germline P granules have also been shown to prevent accumulation of soma-specific transcripts.[22]

Our unpublished data reveal that more than 80% of ENDU-2 bound and repressed transcripts in the germline are also up-regulated in P granule depleted animals, suggesting a similarity between ENDU-2 and germline P granule-mediated functions. Notably, ENDU-2 proteins in the germline are localized in cytosolic puncta-like structures with enrichment in the perinuclear region. Therefore, ENDU-2 might be partially located in P granules and contribute to some of the P granule mediated functions to confine soma-specific transcripts. (4) How are the mRNA-cleavage and mRNA-binding activities separately regulated? Is this controlled by association of distinct protein complexes? It has been shown that only RNA-cleavage but not RNA-binding activity of XendoU is Mn2+ dependent.[21] Hence, control of ENDU-2’s access to Mn2+ might be crucial for coordinating the two aspects of ENDU-2 function. (5) Which transcripts are cleaved directly by ENDU-2? Although results from us and the others suggest an unspecific RNA cleavage activity of ENDU-2 in the lysate and ENDU-2 binds to about 25% of protein-coding transcripts, lack of ENDU-2 only affects the level of less than 10% of its binding transcripts. One way to identify cleavage substrates of ENDU-2 is sequencing the cleavage products which contain a 2′, 3′ cyclic phosphate (cP) at their 3′ termini. The 3′ terminal cP, however, inhibits adaptor ligation, thus preventing their capture with standard RNA-seq methods. A recently developed cP-RNA-seq method enables the identification of cP-containing RNAs.[23] The cP-containing transcripts with significantly lower abundant in endu-2(lf) mutants than in wild type animals are potential cleavage products of ENDU-2. (6) The most unique feature of ENDU-2 is it’s secretory property which might mediate communication between cells. Why has evolution evolved such a mechanism to combine somatic expression and secretion of ENDU-2 instead of expressing ENDU-2 directly in the germline? We anticipate that differentiated somatic cells, especially neurons, are more adequate to sense environmental alterations, while germline stem cell should sustain their totipotency and stay in the undifferentiated state. In such a scenario, secreting a somatic factor to adjust germline gene expression enables a quick response without expressing diverse receptors and signaling molecules in the germline. If this hypothesis is true, exploring regulators or signaling pathways affecting secretion of ENDU-2 would significantly contribute to understand how intercellular communication coordinates task assignment between the soma and the germline in multicellular organisms.

CONCLUSION

In summary, C. elegans ENDU-2 can regulate gene expression in tissues located distant from those expressing it, especially in response to environmental stimuli such as temperature elevation. Therefore, ENDU-2 mediates a novel non-cell-autonomous mechanism across tissue boundaries, most notably from the soma to the germline. The presence of a putative ER targeting peptide for secretion in the other EndoU homologs (zebra fish, mouse and human) strongly suggests a possible conserved function of this family of proteins in exerting intercellular communication. Notably, human ENDU is highly expressed in the placenta, raising the speculation whether human ENDOU could be
involved in maternal control of inherited traits. In addition, given that tumor cells frequently display misregulated expression of ENDOU, it will be of interest to study the function of ENDOU in tumor biology, in particular its potential role in affecting communication between tumor cells and their microenvironment.

AUTHOR CONTRIBUTIONS
Wenjing Qi, Thomas Heimbucher, and Ralf Baumeister wrote the manuscript, Fan Xu generated the graphical abstract figure.

CONFLICT OF INTEREST
The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are openly available by Nature Communications at https://www.nature.com/articles/s41467-021-21516-6.

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