Mechanisms and developmental roles of XRN-2 mediated RNA regulation in *Caenorhabditis elegans*

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ABSTRACT

This research offers an in-depth exploration of developmental roles and RNA regulationmechanisms of XRN-2, a 5'-to-3' exoribonuclease primarily found in the nucleus. This enzymeplays multifaceted roles in gene expression and development in the nematode *Caenorhabditis elegans* (*C. elegans)*. XRN-2 is involved in degrading and trimming various classes of RNA, significantly affecting processes such as transcription termination and ribosome biogenesis. Although it exhibits broad substrate specificity *in vitro*, XRN-2 selectively targets specific RNA subsets through interactions with other proteins *in vivo*. This research emphasizes that while XRN-2 is indispensable for critical developmental stages in *C. elegans*, the precise molecular pathways through which it operates remain enigmatic due to its widespread expression patternthroughout development.

To gain a deeper understanding of XRN-2's role in animal development, this study details two investigative strategies. The first strategy focused on identifying genetic suppressorslinked to XRN-2 during development, with the aim of uncovering the specific role played by XRN-2 in this context. The second approach involved identifying synthetic lethality partners of XRN-2. By finding synthetic lethality partners of XRN-2, the study aimed to uncover the functional interplay between XRN-2 and these partners, thereby providing a more comprehensive understanding of XRN-2's developmental roles within specific molecular pathways.

In the first part of this research, the developmental roles of XRN-2 in the germline of *C. elegans* were explored using a germline-specific conditional mutant (*xrn-2ts*^{germ}) of XRN- 2, which had been created for the purpose of identifying genetic suppressors of sterility. Four genes, *dpy-10*, *osr-1*, *ptr-6*, and *C34C12.2*, were found to possess loss-of-function alleles, andtheir roles in supressing sterility in the mutant were investigated. Among these genes, *dpy-10*, *osr-1*, and *ptr-6* were identified as positive regulators of *gpdh-1*, a crucial enzyme in glycerol production. When these genes were knocked down, elevated *gpdh-1* mRNA levels were observed, and fertility in *C. elegans* was restored. Furthermore, C34C12.2, a nuclear protein, found as sharing homology with S. cerevisiae Net1, was implicated in the potential regulation of rRNA maturation. Moreover, the restoration of fertility in *xrn-2ts*^{germ} animals was observed as a result of the depletion of NRDE-2, which is a component of the nuclear RNAi machinery and serves as an interacting partner of C34C12.2 in *C. elegans*. These findings suggested thecritical role of XRN-2 in germline development, particularly in the context of rRNA maturation.

In the second part of the study, PUF-9 was identified as a synthetic lethality partner of XRN-2 in *C. elegans*. This discovery underscores the crucial role of PUF-9 in the development of this organism. When *puf-9* was knocked down in *xrn-2* temperature sensitive mutant (*xrn- 2ts*) animals, the worms exhibited a significant decline in vitality, sluggish behaviour, and exacerbated phenotypes such as blister formation and molting defects. Importantly, PUF-9 wasfound to be indispensable for germline development. *puf-9* mutant worms displayed reduced brood size and significant germline deficiencies, including abnormal oocyte development, impaired gonad migration, and sterility. Furthermore, *puf-9* exhibited synthetic lethality with *puf-3* and *puf-8*, indicating a potential genetic interaction between these proteins. Knockdown of either *puf-3* or *puf-8* in *puf-9* mutant animals resulted in significant germline developmental abnormalities, further highlighting the pivotal role of PUF-9 in this critical biological process. In addition to this, the observation of a diminished brood size in the *xrn-2*;*puf-9* double mutant, in contrast to the effects seen in each single mutant, implies a shared functionality between PUF-9 and XRN-2 in the regulation of *C. elegans* germline development.

Finally, the specific mRNA targets regulated by XRN-2 and the mechanisms employed for RNA degradation were sought to be identified. To achieve this, a dataset derived from a

comprehensive time-course RNA expression analysis conducted using a temperature-sensitive *xrn-2* mutant was employed. As a result of the inactivation of *xrn-2*, distinct sets of upregulated and downregulated RNAs were observed. It was noted that the majority of upregulated RNAs appeared to be influenced by transcription read-through events originating from their upstream gene counterparts, while the subsets of downregulated RNAs resulted from collisions between RNA polymerase II (RNAPII) molecules. Upon an in-depth examination of the *xrn-2ts* RNA sequencing data, a significant discovery was made concerning a gene designated as *ceh-99*. Interestingly, following *xrn-2* inactivation, an increase in *ceh-99* expression was observed, although this increase could not be attributed to transcription read-through from its upstream gene. This intriguing observation aroused our interest, leading to the selection of *ceh-99* as a prime candidate for uncovering the mechanisms underlying XRN-2-mediated regulation. It was demonstrated that XRN-2 represses the expression of *ceh-99* through premature termination of RNAPII and the selective repression of a *ceh-99* isoform with a longer 5' UTR. Additionally, an insertion of the TC1 autonomous DNA transposon was identified in the first intron region of the *ceh-99* gene. Furthermore, it was observed that TC1 expression increased following XRN-2 inactivation, and the overexpression of *ceh-99* resulted in an elevated TC1 expression, suggesting that XRN-2 regulates the TC1 expression partially through the *ceh-99* locus.