Translational activation maintains germline tissue homeostasis during adulthood

Marco Nousch1,* and Christian R Eckmann1,2

1Division of Genetics; Institute of Biology; Martin Luther University, Halle-Wittenberg; Halle, Saale, Germany; 2Max Planck Institute of Molecular Cell Biology and Genetics; Dresden, Germany

Adult tissue maintenance is achieved through a tightly controlled equilibrium of 2 opposing cell fates: stem cell proliferation and differentiation. In recent years, the germ line emerged as a powerful in vivo model tissue to investigate the underlying gene expression mechanisms regulating this balance. Studies in numerous organisms highlighted the prevalence of post-transcriptional mRNA regulation, which relies on RNA-targeting factors that influence mRNA fates (e.g. decay or translational efficiency). Conserved translational repressors were identified that build negative feedback loops to ensure one or the other cell fate. However, to facilitate a fast and efficient transition between 2 opposing cell fates, translational repression per se appears not to be sufficient, suggesting the involvement of additional modes of gene expression regulation. Cytoplasmic poly(A) polymerases (cytoPAPs) represent a unique class of post-transcriptional mRNA regulators that modify mRNA 3’ ends and positively influence cytoplasmic mRNA fates. We recently discovered that the 2 main cytoPAPs, GLD-2 and GLD-4, use distinct mechanisms to promote gene expression and that cytoPAP-mediated mRNA activation is important for regulating the size of the proliferative germ cell pool in the adult Caenorhabditis elegans gonad. Here, we comment on the different mechanisms of the 2 cytoPAPs as translational activators in germ cell development and focus on their biological roles in maintaining the balance between germline stem cell proliferation and differentiation in the Caenorhabditis elegans gonad.

Introduction

Maintenance of a healthy balance between proliferation and differentiation is an essential aspect during adult tissue homeostasis. Increased proliferation at the expense of differentiation leads to tissue overgrowth, and conversely, increased differentiation at the expense of proliferation leads to tissue shrinkage; both scenarios will eventually produce a non-functional tissue. The Caenorhabditis elegans (C. elegans) germ line is a powerful model system to study the molecular mechanisms underlying this balance of opposing cell fates and tissue maintenance.1-3 In the adult gonad, germ cells form a syncytial tissue and are arranged in a distal-to-proximal organization that reflects subsequent developmental germ cell stages. Most distally and in close proximity to the germ-line niche (i.e. the somatic gonadal distal tip cell), proliferative germ cells are located that constitute the proliferative zone. At a defined distance from the distal tip, germ cells exit the mitotic cell cycle and further proximally start differentiation by entering prophase I of meiosis. This switch from proliferation-to-differentiation has been termed the mitosis-to-meiosis decision.2,4

Across species, gene expression regulation in germ cells occurs to a large extent at the post-transcriptional level. Hence, many conserved post-transcriptional RNA regulators have been identified to function in germ cell development.1 Especially mRNA-associated translational repressors, i.e., RNA-binding proteins and miRNA-containing protein complexes, were found to form self-enforcing negative feedback loops to maintain germ cell fate

Keywords: germ cells, RNA regulation, poly(A) polymerases, transit-amplifying cells, C. elegans

© Marco Nousch and Christian R Eckmann
*Correspondence to: Marco Nousch; Email: marco.nousch@genetik.uni-halle.de
Submitted: 03/06/2015
Revised: 03/30/2015
Accepted: 04/10/2015
http://dx.doi.org/10.1080/21624054.2015.1042644
This is an Open Access article distributed under the terms of the Creative Commons Attribution-Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The moral rights of the named author(s) have been asserted.
In C. elegans, although no clear involvement of miRNAs has been revealed to date, representatives of 2 translational repressor protein families influence the mitosis-to-meiosis decision: FBF-1 and FBF-2, 2 nearly identical PUF protein family members (commonly referred to as FBF), facilitate proliferation; the STAR protein family member, GLD-1, facilitates differentiation. To prevent the switch from proliferation to differentiation, FBF limits among many differentiation-promoting mRNA targets GLD-1 protein synthesis to post-mitotic cells by selectively binding to gld-1 mRNA in pre-meiotic cells. Also in other germ line stem cell model systems, translational repression emerged as a key mode of gene expression regulation.

The poly(A) tail of mRNAs is a dynamic structure and changes of tail lengths in the cytoplasm are indicative of gene expression regulation. Especially in developmental contexts, the length of the poly(A) tail correlates with mRNA stability and the amount of protein produced: long-tailed mRNAs are more stable and can attract a larger number of ribosomes per mRNA (termed polysomes); short-tailed mRNAs are more prone to degradation and attract less ribosomes. Hence, translational repressors that recruit A-tail-shorting enzymes (i.e. deadenylases) generate mRNA species with trimmed poly(A) tails, which upon translational de-repression probably require additional help to remain stable and efficiently engage in protein production. This help is likely provided in the form of cytoplasmic poly(A) polymerases (cytoPAPs). These conserved A-tailing enzymes are expected to counteract mRNA deadenylation, yet their mode of action and target mRNA repertoire remained unknown. Moreover, cytoPAPs lack common RNA-binding domains and, therefore, are hypothesized to recognize their mRNA targets via interactions with other RNA-binding proteins. Recently, we investigated the underlying mRNA-regulatory mechanisms of the 2 distinct C. elegans cytoPAPs, GLD-2 and GLD-4, and how both represent 2 opposing forces in fine-tuning the mitosis-to-meiosis decision.

GLD-4 promotes translational efficiency
GLD-4 is in its enzymatic domain evolutionarily most similar to that of non-canonical TRF4-type poly(A) polymerase family members. Yet, in many organisms TRF4 proteins are nuclear enzymes that primarily target a variety of non-coding RNA substrates by adding short adenosine stretches (~10–20 nts) for exosome-mediated degradation. By contrast, GLD-4 is predominantly located in the cytoplasm, presumably targeting mRNAs for enhanced expression. GLD-4 shows poly(A) polymerase activity in a heterologous in vivo tethering system, but its endogenous activity on gene-specific A-tail length extension appears rather moderate at steady state. Importantly, GLD-4 depends on its co-factor GLS-1 for efficient PAP activity in tethering assays, suggesting that the GLD-4/GLS-1 complex comprises the active cytoPAP. To reveal the impact of GLD-4 on global poly(A) tail metabolism, we recently measured A-tail lengths on bulk RNA and found that poly(A)-tails were only mildly reduced in the absence of GLD-4 or GLS-1 (unpublished results), arguing that GLD-4 cytoPAP may have an intrinsic low enzymatic activity that is similar to that of its nuclear counterparts. A-tailing in the nucleus by TRF4 proteins leads to the degradation of the respective RNA substrates by the exosome. In our transcriptome analysis of gld-4–compromised animals, we detected only minor changes in mRNA abundance, arguing that GLD-4 has no major role in promoting cytoplasmic mRNA degradation. Taken together, this suggests that GLD-4–mediated polyadenylation in the cytoplasm might fulfill a different function.

GLD-4 function is linked to general translation (Fig. 1A). Our sucrose gradient analysis revealed a severe reduction of
poly-ribosomes in \textit{gld-4}–compromised animals. Moreover, GLD-4 protein co-migrates with polysomes, suggesting a role in promoting efficient translation.\textsuperscript{16} Although it still needs to be shown that GLD-4’s polymerase activity is needed for modifying mRNAs before or during polysome formation, it is tempting to speculate that GLD-4–mediated polyadenylation may counteract the proposed gradual erosion of mRNA poly(A) tails in polysomes.\textsuperscript{25} Also in favor of this idea is our finding that GLS-1, a strong potentiator of GLD-4 cytoPAP activity, co-migrates with polysomes in density gradient analyses and enhances polysome formation.\textsuperscript{16} Alternatively, the GLD-4/ GLS-1 complex might support bulk polysome formation in an enzyme-independent manner by a yet to be identified mechanism.

GLD-4 promotes proliferation

Many aspects of germ line development require GLD-4 function.\textsuperscript{18} However, a role in adult tissue homeostasis, regulating the mitosis-to-meiosis decision had remained uncharacterized. By following aging hermaphrodites, we found that GLD-4 maintains the normal size of the proliferative zone and prevents its progressive shrinkage.\textsuperscript{17} The lab of Judith Kimble had recently provided convincing evidence that the proliferative zone contains 2 pools of cells with distinct properties; a distal pool of about 30–70 germline stem cells (GSCs) that primarily proliferate to self-renew, and a proximal pool of transit-amplifying cells with presumably limited proliferation potential that are primed for differentiation.\textsuperscript{26,27} Interestingly, the extent of the proliferative zone that remains in \textit{gld-4} mutants is similar to the distal GSC pool.\textsuperscript{17} Hence, GLD-4 may function primarily in transit-amplifying cells to control their proliferative capacity. Alternatively, due to a global requirement of GLD-4 in maintaining the expression of broadly acting proliferative factors, both pools of cells may have shrunk in \textit{gld-4} mutants.

Which proliferative factors might be targets of GLD-4–mediated translational activation? Initially, we suspected that FBF represents a suitable candidate. However, we found no convincing evidence that FBF expression depends on GLD-4 activity. By contrast, we discovered the Notch receptor-encoding mRNA \textit{glp-1} as an important target of GLD-4 (Fig. 1C).\textsuperscript{17} GLP-1 keeps germ cells undifferentiated and proliferative by sensing niche-produced Notch ligands.\textsuperscript{28,29} GLP-1 protein expression is restricted to post-mitotic differentiation factors. Irrespective of a possible global nature of GLD-4–mediated enhanced translation in stem cell systems, GLD-4 cytoPAP is important for specific mRNAs to promote protein amounts that are required to execute a specific cell fate decision.

GLD-2 stabilizes translationally repressed mRNAs

\textit{C. elegans} GLD-2 is the founding member of the GLD-2–type cytoPAP family, whose representatives are in many species central determinants of cytoplasmic poly(A) metabolism.\textsuperscript{14,19} Its poly (A) polymerase capability was also demonstrated in a heterologous \textit{in vivo} assay, by artificially tethering GLD-2 to an mRNA substrate.\textsuperscript{36} A few gene-specific poly(A) tail measurements on putative GLD-2 targets also supported a role in A-tail extension of endogenous germ cell-specific mRNAs.\textsuperscript{21,37-39} Recently, we analyzed the magnitude of GLD-2–mediated A-tailing by performing bulk poly(A) tail measurements, which provides a snapshot of the polyadenylation status of all mRNAs rather than that of individual mRNAs. With this assay, we detected a general shortening of tails in \textit{gld-2}–compromised animals,\textsuperscript{18} arguing that GLD-2 cytoPAP polyadenylates many germline mRNAs rather than a small subset of individual targets to control their expression.

Cytoplasmic polyadenylation is proposed to be important for translational activation of target mRNAs in many tissues across species.\textsuperscript{11,40,41} To identify GLD-2 targets in \textit{C. elegans} and reveal how GLD-2 promotes gene expression at the functional level in the worm, we analyzed the abundance and translation efficiency of mRNAs at the global scale, comparing \textit{gld-2}–deficient animals to wild type in RNA deep sequencing and polysome profiling experiments. Our transcriptome analysis revealed that high, wild-type levels of many germline mRNAs depend on GLD-2. Interestingly, we could not detect a significant impact of
GLD-2 on the translatability of mRNAs in our polysome analysis. This could be explained by our experimental set up, which was geared toward the detection of strong changes in mRNA translation efficiency; moderate or weak changes would have gone unnoticed. Alternatively, its initial role in mRNA stabilization may have overshadowed a subsequent role in promoting translation. A detection of the second GLD-2 role would have been masked in our data set due to the premature degradation of GLD-2 targets. Hence, we concluded that GLD-2 cytopPAP primarily stabilizes or promotes the initial expression of its target mRNAs (Fig. 1B). Therefore, we defined less abundant mRNAs as GLD-2 targets. Obviously, some mRNAs will also have been indirectly down-regulated as a consequence of GLD-2–dependent developmental changes in the mutant. Nonetheless, we think that our list of GLD-2–stabilized germline genes is highly enriched for direct targets and provides a valuable resource for studying GLD-2–regulated mRNAs.

GLD-2–mediated polyadenylation appears to be a major part of post-transcriptional gene expression networks in the germ line. Among all GLD-2–stabilized mRNAs, ~70% have been reported to be likely targets of several translational repressors: FBF-1, GLD-1, and OMA-1, a TIS11 zinc-finger protein family member. This suggests that GLD-2–mediated mRNA stabilization is primarily important for mRNAs that are subject to prior or continued translational repression. In this respect, it is important to note that mRNA deadenylation represents the first step of the major mRNA degradation pathway, and that numerous translational repressors, such as members of the PUF protein family, recruit deadenylases as part of their repressive activity. Hence, GLD-2–mediated cytoplasmic polyadenylation appears to represent an effective counterforce to RNA decay-inducing poly(A) tail removal. Due to the large mRNA target overlap with various translational repressors, we propose that GLD-2 is a major mRNA stabilizer that protects many—if not all—translationally repressed genes in germ cells (Fig. 1B).

GLD-2 promotes differentiation

GLD-2 family members are important for late stages of female germ cell development in many organisms. In C. elegans, also an earlier role during oogenesis had been revealed, in which GLD-2 promotes the expression of the FBF-target GLD-1 to facilitate differentiation in the mitosis-meiosis decision. Based on the large overlap between GLD-2 and FBF targets, we think that GLD-2 enhances the production of proteins from a broad range of differentiation-promoting mRNAs, most likely after their release from repression. In our recent work, we were able to show that GLD-2 protein production is actively repressed in the proliferative zone: GLD-2 protein abundance is very low in the distal half of the proliferative zone (i.e., GSCs), gradually increases further proximal in transit-amplifying cells, and reaches its peak expression in cells that have entered meiotic prophase. We found that gld-2 mRNA is targeted for translational repression by FBF in the proliferative zone. The 3' UTR of gld-2 carries an active FBF-binding site and RNAi-mediated down regulation of FBF leads to an up regulation of GLD-2 protein in distal germ cells. Although, it is at the moment unclear whether upregulated GLD-2 is actively promoting differentiation and how strongly other FBF targets, such as GLD-1, contribute to differentiation, the loss of FBF correlates with a complete loss of the proliferative cell fate. This suggests that GLD-2 levels are kept low in proliferating cells by FBF to prevent premature entry into differentiation, and substantiates GLD-2's role as a major positive mRNA regulator that ensures a swift and efficient transition from proliferation to differentiation (Fig. 1C).

The relationship between GLD-2 and GLD-4

In the mitosis-to-meiosis decision, the 2 translational activators represent opposing forces. As described above, GLD-4 promotes the proliferative cell fate and loss of GLD-4 shrinks the proliferative zone. By contrast, GLD-2 promotes the differentiation fate and loss of GLD-2 expands the proliferative zone. Our analysis of adult gld-2 gld-4 double mutants showed that the extent of the proliferative zone resembles wild-type size. Although it is not clear at the moment whether the proliferative zone in the double mutant resembles a wild-type composition of GSCs and transit-amplifying cells, this result suggests that either single mutant defect reflects an imbalance between the 2 translational activators. How this apparent balance between GLD-4 and GLD-2 may be achieved at the functional level is currently unknown. One simple idea is that the mRNA target activation strength of both cytopPAPs sums up to be equal. Alternatively the 2 enzymes may be tightly connected and limit each other’s activities, either directly as part of the same RNA-protein complexes or via indirect mechanisms, such as competing for similar co-factors. Nonetheless, our findings illustrate that counteracting translational activators of most likely equal strength, yet with different mechanistic properties, are involved in setting the boundary of proliferation and differentiation.

Translational activation vs. repression in germine homeostasis

GLD-2 and GLD-4 are part of an ever-expanding, highly redundant post-transcriptional RNA regulatory network that governs, downstream of niche signaling, the switch from mitosis to meiosis. But what is the contribution of translational activation and repression? A combined removal of translational repressors (i.e. FBF and GLD-1) leads to the loss of differentiation and a germline tumor forms. By contrast, a combined removal of both translational activators (i.e., GLD-4 and GLD-2) leads to differentiation onset in a superficially comparable manner to wild type. This suggests that, in combination with GLP-1 signaling, translational repression may be sufficient to organize the mitosis-to-meiosis decision, and that cytopPAP-mediated translational activation by itself cannot initiate the switch to meiosis. However, this simplistic interpretation may be incorrect, given that translational activation may be mechanistically coupled to repression. Support for this idea comes also from the findings that many GLD-2 target mRNAs of early differentiation are FBF targets, and that
FBF binds and stimulates GLD-2 cytoplasmic polyadenylation activity. Therefore, the effectiveness of translational activation may depend on prior repression and is hidden in its absence. Moreover, it remains possible that, next to GLD-2 and GLD-4, additional, yet to be discovered poly(A) polymerases or other translational activators may exist that function in the mitosis-to-meiosis decision.

Clearly, basic translation repression activity of GLD-1 is not enough to sustain the cell fate switch to meiotic prophase and commitment to meiosis relies on translational activation. This differential requirement of cytoplasmic polyadenylation can be explained by a differential dose-dependent requirement of meiosis-promoting factors for initiation and maintenance. With respect to the differential dose-dependent requirement of cytoplasmic poly(A) polymerases for successive stages of differentiation is explained by a choreography of both molecular mechanisms and timing. Therefore, it is possible that, next to GLD-2 and GLD-4, other regulators of opposing cell fate decisions may exist that function in the mitosis-to-meiosis decision.

In summary, we propose that translational repres sors of opposing cell fate decisions in GSCs.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Tosin Oyewale and Sophia Millonig for comments on the manuscript.

Funding

Funding was provided by the Max Planck Society (MPI-CBG), Deutsche Forschungsgemeinschaft (DFG grant EC369-2/3 and EC369-3/1), and an EFRE start-up grant from Sachsen-Anhalt to C.R.E.

References

1. Nousch M, Eckmann CR. Translational control in the Caenorhabditis elegans germ line. Adv Exp Med Biol 2013; 757:205-47; PMID:22872479; http://dx.doi.org/10.1007/978-1-4614-4015-4_8
2. Kershen A, Crittenden SL, Friend K, Sorensen EB, Porter DF, McCall MM, Kimble J. Developmental regulation of the mitotic/meiotic decision in the Caenorhabditis elegans nematode. Adv Exp Med Biol 2013; 757:71-99; PMID:22872475; http://dx.doi.org/10.1007/978-1-4614-4015-4_4
3. Crittenden SL, Eckmann CR, Wang L, Bernstein DS, Wikmans K, Kucher N. Regulation of the mitotic/meiotic decision in the Caenorhabditis elegans nematode. Philos Trans R Soc Lond B Biol Sci 2003; 358:1359-62; PMID:14511482; http://dx.doi.org/10.1098/ rstb.2003.1333
4. Sladina M, Lehmann R. Translational control in germ-line stem cell development. J Cell Biol 2014; 207:13-21; PMID:25351405; http://dx.doi.org/10.1083/jcb.201407102
5. Jones AR, Francis R, Schelle T. GLD-1, a cytoplasmic protein essential for oocyte differentiation, shows stage- and sex-specific expression during Caenorhabditis elegans germ line development. Dev Biol 1996; 180:165-83; PMID:8948533; http://dx.doi.org/10.1006/dbio.1996.0283
6. Critten SL, Bernstein DS, Bachorik JL, Thompson BE, Gallegos MA, Moulder G, Barstead R, Wikmans K, Kimble J. A conserved RNA-binding protein controls germline stem cells in Caenorhabditis elegans. Nature 2002; 417:660-3; PMID:12059606; http://dx.doi.org/10.1038/nature00754
7. Kershen A, Kimble J. Genome-wide analysis of mRNA targets for Caenorhabditis elegans FBF, a conserved stem cell regulator. Proc Natl Acad Sci U S A 2010; 107:3936-41; PMID:20412496; http://dx.doi.org/10.1073/pnas.100495107
8. Insco ML, Bailey AS, Kim J, Olivas GE, Wapinski OJ, Tam CH, Fuller MT. A self-limiting switch based on translational control regulates the transition from proliferation to differentiation in an adult stem cell lineage. Cell Stem Cell 2012; 11:689-700; PMID:23122292; http://dx.doi.org/10.1016/j.stem.2012.08.012
9. Wang Y, Stave JM, Wilhelm JE, Newmark PA. A functional genomic screen in planarians identifies novel regulators of germ cell development. Genes Dev 2010; 24:2081-92; PMID:20844018; http://dx.doi.org/10.1101/gad.1951010
10. Wodell L, Belloc F, Bava FA, Mendes R. Translational control by changes in poly(A) tail length: recycling mRNAs. Nat Struct Mol Biol 2012; 19:577-85; PMID:22664985; http://dx.doi.org/10.1038/nsmb.2311
11. Subchenly AO, Eichorn SW, Chen GR, Sive H, Bartel DP. Poly(A)-tail profiling reveals an embryonic switch in translational control. Nature 2014; 508:66-71; PMID:24476825; http://dx.doi.org/10.1038/nature13007
12. Goldstrohm AC, Wickens M. Multifunctional deadenylases complexes diversify mRNA control. Nat Rev Mol Cell Biol 2008; 9:357-44; PMID:18334997; http://dx.doi.org/10.1038/nrm2370
13. Wang L, Eckmann CR, Sladina M, Wikmans K, Kimble J. A regulatory cytoplasmic poly(A) polymerase in Caenorhabditis elegans. Nature 2002; 419:312-6; PMID:12239571; http://dx.doi.org/10.1038/nature01039
14. Martin G, Doublet S, Keller W. Determinants of subcellular specificity in RNA-dependent nucleolytic trans- fersases. Biochem Biophys Acta 2008; 1779:206-16; PMID:18177575; http://dx.doi.org/10.1016/j.bbamcr.2007.12.003
15. Millonig S, Minasaki R, Nousch M, Eckmann CR. GLD-4-mediated translational activation regulates the size of the proliferative germ cell pool in the adult C. elegans germ line. PLoS Genet 2010; 6:e1000467; PMID:205254367; http://dx.doi.org/10.1371/journal. pgen.1000467
16. Nousch M, Yersovska A, Habermann B, Eckmann CR. The cytoplasmic poly(A) polymerases GLD-2 and GLD-4 promote gene expression via distinct mechanisms. Nucleic Acids Res 2014; 42:11622-33; PMID:25217583; http://dx.doi.org/10.1093/nar/ gku838
17. Minasaki R, Eckmann CR. Subcellular specialization of multifaceted 3′end modifying RNA polymerases. Genes Dev 2009; 23:824-36; PMID:19339688; http://dx.doi.org/10.1101/gad.494009
18. Schmidt M, Kucher B, Eckmann CR. Two conserved regulatory cytoplasmic poly(A) polymerases, GLD-4 and GLD-2, regulate meiotic progression in C. elegans. Genes Dev 2009; 23:824-36; PMID:19339688; http://dx.doi.org/10.1101/gad.494009
19. Minasaki R, Eckmann CR. Subcellular specialization of multifaceted 3′end modifying nucleolytic trans- fersases. Cell Stem Cell 2012; 11:689-700; PMID:22551970; http://dx.doi.org/10.1016/j.stem.2012.03.011
20. Schmidt K, Butler JS. Nuclear RNA surveillance: role of TRAMP in controlling exosome specificity. Wiley Interdiscip Rev RNA 2013; 4:217-31; PMID:23417976; http://dx.doi.org/10.1002/wrna.1155
21. Minasaki R, Rudel D, Eckmann CR. Increased sensitiv- ity and accuracy of a single-stranded DNA splint-mediated ligation assay (sPAT) reveals poly(A) tail length dynamics of developmentally regulated mRNA. RNA Biol 2014; 11:111-23; PMID:24526206; http://dx.doi.org/10.4161/rna.27992
22. Housseley J, Tollevery D. Yeast Trf5p is a nuclear poly(A) polymerase. EMBO Rep 2003; 4:217-31; PMID:12239571; http://dx.doi.org/10.1038/nrm.2003.29
23. Vanacova S, Wolf J, Marin G, Blank D, Dertternier S, Friedlein A, Langen H, Keith G, Keller W. A new yeast poly(A) polymerase complex involved in RNA quality control. PLoS Biol 2005; 3:e189; PMID:15828860; http://dx.doi.org/10.1371/journal.pbio.0030189
24. LaCava J, Housseley J, Savaveva C, Petefski E, Thomp- son E, Jaquere A, Tollevery D. RNA degradation by the exosome is promoted by a nuclear polyadenylation
32. Austin J, Kimble J. glp-1 is required in the germ line for progression from a stem cell-like state to early differentiation in the C. elegans germ line. Proc Natl Acad Sci U S A 2010; 107:2048-53; PMID:20080700; http://dx.doi.org/10.1073/pnas.0912704107

25. Funakoshi Y, Doi Y, Hosoda N, Uchida N, Osawa M, Shimada I, Tsujimoto M, Suzuki T, Katada T, Hoshino S. Mechanism of mRNA deadenylation: evidence for a molecular interplay between translation termination factor eRF3 and mRNA deadenylases. Genes Dev 2007; 21:3135-48; PMID:18056425; http://dx.doi.org/10.1101/gad.1597707

27. Byrd DT, Knobel K, Affeldt K, Crittenden SL, Kimble J. Cyclin E and CDK-2 regulate proliferative cell fate and cell cycle progression in the C. elegans germline. Development 2011; 138:2223-34; PMID:2153871; http://dx.doi.org/10.1242/dev.059555

26. Cinquin O, Crittenden SL, Morgan DE, Kimble J. The GLD-2 poly(A) polymerase activates gld-1 mRNA in the C. elegans germ line. Proc Natl Acad Sci U S A 2006; 103:15108-12; PMID:17012378; http://dx.doi.org/10.1073/pnas.0607050103

37. Suh N, Jedamzik B, Eckmann CR, Wickens M, Kimble J. The GLD-2 poly(A) polymerase activates gld-1 mRNA in the C. elegans germ line. Proc Natl Acad Sci U S A 2006; 103:15108-12; PMID:17012378; http://dx.doi.org/10.1073/pnas.0607050103

43. Wright JE, Gaidatzis D, Nishi Y, Guven-Oztan T, Oldenbroek M, Yamamoto I, Lin R, Greenstein D. Translational control of the oogonic program by components of OMA ribonucleoprotein particles in Caenorhabditis elegans. Genetics 2014; 198:1513-33; PMID:25261679; http://dx.doi.org/10.1534/genetics.114.168823

46. Suh N, Crittenden SL, Suh N, Kimble J. GLD-3 and control of the mitosis/meiosis decision in the germline of Caenorhabditis elegans. Genetics 2004; 168:147-60; PMID:15454534; http://dx.doi.org/10.1534/genetics.104.029264

49. Norbury CJ. Cytoplasmic RNA: a case of the tail wagging the dog. Nat Rev Mol Cell Biol 2013; 14:643-53; PMID:23989958; http://dx.doi.org/10.1038/nrm3645

54. Joly W, Chartier A, Rojas-Rios P, Bause R, Simonelig M. The CCRII deadenylase acts with Nanos and Pumilio in the fine-tuning of Mei-P26 expression to promote germline stem cell self-renewal. Stem Cell Rep 2013; 1:411-24; PMID:24286029; http://dx.doi.org/10.1016/j.stemcr.2013.09.007

53. Marin VA, Evans TC. Translational repression of a C. elegans Notch mRNA by the STAR/KH domain protein GLD-1. Development 2003; 130:2623-32; PMID:12736207; http://dx.doi.org/10.1242/dev.00486

48. Joly W, Chartier A, Rojas-Rios P, Bause R, Simonelig M. The CCRII deadenylase acts with Nanos and Pumilio in the fine-tuning of Mei-P26 expression to promote germline stem cell self-renewal. Stem Cell Rep 2013; 1:411-24; PMID:24286029; http://dx.doi.org/10.1016/j.stemcr.2013.09.007

42. Jungkamp AC, Strockius M, Mecenas D, Grun D, Mastrobuoni G, Kempa S, Rajewsky N. In vivo and transcriptome-wide identification of RNA binding protein target sites. Mol Cell 2011; 44:828-40; PMID:22152485; http://dx.doi.org/10.1016/j.molcel.2011.11.009

41. Charlesworth A, Meijer HA, de Moor CH. Specificity factors in cytoplasmic polyadenylation. Wiley Interdiscip Rev RNA 2013; 4:477-61; PMID:23776146; http://dx.doi.org/10.1002/wrna.1171