RNA BIOLOGY
2017, VOL. 14, NO. 10, 1320–1325
https://doi.org/10.1080/15476286.2017.1300222

Taylor & Francis
Taylor & Francis Group

POINT OF VIEW

Expanding the repertoire of deadenylases

Ilias Skeparnias*, Dimitrios Anastasakis*,**, Athanasios-Nasir Shaukat, Katerina Grafanaki, and Constantinos Stathopoulos

Department of Biochemistry, School of Medicine, University of Patras, Greece

ABSTRACT

Deadenylases belong to an expanding family of exoribonucleases involved mainly in mRNA stability and turnover, with the exception of PARN which has additional roles in the biogenesis of several important non-coding RNAs, including miRNAs and piRNAs. Recently, PARN in *C. elegans* and its homolog PNLDC1 in *B. mori* were reported as the elusive trimmers mediating piRNA biogenesis. In addition, characterization of mammalian PNLDC1 in comparison to PARN, showed that is specifically expressed in embryonic stem and germ cells, as well as during early embryo development. Moreover, its expression is correlated with epigenetic events mediated by the *de novo* DNMT3b methyltransferase and knockdown in stem cells upregulates important genes that regulate multipotency. The recent data suggest that at least some new deadenylases may have expanded roles in cell metabolism as regulators of gene expression, through mRNA deadenylation, ncRNAs biogenesis and ncRNA-mediated mRNA targeting, linking essential mechanisms that regulate epigenetic control and transition events during differentiation. The possible roles of mammalian PNLDC1 along those dynamic networks are discussed in the light of new extremely important findings.

Abbreviations: DEDD, Asp-Glu-Asp-Asp residues; EEP, Endoculease/Exonulcease/Phosphatase; PARN, Poly(A) specific Ribonuclease; PNLDC1, PARN-like Domain Containing Protein-1; ESC, Embryonic Stam Cell; MZT, Maternal to Zygotic Transition; EGA, Embryonic Genome Activation; TE, Transposon Element

ARTICLE HISTORY
Received 18 January 2017
Revised 13 February 2017
Accepted 23 February 2017

KEYWORDS
Deadenylases; mRNA turnover; non-coding RNAs; PARN; piRNAs; PNLDC1; poly (A) tail; stem cells

Introduction

Developmental process in mammals, from oocyte fertilization to early embryogenesis, depends on the stability and translation rate of maternal mRNAs or mRNAs produced upon embryonic genome transcription activation.[1] Posttranscriptional gene expression regulation (PTGR) through translational control and stability of specific messages, either maintains pluripotency or eventually drives differentiation and proliferation.[2] During these transitions, the stability and decay of mRNAs depend on the length of their 3′ poly(A) tails, which are shaped by opposing activities from poly(A) polymerases and specific exoribonucleases, known as deadenylases.[3] The first deadenylases were discovered during early studies on essential factors which drive oocyte maturation and early development.[4] Today, many deadenylases are considered not only passive mediators of mRNA decay, but rather dynamic and highly specific modulators of gene expression, in each step of development and differentiation.[5]

Deadenylases: mRNA decay and beyond

Deadenylases are 3′–5′ exoribonucleases responsible for the removal of the 3′ poly(A) tail of mRNAs and are divided in 2 major subclasses (DEDD: CAF1, PAN2, PARN and PNLDC1 and EEP: CCR4, Nocturnin, ANGEL and PDE12). Many deadenylase representatives have been studied in model organisms like *S. cerevisiae, C. elegans, Drosophila, Xenopus*, zebrafish, mouse and human. However, the genomes of many organisms contain homologues genes encoding putative and still uncharacterized deadenylases.[6] The number of active deadenylases in each organism, increases as we climb up the evolutionary ladder. The redundancy of deadenylases in higher eukaryotes reflect their biologic significance and regulatory role in PTGR mechanism, although their basic role is the simple hydrolysis of an ester bond.[7] In mammals, CCR4-CNOT complexes and, in many cases, PAN2-PAN3 complex, are the major deadenylases responsible for mRNA turnover. Their role is prominent, among other functions, in maternal mRNA clearance during MZT and EGA, in the prevention of cell death, in senescence, in DNA damage response, cell cycle control, neuronal development and in the generation of induced iPSCs.[6,8,9] Their expression is generally ubiquitous, but some deadenylases can be tissue-specific and in addition, their deregulation has been linked to pathologic conditions, including carcinogenesis.[10,11] All the known deadenylases shuttle between nucleus (found also in nucleoli) and cytoplasm (found mainly in stress granules, P-bodies and Cajal bodies), with the exception of PDE12

CONTACT Constantinos Stathopoulos cstath@med.upatras.gr Biochemistry, University of Patras, School of Medicine, 1 Asklipiou st., Patras 26500, Greece.
*Joint first authors.
**Present address: RNA Molecular Biology Group, Laboratory of Muscle Stem Cells and Gene Regulation, Building 50, Room 1154, National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, Bethesda, MD 20892, USA.

which is localized in mitochondria.[12] Knowledge on the possible epigenetic or posttranscriptional regulation of deadenylases is extremely limited, but they can be posttranslationally modified *via* phosphorylation or proteolysis (in the case of PARN).[6] Attempts to deplete the expression of each mammalian deadenylase in search of the most essential one, showed that some of them can be dispensable.[13] Deadenylases achieve a high degree of activity modulation, and in some cases specificity, through interactions with a diverse group of RNA binding proteins and ncRNAs (like miRNAs and recently piRNAs) which can be guided by characteristic 3′ UTR sequence signatures.[14] The dynamic interplay between deadenylases and their regulators is constant during embryogenesis and differentiation, and leads to fine-tune post-transcriptional regulation of gene expression.[15] Most notably, PABP and the BTG/TOB family can either stimulate or inhibit deadenylation.[16,17,18,19] In addition, combinations of RBPs that belong to CPSF, CPEB and PUF families, regulate localization, stability and translation of mRNAs during MZT, self-renewal of stem cells and differentiation.[14,15] Finally, important *trans*-acting factors such as SMAUG-response elements (SREs) are involved on mRNA stability and degradation in embryos during MZT.[19] On top of the above, 2 of the most important and highly dynamic RNA binding complexes related to targeting of 3′ UTRs are the miRISC and piRISC, which inhibit translation and promote mRNA decay through deadenylation.[20] Recent advances that allowed measurement of poly(A) tails, revealed that during embryogenesis the length of poly(A) tail of mRNAs is variable and regulates translational efficiencies which mark specific transitions during development.[21] A similar correlation between poly(A) tail length and translational efficiencies was recently correlated with the progression of cell cycle in somatic cells.[22]

PARN and PNLDC1: 2 sides of the same coin?

PARN was among the first mammalian deadenylases described to play role in poly(A) shortening.[23] It was initially considered as the major deadenylase with role in maternal mRNA clearance during *Xenopus* oocyte maturation.[24] PARN is the only deadenylase which interacts with both the 5′m7G cap and the poly(A) tail. It is modulated by a large number of 3′ *cis*-elements and *trans*-acting factors and, until recently, it was the only deadenylase which extended its activity beyond mRNA decay.[23] PARN is expressed ubiquity and its subcellular localization to nucleoli and Cajal bodies lead to the observation that besides mRNAs, deadenylates also snoRNAs and scaRNAs of the H/ACA box type.[25] Moreover, human PARN participates in the maturation of small rRNA subunits and the telomerase RNA (TERC), linking PARN mutations with telomere diseases such as familial idiopathic pulmonary fibrosis (IPF) and dyskeratosis congenital.[26,27] Finally, mammalian PARN catalyzes miRNA-mediated degradation of TP53 and the Dicer-independent 3′ end trimming of the erythorpoietic pre-miR-451 which is conserved in all vertebrates.[28,29] These additional substrate specificities of PARN highlight not only the essential role of deadenylases in additional regulatory pathways, but also strongly suggest that depending on the trigger signal, deadenylases could more widely contribute in the regulation of gene expression.[30]

Recently, Mello's laboratory identified in *C. elegans* PARN-1 as the elusive exonuclease that trims pre-piRNAs, an essential and very dynamic class of small non coding RNAs (26–31nt), first discovered in *Drosophila* germline.[31] Acting together with PIWI proteins, piRNAs are mainly responsible for TEs repression through epigenetic modifications (H3K9me3 or CpG methylation) thus preserving genome integrity during reprogramming.[32,33,34] Besides germline, the presence of piRNAs and/or piRNAs/PIWI complexes has been reported in somatic cells (*Drososphila*) and in mammalian stem cells, oocytes and early embryos.[35,36,37] Besides their main role, piRNAs can trigger maternal mRNAs clearance through deadenylation during late spermiogenesis and in the early *Drosophila* embryo.[38,39,40] Current knowledge on the evolutionary origin, the exact pathways of biogenesis and the specific roles of piRNAs in various organisms, is still limited.[41,42]

PARN-1 trimming activity was detected independently of interactions with auxiliary proteins and *in vitro* biochemical characterization showed that recombinant PARN-1 is a *bona fide* deadenylase localized in the germline and in P granules.[31] In a concurrent study published in the same issue of *Cell* by Tomari's laboratory, the same Trimmer activity in *B. mori* was attributed to the putative deadenylase PNLDC1, a homolog of PARN.[43] Either one or both genes exist in almost all higher eukaryotes, with the exception of flies.[44,45] The co-existence of PNLDC1 and PARN is evident in all amniotic vertebrates but, it is also obvious that they form 2 distinct evolutionary branches, possibly separated after an early gene duplication event, when multipotency and cell differentiation mechanisms become the driving force for multicellular organisms. Although PNLDC1 was named after its homology to PARN, it remained until recently uncharacterized for its putative deadenylase activity. It must be noted, that in few databases PNLDC1 protein sequence (annotated also in some cases as PARN-like) is confused with that of PARN and therefore, for a long time PNLDC1 was overlooked. In the study by Tomari's laboratory, PNLDC1 knockdown inhibited pre-piRNA trimming leading to accumulation of piRNAs with 3′ extended ends. However, PNLDC1 pre-piRNA trimming activity was entirely depended on the presence of Papi (a Tudor protein homolog) which, anchored on the mitochondrial surface, supplies MIWI-loaded pre-piRNAs to PNLDC1. It must be noted, that in contrast to PARN-1 from *C. elegans*, recombinant BmPNLDC1 was insoluble and therefore its putative deadenylase activity on mRNA-like substrates was not verified. In addition, the antibody used was unsuitable for subcellular localization studies and therefore the presence of BmPNLDC1 in the mitochondrial fraction was verified based on the separation of cell extracts and subsequent pre-piRNA trimming activity assays of the fractions. Both studies in their conclusive remarks suggested that either PARN or PNLDC1 could be responsible for pre-piRNA trimming activity in mammals and more specifically in mouse. The suggestions were based on the reported elevated PNLDC1 expression in mouse testis (annotated in relative databases) and the reconstitution of the pre-piRNA trimming activity after expression of both mouse PNLDC1 and Tdrkh (the mouse homolog of

BmPapi) in HEK293T cells.[43] In a very thorough report by Brennecke's laboratory that followed, Nibbler was identified as the 3′–5′ exonuclease which besides miRNAs, trims also pre-piRNAs in *Drosophila*, where both PARN and PNLDC1 are absent.[45,46,47] Interestingly, in *C. elegans*, Nibbler is required for 22G siRNA biogenesis.[48] All the described previously reports shed light on the largely unknown mechanisms of piRNA biogenesis, but the possible role of PARN-2 in *C. elegans* and of PARN in *B. mori* await further investigation. Given that the 2 fundamental functions of deadenylases is the balance of poly (A) tail length and the swift response to translational control of specific signaling pathways through decay of specific subsets of mRNAs, the recruitment of either PARN or PNLDC1 could facilitate specific adaptations during early development including, but not restricted to, piRNA biogenesis.

Mammalian PNLDC1: A link between epigenetic regulation, piRNA biogenesis and mRNA turnover?

Although PNLDC1 and PARN preserve the DEDD deadenylase subclass signature, they display limited sequence similarity.[44,49] As a consequence, it is not surprising that mammalian PNLDC1 exhibits different biochemical characteristics compared with PARN. It degrades poly(A) in a cap-independent fashion and has very strict poly(A) substrate specificity (Fig. 1). The differences with PARN extend also to the intracellular localization. PARN has ubiquitous expression and shuttles between nucleus and cytoplasm while mammalian PNLDC1 was found localized in the cytoplasm and mainly in the ER. Although clear and direct localization of PNLDC1 into mitochondrial surface was not detected, the possibility that PNLDC1 under specific circumstances can be localized there as well, cannot be excluded. Its *B. mori* homolog relies exactly on this localization for pre-piRNA trimming with the association of auxiliary proteins. Preliminary fractionation experiments in mESCs also verify this possibility (data not shown). The strong signal of PNLDC1 outside nucleus makes it the only known deadenylase to be exclusively localized in the cytoplasm, an observation suggesting a possible role in posttranscriptional regulation (discussed below).

Mammalian PNLDC1 is also the first deadenylase to be regulated at the transcription level through epigenetic modifications introduced by the DNMT family of DNA methylatransferases.[50] The observation that PNLDC1 expression is reversibly linked to the expression of the *de novo* DNMT3b methyltrasnsferase, brings it in the center of extremely important events known to be regulated by DNMT methylatransferases, including development and genome reprogramming.[51,52,53] Interestingly, in cancer DNMTs appear deregulated and they are targeted by nucleoside analogs which act as demethylating agents, like 5′AZACdR, which in the case of PNLDC1, released its expression in HEK293 cells.[54] A more intriguing linked emerges from the fact that members of the DNMT family (including DNMT3b) are important in both germ and somatic cells and in addition, they are involved in the TEs silencing by CpG *de novo* methylation, in the mouse embryo germline *via* a piRNA related pathway which involves MIWI2 loaded with secondary piRNAs.[32,33] This is the first evidence of piRNA-mediated transcriptional silencing which involves DNMTs and correlates directly with the regulation of a deadenylase expression (Fig. 1). This hypothesis is substantially supported by the fact that PNLDC1 is practically undetectable in differentiated cells and is expressed only in stem or germ cells. Even more interestingly, the observation that PNLDC1 is highly expressed in human and mouse meiotic spermatocytes, where pachytene piRNAs drive a massive elimination of mRNAs through deadenylation, are supportive of the hypothesis that PNLDC1 is an additional regulator during this process.[38] Further experimentations is required to clarify whether PNLDC1 mediates piRNAs biogenesis *per se* or participates also in mRNA decay as deadenylase which is recruited by piRNA-mediated complexes. Given the strict *in vitro* specificity of mammalian PNLDC1 [piRNAs do not possess conserved 3′ poly(A) trailing signatures] and the fact that previous immunoprecipitation of TDRKH, MIWI and MILI proteins (all associated with piRNA binding) from mouse testes failed to co-precipitate PNLDC1, the second option is more favorable (Fig. 1).[55,56] However, the involvement of PNLDC1 in pre-piRNA trimming in early mammalian embryos and germ cell linage commitment mechanisms cannot be excluded, especially given a recent report showing that PIWI proteins and piRNAs bearing characteristic poly(A) marks exist in mammalian oocytes and early (2 to 4 cells) bovine embryos.[37]

The intrinsic expression of PNLDC1 in mESCs allowed its effective knockdown which showed that depletion of PNLDC1 expression does not affect the remaining deadenylases, as previously observed.[13] Downregulation of PNLDC1 was always correlated with DNMT3b expression during mESCs differentiation, an observation that suggest PNLDC1 participation at least in some of the pathways that maintain multipotency during early development and/or pathways that require or induce genome reprogramming and are piRNA-mediated.[57,58] Accordingly, transcriptomic analysis showed significant up- or downregulation of genes that shape cell cycle, are important for DNA replication repair and chromatin remodeling, transcription modification and translational regulation (Fig. 2).[59,60] All these events occur also during germline biogenesis and depend on dynamic processes from a very complex molecular landscape, which awaits further investigation. Given the *in vitro* biochemical character of PNLDC1, its putative participation in more than one events that affect transcriptome and epigenome during early differentiation, must be also considered.

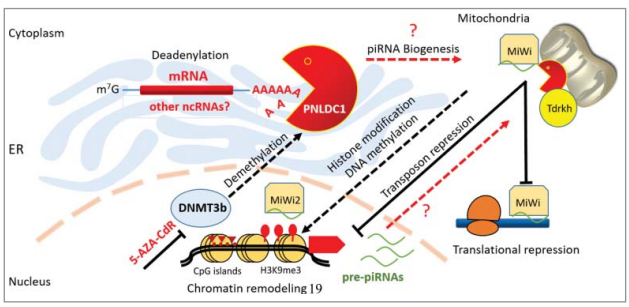

Figure 1. Illustration of verified and hypothetical roles of mammalian PNLDC1.

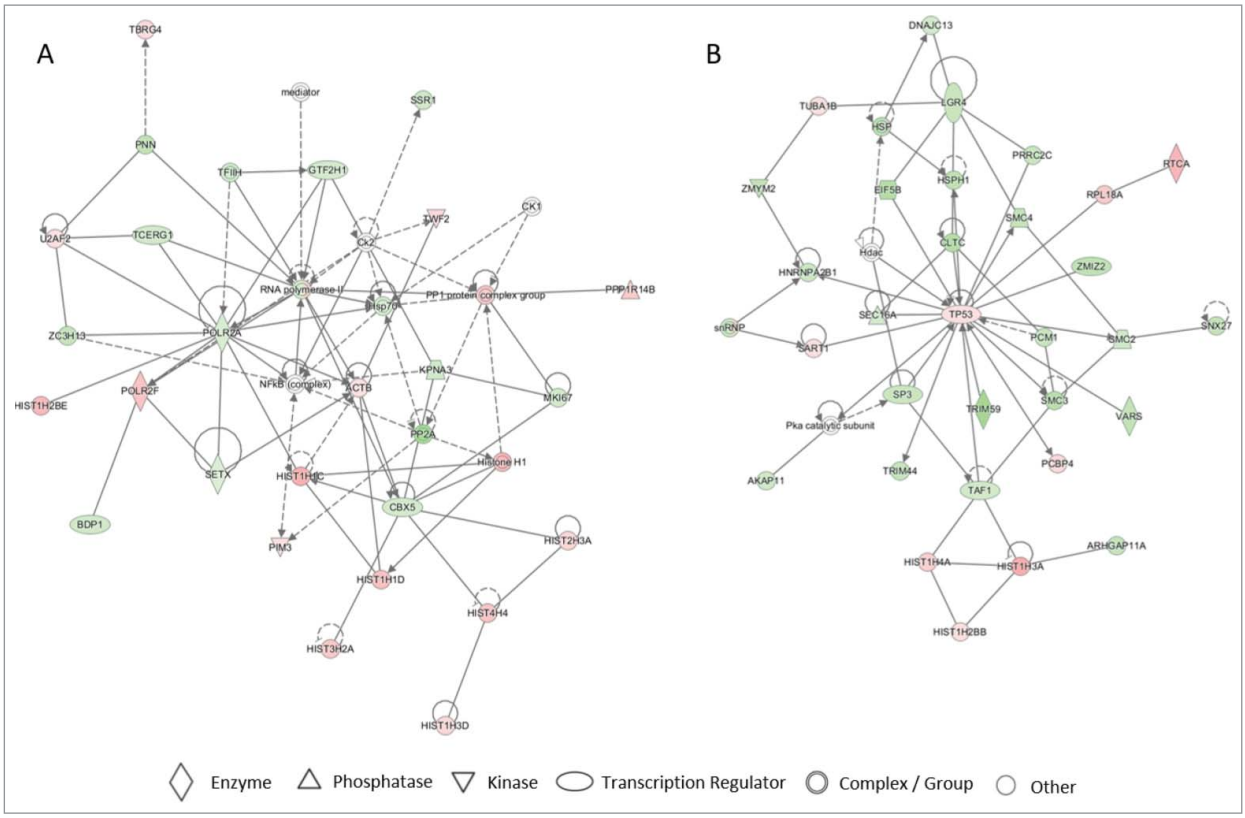

Figure 2. Networks related to cell assembly and organization, DNA replication, recombination and repair, and posttranscriptional modification (A) and cell-cycle regulation (B) according to Ingenuity Pathway (IPA). Differentially expressed genes after PNLDC1 knockdown experiments were uploaded into IPA and the most significant enriched pathways were identified. Genes were overlaid onto a global molecular network developed from information contained in the Ingenuity® Knowledge Base. The obtained networks were algorithmically generated based on the connectivity of differentially expressed genes. Red symbols denote upregulated genes and green symbols denote downregulated genes.

Conclusions

Deadenylases are evolutionary old and important enzymes that have recently expanded their repertoire beyond mRNA turnover. Among them, mammalian PNLDC1 represents a new and unique member which does not act as PARN's *alter ego*. Its exclusive expression in stem and germ cells, its tight epigenetic regulation and strict substrate specificity suggest that PNLDC1 could be a master regulator of early developmental pathways that include, but not limited to, piRNA-mediated epigenetic silencing, piRNA-triggered mRNA decay and in certain cases, piRNA biogenesis. Given that piRNAs are not only present in germline but also in embryonic and adult stem cells, in mammalian oocytes and early embryos, the possible roles of mammalian PNLDC1 could shift according to specific cellular needs during development. The roles of mammalian PNLDC1 as a possible link between common mechanisms in germ and stem cells have just started to unveil.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This work was supported in part and implemented under the "ARISTEIA I" (EXCELLENCE I) Action of the "OPERATIONAL PROGRAMME EDUCATION AND LIFELONG LEARNING" and is co-funded by the European Social Fund (ESF) and National Resources (MIS 1225, No D608 to C.S.). A-N.S is a recipient of "Bodossakis Foundation" postgraduate fellowship, which is gratefully acknowledged.

References

1. Wennekamp S, Mesecke S, Nédélec F, Hiiragi T. A self-organization framework for symmetry breaking in the mammalian embryo. Nat Rev Mol Cell Biol 2013; 14(7):452-459; PMID:23778971; https://doi.org/10.1038/nrm3602
2. Rossant J, Tam PP. New insights into early human development: lessons for stem cell derivation and differentiation. Cell Stem Cell 2017; 20 (1):18-28; PMID:28061351; https://doi.org/10.1016/j.stem.2016.12.004
3. Eckmann CR, Rammelt C, Wahle E. Control of poly(A) tail length. Wiley Interdiscip Rev RNA 2011; 2(3):348-361; PMID:21957022; https://doi.org/10.1002/wrna.56
4. Richter JD, Lasko P. Translational control in oocyte development. Cold Spring Harb Perspect Biol 2011; 3(9):a002758; PMID:21690213; https://doi.org/10.1101/cshperspect.a002758
5. Harnisch C, Moritz B, Rammelt C, Temme C, Wahle E. Activity and function of deadenylases. Enzymes 2012; 31:181-211; PMID:27166446; https://doi.org/10.1016/B978-0-12-404740-2.00009-4
6. Yan YB. Deadenylation: enzymes, regulation, and functional implications. Wiley Interdiscip Rev RNA 2014; 5(3):421-443; PMID:24523229; https://doi.org/10.1002/wrna.1221
7. Goldstrohm AC, Wickens M. Multifunctional deadenylase complexes diversify mRNA control. Nat Rev Mol Cell Biol 2008; 9(4):337-344; PMID:18334997; https://doi.org/10.1038/nrm2370
8. Mittal S, Aslam A, Doidge R, Medica R, Winkler GS. The Ccr4a (CNOT6) and Ccr4b (CNOT6L) deadenylase subunits of the human Ccr4-Not complex contribute to the prevention of cell death and senescence. Mol Biol Cell 2011; 22(6):748-758; PMID:21233283; https://doi.org/10.1091/mbc.E10-11-0898
9. Barckmann B, Simonelig M. Control of maternal mRNA stability in germ cells and early embryos. Biochim Biophys Acta 2013; 1829(6-7):714-724; PMID:23298642; https://doi.org/10.1016/j.bbagrm.2012.12.011
10. Tummala H, Walne A, Collopy L, Cardoso S, de la Fuente J, Lawson S, Powell J, Cooper N, Foster A, Mohammed S, et al. Poly(A)-specific

ribonuclease deficiency impacts telomere biology and causes dyskeratosis congenita. J Clin Invest 2015; 125(5):2151-2160; PMID:25893599; https://doi.org/10.1172/JCI78963
11. Maragozidis P, Papanastasi E, Scutelnic D, Totomi A, Kokkori I, Zarogiannis SG, Kerenidi T, Gourgoulianis KI, Balatsos NA. Poly(A)-specific ribonuclease and Nocturnin in squamous cell lung cancer: prognostic value and impact on gene expression. Mol Cancer 2015; 14:187; PMID:26541675; https://doi.org/10.1186/s12943-015-0457-3
12. Rorbach J, Nicholls TJ, Minczuk M. PDE12 removes mitochondrial RNA poly(A) tails and controls translation in human mitochondria. Nucleic Acids Res 2011; 39(17):7750-7763; PMID:21666256; https://doi.org/10.1093/nar/gkr470
13. Yamashita A, Chang TC, Yamashita Y, Zhu W, Zhong Z, Chen CY, Shyu AB. Concerted action of poly(A) nucleases and decapping enzyme in mammalian mRNA turnover. Nat Struct Mol Biol 2005; 12 (12):1054-1063; PMID:16284618; https://doi.org/10.1038/nsmb1016
14. Matoulkova E, Michalova E, Vojtesek B, Hrstka R. The role of the 3′ untranslated region in post-transcriptional regulation of protein expression in mammalian cells. RNA Biol 2012; 9(5):563–76; PMID:22614827; https://doi.org/10.4161/rna.20231
15. Ivshina M, Lasko P, Richter JD. Cytoplasmic polyadenylation element binding proteins in development, health, and disease. Annu Rev Cell Dev Biol 2014; 30:393-415; PMID:25068488; https://doi.org/10.1146/annurev-cellbio-101011-155831
16. Mauxion F, Chen CY, Séraphin B, Shyu AB. BTG/TOB factors impact deadenylases. Trends Biochem Sci 2009; 34(12):640-647; PMID:19828319; https://doi.org/10.1016/j.tibs.2009.07.008
17. Stupfler B, Birck C, Séraphin B, Mauxion F. BTG2 bridges PABPC1 RNA-binding domains and CAF1 deadenylase to control cell proliferation. Nat Commun 2016; 7:10811; PMID:26912148; https://doi.org/10.1038/ncomms10811
18. Yu C, Ji SY, Sha QQ, Dang Y, Zhou JJ, Zhang YL, Liu Y, Wang ZW, Hu B, Sun QY, et al. BTG4 is a meiotic cell cycle-coupledmaternal-zygotic-transition licensing factor in oocytes. Nat Struct Mol Biol 2016; 23(5):387-394; PMID:27065194; https://doi.org/10.1038/nsmb.3204
19. Liu Y, Lu X, Shi J, Yu X, Zhang X, Zhu K, Yi Z, Duan E, Li L. BTG4 is a keyregulator for maternal mRNA clearance during mouse early embryogenesis. J Mol Cell Biol 2016; 8(4):366-368; PMID:27190313; https://doi.org/10.1093/jmcb/mjw023
20. Ye J, Blelloch R. Regulation of pluripotency by RNA binding proteins. Cell Stem Cell 2015; 15(3):271-280; PMID:25192462; https://doi.org/10.1016/j.stem.2014.08.010
21. Subtelny AO, Eichhorn SW, Chen GR, Sive H, Bartel DP. Poly(A)-tail profiling reveals an embryonic switch in translational control. Nature 2014; 508(7494):66-71; PMID:24476825; https://doi.org/10.1038/nature13007
22. Park JE, Yi H, Kim Y, Chang H, Kim VN. Regulation of Poly(A) tail and translation during the somatic cell cycle. Mol Cell 2016; 62(3):462-471; PMID:27153541; https://doi.org/10.1016/j.molcel.2016.04.007
23. Virtanen A, Henriksson N, Nilsson P, Nissbeck M. Poly(A)-specific ribonuclease (PARN): an allosterically regulated, processive and mRNA cap-interacting deadenylase. Crit Rev Biochem Mol Biol 2013; 48(2):192-209; PMID:23496118; https://doi.org/10.3109/10409238.2013.771132
24. Copeland PR, Wormington M. The mechanism and regulation of deadenylation: identification and characterization of Xenopus PARN. RNA 2001; 7(6):875-886; PMID:11424938; https://doi.org/10.1017/S1355838201010020
25. Berndt H, Harnisch C, Rammelt C, Stöhr N, Zirkel A, Dohm JC, Himmelbauer H, Tavanez JP, Hüttelmaier S, Wahle E. Maturation of mammalian H/ACA box snoRNAs: PAPD5-dependent adenylation and PARN-dependent trimming. RNA 2012; 18(5):958-972; PMID:22442037; https://doi.org/10.1261/rna.032292.112
26. Stuart BD, Choi J, Zaidi S, Xing C, Holohan B, Chen R, Choi M, Dharwadkar P, Torres F, Girod CE, et al. Exome sequencing links mutations in PARN and RTEL1 with familial pulmonary fibrosis and telomere shortening. Nat Genet 2015; 47(5):512-517; PMID:25848748; https://doi.org/10.1038/ng.3278
27. Moon DH, Segal M, Boyraz B, Guinan E, Hofmann I, Cahan P, Tai AK, Agarwal S. Poly(A)-specific ribonuclease (PARN) mediates 3′-end maturation of the telomerase RNA component. Nat Genet 2015; 47(12):1482-1488; PMID:26482878; https://doi.org/10.1038/ng.3423
28. Zhang X, Devany E, Murphy MR, Glazman G, Persaud M, Kleiman FE. PARN deadenylase is involved in miRNA-dependent degradation of TP53 mRNA in mammalian cells. Nucleic Acids Res 2015; 43 (22):10925-10938; PMID:26400160; https://doi.org/10.1093/nar/gkv959
29. Yoda M, Cifuentes D, Izumi N, Sakaguchi Y, Suzuki T, Giraldez AJ, Tomari Y. Poly(A)-specific ribonuclease mediates 3′-end trimming of Argonaute2-cleaved precursor microRNAs. Cell Rep 2013; 5(3):715-726; PMID:24209750; https://doi.org/10.1016/j.celrep.2013.09.029
30. Balatsos NA, Maragozidis P, Anastasakis D, Stathopoulos C. Modulation of poly(A)-specific ribonuclease (PARN): current knowledge and perspectives. Curr Med Chem 2012; 19(28):4838-4849; PMID:22834816; https://doi.org/10.2174/092986712803341539
31. Tang W, Tu S, Lee HC, Weng Z, Mello CC. The RNase PARN-1 Trims piRNA 3′ ends to promote transcriptome surveillance in C. elegans. Cell 2016; 164(5):974-984; PMID:26919432; https://doi.org/10.1016/j.cell.2016.02.008
32. Weick EM, Miska EA. piRNAs: from biogenesis to function. Development 2014; 141(18):3458-3471; PMID:25183868; https://doi.org/10.1242/dev.094037
33. Aravin AA, Sachidanandam R, Bourc'his D, Schaefer C, Pezic D, Toth KF, Bestor T, Hannon GJ. A piRNA pathway primed by individual transposons is linked to de novo DNA methylation in mice. Mol Cell 2008; 31(6):785-799; PMID:18922463; https://doi.org/10.1016/j.molcel.2008.09.003
34. Ishiuchi T, Torres-Padilla ME. LINEing germ and embryonic stem cells' silencing of retrotransposons. Genes Dev 2014; 28(13):1381-1383; PMID:24990961; https://doi.org/10.1101/gad.246462.114
35. Ross RJ, Weiner MM, Lin H. PIWI proteins and PIWI-interacting RNAs in the soma. Nature 2014; 505(7483):353-359; PMID:24429634; https://doi.org/10.1038/nature12987
36. Fu Q, Wang PJ. Mammalian piRNAs: Biogenesis, function, and mysteries. Spermatogenesis 2014; 4:e27889; PMID:25077039; https://doi.org/10.4161/spmg.27889
37. Roovers EF, Rosenkranz D, Mahdipour M, Han CT, He N, Chuva de Sousa Lopes SM, van der Westerlaken LA, Zischler H, Butter F, Roelen BA, et al. Piwi proteins and piRNAs in mammalian oocytes and early embryos. Cell Rep 2015; 10(12):2069-2082; PMID:25818294; https://doi.org/10.1016/j.celrep.2015.02.062
38. Gou LT, Dai P, Yang JH, Xue Y, Hu YP, Zhou Y, Kang JY, Wang X, Li H, Hua MM, et al. Pachytene piRNAs instruct massive mRNA elimination during late spermiogenesis. Cell Res 2014; 24(6):680-700; PMID:24787618; https://doi.org/10.1038/cr.2014.41
39. Rouget C, Papin C, Boureux A, Meunier AC, Franco B, Robine N, Lai EC, Pelisson A, Simonelig M. Maternal mRNA deadenylation and decay by the piRNA pathway in the early Drosophila embryo. Nature 2010 Oct; 467(7319):1128-1132; PMID:20953170; https://doi.org/10.1038/nature09465
40. Vourekas A, Zheng Q, Alexiou P, Maragkakis M, Kirino Y, Gregory BD, Mourelatos Z. Mili and Miwi target RNA repertoire reveals piRNA biogenesis and function of Miwi in spermiogenesis. Nat Struct Mol Biol 2012; 19(8):773-781; PMID:22842725; https://doi.org/10.1038/nsmb.2347
41. Grimson A, Srivastava M, Fahey B, Woodcroft BJ, Chiang HR, King N, Degnan BM, Rokhsar DS, Bartel DP. Early origins and evolution of microRNAs andPiwi-interacting RNAs in animals. Nature 2008; 455(7217):1193-1197; PMID:18830242; https://doi.org/10.1038/nature07415
42. Iwasaki YW, Siomi MC, Siomi H. PIWI-Interacting RNA: Its Biogenesis and Functions. Annu Rev Biochem 2015; 84:405-433; PMID:25747396; https://doi.org/10.1146/annurev-biochem-060614-034258
43. Izumi N, Shoji K, Sakaguchi Y, Honda S, Kirino Y, Suzuki T, Katsuma S, Tomari Y. Identification and Functional Analysis of the Pre-piRNA 3′ Trimmer in Silkworms. Cell 2016; 164(5):962-973; PMID: 26919431; https://doi.org/10.1016/j.cell.2016.01.008
44. Anastasakis D, Skeparnias I, Shaukat AN, Grafanaki K, Kanellou A, Taraviras S, Papachristou DJ, Papakyriakou A, Stathopoulos C. Mammalian PNLDC1 is a novel poly(A) specific exonuclease with

discrete expression during early development. Nucleic Acids Res 2016; 44(18):8908-8920; PMID:27515512; https://doi.org/10.1093/nar/gkw709
45. Hayashi R, Schnabl J, Handler D, Mohn F, Ameres SL, Brennecke J. Genetic and mechanistic diversity of piRNA 3′-end formation. Nature 2016; 539(7630):588-592; PMID:27851737; https://doi.org/10.1038/nature20162
46. Han BW, Hung JH, Weng Z, Zamore PD, Ameres SL. The 3′-to-5′ exoribonuclease Nibbler shapes the 3′ ends of microRNAs bound to Drosophila Argonaute1. Curr Biol 2011; 21(22):1878-1887; PMID:22055293; https://doi.org/10.1016/j.cub.2011.09.034
47. Liu N, Abe M, Sabin LR, Hendriks GJ, Naqvi AS, Yu Z, Cherry S, Bonini NM. The exoribonuclease Nibbler controls 3′ end processing of microRNAs in Drosophila. Curr Biol 2011; 21(22):1888-1893; PMID:22055292; https://doi.org/10.1016/j.cub.2011.10.006
48. Gu W, Shirayama M, Conte D Jr, Vasale J, Batista PJ, Claycomb JM, Moresco JJ, Youngman EM, Keys J, Stoltz MJ, et al. Distinct argonaute-mediated 22G-RNA pathways direct genome surveillance in the C. elegans germline. Mol Cell 2009; 36(2):231-244; PMID:19800275; https://doi.org/10.1016/j.molcel.2009.09.020
49. Balatsos NA, Vlachakis D, Maragozidis P, Manta S, Anastasakis D, Kyritsis A, Vlassi M, Komiotis D, Stathopoulos C. Competitive inhibition of human poly(A)-specific ribonuclease (PARN) by synthetic fluoro-pyranosyl nucleosides. Biochemistry 2009; 48(26):6044-6051; PMID:19472977; https://doi.org/10.1021/bi900236k
50. Ku HY, Lin H. PIWI proteins and their interactors in piRNA biogenesis, germline development and gene expression. Natl Sci Rev 2014; 1 (2):205-218; PMID:25512877; https://doi.org/10.1093/nsr/nwu014
51. Tessema M, Willink R, Do K, Yu YY, Yu W, Machida EO, Brock M, Van Neste L, Stidley CA, Baylin SB, et al. Promoter methylation of genes in and around the candidate lung cancer susceptibility locus 6q23-25. Cancer Res 2008; 68(6):1707-1714; https://doi.org/10.1158/0008-5472.CAN-07-6325
52. Hlady RA, Novakova S, Opavska J, Klinkebiel D, Peters SL, Bies J, Hannah J, Iqbal J, Anderson KM, Siebler HM et al. Loss of Dnmt3b function upregulates the tumor modifier Ment and accelerates mouse lymphomagenesis. J Clin Invest 2012; 122(1):163-177; PMID:22133874; https://doi.org/10.1172/JCI57292
53. Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 1999; 99(3):247-257; PMID:10555141; https://doi.org/10.1016/S0092-8674(00)81656-6
54. Rhee I, Bachman KE, Park BH, Jair KW, Yen RW, Schuebel KE, Cui H, Feinberg AP, Lengauer C, Kinzler KW, et al. DNMT1 and DNMT3b cooperate to silence genes in human cancer cells. Nature 2002; 416(6880):552-556; PMID:11932749; https://doi.org/10.1038/416552a
55. Vagin VV, Wohlschlegel J, Qu J, Jonsson Z, Huang X, Chuma S, Girard A, Sachidanandam R, Hannon GJ, Aravin AA. Proteomic analysis of murine Piwi proteins reveals a role for arginine methylation in specifying interaction with Tudor family members. Genes Dev 2009; 23(15):1749-1762; PMID:19584108; https://doi.org/10.1101/gad.1814809
56. Chen C, Jin J, James DA, Adams Cioaba MA, Park JG, Guo Y, Tenaglia E, Xu C, Gish G, Min J, et al. Mouse Piwi interactome identifies binding mechanism of Tdrkh Tudor domain to arginine methylated Miwi. Proc Natl Acad Sci U S A 2009; 106(48):20336-20341; PMID:19918066; https://doi.org/10.1073/pnas.0911640106
57. Wang H, Morita M, Yang X, Suzuki T, Yang W, Wang J, Ito K, Wang Q, Zhao C, Bartlam M, et al. Crystal structure of the human CNOT6L nuclease domain reveals strict poly(A) substrate specificity. EMBO J 2010; 29(15):2566-2576; PMID:20628353; https://doi.org/10.1038/emboj.2010.152
58. Ku HY, Lin H. PIWI proteins and their interactors in piRNA biogenesis, germline development and gene expression. Natl Sci Rev 2014; 1 (2):205-218; PMID:25512877; https://doi.org/10.1093/nsr/nwu014
59. He S, Nakada D, Morrison SJ. Mechanisms of stem cell self-renewal. Annu Rev Cell Dev Biol 2009; 25:377-406; PMID:19575646; https://doi.org/10.1146/annurev.cellbio.042308.113248
60. Martello G, Smith A. The nature of embryonic stem cells. Annu Rev Cell Dev Biol 2014; 30:647-675; PMID:25288119; https://doi.org/10.1146/annurev-cellbio-100913-013116