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Five questions toward mRNA degradation in oocytes and preimplantation embryos: when, who, to whom, how, and why?†

Zhi-Yan Jiang and Heng-Yu Fan*

Life Sciences Institute, Zhejiang University, Hangzhou, China

***Correspondence:** Life Sciences Institute, Zhejiang University, 866 Yu Hang Tang Rd., Hangzhou 310058, China. Tel: +8657188981370; E-mail: hyfan@zju.edu.cn

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Abstract

RNA—the primary product of the genome—is subject to various biological events during its lifetime. During mammalian gametogenesis and early embryogenesis, germ cells and preimplantation embryos undergo marked changes in the transcriptome, including mRNA turnover. Various factors, including specialized proteins, RNAs, and organelles, function in an intricate degradation system, and the degradation selectivity is determined by effectors and their target mRNAs. RNA homeostasis regulators and surveillance factors function in the global transcriptome of oocytes and somatic cells. Other factors, including BTG4, PABPN1L, the CCR4-NOT subunits, CNOT6L and CNOT7, and TUTs, are responsible for two maternal mRNA avalanches: M- and Z-decay. In this review, we discuss recent advances in mRNA degradation mechanisms in mammalian oocytes and preimplantation embryos. We focused on the studies in mice, as a model mammalian species, and on RNA turnover effectors and the *cis*-elements in targeting RNAs.

Keywords: RNA-binding proteins, meiosis, mRNA homeostasis, oocyte, zygote, maternal-tozygotic, transition

Introduction

Multiple macromolecules comprise a single cell, an organism, and biological systems. Among these, RNA—the direct product of the genome—is produced through transcription and functions in various biological processes. Of all the different types of RNAs, ribosomal RNAs (rRNAs) composite ribosome complexes and transfer RNAs (tRNAs) are responsible for amino acid translocation; long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) participate in the post-transcriptional regulation of RNAs; and messenger RNAs (mRNAs) provide instructions for protein coding.

To date, novel sequencing technologies have supported research on cellular transcriptomes. Although vigorous transcription activities may occur within a cell, the transcriptome remains relatively stable, being in equilibrium. Mature eukaryotic mRNAs, with a few exceptions such as histone mRNAs, contain a 7-methylguanosine (m7G) cap structure at the 5′-end and a poly(A) tail at the 3′-end [1]. These structures stabilize mRNAs in the cytoplasm and facilitate their translation. In order to avoid aberrant RNA accumulation, RNA turnover is vital for dynamic homeostasis. Researchers have shown that RNA degradation is an intricate and integrated system: a mature mRNA undergoes decapping by DCP1/2 (decapping protein 1/2) and subsequent digestion by a 5′–3′ exoribonuclease 1 (XRN1) [2]; or deadenylation by deadenlyases, including the poly(A)-nuclease (PAN2/3) complex and the CCR4-NOT complex [3], followed by degradation by the exosome [4]. Diverse RNA-binding proteins (RBPs) are also involved in these activities, and function as adaptors for the degradation machinery or help RNA translocation to specialized degradation complexes. RNA modifications also participate in the overall decay system: 3′-oligo-uridylation guards the degradation of 10% oocyte mRNAs during mouse folliculogenesis [5], whereas the *N*-6-methylated adenosine (m^6A) modification serves as a decay signal in various cell types [6–8].

Germ cells possess a unique mission: inheritance. Female germ cells, namely oocytes, have trudged a long journey to become what they are. After the activation of primordial follicles, it takes a long time for oocytes to undergo folliculogenesis and meiotic maturation. Finally, female mice ovulate and release mature metaphase II (MII) oocytes. During these periods, the global oocyte transcriptome undergoes notable changes. The massive amount of maternal mRNAs are degraded in two "avalanches"; the first wave is termed M-decay and occurs during meiotic maturation [9], and the second, termed Z-decay, is accompanied by zygotic genome activation (ZGA; [10]). M-decay, which represents maternal factor-regulated decay, initiates after germinal vesicle breakdown (GVBD) and is sustained until the MII stage. Z-decay is guided by zygotic factors, and mainly occurs between the two- and four-cell stages in mouse preimplantation embryos. Principally serving as a cradle for embryogenesis, oocytes provide maternal factors for zygotes, and the re-sculpture of the oocyte transcriptomes is vital for the offspring [11].

In this review, we summarize the progress in research on mRNA degradation during the development of mammalian oocytes and preimplantation embryos. We categorize

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mechanisms of mRNA degradation into three subgroups based on the goals and occurrence: for RNA global homeostasis, for RNA surveillance, and for massive maternal RNA removal ("avalanches"). We propose a generalized explanation of each degradation pathway that is essential for female fertility and reproduction, including the main functional complexes, associated factors, calling and/or responding elements in mRNAs, and an overall working model.

To highlight key studies, we focused on papers published in the last 5 years. We discuss the common decay pathways in various cell types, such as the CCR4-NOT complex-mediated deadenylation, and oocyte-specific degradation mechanisms, such as cytoplasmic lattices (CPLs). A few studies investigated mRNA degradation in ovarian granulosa cells and spermatogonia are involved. To trace and compare the evolution of such mechanisms, we focused on studies performed in mice; meanwhile, studies in other animal models, including *Xenopus* and zebrafish, were also included.

WHEN does it happen: continuous "microwaves" and two avalanches

In somatic cells, transcription occurs throughout the life cycle of the cell, except when the cell enters mitosis. During S phase, the genome is occupied by DNA polymerases. After progression into M phase, RNA polymerases are unable to load onto chromatids because they are condensed into chromosomes [12]. However, the abundance of total mRNA remains relatively stable during the long G0 stage. These regulatory processes are conserved among different cell types. In mice, before developing into fully grown germinal vesicle oocytes (FGOs), vigorous transcription activities occur in growing germinal vesicle oocytes (GGOs), while the translation level is relatively low. Despite mounting natal transcripts, the overall oocyte transcriptome is stable, indicating a pivotal role for mRNA degradation mechanisms. In general, the clearance of maternal transcripts in oocytes can be subdivided into three stages: (1) during oocyte growth, transcript degradation in micro waves maintains the overall mRNA equilibrium, which is categorized as mRNA homeostasis and surveillance; (2) during meiotic maturation and early embryogenesis, the removal of a massive amount of maternal transcripts is triggered by maternal factors (M-decay, the first avalanche); and (3) after fertilization, a few remaining maternal transcripts are degraded by zygotic factors, accompanying ZGA (Z-decay, the second avalanche; Table 1, Figure 1A and B).

Homeostasis and surveillance factors are considered as guards for overall transcript levels during the growing stage. The former ones are aimed at supportive RNAs, which monitor the synthesis and decay of targets, and help maintain them in a dynamic equilibrium. Terminal uridylyl transferase (TUT) is the main factor in homeostasis. TUT4 and TUT7 (also known as ZCCHC11 and ZCCHC6, respectively) are ubiquitously expressed during mammalian gametogenesis. Oocytes contain a divergent pattern of mRNA 3′-UTRs compared with other somatic cells, with shorter poly(A) tails and a higher ratio of oligo- to mono-uridylation [5] (Figure 1C). Of the transcripts requiring removal during oocyte maturation and follicle development, ~10% are uridylated under the control of these two TUTs, calling for further degradation and partially maintaining the global transcriptome. When this machinery is lacking, as in *Tut4/7*$^{fl/fl}$; *Zp3*-Cre mice, there is aberrant accumulation of maternal transcripts in oocytes, with defects in meiotic maturation.

However, surveillance factors govern the degradation of detrimental RNAs [13]. To avoid ectopic expression or other potential hindrances caused by incompatible transcripts, surveillance factors, such as the exosome and MARF1, are involved in this regulation. To become a key factor involved in monitoring the status of various RNA biotypes, the exosome associates with divergent ribonucleases, gains 3′–5′ exo- and endonuclease activity, and collaborates with diverse adaptors to specific targets [4]. The resumption of meiosis—a hallmark of oocyte maturation—is controlled by the oocyte maturation-promoting factor (MPF). A predicted ribonuclease, meiosis arrest female 1 (MARF1), represses the protein phosphatase 2 catalytic subunit *Ppp2cb* mRNA. *Marf1* mutant mice were found to present a female-only infertile phenotype, with a massive amount of transcripts accumulating in FGOs, including those of *Ppp2cb* [14]. The aberrant existence of *Pp2cb* mRNAs resulted in endured PP2C assembly, leading to the inactivation of the MPF and the failure of GVBD [14–16]. Overall, evidence indicates that post-transcription processing and degradation of mRNAs over time are vital for female reproduction (Figure 1D).

When oocytes reach the FGO stage, RNA polymerase II (Pol II) is excluded from the genome, and the transcription activity ceases [17, 18]. Oocytes retain transcriptional silencing until ZGA occurs around the late one-cell to two-cell stage. Meanwhile, the oocyte-specific RNA clearance machinery is initiated. M-decay, the maternal-decay pathway, involves the elimination of maternal transcripts that are exclusively guarded by maternal factors [9]. Such machinery is activated after the resumption of meiosis, as multiple decay-related factors, including BTG4 [19] and CNOT6L [20], are translated during this period (Figure 1B and D). Over 75% of the maternal mRNAs are degraded by the end of the MII stage [19]. Z-decay, namely the zygotic-decay pathway, involves the clearance of maternal transcripts and is dependent on de novo zygotic transcription products [10] (Figure 1E). A few maternal factors, such as BTG4, are also responsible for this process. Up to 90% of the total maternal transcripts are removed at the end of the two-cell stage. The reliance of zygotic transcripts might explain the coincidence between Z-decay and ZGA, whereas Z-decay in humans governs a larger amount of transcripts than that in mice [10, 21]. These zygotic products serve as core degradation factors or activators of RNA decay machinery, and a conserved TUT4/7-driven oligo-uridylation pathway, together with maternal YAP1 and zygotic TEAD4, have been reported among species. These two avalanches sculpt and determine the oocyte- and embryo-hosting RNAs, prohibiting the production of incompatible proteins, leading to the rearrangement of a pluripotent cell transcriptome and giving rise to the offspring.

WHO and HOW: the hunter says "yes"

Heterogeneous nuclear RNAs (hnRNAs), the primary product of DNA transcription [3], become mature mRNAs only after a series of post-transcription processes. In contrast to transcription products in prokaryotes, eukaryotic transcripts undergo the removal of introns within splicing complexes, 5′-capping and sequential methylation, and 3′-end polyadenylation by poly(A) polymerases (PAPs; [1]). Owing to their long

Table 1. Key maternal factors in maternal mRNA degradation

No.	Gene name	Subgroup of degradation	Phenotype(s) in knockout/conditional-knockout female mice	Molecular functions	Reference(s)
1	*Tut4/7*	Homeostasis system, Z-decay	Defects in meiotic maturation	Oligo-uridylation on short-poly(A) tailing mRNAs	[5]
2	*Marf1*	Surveillance	Defects in meiotic maturation, GVBD failure	A ribonuclease to degrade mRNAs	[14–16]
3	*Cnot7*	M-decay and Z-decay	Fertile (in *Cnot7*$^{-/-}$ mice)	A catalytic subunit of CCR4-NOT, CAF1 homologue in mammals	[28]
4	*Btg4*	M-decay and Z-decay	Defects in embryogenesis	An adaptor of CCR4-NOT^{CNOT7}	[19, 29, 49]
5	*Cnot6l*	M-decay	Defects in meiotic maturation	A catalytic subunit of CCR4-NOT, CCR homologue in mammals	[20]
6	*Zfp36l2*	M-decay	Defects in meiotic maturation	An adaptor of CCR4-NOT^{CNOT6L}, targeting ARE-containing mRNAs	[20, 32, 33, 35]
7	*Pabpn1l*	M-decay	Defects in embryogenesis	(1) An adaptor of BTG4-CCR4-NOT^{CNOT7}, facilitating its binding to mRNAs; (2) A stabilizer of BTG4, avoiding its polyubiquitination by β-TrCP1	[46]
8	*Zar1/2*	M-decay	Defects in meiotic maturation and embryogenesis (in *Zar1*$^{-/-}$ and *Zar1/2*$^{-/-}$ females)	(1) Collaborating with CPLs, guarding mRNA removal during the growing stage and M-decay (2) Activating translation during meiosis resumption	[48, 49]
9	*Yap1*	Z-decay	Defects in embryogenesis	Activating the transcription of *Tead4*, directing Z-decay	[10, 21, 57]
10	*Tead4*	Z-decay		A transcription factor promoting the transcription of *Tut4/7*, directing Z-decay	[10, 21]
11	*Exosc10*	Surveillance	Defects in meiotic maturation	An RNase associated with the exosome	[62]

GVBD, germinal vesicle breakdown; ARE, AU-rich elements; CPL, cytoplasmic lattice.

tails, poly(A)-containing RNAs can be transported into the cytoplasm, stabilized, and therefore translated. In contrast, deadenylation functions as a reverse process in poly(A) tail shortening, leading to host mRNA degradation.

In classical views, mRNA deadenylation begins with the activation of the poly(A)-nuclease (PAN) deadenylation complex and the CCR4-NOT complex [3]. The shortening of poly(A) tails resulting from deadenylation leads to the dissociation of PABPs [22]. These mRNAs are further uridylated by uridylyl transferases. LSm (like-Sm) proteins are subsequently recruited to the terminal uridylyl residues and drive mRNA targets to undergo 5′–3′ exonucleolytic digestion [23]. Other factors, including specialized RNAs, functional proteins, and organelles, participate in these transcriptome avalanches. These modules either exist in most mammalian cell types, similar to mRNA degradation machinery in gametes, taking the exosome as an example; or merely emerge in reproductive systems during evolution, such as the CPLs.

Combination of CNOT7 and BTG4 within M-decay

CCR4-NOT comprises a scaffold subunit, CNOT1, catalytic subunits (CNOT6/6L and CNOT7/8), and multiple regulatory modules, including CNOT4 and CNOT9 [24, 25] (Table 2). Intriguingly, these core enzymatic subunits are attributed to different ribonuclease families: CNOT6/6L belong to the EEP type, whereas the latter ones are DEDD-type members [26].

The physiological functions of CNOT7 have been previously reported [27, 28]. Phenotypes of knockout (KO) mouse models indicate that CNOT7 is not essential for animal survival, but is indispensable for gametogenesis. Removal of *Cnot7* resulted in sterility in male mice, with disorganization of seminiferous tubules and loss of germ cells. *Cnot7*$^{-/-}$ female mice are fertile; this does not indicate that CNOT7/8 are irresponsible for female fertility because *Cnot8* presumably plays a redundant role in female germ cells, as co-depletion of *Cnot7/8* resulted in a release from the MII arrest [28, 29]. B-cell translocation gene-4 (BTG4), a member of the BTG and TOB family, interacts with CNOT7 and serves as a novel maternal-effect and meiosis-associated factor [19]. In contrast to KO mice for other members of the BTG and TOB family, Yu et al. illustrated that *Btg4*$^{-/-}$ females were sterile, and their embryos presented the one- to two-cell arrest, clarifying the conserved regulatory effect of BTG and TOB family members in reproduction. BTG4 can bind CNOT7 through the conserved BTG domain, whereas tryptophan 95 in the BTG domain is essential for this interaction [19, 29]. This facilitates the interaction between CNOT7 and bait mRNAs through the binding of BTG4 to eukaryotic translation initiation factor 4E (eIF4E), as BTG4^{boxB} has affinity toward eIF4E^{CTD} and functions as an adaptor to eIF4E-docking mRNAs (Figure 2A). This complex is essential for the deadenylase activity of CNOT7, since the poly(A) tails of maternal transcripts were not shortened in *Btg4*$^{-/-}$ and *Btg4*$^{W95A/-}$ oocytes [19]. The accumulation of BTG4 protein from the MII stage to early embryos provides a time frame for CCR4-NOT^{CNOT7}-instructed deadenylation.

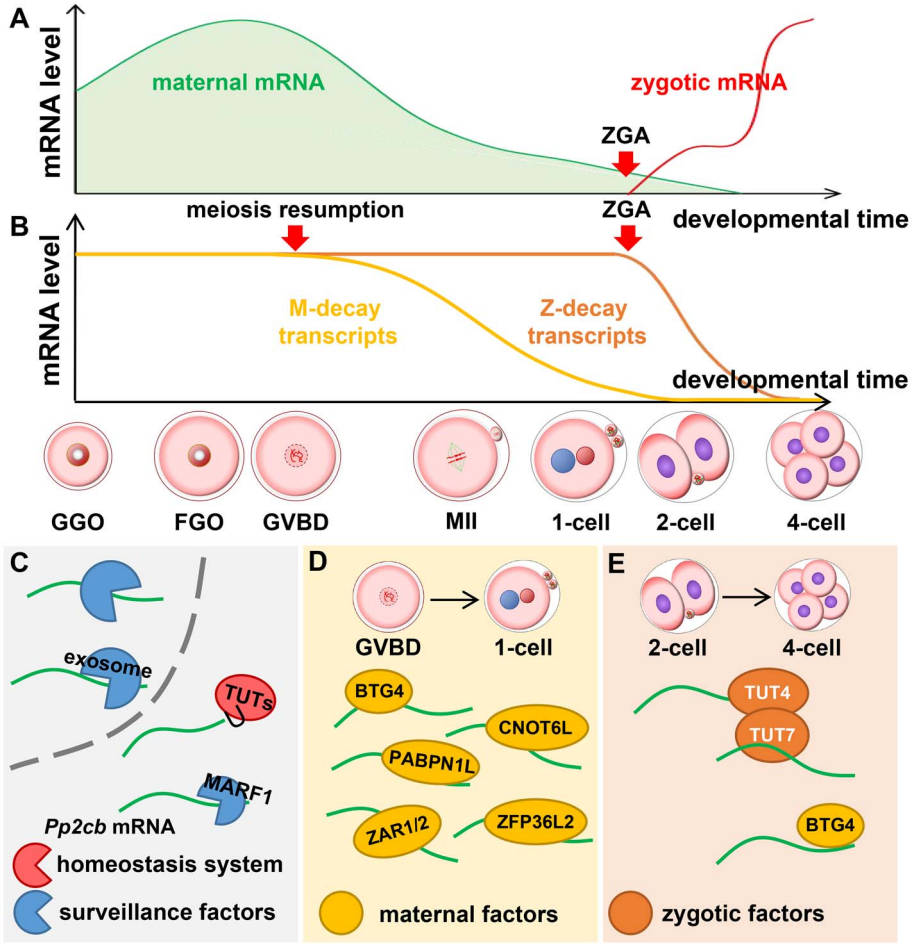

Figure 1. Dynamics of maternal mRNAs in mouse oocytes and preimplantation embryos. (A) Growing oocytes during folliculogenesis are under robust transcription, whereas this activity ceases when reaching the fully-grown oocyte (FGO) stage. Zygotic genome activation (ZGA) occurs around the 2-cell stage in mice with natal zygotic transcripts. (B) M-decay occurs when meiotic resumes. A majority of maternal mRNAs are removed till the end of the MII stage in mice. Z-decay occurs shortly after ZGA and eliminates a minority of maternal mRNAs. (C) The homeostasis system and surveillance factors are responsible for the dynamic equilibration of the transcripts in growing oocytes (GGOs). The homeostasis performers, such as terminal uridylyl transferases (TUTs), monitor the production and decay of beneficial transcripts, whereas the surveillance factors, such as meiosis arrest female 1 (MARF1) and the exosome, help remove the detrimental ones. (D) Multiple maternal factors are responsible for M-decay, including B-cell translocation gene-4 (BTG4), nuclear poly(A) binding protein 1-like (PABPN1L), zygotic arrest-1 (ZAR1) and ZAR1-like (ZAR2), CNOT6L, and zinc finger protein 36-like 2 (ZFP36L2). (E) Z-decay relies on de novo zygotic gene products, including TUT4/7. A few maternal factors, including BTG4, participate in this progress.

Maternal transcript removal directed by BTG4-CCR4-NOT^{CNOT7} is conserved among species. Recently, four women suffering from infertility were identified as carriers of homozygous pathogenic *BTG4* variants [30]. Two mutations in the BTG domain, $BTG4^{c.1A>G}$ and $BTG4^{c.73C>T}$, harbored by two individuals, led to BTG4 protein loss due to alternation of the first amino acid assembly and a premature translation stop codon, respectively. Another variant, $BTG4^{c.475\_478del}$, which harbored a mutation in the C-terminal domain, resulted in BTG4 protein truncation. All three variants resulted in severe impairment of BTG4 protein. The other variant, $BTG4^{c.166G>A}$, resulted in a missense mutation from alanine 56 to threonine in the BTG domain. The $BTG4^{c.166G>A}$ mutation did not affect the distribution or expression of BTG4. Numerous transcripts remained in $BTG4^{c.166G>A}$-variant zygotes, with relatively longer poly(A) tails, compared with $TUBB8^{c.922G>A}$-variant zygotes, which did not influence mRNA decay [21]. Moreover, the interaction between BTG4 and CNOT7 was lost in BTG4^{A56T}, suggesting that A56, in addition to W95, is another key residue for this collaboration and accounts for maternal mRNA cleansing. Therefore, these data indicate that *BTG4* is responsible for the failure of zygotic cleavage, and serves as a genetic marker for female infertility in clinical diagnosis.

Coordination of CNOT6L and ZFP36L2 within M-decay

Murine and human oocytes harbor high levels of *Cnot6* and *Cnot6l* mRNAs. *Cnot6* is not involved in reproduction, as $Cnot6^{-/-}$ female and male mice are fertile [31]. *Cnot6l*-null females were subfertile, providing direct genetic evidence for the role of the CCR4-NOT$^{CNOT6/6L}$ complex in maternal mRNA degradation [20]. Sha et al. reported that over 50% of ovulated $Cnot6l^{-/-}$ oocytes failed to exclude poly body 1 (PB1) and harbored multipolar spindles. CNOT6L, with the help of an adaptor, zinc-finger protein 36-like 2 (ZFP36L2), specifically bound to the (A+U)-rich element (ARE)-containing mRNA 3′-UTRs, and carried out deadenylation and further degradation (Figure 2B). This ZFP36L2-CCR4-NOT^{CNOT6L}-directed degradation occurred in prometaphase

Table 2. Biochemical properties and biological functions of subunits in the CCR4-NOT complex

Gene name	Function in CCR4-NOT complex	Phenotype(s) of KO mice	Phenotype(s) of germ cell-specific cKO mice
Cnot1	A scaffold subunit	Embryonic lethal [82]	Not reported
Cnot2	A scaffold subunit	Not reported	Not reported
Cnot3	A scaffold subunit	Embryonic lethal [83]	Not reported
Cnot4	A regulatory subunit, functioning as an E3 ubiquitin ligase and an mRNA adaptor	Embryonic lethal [61]	Male-specific infertility [61]
Cnot6	A catalytic subunit, a CCR homologue in mammals, belonging to the EEP-type ribonuclease family	Female-specific infertility in double KO of *Cnot6/6 l* [31]	Not reported
Cnot6l	A catalytic subunit, CCR homologue in mammals, belonging to the EEP-type ribonuclease family	Female-specific subfertility [20]	Not reported
Cnot7	A catalytic subunit, CAF1 homologue in mammals, belonging to the DEDD-type ribonuclease family	Not reported	Male-specific infertility [28]
Cnot8	A catalytic subunit, CAF1 homologue in mammals, belonging to the DEDD-type ribonuclease family	Embryonic lethal [84]	Not reported
Cnot9	A regulatory subunit, CAF40 homologue in mammals, interacting with multiple RBPs	Caudal body truncation during embryogenesis [85]	Not reported
Cnot10	A genuine subunit in mammals, forming the CNOT10-CNOT11 module	Not reported	Not reported
Cnot11	A genuine subunit in mammals, forming the CNOT10-CNOT11 module	Not reported	Not reported

Subunits mentioned in this review are listed, with the ones discussed in detail present in bold fonts. KO, knockout; cKO, conditional knockout; RBP, RNA-binding protein.

I, prior to the degradation triggered by BTG4. This process also managed the clearance of a larger transcript population than that of BTG4-CCR4-NOT^{CNOT7} (for the transcripts degraded during GV-to-MII transition in WT oocytes, ~58.9% were not removed in *Btg4*$^{-/-}$ oocytes, whereas 96.6% remained in *Cnot6l*$^{-/-}$ oocytes). These mRNAs, including *Cpeb1* (cytoplasmic polyadenylation element binding protein 1) and *Tubb4b* (tubulin beta 4 B class IVb, encoding a structural basis for spindle microtubules), were degraded and could no longer be loaded with polysomes, further governing maternal translation and spindle assembly. CNOT6L may partially function independently of other CCR4-NOT subunits; the MI arrest phenotype of *Cnot6l*$^{-/-}$ oocytes could be partially rescued by the overexpression of CNOT6L$^{\triangle LRR}$, a mutant lacking the interaction region toward CNOT7. Hence, a stepwise adaptor-recruiting system monitors the deadenylase activity of the CCR4-NOT complex, further determining stage-specific mRNA turnover.

Similarly, ZFP36L2 was identified as a maternal mRNA decay factor. Female mice carrying the oocyte-specific depletion of *Zfp36l2* (in *Zfp36l2*$^{fl/fl}$*; Zp3*-Cre mice) [32], and those carrying an N-terminal-truncated ZFP36L2, *△N-Zfp36l2* [33], were reported to be sterile. Unlike *Btg4*$^{-/-}$ females, relatively few transcripts accumulated in both mouse strains, including *Lhcgr* and mRNAs encoding histone demethylases. During the transition from the non-surrounded nucleus (NSN) to surrounded nucleus (SN), ZFP36L2 recognized *Kdm5a* and other histone demethylase transcripts, guarded their degradation, and reduced their protein levels [32]. The levels of H3K4me3 and H3K9me3 were subsequently elevated, enabling transcription silencing in FGOs. Upon luteinizing hormone (LH) surge, expression of the membrane receptor LHR is induced, and adenylyl cyclase is activated through the interconnection between LHR and $G_{\alpha S}$-GTP [34]. Thus, cyclic AMP level was increased to help maintain oocytes within prophase I. ZFP36L2 bound to one of the AREs residing in *Lhcgr* mRNA 3′-UTR [33], removed this target, and was thus vital for the resumption of meiosis.

The coordination between ZFP36L2 and CNOT6L has been demonstrated in humans. Recently, Zheng et al. reported two patients carrying biallelic variants of *ZFP36L2*, both of whom were infertile females suffering from recurrent preimplantation embryo developmental arrest [35]. Both patients were found to harbor mutations in ZFP36L2^{S308}; Patient 1 harbored a homozygous non-frameshift deletion from Ser308 to Ser310, whereas Patient 2 harbored two heterozygous variants, one of which was identical to Patient 1, and the other was a missense variant (Ser 308 Ala). The inheritance of these variants followed a recessive pattern. Mutated ZFP36L2 resulted in massive accumulation of ARE-containing transcripts in zygotes from these patients. Human ZFP36L2 interconnects with CNOT6L, suggesting that maternal mRNA removal guided by ZFP36L2-CCR4-NOT^{CNOT6L} is conserved in mammals.

Assistance of PABPN1L in the BTG4-CNOT7 guarding M-decay

The transportation of mRNAs from the nucleus to the cytoplasm, as well as the removal of cytoplasmic transcripts, is accompanied by PABPs [22]. PABPs can be categorized into two subgroups: nuclear PABPs (PABPNs) and cytoplasmic PABPs (PABPCs). Nuclear and cytosolic PABPs differ in the number of RNA-recognition motifs: PABPN1 and PABPN1-like (PABPN1L) specifically localize in the nucleus with a single RNA recognition motif (RRM) in the central region [36, 37], whereas cytoplasmic PABPs (PABPCs) harbor four RRMs and a PABP domain [38]. PABPC—a PABP prototype—is widely distributed among various mammalian cell types, including somatic cells and germ cells [39]. PABPC1 participates in divergent post-transcriptional processes in the cytoplasm, including CNOT6-mediated mRNA deadenylation [22] and nonsense-mediated decay toward transcripts with premature translational termination codons [40]. PABPC1L—also known as embryonic poly(A)-binding protein (EPAB)—recognizes elongated poly(A) tails

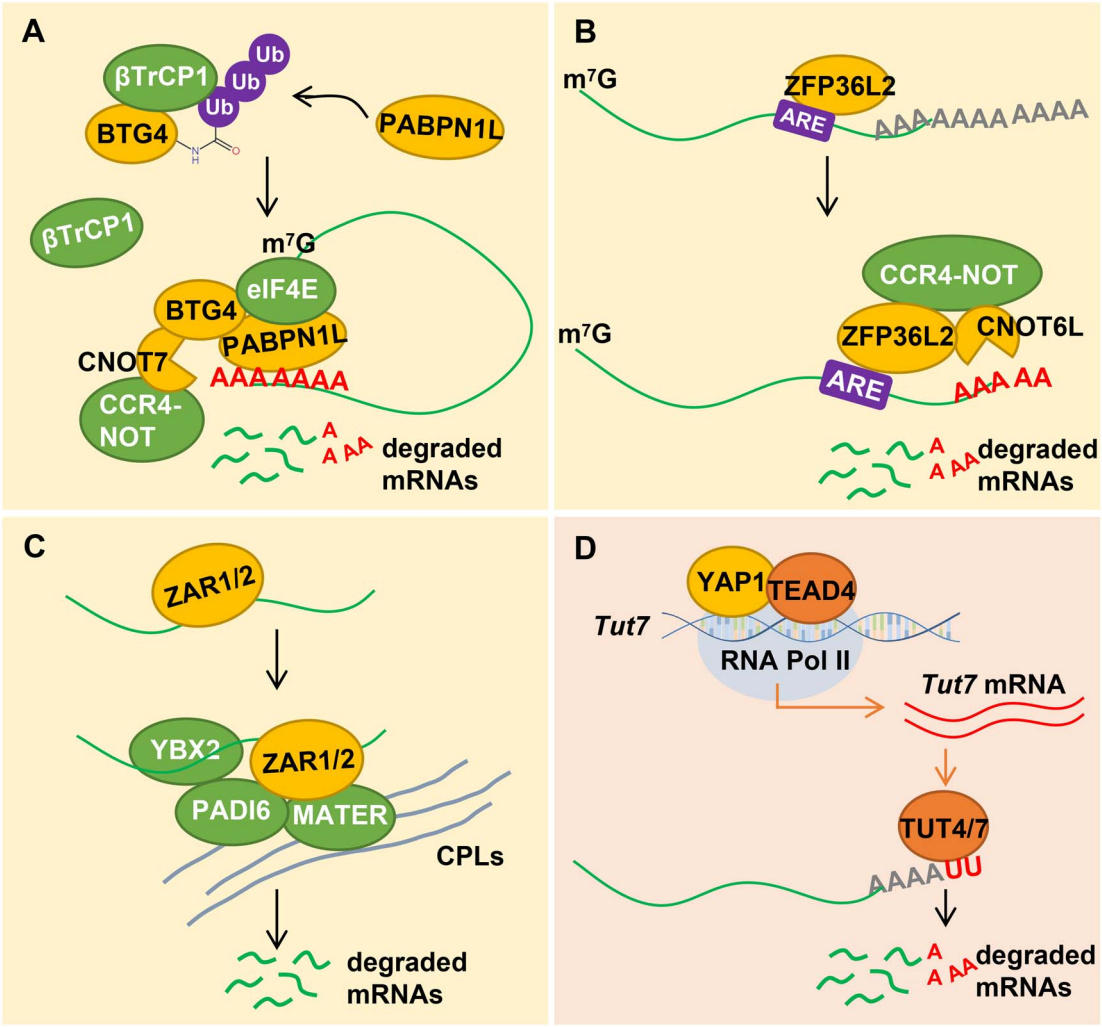

Figure 2. Functional factors in maternal mRNA elimination. (A) Combination of CNOT7 and BTG4 guards M-decay with the assistance of PABPN1L. BTG4 recruits CNOT7 to eIF4e docking transcripts in MII oocytes, whereas PABPN1L enhances this interaction and helps stabilize BTG4 against ubiquitination. (B) Coordination of CNOT6L and ZFP36L2 modulates M-decay. CCR4-NOT^{CNOT6L} is tethered towards ARE-containing transcripts by ZFP36L2 and triggers target deadenylation during the meiotic maturation. (C) Collaboration between ZAR1/2 and cytoplasmic lattices (CPLs) monitors M-decay. Zygotic arrest-1 and ZAR2 directly interact with two of the CPL members, maternal antigen that embryos require (MATER) and peptidylarginine deiminase 6 (PADI6), and indirectly interact with Y-box protein 2 (YBX2), thus guarding M-decay. (D) Maternal yes-associated protein 1 (YAP1)-zygotic TEAD4-triggered TUT4/7 in Z-decay. At around the 2-cell stage in mice and the 4-cell stage in human, zygotic *Tead4* is transcribed upon ZGA. The combination of maternal YAP1 and zygotic TEAD4 induces the expression of zygotic TUT4/7, which govern Z-decay through oligo-uridylation.

for further polyadenylation [41]. In addition, PABPC1L initiates translation by enhancing the transcript affinity of eIF4G–eIF4e and triggering Maskin dissociation, a translational suppressor of the cap-binding complex [42]. Meanwhile, *Xenopus* PABPC1L associates with the DAZL-PUM1/2 complex and promotes target translation [43]. In mice, PABPC1L is primarily expressed during oocyte growth [39] and regulates oocyte-cumulus cell communication. *Pabpc1l*$^{-/-}$ females were infertile, and their oocytes were small and loosely connected to cumulus cells. Impaired formation of transzonal processes and disrupted signal transmission were reported in *Pabpc1l*$^{-/-}$ cumulus-oocyte complexes [44, 45].

However, PABPNs work via different mechanisms. The PABPN1 is ubiquitously expressed in all cell types [38], and is required for poly(A) tail elongation and mRNA stabilization; its function in oocytes remains unknown. In contrast, PABPN1-like (PABPN1L) is involved in the elimination of maternal transcripts by recruiting BTG4 to the poly(A) tails of target mRNAs [46]. Substantial amounts of mRNAs, which would be degraded in WT embryos, accumulated in embryos derived from *Pabpn1l*$^{-/-}$ oocytes. This accumulation of transcripts was similar to the upregulation of transcripts in *Btg4*$^{\male+/\female-}$ embryos. Furthermore, ~20–30 bases of the poly(A) tails were occupied by a single PABPN1L molecule, and this binding affinity required its RRM. In contrast, the interplay between PABPN1L and BTG4 requires another domain located at its C-terminus. Hence, PABPN1L helps strengthen the binding of BTG4-CCR4-NOT^{CNOT7} to mRNAs (Figure 2A).

Moreover, PABPN1L has a second cytoplasmic function for the maternal-zygotic transition (MZT) by being a BTG4 stabilizer. In somatic cells, BTG family members are short-lived, since the SKP1-Cullin 1-F-box protein (SCF) ubiquitin E3 ligase promptly ubiquitinates BTGs, including BTG4 [47]. However, the presence of PABPN1L blocks the binding of βTrCP1

(β-transducin repeat-containing protein1), a substrate adaptor of SCF in polyubiquitination, to BTG4 (Figure 2A). The aberrant reduction of BTG4 protein levels in *Pabpn1l*$^{-/-}$ MII oocytes was found to be reversed upon ectopic PABPN1L expression with microinjection. As the PABPN1L protein is transiently expressed during the MII stage and will not be removed until the two-cell stage, PABPN1L maintains the existence of BTG4 and facilitates maternal mRNA clearance during this period, aiding for subsequent ZGA.

Collaboration of ZAR1/2 and CPLs inside GGOs and FGOs

Zygotic arrest-1 (*Zar1*) is one of the earliest identified maternal-effect genes specifically expressed in oocytes [48]. Its homologue, *Zar2* (also known as *Zar1-like* and *Zar1l*) shares identity with *Zar1*, as it encodes a conserved C-terminal RNA-binding domain and presents a similar expression dominance in oocytes and preimplantation embryos. Rong et al. reported that functional redundancy exists between ZAR1 and ZAR2 [49]. Both *Zar1*$^{-/-}$ and *Zar1/2*$^{-/-}$ female mice were sterile, whereas females lacking *Zar2* were fertile. Zygotic arrest-1-null oocyte-derived embryos presented the one- and two-cell arrest, with a prolonged period of polar body emission in MII oocytes. However, *Zar1/2*$^{-/-}$ oocytes were unable to emit normal polar bodies and were primarily arrested around metaphase I or telophase I. Taken together, these data suggest that ZAR2 enhances the function of ZAR1, since double KO mice exhibited more severe impairment during meiotic maturation than *Zar1*$^{-/-}$ females.

Zygotic arrest-1/2 are expressed in GGOs and FGOs, and possess at least two distinct functions in oocyte growth and meiotic maturation. The predominant function of ZAR1/2 is the direction of mRNA decay through CPLs. Cytoplasmic lattice is a specialized organelle in oocytes that appears following the activation of primordial follicles and exists throughout folliculogenesis and early embryogenesis [50, 51]. Cytoplasmic lattices are composed of ribosomes that are not incorporated into polysomes and, therefore, are not involved in the translation process. Multiple maternal factors, including maternal antigen that embryos require (MATER; [52]), peptidylarginine deiminase 6 (PADI6; [53]), and Y-box protein 2 (YBX2, formally known as MSY2; [53, 54]), have been reported as CPL components. Knockdown of *Ybx2* led to the failure of the NSN to SN transition. Conversely, *Mater*- and *Padi6*-deficient female mice could ovulate but displayed an embryo development arrest at the one- to two-cell stage, as reported in *Padi6*$^{-/-}$ and *Mater*$^{tm/tm}$ (tm stands for targeted mutation) female mice, respectively [55, 56]. Zygotic arrest-1 directly interacted with MATER and PADI6, and indirectly interacted with YBX2, based primarily on their binding RNAs (Figure 2C). Although the specificity of ZAR1/2 bait mRNAs remains elusive, ZAR1/2 were responsible for removing a cohort of transcripts during the growing stage and M-decay. Second, ZAR1/2 activated translation accompanied by the resumption of meiosis. A decrease in global translation was observed in *Zar1/2*$^{-/-}$ oocytes from the GVBD to MII stage. In addition, transcripts of multiple maternal genes were not polyadenylated during the FGO to MI transition in these oocytes, including factors involved in the progression of meiosis, such as *Btg4* and *Ccnb1*, and spindle assembly guards, such as *Tpx2*. Therefore, the translation of these mRNAs was compromised in the absence of ZAR1/2, resulting in the failure of meiotic maturation and a secondary zygotic arrest phenotype.

TUT4/7-directed oligo-uridylation in Z-decay

TUT4/7 guard the 3′-terminal uridylation of short-tailed mRNAs. TUT4/7 are highly conserved in mammals, with a core nucleotidyltransferase domain embedded within three CCHC-type zinc finger domains. Apart from their roles in homeostasis, a YAP1-TEAD4-TUT7 degradation pathway has been reported in Z-decay [10, 21]. *Tut7* mRNAs, as M-decay transcripts, were removed before fertilization. Meanwhile, *Tut4/7* are early zygotic genes expressed shortly after ZGA. Yes-associated protein 1 (YAP1) is a transcriptional co-activator of TEAD family transcription factors that are expressed both maternally and zygotically. An oocyte deficient in *Yap1* was able to undergo meiotic maturation; however, its derived embryos exhibited a ZGA defect [57]. *Yap1*$^{♂+/♀-}$ embryos presented a prolonged two-cell stage and delayed four-cell entry. In contrast, *Tead4* is a zygotic gene. Upon minor ZGA, natal TEAD4 associates with maternal YAP1 and activates the transcription of zygotic genes. The *Tut7* gene promoter also harbors a putative TEAD-binding site, ~1000 bp upstream of its transcription start site. Thus, *Tut7* is an early ZGA gene, and maternal YAP1-zygotic TEAD4-TUT7 modulates the degradation of over 60% of Z-decay transcripts [10] (Figure 2D). This mechanism is also involved in Z-decay in humans. After maternal *Tut7* mRNA was cleared in M-decay, zygotic *Tut7* transcripts were found to accumulate around the eight-cell stage, whereas *TEAD4* was transcribed in minor ZGA around the four-cell stage [21]. During in vitro fertilization, lower levels of *TEAD4* and *TUT7* transcripts have been frequently reported in patient-derived embryos, which are frequently arrested at the eight-cell stage, highlighting the importance of *TEAD4* and *TUT7* in embryogenesis.

TUT4/7-directed degradation also occurs during male gametogenesis. TUT4/7 are expressed throughout spermatogenesis and have redundant functions [58]. Generally, TUT4/7 function redundantly during the 3′-uridylation of precursor-miRNA (pre-miRNA) and are involved in miRNA biogenesis in cultured cells [59, 60]. Similarly, in mouse testes, TUT4/7 are involved in miRNA oligo-uridylation, but they do not alter the level of targeting miRNAs. Although *Tut4*$^{-/-}$ and *Tut7*$^{-/-}$ males were fertile, *Tut4/7*$^{fl/fl}$*; Stra8*-Cre males were sterile, with atrophic testes and developmental arrest at the late pachytene stage. During the transition from the leptotene to pachytene stage, TUT4/7 recognize short-tailed transcripts enriched in AREs, uridylate them, and facilitate their turnover, leading to an avalanche of paternal mRNAs.

Specialized functional proteins and subcellular organelles: multiple choices

In addition to the catalytic subunits, other regulatory subunits of CCR4-NOT function in RNA decay mediation. CNOT4—a CCR4-NOT subunit conserved from yeast to humans—primarily interacts with several ubiquitin-conjugating enzymes with its RING domain at the N-terminus. CNOT4 harbors an RRM, and may serve as an mRNA adaptor of CNOT7. CNOT4 is highly expressed in mouse testes, and its expression level is maintained throughout spermatogenesis. The CNOT4-CCR4-NOT^{CNOT7} combination is essential for mRNA degradation between the

zygotene and pachytene stage [61]. Dai et al. recently reported defective DNA damage repair and impediment in homologous crossover between X and Y chromosomes in *Cnot4*$^{fl/fl}$*; Stra8*-Cre males, leading to spermatocyte developmental arrest at metaphase I, and male-specific sterility. These results suggest a correlation between mRNA degradation and timely repair of double strand breaks (DSBs), as well as the formation of crossovers during meiosis in mammals. However, conditional knockout of *Cnot4* in oocytes using *Gdf9-Cre* did not affect oocyte meiosis and MZT, indicating that CCR4-NOT substrates are recruited by different mRNA adaptors to CNOT7 during oogenesis and spermatogenesis.

The exosome is a complex that promotes 3′–5′ degradation of mRNAs, and can reside in the nucleoli, nucleoplasm, and cytoplasm. Oocyte-specific depletion of *Exosc10*, an RNase associated with the exosome, revealed that the exosome was also involved in the resumption of meiosis. *Exosc10*$^{fl/fl}$*; Zp3*-Cre females were subfertile, with fewer ovulated eggs than that of wild-type females and with GVBD failure [62]. Based on the exosome decay pathway, *Exosc10*-null oocytes accumulated transcripts during meiotic resumption, including *Wee1* mRNAs. Transcript accumulation resulted in the upregulation of WEE1 protein expression, maintaining this repressive effect upon CDK1 phosphorylation and impeding meiotic maturation.

To WHOM: the prey also has a voice

The maternal mRNAs, which are removed during M- and Z-decay, are not removed without any reason. Indeed, multiple mRNA elements have been defined as stabilization signals, including traditional motifs such as AAUAAA polyadenylation signals (PASs) and AREs, post-transcriptional modifications such as *N*-6-methyladenosine (m^{6}A), and other novel *cis*-elements (Figure 3A). These elements are recognized by specific RBPs and recruited to the degradation machinery, thereby affecting host stability.

ARE, the *cis*-element in RNA 3′-UTRs [63], is a host-destabilizing signal. A group of RBPs, including AUF1 (also known as heterogeneous nuclear ribonucleoprotein D, HNRNPD), HuR, and tristetraprolin (TTP) interact with AREs and assist in the decay process in cultured cells [64]. ARE-guarded mRNA removal is also vital in mouse oocytes and ovarian granulosa cells. In oocytes, ZFP36L2 can bridge the contact between CCR4-NOT^{CNOT6L} and ARE-harboring mRNAs, resulting in M-decay [20, 33, 35] (Figure 3B, upper box). In granulosa cells, its homologue, ZFP36, also guides the degradation of specific mRNAs. Inside preovulatory follicles, upon LH surge, transcriptionally induced ZFP36 can recognize transcripts including natriuretic peptide precursor C (*Nppc*) mRNAs and govern their degradation. These transcripts harbor non-canonical AREs in their 3′-UTRs, with UU/UA dinucleotide clusters [65]. The mRNA removal guided by ZFP36 requires its coordination with CCR4-NOT$^{CNOT6/6L}$, as recently reported by Dai et al. [31]. Although *Cnot6*- and *Cnot6l*-null females could undergo normal folliculogenesis, *Cnot6/6l*$^{-/-}$ females presented a lower transition rate from preantral to antral follicles, irregular estrus cycles, and ovulation defects. Such reproductive events are regulated by pituitary gonadotropin follicle-stimulating hormone (FSH). The FSH activates cyclic AMP/protein kinase A (cAMP/PKA) and phosphoinositide 3-kinase (PI3K) signaling pathways [66], initiates the transcription of genes including *Cnot6* and *Cnot6l*, and enhances the translation process, including of CNOT6L. While recruited by ZFP36, CNOT6/6L catalyzed the deadenylation and degradation of a broad spectrum of ARE-containing transcripts, which encoded repressors of follicle growth and endocrine activities (Figure 3B, lower box).

Other motifs in 3′-UTRs, including PASs and cytoplasmic polyadenylation elements (CPEs), can stabilize their hosts, and therefore have an opposite effect on clearance. These two elements are common prerequisites for cytoplasmic polyadenylation; in *Xenopus* eggs, CPEs recruit phosphorylated CPEB1s and increase the affinity between their neighboring PASs and cleavage and polyadenylation specificity factors (CPSFs), further recruiting the poly(A) polymerase (PAP) GLD2 [67, 68]. However, the canonical PAP family member, PAPα, contributed to the major polyadenylation activity in mouse FGOs during meiotic resumption [69]. Furthermore, a single proximal PAS could recruit CPSF4, promote polyadenylation, and enhance the translation of its host mRNA [70]. A flanking CPE, together with its binding to CPEB1, counteracted this interaction, blocked translation, and partially hampered its host stability (Figure 3C). Upon the resumption of meiosis, CPEB1 was phosphorylated by extracellular regulated protein kinase 1/2 (ERK1/2) and was thus degraded through the MAPK cascade [71], leading to the cooperation of CPSF4 and poly(A) polymerases upon PASs [70]. In this way, the translation of the host RNA can either be initiated or elevated. Here, CPEs played a dual role in oocyte mRNAs: inside FGOs, CPEs adjacent to PASs served as a destabilizing signal, which can convert to a translation-promoting signal after GVBD.

The length of the 3′-UTR, poly(A)s, and oligo-Us can also provide instructions for clearance activity. In mouse and human FGOs, M-decay transcripts have shorter 3′-UTRs than Z-decay transcripts [10, 21]. Fewer PASs and CPEs resided in the former mRNAs, which correlated with the lengths of the 3′-UTRs. In addition, M-decay transcripts harbored longer poly(A) tails during the FGO stage. Their incorporation into polysomes was also higher than that of other maternal mRNAs. Once meiosis resumed, marked changes are observed in the 3′-end, as their poly(A) tails were shortened, and the uridylated levels were elevated. In contrast, the relatively short-tailed Z-decay transcripts were adenylated during meiotic maturation, tethered to polysomes in MII oocytes, whereas their rates of uridylation remained stable until the one-cell stage. A marked increase in the uridylation level of these transcripts occurred until the two-cell stage, which provided a time frame for their coding proteins between the MII and one-cell stages and facilitated the development of early embryos.

Recently, studies on the epitranscriptome have defined various RNA modifications that affect host stability, primarily assisting in post-transcriptional regulation. *N*-6-methyladenosine is the most prevalent modification of eukaryotic transcripts. According to meRIP-seq data, m^{6}A mainly occurs in the coding sequences (CDS) and 3′-UTRs of mRNAs and is usually located in the GGARH motif [72]. M^{6}A readers recognize and bind to this consensus sequence as well as the embedded m^{6}A site, thus carrying the host transcript to its destination. Among them, YTH-domain factor 2 (YTHDF2) serves as a cytoplasmic reader, selectively anchoring m^{6}A-containing transcripts and transferring them into P-bodies [7]. In zebrafish, maternal *Ythdf2*-deficient embryos presented retarded development and ZGA failure

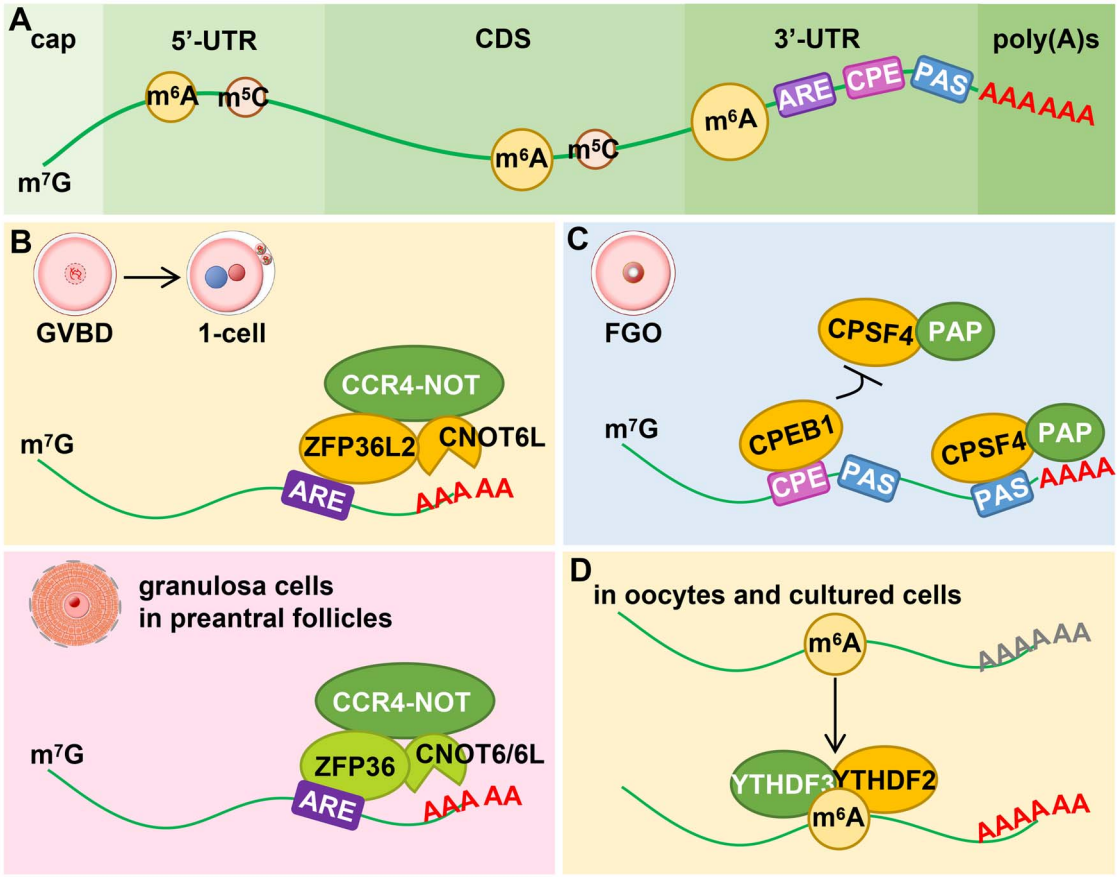

Figure 3. *Cis*-elements serving as degradation signals in eukaryotic mRNAs. (A) Overview of *cis*-elements and structure of eukaryotic mRNAs. Mature mRNAs contain an m7G cap structure at the 5′-end and a poly(A) tail at the 3′-end. Motifs in 3′-UTRs, the lengths of 3′-UTRs and RNA modifications are responsible for the host stability. (B) AU-rich elements (AREs) guard host transcript degradation in a conserved way. Respectively, ZFP36L2 and ZFP36 recognize AREs in transcripts and recruit CNOT6L and CNOT6/6L in MII oocytes and ovarian granulosa cells. These deadenylases thus eliminate the transcripts. (C) Cytoplasmic polyadenylation elements (CPEs) destabilize their residing mRNAs. Inside FGOs, CPE binding protein 1 (CPEB1) binds CPEs and counteracts the binding of cleavage and polyadenylation specificity factor 4 (CPSF4) to a flanking cleavage/polyadenylation site (PAS), further blocking the polyadenylation and translation process. (D) *N*-6-methyladenosine serves as a destabilizing signal in both cultured cells and oocytes. YTH-domain factor-2 (YTHDF2), one of the m^6A readers, recognizes m^6As and tethers the mRNAs into P-bodies. Other readers present diverse functions: YTH-domain factor-3 (YTHDF3) facilitates the transportation to P-bodies, whereas insulin growth factor 2 mRNA-binding proteins (IGF2BPs) counteract with YTHDF2.

owing to the aberrant accumulation of maternal mRNAs [8]. Similarly, in mammals, the lack of YTHDF2 resulted in female-specific sterility. *Ythdf2*$^{-/-}$ MII oocytes restored a cohort of m^6A-harboring mRNAs that failed to be degenerated during M-decay, and their derived embryos met with ZGA failure and loss of embryonic developmental competence [6, 73]. Other cytoplasmic m^6A readers work synergistically or antagonistically with YTHDF2 during this process: YTHDF3 interacts with YTHDF2 and affects the partitioning of their decay-targeted targets (Figure 3D), whereas the insulin growth factor 2 mRNA-binding proteins (IGF2BPs, consisting of IGF2BP1/2/3) recruit matrin 3 (MATR3) and other RNA stabilizers to protect targeting methylated transcripts [74, 75].

WHY: it should be and it will be

The mRNA degradation system involves interconnected machinery with various complexes that function in deadenylation and oligo-uridylation, as well as specialized organelles such as CPLs. Questions have emerged for in-depth research: for example, what determines the precise control of these "avalanches"; what makes the mRNA-specific degradation network important; what happens when part of the system, or even the whole network, loses its function and what are the similarities and differences of maternal mRNA degradation between mice and humans?

Why are the avalanches destined to occur at this point?

A fundamental determinant is the expression of functional factors within a specific timeframe. Although most degradation machinery proteins remain undetectable in FGOs, levels of their coding mRNAs remain high during folliculogenesis, such as *Btg4*. Multiple maternal proteins, including BTG4, PABPN1L, and CNOT7, are translated during meiotic maturation, and their levels peak around the MII stage [19, 46], assuring M-decay (Figure 4). With re-establishment of the zygotic genome, de novo gene products start to be generated at the late one-cell stage, as the transcription activity recovers around PN3. For example, during the two- to four-cell stage, zygotic TEAD4 recognizes the *Tut7* promoter and activates its transcription, initiating zygotic oligo-uridylation-driven degradation [10].

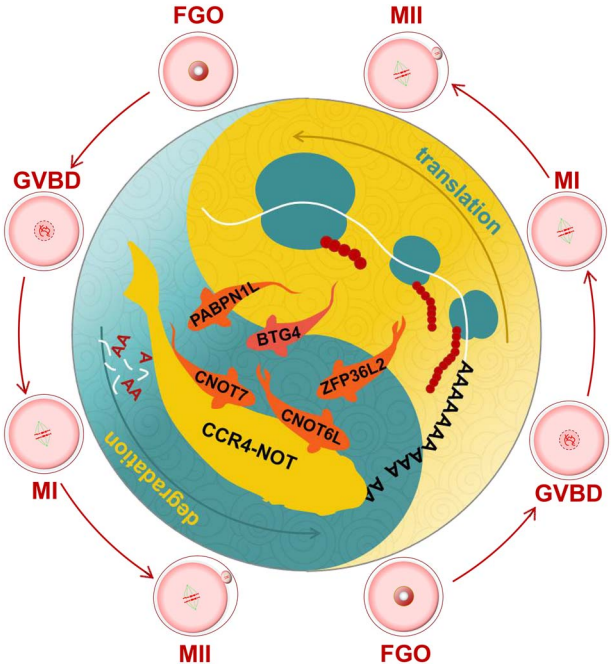

Figure 4. Dynamics of maternal mRNA translation and degradation in mammalian oocytes during meiotic maturation. As meiosis resumes, multiple maternal factors, including BTG4, CNOT6L, and PABPN1L, are translated and further facilitate mRNA degradation, which is initiated by CCR4-NOT-driven deadenylation.

Another key factor controlling timing is the temporal–spatial regulation of the degradation machinery and their target RNAs. In oocytes and somatic cells, only transcripts that have completed post-transcriptional processing, including splicing and polyadenylation, are regarded as mature mRNAs. These mRNAs are transported into the cytoplasm where a majority of the degradation complexes are localized, such as CCR4-NOT and TUTs, whereas only a minority of the RNA decay machinery resides in the nucleus, such as the exosome. Therefore, from a canonical viewpoint, mRNA clearance occurs in the cytosol. However, in FGOs, a large number of transcripts are stored in the nucleoplasm for utilization in later stages when they are translated within MII oocytes. Hence, removal of the nuclear membrane facilitates contact between nuclear mRNAs and the cytoplasmic degradation machinery. As meiosis resumes, the nuclear envelope breaks down, which provides a timeframe for the removal of previously nucleoplasm-located mRNAs from the MII stage to the one-cell stage, before zygotic nuclear re-formation.

Why is the mRNA-specific degradation system important?

Excessive transcript accumulation can hamper host cell metabolism in various ways. For example, M-decay failure leads to ZGA failure. Although it remains unclear how these aberrantly piled transcripts impede ZGA in oocytes, embryos from multiple mouse models, in which mRNA degradation factor-coding genes were mutated or knocked out, presented developmental arrests around the two-cell stage. Loss of function of CCR4-NOT complex members resulted in female infertility, and embryos with maternal loss of *Cnot6l* and CCR4-NOT^{CNOT7} adaptors, including *Btg4*$^{-/-}$ and *Pabpn1l*$^{-/-}$ mice, presented developmental arrest at the one-to two-cell stage [19, 20, 46], just ahead of ZGA. The CPL also governs this process, with *Mater*$^{tm/tm}$ mutants, *Padi6*- and *Msy2*-null females presenting impaired M-decay, resulting in two-cell arrest [53–55]. Comparatively, an undermined Z-decay could establish obstacles toward embryonic totipotency [57]. Oocytes deficient in YAP1 can undergo successful meiotic maturation during meiosis. However, the maternal YAP1-zygotic TEAD4-TUT4/7-driven Z-decay was lost in *Yap1*$^{♂+/♀-}$ embryos, which had a prolonged two-cell stage and a retarded four-cell entry, as well as impaired differentiation of the trophectoderm.

Impaired degradation during folliculogenesis also impedes oocyte and follicle growth. During folliculogenesis, oocyte gene products accumulate as the volume of oocytes increases, and the surrounding granulosa cells proliferate. Once FGOs enter the SN stage, the transcriptional activity halts, and these oocytes possess a comparatively stable transcriptome. When RNA homeostasis is impaired, such as in *Tut4/7*$^{fl/fl}$*; Zp3*-Cre females [5], unhealthy MII oocytes were ovulated, and these mice failed to produce pups. RNA surveillance factors are also vital for the removal of excess transcripts. When the exosome was in trauma, for example, lacking its enzymatic subunit EXOSC10, female mice failed to ovulate, and the FGOs of *Exosc10*$^{fl/fl}$*; Zp3*-Cre females could not develop with normal sizes [62].

A secondary consequence of the increase in mRNA expression is the ectopic translation of their encoded proteins. In *Cnot6l*$^{-/-}$ MII oocytes, a cohort of transcripts normally removed during M-decay were aberrantly incorporated into the polysomes, such as *Tubb4b*, resulting in disordered multipolar spindles [20]. For MPF activation to succeed, various factors that inhibit CDK1 should be removed or downregulated, either at the mRNA or protein level. Among them, WEE1 serves as a CDK-inhibitory kinase, and its transcripts were degraded upon initiation of meiotic maturation under normal conditions. Inside *Exosc10*$^{fl/fl}$*; Zp3*-Cre oocytes, mounted *Wee1* mRNAs resulted in the translation of WEE1 proteins, further impeding CDK1 activation [62]. The protein phosphatase 2 catalytic subunit (PPP2CB) suppresses MPF activation. As a target of MARF1, *Pp2cb* mRNA levels were elevated in *Marf1*$^{ENU/ENU}$ oocytes, and subsequently, PP2CB proteins sustained MPF in an inactive form and hampered the re-initiation of meiosis [16].

What are the similarities and differences in maternal mRNA removal among species?

The timely clearance of maternal transcripts gives rise to healthy offspring. As shown in various KO mice and in vitro fertilization (IVF) patients, impaired mRNA degradation system in oocytes finally led to defects in meiotic maturation as well as in ZGAs, indicating its pivotal roles among species. Multiple maternal factors and their regulatory pathways are conserved in both mice and humans. For example, deadenylation guided by the CCR4-NOT complex governs M-decay in both mouse and human oocytes, as the interconnection between BTG4 and CNOT7, as well as the collaboration between ZFP36L2 and CNOT6L remains between these two species. Although *Btg4*$^{-/-}$, *Cnot6l*$^{-/-}$, and *Zfl36l2*$^{fl/fl}$; *Zp3*-Cre female mice presented defects in meiotic maturation and early embryogenesis [20, 33, 57], patients carrying pathogenic *BTG4* and *ZFP36L2* variants also suffered from infertility. Similarly, zygotic transcription activation guided by

maternal YAP1 and zygotic TEAD4 promotes the expression of zygotic TUT4/7 in mouse and human embryos, initiating oligo-uridylation-mediated Z-decay [10, 21]. Although knockdown of *Tut4/7* led to aberrant transcript accumulation in mouse embryos, a lower expression level of *TUT7* was also found in patient-derived preimplantation embryos suffering from developmental arrest.

A second similarity is the characteristics of the transcripts cleansed in "avalanches." Compared with Z-decay transcripts, M-decay transcripts harbor relatively short 3′-UTRs, with a small number of PASs and CPEs in both mice and humans [21]. This could be attributed to conservation shared by the corresponding degradation factors, which recognize particular elements and direct the degradation of the mRNA hosts, for example, ZFP36L2 and ARE-harboring mRNAs. The resemblance among species could also resulted from a conserved mRNA stabilizing system. Accompanying meiotic resumption, PAP family members, GLD2 in *Xenopus*, and PAPα in mice, are recruited by CPSFs to promote polyadenylation [68, 69]. The affinity between CPSFs and PASs is enhanced by the collaboration of phosphorylated CPEB1 and a neighboring CPE [67, 70]. During meiotic maturation, transcripts with longer 3′-UTRs and larger numbers of PASs and CPEs are polyadenylated and stabilized, which are more likely to be translated, including *Btg4* and *Cnot6l* mRNAs.

A pivotal difference of Z-decay between mice and humans is the starting point and the duration. Owing to its reliance on de novo zygotic gene products, Z-decay primarily occurs shortly after the initiation of ZGA. Although mouse Z-decay mainly occurs at the two-cell stage, human Z-decay occurs during the four- to eight-cell stage [10, 21]. Moreover, zygotic transcription plays a more important role in Z-decay of human maternal transcripts than that of mouse transcripts. When zygotic transcription was inhibited, only half of the Z-decay transcripts failed to be degraded in mouse embryos, whereas >90% of those in humans were stabilized. This is probably due to the longer duration of human Z-decay completion, although the intrinsic mechanisms remain unclear.

Perspective: WHAT ELSE shall we resolve and identify?

Parents confer the recombined genome to their offspring, as well as various parental factors, including mRNAs and proteins. Re-sculpture of the oocyte transcriptome is vital for fertility. Despite the recent progress discussed in this review, open questions remain to be resolved. For example:

1) Do other factors involved in mRNA degradation in somatic cells have similar functions in oocytes? Common decay pathways, including deadenylation upon the CCR4-NOT complex and oligo-uridylation upon TUTs, possess highly conserved functions in both in vitro cultured cell lines and oocytes. The functions of other factors related to mRNA decay in somatic cells, such as PAN2/3, have not yet been elucidated in transcriptome regulation during gametogenesis.
2) Do divergent mRNA degradation mechanisms work synergistically or antagonistically? For example, deadenylation is always followed by oligo-uridylation, since shortened poly(A) tails recruit TUTs. Interconnections among other pathways have not yet been elucidated. In addition, RNAs are degraded by exonucleases or endonucleases. Various complexes are involved in the cleavage of long transcripts. The 5′–3′ exonuclease XRN1 is associated with the 5′-decapping process, while the exosome functions in the 3′–5′ digestion downstream of deadenylation. It remains unknown whether these bilateral digestions concur or counteract each other, whether one takes priority, and what the determinant is.
3) Do other destabilizing elements present in mRNAs? Various mRNA modifications have been identified, in addition to m^6A. Most currently defined modifications promote host stability; for example, 5-methycytosine (m^5C) exists in non-coding RNAs and mRNAs. A transcript harboring m^5C was recognized by YBX1, an MSY2 homologue, and was protected together with the YBX1-PABPC1A complex during zebrafish embryogenesis [76]. Pseudouridylation mainly occurs at the base of the stem-loop structure and enhances host RNA stability [77]. The existence and mechanisms of other modifications involved in degradation remain to be determined. Moreover, a stem-loop inside the 5′-UTR enhances the stability of the host mRNA, as it produces steric hindrance against a degradative ribonuclease [78]. Would there be secondary RNA structures, such as the R-loop, a triple-stranded DNA–RNA structure, that enhances the affinity between its host transcript and the degradation machinery?
4) Would there be consistency and analogies upon evolution of the mRNA degradation system between female and male reproduction? A group of factors function in both oocytes and spermatocytes, though with different subunits and at different stages. CNOT7 is essential for male fertility and M-decay in mice, whereas CNOT6L only functions in maternal mRNA clearance. Although the PIWI-piRNA pathway exists as a male-specific degradation system in mice, in other mammals, such as golden hamsters [79–81], such pathway functions in RNA clearance in both females and males. Would other factors be responsible for mRNA degradation in both genders?

With advances in technology, it is now possible to address these questions. Using a combination of sequencing techniques, we are able to compare sequences and address research questions in detail, such as whether identity exists between the elements in targeting RNAs and the functional factors upon RNA-immunoprecipitation sequencing (RIP-seq) techniques, whether there is a synergy among multiple mRNA elements upon novel single-base analysis, and whether there is a protein interaction upon a single element or RBP-binding site upon comparison of RIP-seq multiplex. The wide application of such methods would facilitate further research into transcriptome regulatory mechanisms as well as the re-sculpture of mRNA species toward reproduction.

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Conflict of Interest

The authors declear no conflict of interest.

References

1. Furuichi Y. Discovery of m(7)G-cap in eukaryotic mRNAs. *Proc Jpn Acad Ser B Phys Biol Sci* 2015; **91**:394–409.
2. Song MG, Kiledjian M. 3′ Terminal oligo U-tract-mediated stimulation of decapping. *RNA* 2007; **13**:2356–2365.
3. 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**:3135–3148.
4. Meola N, Domanski M, Karadoulama E, Chen Y, Gentil C, Pultz D, Vitting-Seerup K, Lykke-Andersen S, Andersen JS, Sandelin A, Jensen TH. Identification of a nuclear exosome decay pathway for processed transcripts. *Mol Cell* 2016; **64**:520–533.
5. Morgan M, Much C, DiGiacomo M, Azzi C, Ivanova I, Vitsios DM, Pistolic J, Collier P, Moreira PN, Benes V, Enright AJ, O'Carroll D. mRNA 3′ uridylation and poly(A) tail length sculpt the mammalian maternal transcriptome. *Nature* 2017; **548**:347–351.
6. Ivanova I, Much C, Di Giacomo M, Azzi C, Morgan M, Moreira PN, Monahan J, Carrieri C, Enright AJ, O'Carroll D. The RNA m(6)A reader YTHDF2 is essential for the post-transcriptional regulation of the maternal transcriptome and oocyte competence. *Mol Cell* 2017; **67**:1059–1067.e1054.
7. Wang X, Lu Z, Gomez A, Hon GC, Yue Y, Han D, Fu Y, Parisien M, Dai Q, Jia G, Ren B, Pan T *et al.* N6-methyladenosine-dependent regulation of messenger RNA stability. *Nature* 2014; **505**:117–120.
8. Zhao BS, Wang X, Beadell AV, Lu Z, Shi H, Kuuspalu A, Ho RK, He C. m(6)A-dependent maternal mRNA clearance facilitates zebrafish maternal-to-zygotic transition. *Nature* 2017; **542**:475–478.
9. Walser CB, Lipshitz HD. Transcript clearance during the maternal-to-zygotic transition. *Curr Opin Genet Dev* 2011; **21**:431–443.
10. Sha QQ, Zhu YZ, Li S, Jiang Y, Chen L, Sun XH, Shen L, Ou XH, Fan HY. Characterization of zygotic genome activation-dependent maternal mRNA clearance in mouse. *Nucleic Acids Res* 2020; **48**:879–894.
11. Sha QQ, Zhang J, Fan HY. A story of birth and death: mRNA translation and clearance at the onset of maternal-to-zygotic transition in mammals†. *Biol Reprod* 2019; **101**:579–590.
12. Leresche A, Wolf VJ, Gottesfeld JM. Repression of RNA polymerase II and III transcription during M phase of the cell cycle. *Exp Cell Res* 1996; **229**:282–288.
13. Moraes KC. RNA surveillance: molecular approaches in transcript quality control and their implications in clinical diseases. *Mol Med* 2010; **16**:53–68.
14. Su YQ, Sun F, Handel MA, Schimenti JC, Eppig JJ. Meiosis arrest female 1 (MARF1) has nuage-like function in mammalian oocytes. *Proc Natl Acad Sci U S A* 2012; **109**:18653–18660.
15. Yao Q, Cao G, Li M, Wu B, Zhang X, Zhang T, Guo J, Yin H, Shi L, Chen J, Yu X, Zheng L *et al.* Ribonuclease activity of MARF1 controls oocyte RNA homeostasis and genome integrity in mice. *Proc Natl Acad Sci U S A* 2018; **115**:11250–11255.
16. Su YQ, Sugiura K, Sun F, Pendola JK, Cox GA, Handel MA, Schimenti JC, Eppig JJ. MARF1 regulates essential oogenic processes in mice. *Science* 2012; **335**:1496–1499.
17. Fulka H, Novakova Z, Mosko T, Fulka J Jr. The inability of fully grown germinal vesicle stage oocyte cytoplasm to transcriptionally silence transferred transcribing nuclei. *Histochem Cell Biol* 2009; **132**:457–468.
18. Abe K, Inoue A, Suzuki MG, Aoki F. Global gene silencing is caused by the dissociation of RNA polymerase II from DNA in mouse oocytes. *J Reprod Dev* 2010; **56**:502–507.
19. Yu C, Ji SY, Sha QQ, Dang Y, Zhou JJ, Zhang YL, Liu Y, Wang ZW, Hu B, Sun QY, Sun SC, Tang F *et al.* BTG4 is a meiotic cell cycle-coupled maternal-zygotic-transition licensing factor in oocytes. *Nat Struct Mol Biol* 2016; **23**:387–394.
20. Sha QQ, Yu JL, Guo JX, Dai XX, Jiang JC, Zhang YL, Yu C, Ji SY, Jiang Y, Zhang SY, Shen L, Ou XH *et al.* CNOT6L couples the selective degradation of maternal transcripts to meiotic cell cycle progression in mouse oocyte. *EMBO J* 2018; **37**.
21. Sha QQ, Zheng W, Wu YW, Li S, Guo L, Zhang S, Lin G, Ou XH, Fan HY. Dynamics and clinical relevance of maternal mRNA clearance during the oocyte-to-embryo transition in humans. *Nat Commun* 2020; **11**:4917.
22. Yi H, Park J, Ha M, Lim J, Chang H, Kim VN. PABP cooperates with the CCR4-NOT complex to promote mRNA Deadenylation and block precocious decay. *Mol Cell* 2018; **70**:1081–1088 e1085.
23. Chowdhury A, Mukhopadhyay J, Tharun S. The decapping activator Lsm1p-7p-Pat1p complex has the intrinsic ability to distinguish between oligoadenylated and polyadenylated RNAs. *RNA* 2007; **13**:998–1016.
24. Collart MA. The Ccr4-not complex is a key regulator of eukaryotic gene expression. *Wiley Interdiscip Rev RNA* 2016; **7**:438–454.
25. Collart MA, Panasenko OO, Nikolaev SI. The Not3/5 subunit of the Ccr4-not complex: a central regulator of gene expression that integrates signals between the cytoplasm and the nucleus in eukaryotic cells. *Cell Signal* 2013; **25**:743–751.
26. Winkler GS, Balacco DL. Heterogeneity and complexity within the nuclease module of the Ccr4-not complex. *Front Genet* 2013; **4**:296.
27. Berthet C, Morera AM, Asensio MJ, Chauvin MA, Morel AP, Dijoud F, Magaud JP, Durand P, Rouault JP. CCR4-associated factor CAF1 is an essential factor for spermatogenesis. *Mol Cell Biol* 2004; **24**:5808–5820.
28. Aslam A, Mittal S, Koch F, Andrau JC, Winkler GS. The Ccr4-NOT deadenylase subunits CNOT7 and CNOT8 have overlapping roles and modulate cell proliferation. *Mol Biol Cell* 2009; **20**:3840–3850.
29. Pasternak M, Pfender S, Santhanam B, Schuh M. The BTG4 and CAF1 complex prevents the spontaneous activation of eggs by deadenylating maternal mRNAs. *Open Biol* 2016; **6**:160184. doi: 10.1098/rsob.160184.
30. Zheng W, Zhou Z, Sha Q, Niu X, Sun X, Shi J, Zhao L, Zhang S, Dai J, Cai S, Meng F, Hu L *et al.* Homozygous mutations in BTG4 cause zygotic cleavage failure and female infertility. *Am J Hum Genet* 2020; **107**:24–33.
31. Dai XX, Jiang ZY, Wu YW, Sha QQ, Liu Y, Ding JY, Xi WD, Li J, Fan HY. CNOT6/6L-mediated mRNA degradation in ovarian granulosa cells is a key mechanism of gonadotropin-triggered follicle development. *Cell Rep* 2021; **37**:110007.
32. Dumdie JN, Cho K, Ramaiah M, Skarbrevik D, Mora-Castilla S, Stumpo DJ, Lykke-Andersen J, Laurent LC, Blackshear PJ, Wilkinson MF, Cook-Andersen H. Chromatin modification and global transcriptional silencing in the oocyte mediated by the mRNA decay activator ZFP36L2. *Dev Cell* 2018; **44**:392–402.e397.
33. Ball CB, Rodriguez KF, Stumpo DJ, Ribeiro-Neto F, Korach KS, Blackshear PJ, Birnbaumer L, Ramos SB. The RNA-binding protein, ZFP36L2, influences ovulation and oocyte maturation. *PLoS One* 2014; **9**:e97324.
34. Puett D, Li Y, Angelova K, Demars G, Meehan TP, Fanelli F, Narayan P. Structure-function relationships of the luteinizing hormone receptor. *Ann N Y Acad Sci* 2005; **1061**:41–54.
35. Zheng W, Sha QQ, Hu H, Meng F, Zhou Q, Chen X, Zhang S, Gu Y, Yan X, Zhao L, Zong Y, Hu L *et al.* Biallelic variants in ZFP36L2 cause female infertility characterised by recurrent preimplantation embryo arrest. *J Med Genet* 2021.
36. Mangus DA, Evans MC, Jacobson A. Poly(a)-binding proteins: multifunctional scaffolds for the post-transcriptional control of gene expression. *Genome Biol* 2003; **4**:223.
37. Zhao LW, Fan HY. Revisiting poly(a)-binding proteins: multifaceted regulators during gametogenesis and early embryogenesis. *Bioessays* 2021; **43**:e2000335.
38. Gorgoni B, Gray NK. The roles of cytoplasmic poly(a)-binding proteins in regulating gene expression: a developmental perspective. *Brief Funct Genomic Proteomic* 2004; **3**:125–141.

39. Uysal F, Ozturk S. Embryonic poly(a)-binding protein is differently expressed and interacts with the messenger RNAs in the mouse oocytes and early embryos. *J Cell Biochem* 2019; **120**:4694–4709.
40. Peixeiro I, Inácio Â, Barbosa C, Silva AL, Liebhaber SA, Romão L. Interaction of PABPC1 with the translation initiation complex is critical to the NMD resistance of AUG-proximal nonsense mutations. *Nucleic Acids Res* 2012; **40**:1160–1173.
41. Kim JH, Richter JD. Opposing polymerase-deadenylase activities regulate cytoplasmic polyadenylation. *Mol Cell* 2006; **24**:173–183.
42. Cao Q, Richter JD. Dissolution of the maskin-eIF4E complex by cytoplasmic polyadenylation and poly(A)-binding protein controls cyclin B1 mRNA translation and oocyte maturation. *EMBO J* 2002; **21**:3852–3862.
43. Esencan E, Kallen A, Zhang M, Seli E. Translational activation of maternally derived mRNAs in oocytes and early embryos and the role of embryonic poly(A) binding protein (EPAB). *Biol Reprod* 2019; **100**:1147–1157.
44. Lowther KM, Favero F, Yang CR, Taylor HS, Seli E. Embryonic poly(A)-binding protein is required at the preantral stage of mouse folliculogenesis for oocyte-somatic communication. *Biol Reprod* 2017; **96**:341–351.
45. Lowther KM, Mehlmann LM. Embryonic poly(A)-binding protein is required during early stages of mouse oocyte development for chromatin organization, transcriptional silencing, and meiotic competence. *Biol Reprod* 2015; **93**:43.
46. Zhao LW, Zhu YZ, Chen H, Wu YW, Pi SB, Chen L, Shen L, Fan HY. PABPN1L mediates cytoplasmic mRNA decay as a placeholder during the maternal-to-zygotic transition. *EMBO Rep* 2020; **21**:e49956.
47. Sasajima H, Nakagawa K, Kashiwayanagi M, Yokosawa H. Polyubiquitination of the B-cell translocation gene 1 and 2 proteins is promoted by the SCF ubiquitin ligase complex containing βTrCP. *Biol Pharm Bull* 2012; **35**:1539–1545.
48. Wu X, Viveiros MM, Eppig JJ, Bai Y, Fitzpatrick SL, Matzuk MM. Zygote arrest 1 (Zar1) is a novel maternal-effect gene critical for the oocyte-to-embryo transition. *Nat Genet* 2003; **33**:187–191.
49. Rong Y, Ji SY, Zhu YZ, Wu YW, Shen L, Fan HY. ZAR1 and ZAR2 are required for oocyte meiotic maturation by regulating the maternal transcriptome and mRNA translational activation. *Nucleic Acids Res* 2019; **47**:11387–11402.
50. Bachvarova R, De Leon V. Stored and polysomal ribosomes of mouse ova. *Dev Biol* 1977; **58**:248–254.
51. Gallicano GI, McGaughey RW, Capco DG. Cytoskeletal sheets appear as universal components of mammalian eggs. *J Exp Zool* 1992; **263**:194–203.
52. Morency E, Anguish L, Coonrod S. Subcellular localization of cytoplasmic lattice-associated proteins is dependent upon fixation and processing procedures. *PLoS One* 2011; **6**:e17226.
53. Liu X, Morency E, Li T, Qin H, Zhang X, Zhang X, Coonrod S. Role for PADI6 in securing the mRNA-MSY2 complex to the oocyte cytoplasmic lattices. *Cell Cycle* 2017; **16**:360–366.
54. Medvedev S, Pan H, Schultz RM. Absence of MSY2 in mouse oocytes perturbs oocyte growth and maturation, RNA stability, and the transcriptome. *Biol Reprod* 2011; **85**:575–583.
55. Tong ZB, Gold L, De Pol A, Vanevski K, Dorward H, Sena P, Palumbo C, Bondy CA, Nelson LM. Developmental expression and subcellular localization of mouse MATER, an oocyte-specific protein essential for early development. *Endocrinology* 2004; **145**:1427–1434.
56. Esposito G, Vitale AM, Leijten FP, Strik AM, Koonen-Reemst AM, Yurttas P, Robben TJ, Coonrod S, Gossen JA. Peptidylarginine deiminase (PAD) 6 is essential for oocyte cytoskeletal sheet formation and female fertility. *Mol Cell Endocrinol* 2007; **273**:25–31.
57. Yu C, Ji SY, Dang YJ, Sha QQ, Yuan YF, Zhou JJ, Yan LY, Qiao J, Tang F, Fan HY. Oocyte-expressed yes-associated protein is a key activator of the early zygotic genome in mouse. *Cell Res* 2016; **26**:275–287.
58. Morgan M, Kabayama Y, Much C, Ivanova I, Di Giacomo M, Auchynnikava T, Monahan JM, Vitsios DM, Vasiliauskaitė L, Comazzetto S, Rappsilber J, Allshire RC *et al.* A programmed wave of uridylation-primed mRNA degradation is essential for meiotic progression and mammalian spermatogenesis. *Cell Res* 2019; **29**:221–232.
59. Heo I, Joo C, Kim YK, Ha M, Yoon MJ, Cho J, Yeom KH, Han J, Kim VN. TUT4 in concert with Lin28 suppresses microRNA biogenesis through pre-microRNA uridylation. *Cell* 2009; **138**:696–708.
60. Faehnle CR, Walleshauser J, Joshua-Tor L. Multi-domain utilization by TUT4 and TUT7 in control of let-7 biogenesis. *Nat Struct Mol Biol* 2017; **24**:658–665.
61. Dai XX, Jiang Y, Gu JH, Jiang ZY, Wu YW, Yu C, Yin H, Zhang J, Shi QH, Shen L, Sha QQ, Fan HY. The CNOT4 subunit of the CCR4-NOT complex is involved in mRNA degradation, efficient DNA damage repair, and XY chromosome crossover during male germ cell meiosis. *Adv Sci (Weinh)* 2021; **8**:2003636.
62. Wu D, Dean J. EXOSC10 sculpts the transcriptome during the growth-to-maturation transition in mouse oocytes. *Nucleic Acids Res* 2020; **48**:5349–5365.
63. Barreau C, Paillard L, Osborne HB. AU-rich elements and associated factors: are there unifying principles? *Nucleic Acids Res* 2005; **33**:7138–7150.
64. Clement SL, Scheckel C, Stoecklin G, Lykke-Andersen J. Phosphorylation of tristetraprolin by MK2 impairs AU-rich element mRNA decay by preventing deadenylase recruitment. *Mol Cell Biol* 2011; **31**:256–266.
65. Xi G, An L, Wang W, Hao J, Yang Q, Ma L, Lu J, Wang Y, Wang W, Zhao W, Liu J, Yang M *et al.* The mRNA-destabilizing protein Tristetraprolin targets "meiosis arrester" Nppc mRNA in mammalian preovulatory follicles. *Proc Natl Acad Sci U S A* 2021; **118**:e2018345118. doi: 10.1073/pnas.2018345118.
66. Casarini L, Crépieux P. Molecular mechanisms of action of FSH. *Front Endocrinol (Lausanne)* 2019; **10**:305.
67. Richter JD. CPEB: a life in translation. *Trends Biochem Sci* 2007; **32**:279–285.
68. 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.
69. Jiang JC, Zhang H, Cao LR, Dai XX, Zhao LW, Liu HB, Fan HY. Oocyte meiosis-coupled poly(A) polymerase α phosphorylation and activation trigger maternal mRNA translation in mice. *Nucleic Acids Res* 2021; **49**:5867–5880.
70. Dai XX, Jiang JC, Sha QQ, Jiang Y, Ou XH, Fan HY. A combinatorial code for mRNA 3'-UTR-mediated translational control in the mouse oocyte. *Nucleic Acids Res* 2019; **47**:328–340.
71. Sha QQ, Dai XX, Dang Y, Tang F, Liu J, Zhang YL, Fan HY. A MAPK cascade couples maternal mRNA translation and degradation to meiotic cell cycle progression in mouse oocytes. *Development* 2017; **144**:452–463.
72. Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, Osenberg S, Cesarkas K, Jacob-Hirsch J, Amariglio N, Kupiec M, Sorek R, Rechavi G. Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. *Nature* 2012; **485**:201–206.
73. Shi H, Wang X, Lu Z, Zhao BS, Ma H, Hsu PJ, Liu C, He C. YTHDF3 facilitates translation and decay of N(6)-methyladenosine-modified RNA. *Cell Res* 2017; **27**:315–328.
74. Liu HB, Muhammad T, Guo Y, Li MJ, Sha QQ, Zhang CX, Liu H, Zhao SG, Zhao H, Zhang H, Du YZ, Sun K *et al.* RNA-binding protein IGF2BP2/IMP2 is a critical maternal activator in early zygotic genome activation. *Adv Sci (Weinh)* 2019; **6**:1900295.
75. Huang H, Weng H, Sun W, Qin X, Shi H, Wu H, Zhao BS, Mesquita A, Liu C, Yuan CL, Hu YC, Hüttelmaier S *et al.* Recognition of RNA N(6)-methyladenosine by IGF2BP proteins enhances mRNA stability and translation. *Nat Cell Biol* 2018; **20**:285–295.

76. Zou F, Tu R, Duan B, Yang Z, Ping Z, Song X, Chen S, Price A, Li H, Scott A, Perera A, Li S *et al.* Drosophila YBX1 homolog YPS promotes ovarian germ line stem cell development by preferentially recognizing 5-methylcytosine RNAs. *Proc Natl Acad Sci U S A* 2020; **117**: 3603–3609.
77. Nakamoto MA, Lovejoy AF, Cygan AM, Boothroyd JC. mRNA pseudouridylation affects RNA metabolism in the parasite Toxoplasma gondii. *RNA* 2017; **23**:1834–1849.
78. Emory SA, Bouvet P, Belasco JG. A 5′-terminal stem-loop structure can stabilize mRNA in Escherichia coli. *Genes Dev* 1992; **6**: 135–148.
79. Zhang H, Zhang F, Chen Q, Li M, Lv X, Xiao Y, Zhang Z, Hou L, Lai Y, Zhang Y, Zhang A, Gao S *et al.* The piRNA pathway is essential for generating functional oocytes in golden hamsters. *Nat Cell Biol* 2021; **23**:1013–1022.
80. Loubalova Z, Fulka H, Horvat F, Pasulka J, Malik R, Hirose M, Ogura A, Svoboda P. Formation of spermatogonia and fertile oocytes in golden hamsters requires piRNAs. *Nat Cell Biol* 2021; **23**:992–1001.
81. Hasuwa H, Iwasaki YW, Au Yeung WK, Ishino K, Masuda H, Sasaki H, Siomi H. Production of functional oocytes requires maternally expressed PIWI genes and piRNAs in golden hamsters. *Nat Cell Biol* 2021; **23**:1002–1012.
82. Shirai YT, Suzuki T, Morita M, Takahashi A, Yamamoto T. Multifunctional roles of the mammalian CCR4-NOT complex in physiological phenomena. *Front Genet* 2014; **5**:286.
83. Neely GG, Kuba K, Cammarato A, Isobe K, Amann S, Zhang L, Murata M, Elmén L, Gupta V, Arora S, Sarangi R, Dan D *et al.* A global in vivo Drosophila RNAi screen identifies NOT3 as a conserved regulator of heart function. *Cell* 2010; **141**:142–153.
84. Mostafa D, Takahashi A, Yanagiya A, Yamaguchi T, Abe T, Kureha T, Kuba K, Kanegae Y, Furuta Y, Yamamoto T, Suzuki T. Essential functions of the CNOT7/8 catalytic subunits of the CCR4-NOT complex in mRNA regulation and cell viability. *RNA Biol* 2020; **17**:403–416.
85. Sun M, Mondal K, Patel V, Horner VL, Long AB, Cutler DJ, Caspary T, Zwick ME. Multiplex chromosomal exome sequencing accelerates identification of ENU-induced mutations in the mouse. *G3 (Bethesda)* 2012; **2**:143–150.