CHAPTER SIX

The Maternal-to-Zygotic Transition During Vertebrate Development: A Model for Reprogramming

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Current Topics in Developmental Biology, Volume 113
ISSN 0070-2153
http://dx.doi.org/10.1016/bs.ctdb.2015.07.020

Abstract

Cellular transitions occur at all stages of organismal life from conception to adult regeneration. Changing cellular state involves three main features: activating gene expression necessary to install the new cellular state, modifying the chromatin status to stabilize the new gene expression program, and removing existing gene products to clear out the previous cellular program. The maternal-to-zygotic transition (MZT) is one of the most profound changes in the life of an organism. It involves gene expression remodeling at all levels, including the active clearance of the maternal oocyte program to adopt the embryonic totipotency. In this chapter, we provide an overview of molecular mechanisms driving maternal mRNA clearance during the MZT, describe the developmental consequences of losing components of this gene regulation, and illustrate how remodeling of gene expression during the MZT is common to other cellular transitions with parallels to cellular reprogramming.

1. INTRODUCTION

The debate regarding the origin of life complexity dates back to Greek philosophical writings. Through observation of chick embryos, Aristotle postulated that development starts with a uniform egg that gradually develops complexity. However, the seventeenth century was dominated by the preformationist theory of heredity, which favored the idea that the sperm and/or the egg contained a small, but fully formed, individual called the "homunculus" that grew over time (reviewed in Maienschein, 2012). In fact, early microscopists reported observing homunculi, supporting the preformationist theory of heredity (reviewed in Magner, 2002).

These contrasting ideas prompted nineteenth Century embryologists to experimentally test each model. In his seminal experiment, Wilhelm Roux, credited as one of the founders of embryology, destroyed two of the four cells in an early frog blastula and found that only half of the embryo eventually formed, supporting the idea that early embryonic cell fate is predetermined (reviewed in Maienschein, 2012). However, when Hans Driesch separated two-cell sea urchin embryos, two normal, but smaller urchins formed, demonstrating instead that the earliest embryonic cell fate

is undetermined (reviewed in Maienschein, 2012). This work led to the current paradigm that life starts with a naïve state that gradually develops complexity through sequentially transitioning to more differentiated cell types, giving rise to tissues and organs in the embryo.

The paradigm that life starts from a naïve state is seemingly paradoxical given that embryos derive from a union of two rather specialized differentiated cells, an egg and a spermatozoon. Thus the first step of development requires the reprogramming of differentiated gametes to a transiently totipotent zygote. This reprogramming event during embryonic development occurs when fertilization triggers the maternal-to-zygotic transition (MZT).

The foundation toward understanding how such reprogramming occurs was facilitated with the discoveries that both nuclear and cytoplasmic activities control embryonic development. To investigate whether nuclear or cytoplasmic information drives development, Theodor Boveri fertilized enucleate urchin oocytes with sperm of a different species and showed that resulting larvae possesses features of both parents (Laubichler & Davidson, 2008). However, it was unknown whether DNA information in gametes is specialized and was lost as cells differentiated during development. To address this question, Sir John Gurdon transplanted the nucleus of a differentiated intestinal epithelial cell into an enucleate oocyte and showed that a mature frog develops (Gurdon, 1962). This experiment demonstrated that DNA in all cells within an individual remains the same as differentiation proceeds and additionally demonstrated that oocyte cytoplasm is endowed with factors capable of reprogramming a somatic nucleus back to its naïve state. Together, the concerted efforts between nuclear information and cytoplasmic material establish the totipotent cellular state required to make new life.

In retrospect, Boveri's observations also explain the two hallmarks of MZT during early embryonic development. Initially, the transcriptionally silent zygote utilizes mRNAs and proteins inherited in the egg cytoplasm to carry out cellular functions. Subsequently, the zygotic genome begins transcription, maternal mRNAs are actively cleared, and developmental control is transferred to the nucleus. Together, cytoplasmic and nuclear activities enable oocyte reprogramming during the maternal-to-zygotic transition (Lee, Bonneau, & Giraldez, 2014).

Oocyte reprogramming during embryogenesis is analogous to somatic cell reprogramming to pluripotency *in vitro*; both involve transitioning from differentiated to pluripotent identity (Giraldez, 2010), summarized in Fig. 1. During the MZT, the previously silenced zygotic genome starts transcription to activate the new genetic program (Lee, Bonneau, et al., 2014),

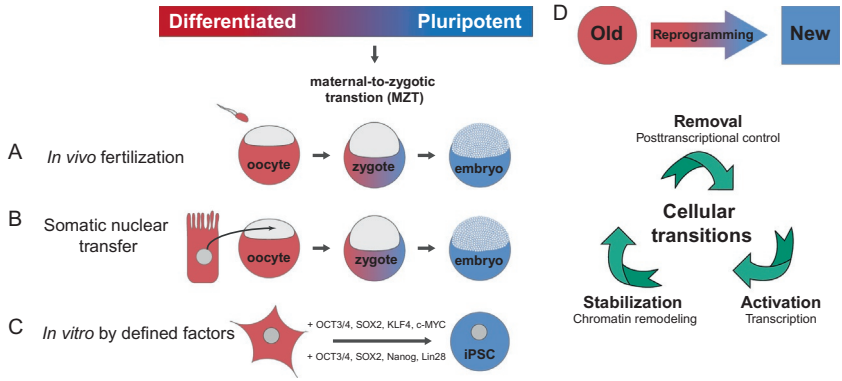

Figure 1 Features of cellular reprogramming. (A–C) Types of cellular reprogramming to pluripotency. (A) *In vivo* fusion of oocyte and spermatazoon initiates the MZT during which the zygote is reprogrammed to a transiently totipotent embryo. (B) Nucleus from a differentiated cell is reprogrammed to a totipotent embryo when transplanted into enucleate fertilized oocyte (Gurdon, 1962). (C) *In vitro*, forced expression of four transcription factors OCT3/4, SOX2, KLF4, and c-MYC (Takahashi & Yamanaka, 2006) or OCT3/4, SOX2, Nanog, and Lin28 (Yu et al., 2007) in differentiated cells induces a fraction of cells to reprogram to a pluripotent-like state called induced pluripotent stem cell (iPSC). (D) Model of cellular reprogramming: reprogramming between two cellular states involves (1) activation of the new program through gene transcription, (2) stabilization of that program through chromatin remodeling, and (3) removal of the previous state by posttranscriptional mechanisms.

the chromatin is remodeled to stabilize the pluripotent state (Zhou & Dean, 2015), and maternal instructions in the form of mRNAs and proteins are actively cleared to remove the previous cellular identify (Giraldez, 2010; Tadros & Lipshitz, 2009; Walser & Lipshitz, 2011). Likewise, reprogramming to pluripotency *in vitro* requires activation of the pluripotency program (Takahashi & Yamanaka, 2006; Yu et al., 2007), chromatin remodeling (Apostolou & Hochedlinger, 2013), and less well-understood posttranscriptional mechanisms to erase the differentiated gene expression program.

Recent reviews on maternal mRNA clearance during MZT within this book and elsewhere (Barckmann & Simonelig, 2013; Colegrove-Otero, Minshall, & Standart, 2005; Langley, Smith, Stemple, & Harvey, 2014; Walser & Lipshitz, 2011) highlight known factors involved in maternal mRNA clearance. Here, we focus on recent advances in the field, common themes in the mechanisms of maternal mRNA clearance across animals, and how this process closely parallels other cellular reprogramming events. We end by describing developmental contexts where maternal clearance is

compromised. We propose that maternal mRNA clearance is a requirement to enable the acquisition of the pluripotent state and may even be a common feature of many cellular transitions.

2. MECHANISMS OF MATERNAL mRNA CLEARANCE DURING THE MZT

2.1 Scope of Maternal mRNA Destabilization During the MZT

The maternal-to-zygotic transition occurs in all animals (Tadros & Lipshitz, 2009) and in plants (Baroux, Autran, Gillmor, Grimanelli, & Grossniklaus, 2008; Xin, Zhao, & Sun, 2012), indicating that this transition may be a universal feature of multicellular life. Beginning with a mostly transcriptionally silent embryo, the MZT involves the activation of the zygotic genome and the clearance of maternal mRNAs. Mechanisms regulating the activation of the zygotic genome were recently reviewed (Lee, Bonneau, et al., 2014) and highlight the interplay between zygotic transcription and maternal mRNA clearance. Maternal mRNA clearance during the MZT is a dramatic remodeling of the transcriptional landscape with 30–40% maternal mRNAs eliminated in different species (Baugh, Hill, Slonim, Brown, & Hunter, 2003; De Renzis, Elemento, Tavazoie, & Wieschaus, 2007; Hamatani, Carter, Sharov, & Ko, 2004) and up to 60% of maternal mRNA levels are considerably reduced (Thomsen, Anders, Janga, Huber, & Alonso, 2010). In order to understand how maternal mRNAs are regulated during MZT, it is useful to first review which mRNA features impact its stability.

2.2 Steps in Eukaryotic mRNA Regulation

Following transcription, gene expression in the cytoplasm depends on protein synthesis rate and on the stability of the cognate mRNA. Protein synthesis rate and mRNA stability are influenced by a combination of three main mRNA features: the mRNA sequence, the 7-methylguanylate (m^7G) cap at the 5′ end, and the length of the 3′ poly(A) tail.

Sequences and chemical modifications within the mRNA encode recognition sites for factors that positively and negatively regulate mRNA stability, translation, and localization to permit cell-specific gene expression, recently reviewed in Fu, Dominissini, Rechavi, and He (2014), Gebauer, Preiss, and Hentze (2012), and Medioni, Mowry, and Besse (2012). Mechanistically, binding factors either lead to endonucleolytic cleavage, followed by XRN1 and Exosome complex-mediated hydrolysis from both

unprotected mRNA ends, or recruit PARN or CCR4–NOT1 complex to stimulate deadenylation (Beelman & Parker, 1995; Decker & Parker, 1994; Schoenberg & Maquat, 2012), which leads to decapping for some mRNAs (Decker & Parker, 1994) and serves as the rate-limiting step for many mRNA degradation pathways (Wahle & Winkler, 2013).

The poly(A) tail, situated at the 3′ extremity of mRNAs, is bound by poly(A)-binding proteins (PABPs) to stabilize the 3′ end (Bernstein, Peltz, & Ross, 1989) and interacts with translation initiation factor eIF4G bound to the 5′ cap to stimulate translation (Weill, Belloc, Bava, & Méndez, 2012). Proteins bound to 3′UTR elements regulate poly(A) tail length (Charlesworth, Meijer, & De Moor, 2013) and they are dynamically regulated during embryonic development (Richter, 1996, 1999; Richter & Lasko, 2011).

Finally, capped mRNAs are protected from 5′-to-3′ XRN1-mediated exonucleolytic decay (Murthy, Park, & Manley, 1991). Cap hydrolysis via DCP2 leads to mRNA destabilization and can be regulated globally or for a subset of mRNAs (Cowling, 2010; Franks & Lykke-Andersen, 2009; Liu & Kiledjian, 2006). In some cases, 5′-to-3′ mRNA degradation occurs cotranslationally (Hu, Sweet, Chamnongpol, Baker, & Coller, 2009; Pelechano, Wei, & Steinmetz, 2015). Additionally, efficient translation requires m^7G cap interaction with translation initiation factor eIF4E, which is dynamically regulated in development and disease (Richter & Sonenberg, 2005).

Together these mRNA features are mechanistically linked to coordinate posttranscriptional gene regulation for individual mRNAs as well as for coregulated groups of transcripts. This network of mRNA regulation dominates gene control during oogenesis and early embryogenesis, occurring in the absence of transcription. Deadenylation of target mRNAs is a common convergence point of many maternal mRNA clearance mechanisms and will be discussed first.

2.3 Maternal Clearance Mechanisms Involving Poly(A) Tail: Smaug

In *Drosophila*, Smaug is a multifunctional, highly conserved protein that is translationally activated by the Pan gu (PNG) Ser/Thr kinase following egg activation (Tadros et al., 2007). Smaug binds RNA via a sterile alpha motif (SAM) domain, with specificity that is shared with yeast Vts1 and is likely conserved from yeast to humans (Aviv et al., 2003). Smaug recognizes its targets via binding stem loop structures called Smaug recognition

elements (SREs) (Dahanukar, Walker, & Wharton, 1999; Smibert, Lie, Shillinglaw, Henzel, & Macdonald, 1999) and recruits the CCR4/POP2/NOT-deadenylase complex to initiate poly(A) tail shortening and consequent mRNA elimination (Semotok et al., 2005). Interestingly, SREs in the *nanos* 3′UTR result in translational repression (Nelson, Leidal, & Smibert, 2004), while for *Hsp83*, which has several SREs in the coding region, the result is deadenylation via CCR4/POP2/NOT, followed by mRNA elimination (Semotok et al., 2005, 2008), suggesting that SRE location on the transcript may influence target mRNA fate. However, transcriptome-wide mapping of Smaug binding in *Drosophila* embryos revealed that the overwhelming majority of SREs are in coding regions, regardless of whether the mRNA is translationally repressed but not destabilized by Smaug (like *nanos*) or destabilized but not repressed (like *Hsp83*) (Chen et al., 2014). Interestingly, *nanos* SREs associate with an ATP-dependent complex (Jeske, Moritz, Anders, & Wahle, 2011) that requires Smaug-mediated microRNA-independent recruitment of Ago1 for repression (Pinder & Smibert, 2013), indicating that additional features distinguish these two modes of posttranscriptional regulation.

2.4 Maternal Clearance Mechanisms Involving Poly(A) Tail: Pumilio

The RNA-binding protein Pumilio has been implicated in deadenylation and clearance of maternal mRNAs. In developmental contexts, Puf-domain family members remain most appreciated for their diverse roles in translational repression (Quenault, Lithgow, & Traven, 2011; Vardy & Orr-Weaver, 2007). In *Drosophila*, the Pumilio-binding element (PBE) sequence is enriched in destabilized maternal mRNAs (De Renzis et al., 2007; Thomsen et al., 2010), implicating Pumilio as a regulator of maternal mRNA clearance in this species. In fact, when Pumilio's RNA-binding domain is expressed in the female germline, it is found to interact with over 900 mRNAs, yet only 130 of these increased and 243 decreased in abundance in a *pumilio* mutants (Gerber, Luschnig, Krasnow, Brown, & Herschlag, 2006), a globally modest effect on mRNA stability. However, a recent analysis of endogenous Pumilio identified over 600 bound mRNAs and found that these are highly enriched for transcripts that are translationally repressed and degraded during the MZT (Laver et al., 2015). Mechanistically, Pumilio binds POP2 of the CCR4–POP2–NOT–deadenylase complex, accelerates reporter mRNA deadenylation, and antagonizes poly(A)-binding protein (PABP) activity (Weidmann, Raynard, Blewett,

Van Etten, & Goldstrohm, 2014). Interestingly, full length Pumilio represses and destabilizes reporter mRNA, while the N-terminal portion predominantly causes repression (Weidmann & Goldstrohm, 2012), suggesting that its role in mRNA destabilization relates to the C-terminal domain's binding partners or activity. Despite the extensive work on Pumilio function in *Drosphila* as well as its stem cell function (discussed below), the extent of Pumilio-mediated maternal mRNA clearance across species remains to be determined.

2.5 Maternal Clearance Mechanisms Involving Poly(A) Tail: EDEN-BP

In *Xenopus*, fertilization triggers sequence-specific and regulated deadenylation of target maternal mRNAs. Embryonic deadenylation element (EDEN) is a U(A/G) dinucleotide repeat and serves as the recognition site for EDEN-binding protein (EDEN-BP) (Paillard et al., 1998) to deadenylate select target mRNAs. EDEN deadenylation capacity is enhanced by an $(AUU)_3$ sequence located in close proximity to the poly(A) tail (Audic, Omilli, & Osborne, 1998) and by the AUUUA sequence (Ueno & Sagata, 2002), suggesting the existence of a combinatorial sequence code to allow for target-specific deadenylation dynamics. Additionally, while EDEN-BP levels remain constant from fertilization to the tadpole stage in *Xenopus* (Gautier-Courteille et al., 2004), it is dephosphorylated following fertilization, which corresponds to target deadenylation (Detivaud, Pascreau, Karaiskou, Osborne, & Kubiak, 2003), indicating that fertilization-dependent posttranslational control is required for EDEN-BP activity. EDEN-BP oligomerization via a 27-amino acid region is also required for target mRNA binding and deadenylation (Cosson et al., 2006), implicating posttranslational control as an additional layer of regulating maternal mRNA deadenylation.

EDEN-BP is homologous to human CUG-BP (Timchenko et al., 1996), which recruits PARN to deadenylate and destabilize specific target mRNAs (Moraes, Wilusz, & Wilusz, 2006). Additionally, the action of embryonic poly(A)-binding protein (ePAB), the predominant poly(A)-binding protein in *Xenopus* oocytes and early embryos, is critical for deadenylation rate in *Xenopus* embryos. ePAB immunodepletion results in increased deadenylation, while overexpression inhibits it (Voeltz, Ongkasuwan, Standart, & Steitz, 2001), suggesting that ePAB stabilizes poly(A) tails and that deadenylation requires antagonizing ePAB binding to its targets or its activity.

The role of EDEN-BP for deadenylation varies across species. EDEN-BP binds 158 maternal mRNAs in *Xenopus* egg extracts that are enriched for genes involved in cell cycle and oocyte maturation (Graindorge et al., 2008), suggesting that EDEN-BP functions to repress the oogenesis program. Interestingly, in aged *Xenopus* oocytes mRNAs that are normally deadenylated after fertilization, undergo precocious deadenylation during oocyte maturation (Kosubek, Klein-Hitpass, Rademacher, Horsthemke, & Ryffel, 2010). This suggests that decreased fertility with age involves dysregulation of molecular mechanisms mediating deadenylation during early embryogenesis. EDEN-dependent translational repression activity is conserved between *Xenopus* and *Drosophila*. However, EDEN reporters are not deadenylated in *Drosophila* (Ezzeddine et al., 2002), indicating species-specific usage of the same sequences for different modes of regulation. In fact, *Drosophila* Bruno, which resembles human CUG-BP (Webster, Liang, Berg, Lasko, & Macdonald, 1997), recruits Cup to translationally repress, but not deadenylate *oskar* mRNA (Nakamura, Sato, & Hanyu-Nakamura, 2004; Wilhelm, Hilton, Amos, & Henzel, 2003), suggesting that the role of EDEN-BP in deadenylation has diverged in vertebrates.

2.6 Maternal Clearance Mechanisms Involving Poly(A) Tail: microRNAs

The interaction of microRNAs with target mRNAs causes translational repression, deadenylation, and mRNA destabilization (Bartel, 2009). Investigating microRNA action in a developmental context revealed detailed features of this mRNA destabilization mechanism. In zebrafish, *miR-430* is transcribed zygotically (Giraldez et al., 2005; Lee et al., 2013) and induces deadenylation and mRNA destabilization of several hundred transcripts (Giraldez et al., 2006). Identification of the temporal sequence of events for microRNA-mediated regulation was made possible by ribosome profiling (Ingolia, Ghaemmaghami, Newman, & Weissman, 2009) to measure the precise location and density of ribosome occupancy genome-wide during the MZT (Bazzini, Lee, & Giraldez, 2012). These experiments showed that *miR-430* reduces ribosome occupancy on target mRNAs before inducing complete deadenylation and mRNA destabilization. Indeed, *miR-430* reduces ribosome density uniformly, consistent with inhibition of translation initiation rather than ribosome drop-off (Bazzini et al., 2012). While *miR-430* ultimately triggers deadenylation of its targets, the nonsteady state context of the developing embryo allows the uncoupling of translational repression from the decay of these mRNAs. However, it appears that many

of these mRNAs are being deadenylated soon after the microRNA is expressed (Subtelny, Eichhorn, Chen, Sive, & Bartel, 2014). microRNAs have also been found to play a role during the MZT in *Xenopus*, *Drosophila* and, possibly, mammals (discussed below).

2.7 Methods of Measuring Poly(A) Tail Length

The importance of polyadenylation for mRNA regulation mechanisms during early embryogenesis motivated the development of methods to accurately measure poly(A) tail length for large numbers of mRNAs. Poly(A) tail length measurement for individual mRNAs was initially achieved by cDNA synthesis with oligo(dT) or cleavage of upstream sequence to release the poly(A) tail (Murray & Schoenberg, 2008). The high-resolution poly(A) tail (Hire-PAT) assay enables single nucleotide resolution and quantification of polyA tail length for individual mRNAs and, using this technique (Bazzini et al., 2012), it was shown that microRNA-mediated translational repression takes place before complete mRNA deadenylation. Estimation of poly(A) tail length in a population of mRNAs is possible through affinity chromatography of RNA on poly(U) beads, its differential elution at increasing temperatures or salt concentrations, followed by microarray or sequencing (Beilharz & Preiss, 2007; Du & Richter, 2005; Meijer et al., 2007).

More recently, high-throughput methods have enabled poly(A) tail measurements transcriptome wide. First, poly(A)-tail length profiling by sequencing (PAL-seq) measures fluorescence signal after reverse transcription of the poly(A) tail as a proxy for its length (Subtelny et al., 2014). This method identified a positive correlation between poly(A) tail length and translation efficiency during early embryonic development in several species, which diminished at gastrulation, suggesting a developmental switch in translational control during the MZT. Second, TAIL-seq directly sequences the 3′ mRNA ends to determine the position of the poly(A) tail start, allowing highly accurate and high-throughput measurement of mRNA poly(A) tail lengths (Chang, Lim, Ha, & Kim, 2014). This method identified widespread uridylation and guanylation of poly(A) tails in cells, but the function of these modifications during development awaits further analysis. Polyadenylation is a highly dynamic process during embryogenesis that is directly linked to translational regulation and mRNA stability. These new methods make it possible to investigate the mechanisms that dictate poly(A) tail length during embryonic development.

2.8 Role of Decapping in Maternal mRNA Clearance

In somatic cells, mRNA decapping and decay are tightly coupled to deadenylation (Parker & Song, 2004); however, this is not always the case during early development. In *Xenopus*, maternal mRNAs with AU-rich elements (ARE) in their 3′UTRs are deadenylated following egg activation, but are eliminated only after the mid-blastula transition (MBT) (Audic, Omilli, & Osborne, 1997; Voeltz & Steitz, 1998). This indicates that deadenylation and decay are uncoupled during early embryonic development. What developmental cue prevents mRNAs primed for destabilization to remain stable until the MBT? Interestingly decapping activity in *Xenopus* is first detected at the MBT (Gillian-Daniel, Gray, Aström, Barkoff, & Wickens, 1998; Zhang, Williams, Wormington, Stevens, & Peltz, 1999), suggesting that for maternal mRNAs that are deadenylated maternally via AU-rich elements, zygotic activation of decapping activity could be the trigger for mRNA destabilization.

Decapping enzymes are regulated at the level of their catalytic activity and localization. The catalytic activity of Dcp2 is stimulated by several enhancers of decapping (Arribas-Layton, Wu, Lykke-Andersen, & Song, 2013; Jonas & Izaurralde, 2013; Ling, Qamra, & Song, 2011), suggesting the potential for developmental regulation. Metazoan-specific EDC4 functions as a scaffold to facilitate Dcp2 interaction with its cofactor, Dcp1, to activate decapping (Chang, Bercovich, Loh, Jonas, & Izaurralde, 2014). Zygotic synthesis of decapping enhancers could be a feasible developmental strategy to trigger destabilization of maternal mRNAs with AREs at the MBT, but maintain their stability during oogenesis. Additionally, in *C. elegans*, Dcp2 localizes to cytoplasmic foci, potential sites of mRNA decay, while DcpS is distributed throughout the cytoplasm (Lall, Piano, & Davis, 2005). Thus, localization of mRNAs and of decapping proteins may be regulated to initiate mRNA degradation.

It remains to be discovered what mechanisms activate decapping and what their contribution is during maternal mRNA clearance. However, a large fraction of destabilized maternal mRNAs first require a zygotic deadenylation trigger such as *miR-430* (Bazzini et al., 2012; Giraldez et al., 2006), indicating that decapping is not the limiting factor in global degradation of maternal mRNAs.

2.9 Maternal and Zygotic Modes of Maternal mRNA Clearance

A major segregating factor in the mechanisms of maternal mRNA clearance is their dependence on zygotic transcription (Fig. 2). This observation was

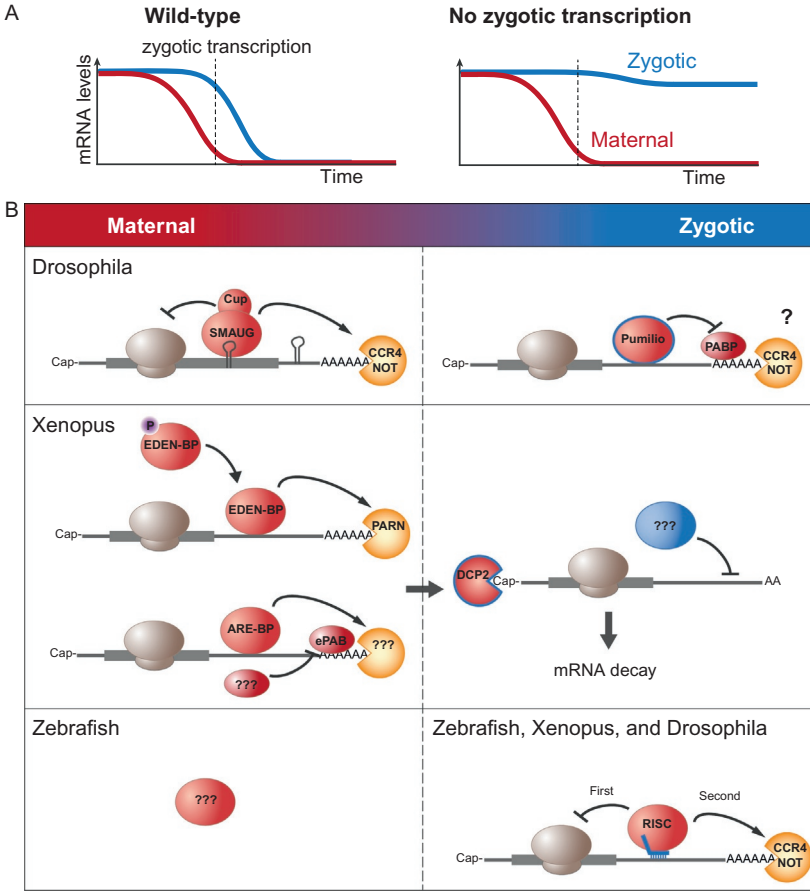

Figure 2 Maternal and zygotic mechanisms of maternal mRNA clearance. (A) Maternal mRNAs under the regulation of the "maternal mode" mechanisms (red) will be destabilized independently of zygotic transcription, while mRNAs under the "zygotic mode" mechanisms (blue) will be stable in the absence of zygotic transcription. (B) Examples of characterized pathways of maternal and zygotic mode mechanisms across species. Maternal mode factors (red), zygotic mode factors (blue), and maternal factors activated after zygotic transcription (red with blue outline).

first possible in *Drosophila*, where egg activation and fertilization are uncoupled processes (Bashirullah et al., 1999; Tadros et al., 2003). For example, *nanos* mRNAs is degraded upon egg activation, indicating that this decay mechanism uses only maternal instructions (maternal mode). Conversely, *bicoid* mRNA is degraded only after zygotic transcription (zygotic

mode), while *Hsp83* mRNA utilizes both clearance mechanisms (Bashirullah et al., 1999). In other species, maternal and zygotic modes of regulation can be distinguished either temporally or by blocking zygotic transcription (Ferg et al., 2007; Hamatani et al., 2004). The maternal mode of mRNA decay occurs before or independently of zygotic transcription. While the zygotic mode occurs after zygotic transcription and is blocked when zygotic transcription is inhibited, summarized in Fig. 2A. This indicates that some pathways of maternal mRNA clearance are inherited in the oocyte cytoplasm, while others are synthesized *de novo*, further highlighting the contributions of both the maternal cytoplasm and zygotic nucleus to the MZT.

Zygotic transcription can be detected using multiple methods. Application of RNA Polymerase II inhibitors such as alpha-amanitin (Lindell, Weinberg, Morris, Roeder, & Rutter, 1970; reviewed in Bensaude, 2011), cause embryonic arrest soon after the onset of zygotic transcription. Using this method, it was determined that zygotic transcription is required beyond the two-cell stage in mouse (Flach, Hjohnson, Braude, Taylor, & Bolton, 1982; Golbus, Calarco, & Epstein, 1973; Warner & Versteegh, 1974), 4–8 cell stage in human (Braude, Bolton, & Moore, 1988), 5–8 cell stage in cat (Hoffert, Anderson, Wildt, & Roth, 1997), 8–16 cell stage in rabbit (Manes, 1973), 100-cell stage in *C. elegans* (Edgar, Wolf, & Wood, 1994), following nuclear cycle 13 in *Drosophila* (Edgar, Kiehle, & Schubiger, 1986) and at the mid-blastula transition (MBT) in Zebrafish and *Xenopus* (Kane et al., 1996; Newport & Kirschner, 1982). More sensitive methods to identify the onset and the identity of early zygotic transcripts includes measuring (1) RNA accumulation containing paternal single-nucleotide polymorphisms (SNPs) (Harvey et al., 2013; Sawicki, Magnuson, & Epstein, 1981), (2) intronic sequences in unprocessed zygotic mRNAs (Lee et al., 2013), and (3) new transcripts after labeling with 4-thiouridine (4SU) (Heyn et al., 2014). Defining the timing of zygotic transcription across species facilitated distinguishing mRNAs under maternal or zygotic modes of clearance across species.

2.10 Proportion of Maternal and Zygotic Modes Across Species

While most animals experience both maternal and zygotic modes of maternal mRNA clearance, the proportion of each mode of clearance utilized varies across animals (Tadros & Lipshitz, 2009; Walser & Lipshitz, 2011). High-throughput mRNA profiling at different developmental stages

or coupled with chemical inhibitors of *de novo* zygotic transcription enabled the identification of mRNAs under these distinct modes of clearance across species. In *Drosophila*, over 1000 maternal mRNAs are cleared following egg activation, two-thirds of which depend on Smaug for elimination (Tadros et al., 2007). An additional 563 stabilized mRNAs were discovered in the background of specific chromosomal arm deletions (De Renzis et al., 2007), suggesting that these mRNAs are also subject to the zygotic mode of clearance and require genes encoded within these deletions for degradation.

In mouse, the majority of mRNAs are destabilized prior to zygotic transcription. Oocyte maturation between the germinal vesicle (GV) breakdown and Meiosis II (MII) stages triggers destabilization of almost 3000 mRNAs (Su et al., 2007). These transcripts are enriched for genes involved in ATP production such as oxidative phosphorylation and ubiquinone biosynthesis (Su et al., 2007; Zeng, Baldwin, & Schultz, 2004), likely reflecting the changing metabolic needs of the maturing oocytes. Degradation of maternal mRNA during oocyte maturation is carefully regulated and evidence for this is that over 9200 mRNAs remain remarkably stable during this time (Su et al., 2007), potentially implicating coexistence of stabilization mechanisms to protect mRNAs utilized after fertilization. Interestingly mammalian oocytes require high levels of cAMP to remain arrested in the GV stage (Horner et al., 2003). Selective degradation of the ATP production machinery in order to generate cAMP could be a mechanism to enable oocytes to progress to MII stage (Su et al., 2007). An additional almost 2300 maternal mRNAs are eliminated immediately following fertilization, consistent with a maternal mode of clearance, while almost 500 mRNAs are cleared at the two-cell stage, consistent with a zygotic mode of regulation (Hamatani et al., 2004). It would be interesting to investigate whether alpha-amanitin treatment selectively stabilizes these mRNAs to determine whether this cluster requires zygotic transcription for clearance.

Comprehensive analysis of the *C. elegans* transcriptome during the oocyte-to-embryo transition (OET) showed that roughly 25% of the maternal mRNA pool (~1900 mRNAs) is eliminated between the mature oocyte and one-cell stage (Stoeckius et al., 2014), indicating dramatic mRNA turnover during the MZT, and prior to the MBT, similar to that in *Drosophila* (Tadros et al., 2007). Destabilized mRNAs are enriched for a poly(C) motif, which is also sufficient to destabilize reporter mRNAs, and binds poly(C)-binding protein (PCBP). Additionally, endo-siRNAs but not microRNAs have been implicated in this regulation (Stoeckius et al., 2014). An additional ~30% of the remaining maternal mRNAs is destabilized around the four-cell stage (Baugh et al., 2003). MicroRNAs are implicated in

maternal and zygotic mode deadenylation, but not the decay of maternal mRNAs in this species (Alvarez-Saavedra & Horvitz, 2010; Wu et al., 2010), which parallels EDEN-BP in *Xenopus*.

In Zebrafish, the zygotic modes of maternal mRNA clearance likely dominate. The zygotic expression of *miR-430* triggers repression, deadenylation, and clearance of several hundred maternal mRNAs (Bazzini et al., 2012; Giraldez et al., 2006) that have *miR-430* target sites; up to 40% of maternal mRNAs are potentially regulated by this mechanism (Giraldez et al., 2006). Interestingly, several studies have reported early sizable destabilization of maternal mRNAs in Zebrafish prior to zygotic transcription (Aanes et al., 2011; Mathavan et al., 2005; Rabani et al., 2014), suggesting the action of early-acting maternal-mode decay mechanisms. However, these studies rely on poly(A) selected RNA-seq (Aanes et al., 2011; Mathavan et al., 2005; Rabani et al., 2014) to draw these conclusions. Polyadenylated mRNAs are more efficiently captured during poly(A) selection protocols (Harvey et al., 2013). Because it is likely that the poly(A) tail length of different maternal mRNAs varies (see above), conclusions about early decay or transcription can be misleading. Leveraging ribosome depletion to sequence total RNA (Lee et al., 2013) and taking advantage of exogenous spike-ins (Lovén et al., 2012) will offer accurate measurement of the timing and the dynamics of mRNA clearance.

2.11 Shared Features of Maternal mRNA Clearance Mechanisms Across Animals

We have seen that maternal mRNA clearance is universal yet diverse across animals. This diversity likely stems from different developmental requirements such as timing and unique physiological environments. While the regulated targets may vary across species, the underlying mechanisms are likely conserved. For example, in the parasitic worm *Ascaris suum* maternal mRNA clearance is temporally coordinated despite it lacking a transcriptionally quiescent maternal stage. Unlike other known metazoans, favorable environmental cues rather than fertilization triggers pronuclear fusion and embryonic progression in this species (Wang, Garrey, & Davis, 2014). However, maternal mRNA clearance still occurs in distinct waves in *Ascaris suum*, with over 1100 genes degraded immediately after fertilization and an additional 1662 mRNAs degraded by the 10-cell stage (Wang, Garrey, et al., 2014). The protracted development in this species likely accounts for its unique MZT features. However, the temporal coordination of maternal mRNA clearance in this species suggests that the same mechanisms are likely in place as in other animals.

Additionally, zygotic expression of microRNAs to clear maternal mRNAs is a common strategy used by most animals and highlights the conserved nature of maternal clearance mechanisms. The first microRNA identified to function in the clearance of maternal mRNAs was zebrafish *miR-430* (Giraldez et al., 2006). Interestingly, *Xenopus* exhibits zygotic expression of *miR-427*, which shares its seed sequence with zebrafish *miR-430*, and also destabilizes maternal mRNAs (Lund, Liu, Hartley, Sheets, & Dahlberg, 2009; Rosa, Spagnoli, & Brivanlou, 2009). In *Drosophila*, a different set of miRNAs (the *miR-309* family) plays an analogous role in maternal mRNA clearance (Bushati, Stark, Brennecke, & Cohen, 2008), suggesting convergent evolution of the same mechanism.

In mammals, the scope and the diversity of microRNA function for maternal mRNA clearance is still poorly understood. In mouse, *miR-290* shares its seed sequence with *miR-430/427/302* family microRNAs and is expressed at high levels in early embryos and in embryonic stem cells (Tang et al., 2007; Zeng & Schultz, 2005), suggesting a role in maternal mRNA clearance similar to other vertebrates. Genetic inactivation of the *miR-290/295* cluster in mouse results in partially penetrant embryonic lethality, primordial germ cell migration defects, and female sterility for surviving homozygous mutants (Medeiros et al., 2011), pointing to a predominant role for *miR-290/295* cluster in germ line development. Interestingly, while genetic inactivation of *miR-302*, which shares its seed with *miR-290*, results in failure in neural tube closure, *miR-302/miR-209* double mutant is early embryonic lethal (Parchem et al., 2015). This suggests that the *miR-290/302* family microRNAs have a redundant role for early embryonic development in mouse and specific roles during later embryogenesis. In bovine embryos, early zygotic expression of *miR-212* negatively regulates *FIGLA* mRNA levels (Tripurani et al., 2013), suggesting that microRNAs have a role in maternal mRNA clearance in other mammals. The extent of *miR-212* and other mammalian microRNA involvement in maternal mRNA clearance awaits further investigation.

2.12 Regulation of microRNAs During the MZT

We have seen that embryogenesis depends heavily on coordinated control of maternally provided mRNAs, but the importance of microRNA regulation during the MZT has only recently come to light. For example, *miR-430* in zebrafish undergoes rapid synthesis soon after zygotic genome activation initiates, but precursor levels stop accumulating after gastrulation (Giraldez et al., 2005), indicating that *miR-430* locus transcription is suppressed at this

stage. Nanog binds the *miR-430* promoter and is required for *miR-430* expression (Lee et al., 2013), but it remains to be discovered how Nanog function is blocked after the MZT.

Interestingly, mature *miR-430* persists up to 2 days of development (Giraldez et al., 2005), and its activity is regulated in a tissue specific manner. *miR-430* balances the nodal signaling pathway through regulation of both the pathway agonist, *squint*, and the antagonist, *lefty* (Choi, Giraldez, & Schier, 2007). *miR-430* also regulates Sdf1 chemokine signaling to ensure accurate primordial germ cell (PGC) migration (Staton, Knaut, & Giraldez, 2011). In the germ line, the RNA-binding proteins, Dead end 1 (Dnd1) (Kedde et al., 2007) and Deleted in azoospermia like (Dazl) (Takeda, Mishima, Fujiwara, Sakamoto, & Inoue, 2009), inhibit *miR-430* action to set up differential gene expression between the soma and germ line (Mishima et al., 2006). Additionally, the RNA-binding protein, TDP-43, disrupts microRNA incorporation into RISC to limit its activity (King et al., 2014). However, it remains to be elucidated exactly how microRNA activity is regulated during development.

At the posttranscriptional level, maternal microRNAs undergo regulated clearance. Maternal microRNAs are heavily adenylated at the 3′ end from invertebrates to mammals (Lee, Choi, et al., 2014), suggesting a deeply conserved function of this mechanism for embryonic development. In *Drosophila* oocytes and embryos, microRNAs are polyadenylated by a noncanonical poly(A) polymerase, Wispy, which directs their destabilization after fertilization (Lee, Choi, et al., 2014). Maternal *wispy* mutants arrest prior to pronuclear fusion in *Drosophila* (Brent, MacQueen, & Hazelrigg, 2000), and Wispy's role in active clearance of microRNAs via deadenylation may be required for appropriate gene expression during embryogenesis (Lee, Choi, et al., 2014). Mechanistically, Wispy interacts with Ago1, which may allow selective adenylation of microRNAs (Lee, Choi, et al., 2014). Interestingly, zygotic microRNAs such as *miR-430* in zebrafish seem to have a long half-life (Giraldez et al., 2005) and serve important functions during embryonic development (as discussed earlier), indicating that zygotic microRNAs are either protected from Wispy-mediated polyadenylation or that this pathway is no longer active after zygotic transcription. Together these studies illustrate how microRNA activity and stability is tightly regulated during development.

2.13 Endonucleolytic Cleavage During the MZT

Endonucleolytic activity exists in *Xenopus* and *Drosophila* embryos; however, its role during embryogenesis has not been determined. It is clear that the

Xlhbox2/HoxB7 mRNA undergoes endonucleolytic cleavage in *Xenopus* oocytes because cleavage intermediates have been detected using Northern and RNAseH assays (Brown & Harland, 1990). The poly(A) tail length of the mRNA remains constant in these degradation intermediates (Brown & Harland, 1990) and decay persists in the presence of cycloheximide (Brown, Zipkin, & Harland, 1993), indicating that the mechanism neither involves deadenylation nor requires translation, respectively. A 90-nucleotide region in the *Xlhbox2/HoxB7* 3′UTR is sufficient for cleavage (Brown & Harland, 1990), indicating that this mechanism is sequence specific. This endonucleolytic activity is also present in *Drosophila* embryo lysates (Brown et al., 1993), suggesting conservation between distantly related species. Interestingly, injecting RNA corresponding to the endonuclease recognition site and nearby flanking region in excess accelerates cleavage in endogenous and reporter mRNA (Brown et al., 1993), indicative of endonuclease inhibitor presence. It is possible that active silencing of endonuclease activity plays a role during embryogenesis. Widespread action of endonucleolytic cleavage in transcriptionally silent oocytes and early embryos could result in precocious mRNA decay, eliminate the potential to reuse mRNAs, and be harmful for embryogenesis. Specific endonuclease inhibitor(s) that are active during embryonic development have yet to be identified.

Endonucleolytic cleavage mechanisms have also been proposed to regulate maternal mRNAs. In mouse, genetic inactivation of *Dicer*, an enzyme required for biogenesis of microRNAs and processing of dsRNAs, results in the failure to complete Meiosis I, stabilization of 1300 mRNAs, and upregulation of retrotransposons (Murchison et al., 2007). However, DGCR8, required for processing primary (pri-) microRNAs, is dispensable for mouse oocyte (Ma et al., 2010; Suh et al., 2010) and preimplantation development (Suh et al., 2010). The phenotype of Dicer mutants is independent of microRNAs and likely results from misregulation of transposons (Murchison et al., 2007). Additionally, in *C. elegans* embryos, endo-siRNAs regulate ~10% of destabilized maternal mRNAs while microRNAs do not appear to contribute to maternal mRNA destabilization (Stoeckius et al., 2014).

2.14 Role of Coding Sequence in mRNA Decay

Recent evidence suggests that the coding sequence can have an impact on mRNA stability. Transcriptome-wide analysis in yeast found that mRNAs that utilize optimal codons have increased half-life and, strikingly, that

optimizing codon usage in an unstable mRNA, *LSM8*, increases its half-life nearly eightfold, from 2.5 to 18.7 min (Presnyak et al., 2015). This finding is intriguing because it offers an explanation of how groups of mRNAs could be coregulated independently of harboring common *cis*-regulatory elements. Additionally, genes in common pathways share codon usage characteristics. For example, stable mRNAs encoding glycolytic enzymes have 86% optimized codons, a disproportionately large fraction, while unstable mRNAs, such as those encoding factors involved in the pheromone response have 43% optimal codons (Presnyak et al., 2015), prompting the authors to speculate that species could evolve specific codon content in related gene groups to enable their coregulation. In fact, common *cis*-regulatory elements are not enriched in unstable mRNAs in yeast (Geisberg, Moqtaderi, Fan, Ozsolak, & Struhl, 2014); usage of rare codons could, therefore, explain the instability of mRNAs with seemingly unrelated sequence signatures.

However, that study does not exclude the possibility that primary sequence determinants drive mRNA destabilization in this context. Coding sequence is known to harbor destabilization elements. For example, highly unstable *c-fos* mRNA harbors several destabilization elements within its coding region since specific deletions prolong mRNA half-life (Schiavi et al., 1994). These coding-region destabilization elements require translation and involve deadenylation since inhibiting translation with Anisomycin increases mRNA half-life and increases the poly(A) tail length (Schiavi et al., 1994). Interestingly, the sequence of the *c-fos* coding region, not its amino acid composition, encodes the destabilization element because an out-of-frame *c-fos* variant is equally unstable (Wellington, Greenberg, & Belasco, 1993). While the authors of the yeast codon-usage study showed that the correlation between mRNA stability and optimal codon usage disappears when the same mRNAs are translated out-of-frame *in silico* (Presnyak et al., 2015), half-life measurements for out-of-frame translated mRNAs were not reported. It is, thus, possible that the alteration to the primary sequence necessary to generate codon-optimized mRNAs in this study resulted in increased mRNA stability. Comparing half-lives of codon optimized, nonoptimized, and out-of-frame translated optimized mRNAs will help to uncover the contribution of this mechanism to maternal mRNA regulation.

Evidence of a global correlation of rare codons with mRNA instability motivates investigation of whether unstable maternal mRNAs correlate with suboptimal codon usage across species. It is possible that maternal mRNAs destined for clearance utilize rare codons to favor mRNA destabilization during the MZT.

2.15 Cooperativity and Redundancy in Maternal mRNA Clearance Mechanisms

Destabilized maternal mRNAs are regulated by multiple cooperating mechanisms. In *Drosophila*, *nanos* mRNA harbors Smaug recognition elements (SREs) (Dahanukar et al., 1999; Smibert et al., 1999) and predicted piRNA-binding sites in different regions of its 3′UTR (Rouget et al., 2010), suggesting that piRNAs and Smaug could cooperate in maternal mRNA clearance. Indeed, mutants for Smaug or the piRNA effector protein, Aubergine, exhibit stabilization of *nanos* mRNA (Rouget et al., 2010; Tadros et al., 2007). Additionally, in zebrafish, *miR-430* is zygotically encoded and targets several hundred mRNAs for clearance (Bazzini et al., 2012; Giraldez et al., 2006), but evidence suggests that it does not always function in isolation. Knockdown of TATA-binding factor (TBP), a transcription preinitiation complex component, using a translation-blocking morpholino has no effect on *miR-430* transcription or processing, but results in stabilization of a subset of *miR-430*-target mRNAs (Ferg et al., 2007). This observation demonstrates that, to destabilize a subset of its target mRNAs, *miR-430* cooperates with an additional, as-of-yet unidentified, factor(s) that require zygotic activation. In *Xenopus*, EDEN-dependent deadenylation is necessary, but not sufficient for *c-mos* mRNA deadenylation, and ARE-like sequences enhance deadenylation in a position-dependent manner relative to EDEN (Audic et al., 1998; Ueno & Sagata, 2002). Cooperative mechanisms of mRNA clearance may enable specificity or precise timing for target mRNA destabilization.

Additionally, redundancy is built into maternal mRNA clearance during the MZT. Pumilio directly interacts with hundreds of mRNAs, yet *pumilio* mutants have been reported not to exhibit global defects in mRNA destabilization (Gerber et al., 2006), implicating the action of a redundant factor for Pumilio target mRNAs. Similarly, in *C. elegans*, maternal mRNAs destabilized during the oocyte-to-embryo transition are enriched for a poly(C) motif (Stoeckius et al., 2014). This motif is necessary and sufficient for destabilization in reporter assays, but knockdown of all poly(C)-binding protein (PCBP) paralogs does not result in stabilization of mRNAs harboring the poly(C) motif (Stoeckius et al., 2014). Likewise, the RNA-binding protein, Brain Tumor (Brat), associates with nearly 1100 mRNAs in *Drosophila* embryos and its binding motif is sufficient to trigger mRNA destabilization in Brat-dependent manner using reporters, but the *brat* mutant exhibits stabilization of only a subset of its target mRNAs (Laver et al., 2015). The absence of widespread stabilization of targets mRNAs in mutants

or knockdown of the corresponding *trans*-factors, suggests redundancy in maternal mRNA clearance mechanisms.

2.16 Combinatorial Code in Maternal mRNA Clearance

Cooperativity and redundancy in maternal mRNA clearance mechanisms suggests the existence of a combinatorial code that governs mRNA fate during development (Fig. 3). While some elements of this regulatory code have been identified during the MZT reviewed in (Walser & Lipshitz, 2011) the complexity of this regulatory mechanisms suggest that there are additional elements of this code that likely regulate mRNA stability. Large-scale approaches have allowed probing of mRNA structure (Ding et al., 2014;

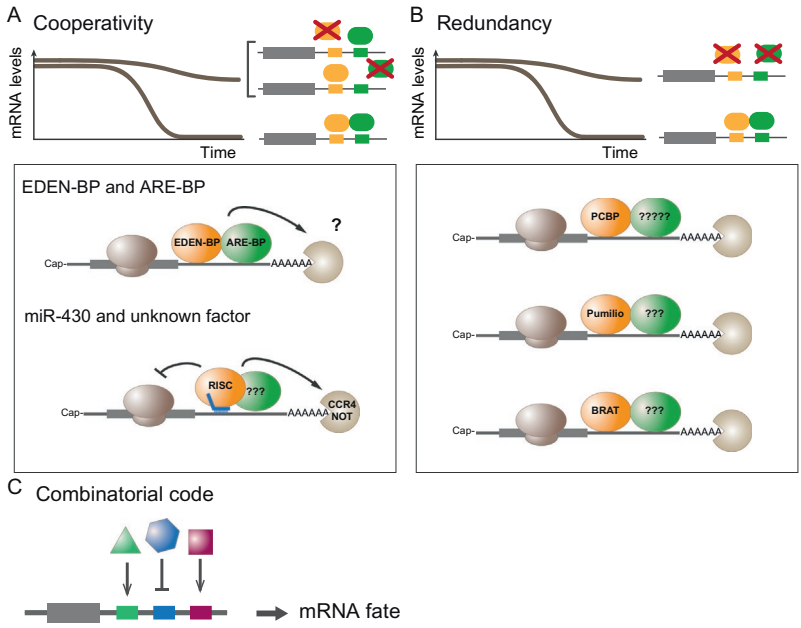

Figure 3 Combinatorial code in maternal mRNA clearance. (A) Cooperative mechanisms require both factors to destabilize mRNA, such that depletion of either one results in stabilization of the target mRNAs. Examples include EDEN-BP together with ARE-BP and miR-430 together with as-of-yet unidentified factor(s) (Ferg et al., 2007; Ueno & Sagata, 2002). (B) Redundant mechanisms require either factor, such that depletion of one of the factors does not affect the stability of target mRNAs. Examples include PCBP in *C. elegans* (Stoeckius et al., 2014) and Pumilio (Gerber et al., 2006) and BRAT (Laver et al., 2015) in Drosophila. Depleting both factors is required to block mRNA destabilization. (C) Model of combinatorial code for maternal mRNA clearance. Individual transcripts harbor multiple regulatory elements that affect mRNA stability. The combination of all signals acting on the mRNA determines mRNA fate.

Rouskin, Zubradt, Washietl, Kellis, & Weissman, 2014; Spitale et al., 2015; Wan et al., 2014), RNA modifications (Batista et al., 2014; Geula et al., 2015), and identification of sequence elements that cause mRNA decay in cell culture (Goodarzi et al., 2012; Oikonomou, Goodarzi, & Tavazoie, 2014) and in yeast (Geisberg et al., 2014). Technological advances that enable the application of these methods *in vivo* within the embryo will define the function of individual elements and the regulatory code (sequence, structure and RNA modifications) to understand the posttranscriptional regulatory networks driving the embryonic transition to pluripotency.

3. CONSEQUENCES OF FAILURE OF MATERNAL mRNA CLEARANCE

There is a growing consensus that degradation of maternal mRNAs is instructive for development and essential to successfully undergoing the MZT (DeRenzo & Seydoux, 2004; Giraldez, 2010; Stitzel & Seydoux, 2007; Tadros & Lipshitz, 2009). Model organisms have offered tractable means to investigate the functional relevance of maternal mRNA clearance during embryonic development (Table 1). Additionally, high-throughput gene expression profiling experiments support the relevance of maternal mRNA clearance for early development.

3.1 Loss of Maternal mRNA Clearance in Model Organisms

Components of the maternal mRNA clearance machinery are required for normal embryogenesis. In *Drosophila*, *smaug* mutants exhibit stabilization of ~1000 mRNAs, which is two-thirds of the transcripts that undergo the maternal mode maternal mRNA clearance in this species (Tadros et al., 2007). *smaug* mutants fail to undergo cellularization at the MBT and do not activate high levels of zygotic transcription (Benoit et al., 2009), suggesting that maternal mRNA clearance is critical for these developmental processes. Additionally, mutants for the piRNA effector protein, Aubergine, exhibit stabilization of *nanos* mRNA and defects in head development (Rouget et al., 2010), suggesting a function of piRNAs in the regulation of maternal mRNAs. However, given the complexity of piRNA populations in the embryo, it is unclear whether the effects of Aubergine loss could be secondary to disrupting other processes, such as DNA damage or the activation of the zygotic program. In mouse, mutations in CCCH tandem zinc finger protein, Zfp36l2, results in embryonic arrest at the

Table 1 Loss of Function Phenotypes of Maternal mRNA Clearance Machinery

Species	Factor	Phenotype	Reference
C. elegans	PCBP	No phenotype	Stoeckius et al. (2014)
	miR-35–42, miR-51–56, miR-58/80–82	Embryonic lethality; locomotion, body size, and reproductive defects	Alvarez-Saavedra & Horvitz (2010) and Wu et al. (2010)
Drosophila	Smaug	Stabilization of ~1000 maternal mRNAs, failure to undergo cellularization, failure to activate zygotic genes	Tadros et al. (2007) and Benoit et al. (2009)
	Aubergine	Stabilization of nanos, head development defects	Rouget et al. (2010)
	Brain tumor (BRAT)	Stabilization of ~20% target mRNAs	Laver et al. (2015)
	Pumilio	Targets mRNAs ~10% stabilized; failure to maintain female germ line	Forbes and Lehmann (1998), Lin and Spradling (1997), and Gerber et al. (2006)
	miR-309	20% embryonic lethality	Bushati et al. (2008)
Zebrafish	miR-430	Stabilization of several hundred mRNAs, abnormal cell movements during morphogenesis (in MZDicer mutant)	Giraldez et al. (2006) and Bazzini et al. (2012)
Xenopus	EDEN-BP	Somitogenesis defects (in morpholino knockdown)	Paillard et al. (1998) and Gautier-Courteille et al. (2004)
	miR-427	Axis formation defects (in morpholino knockdown)	Rosa et al. (2009) and Lund et al. (2009)
	ARE-BP	Unknown	Voeltz and Steitz (1998)
	ePABP	Increased deadenylation of target mRNAs (immunodepletion)	Voeltz et al. (2001)
Mouse	ZFP36L2	Embryonic lethal at two-cell stage	Ramos et al. (2004)
	miR-290/295	Partially penetrant embryonic lethality, female sterilty in surviving mutants	Medeiros et al. (2011)
	miR-302a-d; miR-302/ miR-209	failure in neural tube closure; early embryonic arrest	Parchem et al. (2015)
Cow	miR-212	Unknown	Tripurani et al. (2013)

two-cell stage (Ramos et al., 2004), indicating a failure to complete the MZT. Zfp36l2 is an RNA-binding protein that recognizes AREs in the 3′UTRs of its target mRNAs to initiate mRNA degradation (Lai, Carballo, Thorn, Kennington, & Blackshear, 2000), thus it is conceivable that this protein functions to target maternal mRNAs for clearance during MZT in mouse. The targets of Zfp36l2 during mouse preimplantation development remain to be identified, as well as the sequence motifs it recognizes, and whether the embryonic lethal mutant phenotype results directly from the loss of its maternal mRNA clearance function.

MicroRNAs are directly involved in the clearance of their target mRNAs and the loss of microRNA-mediated maternal mRNA clearance results in developmental defects across different species. The first microRNA involved in maternal mRNA clearance was discovered using a maternal-zygotic mutant for Dicer (MZ*dicer*), an enzyme required for canonical microRNA biogenesis (Giraldez et al., 2006). Absence of *miR-430*-mediated mRNA clearance in the MZ*dicer* results in cell movement defects during gastrulation (Giraldez et al., 2006), implicating maternal mRNA clearance to be important for early embryonic processes. In *Xenopus*, *miR-427* has the same seed sequence as zebrafish *miR-430* (Rosa et al., 2009), is highly transcribed at the MBT, and causes deadenylation and decay of *cyclin A1* and *cyclin B2* mRNAs (Lund et al., 2009). Inhibition of *miR-427* function with anti-*miR-427* morpholinos causes dramatic defects in axis formation resulting, at least in part, from misregulation of the nodal pathway (Rosa et al., 2009), but it is possible that additional target mRNAs contribute to this phenotype. In *Drosophila*, a cluster of *miR-309* microRNAs regulates maternal mRNA clearance and genetic deletion of this microRNA cluster results in 20% embryonic lethality that is rescued with a transgene encoding this genomic locus (Bushati et al., 2008). MicroRNAs are directly involved in destabilizing target mRNAs, thus developmental defects resulting from loss of microRNA-mediated maternal mRNA clearance demonstrate the functional importance of maternal mRNA clearance for animal development.

3.2 Maternal mRNA Clearance During Human Preimplantation Development

Maternal mRNA clearance likely plays an instructive role in human preimplantation development. This step involves several waves of maternal mRNA clearance with ~1700 mRNAs eliminated by day 2 (four-cell stage), ~700 mRNAs between day 2 and 3 (four- to eight-cell stage), and additional ~2700 mRNAs by day 5 (blastocyst stage) (Zhang et al.,

2009). Additionally, single-cell RNA sequencing of preimplantation human embryos identified that ~10% of the expressed maternal mRNAs (1941 out of 22,687) and 2% of the lncRNAs (185 out of 8701) are eliminated between the four- and eight-cell stage (Yan et al., 2013). These studies demonstrate that early human development is characterized by dramatic turnover of mRNAs and ncRNAs and that this occurs in distinct waves, suggesting that this is a regulated process. Indeed, maternal mRNAs that are eliminated early, by the two-cell stage, are enriched for different gene categories (cell cycle, transcription regulation) than mRNAs eliminated at later stages (protein phosphorylation, cell morphogenesis) (Vassena et al., 2011; Yan et al., 2013), arguing for specificity of maternal clearance during each successive wave. Furthermore, clearance of maternal mRNAs is likely a requirement for preimplantation human development because IVF-derived human embryos that fail show evidence of zygotic gene expression but failure to downregulate maternal transcripts (Dobson et al., 2004; Wong et al., 2010). These studies demonstrate that successful reprogramming corresponds with clearance of maternal mRNAs and suggest that this process plays an instructive role during reprogramming.

3.3 The MZT in Interspecies Somatic Nuclear Transfer Embryos

Somatic nuclear transfer (Fig. 1A) within a species (Gurdon, 1962; Wilmut, Schnieke, McWhir, Kind, & Campbell, 1997) allows successful reprogramming of the differentiated nucleus and the completion of the MZT. However, the efforts to clone animals combining an oocyte and a nucleus from different species (interspecies somatic nuclear transfer, or iSNT) have shown limited success. iSNT was reported as early as 1886 to not be possible between toad and frog in either direction (reviewed in Laubichler & Davidson, 2008). The only successful example of iSNT was the cloning of the endangered gaur bull (*Bos gaurus*) using enucleated oocytes of domestic cow (*Bos taurus*) (Lanza et al., 2000). What limits the reprogramming potential when a nucleus is in a foreign oocyte?

Transcriptome analyses show that iSNT embryos fail prior to completing the MZT and that incomplete maternal mRNA clearance may be the culprit. Development could only be recapitulated until the 8–16 cell stage when a rhesus fibroblast nucleus was fused with bovine enucleated oocytes (Wang et al., 2011). Zygotic genome activation occurs during the 6–8 cell stage in rhesus (Schramm & Bavister, 1999) and 8–16 cell stage in cow (Camous, Kopecny, & Flechon, 1986) indicating that developmental arrest in

rhesus-bovine iSNT embryos occurs prior to MZT completion. All of these defects could be due to a complete failure to activate the zygotic genome. However, comparison of gene expression between cow embryos produced by *in vitro* fertilization (IVF) and failed rhesus iSNT embryos using microarrays showed that zygotic genes are activated, but over 1500 maternal mRNAs are not cleared (Wang et al., 2011). A similar approach was used in efforts to clone the endangered Przewalski gazelle, but no viable embryos developed (Zuo et al., 2014). Transcriptome analysis showed that successful IVF-derived cow embryos cleared 1515 mRNAs, while failed gazelle iSCNT embryos cleared only 343 mRNAs (Zuo et al., 2014), demonstrating a dramatic defect in maternal mRNA clearance. While these defects could result from the failed transcription of key zygotic genes, failed recognition and clearance of the maternal mRNAs by the heterologous maternal and zygotic programs could also contribute to embryonic reprogramming during the MZT.

4. MZT CONNECTION TO OTHER TRANSITIONS AND REPROGRAMMING

4.1 Unicellular to Multicellular Transition

Active clearance of the previous mRNA landscape may be a general feature of cellular transitions in multicellular organisms. For example, differentiation in a simple model of multicellularity, the slime mold *Dictyostelium discoideum*, involves dramatic mRNA turnover. The life cycle of this organism involves a unicellular growth state and differentiation to multicellular, aggregated stage consisting of two cell types (Kessin, 2001). Starvation triggers the unicellular to multicellular transition in *D. discoideum* and corresponds to changes in cell fate decisions and morphology (Clarke & Gomer, 1995). Time-course transcriptional profiling using microarrays following starvation in *D. discoideum* showed that the unicellular to multicellular transition involves dramatic changes in gene expression within 6–8 h poststarvation and corresponds to the multicellular transition (Van Driessche et al., 2002). Several hundred mRNAs are dramatically downregulated during this transition and the authors speculate that these genes may function to repress the differentiated, multicellular state (Van Driessche et al., 2002).

Active mRNA clearance mechanisms likely control the unicellular to multicellular transition in *D. discoideum*. mRNA half-lives range from 50 min to 10 h in this species (Casey, Palnik, & Jacobson, 1983). Given that several hundred mRNAs are eliminated within 6–8 h after starvation, a

subset of these mRNAs likely undergoes regulated mRNA destabilization during the unicellular to multicellular transition. Distinguishing between active and passive mechanisms of mRNA clearance requires comparing mRNA half-lives in the unicellular state versus the starvation-induced differentiated state in the presence of transcription inhibitors. Identification of molecular triggers of mRNA destabilization in such a simple system will reveal mechanisms regulating multicellular transitions and advance our understanding of how similar mechanisms may be involved in cellular transitions in metazoans.

4.2 Maternal mRNA Clearance Is Analogous to Reprogramming *in Vitro*

The MZT is in many ways analogous to cellular reprogramming during the induced pluripotency transition *in vitro* (Giraldez, 2010; Lee, Bonneau, et al., 2014) and summarized in Fig. 4. During the MZT, endogenous factors activate zygotic genes and maternal instructions are cleared to facilitate the transition of differentiated gametes to a totipotent state. *In vitro*, forced expression of reprogramming factors, induces the transition of differentiated somatic cells to adopt a pluripotent identity (Takahashi & Yamanaka, 2006;

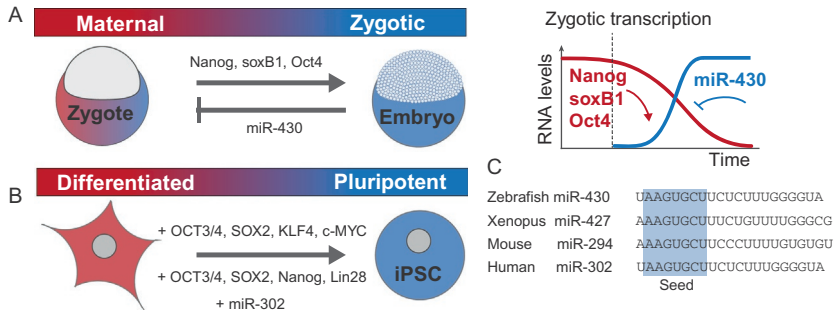

Figure 4 The MZT is analogous to *in vitro* pluripotency reprogramming. (A) In zebrafish, the pluripotency factors Nanog, SoxB1, and Oct4 activate zygotic gene transcription, including miR-430, which clears maternal mRNAs (Giraldez et al., 2006; Lee et al., 2013). Together, the activation of the zygotic genome and the clearance of maternal mRNAs facilitate oocyte reprogramming to the zygotic state. (B) Forced expression of pluripotency factors reprograms somatic cells to induced pluripotent cells (Takahashi & Yamanaka, 2006; Yu et al., 2007) and miR-302 (orthologous to miR-430) is sufficient for reprogramming (Anokye-Danso et al., 2011; Miyoshi et al., 2011). (C) miR-430/302/294 family microRNAs are highly conserved, share seed sequence, and are expressed in stem cells and early embryos (Houbaviy, Murray, & Sharp, 2003; Suh et al., 2004).

Yu et al., 2007). Below, we highlight recent studies that describe shared features of posttranscriptional regulation for pluripotent cells and embryos during the MZT.

4.3 microRNA Function in Reprogramming

Reprogramming during development and *in vitro* both exploit the highly conserved *miR430/290/302* family of microRNAs to erase the previous transcriptional landscape. Orthologs of these microRNAs are abundantly expressed in early embryogenesis in zebrafish (Giraldez et al., 2005), *Xenopus* (Lund et al., 2009), and in mammalian stem cells and embryos (Houbaviy et al., 2003; Suh et al., 2004), suggesting that they influence early developmental events. In the context of iPSC reprogramming, the addition of *miR-302/294* together with Oct4, Sox2, and Klf4 increases fibroblast reprogramming efficiency by 10-fold in mouse (Judson, Babiarz, Venere, & Blelloch, 2009) and in human fibroblasts (Subramanyam et al., 2011), implicating this microRNA as a core component of the pluripotency network. In fact, expression of the *miR302/367* cluster alone appears sufficient to reprogram mouse and human fibroblasts to iPSCs two orders of magnitude more efficiently than the OSKM cocktail (Anokye-Danso et al., 2011; Lin et al., 2011; Miyoshi et al., 2011).

What makes this microRNA family such a potent reprogramming factor? *miR-430/302/294* family members rescue the Dgcr8 mouse knockout ES cell proliferation defect through downregulation of several G1/S transition regulators (Wang et al., 2008), implicating a role in cell proliferation for reprogramming regulation. However, in addition to proliferation these reprogramming microRNAs also regulate apoptosis, chromatin remodelers, and the mesenchymal to epithelial transition (MET) (Anokye-Danso, Snitow, & Morrisey, 2012). Additionally, *miR-181* family microRNAs enhances OSK-mediated fibroblast reprogramming efficiency by three fold in mouse (Judson, Greve, Parchem, & Blelloch, 2013). Interestingly, no synergistic increase in reprogramming was observed for the combination of *miR-294* and *miR-181*, suggesting that these microRNA converge on common pathways downstream of their direct targets (Judson et al., 2013). Likewise *miR-430* targets several hundred different mRNAs for clearance during development (Giraldez et al., 2006). The diversity and the scope of regulation exerted by these microRNA families suggest that its function may be in erasing the preexisting transcriptional landscape as an instructive strategy to facilitate the installation of the pluripotency program.

4.4 Pumilio Function in Stem Cell Maintenance

Across metazoans species, Pumilio has a role in repressing differentiation. In *Drosophila pumilio* mutants, germline stem cells fail to undergo asymmetric divisions and consequently differentiate into egg chambers, indicating a failure to maintain the self-renewal potential in the germline stem cells (Forbes & Lehmann, 1998; Lin & Spradling, 1997). Likewise, genetic inactivation of both Pumilio homologs, *fbf-1* and *fbf-2*, in *C. elegans* leads to failure in germline maintenance, manifested in the adult germline consisting exclusively of sperm (Crittenden et al., 2002). Human Pumilio2 is expressed in embryonic stem cells, in ovary and testis, while Pumilio1 is expressed ubiquitously (Moore et al., 2003), suggesting a conserved function for Pumilio2 in stem cell maintenance. Drawing on these examples, and on function of Pumilio in posttranscriptional regulation of maternal mRNA in *Drosophila* (discussed above), this multipurpose protein could play a role in clearing the oocyte's transcriptional history to enable a transient, pluripotent state.

4.5 RNA Modifications as Markers of Decay in Stem Cells

RNA modification has recently been implicated in mediating mRNA turnover in embryonic stem cells and for maintaining the pluripotent identify. N^6-methyl-adenosine (m^6A) modification in RNA is a substrate for YTHDF proteins (Dominissini et al., 2012; Wang, Lu, et al., 2014), which direct mRNAs to processing bodies (P-bodies) (Wang, Lu, et al., 2014) and directly interact with Pop2 in the Pop2–Ccr4–Not1-deadenylase complex to direct mRNA deadenylation and destabilization (Kang et al., 2014). Global mapping of m^6A modification in mouse and human ES cells showed that thousands of mRNAs, including components of the pluripotency network, and ncRNAs are modified with m^6A and that this correlates with mRNA instability (Batista et al., 2014; Geula et al., 2015). Genetic inactivation of the methylation "writer," Mettl3, resulted in a global decrease in m^6A, stabilization of pluripotency factors such as Nanog, and prevented exit from self-renewal (Batista et al., 2014). Likewise, Mettl3 mouse knockout ES cells have prolonged expression of pluripotency factors and fail to undergo proper lineage priming, which consequently leads to embryonic lethality (Geula et al., 2015). RNA modification provides a potentially effective way to mark maternal mRNA to facilitate selective destabilization of these transcripts at MZT. In some cases, m^6A modification even alters the binding affinity of RNA-binding proteins (Liu et al., 2015), providing a dynamic strategy to regulate large numbers of transcripts.

4.6 Poly(C) Destabilization Motif in Stem Cells and Embryos

Embryos and pluripotent stem cells share unique features of posttranscriptional regulatory mechanisms that are distinct from somatic cells. Global analysis of mRNA decay rates reveals that posttranscriptional regulation is different between differentiated mouse embryonic fibroblasts (MEFs) and induced pluripotent stem cells (iPSCs) derived from these MEFs. Transcripts bearing 3′UTR C-rich sequence elements, many of which encode transcription factors, are significantly less stable in iPS cells than in MEFs. Intriguingly, two poly(C)-binding proteins that recognize this type of element are reciprocally expressed in iPS and HFF cells (Neff, Lee, Wilusz, Tian, & Wilusz, 2012), suggesting that poly(C)-binding proteins direct mRNA instability in iPSC. Interestingly, the poly(C) motif is also enriched in unstable maternal mRNAs during the oocyte-to-embryo transition (OET) in *C. elegans* (Stoeckius et al., 2014). However, depletion of all poly(C)-binding proteins in this species does not globally affect the stability of mRNAs harboring the poly(C) motif (Stoeckius et al., 2014), indicating that this mRNA destabilization mechanism requires additional component(s). Identification of these additional components will likely also elucidate the unique features of posttranscriptional mRNA regulation in pluripotent cells that permit selective destabilization of mRNAs in pluripotent stem cells, but not in differentiated somatic cells. This further suggests that posttranscriptional mRNA regulatory machinery could be leveraged to improve reprogramming efficiency *in vitro* by pushing somatic cells toward pluripotency.

5. CONCLUDING REMARKS

In this chapter, we have described the current understanding of the molecular mechanisms regulating maternal mRNA clearance across species. Through some of these examples we describe the parallels that exist between cellular and developmental reprograming, including microRNA regulation (*miR-430/302/295*), RNA-binding proteins (Pumilio and PCBP), and even transcription factors such as Nanog, Oct4, Sox2 (Fig. 4). Based on the key parallels between cellular and developmental reprograming, we postulate that many of these mechanisms might be shared during the maternal-to-zygotic transition to clear the previous maternal program and to help establish the new zygotic program of development.

The advent of new methods to measure RNA and protein modifications genome-wide will allow us to investigate their role in the regulation of maternal mRNAs and the modulation of RNA-binding affinity. Likewise, methods to determine RNA structure, dynamics of poly(A) tail length, translational regulation, and RNA-binding protein activity using high-throughput methods is opening exciting avenues to identify the code that mediates the posttranscriptional regulation of maternal instructions during the MZT. Finally, translational activation following fertilization could modify mRNA half-life based on codon usage (Presnyak et al., 2015) to regulate mRNA stability during the MZT. Future studies in these areas are likely to provide not only the key mechanisms regulating gene expression during cellular transitions but also during reprogramming and cell differentiation.

While all animals undergo the MZT, there is a surprising degree of variation in the targets that undergo mRNA clearance during development. Variation in targets across different species likely reflects different reproductive strategies and developmental requirements. Species employ a diverse, yet conserved set of RNA-binding proteins and noncoding RNAs to regulate maternal mRNAs during the MZT. Several questions remain to be answered. What mechanisms activate the different pathways of mRNA clearance and translational regulation? What instructive roles does maternal clearance play in development? To what extent does translational and post-translational control contribute to regulating each step?

We also lack an understanding of how different decay pathways interact to facilitate mRNA clearance in a coordinated and specific manner. We have seen examples of mRNAs under the regulation of cooperative and redundant mechanisms (Fig. 3). Therefore, mRNA fate can be calculated according to the combined action of several stabilizing and destabilizing factors acting on the same transcript. Understanding how this regulatory code is organized and interpreted to allow for the coordinated and specific elimination of hundreds of mRNAs, remain fundamental challenges for the field.

Change is a key constant in development. Multicellular life continually uses cellular transitions to generate tissue complexity during development and regeneration in adulthood. The MZT is a dramatic example of a cellular transition of a differentiated oocyte toward a transient totipotent embryo. Across animals, hundreds of mRNAs are regulated in a coordinated manner during the MZT, making this an ideal context to identify posttranscriptional mechanisms that shape gene expression.

ACKNOWLEDGMENTS

We thank Dr. Miler T. Lee, Dr. Carter M. Takacs, and Dr. Howard D. Lipshitz for critical reading of the manuscript and all members of the Giraldez Laboratory for discussion. V.Y. has been supported by the NIH Training Grants T32 GM007223 and T32 HD007149, the Edward L. Tatum Fellowship (Yale University), and the Yale Medical Research Scholars Program. The Giraldez Lab is supported by National Institutes of Health grants R01-GM081602-07, R01-GM101108-4, R01-GM102251-4, R01-GM103789-3, R01-HD074078-4, and R21-HD073768-2.

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