RNA Methylation Clears the Way

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During the maternal-to-zygotic transition, maternal mRNAs are cleared by multiple distinct but interrelated pathways. A recent study in *Nature* by Zhao et al. (2017) finds that YTHDF2, a reader of N⁶- methylation, facilitates maternal mRNA decay, introducing an additional facet of control over transcript fate and developmental reprogramming.

The maternal-to-zygotic transition (MZT) is a critical first step of animal development in which two differentiated gametes are reprogrammed into a totipotent embryo. Similar to other cellular transitions, two concurrent steps define reprogramming during MZT: removal of the previous cellular identity and establishment of pluripotency through new transcription. During MZT, these steps are realized as clearance of maternal mRNAs and zygotic genome activation (ZGA). During clearance, maternal products are actively eliminated by multiple, complementary post-transcriptional mechanisms (Yartseva and Giraldez, 2015). Because of the diversity of mechanisms regulating clearance, understanding the specific factors and contributions of different decay pathways remains a challenge.

Two main types of degradation activity coordinate clearance. The zygotic mode requires zygotic transcription, whereas the maternal mode is exclusively maternally encoded (Tadros and Lipshitz, 2009). In zebrafish, several mechanisms coordinate clearance of the maternal mRNAs (Figure 1A). One key zygotic player is the microRNA miR-430, which promotes degradation of several hundred mRNAs through translational repression and deadenylation (Giraldez et al., 2006; Bazzini et al., 2012). One maternal mechanism is codon optimality, in which stabilizing or destabilizing codons affect mRNA translation and polyadenylation status to influence transcript stability (Bazzini et al., 2016; Mishima and Tomari, 2016). Together, the combined action of the maternal and zygotic programs modulates the stability of thousands of mRNAs during MZT. However, some transcripts are cleared independently of these mechanisms, suggesting that additional pathways facilitating maternal mRNA decay are yet to be uncovered.

Recent work by Zhao et al. (2017) explores the potential for the RNA modification, N6-adenosine methylation (m⁶A), to direct maternal clearance. m⁶A is a known regulator of transcript stability, especially during key cellular transitions such as the transition from naive pluripotency to differentiation in mouse embryonic stem cells (Geula et al., 2015). The consequences of methylation on mRNA fate are interpreted by "reader" proteins, including YTHDF2, which has been shown to facilitate degradation of methylated transcripts in humans (Wang et al., 2014). To determine whether m⁶A controls maternal mRNA fate and promotes embryonic pluripotency. Zhao et al. (2017) generated zebrafish mutants lacking the m⁶A reader, YTHDF2. Embryos from mothers lacking YTHDF2 (maternal mutants) exhibit developmental delay and are correspondingly delayed in both ZGA and maternal clearance, consistent with a role for methylation in regulating MZT.

To explore the mechanisms effecting maternal mRNA decay, the authors used mRNA sequencing to identify degraded transcripts and divided them into three gene classes—decayed maternal mRNAs, stable maternal mRNAs, and zygotically transcribed mRNAs—based on expression through MZT. To determine which of these mRNAs are modified, the authors performed high-resolution m⁶A mapping. When the authors inspected the RNA levels of the different transcript classes in embryos lacking maternal YTHDF2, they found that maternal mRNAs are stabilized, while

zygotic mRNAs are downregulated. These results indicate a YTHDF2-dependent defect in both ZGA and clearance.

To further dissect the relationship among YTHDF2, m⁶A, and decay, the authors injected methylated and nonmethylated reporters into maternal ythdf $2^{-/-}$ and wild-type embryos. The m⁶A reporters degraded more rapidly than the non-methylated control in wildtype embryos but were stabilized in the ythdf2^{-/-} mutants. This pattern of differential decay implies that YTHDF2 may specifically promote degradation of modified mRNAs. Indeed. in wild-type embryos. methylated maternal transcripts exhibited a greater magnitude of degradation than non-methylated transcripts, though this difference may be due to the initially higher expression levels of m⁶A mRNAs. However, it remains possible that YTHDF2 directs transcript fate regardless of methylation status, as the authors did not investigate whether stabilization of maternal mRNAs in YTHDF2 mutants is exclusively limited to those that are methylated. Notably, of the 2,327 mRNAs mis-regulated in ythdf2-/- embryos, only 852, or ~37%, are methylated. Similarly, there are 2,653 m⁶A mRNAs that are not affected by loss of YTHDF2 function. This suggests either an indirect effect of YTHDF2 on non-methylated transcript stability or that YTHDF2 function extends beyond modified transcripts. Nevertheless, the study by Zhao et al. (2017) provides evidence that methylation of some maternal mRNAs marks them for YTHDF2-controlled decay, uncovering both m⁶A and YTHDF2 as regulators of maternal clearance during MZT. Loss of maternal, or both maternal and zygotic, YTHDF2 also disrupted



global zebrafish development. Such

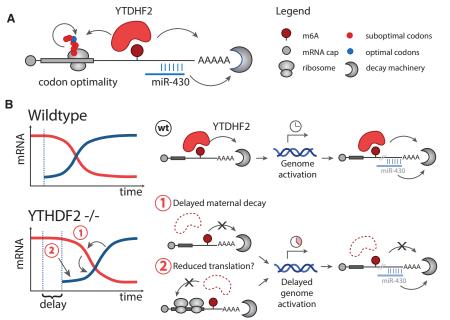


Figure 1. Multiple Pathways Promote Maternal Clearance during MZT

(A) Multiple mechanisms, including maternally coded codon optimality and miRNA-mediated decay via the zygotic factor miR-430, promote maternal mRNA decay. YTHDF2 also facilitates degradation of maternal transcripts. (B) Two possible pathways explain how YTHDF2 contributes to maternal mRNA clearance. In both scenarios, transcript decay may be co-regulated by methylation and miR-430. (1) YTHDF2 is directly required for maternal mRNA decay. Delayed destabilization of some transcripts in *ythdf2^{-/-}* mutants impairs zygotic genome activation (ZGA), further enhancing the delay in clearance. (2) YTHDF2 is indirectly required for maternal mRNA decay. YTHDF2 may facilitate translation of transcriptional activators, such that loss of YTHDF2 slows clearance via delayed ZGA and delayed transcription of miR-430. For (B), maternal products and pathways are colored in red and zygotic ones are in blue.

ythdf $2^{-/-}$ mutants exhibited a delay beginning at the mid-blastula transition, though both maternal and maternal-zygotic mutant embryos recovered by day 3 and showed no adult defects. It is notable that normal developmental progression was dependent strictly on the maternal contribution of YTHDF2, as it was observed in embryos from ythdf $2^{-/-}$ females regardless of paternal genotype, suggesting that the zygotic contribution of YTHDF2 cannot rescue the loss of maternal protein.

How does m⁶A-dependent disruption of maternal mRNA clearance lead to delayed development? The authors propose that failure to properly clear maternal mRNAs may impede zygotic genome activation (Figure 1B). They note that stabilized, methylated, maternal genes include cell-cycle regulators and that embryos temporarily arrest upon completion of the cell cycle immediately prior to ZGA. It is possible that stabilization of these mRNAs, or other key pluripotency factors, could prevent timely ZGA. Alternatively, a possibility that is not explored by the authors is that disrupted maternal clearance could be a consequence of delayed zygotic genome activation (Figure 1B). m⁶A is known to globally promote translation (Wang et al., 2015); in the context of MZT, m⁶A could boost translation of key activators of zygotic transcription. Interestingly, the authors note that many transcripts dependent on YTHDF2 for decay are also targets of the zygotically supplied microRNA miR-430. Delayed ZGA would also lessen miR-430 transcription, causing a lag in maternal clearance that could be misattributed to loss of YTHDF2. One cannot exclude the possibility that the effects observed in YTHDF2 mutants are due to translational changes that hinder ZGA and thus indirectly disrupt maternal clearance.

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It remains to be determined how methylation-driven decay functions in the context of the previously established modes of maternal mRNA clearance (Figure 1A). It is known that mechanisms of maternal and zygotic decay can either cooperate or act redundantly to remove mRNAs, and methylation may augment the effects of existing pathways. For example, m⁶A may enhance the effects of codon optimality, possibly by contributing to ribosome pausing. Similarly, the authors note that mRNAs targeted by both zygotic miR-430 and maternal YTHDF2 degrade more rapidly than transcripts regulated by either mechanism alone, potentially due to cooperative decay. By facilitating interplay between maternal and zygotic pathways, methylation may allow acute temporal control over mRNA removal. Given that m⁶A regulates gene expression during other developmental transitions, RNA methylation may serve as a universal mechanism to promote reprogramming.

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