“Father knows best?”

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Following fertilization, activation of a complex developmental program requires the differential expression of key genes. In most metazoa, the prevailing view is that early differential gene expression occurs primarily through post-transcriptional regulation of maternally deposited products in the oocyte. Two novel studies published from the Rajewsky laboratory in this issue of *The EMBO Journal* add significantly to the emerging notion that this process may be more complex than previously appreciated.

See also: **M Stoeckius *et al*** (August 2014a) and **M Stoeckius *et al*** (August 2014b)

In the first study from Rajewsky and co-workers (Stoeckius *et al*, 2014a), global approaches including metabolic labeling and RNA-seq of 1-cell stage embryos are employed to discover significant deposition of mRNAs and small non-coding RNAs of paternal origin into *Caenorhabditis elegans* oocytes. In their second paper, the authors comprehensively surveyed transcriptome and proteome changes that occur at one of the earliest steps in animal development: the oocyte-to-embryo transition (OET). The results thus establish a nearly complete inventory of one of the most fundamental transition periods in embryonic development. The most prominent finding here is the discovery of a remarkable wave of mRNA turnover that immediately follows fertilization. They go further to characterize an mRNA clearance mechanism involving a 3' UTR polyC motif that allows coordinated and rapid turnover of maternal mRNAs prior to what is generally considered to be the maternal-to-zygotic transition in *C. elegans* (Stoeckius *et al*, 2014b). Taken together, these rather unexpected results offer a glimpse into the role paternal RNAs might play during early developmental decisions.

The tightly controlled and fertilization-induced mRNA turnover begs many questions with regard to our current perception and understanding of early developmental processes in general (Fig 1).

To investigate the contribution of paternal RNAs to zygotes in *C. elegans*, Stoeckius *et al* crossed two divergent strains that had undergone uniparental metabolic RNA labeling. They found that 10% of the total RNA (including mRNAs and small RNAs) in F1 early embryos is of paternal origin (Stoeckius *et al*, 2014a). UV crosslinking of these paternal RNAs in early embryos resulted in embryonic lethality, suggesting that these RNAs can be functionally important for early development. SNP analysis of mRNAs in the F1 embryos indicated that hundreds of different mRNAs (as well as numerous small non-coding RNAs) are of paternal origin. Corroborating potential functionality, the majority of these paternal mRNAs overlap with transcripts that become up-regulated in 1-cell embryos when compared to oocytes. Since transcription in *C. elegans* is thought to be silent until the 4-cell stage, and since the authors show that paternal RNA is transmitted into the 1-cell embryo, the authors built a case for the deposition of significant amounts of sperm-derived RNAs into oocytes upon fertilization. This is further corroborated by the fact that the very same RNAs can be detected in sperm. While these are valid and intellectually challenging conclusions, there is increasing evidence that suggests transcription does occur earlier than previously thought, at least in some nematodes (Wang *et al*, 2014) and *Drosophila* embryos (Ali-Murthy *et al*, 2013). Thus, the origin of some of these RNAs and certainly their potential biological function(s) may warrant further exploration.

A few recent studies already suggested roles for individually contributed, paternal RNAs during early development: The

transmission of small RNAs from sperm has been linked to *C. elegans* male fertility and to establish an epigenetic memory of male-specific germline gene expression (Conine *et al*, 2013). In mouse embryos, a sperm-derived miRNA is required for the first cleavage (Liu *et al*, 2012) and even behavioral traits can be passed along in a trans-generational manner upon transfer of sperm RNA to fertilized oocytes (Gapp *et al*, 2014). Significant roles for paternally deposited RNAs, as further suggested by the results from Rajewsky *et al*, indicate a so far overlooked contribution to the early embryonic transcriptome landscape that deserves much further in-depth investigation. Intriguing questions include: Will additional high-throughput sequencing and other approaches now show that functional contributions of paternal RNAs are common in many organisms? Do the different kinds of paternally contributed RNAs have specific functional roles during development?

In the second paper, another key process in early development comes into focus: the activation of the zygotic genome tightly intertwined with the clearance of maternal RNAs, commonly described as the maternal-to-zygotic transition (MZT). In *C. elegans*, the MZT is thought to initiate not before the 4-cell stage (Seydoux & Dunn, 1997). One of the most surprising findings in the second paper from the Rajewsky laboratory is the notion of widespread RNA degradation much earlier than described before, namely immediately following (and thus possibly closely linked to) fertilization (Stoeckius *et al*, 2014b). Some support for this observation (and suggesting a certain degree of conservation) comes from a study that revealed widespread degradation of transcripts immediately following fertilization in *Ascaris*, a divergent nematode. In this context, however (and distinct from the situation in *C. elegans*), *Ascaris* initiates

Figure 1. The evolving model of transcriptome dynamics during *Caenorhabditis elegans* early development.

(A) Prevailing view. Fertilization of an oocyte by an amoeboid sperm gives rise to a zygote comprised of both parent genomes and maternally contributed RNAs. Gene expression in zygotes and early embryos is driven by maternally contributed RNAs. Activation of the maternal-to-zygotic transition (MZT) initiates widespread degradation of maternal RNAs and transcription of the zygotic genome. Transcription in the germ cell remains inactive at MZT. (B) Adapted view. Paternal RNAs are deposited into the oocyte, contribute to development, and these RNAs are present through at least the 1-cell stage. A subset of maternal RNAs undergoes coordinated degradation immediately following fertilization during the oocyte-to-embryo transition (OET).

substantial zygotic transcription immediately following fertilization and prior to pronuclear fusion (Wang *et al*, 2014). This may suggest an uncoupling mechanism that operates during MZT, at least in *C. elegans*, so that mRNA degradation begins much earlier than zygotic transcription. Although much remains to be learned regarding how the MZT is regulated, post-transcriptional mechanisms for maternal RNA clearance have been identified in some organisms including zebrafish where a miRNA guides deadenylation and degradation of maternal RNAs (Giraldez *et al*, 2006). In contrast, Stoeckius *et al* do not implicate miRNAs but convincingly show that degradation of a large subset of maternal mRNAs at the OET is regulated through a *cis*-acting polyC motif in the 3' UTR of transcripts. The authors proceed to identify homologues of the polyC-binding protein (PCBP) family that recognize this element and elicit transcript decay. Using a targeted, single-copy insertion of reporter constructs, the authors determine both requirement and sufficiency of the polyC motif for mRNA degradation.

Since not all maternal mRNAs that are cleared at the OET contain a polyC motif, other mechanisms are likely to contribute to this process. The authors note that endogenous siRNAs may target approximately 10% of the down-regulated maternal mRNAs. It thus remains to be determined whether and what role siRNAs might play during the degradation of maternal mRNAs in other developmental systems.

Together, these studies offer compelling evidence that RNAs of paternal origin can be transferred to oocytes and play functional roles that need to be more specifically determined in the future. Further, the timing of maternal mRNA clearance in some organisms may start much earlier than previously appreciated and maternal mRNAs can be cleared in a rather coordinated manner employing a common set of *cis*- and *trans*-acting factor(s). These data significantly advance our understanding of early embryogenesis in *C. elegans* and spark many questions as how far this translates to higher organisms. Additionally, high-throughput sequencing, nascent RNA sequencing, and

ribosomal profiling of well-defined stages of early development and carefully conducted single-cell profiling are likely to provide further insights into both commonly used and distinct mechanisms of post-transcriptional gene regulation during various steps of embryonic development.

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