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Contribution of transcription to animal early development

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In mature gametes and during the oocyte-to-embryo transition, transcription is generally silenced and gene expression is post-transcriptionally regulated. However, we recently discovered that major transcription can occur immediately after fertilization, prior to pronuclear fusion, and in the first cell division of the oocyte-to-embryo transition in the nematode *Ascaris suum*. We postulate that the balance between transcriptional and post-transcriptional regulation during the oocyte-to-embryo transition may largely be determined by cell cycle length and thus the time available for the genome to be transcribed.

Keywords: early development, maternal deposition, oocyte-to-embryo transition, post-transcriptional regulation, zygotic transcription

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Maternal Deposition and Post-transcriptional Regulation During Development

During late gametogenesis, the oocyte-to-embryo transition, and early embryogenesis, transcription is thought to be largely quiescent, yet differential gene expression is thought to be necessary for these processes. How then could development proceed without transcription? During animal oogenesis, significant transcription occurs in the diplotene stage of meiosis before the genome is silenced, and large amounts of RNAs and proteins are produced and accumulate in the mature oocyte. A variety of adaptations in animals ensure that the mature oocyte has the necessary RNAs, proteins and nutritional components to develop and navigate through the oocyte-to-embryo transition and early embryogenesis, before zygotic transcription is re-activated for subsequent development. For example, lampbrush chromosomes are often formed to allow massive transcription during meiosis; excess germ cells in *C. elegans*[1] or nurse cells in *Drosophila*[2] contribute RNAs and proteins to the maturing oocyte; and rRNA genes are amplified up to ~1,000-fold in *Xenopus*[3] to enable massive rRNA transcription and accumulation.

The deposited RNAs and proteins are differentially and coordinately used to drive developmental processes. This is mediated by a variety of post-transcriptional regulatory mechanisms including translational repression and activation, mRNA clearance, post-translational modifications, and protein degradation.[4,5] Translational repression of mRNAs is often achieved through interaction of RNA-binding proteins with the 3′-UTR of messages.[6] For example, in a conserved mechanism first discovered in *Xenopus*[7] oocytes, maternal mRNAs are translationally repressed through binding of RNA-binding proteins, such as Cytoplasmic Polyadenylation Element Binding proteins (CPEBs), to sequence motifs known as Cytoplasmic Polyadenylation Elements (CPEs) in their 3′-UTRs. During the oocyte-to-embryo transition, phosphorylation of CPEBs activates mRNA translation by promoting polyadenylation.[8] In addition to repression, degradation of maternally contributed mRNAs can also be achieved through the interaction of RNA-binding proteins with 3′-UTR cis-elements. RNA-binding proteins that elicit decay of maternal mRNAs include SMAUG in *Drosophila*[9] and PolyC-Binding Proteins (PCBPs) in *C. elegans*.[10] There is also evidence that miRNAs can promote degradation of maternal transcripts, as seen in zebrafish embryos where miR-430 targets the 3′-UTR of mRNAs destined for decay.[11] Proteins are also targeted for degradation during early development through the ubiquitin-proteasome pathway and macroautophagy (see reviews[12,13]).

Zygotic Transcriptional Landscapes in Model Organisms

A prevailing view in developmental biology is that after fertilization, the zygotic genome remains transcriptionally silenced until the onset of the maternal-to-zygotic transition, a developmental period where maternally contributed RNAs are degraded and new transcription is required for development to proceed.[14] Studies in multiple organisms have revealed that the activation of transcription during early embryo development occurs in 2 waves. The first and minor wave transcribes only a few dozen to hundreds of genes. The second and major wave transcribes thousands of genes.[14] The timing of these transcription waves varies between organisms and has been described using multiple methods. Early studies used radioactive or bromouridine labeling of RNA to define the onset and amount of transcribed RNAs. Northern blots, quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR), and in situ hybridization were used later to trace individual mRNA changes during development. More recently, high-throughput approaches such as microarray and RNA-seq combined with advanced sampling technologies were used to systematically examine gene transcription and RNA changes in early development (**Table 1**). These genome-wide studies not only revealed the dynamic nature of transcription and RNA turnover during early development, but have also provided valuable resources for evolutionary comparisons of early development in various organisms.[23,30-32] Interestingly, these newer studies observed that transcription occurs in much earlier developmental stages than previously thought in several organisms, including *Drosophila*,[19] zebrafish,[23] and mammals.[29] This is likely due to the use of more sensitive methods that can identify the minor wave of early transcription.

Although transcription is now known to occur very early in development in many organisms (**Table 1**), its specific contributions to early development are largely unknown. Furthermore, in many cases where early transcription occurs, transcriptional inhibition using α-amanitin does not immediately lead to inhibition of development (see review[14]). Thus, additional studies are needed to unveil the functions of early transcription during embryogenesis.[19,33]

Variation in Gene Regulation Programs During Early Development

Variations in the onset of major transcription in different organisms are observed ranging from the 2-cell stage in mouse to > 4000-cell stage in frog and fruit fly.[14] In addition, large differences in the overall contributions of post-transcriptional versus transcriptional gene regulation driving early development can occur in related species. For example, significant differences in the maternal contribution and requirements for early transcription have been observed in nematodes.[34] *C. elegans* embryos can develop to 100–150 cells when zygotic transcription is blocked by α-amanitin[34] or a mutant RNA polymerase.[35] In contrast, *Acrobeloides nanus* embryos are arrested at the 5-cell stage when transcription is blocked by α-amanitin.[34] *C. elegans* develops very rapidly with an average cell cycle length of 20–25 min, while *A. nanus* develops 4–5 times slower. The differences in requirements for transcription in these nematode embryos may be due to differences in cell cycle length and thus the speed of early development, as fast development and progression through mitosis can cause abortion of nascent transcripts.[36] Although differences in embryonic pattern formation and cell-specification may also account for these differences,[34] the slower developing *A. nanus* may be more dependent on new transcription due to smaller contributions from maternal deposition. We recently compared the developmental transcriptome dynamics of *C. elegans* with an extremely slow-developing nematode, *Ascaris suum*.[32] *A. suum* and *C. elegans* appear to have identical early cleavage and developmental patterns, but the early cell cycle lengths of *A. suum* (1,200 min) are ~50-fold longer than those in *C. elegans* (20–25 min) (**Fig. 1**). In *C. elegans*, the

Table 1. Genome-wide studies on early developmental transcriptomes in model organisms

Organism	Embryo Samples	Genomic Approaches	Reference
S. purpuratus	Staged embryos	Microarray	Wei et al, 2006[15]
	Staged embryos	Whole-genome tiling array	Samanta et al, 2006[16]
C. elegans	Handpicked embryos	Microarray	Baugh et al, 2003[17]
	Picked or cell sorting	RNA-seq	Stoeckius et al, 2014[10]
D. melanogaster	Chromosomal ablation	Microarray	De Renzis et al, 2007[18]
	Genetic cross	RNA-seq & SNP analysis	Ali-Murthy et al, 2013[19]
D. rerio	Staged embryos	RNA-seq (SOLiD)	Aanes et al, 2011[20]
	Staged embryos	RNA-seq and lncRNA analysis	Pauli et al, 2012[21]
	Genetic cross	RNA-seq & SNP analysis	Harvey et al, 2013[22]
	Metabolic RNA labeling	RNA-seq	Heyn et al, 2014[23]
X. tropicalis	Staged embryos	Microarray	Yanai et al, 2011[24]
	Staged embryos	RNA-seq	Tan et al, 2013[25]
	Staged embryos	RNA-seq (poly-A and ribo-zero)	Paranjpe et al, 2013[26]
M. musculus	Staged embryos	Microarray	Hamatani et al, 2004[27]
	Staged embryos	Microarray	Xie et al, 2010[28]
	Staged embryos	Single-cell RNA-seq & SNP analysis	Xue et al 2013[29]

minor, initial wave of transcription is thought to begin at the 4-cell stage and the major wave does not start until the ~100-cell stage. In contrast, *A. suum* major transcription initiates immediately following fertilization prior to pronuclear fusion and appears to drive early development (**Fig. 1**). Developmental polysome profiles revealed little translational regulation in *A. suum*, further supporting the notion that transcriptional regulation drives *A. suum* early development.[32]

Zygotic Transcription May Be Dependent on the Cell Cycle Length

The cell cycle length and thus the time available for the genome to be transcribed may be a key determinant for the contributions of transcriptional vs. post-transcriptional regulation of gene expression in early embryogenesis.[36,37] We speculate that the predominant use of newly transcribed genes in *A. suum* may have been enabled by its unusually long cell cycles and protracted early development. Indeed, in organisms with long cell cycle times during early cell divisions, such as mouse and human, significant numbers of genes are transcribed during the 1–4 cell stages.[27,29] A comparison of organisms with fast or slow cell cycles during early development suggests that the time when the genome is transcriptionally activated correlates well with the length of cell cycle during early cleavages (**Fig. 2**). As many model organisms have a short life cycle and are fast-developing (except mammals), features that make them favorable for research, many studies have found that these fast-developing animals use a gene regulation program that primarily depends on post-transcriptional control during early development.

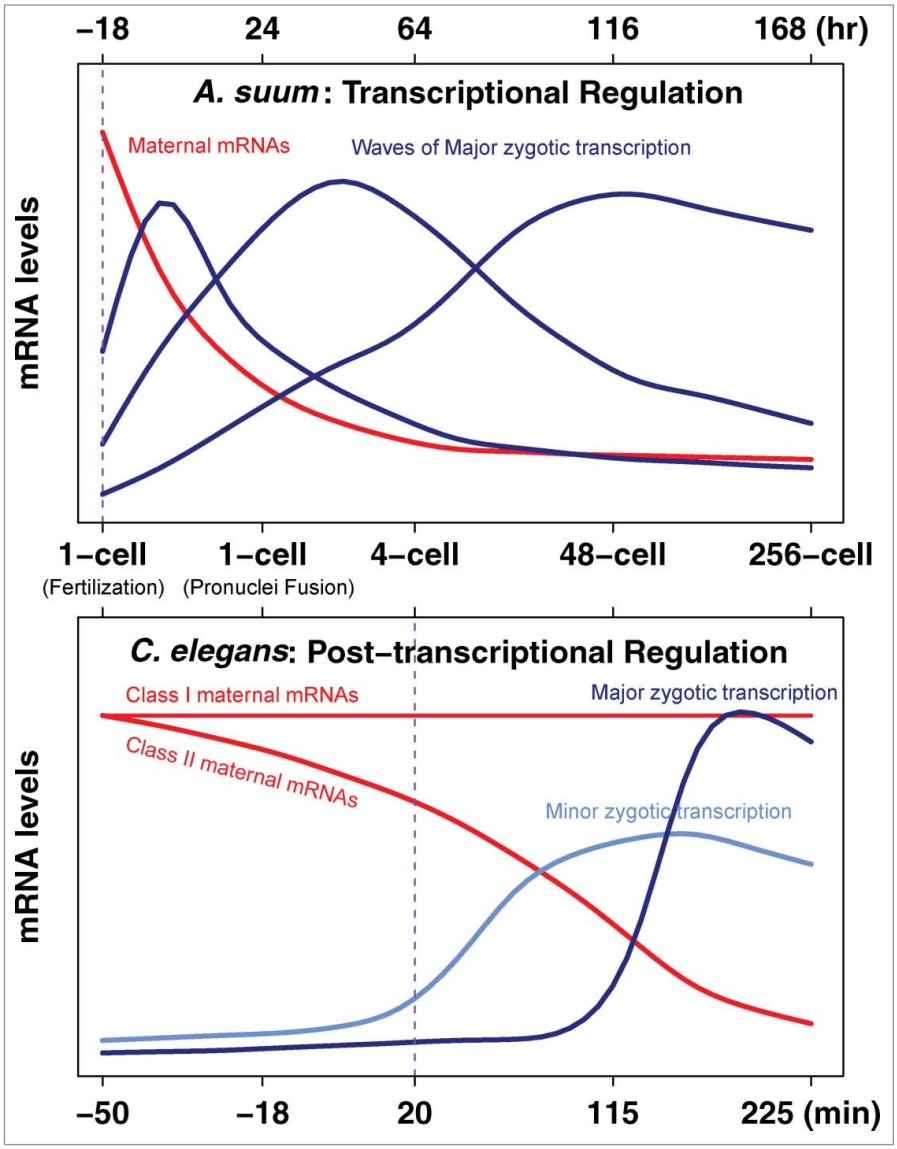

Figure 1. Rewiring of nematode gene expression program during early development. In *A. suum*, maternal mRNA degradation and zygotic transcription initiates immediately after fertilization and continues prior to pronuclear fusion. Thus, the maternal-to-zygotic transition in *A. suum* occurs right after fertilization (vertical purple line). Very little maternal RNA remains after the 4-cell stage suggesting that de novo transcription drives *A. suum* early development. In contrast, the *C. elegans* maternal-to-zygotic transition starts at ~4-cell stage (vertical purple line) and major transcription does not occur until ~100 cells. Maternal mRNA levels remain high during *C. elegans* early development and differential gene expression is post-transcriptionally regulated.

The lack of transcriptional activity during early development has been explained by several non-mutually exclusive mechanisms (see reviews[14,38]), including: (1). There is an excess of a repressor that maintains the repressed chromatin status. This repressor is diluted out during developmental cleavages to re-activate transcription (the "excess repressor model"); (2). The transcription machinery is incomplete and the missing components need to be expressed in development to assemble the functional transcription complex (the "limited machinery model"); (3). There is a maternal clock that is triggered by egg activation or fertilization that is independent of cell cycle and nucleo-cytoplasmic ratio but dependent on the absolute time of development (the "maternal clock model"); (4). The chromatin from early cell stages is not competent or ready for transcription (the "incompetent chromatin model"); and (5). Rapid cell cycle and DNA replication without G1 and G2 during embryo cleavage leads to transcript abortion (the "rapid cell cycle model"). Since major transcription is seen before pronuclear fusion and in 1- to 4-cell embryos in *A. suum* and mammals, this argues against the excess repressor model, the limited machinery model, and the incompetent chromatin model in these

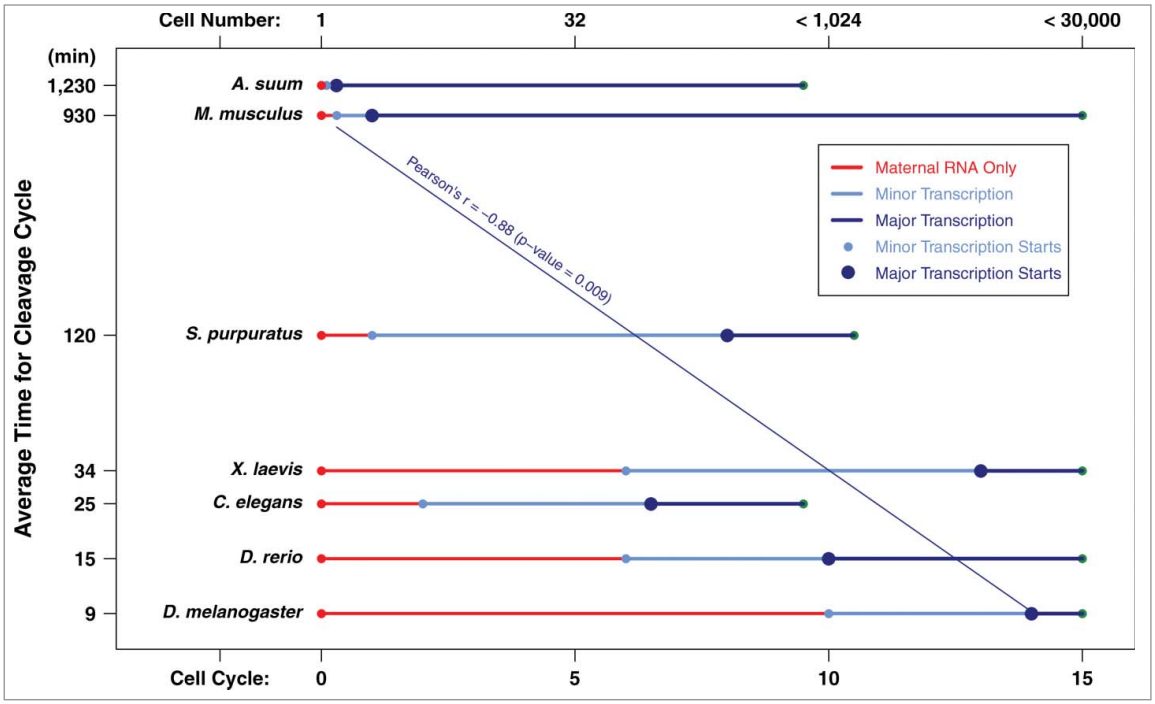

Figure 2. Zygotic genome activation is correlated with early development cell cycle length. The average cell-cycle length for each organism's early cell divisions was calculated from 1 to 128 cells. The cell cycle (labeled at the bottom) and cell number (labeled at the top) for minor and major transcription in model organisms were derived from Tadros and Lipshitz.[14] Data for *A. suum* was from our recent study.[32] Note the y-axis is in log scale.

organisms. While a maternal clock cannot be ruled out, the correlation between zygotic genome activation and cell cycle length (**Fig. 2**) strongly suggests organisms with longer cell cycles transcribe significantly more genes during early development. Consistent with this, significant transcription occurs in fast-developing animals when the cell cycle length increases during late cleavage and gastrulation. In addition, there appears to be a bias for transcription of relatively short genes (such as intron poor or intronless genes) in these organisms.[23] Thus, one determining factor for the overall level of transcription in early development may be related to the time available for the genome to be accessed by transcription machinery, supporting the rapid cell cycle model.

The Balance Between Maternal Deposition, Transcriptional and Post-transcriptional Regulation During Development

The amount of maternal deposition and the relative contributions of transcriptional versus post-transcriptional regulation to early development are ultimately determined by the life cycles and the biology of the organisms. Adaptions for massive maternal contribution during meiosis seem to have co-evolved with the requirement for the maternal deposition. For example, the late onset of transcription during *Xenopus* embryogenesis requires a large amount of maternal deposition and complex gene regulation during development. To achieve this, *Xenopus* has evolved months-long diplotene stages, developed an rRNA gene amplification mechanism,[3] and diverse mechanisms of post-transcriptional gene regulation during oogenesis and embryo development.[39,40] Increasing evidence suggests that paternal contributions may also play an important role during early development.[41] With the advancement of new technologies enabling the use of limited amounts of samples (single-cell technology) and the whole genome identification of nascent transcription (such as GRO-seq and RNA polymerase II ChIP-seq), additional examples of the dynamic balance between maternal deposition, post-transcriptional regulation, and new transcription are likely to be defined at high-resolution in various organisms. Recent studies have also demonstrated the importance of transcriptional regulation in embryonic stem cell maintenance and its differentiation in early development in mammals.[42] Transcription is regulated by the dynamic structural changes of chromatin, such as histone modifications, DNA methylation, and chromatin remodeling, that effect access of RNA polymerase and general and specific transcription factors.[43] With differences in the timing and contributions of transcription in early development, chromatin regulatory mechanisms must also change, be dynamic, and be specifically adapted to each organism. Understanding the balance and interplay of transcriptional and post-transcriptional regulation during early development will shed new light on one of the most complex transformations in biology: the fusion of oocyte and sperm and subsequent development to generate a new life.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Note

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