miRNA Regulatory Ecosystem in Early Development

Yuka W. Iwasaki[1] and Haruhiko Siomi[1,*]
[1]Department of Molecular Biology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku, Tokyo 160-8582, Japan
*Correspondence: awa403@z2.keio.jp
http://dx.doi.org/10.1016/j.molcel.2014.11.010

MicroRNAs (miRNAs) reshape spatiotemporal gene expression by both modulating the levels of actively transcribed genes and accelerating the clearance of previously transcribed messages, thereby promoting the transition from a preceding stage to subsequent processes during development. Lee et al. (2014) now demonstrate that maternal miRNAs are adenylated by Wispy, which leads to clearing of maternal miRNAs during early embryogenesis.

> I knew the moment had arrived
> For killing the past and coming back to life
>
> —David Gilmour (*The Division Bell*)

Maternally deposited mRNAs are involved in early animal development. Maternal mRNAs are translationally activated by egg activation following fertilization, which correlates with an increase in the length of their poly(A) tails. Subsequently, maternal mRNAs are destroyed, and genetic control is switched from the maternal genome to the zygotic genome, in an early embryogenesis process called the maternal-to-zygotic transition (MZT) (Giraldez 2010). Two degradation pathways function to eliminate maternal mRNA. First, maternal factors initiate the destruction of a subset of maternal mRNAs, which enables zygotic transcription. Second, de novo zygotic transcription of microRNAs (miRNAs) clears maternal mRNAs by promoting their deadenylation. For example, in *Drosophila*, the maternally provided RNA-binding protein Smaug directs the degradation of a subset of maternal transcripts following egg activation. Smaug does this by recruiting the CCR4/POP2/NOT deadenylase complex to its target transcripts, which induces poly(A) tail shortening and transcript destruction (Semotok et al., 2005; Benoit et al., 2009). This Smaug-mediated clearance of maternal mRNAs activates the second and more rapid phase of maternal mRNA clearance by enabling zygotic expression of miRNAs (Bushati et al., 2008). Zygotic transcription may be activated following the destruction of maternal mRNA by the subsequent downregulation of maternal proteins that repress the zygote genome (Figure 1).

miRNAs are typically produced from long primary transcripts by two sequential processing reactions. The first process includes the enzyme Drosha, which is found in the nucleus, producing pre-miRNAs. The second process consists of the enzyme Dicer, which is found in the cytoplasm, generating mature miRNAs. Following Dicer processing, miRNAs are loaded onto Argonaute proteins to form miRISCs, which control mRNA expression by promoting degradation or repressing translation. Thus, miRNAs play an important role in establishing specific cellular states by shaping specific transcriptomes and proteomes. A large fraction of miRNAs exhibit developmental, stage-specific, and tissue-specific expression patterns, which often involves posttranscriptional regulation of miRNA production and turnover (Ha and Kim, 2014). One pathway that controls the abundance and/or activity of some miRNAs involves the nontemplated addition of nucleotides (i.e., not found in genomic sequences) to their 3′ ends. This is performed by members of the Pol β superfamily, including poly(A) polymerases (PAPs), noncanonical PAPs, and terminal uridylyl transferases (TUTs) with distinct nucleotidyl transferase activities and biological functions. The consequences of nucleotide addition to the 3′ ends of miRNAs varies and depends on substrates, numbers (mono or oligo) and modifications (adenylation or uridylation) of added nucleotides. For example, oligouridylation of pre-let-7 by TUT4 induces its degradation in embryonic stem cells, whereas monouridylation by the same enzyme promotes processing by Dicer in somatic cells.

The nontemplated 3′ nucleotide additions occur only on specific miRNAs and are cell-type and developmental-stage specific. It has been shown that in early fly embryos, a large number of miRNAs exhibit nontemplate 3′ end additions of adenosines (Fernandez-Valverde et al., 2010). Deep sequencing performed by Lee and colleagues (2014) also showed that over 30% of miRNAs from *Drosophila*-activated eggs exhibited nontemplate 3′ end adenosine additions. However, adenylation was found to be reduced in early embryos, which suggests the adenylation is restricted to maternal miRNAs. This dynamic adenylation pattern during early embryogenesis was also evident in sea urchin and mice.

To identify the enzyme responsible for maternal miRNA adenylation, Lee et al. (2014) examined expression patterns of several PAPs in *Drosophila* and found that Wispy (Wisp), a noncanonical PAP, is highly expressed in egg cells and early embryos and decreases following zygotic genome activation. Wisp mutant eggs have decreased adenylation of miRNAs and subsequently increased miRNA levels. Upregulating Wisp in cultured S2 cells results in increased adenylation with a concomitant reduction of mature miRNAs, without modification or accumulation of pre-miRNAs. Consistent with this, close examination of individual sequencing reads show that both the 5p and 3p strands of miRINAs are adenylated. This indicates that Wisp acts downstream of Dicer processing, which exposes the 3′ end of the 5p strand.

Previous studies showed that Wisp is required for poly(A) tail elongation of several maternal mRNAs upon egg activation, which initiates the translation of

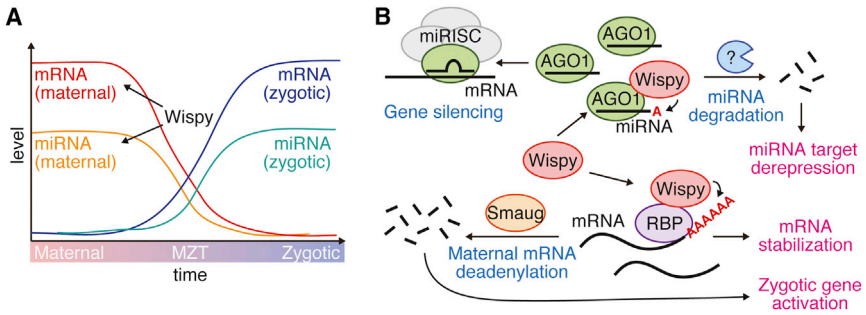

Figure 1. Function of Wispy during Maternal-to-Zygotic Transition

(A) General diagram of maternal and zygotic mRNA and miRNA levels during the MZT. Wispy plays a role in maternal clearance of transcripts and activation of zygotic translation.

(B) miRNA and mRNA levels are regulated by Wispy-mediated adenylation. Wispy associates with AGO1, leading to adenylation of miRNAs. Adenylation triggers degradation of miRNAs by an unknown nuclease, resulting in depression of miRNA target genes. AGO1-miRNA complexes (miRISCs), which are unrecognized by Wispy, are capable of silencing their target genes. Wispy, together with RNA-binding proteins (RBP), polyadenylates some population of maternal mRNAs and stabilizes them. However, some adenylated mRNAs are recognized by a translational repressor, Smaug, which results in deadenylation and destabilization of the mRNA. Consequently, regulation of maternal mRNAs leads to activation of zygotic genes.

these mRNAs (Cui et al., 2008). Because Wisp lacks an RNA-recognition motif, it is recruited to target maternal mRNAs through direct interaction with RNA-binding proteins, including Bicaudal-C. Lee et al. (2014) now demonstrate that Wisp physically interacts with AGO1, on which miRNAs are loaded, to form miRISCs after Dicer processing (Okamura et al., 2004). Wisp directly adenylates AGO1-associated miRNAs. These findings suggest that Wisp has two functions in the activation of zygotic transcription: it triggers the clearance of specific maternal miRNA subpopulations by adenylating them during MZT and stabilizes some maternal mRNA by poly(A) elongation. Together with maternal mRNA clearance, these functions activate zygotic genome transcription (Figure 1).

miRNA adenylation by GLD2, a possible Wisp ortholog, is required to stabilize miRNAs in somatic cells (Katoh et al., 2009; D'Ambrogio et al., 2012). This function is the exact opposite to the proposed role of Wisp. In addition to causing miRNA destruction, Wisp is also required for maternal mRNA poly(A) tail elongation upon egg activation. Therefore, if there is a limited amount of Wisp, competition between miRNA destruction and mRNA poly(A) elongation must occur. The study by Lee et al. (2014) raises several important questions, including how miRNA adenylation leads to opposite effects in different cell types, how Wisp is regulated to add long poly(A) tails on mRNAs and mono or oligo adenines on miRNAs, and how specific miRNAs are selected for adenylation given that Wisp adenylates miRNAs through direct association with AGO1.

REFERENCES

Benoit, B., He, C.H., Zhang, F., Votruba, S.M., Tadros, W., Westwood, J.T., Smibert, C.A., Lipshitz, H.D., and Theurkauf, W.E. (2009). Development *136*, 923–932.

Bushati, N., Stark, A., Brennecke, J., and Cohen, S.M. (2008). Curr. Biol. *18*, 501–506.

Cui, J., Sackton, K.L., Horner, V.L., Kumar, K.E., and Wolfner, M.F. (2008). Genetics *178*, 2017–2029.

D'Ambrogio, A., Gu, W., Udagawa, T., Mello, C.C., and Richter, J.D. (2012). Cell Rep. *2*, 1537–1545.

Fernandez-Valverde, S.L., Taft, R.J., and Mattick, J.S. (2010). RNA *16*, 1881–1888.

Giraldez, A.J. (2010). Curr. Opin. Genet. Dev. *20*, 369–375.

Ha, M., and Kim, V.N. (2014). Nat. Rev. Mol. Cell Biol. *15*, 509–524.

Katoh, T., Sakaguchi, Y., Miyauchi, K., Suzuki, T., Kashiwabara, S., Baba, T., and Suzuki, T. (2009). Genes Dev. *23*, 433–438.

Lee, M., Choi, Y., Kim, K., Jin, H., Lim, J., Nguyen, T.A., Yang, J., Jeong, M., Giraldez, A.J., Yang, H., et al. (2014). Mol. Cell *56*, this issue, 696–707.

Okamura, K., Ishizuka, A., Siomi, H., and Siomi, M.C. (2004). Genes Dev. *18*, 1655–1666.

Semotok, J.L., Cooperstock, R.L., Pinder, B.D., Vari, H.K., Lipshitz, H.D., and Smibert, C.A. (2005). Curr. Biol. *15*, 284–294.