in the endosomal trafficking and retrieval of Atp7b to the *trans*-Golgi network. Intriguingly, increasing copper concentrations disrupt this AP-1 CCV interaction, suggesting additional molecular mechanisms whereby Atp7b maintains intracellular copper homeostasis. Further work should now focus on linking these observations to develop a clearer picture of how these copper-containing compartments are maintained in the late secretory pathway, what the precise molecular nature is of the copper within the compartments, and what role if any Atp7b plays in this process directly at the site of biliary excretion. Although the authors suggest that there may be differences in excretion mechanisms dependent upon the hepatic copper content, physiological data suggest that this process is most likely a continuum that is constantly adapting to changes in intracellular copper to maintain physiologic organismal homeostasis.

While providing new insight into the cellular pathways affected in Wilson disease, the true importance of this work is in the broad recognition that a unique aspect of late endosome/lysosome function can be adapted in a highly differentiated cell to permit the regulation of homeostasis of a specific metal. This is consistent with our evolving understanding of the specialized roles of the late secretory pathway and is unlikely to be the only example of such a process in the liver, an organ that is critical for the metabolism and excretion of a myriad of metabolites and xenobiotics. As such, the observations of Polishchuk et al. (2014) suggest an exciting new venue for investigation into the role of the late secretory pathway in hepatic specific cell biological processes and the pathophysiology of disease; these observations also have important therapeutic implications for understanding the mechanisms resulting in the stimulation of hepatic lysosomal exocytosis.

REFERENCES

Appelqvist, H., Wäster, P., Kågedal, K., and Öllinger, K. (2013). J. Mol. Cell Biol. *5*, 214–226.

Gitlin, J.D. (2003). Gastroenterology *125*, 1868–1877.

Hirst, J., Borner, G.H., Antrobus, R., Peden, A.A., Hodson, N.A., Sahlender, D.A., and Robinson, M.S. (2012). Curr. Biol. *22*, 1711–1716.

Holloway, Z.G., Velayos-Baeza, A., Howell, G.J., Levecque, C., Ponnambalam, S., Sztul, E., and Monaco, A.P. (2013). Mol. Biol. Cell *24*, 1735–1748, S1–S8.

Ishizaki, H., Spitzer, M., Wildenhain, J., Anastasaki, C., Zeng, Z., Dolma, S., Shaw, M., Madsen, E., Gitlin, J., Marais, R., et al. (2010). Dis. Model. Mech. *3*, 639–651.

Madsen, E., and Gitlin, J.D. (2007). Annu. Rev. Neurosci. *30*, 317–337.

Martinelli, D., and Dionisi-Vici, C. (2014). Ann. N Y Acad. Sci. *1314*, 55–63.

Polishchuk, E.V., Concilli, M., Iacobacci, S., Chesi, G., Pastore, N., Piccolo, P., Paladino, S., Baldantoni, D., van IJzendoorn, S.C.D., Chan, J., et al. (2014). Dev. Cell *29*, this issue, 686–700.

Emerging from the Clouds: Vasa Helicase Sheds Light on piRNA Amplification

Julie M. Claycomb[1,*]
[1]Department of Molecular Genetics, University of Toronto, Toronto, ON M5S 1A8, Canada
*Correspondence: julie.claycomb@utoronto.ca
http://dx.doi.org/10.1016/j.devcel.2014.06.009

Reporting in a recent study in *Cell*, Xiol and colleagues (2014) identify the conserved DEAD box helicase Vasa as a platform for secondary Piwi-interacting RNA (piRNA) biogenesis in the insect nuage. This study represents a substantial breakthrough in understanding the mechanism and location of piRNA amplification by the "ping-pong" cycle.

Small RNA pathways have long been hailed as guardians of the genome and are thought to carry out this role by repressing deleterious transcripts. Perhaps in no tissue is this activity more important than in the gonad, which holds the potential to dictate the integrity of a species. In animal gonads, the Piwi-interacting RNA (piRNA) pathway is the major small RNA-mediated protector of the genome. This pathway utilizes a particular subset of Argonaute effectors, the Piwi clade, that are guided by a diverse repertoire of small RNA sequences, the piRNAs (24–30 nt), to silence transposable elements (TEs). piRNA regulation of TEs occurs at both the transcriptional and posttranscriptional levels and aims to prevent devastating effects due to TE mobilization, such as mutations in protein coding genes and double-strand breaks that activate the DNA damage response and lead to sterility (for review, see Luteijn and Ketting, 2013). While the importance of the piRNA pathway is readily evident in animals, the mechanisms by which piRNAs are produced have remained relatively elusive, especially in comparison to other types of small RNAs, such as microRNAs or small interfering RNAs. In a landmark study published in *Cell*, Xiol et al. (2014) close key gaps in our understanding of where and how piRNAs are generated by making clever use of a mutation that fortuitously decreases the in vivo dynamics of Vasa helicase, a key factor in the piRNA pathway.

piRNA biogenesis begins in the nucleus with the transcription of clusters

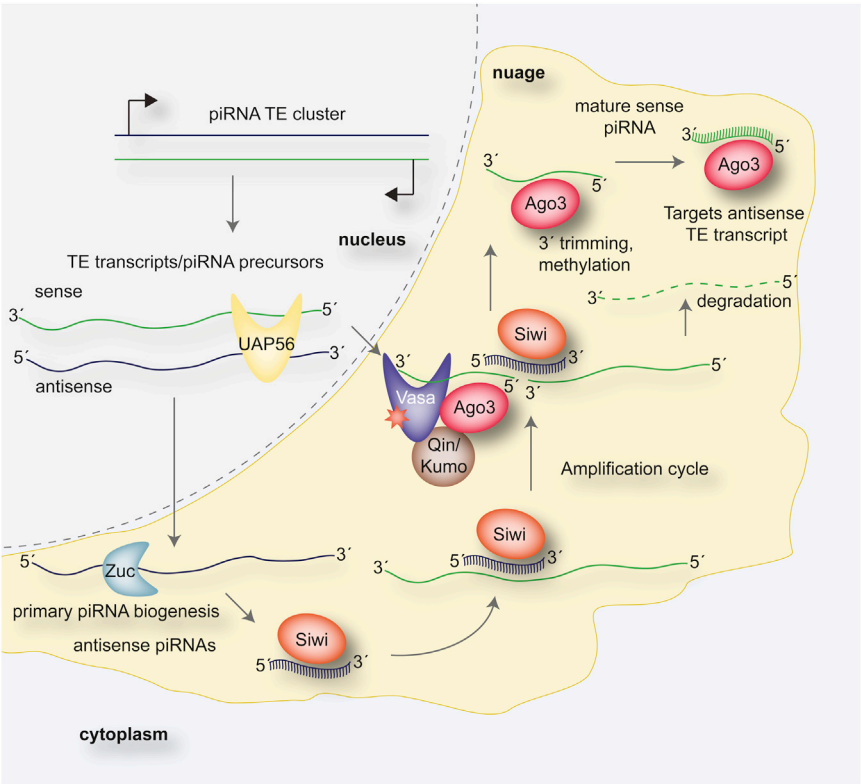

Figure 1. Model of the Amplifier Complex Function in piRNA Biogenesis
TE clusters are transcribed, and transcripts are exported with the aid of UAP56, then converted to primary antisense piRNAs by the nuclease Zucchini. Antisense piRNAs are loaded into Aub/Siwi complexes, which target sense TE transcripts (piRNA precursors). Vasa interacts with the sense piRNA precursor and Ago3, Qin/Kumo, and Siwi. Siwi slices the piRNA precursor, liberating a portion for degradation and creating a piRNA intermediate associated with Ago3. Ago3 and mature sense piRNAs (trimmed and methylated) then target antisense transcripts in a similar manner during the ping-pong cycle.

of TE fragments (piRNA clusters). These transcripts are directed to perinuclear germ granules known as nuage (French for "cloud") in *Drosophila*. Germ granules such as nuage are ribonucleoprotein (RNP) organelles that are nonmembrane bound and that play key roles in germline identity and metabolism (for review, see Voronina et al., 2011). The majority of factors involved in the piRNA pathway localize to nuage, suggesting that these serve as sites of piRNA biogenesis. One arm of the piRNA biogenesis pathway utilizes TE transcripts to generate piRNAs discretely, while a second arm employs two Argonautes, Aubergine (Aub in *Drosophila*, Siwi in silkworm) and Ago3, with complementary TE transcripts (sense and antisense) to amplify piRNAs. This feedforward mechanism, called the ping-pong cycle, leads to the selective amplification of particular piRNA sequences to rally a cellular adaptive immune response and combat rogue TEs (Figure 1) (for review, see Siomi et al., 2011).

There are a number of outstanding questions regarding the ping-pong cycle and the molecular mechanisms of piRNA amplification. In particular, during this cycle, Aub/Siwi is loaded with antisense piRNAs and cleaves the sense strand of a complementary target in order to generate a substrate for the production of a sense piRNA that is loaded into Ago3. How Aub/Siwi ensures that the piRNA precursor is cleaved only once, without degrading the entire transcript (as is the outcome during RNAi), is a lingering question. Likewise, how a handoff between Ago3 and Aub/Siwi is orchestrated has remained a mystery. Making use of a silkworm cell line and a mutation in the Vasa DEAD motif (DQAD) that abrogates its ATPase activity, Xiol et al. (2014) now provide answers to these two questions.

Vasa protein localizes to nuage in *Drosophila* egg chambers (Wilsch-Bräuninger et al., 1997) and similarly in silk worm cells, as revealed by the authors' live-imaging analysis. Wild-type silkworm Vasa exhibited a dynamic localization within nuage-like particles. Notably, Vasa^{DQAD} altered the dynamics of nuage, leading to the retention of the normally cytoplasmic protein, Siwi, and to the formation of larger nuage-like particles. Immunoprecipitation of Vasa^{DQAD} recovered Ago3, Siwi, and Qin/Kumo (a Tudor domain protein implicated in the piRNA pathway), interactions that are not observed with wild-type Vasa. Thus, while wild-type Vasa is part of dynamic complexes, Vasa^{DQAD} is trapped in a conformation that enabled an in vivo snapshot of its activity in the piRNA pathway. These observations pointed to Vasa as a "biochemical platform" for uniting key components of the piRNA amplification machinery (the Amplifier complex) in nuage.

How does Vasa function in piRNA amplification? The authors found that 27–30 nt piRNAs antisense to TE, and previously shown to be associated with Siwi complexes, are bound to Vasa^{DQAD} complexes. A second population of ~12 nt RNAs derived from TE sense transcripts was also associated with Vasa^{DQAD}, and sequencing of longer RNA associated with the complex also confirmed sense-oriented TE transcripts. These observations implicated Vasa mechanistically in the handoff of piRNA intermediates between Ago3 and Siwi.

Using an elegant artificial piRNA precursor, the authors were able to unambiguously identify piRNA intermediates within the Amplifier complex. This piRNA precursor combined GFP sequences with target sites complementary to abundant antisense piRNAs. The artificial precursor associated with Vasa^{DQAD} complexes and gave rise to piRNAs that associated with Ago3 (sense) and Siwi (antisense). Importantly, RNA cleavage products identified in the Amplifier complex corresponded to the (sense) piRNA precursor. In fact, the piRNA precursors were cleaved at sites to which antisense piRNAs (associated with Siwi) were complementary. Furthermore, the cleaved intermediate overlapped with Ago3 piRNA sequences, indicating that it could give rise to mature piRNAs. Structural studies of the Vasa^{DQAD} helicase domain pointed to a model whereby the helicase domain of Vasa binds Ago3 and Siwi when it is

clamped onto piRNA precursors. Siwi cleavage liberates two RNA fragments, one of which is the piRNA intermediate, and ATP hydrolysis by Vasa subsequently triggers the handoff and release of the two cleaved products. In summary, this work illuminates Vasa as the stage on which the cleavage activity of Siwi is coordinated with the loading of a piRNA transcript into Ago3.

The work of Xiol et al. (2014) is a great leap forward in our understanding of the piRNA amplification cycle and provides exciting prospects for dissecting other small RNA pathways. Vasa is a member of the largest class of RNA helicases, and a number of other DEAD box helicase family members have been implicated in small RNA pathways across various species (for review, see Linder and Jankowsky, 2011). Could this particular role for DEAD box helicases, or Vasa in particular, be conserved in small RNA processing in other organisms? Other recent work has demonstrated roles for three DEAD box helicases that localize to RNA granules in the germline 22G-small RNA pathway and exogenous RNAi in *Caenorhabditis elegans* (Phillips et al., 2014; Yang et al., 2014; Shirayama et al., 2014). 22G-RNAs differ from piRNAs in their amplification mechanism, but nonetheless, specific DEAD box helicases could be utilized in a manner similar to Vasa to load Argonaute in germ granules. Finally, their association with nuclear pores places nuage and other perinuclear RNP granules at an unparalleled location for sorting and directing transcripts into various small RNA machineries as they exit the nucleus. Thus, these studies forecast a deluge of discoveries unraveling the molecular mechanisms by which DEAD box helicases and germ granules coordinate target specificity and small RNA amplification in multiple small RNA biogenesis pathways.

REFERENCES

Linder, P., and Jankowsky, E. (2011). Nat. Rev. Mol. Cell Biol. *12*, 505–516.

Luteijn, M.J., and Ketting, R.F. (2013). Nat. Rev. Genet. *14*, 523–534.

Phillips, C.M., Montgomery, B.E., Breen, P.C., Roovers, E.F., Rim, Y.S., Ohsumi, T.K., Newman, M.A., van Wolfswinkel, J.C., Ketting, R.F., Ruvkun, G., and Montgomery, T.A. (2014). Curr. Biol. *24*, 839–844.

Shirayama, M., Stanney, W., 3rd, Gu, W., Seth, M., and Mello, C.C. (2014). Curr. Biol. *24*, 845–851.

Siomi, M.C., Sato, K., Pezic, D., and Aravin, A.A. (2011). Nat. Rev. Mol. Cell Biol. *12*, 246–258.

Voronina, E., Seydoux, G., Sassone-Corsi, P., and Nagamori, I. (2011). Cold Spring Harb. Perspect. Biol. *3*, pii: a002774.

Wilsch-Bräuninger, M., Schwarz, H., and Nüsslein-Volhard, C. (1997). J. Cell Biol. *139*, 817–829.

Xiol, J., Spinelli, P., Laussmann, M.A., Homolka, D., Yang, Z., Cora, E., Couté, Y., Conn, S., Kadlec, J., Sachidanandam, R., et al. (2014). Cell *157*, in press. Published online June 5, 2013.

Yang, H., Vallandingham, J., Shiu, P., Li, H., Hunter, C.P., and Mak, H.Y. (2014). Curr. Biol. *24*, 832–838.