Developmental Cell
Previews

Copper Homeostasis: Specialized Functions of the Late Secretory Pathway

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http://dx.doi.org/10.1016/j.devcel.2014.06.002

Differentiated cells have evolved mechanisms to adapt the functions of the late secretory pathway to the specific needs of the organism. Reporting in this issue of *Developmental Cell*, Polishchuk et al. (2014) demonstrate that hepatocytes utilize a unique exocytic aspect of the late endosomal/lysosomal compartment to maintain organismal copper homeostasis.

Although originally characterized as a specialized digestive compartment of the cell, lysosomes are now recognized to have critical roles in membrane trafficking, endocytosis, and intracellular protein sorting. As might be anticipated from these essential functions in cellular physiology, these organelles are also involved in the pathophysiology of numerous metabolic and storage diseases. Within differentiated cell types, lysosomes assume unique roles specific to the functions of organs or tissues, for example pigment formation in melanosomes (Appelqvist et al., 2013). Within the late secretory pathway, lysosomes participate in highly specialized functions such as bone resorption and extracellular signaling through a process of exocytosis that involves fusion with the plasma membrane. In this issue of *Developmental Cell*, Polishchuk and colleagues (2014) show that hepatocytes utilize this unique exocytic aspect of the late endosomal/lysosomal compartment to maintain organismal copper homeostasis.

Copper is an essential transition element that plays a fundamental role as a cofactor in a select number of enzymatic pathways essential for cellular respiration, iron homeostasis, pigment formation, neurotransmitter production, peptide biogenesis, connective tissue biosynthesis, and antioxidant defense. In mammals, copper homeostasis is maintained entirely by gastrointestinal absorption and biliary excretion. Absorbed copper is efficiently removed from the circulation by the liver, which regulates the storage and excretion of this metal. Biliary excretion is the predominant mechanism determining copper balance, and the amount of copper appearing in bile is directly proportional to the size of the hepatic copper pool. Hepatocytes are therefore the primary site of copper homeostasis as these polarized epithelial cells discern copper status and regulate copper excretion into the bile, depending on the intracellular concentration of this metal (Madsen and Gitlin, 2007).

The molecular mechanisms by which the hepatocyte determines copper homeostasis were clarified with the elucidation of the genetic defect in Wilson disease, a disorder of excess copper accumulation resulting from loss-of-function mutations in the copper-transporting ATPase Atp7b (Gitlin, 2003). Atp7b is localized to the *trans*-Golgi network of the hepatocyte, and, as the copper concentration increases, this protein moves from the *trans*-Golgi network to a cytoplasmic vesicular compartment, which until now has remained uncharacterized. Following sequestration of copper into this compartment by Atp7b, the concomitant decrease in cytosolic copper concentration initiates the return of this ATPase to the *trans*-Golgi network while the copper is discharged at the canalicular (apical) membrane. This novel posttranslational mechanism ensures that excess cytosolic copper will be excreted rapidly into the bile (Gitlin, 2003).

In this current study, Polishchuk et al. (2014) demonstrate that the vesicular compartment containing Atp7b resides within the late endosome/lysosome pathway. Specifically, the authors demonstrate that exposure of hepatocytes to increasing copper concentrations results in direct trafficking of Atp7b from the *trans*-Golgi network to a specific subset of organelles with molecular characteristics of lysosomes, including LAMP1 and CD63. Increasing the intracellular copper concentration resulted in apical (canalicular) localization of Atp7b in a polarized hepatocyte cell line with concomitant lysosomal exocytosis, suggesting that this exocytic process may serve as the primary mechanism for copper excretion into the bile. Consistent with this concept, activation of lysosomal exocytosis with the transcriptional regulator TFEB resulted in increased movement of Atp7b to the apical surface, whereas inhibition of exocytosis with mucolipin-1 decreased this trafficking. In these experiments, the authors measured lysosomal exocytosis utilizing a copper-sensitive dye that detects intracellular concentration of exchangeable copper. Furthermore, abrogating Atp7b expression in this polarized hepatocyte cell line revealed a direct role for Atp7b in copper import into lysosomes and in the apical exocytosis of copper. Additional studies suggest that the direct interaction between Atp7b and the p62 subunit of dynactin, a multi-subunit complex mediating dynein binding and vesicle transport along microtubules, may account for the Atp7b-mediated lysosomal apical trafficking.

Taken together, these new studies provide clear evidence for a direct role of the late endosomal/lysosomal pathway in mediating copper homeostasis through exocytosis at the biliary canalicular membrane. Importantly, these observations must now be placed in the context of recent genetic (Ishizaki et al., 2010; Martinelli and Dionisi-Vici, 2014) and cell biological (Holloway et al., 2013; Hirst et al., 2012) studies that reveal a direct role for clathrin-coated vesicles (CCV) and the adaptor protein 1 (AP-1) complex

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.

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

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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