

RNA-binding Proteins Required to Sort Small RNAs for Secretion in Exosomes from Human Cultured Cells

Randy SCHEKMAN

**Professor, Department of Molecular and Cell Biology, University of California, Berkeley;
Investigator, Howard Hughes Medical Institute;
2013 Nobel Laureate in Physiology or Medicine;
IAS Senior Visiting Fellow
Email: schekman@berkeley.edu**

Highly purified extracellular vesicles (EVs) isolated from human cell lines display a small number of substantially (~ 1000 fold) enriched miRNAs that differ from one cell line to another. In spite of the small number of such species, no single RNA sorting sequence is evident. In order to explore the mechanism of RNA sorting, we established a cell-free reaction that reproduces the selective incorporation of synthetic, mature miRNAs (miR223 and miR122) into vesicles formed in a reaction containing membranes and cytosol from mechanically disrupted HEK293 cells. The sorting reaction requires both membrane and cytosol and is stimulated by hydrolysable ATP and incubation at a physiologic temperature. Using biotinylated derivatives of two different miRNAs, we found different sets of RNA binding proteins incorporated along with each species, among which the proteins Ybx1 and Lupus La are required to sort mir223 and miR122, respectively. EVs also contain more abundant major species of small RNA including full-length tRNA, Y-RNA and vault RNA, and each requires the Ybx1 protein for selective sorting into exosomes secreted by cells and into vesicles in the cell-free reaction. The La antigen binds to both 5' and 3' signals on miR122 to promote packaging of the RNA into vesicles in the cell-free vesicle biogenesis reaction.

References

Shurtleff MJ, Temoche-Diaz MM, Karfilis KV, Ri S, Schekman R (2016). [Y-box protein 1 is required to sort microRNAs into exosomes in cells and in a cell-free reaction](#). *Elife*. Aug 25;5. pii: e19276. doi: 10.7554/eLife.19276. PMID: 27559612

Shurtleff, M., Yao, J., Qin, Y, Nottingham, R., Temoche-Diaz, M., Schekman, R and Lambowitz, A. (2017) A broad role for YBX1 in defining the small non-coding RNA composition of exosomes *PNAS* 2017 October, 114 (43) E8987-E8995. <https://doi.org/10.1073/pnas.1712108114>

Shurtleff, M., Temoche-Diaz, M. and Schekman, R. (2018) Extracellular Vesicles and Cancer: Caveat Lector. *Ann. Rev. Cancer Biology* <https://doi.org/10.1146/annurev-cancerbio-030617-050519>

Temoche-Diaz, M., Shurtleff, M., Nottingham, R., Yao, J., Fadadu, R., Lambowitz, A. and Schekman R. (2019) Distinct mechanisms of microRNA sorting into cancer-derived extracellular vesicle subtypes <https://www.biorxiv.org/content/10.1101/612069v1>