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SMALL RNAS

RISCs hitch a ride

> MVBs are important sites of functional miRISC assembly.

Components of microRNA (miRNA)-mediated post-transcriptional silencing are known to congregate in cytoplasmic foci. New research published in *Nature Cell Biology* suggests that P bodies and GW bodies, cytoplasmic foci that were previously thought to be identical, can be differentiated by the fact that components of the RNA-induced silencing complex (RISC) present in GW bodies can be physically and functionally linked with vesicles of the endocytic pathway, whereas components of P bodies cannot.

Gibbings *et al.* showed that the RISC components Gly-Trp protein of 182 kDa (GW182) and Argonaute 2 (AGO2) are present in GW bodies, and that GW182 is highly enriched in purified secreted vesicles called exosomes, whereas P body components are barely detectable in exosomes. Given that exosomes are secreted by late endosomal compartments known as multivesicular bodies (MVBs), the authors also tested early and late endosomal fractions. They found that GW182 and AGO2 are consistently associated with MVBs, whereas P body components are absent. These results suggest that GW bodies are often associated with MVBs and are distinct from P bodies.

Importantly, mature miRNAs and their target mRNAs also localize to MVBs, but not to exosomes, which raises the possibility that MVBs are sites of miRNA-loaded RISC (miRISC) assembly and/or function. Knockdown of components of the endosomal sorting complex required for transport (ESCRT) machinery (which is responsible for protein sorting into MVBs) causes GW182 to accumulate and compromises miRNA activity. These findings suggest that the sorting of GW182 into MVBs is important for miRNA-mediated gene silencing.

An independent study by Lee *et al.* suggests that the sorting of GW182 into MVBs is important for the efficient loading of miRNAs or small interfering RNAs (siRNAs) onto the *Drosophila melanogaster* Ago proteins. The initial observation that led to this conclusion was the identification of the Hermansky–Pudlak syndrome 4 (*Hps4*) gene in a screen for factors that enhance siRNA-mediated gene silencing. HPS4 is a tethering factor that, when mutated, blocks MVB maturation into lysosomes ('turnover').

The authors showed that the loss of HPS4 also enhances siRNA-mediated gene silencing in human cells, and enhances miRNA-mediated silencing in both *D. melanogaster* and human cells, which correlates with the dispersal of miRISCs from lysosomes. By contrast, blocking MVB formation by disabling the ESCRT machinery results in impaired miRNA silencing and correlates with the concentration of miRISCs in early endosomes. Together, these data suggest that MVBs are important sites of functional miRISC assembly.

By measuring the levels of miRNAs associated with AGO1 in wild-type cells versus HSP4-mutant cells, Lee and colleagues concluded that the loading of miRNA onto RISC is enhanced when MVB turnover is impaired. They found the same correlation between siRISC loading and MVB turnover, whereas siRISC loading was inhibited when MVB formation was blocked.

So, according to both studies, MVBs promote small RNA-mediated gene silencing, and one possible mechanism is that loading of small RNAs onto RISC is coupled to MVB formation. Whether small RNA-mediated gene silencing takes places at MVBs remains an open question.

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ORIGINAL RESEARCH PAPERS Gibbings, D. J. *et al.* Multivesicular bodies associate with components of miRNA effector complexes and modulate miRNA activity. *Nature Cell Biol.* 16 Aug 2009 (doi:10.1038/ncb1929) | Lee, Y. S. *et al.* Silencing by small RNAs is linked to endosomal trafficking. *Nature Cell Biol.* 16 Aug 2009 (doi:10.1038/ncb1930)

FURTHER READING Anderson, P. & Kedersha, N. RNA granules: post-transcriptional and epigenetic modulators of gene expression. *Nature Rev. Mol. Cell Biol.* **10**, 430–436 (2009)