**Introduction**

miRNA mediated gene silencing and ubiquitin mediated protein quality control represents two fundamental mechanisms that control proper gene expression. In this study we unexpectedly discovered that fly and human AGO proteins, key components in the miRNA pathway, undergo lipid-mediated phase separation and condense into RNP granules on the endoplasmic reticulum (ER) membrane to control protein production. Phase separation on the ER is mediated by electrostatic interactions between a conserved lipid binding motif within the AGOs and lipid PI(4,5)P2. ER-localised AGO condensates recruit the E3 ubiquitin ligase Ltn1 to catalyse nascent peptide ubiquitination and coordinate with the VCP-Ufd1-Npl4 complex to process unwanted protein products for proteasomal degradation. Collectively, our study provides insight into the understanding of transcription-translation coupling controlled by AGOs via “lipid-mediated phase separation.”

MicroRNAs (miRNAs), subset of small non-coding RNAs (~21 nt in length) play important roles in post-transcriptional gene expression and thus control diverse biological processes. miRNAs execute its functions by interacting with argonaute (AGO) proteins (e.g., dmAGO1 and hsAGO2) to form RNA induced silencing complex (RISC). RISCs then use miRNAs as guides to bind complementary RNAs in a sequence dependent manner to control mRNA repression and/or decay. Of note, within RISCs, the AGO-miRNA interaction affects miRNA hybridisation kinetics, thus instructing miRNAs to efficiently target mRNAs and thereby profoundly influencing wide variety of biological processes.

Protein quality control (PQC) pathways maintain protein homeostasis by eliminating unwanted proteins, thus controlling cellular fitness Defects in PQC have been linked to various diseases and is a hallmark of neurodegeneration. Ltn1, a conserved E3 ubiquitin ligase acts as a key component in the PQC pathway to ubiquitylate nascent peptides. AGO proteins appear to affect protein synthesis of its target mRNAs via translational repression, however, whether AGOs have a role in directly controlling newly-synthesized peptides at their birthplace remain unknown. Here we identified a conserved lipid-binding motif that specifically interacts with PI(4,5)P2 within the N domain of AGO proteins. Lipid-binding promotes AGOs to condense into phase separated granules on the ER membrane. The ER-localised AGO condensates thus recruits Ltn1 to catalyze nascent peptide ubiquitination and coordinate with the VCP–Ufd1–Npl4 complex to process unwanted protein products for proteasomal degradation. Our results suggests that AGOs on the ER can couple two fundamental cellular processes, post-transcriptional gene silencing and protein quality control, thus ensuring efficient gene silencing.

**Results**

In a effort to investigate the membrane function of AGO proteins, we used *Drosophila* AGO1 (dmAGO1) as a bait to perform a yeast two-hybrid screen, which led to identification of number of dmAGO1-interacting proteins. One candidate VCP (also known as Ter94), attracted our attention, because it is a ER membrane associated protein and it also interacts with dmAGO1. To validate the dmAGO1-VCP interaction, we preformed co immunoprecipitation (co-IP) experiments by using anti dmAGO1 and anti VCP antibodies and found that endogenous dmAGO1 and VCP could form a complex in S2 cells and ovaries. Further domain-mapping analysis revealed that dmAGO1 associated with VCP through its C terminus and VCP associated with dmAGO1 through their ATPase domains (D1 and D2 domains), suggesting that VCP is associated with dmAGO1 in a ‘domain dependent manner.’ Importantly results from cell based reporter assays suggest, that knockdown of *vcp* significantly enhanced the miRNA-mediated gene silencing activity. Of note, we found that knockdown of *vcp* alone had no effect on dmAGO1 stability and miRNA biogenesis. Given that VCP is an ER associated protein, we then tested whether *vcp* knockdown affects ER localisation of dmAGO1 We performed fractionation assays according to previously described methods and found that *vcp* knockdown significantly promoted the accumulation of dmAGO1 on the ER fraction, which was traced by endogenous BiP (an ER marker).

Microscopy imaging assays further supported that a potion of endogenous dmAGO1 could form granules to associate with the ER membrane and *vcp* knockdown promoted dmAGO1 accumulation on the ER. To test, whether the enhanced gene silencing activity induced by *vcp* knockdown is attributed to the increased levels of dmAGO1 on the ER, we generated an ER-localized form of dmAGO1, which enforces majority of the dmAGO1 (~70%) to localize on the ER membrane. Considering both dmAGO1 and hsAGO2 act in the miRNA pathway we also analyzed hsAGO2 in S2 cells and Hela cells and obtained consistent results. It is worth noting that like the behaviour of “wild type hsAGO2,” some of the proteins could also form granules partially overlapped with the ER membrane in Hela cells. These findings indicate that AGOs can efficiently regulate gene silencing on the ER.

**Discussion**

AGO proteins play principal roles in regulating small RNA mediated gene silencing. Interestingly, AGO proteins are present in cytoplasmic RNA granules and associated with membrane bound organelles (e.g., ER). However the functional importance of membrane associated AGO proteins has long been under estimated. In this study, we focussed on studying the membrane function of AGO proteins. Investigation of the *Drosophila* AGO1 revealed a mechanism where ER-localised dmAGO1 forms a complex with the E3 ubiquitin ligase Ltn1 to catalyse the ubiquitination of nascent peptides and then coordinates with the VCP–Ufd1–Npl4 complex to process unwanted new protein products, which are ultimately degraded by the proteasome. Thus, in addition to facilitating miRNA guided repression of RNA translation dmAGO1 also acts in concert with the ribosome quality control machinery to ensure efficient repression of gene products. Given that AGO proteins play evolutionarily conserved roles in gene expression our study provides a critical starting point toward mechanistic understandings of transcription-translation coupling controlled by AGO proteins.

It is well-documented that AGOs form a complex with miRNAs. In this study we uncovered a mechanism by which AGOs form a complex with Ltn1 on the ER. Although we identified more than one hundred high confident targets for dmAGO1/Ltn1, we did not analyze them in-depth in this study. The identified targets are of potential interest. For example a significant portion of targets of dmAGO1/Ltn1 is involved in the secretion pathway, which is consistent with previous findings. Interestingly, we also found that a number of targets of dmAGO1/Ltn1 is mitochondrion proteins. This finding raises a intriguing possibility that Ltn1-mediated protein quality control might contribute to the homeostasis and function of mitochondrion. It would be important to solve this issue in future studies.