Final Report

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Project acronym: miRNA mechanism

1. Final publishable summary report (max. 2 Pages)

Results and Conclusions:

microRNAs (miRNAs) have been implicated in a broad range of diseases, such as the development and progression of cancer. miRNAs are small RNAs that post-transcriptionally regulate gene expression in eukaryotes. In animals, miRNAs bind to complementary sites in messenger RNAs (mRNAs), causing translational repression and mRNA deadenylation and degradation. It has been proposed that miRNAs inhibit translation during the initiation phase of protein synthesis. However, the precise molecular mechanism is still unknown. This study has shed light on the interplay between miRNAs and the translation initiation machinery. mRNAs bearing a poly(A) tail are typically more strongly repressed by miRNAs than their counterparts lacking this 3' modification. Using a Drosophila cell-free system, we analyzed the role of the poly(A)-tail in miRNA-mediated repression in detail. We show that repression increases with an extension of the poly(A)-tail and that the poly(A)-tail contribution to miRNA-mediated repression occurs independently of the number of miRNA-binding sites in the mRNA. We demonstrate that this effect is mediated by the poly(A)-binding protein PABP, since poly(A)-tail contribution is lost in PABP-depleted extracts and can be rescued by addition of recombinant PABP. miRNAs function as part of ribonucleoprotein complexes, miRNPs, with Argonaute (Ago) and GW182 family proteins being the crucial components. The functional regions of GW182 have been mapped and it was shown that tethering the silencing domain (SD) of GW182 to an mRNA causes repression. We show here that tethering GW182-SD faithfully recapitulates all the hallmarks of miRNA-mediated repression in vitro. However, when translation is repressed by GW182-SD tethering rather than by miRISC itself, the stimulatory poly(A) effect is lost. Contrary to current models in which the micro RNA-induced silencing complex (miRISC) interferes with a positive function of PABP in translation, we show that repression positively correlates with poly(A) tail length. These findings are explained by a novel role of PABP as a co-repressor that facilitates the specific association of miRISC with microRNA-regulated mRNAs. Furthermore, we provide evidence

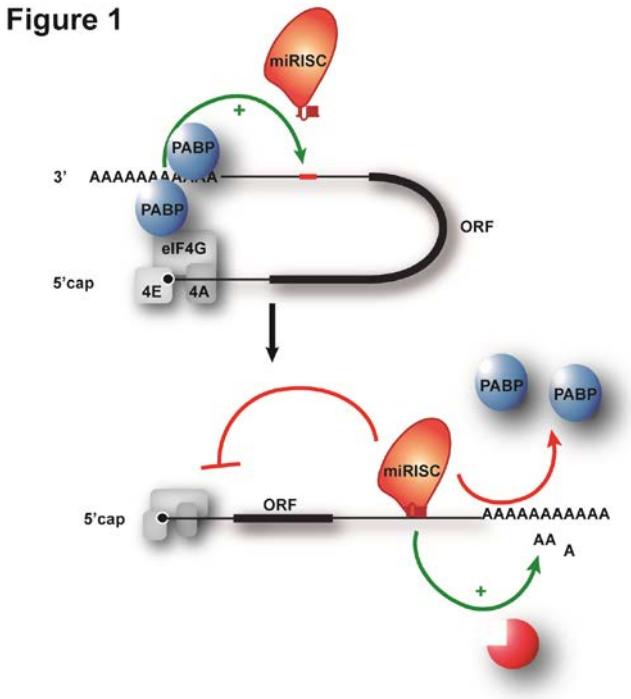

Figure 1. Based on our data we propose the following model: PABP and the poly(A) tail contribute to miRNA-mediated repression by stimulating the association of miRISC to the target mRNA. Upon binding of miRISC, PABP is displaced from the repressed mRNA. Displacement of PABP can be further enhanced through the deadenylation of the repressed mRNA and could contribute to translational repression by miRISC.

that PABP is displaced from a repressed RNA and that this displacement is an early event, which is further enhanced by deadenylation of the target RNA. Therefore, the **work which was funded by this fellowship has provided an important conceptual advancement in form of a revised model (see Figure 1) for the function of PABP and the poly(A) tail in micro RNA-mediated translational repression**.

Socio-Economic Impact:

This research project has a wide-range implication beyond gene regulation with an undeniable socio-economic significance for human health. miRNAs have been implicated in the establishment of various human diseases, such as cancer. However, the molecular mechanism by which they inhibit target gene expression is still incompletely understood, thereby stalling the development of novel diagnostic and therapeutic strategies. Our work has shed light on the interplay of miRISC with the pivotal translation initiation factor PABP. Thus, our work has enhanced our understanding of the fundamental molecular mechanism of miRNAs, and this in the long-term will extend the potential development of pharmaceutical applications to target diseases with aberrant miRNA function.