

Reversibility of microRNAs action and its implications for regulation of gene expression

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Abstract. miRNAs repress translation of their target mRNAs by base pairing with the mRNAs 3' UTR. miRNA repressed mRNAs are localized in P-bodies in human cells. Amino acid starvation induces expression of CAT-1 protein from the miR-122 repressed and P-body localized mRNAs in Huh7 cells and HuR protein is essential for stress induced expression of CAT-1. (www.actabiomedica.it)

Key words: miRNA, Stress, HuR, CAT-1, P-body

Introduction

MicroRNAs, 21-22 nt small RNAs, are negative regulators of gene expression in metazoa and plants. In animals, miRNA repress translation of target genes by base pairing with the mRNA 3'UTR (1). Recently, it has been shown that components of miRNA machinery are concentrated in P-bodies (PBs) (2, 3). Reporter mRNAs repressed by miRNAs also localize to these structures in human cells. PBs were originally discovered in yeast as the sites of mRNA degradation, but recent data (4) indicate that PBs also serve as storage site for translationally repressed mRNAs in yeast. The fate of miRNA repressed mRNAs localized to PBs is not clear. Likewise, it is not known whether miRNA-mediated repression is a reversible process and whether mRNAs localized in PBs can return to active translation in mammalian cells (Fig.1). Different forms of cellular stress lead to the increased expression of genes required for cellular survival under stress. In mammalian cells, expression of a large fraction of the stress induced genes including CAT-1, is also regulated at post-transcription level (5). In a human hepatocellular carcinoma cell line Huh7, expression of the cationic amino acid

transporter 1 (CAT-1), a miR-122 repressed message, was studied where stress induced expression of CAT-1 was associated with reversal of microRNA inhibition in Huh7 cells (6).

Material and methods

Huh7, a hepatocellular carcinoma cell line expressing miR-122, was used for CAT-1 RNA and

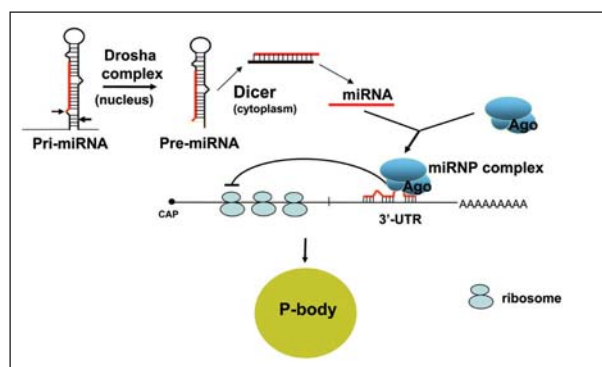


Figure 1. microRNA mediated repression and P-body localization of mRNAs in mammalian cells.

protein analysis. Renilla luciferase reporters with CAT-1 3'UTR were studied. For stress inductions, cells were incubated in amino acid free medium for hours. In situ analysis was performed for localization studies of CAT-1 mRNAs.

Outcomes

Our data demonstrate that a liver specific miRNA, miR-122, represses CAT-1 expression in a human hepatocellular carcinoma cell line Huh7. We further demonstrate that metabolic stress induces expression of the CAT-1 protein in Huh7 by relieving the CAT-1 mRNA from translational repression by miR-122. The CAT-1 mRNA localizes to PBs in a miR-122 dependent manner in Huh7 cells. The stress induced de-repression is accompanied by a re-localization of CAT-1 mRNA from PBs. In addition, it is associated with a recruitment of CAT-1 mRNA to polysomes, consistent with the miR-122 repressing the translation initiation. Chimeric reporter mRNAs with CAT-1 3'UTR showed similar behaviour in Huh7 cells. HuR, a member of ELAV family of proteins, was found to be essential for both derepression of translation and re-localization of CAT-1 mRNA from PBs in stressed cells, indicating that mRNA translation and stability may be regulated by miRNPs and ELAV proteins in a reciprocal way and be modulated by different physiological conditions in mammalian cells (6).

Conclusions

This work support the reversibility of microRNAs action in human cells where binding of HuR induces the relocalization and translation of a microRNA repressed message out of PBs in stressed condition. It also favors a model where multiple elements within the 3'UTR of an mRNA could cumulatively determine the fate of the mRNA by binding different

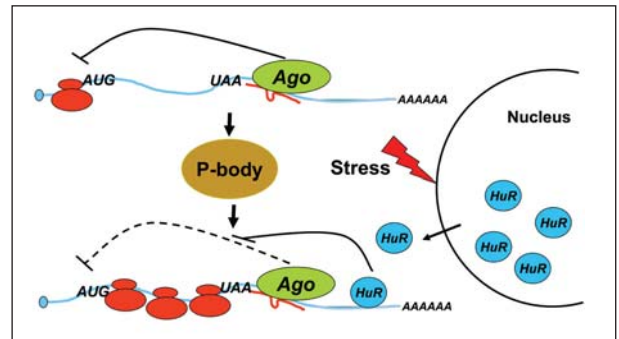


Figure 2. Reversal of microRNAs action in stressed cells

trans-acting factors in a co-operative or antagonistic way (Fig. 2).

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