Abstract

miRNAs are small regulatory RNAs, which function as post-transcriptional mRNA regulators. They direct ribonucleoprotein complexes to cognate mRNA to repress them by translational inhibition and degradation. miRNAs regulate thousands of mRNAs in mammals and have been recognized as regulatory factors in most cellular and developmental processes. Dysregulation of the miRNA pathway can lead to severe defects and diseases. Interestingly, a unique situation exists in mouse oocytes, where all the miRNA pathway components are present, yet the pathway is dispensable and nonfunctional, the molecular foundation of this phenomenon and its significance still remain unclear.

In spite of the pronounced effects of the miRNA pathway in gene regulation in somatic cells, study strategies of the pathway bare limitations. Current methods for studying the activity of the miRNA pathway employ corelative studies (such as NGS) or reporter assays, which have relatively low throughput and are prone to artifacts. Here, I present design and development of a new strategy for directly monitor global miRNA pathway activity and integrity in near physiological conditions in living cells, which could also be employed *in vivo* for studies of mouse oocytes. The strategy is based on fluorescently tagged endogenous proteins of the ribonucleoprotein complex AGO2 and TNRC6C, which would form a biosensor sensing formation and dynamics of the miRNA pathway effector complex. I designed CRISPR/Cas9-based knock-ins of fluorescent protein coding sequences and troubleshooted numerous obstacles. I managed to produce *Ago2* knock-in cell lines and developed an optimized strategy for knock-in of *Tnrc6c*.