From in silico to NMR in-cell studies, microRNA-34a and its targetome

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miRNAs (miRs) are small (~22 nucleotides, nt.) non-coding regulatory RNAs. One miR can target a variety of messengerr RNAs (mRNAs) and one mRNA can be the target of several miRs. This capacity to regulate gene expression is currently seen as a valid therapeutic approach.

miR-34a is a mediator of tumour suppression [1] with ~100 validated (reporter assay, qPCR, western blot) mRNA targets [2]. It is involved in different cancer-related processes, including proliferation, and metastasis, as well as differentiation and apoptosis [1].
miRs, loaded into the RNA induced silencing complex (RISC), bind mRNAs, inhibiting their translation or inducing degradation. The seed region (2-8 nt.) is crucial for mRNA selection (Figure 1a), but it is not yet fully understood how a single miR can modulate directly an entire network of different mRNA targets.

Our aim is to investigate the structures adopted by miR - mRNA complexes in cells, linking these structures to the function carried out in cells by these two binding partners (Figure 1b). In silico (MC-Fold) and biophysical [3] (e.g. Nuclear Magnetic Resonance) methods are used to predict and investigate secondary structures of miR - mRNA complexes in biologically relevant environments (in lysate or in cells) [4, 5]; molecular biology methods are used to elucidate the function - structure connection. Preliminary results indicate an uptake of our transcribed miR-34a by HEK293T, HeLa and neural stem (NE4C) cells. qRT-PCR and fluorescent probes are used for the quantification and visualization of miR-34a. We are currently optimizing parameters for different transfection methods and in different cell lines. Other experiments to detect the targeting of miR-34a are in progress.

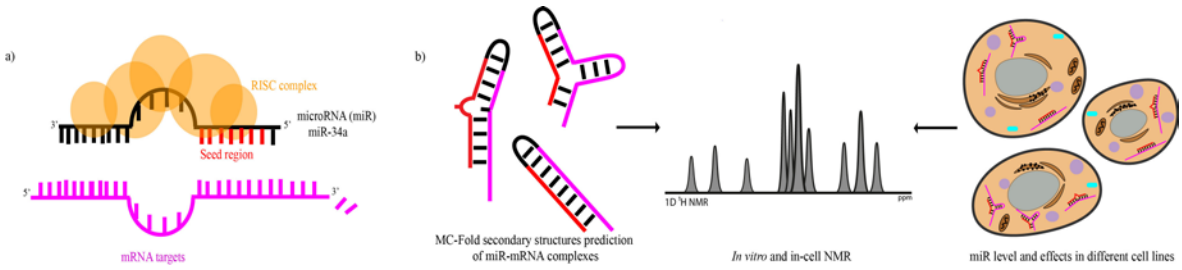

Figure 1: a) Scheme of miR - mRNA interaction, b) Iterative workflow to evaluate the structure and function relationship of miR - mRNA in cells.

References

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