Abstract

This thesis provides biophysical bases of the interactions between two proteins involved in microRNA (miRNA)-mediated silencing: CNOT1 and the silencing domain of GW182. The regulation of gene expression at the post-transcriptional level involves the crucial CCR4-NOT deadenylase complex, which deadenylates mRNA, and can also inhibit translation in an independent fashion. In miRNA-mediated silencing, the CCR4-NOT complex is brought into the vicinity of the target mRNA by the successive actions of the miRNA, the Argonaute protein and finally, the GW182 protein, which interacts directly with CCR4-NOT. In the case of silencing of mRNAs containing AU-rich elements, the same action is performed by the protein called tristetraprolin involved in the regulation of inflammatory processes. The interactions and interplay between all of these high molecular weight proteins are relatively poorly understood. In particular, the interaction sites between GW182 and the CCR4-NOT complex were previously unknown.

Molecular biology experiments allowed the identification of CCR4-NOT interaction motifs on GW182. One of them is crucial for deadenylation, while the other is vital in mediating the interaction with CCR4-NOT *via* CNOT1, the scaffolding subunit of the CCR4-NOT complex. Biophysical experiments based on hydrogen-deuterium exchange mass spectrometry allowed the identification of the corresponding binding site on CNOT1(800-999). Surprisingly, the binding site of the GW182 silencing domain was found to be at the same CNOT1(800-999) surface region as the binding site of tristetraprolin. Biochemical experiments excluded their simultaneous binding to CNOT1. The GW182 and tristetraprolin proteins share a common motif, RLPXϕ, that interacts with CNOT1 in a very similar, but not identical, manner. This sequence has been proposed to act as a short linear motif. Thus, the two different gene silencing pathways: miRNA-mediated silencing and ARE-mediated silencing intersect at CNOT1, which serves as a molecular hub.

The structural dynamics of the GW182 silencing domain and the CNOT1(800-999) fragments were also studied. The GW182 silencing domain was experimentally proved to be natively unstructured except for an RNA-recognition motif (RRM) domain. The GW182 RRM domain was found to be a loose structure, contrary to the CNOT1(800-999) structure that was found to be very rigid.

Experiments performed in this thesis have led to the discovery of the interaction sites between the natively disordered GW182 silencing domain and the helical CNOT1(800-999) protein fragment, contributing to the understanding of the molecular mechanisms of recognition within protein complexes involved in gene silencing in different physiological processes.