Badania strukturalne części rdzeniowej kompleksu odpowiedzialnego za biogenezę miRNA u roślin

MicroRNAs (miRNAs) are small non-coding RNAs of about 21 nt in length, which participate in a wide variety of physiological cellular processes by regulating the gene expression at a sequence-specific manner. Primary transcripts of miRNA genes, called pri-miRNAs, are synthesized by RNA polymerase II, and contain characteristic hairpin-like secondary structures in which sequences of mature miRNAs are embedded. In plants both main miRNA biogenesis steps, pri-miRNA processing and pre-miRNA processing, occur inside the cell nucleus, and are perform by a complex comprising of at least five different proteins: DCL1 (an RNase III ribonuclease), HYL1 (a dsRNA-binding protein), SERRATE (SE) (a zinc-finger-domain protein), and CBC (a nuclear cap-binding protein complex) that is composed of two subunits, CBP20 and CBP80: DCL1 provides nucleolitic activity of the miRNA processing complex. Thus, in plants, in contrary to animal cells, a single RNase III type nuclease, DCL1, is responsible for processing of pri-miRNA into miRNA. However, DCL1 has to closely collaborate with its protein partners to achieve the precision and efficacy of both steps of miRNA biogenesis. The involvement of other proteins in miRNA biogenesis is crucial for the process, since DCL1 must first remove precisely single stranded RNA fragments from both sides of the miRNA containing hairpin, thus forms a correct RNA substrate for the next step of miRNA processing which is also catalyzed by DCL1 itself.

The main but still unsolved problem remains the structural precision and specificity of DCL1 in two steps of plant miRNA processing. Therefore, we plan to use various biophysical approaches to collect data on the structure of complexes of proteins involved in plant miRNA biogenesis. These methods include: small angle X-ray scattering (SAXS), crystallography, as well as cryo electron microscopy (cryo-EM). We want to hire a Ph.D. student who will be able to prepare recombinant proteins, obtain protein/RNA complexes, and analyze them. We will start with a complex of SERRATE and HYL1 attached to a stem-loop RNA structure (SLRNA) containing short single stranded fragments from both sides. The second complex we want to obtain is AtCBP20/AtCBP80 bound to a m^7pppG cap analogue, and SERRATE which interacts with the CBC. We will also try to obtain bigger complexes: the CBP20/CBP80/HYL1/SE protein complex assembled on a capped RNA containing a stem-loop structure, but this seems to be more tricky, and therefore we cannot guarantee now the final output of our attempts. However, after solving the structure of the two above-mentioned subcomplexes: SLRNA/SE/HYL1 and m^7GpppG/CBP20/CBP80/SE, we should be able to merge them to model a reasonable good picture of the whole complex. In addition, we will also try to obtain a complex consists of SE, HYL1 and DCL1 attached to a stem-loop structure resembling a miRNA precursor. This would be the most difficult task since we have not been successful in production of recombinant DCL1 in

bacteria. We are currently trying to express DCL1 in one of eukaryotic systems. We have already got promising results but still the yield of recombinant DCL1 is not satisfactory for structural studies. On the other hand, in our laboratory we can express and purify recombinant SE, HYL1, CBP80 and CBP20. The final analysis of the complexes obtained will be performed in very close collaboration with our scientific partners which provide expertise in structural biology method.

Most of the structural analyses planned in the project will be carried out with help and under supervision of our scientific partners: (1) cryo-EM: Prof. Reinhard Luerhmann from the Max-Planck-Institute for Biophysical Chemistry in Göttingen, Germany ; (2) crystallography: Dr. Marcin Nowotny from the International Institute of Molecular and Cellular Biology in Warsaw, Poland, and Prof. Mariusz Jaskolski from the Adam Mickiewicz University in Poznan, Poland; (3) SAXS: Prof. Maciej Kozak from the Adam Mickiewicz University in Poznan, Poland. All our partners are experts in their fields, and have well documented achievements (articles) in the field of structural biology, and all of them are enthusiastic about carrying out structural studies on the plant miRNA biogenesis complex.