

## **Introduction**

Pollen grains are essential for sexual reproduction of plants. During the final stage of development, pollen grains enter a state of complete or partial developmental arrest that strongly associates with a sequestration of the storage mRNAs pool with non-translating ribosomes and acquisition of desiccation tolerance. Pollen developmental arrest ensures survival during pollen air dispersal whereas accumulation of mRNA facilitates the rapid activation of translation upon acceptance by the stigma. Therefore, pollen grains offers unique chance to analyze the mechanisms of translational control.

## **Research objectives**

We have previously shown that annexin 5 (ANN5) is involved in pollen development in *Arabidopsis thaliana*. Our recent study revealed that ANN5 function might be developmentally regulated. ANN5 is present in the cellular substructures related to rRNA metabolism (nucleolus), it affects rRNA processing and it interacts with the plastidial translational elongation factor. We therefore hypothesize that ANN5 operates at the level of protein synthesis during pollen development.

The control of protein synthesis is a central aspect of plant development and adaptation to environmental stress, but the mechanisms are still poorly understood. There are data showing that metazoan annexin ANXA2 is involved in the regulation of specific mRNAs translation. This opens the possibility that at least some plant annexins may play similar role. We have recently reported that ANN5 participates in the pollen stress response to external factors inducing osmotic or ionic stress. Therefore, guided by the above mention reports and our findings we decide to explore the role of ANN5 in modulation of translation in pollen grains under stress condition.

We induced cellular stress with the excess of manganese and zinc ions. Manganese and zinc are important micronutrient for plant growth and development, however both deficiency and excess of  $Mn^{2+}$  or  $Zn^{2+}$  impair many physiological processes in plants. We found that exogenously applied  $Mn^{2+}$  or  $Zn^{2+}$  to pollen grains significantly changed ANN5 distribution in the pollen cytoplasm and altered spatial organization of pollen RNA. Both metal ions induced formation of ANN5-containing cytoplasmic granules. During time of environmental stress hundreds of RNAs and proteins usually condense to form microscopically visible foci that are commonly referred to as membraneless granules and are linked to translational arrest of mRNAs. Therefore, we hypothesize that by acting on translational machinery ANN5 may play an important role in pollen response to environmental factors. To verify this hypothesis we will apply a combination of biochemical, molecular biology and cell biology approaches.

## **Work plan**

Our experiments will be carried on model plant *A. thaliana*. We divided our proposal into three main tasks: (1) functional analysis of ANN5 during pollen maturation in terms of its role in ribosome biogenesis and impact on mRNA translation; (2) an investigation of the ANN5 role in reshaping of translome in mature pollen in response to the excess metal ions; (3) elucidation of mechanisms that determine multitasking properties of ANN5.

## **Expected impact of the research project on the development of science**

We believe that the research outcome of our studies will significantly contribute to the current knowledge of the pollen physiology and will improve our understanding of the regulation of translational machinery in plants. Moreover, our findings may underpin further research into the pollination biology of other Brassica species, many of which are important agricultural crops (canola, cabbage, cauliflower, Brussels sprouts). The project results will be presented in open access journals and conferences to scientific audience as well and non-specialist using social networking services and presentations at science festivals.