1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan, all in English)

Project title:

The role of epigenetic mechanisms in the regulation of chromatin structure/activity of *Arabidopsis thaliana* female gametophyte cells

1.1. Project goals

1. Identification of small non-coding RNA in *A. thaliana* female gametes (egg cell and central cell).
2. Role of epigenetic control in the regulation of gene expression in *A. thaliana* female gametophyte cells before and after fertilization - analyses of the chromatin state/dynamics and localization of selected small RNAs and epigenetic marks.

1.2. Outline

Small non-coding RNA (sRNAs) have emerged as important molecules in the regulation of gene expression involved in many biological processes in plants including developmental transition and patterning, responses to the environment, the maintaining genome stability and defense against viruses and bacteria. They belonging to two classes: micro RNA (**miRNAs**) and short interfering RNA (**siRNAs**) which act by silencing gene expression on post-transcriptional (PTGS) or transcriptional level (TGS). Additionally, sRNA plays an important role in some **epigenetic modifications** which regulate the structure (remodeling) and activity (DNA methylation) of the chromatin. Chromatin dynamics modify the organization eu- and heterochromatin and influence on accessibility and processivity of the transcription machinery. Two states of chromatin can be recognized: an open, transcriptionally permissive state and compact, transcriptionally repressive state. The knowledge of small RNAs biogenesis and their mechanisms of action has dynamically expanded over the past years, **but their functions in the epigenetic regulation of plant sexual reproduction are still not fully understood**.

In about 70% of angiosperms, including *Arabidopsis thaliana*, a mature female gametophyte (embryo sac) consists of seven cells with different biological function. Two **synergids** attract the pollen tube and one of them becomes the site of delivery of sperm cells. The **egg cell** and the **central cell** are target cells for male gametes and directly participate in the unique process of double fertilization. Three **antipodal cells** on the other hands, perform nutrition functions and often degenerate before fertilization. The effect of fusion of male gamete with an egg cell is **zygote**, from which an embryo develops, whereas nutrition tissue is differentiated in a form of **endosperm** from a fertilized central cell. In the flowering plants the knowledge of the

organization of gene expression in mature female gametophyte cells and during early stages of the embryogenesis is fragmentary. Antipodes and synergids seem to have the chromatin organization similar to the sporophytic cells while egg cell and central cell reveal less condensed chromatin. Transcriptomic analysis of *Arabidopsis*, *Oryza*, *Triticum* has revealed a distinct gene expression pattern of these cells but do not explain the regulatory mechanisms of these differences which reflect their specification and the chromatin state/activity. One of these processes is probably **small non-coding RNA pathway**.

1.3. Work plan

1. Isolation of *A. thaliana* egg cell and central cell – tissue embedding and laser capture microdissection techniques.
2. Deep sequencing to small RNA (RNA isolation, library construction and sequencing).
3. Bioinformatic analyses.
4. Ultrastructural analyses of the chromatin state in *A. thaliana* female gametophyte cells before and after fertilization (electron microscopy).
5. Localization of the selected small RNA in *A. thaliana* female gametophyte cells and during early embryogenesis in the zygote and endosperm using *in situ* hybridization techniques (fluorescence and electron microscopy).
6. Immunocytochemistry localization of the selected epigenetic marks (5-methylcytosine, histone modifications) in *A. thaliana* female gametophyte cells before and after fertilization (fluorescence and electron microscopy).

1.4. Literature

Ashapkin VV, Kutueva LI, Aleksandrushkina NI, Vanyushin BF (2019) Epigenetic Regulation of Plant Gametophyte Development. Int J Mol Sci, 20(12), pii: E3051

Kirkbride RC, Lu J, Zhang C, Mosher RA, Baulcombe DC, Chen ZJ (2019) Maternal small RNAs mediate spatial-temporal regulation of gene expression, imprinting, and seed development in *Arabidopsis*. Proc Natl Acad Sci USA, 116(7): 2761-2766

Wu Y, Yang L, Yu M, Wang J (2017) Identification and expression analysis of microRNAs during ovule development in rice (*Oryza sativa*) by deep sequencing. Plant Cell Rep, 36(11): 1815-1827

Baroux C, Autran D (2015) Chromatin dynamics during cellular differentiation in the female reproductive lineage of flowering plants. Plant J, 83(1): 160-176

1.5. Required initial knowledge and skills of the PhD candidate

- Master degree in biotechnology or molecular biology.
- Experience in working with DNA, RNA and cytological techniques: immunocytochemistry, fluorescence *in situ* hybridization (FISH).

- Good oral and written communication skills in English.
- Ability to perform in-depth and critical data analysis.
- Strong self-motivation, creativity and able to work collaboratively.

1.6. Expected development of the PhD candidate's knowledge and skills

- Experience in research using modern cellular and molecular biology techniques.
- Activity in applying for research grants and experience in managing of own research project funding of other sources than the university.
- Active participation in international and national scientific conferences.
- Experience in preparation and writing manuscripts.