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Paving the way for transcriptomics analysis in the reproduction processes of hexaploid wheat (*Triticum aestivum* L.)

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Albeit Nawaschin discovered double fertilisation, during which two female reproductive cells are fertilised, well, over a hundred years ago (1989), this unique reproduction strategy characteristic of flowering plants has remained elusive and defied attempts to disentangle the molecular and cellular mechanisms that ultimately lead to a life strategy fundamentally different from that of animals. The remarkable feature of this life cycle is the masterly treatment of the alternation of generations between a diploid sporophyte and a haploid gametophyte, the latter allowing for purging the population of deleterious mutations since the phenotype reflects the haploid genotype. Double fertilisation involves a complex series of interactions, a key step of which is the activation of the egg cell produced by the haploid female mega gametophyte. The cascade events of the sperm-induced activation of the angiosperm egg and the concomitantly unfolding zygote development coupled with the switch from maternal to embryonic control of gene activation and resulting in asymmetrical cell cleavage that produces two daughter cells of different cell fate still await deciphering owing mainly to the inaccessibility of the female gametes/zygotes of higher plants as these develop deeply enclosed in surrounding maternal tissues hence rendering their isolation a deterring task. Thus, there is a considerable time lag in studying proembryo formation in plants as compared to animal embryology leaving a number of questions of overriding importance, such as: 1. How and when is cell polarity established in the angiosperm egg cell, 2. How do molecular processes such as cell division, cell expansion, cell maturation and differentiation unfold which bring the zygote (the progenitor cell of the next sporophytic generation) from the unicellular to the multi-cellular stage, 3. What genes are involved in triggering the first asymmetrical division of the zygote and how these genes are regulated, unanswered? Wheat is a staple crop and prevalent alimentation source in many parts of the world which warrants studies aimed at unravelling mechanisms ultimately responsible for seed set, the prerequisite of which is successful fertilisation of the egg as well as normal endosperm development. A micromanipulation technique based on gamete isolation, electro-fusion performed at the gamete cell-pair level and microinjection of isolated wheat egg cells/fusion products was elaborated which permits a complex approach in addressing these issues. Capitalising on this method, one of the earliest marks of fertilisation-associated events, egg activation by the sperm cell through calcium signalling has been dissected together with the in vivo dynamics of the F-actin cytoskeleton shown to be involved in imparting spatial information to the egg cell concerning the micropylar-chalazal axis of the embryo sac. Furthermore, one of the main cell cycle protein kinases, cdc2 kinase, was localised in isolated and in vitro fertilised egg cells of wheat with double labelling the protein of interest and tubulin assisted by fluorescent and confocal microscopy. The importance of the pivotal role played by p34cdc2 in reorganising the intracellular structures of the wheat zygote for rendering it capable of preparing for mitosis that produces the proembryo possessing two cells with distinct cell fates will be discussed as well as the implications of this approach in transcript profiling of isolated wheat female gametes, zygotes and two-celled embryos in the context of studying cell polarity/asymmetrical cell division.

Biography

Aniko Dobosy began her studies in the realm of plant molecular sciences three years ago at the University of Kaposvar, Hungary. As a research student she was involved in several projects led by Zsolt Ponya PhD, focusing on the cellular/molecular aspects in vitro versus in vivo zygotic embryo formation in angiosperms with special regard to comparing zygote formation and egg activation in the plant versus animal kingdom using micromanipulation techniques. She is co-author of the study by Zsolt Ponya, published in IJMS: "When Isolated a Full Receptivity, in Vitro Fertilized Wheat (*Triticum aestivum*, L.) Egg Cells Reveal [Ca²⁺]_{cyt} Oscillation of Intracellular Origin". She is currently the CEO of a software developing company: eSolution Ltd., Hungary, which deals with data processing and evaluation of results gathered in various research projects and in other versatile areas such as engineering.

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