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How gamete micromanipulation and cell engineering can contribute to establishing a time scale for performing transcriptomics studies in wheat (*Triticum aestivum, L.*) during fertilisation and proembryo formation

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Land plants are sessile organisms; consequently they had to evolve powerful genetic and epigenetic tools and strategies to ward off perils of their surroundings and to render them adaptive to environmental stress. Additionally, they have to be capable of fast tracking the adaptive optima imposed by transient selective forces while permanently tuning their body to cope with the challenges posed by the ever-changing environment. Thus, in terms of genetic plasticity, land plants are by far more flexible organisms as compared to animals as they have much more dynamic and plastic genome. Furthermore, unlike in mammals, in angiosperm plants germline cells are not set aside at an early stage during development so cells partaking of reproduction can be viewed as the depositories of "cell memories" which have accumulated due to survival/adaptation strategies having proven successful in the cells of the mother plant that, through numerous cell divisions in the sporophytic generation, ultimately serves as the source of progenitor cells for the gametes produced during the haploid developmental stage. This very fact is thought to be behind the evolutionary success of land plants and also makes them an attractive system to study genetic plasticity. In this sense angiosperm nucellar cells are particularly interesting as they reveal versatile developmental modalities; they can either use or avoid meiosis without compromising subsequent development. Normally, once the molecular "decision" is made, a highly conserved, modular developmental pathway unfolds producing the female gametophyte (FG) with a haploid egg. Occasionally, the egg cell can go through parthenogenesis which is an extraordinary phenomenon whereby the egg cell initiates cell division without paternal genetic contribution. Although the study of parthenogenesis/apomixis in angiosperms has recently leapt ahead, the cellular and molecular events of the activation of the egg foregoing sperm incorporation remains to be elucidated.To explore whether the cascade events of signal transduction triggered by sperm-egg fusion and leading to egg activation can be elicited in lieu of fertilisation, egg cells of wheat isolated at different stagesof in situ development were microinjected with the DefH9-iaaMgene, which had been previously demonstrated to confer auxin synthesis specifically on ovules and derived tissues in other systems as well as induce parthenocarpy in several plant species (tobacco, egg plant, tomato). Furthermore, wheat zygotes were used to study the relationship between cell cycle and morphogenesis, as in the angiosperm zygote cellular differentiation and cell cycle control are closely linked because cytoplasmic reorganisation occurs concomitantly upon fertilisation leading to asymmetrical cell cleavage. By exploiting a protein delivery system facilitating functional studies in living cells, an antibody raised against the 17 amino acids at the C-terminus of a synthetic peptide based on the deduced protein encoded by the cdc2 gene, a key protein in cell cycle regulation isolated previously from wheat, was introduced in in vitro fertilized wheat egg cells at defined times permitting the chronological follow-up of the impact of blocking cdc2 activity on morphogenesis during wheat zygote/proembryo development. The findings of microinjecting DNA encoding Rho GTPases implicated in cellular response to extracellular signals by inducing coordinated changes in the organisation of the actin cytoskeleton and in transcription to drive a wide range of fundamental biological processes such as cell cycle and morphogenesis, will also be presented. In order to ensure the maintenance of in *planta* observed polarity of in vitro fertilised egg cells, which was found to be lost during in *vitro* culture, a micromanipulation-based technique enabling re-implantation of fusion products into maternal tissues (ovules) was elaborated. The relevance to stem cell research of our preliminary results of exploratory cell engineering experiments on the creation of half clones/cybrids via fusion of karyoplasts isolated from unfertilised wheat egg cells and cytoplasts stemming from in *vitro* fertilised female gametes will be addressed. In practical terms research of this kind may ultimately have ramifications in achieving clonal propagation in agriculturally important plants through seeds hence contributing to efforts aimed at fixing desirable gene combinations/hybrid vigour in crops and to defining time windows for isolating egg cells/zygotes/proembryos for trancriptomic studies in wheat.

Biography

Zsolt Ponya has completed his PhD at the age of 32 years from the Eotvos Lorand University of Arts and Sciences, Budapest, Hungary, and following obtaining his degree, he has launched his postdoctoral studies at the University of Siena, Italy, followed by a postdoc research fellowship at the Ben-Gurion University of the Negev, Israel. He is currently a senior scientist at the University of Kaposvár, Hungary. He has published a number of papers in reputed international journals and is a member of the editorial board of several prestigious scientific journals.

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