PLENARY LECTURES

Genomic imprinting in plant sexual development

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Epigenetic modifications of chromatin and DNA, while not altering the DNA's primary sequence, also contain inheritable information. DNA methylation, posttranslational modifications of histone, positioning of nucleosomes and small RNA interference have been described as the mechanisms of chromatin reprogramming. "Epialleles" which are formed after the chromatin reprogramming refers to the transmission of modified genetic material from one generation to the next in both animals and plants. In mammals epigenetic modifications in germline cells are reset during the formation of gametes from 2n cell in this group of organisms, the process of double fertilization occur. There is a lot of genetic evidence that during plant reproduction the epigenome remodeling takes place at the three stages of development: first, during specifications of megaspore mother cells (MMC) (She et al., 2013), second, during initiation and maturation of the female and male gametophyte (Jullien et al., 2012), and third, after fertilization during seed development.

Our previous studies in *Hyacinthus orientalis* have shown that transcriptional activity of the egg and central cell is silenced during embryo sac maturation and that fertilization induces the activation of the zygote and endosperm genomes (Niedojadło et al., 2012). Changes in transcriptional activity and chromatin structure also take place in the male germ cells. The aim of our present investigations is to analyze the distributions pattern of epigenetic factors that are involved in the silencing and condensation of chromatin in germ line cells before and after fertilization.

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Neb-colloostatin induces apoptosis in ovary of *Tenebrio molitor* beetle

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Follicular atresia, defined as the degeneration of the follicle, generally occurs during normal oogenesis in many animal species. Under physiological conditions it plays a significant role in the maturation process during the normal development of eggs and in the removal of abnormal or damaged oocytes before they reach maturity. However, follicular atresia has been massively induced in response to starvation, malfunction of ecdysone signalling, treatment with chemotherapeutic drugs and pathogen infection (Buszczak and Cooley, 2000; Markaki et al., 2004; Medeiros et al., 2011). We tested peptide *Neb*-colloostatin for a sterilizing effect on females of *Tenebrio molitor*. Injection of nanomolar doses of *Neb*-colloostatin into the haemocel of *T. molitor* females during their first reproductive cycle showed that this peptide strongly inhibited ovarian growth and oocyte development. Degeneration of *T. molitor* follicles includes changes in morphology and viability of follicular cells and oosorption as a consequence of these changes. The first observable effect of *Neb*-colloostatin-induced atresia is the disappearance of patency in the follicular epithelium. This probably inhibits the active uptake of vitellogenins by the oocyte. *Neb*-colloostatin injection causes changes in the morphology indicating death of follicular cells. We observed F-actin cytoskeleton disorganization, induction of caspase activity, changes in chromatin organization and autophagic vacuole formation. Our results show that in *T. molitor* females, *Neb*-colloostatin injection resulted in atresia of ovarian follicles both the apoptotic and the autophagic mechanism of programmed cell death. Induction of apoptosis and autophagy of ovarian follicles may contribute to more efficient removal of atretic follicles during ovarian follicular regression in insects. Moreover, the results obtained indicate the possibility of future use of synthetic pseudopeptide and peptidomimetic analogues of *Neb*-colloostatin as bioinsecticides against insects.

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The ovarian characters of Conchostraca (Crustacea: Branchiopoda) and its significance for the Pancrustacea hypothesis

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In contrast to the traditional views, modern molecular phylogenetic analyses strongly suggest a close relationship between Crustacea and Hexapoda and even postulate combining these groups into the Pancrustacea/Tetraconata clade. Crustaceans are highly diversified arthropods with unresolved phylogenetic relationships and it is still contentious which crustacean subgroup may be the sister group to Hexapoda. Recent molecular studies have indicated that such a group might be the crustacean lineage comprising Branchiopoda. To test this hypothesis on the morphological ground, we have investigated the structure of the ovary and ultrastructural aspects of oogenesis in two branchiopod species: *Cyzicus tetracerus* and *Lynceus brachyurus*, representing two separate branchiopod orders: Spinicaudata and Laevicaudata, respectively. The organization of the female gonads has not been investigated in these groups yet. Our analysis has revealed that in both studied species as the sac-shaped ovaries grow they form numerous protrusions sticking out into the hemocoel. Each ovarian protrusion constitutes an ovarian follicle comprising a germline cyst surrounded by a simple somatic (follicular) epithelium, supported by a thin basal lamina. Each germline cyst consists of four germline cells: an oocyte and three supporting nurse cells, and the oocyte differentiates relatively late during ovarian follicle development. The oocyte cytoplasm is filled with numerous cisternae of rough endoplasmic reticulum and Golgi complexes apparently engaged in the synthesis of reserve materials. The follicular cells are penetrated by a characteristic canal system which is most likely involved in transporting nutrients from the hemolymph to the surface of the oocyte. We have found that the structure of the ovary and the ultrastructural characteristic of oogenesis are not only remarkably similar in both *Cyzicus* and *Lynceus*, but also share significant similarities with the female gonads of the basal hexapods: Campodeina and Collembola, which is of interest in the light of the Pancrustacea hypothesis.

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Embryological characters are conservative in a very differentiated *Viola* L. genus (Violaceae)

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Embryological characters are very important in the taxonomy of the angiosperms (Davis, 1966). The taxonomy of Violaceae with 22 genera and 1000–1100 species worldwide is complicated and several classifications and phylogenetic relationships within the family have been proposed based on different traits, including nuclear and chloroplast markers (Tokuoka, 2008; Wahlert et al., 2014). The genus *Viola* L. is the largest within Violaceae occurring on both hemispheres with ~600 species (Ballard et al., 1999; Wahlert et al., 2014). Infrageneric classification has varied depending on the authors and methods of analysis (Ballard et al., 1999) but the current phylogenetically-based classification recognizes two subgenera and 16 sections (Marcussen et al. unpubl.).

Embryological characters of species from several *Viola* sections (*Chamaemelanium, Erpetion, Melanium, Plagiostigma, Viola*) and subsections (*Boreali-Americanae, Chamaemelanium, Dischidieae, Nudicaules, Rostratae, Stolonosae, Viola*) were analyzed. Despite of great morphological, microstructural (stigma), molecular intrageneric differentiation, and a high age (~20 Ma; Marcussen et al., 2012) of the sections, there is a uniformity in ovule characters and female gametophyte, embryo and endosperm pattern of development. Ovules are anatropous, bitegmic, crassinucellar with micropyle formed by both integuments. Female gametophyte develops according to monosporous Polygonum type, suspensorless embryo follows Asterad type, endosperm is nuclear.

The developmental disturbances and abnormalities of female gametophyte observed in investigated species were correlated with the impact of polluted with heavy metals environment and with hybridization. Some specific characters of ovules found in plants from heavy metal polluted areas might be considered as protection against pollution.

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Formation of a stockpile of ova in ovaries of Vertebrates: limited and unlimited oogenesis

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Females belonging to the major extant vertebrate clades represent two basic modes of oocyte stock formation: 1/ cyclic proliferation of primary oogonia followed by meiotic entry (unlimited oogenesis; a source of new oocytes remains "open" during the whole life span of a female), 2/ all oocytes are formed only once for a lifespan and are recruited from the limited stock (limited oogenesis; a source of new oocytes is "closed" after establishing the finite number of further ova during early ontogenesis). Oogenesis in mammals, birds, anuran amphibians, some teleostean fishes, Chondrichthyes, and lampreys is closed, whereas it remains open in some teleostean fishes and Lepidosauria (snakes and lizards). Little or no data exist about formation of oocyte pool in Chelonia, Crocodylia, Chondrostei, Cladistia, and Myxinii, as well as for extant basal sarcopterygians (Crossopterygii and Dipnoi) (literature review: Ogielska et al., 2013). On the basis on available data, we can assume that the closed oogenesis is probably an ancestral state in vertebrate evolution and is ancestral also for the major taxa of vertebrates (Osteichthyes, Sarcopterygii, Amniota, Diapsida, and Archosauromorpha). However, the ancestral mode of oogenesis for all vertebrates should be verified with new and reliable data on hagfishes that constitute the most basal living group of vertebrates. The categorization of oogenesis as closed or open encounter difficulties also because the interpretation of the presence of proliferating oogonia is often confused by several authors as synonymous with completion of meiosis. The closed oogenesis is correlated with gonochorism (non-reversible determination of sex during early ontogenesis). The open oogenesis seems to offer new possibilities in evolutionary flexibility of reproduction (some teleosteans display a sequential hermaphroditism and sex reversal more than once during life span).

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Calcium-binding proteins immunoreactivity during ontogenesis of the limbic structures in the guinea pig

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Calcium binding proteins (CaBPs) such as calbindin D28k (CB), calretinin (CR) and parvalbumin (PV) belong to the EF-hand family proteins, which regulate many cellular processes mediating by calcium ions (Ca2+). The CaBPs are engaged in developmental processes and are considered to be good spatiotemporal markers of developing circuitry and certain subpopulations of neurons. This feature has been applicated to studying development of CaBPs immunoreactivity in the so-called Papez circuit including dentate gyrus – DG, hippocampus proper – HP, mammillary body- MB, anterior thalamus – AT, which are interconnected anatomically and functionally. The brains of the guinea pig were investigated from 30th day of gestation (E30) to adult stage (P80) by immunohistochemistry.

CB. The most striking features were the presence of CB in almost all granule cells in the DG and CB-positive mossy fibers in all the studied stages. CB colocalized with CR in some cells in the pyramidal layer (PL). CB-reactivity is a useful marker to delineate the MB nuclei, especially in a fetal period, whereas in the AT it may be a marker of P20 stage, in which CB appears for the first time in the anteromedial nucleus only in the cells that contain CR.

CR. CR-positive perikarya were present throughout all the studied stages in PL and some of them co-expressed CB. Also single CR-ir perikarya were detected in remaining layers of the DG. The most outstanding feature was an appearance of CR-reactivity in a specific spatiotemporal pattern, thus CR may be use as a marker of E50 and E60 (in the anteromedial and anteroventral nuclei, respectively).

PV. PV-reactivity was the highest between E50 and P0 in DG and HP what may suggest that maturity of inhibitory local circuitry takes place in this structures before a birth.

Conclusions. The pattern of CB and CR colocalization let us suggest that the proteins enhanced neuroprotection and neuronal survival in the studied limbic structures of the guinea pig. CB and CR may be useful markers of some neuronal centers as well as developmental stages in the Papez circuit of the guinea pig.

Ultrastructure, distribution and transovarial transmission of endosymbiotic microorganisms in leafhoppers (Hemiptera, Cicadomorpha: Cicadellidae)

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In leafhoppers, like in other phloem consuming hemipterans, commonly occur obligate endosymbiotic microorganisms (Buchner, 1965; Baumann, 2005). Endosymbionts are responsible for synthesis of essential amino acids missing in the phloem sap (Baumann, 2005). In the host insect body, endosymbiotic microorganisms are harbored in giant cells of mesodermal origin termed bacteriocytes. The bacteriocytes are integrated into large, paired organs termed bacteriomes that are localized in the vicinity of the ovaries. The bacteriocyte cytoplasm is tightly packed with endosymbiotic microorganisms. Histological and ultrastructural studies have shown that in leafhoppers two or more types of morphologically distinct endosymbiotic bacteria are present. These microorganisms may be located in separate bacteriocytes or may co-exist in the same bacteriocyte. In leafhoppers the endosymbionts are transported from the mother to the embryo vertically (=transovarially). Endosymbionts are released from the bacteriocytes, migrate via the hemolymph towards the ovaries and infect the ovarioles containing vitellogenic oocytes. The bacteria transverse the cytoplasm of follicular cells surrounding the posterior pole of the oocyte and gather in the perivitelline space, as a rule in the form of a characteristic "symbiont ball". Until the end of the oocyte growth the endosymbiotic bacteria do not invade the oocyte.

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Flower structures and flowering biology in *Tinantia anomala* (Torr.) CB Clark

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Tinantia anomala flowers exhibit zygomorphic symmetry with distinct dimorphism of the stamens, which vary in shape and size. Moreover, the three shorter upper stamens (PK) have numerous brightly coloured trichomes along the filament, whereas the three lower stamens are characterised by a long, curved filament with a few trichomes at its base. Due to the specific arrangement of the flower elements (face effect), Vogel proposed a hypothesis that this species exhibits mimicry. According to the theory postulated by him, the short stamens, which contain sterile pollen, together with the filament trichomes are a pollinator insect-luring element, whereas functional male gametophytes are only produced in the long anthers. Pollen grain viability tests applied in our investigations have demonstrated a high (60 – 70%) ability of grains from both long and short anthers to germinate into pollen tubes.

The anatomical structure of anthers and the ultrastructure of pollen grains were examined using electron microscopy (TEM and SEM). Dimorphism was also observed in the structure and chemical composition of *T. anomala* male gametophytes originating from different anthers. Microscope images revealed different morphology of pollen grains produced in *T. anomala* anthers. In microsporangia of the long anthers, small pollen grains with a convex-triangular profile and distinct poruses were produced. Pollen grains from the short anthers were larger and had a concave-triangular profile and three poruses as well. Analysis of the protein composition of the two types of anthers performed with the bidirectional electrophoresis (IEF/SDS – PAGE) method showed differences in their protein profiles. Approximately 400 different proteins were identified in the protein profiles obtained, with 40 and 8 proteins specific for the short and for long anthers, respectively. Proteins that were common for both anther types differed only in terms of quantity.

T. anomala is a convenient object of embryological investigations due to its short development cycle, production of fruits within a few weeks after flowering, and the sexual and vegetative strategies of reproduction. Understanding the issue why and how these two reproduction modes emerged is one of the most important challenges of evolutionary biology.

Function of glial cells in the development of the nervous system

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Recent advances in the biology of glial cells, particulary during development of the nervous system have revealed many glial-neuronal interactions that occur both during development and following injuries. During early stages of development most progenitor cells in the ventricular zone of the neural tube proliferate rapidly and they undergo symmetric and asymmetric cell division which is influenced by signals in the local environment.

Beginning from the end of the 19th century appeared many theories of neuronal and neuroglial histogenesis and the separation of glial and neuronal lineages has a long history.

It is now known that the radial glial cells which are important during migration are the earliest morphologically distinguishable cells in the neural epithelium. Radial glial cells are progenitor cells that generate both neurons and glial cells.

During cortical neurogenesis the radial glial cells maintain their apico-basal polarity and undergo asymmetric division to self renew and to produce a daughter which is either the neuron or intermediate progenitor cell which is more restricted and is also named basal progenitor cells within the embryonic subventricular zone. This subventricular zone is a major site of neurogenesis.

The diversity in molecular and morphological characteristics of neurons is determined mainly by intrinsic cellular mechanisms that are established as a new neurons are generated (Kriegstein and Alvares-Buylla, 2009). Radial glial cells may also serve as progenitors of neurons in the adult nervous system.

Important functions of the glial cells during development of the nervous sytem are their role in migration and myelination. The radial glial cells act as a framework which guides the growing neurons to the correct target areas.

Schwann cells in the peripheral nervous system and oligodendrocytes in the central nervous system form the concentric layers of the myelin.

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Outcrossing in selfpollinating crops – how does it influence coexistence of conventional, organic and GM crop production.

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Agriculture operates in an open space, thus accidental appearance of genetically modified crops in fields, where they were not cultivated cannot be excluded. Therefore, there is a need to introduce rules for the coexistence of different cropping systems, according to the legal requirements. "Coexistence" refers to the ability of farmers to make a practical choice between conventional, organic, or genetically modified cropping system. According to the European Commission, consumers and producers should have a real choice between different agricultural products, as well as to the way of their production. One of the important factors taken into consideration when determining the rules of coexistence, is the range and level of outcrossing, which depends on the specifics of generative reproduction system of a particular species or genotype. There are many ways in which pollen can be transferred onto the stigma, and many factors such like weather conditions that can influence the pollination frequency and distance. This interesting aspect of coexistence has been studied for maize, which is an open pollinated crop, and triticale, which can serve as an example of a selfpollinated plant. Genetically modified maize (MON 810) can be cultivated in the EU and the level of outcrossing has been studied in several European environments. Such experiments concerning triticale were conducted in Europe for the first time. The results show that triticale, even though it is considered to be a selfpollinating plant, partially outcrosses. This requires elaboration of GM field isolation strategy to ensure coexistence.