PLENARY LECTURES

Cytoskeleton in plant reproduction

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Antibodies dedicated to separate proteins or cell organelles have made during the last twenty years a career as important tools for localisation of enzymes and metabolites as well as deposition of subtle elements of the cytoskeleton. The cytoskeleton, a three dimensional network of protein filaments, plays a significant role in cellular transport, cytomorphogenesis, developmental patterning of tissues and organs and plant reproduction.

The particular configurations of tubuline and actin filaments function in relation to the stages of reproductive processes. Events such as meiosis, micro- and macrosporogenesis, formation of gametophytes, and fusion of gametes depend on proper transformations of the sizable populations of microtubules and actin microfilaments.

In the young female gametophyte all cells look similar and perform a weak cytoskeleton with sparse microtubules and microfilaments near the cell wall. When the gametophyte reaches maturity, the amount of Factin differs in an egg apparatus, the egg cell contains less actin than synergids which have much more Factin and microtubules distributed mainly near the filiform apparatus.

In Angiosperms, a pollen tube (a male gametophyte) enters the synergid releasing two sperms. As a result, two distinct bands of actin microfilaments are formed to facilitate the access of male gametes to the egg and the central cell for fusion. However, in some plants sexual reproduction does not exist, so the embryo and the endosperm develop autonomously. In the ovules of such species, meiotic divisions are disturbed and do not lead to the formation of haploid megaspores. As a consequence, diploid embryo sacs are developed but they have different configurations of the cytoskeleton. This is to say that there are vital differences between the apomictic and sexual embryo sacs. Apomictic embryo sacs show a lack of the filiform apparatus in synergids and cytoskeletal brush-like structures on the micropylar pole. Such synergids remain in the juvenile phase of ontogeny with a poor cytoplasmic skeleton and weak secretory activity.

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Actin cytoskeleton organization and dynamics in *Drosophila melanogaster* spermatid individualization

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During spermatid individualization in *Drosophila melanogaster* structures called actin cones mediate cellular remodeling that separates the syncytial spermatids into individual sperm cells (Noguhi and Miller, 2003). To accomplish this, actin cones move synchronously from the sperm nuclei to the ends of the tails. As they move, the cytoplasm and most organelles are pushed out of the flagella, and the cell membrane is reorganized and attached to the axonema. Myosin II subfragment 1 (S1) decoration demonstrated the actin cones are composed of two structural domains, a front meshwork and a rear region of parallel bundles. These two domains form separately in time by different polymerization mechanisms, are differentially regulated by actin-associated proteins, and have different roles in individualization (Noguhi et al., 2008). Immunocytochemical studies were shown that one of the key protein required for the final step of *Drosophila* spermatogenesis is molecular motor myosin VI that stabilizes branched actin network at the fronts of the actin cones as they move (Noguchi et al., 2006). This system provides an example of how the basic mechanisms used in cell motility are modified to mediate motile processes within specialized cells.

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Genome modifications in plant development

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Eucaryotic genome can be defined by DNA amount (genome size), chromosome number (karyotype) and the level of polyploidy. These features characterize each species but are modified during plant development. The complex of DNA, proteins and RNA is compacted and organized inside the nucleus as a chromatin which is visible as chromosomes during cell divisions. During plant development cells undergo a series of changes leading to their differentiation. Cell differentiation coincides with large-scale chromatin structure changes and with an increase of the DNA content during endoreduplication process. Different cells of the plant express different sets of genes. During development the chromatin needs to be altered and restructured to regulate gene expression. Beyond the primary DNA sequence of a genome, the higher order chromatin organization plays a key role in determining patterns of genes expression. Proper development of multicellular organism depends on the establishment of differential transcription programs. The regulation of gene expression involves many different processes, among them methylation of the DNA and histone modifications creating defined nuclear structure, which can be studied at cytogenetic and molecular level.

Recent development of biotechnology, molecular cytogenetics and new techniques such as fluorescent in situ hybridization, immunostaining, confocal microscopy, flow cytometry, and computer image analysis open new possibilities for chromatin structure investigation and detection of different genetic and epigenetic changes in particular cells during plant development. These investigations have provided new data on understanding how different pattern of genes expression are established and maintained during subsequent cell divisions. Some current investigations in this area will be presented.

Signal exchange between cells during progamic phase in flowering plants

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In sexual reproduction of flowering plants 3 different organisms are involved: diploidal sporophyte (maternal plant) and 2 haploid gametophytes (male = pollen grain or pollen tube; female = embryo sac). Communication between the 3 partners is crucial for the fertility of the plant. Partners have to exchange signals informing about their genetic and physiological constitution which stimulate or inhibit pollen tube growth and promote or prevent fertilization. The period of progamic phase is relatively long and lasts from one hour to 2 weeks depending on plant species and exogenic factors. During this time the pollen grain is transferred and deposited on the stigma of the diploid plant in the process of pollination. Chemical signals between pollen grain and stigma create the conditions to support germination of the pollen tube. Here, negative recognition between stigma and pollen grain can evoke incompatibility reactions. After positive recognition the pollen tube germinates and grows through the style to the ovary. On the whole way to the egg cell the pollen tube depends on interaction with sporophytic cells – both partners exchange chemical signals. e.g. calcium ions, pectins and arabinogalactan proteins which support the pollen tube adhesion and direct its growth. In the case of negative interaction between stylar tissue and the pollen tube aggressive enzymes are secreted which inhibit the pollen tube growth. If the signals between pollen tube and sporophytic cells of the pistil are positive the pollen tube reaches the ovules. There, the pollen tube has to find the way to the micropyle of the fertile, receptive ovule, penetrate it and reach the egg apparatus of the embryo sac. In the ovule the male and female gamethopytes exchange signals and recognise each other – positive reaction results in the act of fertilization. The chemical nature of the final signals exchanged between sexual partners is not clearly defined. In the newest literature theories of attraction and repulsion of the pollen tube are discussed.

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Ovary organization in Hirudinida

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In leeches oogenesis takes place inside paired, rounded or elongated coelomic sacs (ovisacs). During initial stages of oogenesis the germ line cells form cysts (clones, clusters, nests) of cells interconnected by stable intercellular bridges. Such cysts have specific organization i.e. each germ cell has only one cytoplasmic bridge connecting it to central anuclear cytoplasmic mass (cytophore). Within leech ovaries germ line clusters together with somatic cells form differently organized structures. In Glossiphoniidae, Hirudinidae and Haemipidae the ovary cords (ovary strings) occur. The ovary cords are composed of several huge (Glossiphoniidae) or smaller (Hirudinidae and Haemopidae) clones with dozens germ cells surrounded by flat somatic cells. Growing oocytes gradually protrude into ovary cavity and eventually detach from the cord and flow freely in coelomic fluid. In Hirudinidae and Haemopidae the cords are polarized; their club-shaped end contains oogonia, developing clones of germ cells and one huge apical cell, which has been never observed in Glossiphoniidae. In Piscicolidae each clone of germ cells is embedded in a cytoplasm of a single follicular cell (intermediate cell), which, in turn, is enveloped by several flattened somatic cells. Such organized egg follicles float freely in an ovary cavity. In Erpobdellidae in each ovisac there are several (5–7) spindle-shaped bodies, ovarian bodies. Ovarian bodies are polarized, their top is occupied by oogonia and developing clusters of germ cells, whereas the lower parts are filled with differentiating germ cells and developing oocytes. Oocytes persist within the ovarian bodies as late as they reach I meiotic metaphase. According to literature (Jörgensen, 1908; van Damme, 1974) at the top of *Erpobdella* ovarian bodies the cell resembling the apical cell found in Hirudinidae also occurs. Ovarian bodies found in *Erpobdella* may be compared to short ovary cords of Hirudiniae type.

Because our knowledge about oogenesis and ovary organization in Oligochaeta *sensu stricto* (non-leech Clitellata) and sister taxons to Hirudinida (Branchiobdellida and Acanthobdellida) is poor, the ways of ovaries evolution in leeches (and other Clitellata) are still unknown.

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Development of the human cerebral cortex: cortical patterning and migration

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In embryos at stage 11 (30 days) the rostral neuropore is closed and the single telencephalic vesicle appears. At stage 14 (33 days) the cerebral hemispheres are evident. During 6th week it is possible to distinguish future cortical areas: archipallium, paleopallium, and neopallium.

The most important feature in the development of the cerebral cortex is the appearance of the cortical plate within the primordial plexiform layer in embryos at stage 21 (50 days). The cortical plate separates the plexiform layer into the subpial layer and the subplate. By the end of the embryonic period the cortical plate covers most of the surface of the cerebral hemisphere and consists of several rows of cells which migrate from the ventricular zone. In the first four weeks of fetal period the growing cerebral hemispheres cover the diencephalon and midbrain.

Over the past several years two models were proposed for the development of the cortical patterning: 1) the protomap model, and 2) the protocortex model. In the protomap model, the cortical primordium is patterned as it is generated. Area differences are set up in the ventricular zone. In the protocortex model the cortical primordium is essentially homogenous as it is generated and is patterned later by cues from thalamic afferents. Recent investigations showed that, although the cortical primordium may appear morphologically homogenous, it is highly heterogenous and its early patterning does not depend on thalamic innervation. These studies support rather a protomap model.

Essential role in the development and organization of the cerebral cortex plays cell migration. Defects in neuronal migration lead to mental retardation, epilepsy, motor and sensory impairments, and severe learning disabilities.

Basing on orientation two main types of migration have been identified in the brain: radial migration and tangential migration. The radial migration may be of two types. In the first type the migrating neuroblasts use the processes of radial glial cells to establish their position. A second type of radial migration, named somal translocation, is largely independent of radial glial cells. In tangential migration cells migrate orthogonal to the direction of radial migration. It has to be pointed out that the patterning of the cerebral hemispheres and regional specification of the brain precede cell migration.