ORAL PRESENTATIONS

Molecular basis of sexual plant reproduction in Angiospermae

Elżbieta Bednarska, Szymon Pięciński, Krzysztof Zienkiewicz, Katarzyna Niedojadało, Dariusz J. Smoliński

Department of Cell Biology, Nicolaus Copernicus University, 9 Gagarina St., 87-100 Toruń, Poland, e-mail: ebedn@biol.uni.torun.pl

Sexual reproduction in Angiospermae starts when pollen grain is landing on the stigma. Pollination induces the set of the pollen-pistl interactions. On the stigma (sporophytic incompatibility) or in the style (gametophytic incompatibility) the pollen recognition processes takes place. The rejection of the incompatible male partners occurs before the sperm cells are delivered to the embryo sac. The genetic and molecular aspects of the pollen grain recognition and its rejection in pistil will be discussed.

Another unique process of sexual reproduction in flowering plants is a double fertilization. During microgametogenesis two sperm cells are formed. The egg cell and celntral cell, which are the targeds for male gametes are formed in the female gametophyte (embry sac). The future embryo will be formed from the fertilized egg cell, while the fusion of the sperm cell with the central cell gives arise into the endosperm.

It is known that in most of the studied animal species, an unfertilized egg contains maternal transcripts, which are translated during early embryo development. The embryo's own genome expression is not required for its first divisions. The literature data concerning the metabolic activity of flowering plants gametes as well as of the embryo are few and fragmentary. Given the histochemical and ultrastructural data, the egg cell of angiosperms is thought to have a low metabolic activity. The most of studies focused on gene expression analyses in unfertilized egg cells and zygotes were performed using in vitro methods. In *Zea mays* and *Triticum aestivum* numerous different transcripts were isolated from these cells, and it was shown that their expression was changed after *in vitro* fertilization.

Basing on the literature data and our own investigations we will discuss the genome activity and the level of transcripts in male and female gametes, changes in transcripts after fertilization as well as the period of the embryo genome activation in Angiospermae.

REFERENCES:

Dresselhaus T, Srilunchang K-O, Leljak-Levanić D, Schreiber DN. 2006. The fertilization-induced DNA replication factor MCM6 of maize shuttles between cytoplasm and nucleus, and is essential for plant growth and development. *Plant Physiology* 140: 512–527.

Sprunck S, Baumann U, Edwards K, Langridgs P, Dresselhaus T. 2005. The transcript composition of egg cells changes significantly following fertilization in wheat (*Triticum aestivum* L.). *Plant Journal* 41: 660–672.

A cladistic analysis of leeches (Ciltellata, Hirudinida) based on female reproductive system

Aleksander Bielecki[1], Piotr Świątek[2], Jerzy Klag[2]

[1]*Department of Zoology, University of Warmia and Mazury, Oczapowskiego 5, 10-967 Olsztyn-Kortowo, Poland, e-mail: alekb@moskit.uwm.edu.pl*

[2]*Department of Animal Histology and Embryology, Silesian University, Bankowa 9, 40-007 Katowice, Poland, e-mail: jerzy.klag@us.edu.pl, piotr.swiatek@us.edu.pl*

The phylogenetic relationships of several typical leech species from Glossiphoniidae (*G. heteroclita, G. complanata*); Piscicolidae (*P. geometra, P. pojmanskae*); Hirudinidae (*H. medicinalis*); Haemopidae (*H. sanguisuga*) and Erpobdellidae (*E. octoculata*) were investigated using 18 characters relating to the female reproductive system (e.g. overall morphology, occurrence of a copulatory area and vector tissue, internal ovary organization, details of the oogenesis and cocoon deposition). As an ancestor one species from Oligoacheta (*Eisenia foetida*) was chosen. Data were analyzed using Hennig86 (vers. 1.5, J. S. Farris 1988 (Tree Gardener 2,2). As a result one preferred tree was obtained (CI=26, RI=84, tree length=85), which shows, that Hirudiniformes and Glossiphoniidae have evolved independently. These results are in agreement with previous studies based on morphology and molecular data.

Among Glossiphoniidae 7 apomorphic characters were defined: non-polarized ovary cords; occurrence of hundreds nurse cells per one developing oocyte; formation of extra-DNA bodies, multiple nucleoli and accessory nuclei in the germinal vesicle; deposition of hundreds yolky egg per cocoon; cocoons, without spongy covering secreted ventrally and not cemented to a substrate. Glossiphoniidae and Piscicolidae were found to be sister taxons. The family Erpobdellidae was found paraphyletic on the base of the 4 apomorphic characters (occurrence of cortical granules; karyosome formation; ovaries more than 3 somites in length and tubular) to the Glossiphoniidae and Piscicolidae. Fish leeches (Piscicolidae) having 2 autapomorphies: egg follicles and the vector tissue recovered to be the most specialized group. Hirudiniformes possessed 1 synapomorphy (cocoons with spongy covering) and 3 plesiomorphies (lack of cortical granules; ovaries short and spheroid; cocoons not cemented to a substrate). Hirudinidae and Haemopidae seemed to be the sister-taxons.

Our cladistic analysis of leeches based on the characters relating to the female reproductive system did not support the phylogenetic relationships between Hirudiniformes, Erpodbellidae and Piscicolidae. These conclusions are not consistent with the analysis based on the molecular studies. To further understand the phylogenetic relationships between leech families additional characters (relating to internal and external morphology, as well as molecular ones) should be used.

Role of endopolyploidy in embryonic development in plants

Jerzy Bohdanowicz

Department of Plant Cytology and Embryology, University of Gdańsk, 24 Kładki St., 80-822 Gdańsk, Poland, e-mail: jurboh@biotech.ug.gda.pl

Somatic endopolyploidy is widespread in plants in which most cell types may undergo one or several rounds of endoreplication (duplication of the nuclear genome without condensation of chromosomes and karyokinesis). Endoreplication cycles and ploidy-dependent cell enlargement are associated also with differentiation of some nutritive and/or secretory cells and tissues inside the ovule (embryo-suspensor, endosperm, endosperm haustoria, antipodals, synergids). The present state of published knowledge of the submicroscopical morphology and development of such cells is still rudimentary. This talk will summarize results of studies performed in our department on the functional ultrastructure of highly endopolyploid cells playing important role in the development of embryo.

Ultrastructural studies of spermiogenesis in the earthworm *Dendrobaena veneta* (Rosa) after cadmium intoxication

Marlena Brzozowa

Department of Animal Histology and Embryology, Silesian University, Bankowa 9, 40- 007 Katowice, Poland, e-mail: marlenbrzoz@poczta.onet.pl

Cadmium occurence in habitat adversely affects reproductive processes in earthworms. Due to cadmium influence the animals lay fewer cocoons and fewer individuals manage to hatch. However, it has not been explained whether these cadmium induced changes result from the effect on ovarian structure (Siekierska and Urbańska-Jasik, 2002; Siekierska, 2007) or disturbances of spermatogenesis especially spermiogenesis are also involved. Accordingly it was intended to determine whether cadmium disturbes the process of spermiogenesis in the earthworm *Dendrobaena veneta*. It was also intended to check whether cadmium may induce structural changes in spermatids and in mature spermatozoa and whether those changes were dose- and time- dependent.

The process of spermiogenesis was studied in sexually mature earthworms *Dendrobaena veneta* exposed to cadmium at concentrations: 10 mg and 50 mg Cd kg^{-1} of wet soil for 10 and 20 days.

Electron microscope studies revealed that cadmium in both concentrations caused distinct degenerative changes in spermatids. Those changes occurred after 10 days and became more seriously after 20 days.

Very advanced degenerative changes were observed in young spermatids. Cytoplasm of those spermatids was distinctly shrunk and vacuolised and contained shrunk dictyosomes of Golgi complexes accompanied by vacuoles. Degenerative alterations occurred also in mitochondria. Mitochondrial reactions to cadmium were vacuolisation and formation of lamellar inclusions. Endoplasmic reticulum contained extremely swollen cisternae. Many cell nuclei contained lamellar inclusions and membrane bound vacuoles. In animals exposed to 50 mg Cd kg^{-1} for 20 days degenerative changes occurred also in the oldest spermatids in the form of vesicular swellings and lamellar inclusions in the acrosome.

Cadmium in the experimental substratum induced degenerative changes in spermatids and disturbed the course of spermiogenesis. Those changes were more advanced in earthworms exposed to 50 mg Cd kg^{-1} for 20 days. In experimental earthworms some clusters of spermatids which were not changed by cadmium were also observed. Those spermatids differentiated into normal mature spermatozoa.

REFERENCES:

Siekierska E, and Urbańska-Jasik D. 2002. Cadmium effect on the ovarian structure in earthworm *Dendrobaena veneta* (Rosa). *Environmental Pollution* 120: 289–297.

Siekierska E. 2007. Changes in the ovary cells structure in the earthworm *Dendrobaena veneta* (Rosa) in neurosecretion deficiency and after exposure to cadmium. *European Journal of Soil Biology* 43: 311–315.

Mitochondrial activity in germ and somatic cells of the ovary in the earthworm *Dendrobaena veneta* (Annelida, Clitellata)

Justyna Faron[1], Tytus Bernaś[2]

[1]*Department of Animal Histology and Embryology, Silesian University, Bankowa 9, 40-007 Katowice, Poland, e-mail: jfaron@us.edu.pl*
[2]*Department of Plant Anatomy and Cytology, Silesian University, Jagiellońska 26/28, 40-032 Katowice, Poland, e-mail: tbernas@us.edu.pl*

Activity of mitochondria in the germ line and somatic cells of the ovary in the earthworm *Dendrobaena veneta* (Annelida, Clitellata) was studied by use of a ratiometric confocal microscopy and fluorescence, potential-sensitive dye (JC-1). Images taken using confocal microscope were analyzed with the electron micrographs of the ovary. Results were supposed to verify the "disposable soma" theory (Kirkwood and Holliday, 1979) which claims that germ line as potentially immortal may have higher metabolic rate than somatic cells, to produce enough energy necessary to maintain high level of accuracy in the macromolecules synthesis. Mitochondrial activity in oogenesis was studied mainly in vertebrates so far, on the especially collected single oocytes. In this study the whole of ovary (1mm of approx. length) was observed. Early stages of oogenesis were observed simultaneously, beginning from: oogonia, young previtellogenic oocytes and trophocytes (nurse cells), to the greatest vitellogenic oocytes. Somatic cells of the ovary showed the highest level of mitochondrial activity, whilst germ cells showed lower levels of activity or were inactive. Lack of mitochondrial activity was observed in the vitellogenic oocytes. Mitochondria seemed to be inactive also in previtellogenic oocytes, otherwise than mitochondria in trophocytes which contained JC-1 aggregates. Oogonia and prooocytes exhibited regions with gentle activity. Obtained results didn't confirm the "disposable soma" theory.

REFERENCES:

KIRKWOOD TBL, HOLLIDAY R. 1979. The evolution of ageing and longevity. *Proc R Soc London B* 205: 531–546.

Follicular cell differentiation in polytrophic ovaries of *Osmylus fulvicephalus* (Insecta: Neuroptera: Osmylidae)

Arnold Garbiec, Janusz Kubrakiewicz

Insitute of Zoology, University of Wrocław, Sienkiewicza 21, 50-335 Wrocław, Poland, e-mail: jkubrak@biol.uni.wroc.pl

In *Osmylus* ovariole germarium houses clusters of germ cells (cystocytes) each of which is already diversified into an oocyte and its sibling nurse cells. Two types of somatic prefollicular cells (pFCs) accompany cystocytes. Externally located pFCs adhere to the ovariole basal lamina, while their processes penetrate inwards among the germ cells. Internally located pFCs border on germ cells, whereas their contact with the basal lamina is reduced or none. When the cystocytes grow pFCs transform into follicular cells (FCs) that invest the germ cell cluster. Cystocytes together with their covering follicular epithelium form the egg chamber. In the early previtellogenic egg chamber FCs are few. With the progress of previtellogenic and vitellogenic growth their number increases. Two major subpopulations of FCs can be found in the egg chambers. More numerous FCs are externally supported by the ovariole basal lamina, while a small number of FCs remains located among germ cells inside the ovariole. The former FCs either contact directly the nurse cells or form a cuboidal epithelial cover on the lateral aspects of the oocyte. Internal FCs can be found in different positions within the egg chamber: among the nurse cells, at the nurse cell/oocyte interface or between neighboring egg chambers. In the advanced stages of vitellogenesis cuboidal FCs at both poles of the oocyte slide centripetally. At the anterior pole of the oocyte they separate the nurse cells from the oocyte, while at its posterior pole they migrate in between the egg chambers. Converging cuboidal FCs at both extremities of the oocyte drag behind the basal lamina and retain their epithelial character. In consequence, vitellogenic oocyte becomes gradually encapsulated by the epithelial layer. At late vitellogenesis, FCs at the anterior pole of the oocyte become columnar and form an eccentrically located (dorsal) epithelial fold. The latter comprises FCs that are engaged in the formation of the aeromicropyle, and those that mould the micropylar canals. The latter reside only on the dorsal side of the epithelial fold. Their processes that penetrate inside the forming aeromicropyle are supported by an elaborate cytoskeleton. All the remaining FCs are engaged in the synthesis of the main body chorion. The contribution of the internal FCs to the formation of the egg shell is not clear.

Morphology of the tongue in the cat (*Felis silvestris catus*) in the first half of the prenatal development; preliminary observations

Hanna Jackowiak, Joanna Trzcielińska, Szymon Godynicki

Department of Animal Anatomy, Agricultural University of Poznań, ul. Wojska Polskiego 71 C, 60-625 Poznań, Poland, e-mail: hannaj@au.poznan.pl

The aim of study was to characterise the morphology of the tongue in the cat in the first half of the prenatal period.Investigations were conducted on fetuses about size CRL = 2.5–7.5 cm, i.e 20–35 day of the gestation. Age of the fetuses was determined according growth curve of the domestic cat (Evans, Sack, 1973). Tissues immediately after dissection were fixed in 10% neutral formalin. Paraplast sections were stained with Masson-Goldner with method PAS with dimedone. The part of samples were processed for SEM observations.

All tongues of the studied fetuses were elongated with rounded apex. The length of tongues was from 1.9 mm to 6 mm. In the youngest fetuses at about 22 day of gestation, the surface of the tongue is flat and only in anterior part of the tongue the oval area with rounded primordia of the giant filiform papillae were observed. In the posterior part of the tongue near root of the tongue 3–4 rounded thickening of the epithelium was found, which distribution was similar to the vallate papillae.

In the older fetuses at about 30 day p.c. the outline of the giant filiform papillae is distinct and their surface is convex. On the lateral surfaces of the tongue primordia of fungiform papillae appear. The number of epithelial thickening of vallate papillae is 4–5. In next days rounded primordia of fungiform papillae were observed also on the lingual apex. On the lateral surfaces of the anterior part of the lingual body and on the posterior part of the tongue the primordia of the small filiform papillae were present. At this stage of development around primordia of vallate papillae small clefts appear. The root of the tongue is covered by elongated primordia of the conical papillae.

Embryonic epithelium of lingual mucosa in studied developmental periods consists of 3–6 layers of cells. At about 30 day p.c. the presence of glycogen in basal and superficial cells of epithelium covering the primordia lingual papillae, as well in the epithelium on ventral surface of the tongue was demonstrated.

REFERENCES:

EVANS HE, SACK WO. 1973. Prenatal development of domestic and laboratory mammals, growth curves, external features and selected references. *Anat Histol Embryol* 2: 11–45.

LM and SEM study of the tongue development in the geese

Jackowiak Hanna, Skieresz Kinga

Department of Animal Anatomy, Agricultural University of Poznań, ul.Wojska Polskiego 71C, 60-625 Poznań, Poland, e-mail: hannaj@ au.poznan.pl

The aim of the research was description of the development of the lingual mucosa of the geese from 9 to 24 day of embryonic development.

In 9 day p.c. the tongue has the triangular shape with the low prominence in the middle of the tongue and apex integrated with the mandible. In next days the tongue assumes the oblong shape. The median sulcus is visible on the surface of the prominence in 9 day and on the body of the tongue in 14 day.

The first lateral papillae of the body of the tongue appear in 11 day. The new primordia of lateral papillae develop in direction to the lingual apex, one or two every day, achieved the ultimate number of 15 lateral papillae of the tongue. On 20 day along lateral papillae develop one row of the primordia of the filiform papillae. Before the hatching there are 3 rows of promordia.

The conical papillae on the posterior part of the prominence start to develop about 15 day p.c. and in 23 day p.c. reaches the maximal number of 9 papillae, tilted above the flat root.

Histological observations of the lingual mucosa demonstrated that from 9 to 17 day p.c. epithelium on the apex and body of the tongue consists of a few layered epithelium with flat superficial cells. In next days the epithelium change into flat multilayered parakeratinized epithelium. From 22 day p.c. subepithelial connective tissue forms papillary body penetrating to the epithelium. From 9 to 18 day p.c. lingual prominence is also covered with a multilayered epithelium with rounded superficial cells covered by microvilli. At the 18 of day epithelium on the anterior part of the prominence starts to folding and forming numerous furrows, which are observed to 26 day. The epithelium of the posterior part of the prominence transforms into a flat multi-layered epithelium earlier about 20 day. The process of keratinization of the mechanical papillae starts in 23 day p.c. On the lingual root the epithelium up to 23 day has an embryonic structure.

The epithelium on ventral surface of the lingual apex in the geese form a special layer called "lingual nail", which is a external skeleton for the tip of the tongue. This structure starts to develop on 19 day p.c.

Diversification of the ovarian follicular epithelium in the horse fly, *Haematopota italica* (Diptera, Tabanidae)

Mariusz K. Jaglarz and Szczepan M. Biliński

Department of Systematic Zoology, Institute of Zoology, Jagiellonian University, R. Ingardena 6, 30-060 Krakow, Poland, e-mail: jagla@zuk.iz.uj.edu.pl

The diversification of the follicular cells is best characterized in the model insect, the fruit fly, *Drosophila melanogaster*. Detailed structural, genetic and molecular studies demonstrated that during oogenesis the initially identical follicular cells overlying the germ line cells diversify into a number of subpopulations responsible for the formation of specific eggshell structures (reviewed in Horne-Badovinac and Bilder, 2005). Comparative studies showed that in dipterans the functioning of ovarian follicles is group-specific and may significantly differ from that in *Drosophila* (Kubrakiewicz et al., 2003; Tworzydlo et al., 2005). Here, we present results of the ultrastructural analysis of follicular cell morphogenesis in a distant *Drosophila* relative, the horse fly, *Haematopota italica*. We found that within the previtellogenic ovarian follicles of this species, there are 5 morphologically recognizable follicular cell subpopulations: (1) anterior polar cells (anterior PCs) at the anterior pole of the nurse cells compartment, (2) anterior terminal cells surrounding the anterior PCs, (3) cuboidal cells overlying the lateral aspects of the follicle, (4) posterior polar cells (posterior PCs) situated at the posterior pole of the follicle, and (5) posterior terminal cells surrounding the posterior PCs. At the anterior and posterior poles of *Haematopota* follicles, there are two morphologically distinct clusters of follicular cells. Each cluster consists of centrally located 2–3 polar cells surrounded by several terminal cells. During previtellogenesis, the clusters lose the initial symmetry as their cells differentiate and develop conspicuous cytoplasmic projections comprising cytoskeletal elements. Since the projections of the anterior versus posterior cells differ significantly morphologically, we suggest that they participate in different processes. The cell projections of the anterior cluster support migration activity whereas those of the posterior are involved in the formation of specializations at the eggshell posterior pole.

REFERENCES:

Horne-Badovinac S and Bilder D. 2005. Mass transit: epithelial morphogenesis in the *Drosophila* egg chamber. *Developmental Dynamics* 232: 559–574.

Kubrakiewicz J, Jablonska A, Mazurkiewicz M, Biliński SM. 2003. Differentiation and diversification of the follicular cells in flies: insight from the studies of the lower brachycerans' ovaries. *Genesis* 36: 214–224.

Tworzydlo W, Jablonska A, Kisiel E, Bilinski SM. 2005. Differing strategies of patterning of follicular cells in higher and lower brachycerans (Diptera: Brachycera). *Genesis* 43: 49–58.

The link between ancient and the most modern Actinopterygii: do larvae of Osteoglossid fish possess the yolk syncytial layer?

Marta Jaroszewska[1], Konrad Dabrowski[2], Bogdana Wilczyńska[1]

[1]*Laboratory of Histology and Embryology of Vertebrates, Nicolaus Copernicus University, ul. Gagarina 9, 87-100 Toruń, Poland, e-mail: marja@umk.pl*
[2]*Ohio State University, Columbus, Ohio 43210, U.S.A., e-mail: dabrowski.1@osu.edu*

The yolk syncytial layer (ysl) was thought to be a uniquely characteristic feature of the teleosts. However, recent data indicate that the representative of Semionotiformes, North American garfish (*Lepisosteus*) constitutes the first evolutionary appearance of the ysl in actinopterygian fish (Jaroszewska and Dabrowski, 2008). *Amia*, the closest extant fish to Teleostei, does not have a ysl. There is a question of the origin of the ancestral progenitor tissue for ysl in teleost embryos and if the ysl in garfish can be considered as a homologue of the ysl of teleosts. The studies on the silver arowana, *Osteoglossum bicirrhosum*, the oldest lineage of teleosts, could provide the answer to this question (Cooper and Virta, 2007).

Several larvae of silver arowana were fixed for histological analyses when intense absorption of the yolk was taking place. We examined fish (n=6) of 50–65 mm total length with yolksacs extended up to 11.8 mm, to completely absorbed. Light microscopy techniques with H+E staining method were used.

The larvae possess a yolksac reserves composed of two parts, an external, and an internal component, in the abdominal cavity. There is a structure which can be regarded as a ysl, considering the presence of non-cellular structure of the surface of external yolkx. There are differences in the structure of the yolksac compartments, including the appearance of blood vessels. The internal part of the yolk is poorly vascularized, just with capillary vessels, but no structure analogous to ysl could be found. Some differences in the rate of the utilization of the yolk in two compartments were expected on the basis of morphological studies. It was observed that while the internal part of the yolk has a coalescent appearance, the external compartment seems to have a more heterogeneous structure.

Questions remain regarding the divisions, ultrastructure, and functions of the ysl in silver arowana, and also about possible differences in the chemical composition of the two parts of the yolk.

REFERENCES:

JAROSZEWSKA M, DABROWSKI K. *Lepisosteids* and *Amiids. Development of Non-teleost Fish*, In: Kunz Y [ed.], Science Publishers Inc. (in print).

COOPER MS. and VIRTA VC. 2007. Evolution of gastrulation in the ray-finned (Actinopterygian) fishes. *Journal of Experimental Zoology. Part B. Molecular and Developmental Evolution* 308: 591–608.

Structure and function of yolk nuclei in oocytes of salticid spiders

Izabela Jędrzejowska, Roksana Jankowska

Department of Animal Developmental Biology, Zoological Institute, University of Wroclaw, Sienkiewicza 21, 50-335 Wrocław, Poland, e-mail: ijowska@biol.uni.wroc.pl

Yolk nuclei, as prominent cytoplasmic structures, have been reported in oocytes of many spider species. Although among species they exhibit a significant morphological and behavioral constancy during the oocyte growth and maturation, their functional importance remains unclear. Current research on oocytes of *Evarcha arcuata* (Clerck, 1758), a member of Salticidae, revealed the presence of typical yolk nuclei. Ultrastructural analysis of yolk nuclei in this species shows that during early previtellogenesis they are very poor in organelles, being composed mainly of endoplasmic reticulum, mitochondria and lamellar bodies. It was found that endoplasmic reticulum appeared in the form of labyrinth of interconnected cisterneae. The lumen of the latter is relatively wide and filled with electron dense material. With the progress of previtellogenesis and vitellogenesis, cisternae of endoplasmic reticulum increase in number and show increasingly fused and tight arrangement. During vitellogenesis the yolk nuclei contain structurally modified mitochondria and additional components such as lipid droplets. The most striking feature of the yolk nuclei in advanced vitellogenic stages is the presence of numerous lamellar bodies showing a great heterogeneity, indicating their structural relationship with either mitochondria or lipid droplets. The results of ultrastructural observations support the idea that the yolk nucleus participates in the process of lipid synthesis, whereas the lamellar bodies have been interpreted as a lipid droplets precursors.

The role of *Pax3* – positive muscle progenitor cells during myotomal myogenesis in *Coregonus lavaretus* (Teleostei)

Agata Kacperczyk[1], Teresa Jagla[2] and Małgorzata Daczewska[1]

[1]*Department of General Zoology, Zoological Institute, University of Wrocław, Sienkiewicza 21, 50-335 Wrocław, Poland, e-mail: daczew@biol.uni.wroc.pl*
[2]*INSERM U384, Faculte de Medicine, Clermont-Ferrand, France, e-mail: teresa.jagla@u-clermont1.fr*

In *Coregonus lavaretus* prior to segmentation cells adjacent to the notochord, called adaxial cells, express Myogenic Regulatory Factors (MRFs: *Myod*) and MyHC-slow protein. Shortly after somite formation, adaxial cells migrate towards the lateral part of the myotome and form a monolayer of red muscles on its surface. Following the movement of slow muscle precursors to the surface of the somite, deeper cells differentiate into white muscle fibres.

After somitogenesis the paired-type homeobox gene *Pax-3* come be expressed in a single layer of cells superfacial to the myotome (between primary myotome and epidermis). Their expression profile resembles the "exteranl cells" (revealed in many teleosts species) or dermomyotome described in amniota. During later developmental stages *Pax-3* gene is expressed in cells in intermyotomal space and then subsequently in myotomes between myotubes. In these cells, Pax 7 (marker of satellite cells) protein was also observed.

Pax-3/7 positive cells, which have migrated into the myotomes between myotubes, differentiate into satellite cells/secondary myoblasts (of mesenchymal origin) and form secondary muscle fibres. These muscle fibres are formed in discrete germinal zones at the lateral margins of the myotome, a prosess termed "stratified" hyperplasia.

Reduction of callose amount is accompanied by inhibition of growth and antheridiogenesis and induction of atrophy in *Anemia phyllitidis* gametophytes

Andrzej Kaźmierczak

Department of Cytophysiology, University of Łódź, Pilarskiego 14, 90-231 Łódź, Poland, e-mail: kazma@interia.pl

It has been proposed that walled plant cells are composed of two primary structural elements: a cell body (represented by the nucleus and its radiating microtubules; MTs) and a cell periphery complex (formed by the plasma membrane and its actin cytoskeleton). The cell body cooperates, by perinuclear MTs, with F-actin of the cell periphery to position itself at a cell's geometrical/cytoskelatal centre. When these centres overlap a cell divides equally, however when the cell body moves from the geometrical to cytoskeletal centre a cell divides asymmetrically (Baluška et al., 2001). Callose, a natural component widely distributed in the plant kingdom, accompanies formation and maturation of a cell plate and is associated with formation of septae in several species of multicellular green algae, bryophytes as well as ferns (Scherp et al., 2001).

Fluorescence staining and morphometrical measurements revealed that callose was a component of newly formed cell plates of symmetrically and asymmetrically dividing cells during gibberellic acid-induced antheridiogenesis as well as in walls of young growing cells of *Anemia phyllitidis* gametophytes. Callose in cell walls forms granulations characteristic of pit fields with plasmodesmata. 2-deoxy-D-glucose (DDG), eliminated callose granulations and reduced its amount estimated by measurements of fluorescence intensity. This effect was accompanied by reduction of antheridia and cell numbers as well as size and atrophy of particular cells and whole gametophytes. It is suggested that inhibition of glucose metabolism and/or signalling, might decrease callose synthesis in *A. phyllitidis* gametophytes leading to its elimination from cell plates of dividing cells and from walls of differentiating ones as well as from plasmodesmata resulting in inhibition of cytokinesis, cell growth and disruption of the intercellular communication system, thus disturbing developmental programs and leading to cell death. The diverse sensitivity of gametophyte cells to DDG indicated existence of cellular domains, which overlap with the previously distinguished three zones of gametophytes.

REFERENCES:

BALUŠKA F, VOLKMANN D, BARLOW PW. 2001. Motile plant cell body: a 'bug' within a 'cage'. *Trends Plant Sci* 6: 104–111.

SCHERP P, GROTHA R, KUTSCHERA U. 2001. Occurrence and phylogenetic significance of cytokinesis-related callose in green algae, bryophytes, ferns and seed plants. *Plant Cell Rep* 20: 143–149.

Interaction of myogenic cells in the myogenesis of vertebrate skeletal muscles

Leokadia Kiełbówna

Department of Animal Developmental Biology, Zoological Institute, University of Wrocław, Sienkiewicza 21, 50-335 Wrocław, Poland

Determination and differentiation of cells in the skeletal muscle lineage is positively regulated by cell-cell interactions. In the initial phase of myogenesis mesodermal myogenic cells at the stage of gastrula in *Xenopus* show a particular kind of interaction called "community effect". The community effect was first demonstrated when it was shown that groups of more than 100 *Xenopus* muscle precursor cells were able to differentiate, while smaller groups and single cells had no such ability (Gurdon et al., 1993). This suggested that some muscle promoting influence was being transmitted from cell-to-cell. Standley et al. (2001) have shown that eFGF is able to mediate the community effect in *Xenopus* myogenesis and suggested that it is likely to be the endogenous ability to support expression of the myogenic transcription factor XmyoD and XMyf5 in single muscle precursor cells dissected from the dorsolateral mesoderm of early gastrulae. At advanced stages of myogenesis myoblast interaction is associated with adhesion. Cadherins play a key role in mediating cell-cell contact because they are centrally involved in establishing cell-cell adhesive structures. Cadherins are trasmembrane molecules that interact with similar cadherins on neighboring cells via their ectodomains and with the actin-cytosceleton via their cytoplasmic regions. Several cadherins have been implicated in this process. N-cadherin has been studied most extesively, several lines of evidence indicate that it plays a positive role in skeletal myogenesis. They can directly trigger signaling events, which promote the activation of a myogenic differentiation program (Gaichberg and Geiger, 1998)

REFERENCES:

GAICHBERG P and GEIGER B. 1998. Direct involvement of N-cadherin-mediated signaling in muscle differetiation. *Mol Biol* 9: 3119–3131.

GURDON JB, TILLER F, ROBERTS J and KATO K. 1993. A community effect in muscle development. *Curr Biol* 3: 1-11.

STANDLEY HJ, LORN AM, GURDON JB. 2001. FGF and its mode of action in the community effect during *Xenopus* myogenesis. *Development* 128: 1347–1357.

The role of extracellular matrix during wheat androgenesis

Robert Konieczny[1], Jerzy Bohdanowicz[2], Maria Pilarska[1], Joanna Świerczyńska[2], Andrzej Czaplicki[3]

[1]*Department of Plant Cytology and Embryology, Jagiellonian University, Grodzka 52, 31-044 Kraków, Poland; e-mail: robert.konieczny@uj.edu.pl*

[2]*Department of Genetics and Cytology, University of Gdańsk, Kładki 24, 80-822 Gdańsk, Poland*

[3]*Plant Breeding and Acclimatization Institute, 05-870 Błonie, Poland*

Plant extracellular matrix (cell wall) plays a variety roles in development. Its properties influence cell shape, plane of cell division and direction of growth; some of its compounds serve as signalling molecules and as extracellular domains of membrane receptors. Androgenesis in two Polish wheat cultivars, Apollo and Kaspar is accompanied by changes in cell wall structure and differential distribution of pectic and arabinogalactan protein (AGPs) epitopes. Additionally, the formation of conspicuous extracellular surface matrix network (ECMSN) during regeneration was found. Microscopic observations of the onset of regeneration revealed the presence of two types of cells on surface of Apollo and Kaspar callus: large, parenchymatous cells and small meristematic cells arranged in multicellular clusters. In both cultivars the outer parenchymatous cells displayed features of degeneration, and bore specific pectic epitope LM8. However, only in Kaspar the expression of LM8 coincided with formation of ECMSN on the surface of outer parenchyma cells. Instead, the corresponding cells of Apollo callus displayed expression of several AGPs epitopes (JIM4, JIM14 and JIM16) which were not found in the cell walls of Kaspar. The onset of embryogenesis and caulogenesis in both cultivars coincided with dissociation of outer parenchyma cells, formation of meristematic clumps at callus periphery, and development of inner parenchyma showing strong signal of JIM5 and LM2 binding. Contrary to Kaspar, the surface of meristamic tissue in Apollo callus was coated by a conspicuous network of ECMSN. Notably, the outer cell wall of these cells were highly reactive to anti-pectin JIM7, whilst this epitope was not detected in Kaspar culture, at all. The development of regenerants was accompanied by gradual degradation of ECMSN and fading of JIM7 fluorescence. The enzymic digestion of ECMSN at the early stages of development of regenerative structures was found to inhibit regeneration in Apollo cultivar, but did not influence callus growth. Root meristems which were formed throughout the culture were unlabelled by antibodies used. Nevertheless, LM8 labeled specifically lateral root cap cells in Apollo and Kaspar culture. The changes in cell wall structure are discussed in relation to their possible function in wheat androgenesis.

Development and ultrastructure of suspensor basal cell in *Potamogeton natans* L.

Małgorzata Kozieradzka-Kiszkurno and Jerzy Bohdanowicz

Department of Plant Cytology and Embryology, University of Gdańsk, 24 Kładki St., 80-822 Gdańsk, Poland, e-mail: stokrota@biotech.ug.gda.pl

The embryogenesis in *Potamogeton natans* L. (Helobiae) was investigated using cytochemical methods, light and electron microscopy. Rapid differentiation of the basal cell (BC) begins shortly after zygote division. The fully differentiated BC is nearly spherical and contains huge nucleus. The highly endopolyploid nucleus shows loosely arranged polytene chromosomes. As BC grows, its large vacuoles disappear; its cytoplasm increases in volume and the numbers of organelles increase. At the micropylar apex of the cell a transfer wall of ramifying ingrowths forms. The BC is filled with dense cytoplasm containing numerous ribosomes, mitochondria, plastids, endoplasmic reticulum, dictyosomes, microbodies, lipid droplets and vesicles differing in size and content. The mitochondria, spherical to ellipsoidal in shape, are numerous between the transfer wall ingrowths. Plastids are bigger then mitochondria; may contain a few internal membranous structures, numerous small tubules and starch grains. In ultrastructure the chalazal suspensor cells and the embryo-proper cells are similar. There are no plasmodesmata in the outer walls of the whole embryo, but they are numerous in the inner walls of the chalazal suspensor and embryo-proper. Proteins, insoluble polysaccharides, nucleic acids and lipids are localized in the BC during different phases of embryo proper.

Investigations of the cytochemistry and ultrastructure of the suspensor in *Potamogeton natans* at various stages of the development of the embryo-proper, suggest the BC functions as a synthetically active transfer cell absorbing nutrients from maternal tissues, metabolizing them and translocating through the chalazal suspensor cells to the growing embryo-proper.

Mechanisms regulating spermiogenesis in plants

Maria Kwiatkowska, Katarzyna Popłońska, Agnieszka Wojtczak

Department of Cytophysiology, University of Łódź, Pilarskiego 14, 90-231 Łódź, Poland, e-mail: kwiat@biol.uni.lodz.pl

Spermiogenesis, common in animal kingdom, in plants occurs only in the organisms which need aquaous medium for fertilization (e.g. alga, bryophyta, pteridophyta). Spermatozoids which result from spermiogenesis are resistant to harmful environmental factors, they can move freely, their cells have specific shape and mitochondria and plastids contain starch. The above features (except plastids) are characteristic also of animal spermatozoids. Also the mechanisms of spermatid differentiation are very similar which was shown in the experiments on *Chara* algae (Kwiatkowska, 1996; Kwiatkowska and Popłońska, 2002). The basic process in nuclear chromatin remodeling leading to disappearance of the nucleosomal structure and formation of the extremally condensed fibrillar structure due to the exchange of histones into protamines or other more basic proteins which are synthesized in mid spermiogenesis. We have shown that in *Chara* synthesis and transport of the protamine-type proteins involve endoplasmic reticulum and endocytosis processs (Popłońska et al., 2007; and in prep). In animals the site of protamine synthesis is not known. Both in animals and in *Chara* the ubiquitine/proteasome system is involved in the exchange of histones into protamines. Using the inhibitors of proteasome proteolytic activity, immunocytochemical analyses and EM we showed that this system is indispensable for the removal of histones and for the exchange of the nucleosomal structure into fibrillar one (Wojtczak and Kwiatkowska, 2008). Recently we have shown that spontaneous DNA double breaks are necessary for the correct remodeling process. Moreover DNA double breaks are also involved in chromosomal movement to the proper territory in a nucleus (in prep). Similar phenomenon was described in animal spermiogenesis.

REFERENCES:

KWIATKOWSKA M. 1996. Changes in ultrastructure of cytoplasm and nucleus during spermiogenesis in *Chara vulgaris*. *Folia Histochem Cytobiol* 34: 41–56.

KWIATKOWSKA M, POPŁOŃSKA K. 2002. Further ultrastructural research of Chara vulgaris spermiogenesis: endoplasmic reticulum, structure of chromatin, ^{3}H-lysine and ^{3}H-arginine incorporation. *Folia Histochem Cytobiol* 40: 85–97.

POPŁOŃSKA K, WOJTCZAK A, KWIATKOWSKA M, KAŹMIERCZAK A. 2007. Cytochemical and immunocytochemical studies of the localization of histones and protamine-type proteins in spermatids of *Chara vulgaris* and *Chara tomentosa*. *Folia Histochem Cytobiol* 45: 367–374.

WOJTCZAK A, KWIATKOWSKA M. 2008. Immunocytochemical and ultrastructural analyses of the function of ubiquitin-proteasome system during spermiogenesis with the use of the inhibitors of proteasome proteolytic activity in algae *Chara vulgaris*. *Biol Reprod* (in press). DOI: 10.1095/biolreprod.107.062901.

The structure of testes in *Velia caprai* (Heteroptera, Veliidae)

Antoni Ogorzałek

Department of Animal Developmental Biology, Zoological Institute, University of Wrocław. Sienkiewicza 21, 50-335 Wrocław, Poland, e-mail: ogorzal@biol.uni.wroc.pl

The testes in *Velia caprai* (Heteroptera, Gerromopha) were investigated with TEM, DAPI and rhodamine-phalloidine techniques, and immunohistochemically on β tubuline. They contain cysts in which male germinal cells form spiral bundles and are synchronised in consecutive stages of spermatogenesis. Ultrastructurally spermatocytes, spermatids and spermatozoids are similar to those described in other heteropterans. The sperm heads are elongated, the axoneme of the typical 9+2 structure in the proximal sperm part is covered by two mitochondrial complexes of striated structure.

The cells forming the cyst wall penetrate also inside the cyst lumen and among bundles of germ cells. They contain structural indicators of fagocytosis and endocytosis. The apical parts of spermatids and spermatozoids are anchored in this cells.

The netting cells are elongated, rod-shaped. Two extensions containing an accumulation of fibrillar material, one in the subdistal part of the cell, another in the subproximal part, are observed. In the paraxial part of the cell a bundle of tubular structures, each 30 nm in diameter is located. In the thin part they form a spherical complex and between the tubules an electron dense material is observed. In the subdistal part of the netting cells the bundles inside the accumulatian of granular material become wavy. In the subproximal part the tubules branch and form a brush-like structure. In the thin part of netting cell numerous microtubules, arranged parallelly to the cell long axis, are observed. Both in the netting cells and in those forming the cyst wall numerous secretory granules are often observed.

Somatic embryogenesis in zygotic embryo culture of *Trifolium nigrescens* Viv.

Maria Pilarska[1], Robert Konieczny[1], Jerzy Bohdanowicz[2], Joanna Świerczyńska[2]

[1]*Department of Plant Cytology and Embryology, Jagiellonian University, 52 Grodzka St., 31-044 Kraków, Poland, e-mail: maria.pilarska@uj.edu.pl*

[2]*Department of Plant Cytology and Embryology, University of Gdańsk, 24 Kładki St., 80-822 Gdańsk, Poland, e-mail: jurboh@biotech.ug.gda.pl*

Under specific *in vitro* conditions somatic plant cells can change their developmental pathway and produce embryos. This process, called somatic embryogenesis (SE) can be used as a model system for studying the mechanisms underlying plant growth and differentiation. Previously, the method for whole plant regeneration via SE from seedling-derived callus of *Trifolium nigrescens* (ball clover) was described (Konieczny, 1995). In that study however, the frequency of regeneration was relatively low, thus we developed a new system for high efficient SE using zygotic embryos as explants.

To induce SE, the zygotic embryos (~3 mm) were cultured on MS medium supplemented with 0.5 mg/l NAA and 2 mg/l 2iP for 28 days. After 7–10 days the first somatic embryos arose. Throughout the culture the embryoid formation was concomitant with callus growth. Embryoids at the torpedo stage and older were harvested from maternal tissue and transferred on half-strength MS for further development. About 25% of somatic embryos converted into flowering plants.

Histological observations revealed that after 3 days of culture groups of meristematic cells were formed in outer part of hypocotyl cortex, originating probably from single cortical cell. After 7 days of culture the periclinal divisions of epidermal cells covering cotyledons and hypocotyls coupled with extended mitotic activity of parenchyma led to disruption of epidermis and outgrowth of the callus over explant surface. After 9 days of culture the first bipolar somatic embryos were formed. Many embryoids displayed several morphological abnormalities (fused cotyledons, elongated hypocotyls, poorly developed root meristem) which seem to be related to disturbances in auxin flux. The precocious germination of somatic embryos was frequently noted. Observations in SEM revealed, that early stages of SE were accompanied by the formation of conspicuous fibrillar network called extracellular matrix surface network (ECMSN).

REFERENCES:

KONIECZNY R. 1995. Plant regeneration in callus culture of *Trifolium nigrescens* Viv. *Acta Biologica Cracoviensia Series Botanica* 37: 47–52.

Naked embryo sacs of *Utricularia alpina* Jacq. (Orchidioides, Lentibulariaceae)

Bartosz J. Płachno[1], Piotr Świątek[2]

[1]*Department of Plant Cytology and Embryology, Jagiellonian University, 52 Grodzka St., 31-044 Cracow, Poland, e-mail: bartek78pl@poczta.onet.pl*
[2]*Department of Animal Histology and Embryology, Silesian University, Bankowa 9, 40-007 Katowice, Poland, e-mail: piotr.swiatek@us.edu.pl*

Utricularia alpina Jacq. (section Orchidioides) is an epiphyte, which occurs in Antilles and northern S. America. It forms large up to 6 cm orchid-like flowers (Taylor, 1989). *Utricularia alpina* forms a big, stalked placenta, which is covered with numerous, very small unitegmic and tenuinucellate ovules. The placenta consists mainly of large, highly vacuolated parenchyma cells. Some cells of the placental epidermis form the transmitting tract (obturator) for pollen tubes. The placental nutritive tissue cells, in contrast to placental parenchyma, have prominent thick cell walls. The mature embryo sacs grow outside the ovules. Central cell grows on the surface of the placenta and may grow also on the surface of neighboring ovule. Part of the central cell, which is outside of the ovule, is highly vacuolated. In peripheral cytoplasm there are ER cisternae, mitochondria, dictyosomes and small plastids with starch. Naked embryo sacs are very rare in Angiosperms, however, they were described in *Utricularia*, but they have been never analyzed on the electron microscope level. Future research will be concentrated on the pollen tubes and naked part of embryo sacs interaction.

REFERENCES:

TAYLOR P. 1989. The genus *Utricularia* – A taxonomic monograph. *Kew Bulletin* 14: 1–735.

The level of non-hydrophobic lipids in pig embryos during cleavage

Marek Romek[1], Barbara Gajda[2], Paweł Weber[1], Ewa Krzysztofowicz, Zdzisław Smorąg[2]

[1]*Department of Cytology and Histology, Institute of Zoology, Jagiellonian University, 6 R. Ingardena St., 30-060 Cracow, Poland. e-mail: romek@zuk.iz.uj.edu.pl*
[2]*Department of Biotechnology of Animal Reproduction, National Research Institute of Animal Production, 32-083 Balice/Cracow, Poland. e-mail: bgajda@izoo.krakow.pl*

Lipids play an important role as an energy source vital for proper pig embryo development. It has been demonstrated that total content of lipids decreases during cleavage (Romek et al., 2008) while the level of triglycerides remain unchanged. However, there is no information on the quantity of non-hydrophobic lipids in pig embryos. Therefore, the principal aim of the present work was to fill this gap using scanning confocal microscopy and solvatochromic dye nile red.

The experiment was carried out on pig zygotes, 2–4 cell embryos, morula and blastocysts produced in vivo. All embryos were obtained from superovulated gilts, which were then artificially inseminated with the standard dose of semen. Embryos were collected after flushing oviducts (zygote, 2–4 cell) or uterus (8–16 cell, morula, blastocyst) with phosphate buffered saline supplemented with 20% fetal calf serum at about 30°C (Gajda and Smorąg, 2002). For each stage ten embryos were examined. All selected embryos were fixed with 2% formaldehyde in phosphate buffered saline, washed, stained for 3h with 100 nM solution of nile red and examined in confocal microscope. To evaluate the level of non-hydrophobic lipids, nile red was excited using a 514 nm and 633 nm laser lines. Emission spectrums were recorded and deconvoluted into two bands corresponding to non-polar and polar lipids, respectively.

Total amount of non-hydrophobic lipids evaluated using our semi-quantitative method show statistically significant differences between different stages of development and systematically decreased during cleavage, especially in embryo cytoplasm. The highest differences were found between morula and blastocysts. These results may correlate with total embryo oxygen consumption. In the light of the present findings, non-hydrophobic lipids s may be important energy source of pig embryos during early embryonic development.

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REFERENCES:

GAJDA B, SMORĄG Z. 2002. Vitrification of cultured and non-cultured expanded and hatched porcine blastocysts. *CryoLetters* 23: 385-388.

ROMEK M, GAJDA B, KRZYSZTOFOWICZ E, SMORĄG Z. 2008. Lipid Content of non-cultured and cultured pig embryo. *Reproduction in Domestic Animal* (In press).

The role of cell death in the midgut epithelium of *Allacma fusca* (Insecta, Collembola, Symphypleona)

Magdalena M. Rost-Roszkowska

Department of Animal Histology and Embryology, University of Silesia, Bankowa 9, 40-007 Katowice, Poland

Insect midgut epithelium in connection with its functions, undergoes permanent damage (degeneration) and regeneration. At the end of animals life all processes of degeneration in the insect midgut epithelium increase, while regeneration proceeds less intensively. Midgut epithelial cells might be degenerated due to necrosis or apoptosis (Rost-Roszkowska and Poprawa, 2007; Tettamanti et al., 2007).

In the midgut epithelium of *Allacma fusca*, initially, only apoptosis is observed, then it is followed by necrosis. Described mode of removing of cell content into the midgut lumen is called holocrine secretion - cells undergo transformation and their content is disintegrated during digestion (programmed necrosis). Necrosis is often connected with accidental and uncontrolled cell degeneration. Eventually at the end of lifespan, when all organelles undergo degeneration, necrosis completely replaces apoptosis. The apoptotic and necrotic alterations in midgut epithelial cells of *Allacma fusca* are described at the ultrastructural level. Necrosis as the kind of holocrine secretion, is activated in the older specimens, while apoptotic cells take a part in digestion in younger ones. The apoptotic and necrotic alterations in midgut epithelial cells of *Allacma fusca* are described at the ultrastructural level.

REFERENCES:

ROST-ROSZKOWSKA MM, POPRAWA I. 2007. Apoptosis in the midgut epithelium of *Allacma fusca* (Insecta, Collembola, Symphypleona). *Proceedings 3SCM–2007*, Belgrade, Serbia.

TETTAMANTI G, GRIMALDI A, CASARTELLI M, AMBROSETTI E, PONTI B, CONGIU T, FERRARESE R, RIVAS-PENA ML, PENNACCHIO F, and DE EGUILEOR M 2007. Programmed cell death and stem cell differentiation are responsible for midgut replacement in *Heliothis virescens* during prepupal instar. *Cell Tissue Res* 330: 345–359.

Cell death in the midgut epithelium of *Epilachna* cf. *nylanderi* (Insecta, Coccinellidae)

Magdalena M. Rost-Roszkowska[1], Izabela Poprawa[1], Jerzy Klag[1], Paweł Migula[2], Jolanta Mesjasz-Przybyłowicz[3], Wojciech Przybyłowicz[3]

[1]*Department of Animal Histology and Embryology, University of Silesia, Bankowa 9, 40-007 Katowice, Poland*
[2]*Department of Animal Physiology and Ecotoxicology, University of Silesia, Bankowa 9, 40-007 Katowice, Poland*
[3]*Materials Research Group, iThemba LABS, PO Box 722, 7129 Somerset West, South Africa*

Kerr and co-workers (1972) distinguished two main types of cell death: apoptosis and necrosis. The midgut epithelial cells, which are responsible for major digestive functions, secretion and absorption, proceed the degeneration and regeneration processes. During degeneration, damaged or disrupted epithelial cells are rejected to the midgut lumen, where they proceed disintegration (Rost, 2006).

The midgut epithelial cells are in the first line of defence against excess of metals in insect body. Recently, mechanisms of protection from excess nickel were intensively studied in a chrysomelid beetle, *Chrysolina pardalina*, which efficiently eliminates nickel (Klag et al., 2002; Przybyłowicz et al., 2004) *Epilachna cf. nylanderii* is another recently discovered feeder of South-African Ni-hyperaccumulator *Berkheya coddii* (Asteraceae). The midgut epithelial cells of this species are also the first exposed to extremely high concentrations of toxic nickel. In this study all forms of protection observed in the midgut epithelium of *E. nylanderi* are described. Processes of apoptosis and necrosis were analyzed at the ultrastructural level, and TUNEL reaction was used for detection of apoptotic cells.

REFERENCES:

KERR JFR, WYLLIE AH, and CURRIE AR. 1972. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissues kinetics. *Br J Cancer* 26: 239–257.

KLAG J, MESJASZ-PRZYBYŁOWICZ M, NAKONIECZNY M, and AUGUSTYNIAK M. 2002. Ultrastructure of the midgut of the chrysomelid beetle *Chrysolina pardalina*. 15th ICEM, Durban, South Africa, 1–6.

PRZYBYŁOWICZ WJ, MESJASZ-PRZYBYŁOWICZ J, MIGULA P, TURNAU K, NAKONIECZNY M, AUGUSTYNIAK M, and GŁOWACKA E. 2004. Elemental microanalysis in ecophysiology using ion microbeam. *Nucl Instr Meth Phys B* 219–220: 57–66.

ROST MM. 2006. Ultrastructural changes in the midgut epithelium in *Podura aquatica* L. (Insecta, Collembola, Arthropleona) during regeneration. *Arthropod Struct Dev* 35: 69–76.

Egg capsules organization in tropical stoneflies of the family Perlidae

Elżbieta Rościszewska[1], Stefano Fenoglio[2], Sylwia Bohaczyk[1], Inga Domagała[1], Monika Białowąs[1], Magdalena Gawęda[1]

[1]*Department of Systematic Zoology and Zoogeography, Institute of Zoology, Jagiellonian University, Ingardena 6, 30-060 Kraków, Poland, e-mail: rosc@zuk.iz.uj.edu.pl*
[2]*Universitat di Piemonte Orientale, Alessandria, Italy*

Stoneflies are amphibiotic insects. One up to three years embryonic and larval development takes place in cool, well oxygenated water. A mating period occurs on land. Females lay packets of eggs into water. Each egg is covered by egg capsule which is equipped with a specialized attachment structure. Egg capsule is multilayered, radially and regionally differentiated. Egg capsule organization facilitates crucial physiological functions, in particular efficient gas exchange both in aquatic and land environments. Numerous (multiregional) studies carried in many stoneflies species show that egg capsules of all the investigated stoneflies are species specific (Fenoglio and Rościszewska, 2003). Therefore they can serve as important taxonomical criterion (Knispel et al., 2002).

In this contribution the organization of the egg capsules in several tropical (Equadoran, Nicaraguan and Bolivian) species belonging to the family Perlidae is described. The results and the appropriate results which concern the european species of the mentioned family are compared. Adaptations of the egg capsules to the different environments are discussed.

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REFERENCES:

FENOGLIO S, ROŚCISZEWSKA E. 2003. A characterization of the egg capsules of *Anacroneuria starki* and *A. talamanca* (Plecoptera: Perlidae) with a suggestion about the distribution of stoneflies in the tropics. *Folia biologica (Kraków)* 51: 159–164.

KNISPEL S, ROŚCISZEWSKA E, VINÇON G, LUBINI V. (2002). The status of *Perlodes jurassicus* Aubert, 1946 (Insecta: Plecoptera: Perlodidae). *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 75: 183–189.

The ultrastructural and immunocytochemical studies of the thyroid gland in the grass snake *Natrix natrix* L.* (Lepidosauria, Serpentes) embryos

Weronika Rupik

Department of Animal Histology and Embryology, Silesian University, 9 Bankowa St., 40-007 Katowice, Poland, e-mail: rupik@us.edu.pl

Thyroid hormones are necessary for the normal development of all vertebrate species. During early development, thyroid hormones are of maternal origin. In the case of egg-laying vertebrates, they are deposited in the egg yolk (McNabb and King, 1993). Later in development, embryos synthesize and release thyroid hormones from their own developed thyroid gland. The embryos of *Natrix* were isolated in regular sequence of time from egg lying till hatching. The age of embryos was calculated using the table of species development (Rupik, 2002). The ultrastructural findings show that in the course of differentiation thyroid gland contained follicles of different size. At the stage VI it contained small follicles without lumen or with small irregular lumen. High cuboidal or low columnar cells lined them. They were poor in organelles and were considered hypoactive. At the stage VII to XII thyroid follicles consisted of two types of epithelial cells (dark and light). Light cells occurred one or two per follicles. Epithelial cells varied in shape from columnar to flat. The luminal surface of thyreocytes showed increased size and number of microvilli. The nuclei of epithelial cells were regular in shape. Proliferation of rough endoplasmic reticulum was seen with significantly dilated cisterns containing low electron density material. The Golgi complex was well developed. Follicular epithelial cells contained abundant small vesicles and electron dense granules in the apical cytoplasm. In the middle of embryogenesis the follicular epithelium cells frequently showed apocrine secretion into colloidal lumens. Apocrine protrusions were demonstrated, which dome-like or balloon like structures contained light, granular matrix. Immunocytochemical studies coincide with the ultrastructural studies and indicated that thyroglobulin was found in different areas of thyreocytes. It may occupy the whole cell, the apex, the colloid substance and sometimes extracellular spaces.

REFERENCES:

McNabb FMA, and King DB. 1993. Thyroid hormone effects on growth, development, and metabolism. In: Schreibman MP, Scanes CG, and Pang PKT [eds.], *The Endocrinology of Growth, Development and Metabolism in Vertebrates*, 393–417, Academic Press, New York.

Rupik W. 2002. Normal developmental table in early development of adrenal glands in grass snake *Natrix natrix* L. (Lepidosauria, Serpentes). *Adv Anat Embryol Cell Biol* 164: 24–32.

*All specimens used in experiment were captured according to Polish legal regulations concerned with wild species protection (Dz.U. nr 2 poz. 11 z 1984 r., Dz.U. nr 114 poz. 492 z 1991 r.). Department of Histology and Embryology obtained approval of Polish Ministry of Environment Protection and Forestry for performing studies on protected species (DOPog-4201-02-94/05/aj). The grass snake *Natrix natrix* L. is not included in Washington Convention of 1973, ratified by Poland in 1991 (Dz.U. nr 27 poz.112).

Spermiogenesis in the earthworm *Dendrobaena veneta* (Rosa)

Ewa Siekierska, Marlena Brzozowa

Department of Animal Histology and Embryology, Silesian University, Bankowa 9, 40- 007 Katowice, Poland, e-mail: ewa.siekierska@us.edu.pl

Spermiogenesis is the biological process which corresponds to differentiation of haploid spermatids into mature spermatozoa. The dates about spermiogenesis in earthworms are very poor and limitted only to three species. Thus, the purpose of this study was to describe the process of spermiogenesis in the earthworm *Dendrobaena veneta*. Spermiogenesis in *D. veneta* occures in clusters of cells called morulae. Spermatids are at the same stage of development into each morula. The central part of morula is accellular, annucleate mass of cytoplasm called cytophore. Spermatids are interconnected with the cytophore by cytoplasmic bridges until they differentiate into mature spermatozoa.

In the process of spermiogenesis in *D. veneta* 6 stages were distinguished (A-F). Young spermatids (stage A and B) were rounded, isodiametric cells. Cell nuclei were large and spherical. At the developing posterior end of cell the cytoplasm contained electron dense mitochondria, active Golgi complex, proximal and distal centriole. In close proximity to the Golgi stacks, beneath the plasma membrane a small proacrosomal vesicle and the rudiment of acrosomal tube were observed. At the end of stage A spermatids had flagellum that was formed from the distal centriole. At the end of stage B mitochondria destined to form mid piece were agregated to form the nebenkern at the base of nucleus and began to elongate.

As maturation proceeded to stage C and D the nucleus underwent pronounced elongation and further chromatin condensation occurred. In the cytoplasm surrounded the nucleus and nebenkern microtubules were detected. In older spermatids (stage E and F) the acrosome underwent further elongation and migrated from its position to the tip of spermatid so that by stage F was located anteriorly on the nucleus.

Mature spermatozoa of the earthworm *D. veneta* were long, filiform cells with head, mid piece and flagellum. The spermatozoon had long, straight acrosome with primary acrosome vesicle, acrosomal tube and axial rod. An elongated, highly condensed nucleus was followed by a straight mid piece which consisted of six radially adpressed mitochondria. The axoneme of flagellum had normal pattern of microtubules. The anterior section of axoneme was surrounded by glycogen granules two to each dublet of microtubules.

Structure of ovaries and oogenesis in siphonapterans

Bożena Simiczyjew, Renata Sierżant

Department of Animal Developmental Biology, Zoological Institute, University of Wroclaw, Sienkiewicza 21, 51-335 Wroclaw, Poland, e mail: simicz@biol.uni.wroc.pl, renataj@biol.uni.wroc.pl

Two types of ovaries have been shown to exist in siphonapterans: panoistic (neopanoistic) and meroistic-polytrophic. Panoistic ovaries are found in most studied fleas, while polytrophic organization - only in the genus *Hystrichopsylla* (Hystri-chopsyllidae) and *Stenoponia* (Ctenophthalmidae) (Kunitskaya, 1960; Büning, 1994). Panoistic ovaries have been demonstrated in representatives of Pulicidae, Ceratophyllidae and Ischnopsyllidae. In a ctenophthalmid: *Ctenophthalmus agyrtes*, surprisingly, ovarioles represent also panoistic organization, although other studied members of the family have polytrophic ovaries. Panoistic ovarioles in adult females consist of a terminal filament, degenerating germarium, vitellarium and ovariole pedicel. The vitellarium is composed of developing ovarian follicles (oocytes with their investing follicular epithelium) in a linear arrangement. Two regions of the vitellarium can be distinguished: apical and basal. The apical part houses early previtellogenic oocytes which do not fill the whole width of the ovariole. In the basal part of the vitellarium usually several (in *C. canis* only 4) ovarian follicles can be distinguished. Individual follicles are separated by interfollicle cells. The oocyte nuclei are relatively large and contain extrachromosomal amplified rDNA. Transcription of rDNA genes results in appearance of numerous multiple nucleoli that subsequently disperse throughout the karyoplasm. Polytrophic ovaries are found only in Hystricho-psylloidea. In *Hystrichopsylla talpae* the germ cell cluster contains 32 cells: one oocyte and 31 nurse cells, which are localized between the follicular cells, whereas in *Stenoponia* the number of nurse cells ranges between 8 and 13 per oocyte and the nurse cells are localized in the form of group not fusing with the follicular epithelium (Kunitskaya, 1960; Büning, 1994). Despite of the nurse cells presence, in germinal vesicles multiple nucleoli have been observed indicating that amplification of rDNA occurs also in polytrophic siphonapteran ovarioles. The presented data are discussed in the context of phylogenetic relationships between the families within the order and between Siphonaptera and Mecoptera.

REFERENCES:

BÜNING J. 1994. *The insect ovary. Ultrastructure, previtellogenic growth and evolution*. Chapman Hall, London.

KUNITSKAYA NT. 1960. On the reproductive organs in female fleas and determination of heir physiological age. *Med Parazit* (Moskwa) 29: 688–701.

Pollen heteromorphism in wild and ornamental pansies (Sect. *Melanium*, Violaceae)

Aneta Słomka[1], Elżbieta Kuta[1], Maria Pilarska[1], Kristin Kellner[1], Piotr Kawalec[1], Jerzy Bohdanowicz[2]

[1]*Department of Plant Cytology and Embryology, Jagiellonian University, 52 Grodzka St., 31-044 Cracow, Poland, e-mail: e.kuta@iphils.uj.edu.pl*

[2]*Department of Plant Cytology and Embryology, University of Gdańsk, 24 Kładki St., 80-822 Gdańsk, Poland, e-mail: jurboh@biotech.ug.gda.pl*

Pollen heteromorphism, defined as the formation various pollen morphs differing in aperture number in one flower of a plant, is characteristic for ~30% of angiosperms (Furness, Rudall, 2004). Aperture pattern ontogeny is established in male meiosis by the way in which cytokinesis is completed. Thus the type of meiosis (i.e., orientation of meiotic axes, type of cytokinesis, callose deposition to form cleavage walls) determines the aperture number and distribution. It has been documented in several species representing different modes of aperture formation that additional callose deposits predict future aperture sites (Ressayre et al., 2003).

In the genus *Viola*, 1/3 of species have heteromorphic pollen. Two large sections, *Melanium* Ging. (pansies) and *Viola* L. (violets), differ in the frequency of species with pollen heteromorphism (74% and 14%, respectively) (Nadot et al., 2000). Pollinator activity and environmental conditions influence the proportions of different pollen morphs (Till-Bottraud et al., 1999).

This study examined the correlation between aperture number and (1) environmental conditions, analyzing pollen aperture number in taxa evolved on soils contaminated by heavy metals (obligatory metallophytes *V. lutea* ssp. *westfalica*, *V. lutea* ssp. *calaminaria*; facultative metallophytes *V. tricolor, V. arvensis*); (2) pollen viability estimated by histochemical tests. Differences in aperture number between wild and ornamental (cultivars) pansies were also analyzed.

Flowers for pollen analysis were collected directly in the field (Germany, Belgium, France, Poland). It was found that pollen heteromorphism is favored in harsh environmental conditions, as plants produce both long-lived pollen (3- and 4-colpate) and very competitive fast-germinating pollen (5-colpate). Most heteromorphic pollen with 3-,4-,5-, and 6-apertures was produced by the commercial cultivar *V.* x *Wittrockiana,* an interspecific triple hybrid.

Pollen heteromorphism was not positively correlated with pollen viability. In all investigated taxa, pollen viability estimated by histochemical tests was very high (over 90%) except for *V. lutea* ssp. *westphalica* (77%, an effect of hybrid origin), indicating that the formation of more apertures does not result from disturbances in meiosis leading to the formation of additional pseudomicrospores, as has been suggested (Ressayre et al., 2003).

REFERENCES:

Furness CA, Rudall PJ. 2004. *Trends in Plant Science* 9: 154–158.

Nadot S, Ballard Jr HE, Creach JB, Dajoz I. 2000. *Plant Systematic and Evolution* 223: 155–171.

Ressayre A, Mignot A, Siljak-Yakovlev S, Raquin C. 2003. *Protoplasma* 221: 257–268.

Structure and ultrastructure of the developing epidermis in grass snake *Natrix natrix* L.* (Lepidosauria, Serpentes)

Elwira Swadźba

Department of Animal Histology and Embryology, Silesian University, 9 Bankowa St., 40-007 Katowice, Poland, e-mail: elwira.swadzba@us.edu.pl

The age of embryos was calculated using the table of species development (Rupik, 2002). According to light microscopic studies, the embryonic development of grass snake integument may be divided into four phases. The ultrastructural observation has shown that at the first, embryonic phase striatum germinativum contains columnar cells, which are attached to basal membrane by hemidesmosomes. The cytoplasm of the columnar cells is transparent for electrons, contains bundles of intermediate filaments and glycogen accumulations. At the second phase named dermis and epidermis differentiation, scale, and shield primordia formation, among columnar cells there are isolated pigment cells. The cytoplasm of pigment cells contains many vesicles filled with granules of pigment, mitochondria and smooth endoplasmic reticulum. The layers of cells lying above the columnar layer contain mitochondria and rough endoplasmic reticulum. These cells are joined to the columnar layer by desmosomes. The desmosomes are also visible between lateral surfaces of the flat and columnar cells. At the third phase, named stratification and keratinization one can distinguish respective epidermal layers. The innermost layer of epidermis is the stratum germinativum, which contains many branched pigment cells. Above this single layer are the single mesos layer and then thick β layer (or α and β layers), thin Oberhäuten layer, and the periderm. These layers correspond to the distribution of two different types of keratin. On the inner scale surface and hinge regions the epidermis is thinner than on the outer surface. On the outer surface of scales and shields the β layer is very much ticker than the α layer, but on the inner surface of scale and in the hinge region the α layer is predominant. At the forth phase, named shedding complex formation, the epidermis forms the first embryonic shedding complex and at the end of the developmental stage, XII periderm layer begins to detach. The shedding mechanism in snakes evolved after the hard beta-keratin was compartmentalized beneath the soft alpha-keratin to create an intra-epidermal weakness region. The initial shedding complex is formed under the increasing activation of thyroid gland.

REFERENCES:

RUPIK W. 2002. Normal developmental table in Early development of adrenal glands in grass snake *Natrix natrix* L. (Lepidosauria, Serpentes). *Adv Anat Embryol Cell Biol* 164: 24–32.

*All specimens used in experiment were captured according to Polish legal regulations concerned with wild species protection (Dz.U. nr 2 poz. 11 z 1984 r., Dz.U. nr 114 poz. 492 z 1991 r.). Department of Histology and Embryology obtained approval of Polish Ministry of Environment Protection and Forestry for performing studies on protected species (DOPog-4201-02-94/05/aj). The grass snake *Natrix natrix* L. is not included in Washington Convention of 1973, ratified by Poland in 1991 (Dz.U. nr 27 poz.112).

Germline cysts formation in Clitellata

Piotr Świątek[1], Janusz Kubrakiewicz[2], Jerzy Klag[1]

[1]*Department of Animal Histology and Embryology, Silesian University, Bankowa 9, 40-007 Katowice, Poland, e-mail: piotr.swiatek@us.edu.pl*
[2]*Insitute of Zoology, University of Wroclaw, Sienkiewicza 21, 50-335 Wrocław, Poland, e-mail: jkubrak@biol.uni.wroc.p*

In many invertebrate and vertebrate animals germ cells tend to form groups known as clusters or cysts. Since they arise as a consequence of incomplete cytokineses during germ cell divisions, germ cells within the cyst (cystocytes) are interconnected by intercellular bridges and thus constitute a syncytium. The formation of germline cysts has been most extensively studied in *Drosophila* and other insects. Interestingly, the basic principles that were found to underlie the establishment of cyst architecture and polarity in *Drosophila* apply also to germ cell cluster formation in such diverse organisms as *Xenopus* and mouse.

Germ cell clusters have also been described in worms (annelids, flat worms and nematodes), however their architecture differ significantly from that known in arthropods or vertebrates. In annelids germ cells develop within clones that consist of cystocytes clustered around the central anuclear cytoplasmic mass termed cytophore. Cystocytes are individually connected with the central cytophore by their own intercellular bridges. The mechanisms governing the formation of germ cell clusters with a central cytophore remain completely unclear. By means of light, flouorescence, confocal and electron microscopy we have analyzed the formation and architecture of cystocyte clusters during initial stages of spermatogenesis and oogenesis in several species belonging to clitellate (oligochaetous) annelids. We have found that formation of clusters with a central cytoplasmic mass is conditioned by the specific orientation of mitotic spindles during germ cells divisions. The axis of each mitotic spindle is always oriented tangentially to the cytophore surface. In consequence, the plane of cystocyte division is perpendicular to the plane of the pre-existing intercellular bridge. During cytokinesis the contractile ring underlying the leading edge of the cleavage furrow merges with the wall of the pre-existing intercellular bridge eventually splitting it into two new bridges. Our preliminary immunohistochemical analyses showed that following cystocyte divisions the cysts are equipped with an elaborate microtubular cytoskeleton. Numerous microtubules were found to converge centripetally. This arrangement may facilitate the flow of cytoplasm together with the organelles from the cystocytes through intercellular bridges into the cytophore thus contributing to the increase of its volume.

Ovary structure in primitive and advanced families of aphids (Insecta, Hemiptera)

Teresa Szklarzewicz[1], Szczepan Biliński[1], Anna Michalik[1], Piotr Węgierek[2], Karina Wieczorek[2]

[1]*Department of Systematic Zoology, Jagiellonian University, 6 Ingardena St., 30-060 Kraków, Poland, e-mail: szkla@iz.uj.edu.pl*
[2]*Department of Zoology, Silesian University, 9 Bankowa St., 40-007 Katowice*

Aphids have complex life cycles. In the primitive families all generations are oviparous, whereas in advanced families occur both oviparous and viviparous generations. The ovaries of aphids are composed of a limited number of telotrophic ovarioles. The individual ovariole is subdivided into an inconspicuous terminal filament, tropharium, vitellarium and ovariole stalk that joins the ovariole with the lateral oviduct. The tropharium encloses individual trophocytes and early previtellogenic oocytes. The latter as well as prefollicular cells occupy the basal part of the tropharium. In the vitellaria, one or two oocytes develop at the given moment. All germ cells (oocytes and trophocytes) residing within the ovariole belong to one cluster. Analysis of serial sections has shown that ovarioles of representatives of primitive aphids (Phylloxeridae and Adelgidae) as well as ovarioles of oviparous generations of advanced aphids (Anoeciidae, Pemphigidae, Mindaridae, Thelaxidae, Drepanosiphidae, Lachnidae) contain a large and variable number of germ cells (up to 192 in Anoeciidae). In the oviparous generation of representatives of the most advanced family Aphididae, ovarioles enclose the smallest number of germ cells, i.e. 32. The viviparous generations of advanced aphids are characterized by a stable number of germ cells per ovariole, i.e. 32. The obtained results indicate that: (1) the large number of germ cells per ovariole represents ancestral state within aphids; (2) during the evolution of aphids, similarly like in the scale insects, the gradual reduction of the number of germ cells per ovariole took place.

Eggshell in russian sturgeon, *Acipenser gueldenstaedtii* (Acipenseriformes)

Monika Żelazowska

Department of Systematic Zoology, Institute of Zoology, Jagiellonian University, 6 Ingardena St., 30-060 Cracow, Poland, e-mail: monika.zelazowska@uj.edu.pl

The ovary has been obtained from the specimen bred in artificial pond, cut into pieces and processed for light and transmission electron microscopy. The examined ovarian follicles have been classified into 5 consecutive stages which differed in size.

Each individual ovarian follicle consists of spherical oocyte surrounded by follicular cells. The follicular cells lie on a basal lamina that is encompassed by thecal cells. The deposition of an eggshell on the oocyte surface begins in late previtellogenesis. During this stage the perivitelline space between the oocyte and follicular cells appears. In this space the oocyte plasma membrane (oolemma) forms numerous microvilli that, in some places, form groups oriented towards the apical surfaces of follicular cells. In subsequent stages the oocyte microvilli intermingle with processes that are send out by follicular cells. In the investigated species the eggshell have complex fine structure. The egg envelopes are deposited sequentially between the microvilli on the oocyte surface and processes of follicular cells.

In fish, the egg envelopes are synthesized by the oocyte itself and follicular cells from extraovarian precursors that are produced in the liver, secreted and transported in the blood to the ovary under hormonal control. The main functions of the eggshell in fish are: (1) to allow sperm penetration, (2) to prevent polyspermy, and (3) to protect the developing embryo. In sturgeons the eggshell consists of four distinct layers (Cherr and Clark, 1982; Le Menn and Pelissero, 1991). Sturgeon and paddlefish eggs, as a rule, are perforated by numerous micropyles that are located in the animal region (Cherr and Clark, 1982; Detlaff et al., 1993; Linhart and Kudo, 1997).

REFERENCES:

CHERR GN, and CLARK WH Jr. 1982. Fine structure of the envelope and micropyles in the eggs of the white sturgeon, *Acipenser transmontanus* Richardson. *Develop Growth and Differ* 24 (4): 341–352.

DETLAFF TA, GINSBURG AS, and SCHMALHAUSEN OI. 1993. *Sturgeon Fishes: Developmental Biology and Aquaculture*. Springer-Verlag, New York.

LE MENN F, and PELISSERO C. 1991. Histological and ultrastructural studies of oogenesis of the Siberian sturgeon *Acipenser baeri*. In: Willot P [ed.]. Acipenser. Cemagref Publ.

LINHART O, and KUDO S. 1997. Surface ultrastructure of paddlefish eggs before and after fertilization. *J Fish Biol.* 51: 573–582.