PLENARY LECTURES

Anemia phyllitidis gametophytes: A model for the revealing secrets of role of plant hormones in male sex determination

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Protonemata of homosporus ferns, whose cells are less differentiated than those in higher plants, are particulary useful for developmental, physiological, genetic and molecular research on plant hormones concerning processes as diverse as photomorphogenesis, pattern formation and mechanisms cell differentiation.

A particulary interesting aspect of fern gametophyte development is the male and female sex determination. The male sex in gametophytes is determined by endogenously occuring pheromones showing gibberellin-like nature, known as antheridiogens. The activity of antheridiogens in a few species of *Anemia*, *Lygodium* and *Schizea* genus, could be imitated by gibberellic acid, which is able to induce premature antheridia formation. Many cytomorphological aspects of antheridogenesis were reveled using gibberellic acid and *Anemia phyllitidis* young gametophytes. The obtained results became the basis to create a cytophysiological model of a three-zonal structure of fern gametophytes. This model appeared to be ideal for the investigation of ACC (1-aminocyclopropane-1-carboxylic acid; direct precursor in pathway of ethylene synthesis) in male sex determination. It was found that ACC exerted a synergic effect on the gibberellic acid-induced antheridia formation, and this phenomenon could be related with the specific stimulation of cell growth and activity of their differentiation. Studies performed with AOA (aminooxyacetic acid) inhibitor of ACC synthesis, which restrained antheridia formation *via* inhibition of cell divisions, have revealed that synergic effect of ethylene in antheridia formation is closely connected with induction of transverse antheridial mother cell expansion, which depends on the specific arrangement of cellulose microfibrills. The obtained results clearly indicate that ethylene plays an important role in male sex expression in fern gametophytes and also showe similarity of ethylene synthesis pathway to that in higher plants, with ACC as the key by-product.

Caffeine – amazing phenomenon

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Caffeine (1, 3, 7 - trimethylxanthine) is a naturally occuring compound. The main sources of caffeine is coffee, tea, soft drinks, cocoa and chocolate. Prescription and nonprescripton medications are markable source of caffeine (Nawrot et al, 2003).

A lot of population based studies indicate relation of caffeine intake and blood caffeine concentration during pregnancy to fetal growth (Fenster et al, 1991), but evaluation of the developmental risks of caffeine based on epidemiological studies is difficult because of the findings are inconsistent. Animal studies, conducted in pregnant mice and rats, are designed mainly to produce malformations (Bartel and Gnacikowska, 1972) and reproductive or developmental risks of caffeine (Skalko et al, 1984).

Some researches demonstrate that blood caffeine concentrations during pregnancy are not releated to fetal growth, but caffeine intake is negatively associated with birth weight, with this effect being apparent only in smokers (Cook et al, 1996). Smoking is known to increase caffeine metabolism that is why caffeine is regard as coteratogen (Klebanoff, 2002). One of the probable mechanism is blocking by caffeine adenosine receptor. Caffeine may also potentiate the teratogenic effects of substances such as: ethanol, nicotine and certain drugs: vasoconstricting agents (Eskenzai, 1999).

Another examination suggested that heavy (>300mg per day) caffeine use during pregnancy is associated with reduction infant birth weight (Hinds et al, 1996). Some researchers documented an increased risk of spontaneous abortion (Wise, 1999), IUGR (James et al, 1993), premature labor and delivery, spontaneous abortion and congenital malformations. Three mechanims may be responsible for that effects: caffeine can cross the human placenta and enter the fetal gonad; is structurally similar to adenine and can interfere with cell division and metabolism.

Some researches indicate matural fertility problems that resulted from the caffeine consumption (Caan et al, 1998). Wilcox et al. was first who reported an effect of caffeine on human fertility. He reported a 50% reduction in the per cycle probability of conceiving with intakes 1 cup of coffee per day. Caffeine can block normal oogeneis in adult rats. Normal differentiation of fetal ovaries is inhibited to some degree by the administration of 300 mg/ kg caffeine to pregnant rats. Influence of caffeine on folliculogenesis (Mailhes et at, 1996), apoptosis of follicular cells and cell-cell adhesion in development of zonae pellucida and ovaries is very important (Downs and Eppig, 1986).

Altogether, caffeine is likely mutagen and perhaps teratogen, but experimental reports suggest little teratogenic danger to man. On the other hand influence of caffeine on production and fertility require more examination.

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Differentiation and identity of the *Drosophila* leg muscles: roles of the FGF pathway and homeodomain transcription factor Ladybird

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Since morphological Miller's description (Miller, 1950), *Drosophila* leg musculature and its formation has not been revisited. Here, using a set of GFP markers we provide evidence that the *Drosophila* leg muscle development is tightly associated with the formation of internal tendons. Appendicular myogenesis is initiated in the imaginal leg discs of the second instar larvae by massive proliferation of Twist positive adepithelial cells. In the third instar discs, in the vicinity of tendon progenitors, some Twist positive myoblasts differentiate into *dumbfounded* expressing muscle founders. During evagination of the leg discs, at early pupa stages, founder cells follow the developing precursors of internal tendons, reach positions at which individual muscle fibres have to be generated and fuse with surrounding myoblasts to form muscle precursors. This leads to a stereotyped pattern of multifibre muscles that ensure movements of the adult leg. Interestingly, the appendicular muscles are the only *Drosophila* muscles attached to the internal tendons, thus presenting a vertebrate-like multifibre organisation. Three types of appendicular muscles, the reductor, the levator and the depressor, constitute leg musculature. Importantly, a similar organisation of limb muscles exists in vertebrates making *Drosophila* as a powerful model in which to study mechanisms of appendicular myogenesis.
Moreover, we provide evidence that *dumbfounded*-positive myoblasts represent an obligatory step in the formation of multi-fibre leg muscles, and that the FGF receptor Heartless plays an instructive role in their differentiation. FGF activates homeobox gene *ladybird* whose function appears crucial for determining properties of leg versus flight muscles and for establishing the adult leg muscle pattern. Thus, we demonstrate that the number of *dumbfounded*-positive myoblasts and resulting appendicular fibres is controlled by Heartless transduced FGF signals whereas their identity depends on *ladybird*.

Formation, maturation, and elimination of synapses in the developing human central nervous system

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The formation of synapses in the human central nervous system is a complex process that occurs over a protracted period of development and it is an indication of the function of the nervous system.

Synapse formation is preceded by 1) neuronal fate specification, 2) axon path finding, and 3) target selection. The formation of synapses begins before neurogenesis and is completed and extends well into early postnatal life. During development synaptogenesis is closely related to neuronal differentiation and the establishment of neuronal circuitry. For example, shortly after neurons differentiate and extend axons and dendrites, many of genes encoding synaptic proteins are turned on resulting in the formation, accumulation and directional trafficking of vesicles carrying presynaptic and postsynaptic protein complexes.

An important aspect of synpatogenesis is target recognition. This requires regulation of temporal events and spatial specificity. Recently, significant progress has been made in the identification of target-derived factors that either accelerate neuronal maturation or directly induce synapse formation.

The onset of synaptogenesis occurs according to a remarkable invariant timetable. Synapses appear suddenly and increase very rapidly in number thereafter. A general feature of synaptic development is a prolonged maturation phase during which synapses expand in size. In the spinal cord primordial of synaptic connections appear in the 6th week in the ventral horns of the cervical part. These primordial are characterized by apposing thickenings of membranes without synaptic cleft. The development of synapses progresses in the ventrodorsal and the rostrocaudal directions of the spinal cord. In the brain synapses begin to form during 7 and 8 weeks. First synapses are axodendritic. The functional properties of synapses also change with development. Important developmental process is elimination of synapses. This process is regulated mainly by activity.