Volume 58(1):73-94, 2014
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Discovery of novel fluorochromes for use in plant studies

Soujanya Kuntam

Cellular Imaging Laboratory, Institute of Plant Biology, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary

Plant cells store neutral lipids such as triacylglycerols in distinct cytosolic organelles called oil bodies (OBs) (also referred to as lipid bodies/droplets, spherosomes or oleosomes) Although initially thought to be found only in oleogenic seeds and fruits, where they serve as fuel for the growth and development of seedlings prior to photosynthetic establishment, in recent years, studies have shown that OBs are quite ubiquitous. Their role extends from being static depots for carbon storage to stress response, lipid homeostasis, pathogen resistance, hormone metabolism and signaling and a specialized role in anther development. Plant OBs similar to their counterparts in yeast and mammals, are highly dynamic. Proteomic analyses have revealed that, there are a host of proteins which reside on the surface of these OBs and the exact content is changing under varying conditions. But a lot still remains to be uncovered in the area of OB protein and lipid composition as well as OB transport, mechanism of protein targeting, assembly and regulation. Live cell analysis is thus required to unravel the dynamic regulation of this important organelle.

Live-cell imaging offers a unique opportunity for investigating OB regulation. A large collection of imaging tools based on fluorescence is currently available. Varying colors of photostable genetically-encoded fluorescent proteins can be used in multiplexed tracking of protein remodeling on OBs. Photo-switchable fluorescent proteins as well as fluorescent timers allow quantitative assessment of OB protein dynamics. Fluorescent sensors, such as those based on fluorescence resonance energy transfer (FRET), can be applied to follow protein conformational changes or protein-protein interactions relevant to OBs. These tools, combined with the use of reliable OB markers, represent a versatile scheme for investigating OB biology. However, commercially available live cell dyes are limiting in their ability to penetrate cell walls and those that are permeable, in addition to other drawbacks such as photostability (BODIPY) and broad emission range (Nile Red), also restrict multicolor imaging, as most fluoresce in the green to red region of the visible spectrum.

In the present study, we report new fluorochromes as markers for OB in living plant cells. The fluorochromes, which are thalidomide analogs were in-house synthesized and were tested on live plant suspension cultured cells at various concentrations. The spectral emission range for the fluorochromes was identified and cell viability assays were also performed. We could also observe that the OBs remain highly mobile after staining with these fluorochromes, suggesting that the mobility dynamics were not affected significantly. We expect that these new chemicals will provide a novel approach for microscopy analyses of OBs in live plant cells.

Supervisor: Dr Ferhan Ayaydin
Email: ferhan@brc.hu

Bacterial symbionts enhance photo-fermentative hydrogen evolution of *Chlamydomonas* algae

Gergely Lakatos

Laboratory of Microbial Genomics, Institute of Biochemistry Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary

Chlamydomonas reinhardtii represents a well-established algae model system for biohydrogen generation. Two major methods are known for sustained *Chlamydomonas*-based hydrogen evolution. Hydrogen production using sulfur-deprived photoheterotrophic cultures of *Ch. reinhardtii* is the most widespread approach. Sulfur deprivation leads to complete anoxia via the inactivation of the photosynthetic system, thus, the extremely oxygen-sensitive algal Fe-hydrogenase will be able to become functional. Another algal hydrogen evolution approach is possible through dark fermentation. In dark and anaerobic conditions algae can catabolize endogenous carbohydrates or secondary metabolites generating organic acids, ethanol, CO_2 and H_2. The vast majority of algal hydrogen evolution studies were conducted using pure algae cultures. Our knowledge on the exploitability of algal-bacterial consortia in biohydrogen production is fairly short. Biohydrogen production capacity and growth rate of *Ch. reinhardtii* cc849 or the transgenic strain Iba co-cultured with *Bradyrhizobium japonicum* has been investigated. The sulfur-deprivation method together with *B. japonicum* inoculation resulted in enhanced rate of the oxygen consumption in the cultures, increased growth rate of algae and significantly improved hydrogen evolution rate. Our investigations aimed the elucidation of the nature and dynamics of algal hydrogen evolution observed in various algal-bacterial interactions.

The green algae *Chlamydomonas* sp. strain 549 was investigated for its hydrogen-evolution capability in algal-bacterial mixed cultures. Stable bacterial contaminations were identified during algae cultivation, the symbionts belonged to various genera, mostly *Brevundimonas* sp., *Rhodococcus* sp. and *Leifstonia* sp. All natural symbiotic partners enhanced fermentative algal hydrogen production. This phenomenon was not limited for the natural associations, increased algal hydrogen evolution was achieved by simple artificial algae-bacterium communities as well. Designed algal-bacterial co-cultures were tested in hydrogen evolution experiments, the highest hydrogen yield was obtained

when hydrogenase-deficient *E. coli* was applied as symbiotic bacterium. The results showed that the oxygen elimination process is the most crucial factor for algal hydrogen production, efficient bacterial respiration is essential for the activation of algal Fe-hydrogenase.

Supervisor: Gergely Maróti
E-mail: lakger86@brc.hu

Identification of a novel effector cell type in the cell-mediated immunity of *Drosophila*, the multinucleated giant hemocyte

Zita Lerner

Immunology Unit, Institute of Genetics, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary

Innate immunity is the first line immune defense against microbes, parasites and tumours which is composed of humoral and cell mediated events. In *Drosophila*, three main classes of blood cells, so called hemocytes, are the effector cells of cell mediated immunity. The plasmatocytes engulf microbes, produce extracellular matrix components, and provide systemic signals during microbial infections. Crystal cells contain crystallized prophenoloxidase enzyme, which is necessary for the melanization response. Lamellocytes arise upon immune induction, such as infestation by parasitoid wasps, and are required for the encapsulation reaction by forming a multilayered capsule around the parasitic wasp egg, which later melanizes. The effector hemocytes of the *Drosophila* larva originate from three hematopoietic compartments: the lymph gland which is a compact hematopoietic organ with multiple lobes, the sessile tissue where hemocytes are attached to the wall of the hemocoel, and the circulation. All three compartments contribute to differentiation of the effector hemocyte pool following immune induction.

The cell mediated immunity of *Drosophila melanogaster* is well studied; however, our knowledge on the immune response of other insects, in particular, other members of the *Drosophilidae* family is far from complete . The availability of various *Drosophila* species from different natural habitats allows to study the adaption of the cell mediated immune response to the different parasites. Recent studies show the diversity of the capsule forming cells of different Diptera species. According to these data the pseudopodocytes in *D. affinis* and *D. obscura* from the *obscura* group of *Drosophilidae* are capable of phagocytosis, similarly to plasmatocytes, however they are also involved in the capsule formation around foreign particles.

Our aim was to characterize the hemocyte subsets and the hematopoietic compartments in *Drosophila ananassae* from the *ananassae* subgroup. We identified a special giant hemocyte, which we named MGH (Multinuclear Giant Hemocyte) in *D. ananassae*, that appear after immune induction. To isolate different hemocyte subsets and to define their function, origin and formation, we produced monoclonal antibodies to subclasses of hemocytes and developed a transgenic reporter system which allows *in vivo* detection and manipulation of hemocytes and hematopoietic compartments in *D. ananassae.* As MGHs are similar to mammalian multinuclear giant cells, which play an important role in the formation of granulomas, we believe that *D. ananassae* could serve as a model for a better understanding of the development, structure and function of granulomas and of the multinucleated giant cells.

Supervisors: István Andó, Viktor Honti
E-mail: lerner.zita@brc.mta.hu

Methodology of ancient DNA, and results to date

Endre Neparáczki

Ancient DNA Group, Department of Genetics, University of Szeged, Szeged, Hungary

Our research group isolates and studies ancient DNA (aDNA) from excavated human remains in collaboration with the Department of Anthropology. Sequence data obtained from ancient bones can unravel genetic relatedness of individuals, and populations. From a representative data set one can surmise population movements, and population history. The aDNA research can complement anthropological and archaeological data. For kinship studies routinly matrilinearly inherited mitochondrial DNA sequences, or patrilinearly inherited Y-chromosomal sequences are used, but autosomal loci correlated with known phenotypes can also be examined, such as monogenic disease genes, hair and skin color genes, or FOX2P gene, associated with speech ability.

In addition, aDNA of ancient pathogenes can also be obtained from their deceased carriers, which makes it possible to determine the distribution of prehistoric infectious diseases, such TB caused by *Mycobacterium tuberculosis.*

The preservation of aDNA, largely depends on the environment, and even under best conditions, it is largely degraded and fragmented. Usually trace amounts of 50-200 bp long DNA fragments are left, so classical methods apply PCR amplification, and cloning. The risk of contamination with modern DNA is very high, therefore special sterile laboratories are required for aDNA work. In the last few years the