

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.

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

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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.

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Methodology of ancient DNA, and results to date

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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 routinely matrilineally inherited mitochondrial DNA sequences, or patrilineally 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 pathogens 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

appearance of new generation sequencing techniques opened a new dimension of ancient DNA studies, since from traces of DNA, large amount of sequence data can be obtained with this method.

We have recently created a special sterile aDNA laboratory at the Department of Genetics. This so called pre-PCR laboratory is supplemented with a post-PCR, standard molecular laboratory in a distant part of the building (a requirement to prevent contamination). Both laboratories are equipped, and we have optimized DNA extraction and amplification. In the pre-PCR laboratory, a simple method was adapted for bone's milling. For DNA extraction we also adapted a cheap but reliable modified silica powder affinity purification method. For DNA amplification we are testing various enzyme brands and conditions recommended by the manufacturer.

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Effect of hypoxia on MCF-7 cells' transcriptome and metabolic activities

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The hypoxic condition is prevalent in solid tumours and it is often associated with poor prognosis. Metabolic alterations that make possible for the cancer cells to survive and thrive under hypoxic condition are subject to high interest, however a systems-level understanding is still missing.

In order to emulate the hypoxic state, cells of a well established breast cancer model cell line (MCF-7) were cultured under normal oxygen concentration and subsequently exposed to hypoxia. To detect the cells' response to hypoxia, RNA samples were collected and sequenced from both conditions and mRNA abundances were determined.

With the aim of inferring the metabolic routes that may play important roles in the cancer cells' response to hypoxia, we employed the iMAT method that integrates gene expression data and a human genome-scale metabolic network reconstruction to predict metabolic reactions that are specifically altered in hypoxic condition. Beside, to gain a more global view of the functional changes underlying the hypoxia-response, we carried out a Gene Ontology analysis on the RNASeq data. In addition, to generally assess the predictive capability of the human metabolic network model, we applied an essentiality analysis and compared predictions to available high-throughput data.

The analyses resulted in the identification of 33 metabolic reactions which are specifically activated under hypoxia. The majority of the detected reactions is distributed across 4 modules of cellular metabolism, namely sphingolipid metabolism, pyruvate metabolism, nucleotides metabolism, inositol phosphate metabolism. In addition, C160 fatty acid activation, diacylglycerol phosphate kinase and the arginine/lysine transporter were predicted to be active.

The predicted arginine transported and the reactions of the pyruvate metabolism will be subject to further experimental investigations by our collaborators in order to assess their role in hypoxia.

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Methane inhalation prevents from the quantitative changes in nitrergic myenteric neurons and intestinal motility disorders in a rat model of intestinal ischemic-reperfusion injury

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The gastrointestinal tract is highly susceptible to hypoxia, thus local or systemic circulatory disturbances are often associated with intestinal inflammation and enteric neuropathy. Inflammatory mediators influence the activity of enteric neurons, therefore, development of the intestinal inflammation is frequently associated with gut motility disturbances. Previously, we have demonstrated the anti-inflammatory effects of exogenous methane inhalation after IR. However, the effects of inhaled methane on the IR-related quantitative changes of enteric neurons or on the myoelectrical activity of the gastrointestinal tract were not investigated until now. Therefore, the main focus of this study was to investigate the consequences of intestinal IR and normoxic methane inhalation on the quantitative parameters of myenteric neurons and intestinal motility.

For the study 300-350 g male Sprague-Dawley rats were divided into three groups, these are: sham-operated, IR and methane-treated IR (n=8-8). Ischemia was induced by the occlusion of superior mesenteric artery. The inhalation of normoxic artificial air with 2.2% methane