to accumulate various storage materials. The deposition of storage compounds is a widespread strategy among microbes to survive when the nutrient or energy sources are depleted.

My study focuses on the cytoplasmic Hox1 hydrogenase (Rákhely et al. 2004) which is able to reduce protons and oxidize hydrogen *in vivo* depending on the redox status of the cell and environment. The enzyme consits of five subunits: Hox1Y and Hox1H are the small and large hydrogenase subunits, respectively; Hox1F and U are the diaphorase subunits, while Hox1E subunit is involved in the electron transport. The aim of this work is to clarify the physiological contexts of Hox1 hydrogenase; *i.e.* to examine its molecular/metabolic/redox connection to the storage materials and the bioenergetic membrane.

The Hox1 hydrogenase can produce hydrogen under illumination and in the dark. The hydrogen production requires excess electrons derived from e.g. thiosulfate under continuous illumination. On the basis of experimental and *in silico* data, it is hypothesized that the Hox1 hydrogenase is connected directly to photosynthetic membrane via the membrane-located NADH-ubiquinone oxidoreductase complex. The Hox1EFU subunits have remarkable sequence similarity to the NuoEFG subunits which are dissociable from the membrane and they have $NAD^+$ -reducing activity. According to our model, the Hox1EFU subunits can replace the NuoEFG subunits allowing Hox1 to function as a valve. When the central quinone pool is overreduced, excess electrons can be removed in the form of hydrogen by means of Hox1 hydrogenase, otherwise NADH is produced. In order to prove this model, a proteomic apporoach was chosen and affinity chromatography was used to identify interacting protein partners.

Hydrogen can also be produced in the dark through the Hox1 hydrogenase. In this case, the excess of electrons is supposed to arise from stored materials accumulated during photosynthetic growth. Depending on the nutrient supply during growth, *T. roseopersicina* can accumulate elementar sulphur, polyphosphate poly(3-hydroxyalkanoates) and glycogen. A systematic investigation of the physiology and hydrogen production of the cells indicated glycogen as a potential source of electrons for the Hox1 hydrogenase in the dark. The genome of the strain has been sequenced and genes coding for proteins involved in both glycogen synthesis and catabolism were identified. In order to confirm this metabolic connection, both the glycogen synthesis and breakdown were disrupted by genetic tools and a comparison of the hydrogen production and glycogen content of the mutant and the control strains revealed a metabolic linkage between the glycogen and hydrogen metabolism.

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## Diabetes-related structural, molecular and functional alterations in capillaries running in the vicinity of myenteric plexus in streptozotocin-induced diabetic rats

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It has recently been demonstrated that the nitrergic subpopulation of myenteric neurons is especially susceptible to developing neurodegenerative damage in diabetes. The nitrergic neurons located in different gut segments had different susceptibilities to diabetes. Their different responsiveness to insulin treatment had also been revealed, which suggest that the neuronal microenvironment is critical to evolving diabetic nitrergic neuropathy. Although the relationship between the presence of enteric neuropathy and impaired gastrointestinal motility in humans and also in rodent models are well documented, the impact of diabetes on capillaries within the intestinal wall has been completely overlooked until now.

Since the myenteric ganglia are not vascularizated, accordingly the mesenterial capillaries adjacent to the myenteric plexus play a key role to supply them. Therefore we supposed that diabetes-associated alterations, which influence the permeability of these capillaries, may be critical to developing enteric neuropathy observed in streptozotocin-induced diabetics. The diabetes-related endothelial dysfunction leads to decreased bioavailability of endothelial cell-derived nitric oxide and at the same time to increased amount of toxic free radicals before the clinical symptoms appear.

Therefore, the primary question of our study was whether diabetes influences the structural, molecular and functional properties of capillary endothelium closely related to the myenteric plexus.

Ten weeks after the onset of diabetes, different gut segments of control, streptozotocin-induced diabetic and insulin-treated diabetic rats were processed for electronmicroscopic and molecular studies. The thickness of basement membrane (BM) surrounding blood vessels and the size of the individual caveolar compartments were measured by electronmicroscopic morphometry. The quantitative features of blood-tissue exchange of endogenous albumin were investigated by postembedding immunohistochemistry. The quantitative changes in the expression of endothelial nitric oxide synthase (eNOS) and its negative regulatory protein, Caveolin-1 (CAV-1) were elucidated by postembedding immunohistochemistry, RT-PCR technique and western-blot analysis in the endothelium of microvessels around myenteric plexus.

Although the differences between the intestinal segments are well pronounced, region-specific thickening of BM and enlargement of caveolar compartments was demonstrated in diabetic animals. The amount of serum albumin taken up by the plasmalemmal vesicles

and transported to the interstitium was enhanced in diabetics compared to controls. The overexpression of CAV-1 and eNOS was also documented in diabetic groups suggesting enhanced transendothelial transport and hyperpermeability of these capillaries. In some cases immediate insulin replacement prevented the development of diabetes-related region-specific alterations.

These results indicate a close relationship between the segment-specific diabetic nitrergic neuropathy and vascular dysfunction of mesenterial capillaries running in the vicinity of myenteric plexus in the gut. Our data provide morphological, functional and molecular evidence that the endothelial cells of these vessels are direct targets of diabetic damage. We suggest therefore that these endothelial cells are potential therapeutic targets to prevent the development of the nitrergic neuropathy and the gut motility disorders in diabetic patients.

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## Molecular characterization of the computationally predicted *miR-282* microRNA gene of *Drosophila melanogaster*

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MicroRNAs have been discovered as a new type of regulatory genes whose transcripts are marked by a representative intermedier form, the hairpin structure. Due to this typical secondary structure and the advanced bioinformatic methods, hundreds of new miRNA genes have been identified in animals, plants and even viruses. Hundreds of target genes for every single miRNA also have been predicted. In this way, a huge amount of data has been generated, which is waiting for interpretation and experimental confirmation.

MicroRNAs (miRNAs) are ~22 nucleotide long, single-stranded regulatory RNAs that bind to complementary sequences in the three prime untranslated regions of target mRNAs thereby, negatively regulating (by transcript degradation and translational suppression) the target genes. Although a significant group of miRNA genes is found in the introns or sometimes in exons of protein and non-protein coding genes, most microRNA genes lie in intergenic regions and contain their own promoter and regulatory components. MicroRNA primary transcripts (pri-miRNAs) are synthesized by RNA polymerase II. In this way, pri-miRNAs which range couple thousands of nucleotides in length have 5' m7G cap structure and usually subjected to polyadenylation in their 3' end. However the functional analyses are still in their infancy because they are hampered primarily by redundancy among miRNA genes occurring when different miRNAs share the same 5' seed sequence or their target(s) and if they are coexpressed. Moreover, most miRNA mutants show subtle or low-penetrance defects that may be difficult to identify. As a consequence, in only few cases can lead the lack of miRNA function to robust phenotypes. Despite of these findings, it has become clear today that miRNAs are required for the fine tuning of the regulation of sometimes very complex mechanisms and participate in the regulation of almost every biological processes investigated so far.

While in the fruit fly (*Drosophila melanogaster*) 176 miRNAs has been computationally predicted to date (miRBase release 16), the real target mRNAs and biological function have been assigned to only a dozen of them. We characterized a miRNA gene, *mir-282* of *Drosophila melanogaster* which is evolutionary conserved among insects. The *mir-282* gene is located on the third chromosome within a 13.9 kb genomic region devoid of any protein coding genes and our data strongly suggest an independent *mir-282* gene whose primary transcript has a distinct 5' start with a CAP and a few alternative 3' ends with polyA tail. We have determined the correct size of the pre- and mature *mir-282*. We found that the *mir-282* locus encodes a functional transcript which influences viability, longevity and egg production in Drosophila, most likely through the regulation of cAMP level at pupal stage.

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## Neuroprotection with novel KYNA-amide

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Acute protection and the recovery of neurons from cerebral ischemic insults of whatever nature give rise to the main drive in the development of neuroprotective strategies.

The most widely accepted concept relating to ischemic brain damage is the concept of excitotoxicty.

Treatment with N-methyl-D-aspartate receptor antagonists is a widely accepted method with which to stop the advance of excitotoxic processes and concomitant neuronal death. From a clinical aspect, competitive glycine- and polyamine-site antagonists with relatively low affinity and moderate side-effects are taken into account. Endogenous kynurenic acid (KYNA) acts as an antagonist on the obligatory co-