agonist glycine site, and has long been at the focus of neuroprotective trials. Unfortunately, KYNA is barely able to cross the blood-brain barrier. Accordingly, the development and synthesis of KYNA analogs which can readily cross the BBB have been at the focus of research interest with the aim of neuroprotection.

A novel KYNA analog, 2-(2-N,N-dimethylaminoethylamine-1-carbonyl)-1H-quinolin-4-one hydrochloride (Patent Application No: 104448-1998/Ky/me), recently proved to be neuroactive in several experimental paradigms. The analog effectively reduced c-fos and nNOS activation in an experimental animal model of migraine, effects interpreted as due to NMDA blockade. Moreover, in an *in vitro* comparative electrophysiological study, this compound was found to have the same neuromodulatory attributes as KYNA. NMDA antagonism was also acknowledged. 1 mmol of the analog administered i.p. effectively reduces the amplitudes of hippocampal population spikes. Regarding these properties, we estimated the neuroprotective capability of a novel kynurenic acid analog in transient global forebrain ischemia, measuring the rate of hippocampal CA1 pyramidal cell loss and the preservation of long-term potentiation at Schaffer collateral-CA1 synapses.

The neuroprotective potential was reflected by a significantly diminished hippocampal CA1 cell loss and preserved long-term potentiation expression. The neuroprotective effect was robust in the event of pretreatment, and also when the drug was administered at the time of reperfusion.

A detailed analysis of the behavioral effects of this new compound appeared to be extremely important, and we have therefore investigated it from several aspects.

In a preliminary investigation of the effects of the analog on mice, we performed open-field tests of the locomotor activity and exploratory drive. The influence of the analog on spatial orientation and learning was also assessed in the radial arm maze imprinting test. In the Morris water maze tests we examined its effects on the working memory and long-lasting reference memory of rats.

It emerged that there is a dose of this KYNA-amide which is neuroprotective, but does not worsen the cognitive function of the brain. This result is significant in that a putative neuroprotectant without adverse cognitive side-effects is of great benefit.

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Developmental regulation of brassinosteroid distribution in *Arabidopsis*

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Brassinosteroids (BRs; steroidal phytohormones) are essential regulators of plant growth and development. Unlike most other hormones, BRs are not subject to active transport, but exert their effects locally, in a paracrine manner. Local BR levels are efficiently controlled by the coordinated actions of biosynthetic and degradative gene functions, which ensure both homeostatic and differential regulation. While the transcriptional regulation of BR biosynthetic genes is known in great detail, its direct effects on the hormone production and accumulation are still to be clarified.

The aim of our study is to find out how castasterone and brassionolide, the two biologically active forms of BRs, are distributed in the model plant *Arabidopsis thaliana*. To observe developmental changes in the hormone accumulation, we generated transgenic plants expressing reporter genes under the control of an artificial BR-responsive promoter. The BR response constructs will be used for monitoring developmental BR adjustments during morphogenic events, such as germination and the differentiation of reproductive organs. Parallelly, we determine the bioactive BRs in all *Arabidopsis* organs via CG-MS analyses, in order to construct a comprehensive map of hormone distribution in the adult plant. In another approach, we initiated studies on the role of regulated hormone distribution during embryonic development. This line of research utilizes GFP and LUC reporter-tagged versions of the CYP85A2 enzyme that catalyzes the rate-limiting step of BR biosynthesis. The transgenic lines expressing these chimeric proteins will be helpful in elucidating the induction and spatial pattern of embryonic BR synthesis, and its correlation with the developmental auxin re-distribution that has been well characterized.

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The examination of the telomer protecting *Drosophila melanogaster* gene (*dtl*)

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In *Drosophila melanogaster* chromosome ends consist of retrotransposon arrays, the well-defined, short telomeric repeats, characteristic of human and other telomerase-containing organisms are absent. Consequently, in Drosophila there is no need for the sequence-specific,

repeat-recognising telomere capping complex, called shelterin. Instead a new complex, named terminin has evolved in *Drosophila melanogaster* and closely related species, which has an essential role in telomere capping and maintenance. Genes encoding proteins involved in this complex, appeared recently in the phylogenesis and since then these genes have been quickly changing. The proteins are functional analogues of the members of shelterin complex. Terminin complex (just like shelterin) interacts with proteins involved in the DNA damage signaling pathways. The lack of either capping proteins causes telomere disruption, therefore activates DNA damage checkpoints and fuses the end of the chromosomes.

The bicistronic *Drosophila* gene *dtl/tgs1* (*CG31241*) encodes two proteins; *Drosophila Tat-Like/Drosophila Telomere Lost* (DTL) – is a member of the telomer protecting terminin complex, and *Trimethyl-Guanin Synthase* (TGS1) that catalyzes the trimethyl-guanine (TMG) cap synthesis of sn- and snoRNAs (Komonyi et al 2005. and 2009). Our data indicate, that both products of *dtl/tgs1* gene are essential. Mutations which affected *dtl* (but didn't have any effect on *tgs1)* caused telomere associations (TAs), whereas the absence of TGS1 (but not DTL), resulted defected TMG cap containing RNA synthesis. However, DTL and TGS1 seem to have distinct functions such as telomere maintenance and RNA processing, these two functions might possibly be interconnected as TMG cap containing RNAs play an important role in telomere maintenance.

We payed particular attention to determine the role of the two proteins in the germ line. In the lack of maternal *dtl/tgs1* product, the development of eggs is significantly abnormal. After fertilisation, erratic cleavage division can be observed and only a few embryos reach the zygotic expression stage. For better understanding, we have investigated the unique effect of either the DTL or the TGS1 protein during oogenesis.

Since DTL is a putative member of terminin complex, we have supposed that it interacts with proteins involved in telomere maintenance. Therefore, we have performed yeast two hybrid experiments to find possible interacting partners. We have overexpressed FLAG epitop-labelled DTL in transgenic flyes in order to purify DTL containing complex and other interacting partners.

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The role of Rac1 in stress signaling

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The heat shock response (HSR), one of the most studied cellular homeostatic mechanisms, is involved in the maintenance of cell functionality during stress. Yet, the whole stress signaling pathway has not been elucidated. In line with the membrane thermosensor model, mild stress, or "membrane defects" caused by different disease states, is sensed by changes in the fluidity and microdomain structure of membranes, influencing membrane localized signaling activities. In favor of this model, our group exposed different mammalian cells to various membrane fluidizers or drugs with the ability to interact with certain membrane lipids and found substantial modulation of heat shock protein (Hsp) expression. One possible signaling pathway originating from plasma membrane involves the lipid kinase, PI3kinase, which in turn activates the small GTPase, Rac1. Through downstream signaling cascade to MAP kinases, the main transcription factor, HSF1 is activated leading to Hsp synthesis. It was shown that Rac1 translocation to the plasma membrane is essential for activating downstream effectors and its membrane binding is determined at least in part by membrane lipids. In favor of "membrane stress sensor" model, our working hypothesis was that Rac1 pathway is involved in stress signaling through the effect of stress on membrane microdomain organization.

To study Rac1 involvement in HSR, we created tetracycline inducible stable mutant B16-F10 cells which are either dominant negative or constitutively active for Rac1. Though the antibiotic resistance showed stable clones, Rac1 protein expression was not detectable after induction. Now we use the "Sleeping Beauty Transposon" for stable Rac1 gene transfer in B16F-10cells.

As a proof of concept we have shown that using specific Rac1 inhibitor, NSC23766 and Capsaicin, the heat shock response decreased remarkably in dose dependent manner. Moreover, elevated levels of heat shock response achieved by addition of a Hsp co-inducer, BGP-15 is diminished by Rac1 inhibitors. We also documented that the amount of phosphorylated, active transcription factor, HSF1 decreased in inhibitor treated samples. In order to document the different localization of Rac1 in heat treated B16 cells we made immunofluorescence microscopy and Western blots on isolated membrane fractions.

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