Taken together, the *Arabidopsis* SET protein is a potent inhibitor of animal and plant phosphatases and may have a role in heat shock tolerance as indicated by its altered (nuclear) localization in response to a 1h 45°C treatment. Thus, in the light of our results we can presume that the investigation of SET can be of practical importance, since it might have a role in the stress tolerance of plants. This hypothesis is currently investigated in SET-overexpressing transgenic plants.

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Cross-talk between cannabinoid CB_1 and $GABA_B$ receptors in rat brain hippocampus

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Cannabinoid CB_1 and the metabotropic $GABA_B$ receptors have been shown to display similar pharmacological effects and co-localization in certain brain regions. Previous studies have reported a functional link between the two systems. As a first step to investigate the underlying molecular mechanism, here we show cross-inhibition of G-protein signaling between $GABA_B$ and CB_1 receptors in rat hippocampal membranes. The CB_1 agonists R-Win55,212-2 displayed high potency and efficacy in stimulating Guanosine-5'-*O*-(3-[^{35}S]thio)triphosphate, [^{35}S]GTPγS binding. Its effect was completely blocked by the specific CB_1 antagonists AM251 suggesting that the signaling was via CB_1 receptors. The $GABA_B$ agonist baclofen and SKF97541 also elevated [^{35}S]GTPγS binding by about 60%, with potency values in the micromolar range. Phaclofen behaved as a low potency antagonist with an an $ED_{50} \approx 1$ mM . However, phaclofen at low doses (1 and 10 nM) slightly but significantly attenuated maximal stimulation of [^{35}S]GTPγS binding by the CB_1 agonist Win55,212-2. The observation that higher concentrations of phaclofen had no such effect rule out the possibility of its direct action on CB_1 receptors. The pharmacologically inactive stereoisomer S-Win55,212-3 had no effect either alone or in combination with phaclofen establishing that the interaction is stereospecific in hippocampus. The specific CB_1 antagonist AM251 at a low dose (1 nM) also inhibited the efficacy of G-protein signaling of the $GABA_B$ receptor agonist SKF97541. Cross-talk of the two receptor systems was not detected in either spinal cord or cerebral cortex membranes. It is suggested that the interaction might occur via an allosteric interaction between a subset of $GABA_B$ and CB_1 receptors in rat hippocampal membranes. Supported by NKTH DNT 08/2004 and OTKA TS 049817 research grants.

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Functional analysis of *Drosophila melanogaster* histone H4 specific acetylase complex and its role in regulating chromatin structure

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Numerous enzymes and protein complexes are known to bring about changes in the state of chromatin by different mechanisms with resultant effects on gene expression. One class of complexes including the yeast SWI/SNF and a number of others from various organisms, alter the DNA packaging in an ATP-dependent manner. Another class of chromatin structure regulating factors acts by covalently modifying histone proteins. The various modifications include phosphorylation, ubiquitination, ADP-ribosylation, methylation, sumoylation and frequently acetylation, catalyzed by histone acetyltransferases (HATs). In many cases HAT enzymes are components of complexes which also contain among others, ADA-type adaptors.

Recently our laboratory, in parallel with several others, has showed that contrary to the single ADA2 adaptor protein present in *Saccharomyces cerevisiae*, different GCN5-containing HAT complexes of *Drosophila melanogaster* cells contain two related ADA2 proteins encoded by genes referred to as *dAda2a* and *dAda2b*. In several other metazoan organisms, including mouse, human and Arabidopsis, there

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are also two ADA2-type coactivators. Biochemical separation of ADA2-containing *D. melanogaster* complexes indicated that dADA2a is present in a smaller (0.8 MDa) and dADA2b in a larger (2MDa) complex which corresponds to the Drosophila homologue of yeast SAGA complex. In a number of independent studies it was shown that in the absence of dADA2b or dGCN5, in other words, in the absence of functional SAGA, the acetylation of histone H3K9 and K14 is greatly reduced, while the H4K8 acetylation is not affected.

In this work we provide evidence that the dADA2a protein is a specific component of the smaller *Drosophila* HAT complex which during the course of this work became identified as ATAC. We demonstrate the genetic interaction between *dAda2a* and *dGcn5* genes and show they role in H4 acetylation. Finally, we describe the functional interplay between components of the ATAC complex and ATP-dependent nucleosome remodeling ISWI-containing NURF complex.

We provide several lines of evidence for the functional linkage between dADA2a and dGCN5. We show their physical and genetic interaction by yeast two hybrid assays and by analyzing the phenotype of specific single and double mutants, respectively. The loss of either *dGcn5* or *dAda2a* function results in similar chromosome structural and developmental defects. *dGcn5/dAda2a* double-null mutants or a combination of *dAda2a* and *dGcn5* hypomorph alleles result in a phenotype stronger than that of either of the two mutations alone. The overexpression of dGCN5 protein by the use of an act-GAL4 driver in *dAda2a* mutant background results in a partial rescue. Furthermore, the phenotypic features of *dAda2a* mutants indicate a developmental block at the time of larva-pupa transition similarly as it was shown by others for *dGcn5* mutants. In accord with this, by analyzing the puff formation at sites containing ecdysone induced genes and using RT-PCR and Q-PCR to measure specific mRNA levels we demonstrate that the expression of several ecdysone-induced genes such as BR-C, Eip74 and Eip75 are downregulated in the absence of dADA2a protein.

Immunostaining of *Drosophila* polytene chromosome and Western blot analysis revealed a significantly decreased level of K5 and K12 acetylated histone H4 in *dAda2a* and *dGcn5* mutants, while the acetylation established by dADA2b-containing GCN5 complexes at H3K9 and K14 was unaffected. These results, for the first time in the literature, clearly establish the *D. melanogaster* ATAC as a histone H4-specific HAT complex.

In a set of independent experiments we showed functional interaction between the histone modifying ATAC and the nucleosome remodeling NURF complexe. Using appropriate mutants strains we showed that there is genetic interaction between genes encoding ATAC subunits and the NURF subunit ISWI. In addition, immunostaining of polytene chromosomes with dADA2a-specific Ab revealed that the ADA2a binding to *Iswi* chromosomes was strongly reduced. In agreement with this data, immunoblot analysis and chromosome immunostaining showed a significant decreased of K12 acetylated H4 level of salivary gland polytene chromosomes of *Iswi* and *Nurf301* mutants.

Taken together, these results strongly suggest a functional interaction of nucleosome remodeling and histone acetyltransferase complexes. Our data demonstrate that the function of NURF complex is required for the binding of ATAC to chromatin and for subsequent acetylation of H4K12 residues.

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Study of *Medicago truncatula* RRK1 receptor-like cytoplasmic kinase interacting proteins

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Small GTP-binding proteins of the Rho family play a role as regulators of signal transduction in plants. These proteins called ROP („Rho of plant") participate in key cellular events including the determination of polar growth, vesicular trafficking, stress and hormone responses or cell wall synthesis. ROPs act as molecular switches cycling between a GDP-bound inactive and a GTP-bound active state. In our group an alfalfa receptor-like cytoplasmic kinase, termed RRK1, has been identified by yeast two-hybrid screen as an interacting partner of the active MsROP3 GTPase. RLCKs have no extracellular and/or transmembrane domains and are localized in the cytoplasm. The function of the RLCKs is not well understood; they have hypothetical roles in RLK-dependent signaling. Our finding was among the first indications that Rop GTPases may directly influence kinase activity in plants similarly as in animals.

In order to identify downstream signaling events of RRK1, our group applied the yeast two-hybrid system with a cDNA library made from 4-day-old root nodules on *Medicago truncatula* roots, using RRK1 as bait. Several clones were identified and sequence analyzed. The sequence comparison revealed that one of our clones carries a plant specific guanine nucleotide exchange factor (GEF) domain. Conversion of Rops from the inactive GDP-bound to the active GTP-bound form is catalyzed by GEFs. In *Arabidopsis*, the ROPGEF family has 14 members, which contain a plant-specific central, highly conserved catalytic domain termed PRONE (Plant Specific ROP Nucleotide Exchanger) or formerly DUF315, and variable N-and C terminal regions.