

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 complex. 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.

Why is so important to have a kinase that is capable to interact with a ROP GTPase as well as a ROPGEF? GEF proteins have the potential to transfer signals from receptors to ROP GTPases. A huge family of receptor-like kinases (RLKs) has been found in plants but their downstream signaling events are hardly known. Similarly, it is not known what are the upstream signaling steps resulting in ROP activation. What we currently know is that a tomato protein called KPP (kinase partner protein) has been identified as binding partner of the cytosolic domains of the pollen-specific RLKs, LePRK1 and LePRK2. This KPP protein is a homolog of *Arabidopsis* ROPGEF1 and is phosphorylated *in vitro* by LePRK1. Our results indicate that a further type of kinase (RLCK) might be involved linking ROP- and RLK-mediated signaling pathways.

In order to prove this hypothesis, as a first step, we showed the interaction between the MtGEF and MsROP3 proteins. In our yeast two-hybrid experiments, MtGEF displayed strong interaction with the non-nucleotide bearing wild-type and the constitutive active (CA) mutant of MsROP3. Wild type, CA- and dominant negative (DN) mutants of MBP-fused MsROP3 and His tagged-MtGEF fusion proteins expressed in *E. coli* were used for pull down assay. With this *in vitro* protein-protein interaction assay we were able to confirm our yeast results. Then the expression level of MtGEF was investigated in different *Medicago truncatula* tissue types by QRT-PCR, but it showed very low expression in almost all tissues therefore a correlation with MsROP3 or RRK1 expression could not be made. Recently, the full length MtGEF cDNA sequence has been amplified by PCR from a *Medicago truncatula* cDNA library and cloned into various expression vectors. In the future we would like to confirm our previous observations with this full length form as well as to further characterize the potential signaling interactions. This will include the determination of GEF activity toward MsROP3 and the RRK1 kinase activity towards MtGEF. We suppose that MtGEF could be an elusive link between RLKs and ROPs in a plant-specific signal transduction mechanism that also includes a ROP-dependent feedback regulation of GEF activity through RRK1.

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Phylogeny of Alloxysta (Hymenoptera, Cynipoidea, Figitidae, Charipinae) species – morphology vs. molecules

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Members of the figitid genus *Alloxysta* (Förster 1869) are parasitoids of hymenopteran natural enemies of economically important aphid species. Therefore these hyperparasitoid species have large impact on the biological control of insect pests. Due to their minute size most of the morphological characters which are widely used in the taxonomy of other cynipoid taxa, are variable and highly reduced. Most of the species are hardly distinguishable morphologically and it is impossible to determine if the variability is intra- or interspecific. According to some authors there are only a few, very variable and generalist *Alloxysta* species whereas others suggest that the genus contains much more species which are less variable but more specialized. Current phylogenetic relationships of the genus are based on the same, often questionable morphological characters. So far no studies were carried out using molecular markers determining species limits and resolving the phylogeny of the genus. 20 morphological characters were widely used for *Alloxysta* species determination. On the basis of three characters: presence of the propodeal carina, pronotal carina, radial cell, the genus might be divided into six species groups. Mapping morphological characters on a molecular-based phylogeny enabled examination of character evolution. In this study, 20 morphological characters from western Palaearctic *Alloxysta* were mapped on a phylogenetic tree reconstructed from region of the cytochrome-c-oxidase I (COI) and the ribosomal 28S D2 genes analysed with parsimony Bayesian, maximum-likelihood and distance based methods. The COI and 28S D2 trees were congruent. The above mentioned morphological characters may have evolved in parallel in different species groups of *Alloxysta* and, taken alone, may be unsuitable for a subgeneric division of the genus, however, are suitable for species differentiation.

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