PP2A-33
Oligonucleotide recognition by the RNA-binding protein TIAR in post-transcriptional regulation of gene expression

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Introduction: The RNA-binding protein TIAR [related to TIA (T-cell intracellular antigen) -1] is an mRNA-binding protein that acts as a translational repressor, and is particularly important under conditions of cellular stress. It binds to target mRNA via its RNA recognition motif (RRM) domains and transiently prevents their translation via the formation of 'stress granules'. TIAR has been shown in the past to associate with subsets of mRNAs bearing U-rich sequences in their 3′-untranslated regions (UTRs) but we have recently demonstrated that TIAR also has the capacity to bind to a C-rich motif (Kim *et al*., Mol Cell Biol. 2007; 27 (19), 6806–17). Here we describe our further study of the binding interactions between TIAR and its target RNA sequences.
Methods: We have employed SPR (using Biacore instrumentation) to investigate the role of the separate RRM domains of TIAR in binding target RNA as well as the specific features of the target oligonucleotides that are important for binding.
Results: These studies have overall shown that 1) TIAR interacts with different binding specificities for different target oligonucleotides. 2) Binding occurs pre-dominantly through TIAR's second RRM domain and that a poly-U RNA sequence is the highest affinity target.
Conclusions: A better understanding of the binding mode of TIAR and the basis for its specificity for target oligonucleotides will clarify its biological role and help to predict the fate of given mRNA transcripts.

PP2A-34
Lipin1 is a key factor for the maturation and maintenance of adipocytes in the regulatory network with C/EBP-alpha and PPAR-gamma

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Lipin1 expression was induced at a late stage of differentiation of 3T3-L1 pre-adipocytes and maintained at high level in mature adipocytes. Knockdown of expression of lipin1 by small interfering RNA (siRNA) in 3T3-L1 preadipocytes almost completely inhibited differentiation into adipocytes, whereas overexpression of lipin1 accelerated adipocyte differentiation, demonstrating that lipin1 is required for adipocyte differentiation. In mature adipocytes, transfection of lipin1-siRNA decreased the expression of adipocyte functional genes, indicating the involvement of lipin1 in the maintenance of adipocyte function. Lipin1 increases the transcription-activating function of PPARγ_2 via direct physical interaction, whereas lipin1 did not affect the function of other adipocyte-related transcription factors, such as C/EBPα, LXRα or SREBP-1c. In mature adipocytes, lipin1 was specifically recruited to the PPREs of the PEPCK gene, an adipocyte-specific gene. C/EBPα up-regulates lipin1 transcription by directly binding to the lipin1 promoter. Based on the existence of a positive feedback loop between C/EBPα and PPARγ_2, we propose that lipin1 functions as an amplifier of the network between these factors, resulting in the maintenance of high levels of the specific gene expression that is required for adipogenesis and mature adipocyte functions.

PP2A-35
Glycine-rich RNA-binding proteins function as RNA chaperones in cold adaptation processes in *Arabidopsis thaliana* and *Oryza sativa*

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Introduction: Although glycine-rich RNA-binding proteins (GRPs) have been implicated in plant stress responses their importance and functions remain largely unknown. Here we investigated the functional roles of GRPs in *Arabidopsis thaliana* and *Oryza sativa* under cold or freezing stress conditions.
Methods: Gain-of-function and loss-of-function mutants were analyzed under stress conditions. DNA- and RNA-melting assay, ribozyme folding trap assay, and RNase T1 cleavage assay were employed to examine the roles of GRPs as RNA chaperones.
Results: Analyses of the loss-of-function mutants and over-expression transgenic plants revealed that GRPs have a positive impact on cold tolerance. Heterologous expression of rice GRP in Arbidopsis grp7 knockout plants complemented the cold-sensitive phenotypes, implying that the functions of GRPs are conserved between Arabidopsis and rice. The ability of GRPs to enhance freezing tolerance is closely correlated with their RNA chaperone activities. Nucleic acid-binding capability and RNA chaperone activity vary depending on the N-terminal and C-terminal domains of GRPs.
Conclusions: These results reveal that a particular domain structure of GRPs is a crucial determinant for RNA chaperone activity. These results strongly suggest that GRPs perform a function as RNA chaperone during cold or freezing adaptation processes in cells.

PP2A-36
CA XII expression study in HT 29 colon carcinoma cell line upon cytokine TNF-α and TGF-β stimulations

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Introduction: Carbonic anhydrases (CA, EC 4.2.1.1) are a family of metalloenzymes catalyzing the hydration of CO_2 to bicarbonate and a proton. α-Cas is involved in essential cellular functions such as pH regulation, respiration, gluconeogenesis, lipogenesis, ureagenesis, and tumor progression. So far, 15 catalytically active carbonic anhydrase isozymes are described in mammals. hCA XII is one of the transmembrane human carbonic anhydrase isoforms. hCA XII is present in many normal tissues and over-expressed in some tumors.
Aim: The aim of this study was to investigate CA XII mRNA expression in HT 29 colon carcinoma cell line upon cytokine TNF-α and TGF-β stimulations.
Methods: The mRNA expression of CA XII in colon cells was detected by Real-time fluorescent quantitative PCR.
Results: While TNF-α treatment increased proliferation TGF-β had a significant anti-proliferative effect in cells, there was no significant difference in the mRNA expression level of CA XII between TNF-α and TGF-β treated and non-treated colon carcinoma cells.