for binding Arp2/3 complex. Additionally, we identified that Arp2/3 activator Abp1 may bind near Arp3 and induce the 'closed' primed for nucleation conformation of the complex. Overall, these results reveal similarities and differences in the mechanisms of the three inhibitors. Coronin and Arpin both induce a similar open/inactive conformation; yet have highly distinct binding sites on Arp2/3 complex. In contrast, while GMF and Arpin have neighboring binding sites on Arp2/3 complex, and both compete for binding with VCA, they induce distinct inactive conformations, pointing to differences in their functions. This work was supported by RSF grant (#14-14-00234) to O.S.

P11-004-SP
Allosteric regulation of insulin receptors by membrane lipids

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A number of studies reported an association of lipid alterations and insulin sensitivity, e.g., high cholesterol and glycolipid GM3 correlating with insulin resistance. However, the underlying modulatory mechanisms remain to be shown. Insulin acts through its receptors (IRs), which are membrane-embedded type II receptor tyrosine kinases. It is conceivable that their function is modulated by lateral localization to membrane domains and thus their interactions with the lipidic environment. Owing to their complexity and dynamics, it remains challenging to unambiguously show direct lipid-protein effects in living cells. Therefore, recombinant IR isoforms A and B were affinity-purified and reconstituted into proteoliposomes (i.e., lipid vesicles) with various lipid compositions to screen for effects of different membrane physicochemical properties. Affinity-purified IR excels in purity and exhibits insulin-dependent activation. Proteoliposomes were controlled for proper protein transmembrane insertion and orientation, for vesicle integrity and leakage. We present here a platform, which allows the screening for various effects such as IR activation by different ligands in dependence of specific lipids as well as IR modulation by proteins in the context of distinct lipid environments.

P11-005-SP
Cyclin-dependent kinase 5 is involved in pleiotrophin-induced endothelial cell migration

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Cyclin-dependent kinase 5 (CDK5) is a serine/threonine kinase that requires the regulatory subunits p35 or p39 for activation. CDK5 plays an important role in neuronal migration and neurite outgrowth and there are studies showing its implication in tumor growth and angiogenesis. Pleiotrophin (PTN) is a heparin-binding growth factor that induces cell migration in neuronal, cancer and endothelial cells through its receptor protein tyrosine phosphatase β/ζ (RPTPβ/ζ) and $\alpha_v\beta_3$ integrin leading to activation of c-Src kinase, β_3 Tyr773 phosphorylation and activation of ERK1/2. In the present study, by using immunoprecipitation/Western blot analyses, proximity ligation assays and direct measurement of the kinase activity we showed that PTN increased CDK5 kinase activity and its interaction with p35. Down-regulation of CDK5 by siRNA abolished PTN-induced endothelial cell migration. We also observed that PTN-induced CDK5 activation seemed to be independent of $\alpha_v\beta_3$ but dependent of RPTPβ/ζ expression. Moreover, activation of c-Src kinase was involved in CDK5 activation, while pharmacological inhibition of CDK5 did not affect PTN-induced β_3 Tyr773 phosphorylation and ERK1/2 activation. Collectively, these data suggest that CDK5 is a significant regulator of the PTN/RPTPβ/ζ signaling pathway that contributes to PTN-induced endothelial cell migration.

P11-006-SP
Three to stick with: Interactions of the Bazooka PDZ domains with cell–cell junction molecules

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Almost all cells in the human body display some kind of polarity. This polarity ranges from morphologically highly polarized cells, such as neurons or epithelial cells, to asymmetric round cells, such as rolling macrophages inside blood vessels. Studies of the mechanisms underlying the establishment and maintenance of cell polarity have revealed the PAR complex (PARtitioning defective) as a key player. This complex comprises Par3, Par6 and atypical protein kinase C (aPKC), with Par3 being the central scaffolding protein. Par3 contains three PDZ (postsynaptic density protein-95 kDa/ Disk-large/ Zonula occludens 1) domains that interact with numerous ligands and thereby organize polarity and cell junction complexes. PDZ domains usually bind the C-termini of their ligands via a β-sheet augmentation. It has been reported that the Par3 PDZ domains interact with several proteins involved in cell-cell junction formation, such as cadherins, nectins and JAMs. However, to date there remains a lack of structural data concerning the three PDZ domains of Par3 and their interactions with these ligands. In our work, we focus on the *Drosophila* Par3 homolog Bazooka (Baz) and its interactions with different ligands in the context of cell-cell junctions. To this end, we applied a combination of x-ray crystallography and NMR spectroscopy in order to elucidate the structure–function relationship between Baz and its ligands. Our findings will offer the potential to further investigate the link between cell polarity and cell junctions.

P11-007
Heavy metal resistance of *Bacillus subtilis* AG4 isolated from the Sotk Gold Mine in Armenia

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In some environments, such as the mines and ores, heavy metal concentrations are exceeding the lethal limit for most living organisms. However, bacteria highly adapted to the response of long term stress conditions have evolved elaborate metal resistance mechanisms. The present work concerns growth response, heavy metal accumulation ability and the expression of the *copA* and *nikA* genes of *Bacillus subtilis* AG4 isolated from Sotk Gold Mine in the presence of Cu(II), Ni(II), Zn(II) and Cd(II) metals. The results indicate that *B. subtilis* AG4 showed high resistance to Ni(II) and Cu(II) (up to 4.5 mM concentrations) but was

more sensitive to Cd(II) and Zn(II) (up to 0.5 and 1 mM concentrations, respectively). The concentration of the complex metal ions in the medium was found to be optimal for bacterial grow at 16 μM Cu(II), 17 μM Ni(II), 10 μM Cd(II) and 15 μM Zn(II). Strain AG4 showed a strong ability to accumulate Cu(II) and Zn (II) (up to 7 and 3 mg/g of wet weight, respectively). *B. subtilis* strain AG4 was found to harbor the *nikA* and *copA* Ni(II) and Cu(II) resistance genes. The highest expression of the *nikA* and *copA* genes, assessed using RT-qPCR, was observed in the presence of Cu(II), Ni(II), Cd(II) and Zn(II) in the growth medium. The results indicate that *B. subtilis* strain AG4 has potential for biotechnological and bioremediation purposes. The work was partially supported by ANSEF-2015 microbio-3869 and CPEA-2011/10081.

P11-008

Identification of multiple phosphoforms of the Lymphocyte Phosphatase Associated Phosphoprotein (LPAP) by site-directed mutagenesis and mass spectrometry

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Background: LPAP (Lymphocyte Phosphatase-Associated Phosphoprotein) is a transmembrane protein with unknown function that is tightly associated with the phosphatase CD45. There is evidence that phosphorylation status of LPAP undergoes changes after lymphocyte activation. This indicates that LPAP may be involved in the regulation of immune response. However, the information about the identities of LPAP phosphorylation sites is limited. Our aim was to investigate LPAP phosphorylation in rested and activated lymphocytes, identify individual LPAP phosphoforms, and determine possible transitions between them.
Methods: LPAP phosphorylation was determined by electrophoretic mobility shift assay in SDS-PAGE, phosphate affinity electrophoresis (Mn^{2+}-Phostag SDS-PAGE), Pro-Q Diamond phosphoprotein gel staining, Differential Gel Electrophoresis (*DIGE*), and tandem mass-spectrometry. Site-directed mutagenesis was used to define the contribution of individual phosphorylation sites in total phosphorylation. Wild type and mutated LPAP were either transiently expressed in HEK293T cells or stably transduced in T cell line CEM-CCRF. Endogenous LPAP in CEM cells was knocked out by bacterial endonuclease Cas9.
Results: 2D electrophoresis showed that in CEM cells at least five different phosphoforms existed. Using NetPhos software, we predicted 11 the most probable sites of LPAP phosphorylation. These sites were mutated to alanin in order to determine their impact on protein phosphorylation. LPAP transiently transfected in HEK293T cells was phosphorylated only on the Ser153, whereas LPAP in CEM cells was phosphorylated on additional sites, Ser99 and Thr113. The phosphorylation of Ser99 was decreased after cell activation.

P11-009

Activity of Akt/mTOR pathway depends on type and time of hypertensive stimuli in the heart

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Hypertension induces biomechanical stress which causes pathological left ventricular hypertrophy by reactivation of fetal genes in the heart. Akt signaling plays crucial role in the development of physiological and pathological cardiomyocyte hypertrophy by activation of mTOR pathway followed by elevation of protein synthesis. We used two animal models of pathologic hypertension: Spontaneously Hypertensive Rats (SHR), in which hypertension develops gradually, with age, and rats after abdominal aortic banding (AAB), in which hypertension is caused rapidly, by a surgical procedure. Phosphorylation levels of Thr308 and Ser473 of Akt were increased in SHR rats indicating increased kinase activity, whereas Akt phosphorylation was decreased in AAB group. Downstream targets of Akt, GSK-3 and FOXO1, were highly phosphorylated in SHR whereas their phosphorylation was decreased in AAB rats reaffirming regulation by Akt. Both Akt and GSK-3 as well as another kinase – ERK1/2 activate mTOR pathway, whereas AMPK inhibits mTOR activity. The levels of phosphyralotion of Akt, GSK-3 and ERK1/2 were significantly increased in SHR rats preventing inhibition of mTOR pathway by AMPK. Opposite, in AAB group phosphorylation levels of AKT, GSK-3 and ERK1/2 were decreased allowing AMPK to downregulate mTOR activity. This results in increased phosphorylation of mTOR downstream kinase – S6K in SHR but not in AAB group. This study clearly indicates that activity of Akt/mTOR pathway in hypertrophied cardiomyocytes is highly dependent on the origin and period of biomechanical stress, suggesting that only long-term, gradual increase of hypertension in SHR rats mobilize Akt/mTOR pathway in cardiac remodeling. NCN grants: UMO-2011/01/D/NZ3/04777, UMO-2014/13/B/NZ4/00199

P11-010

Three roles of survivin in differentiation and malignant transformation

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Survivin, member of inhibitor of apoptosis (IAP) protein family, is expressed in most tumour cells and is considered to be promising therapeutic target. In addition to inhibition of apoptosis, it regulates proliferation and promotes migration. We aimed to discover when survivin expression is linked to lack of apoptosis, cell migration or proliferation. We used canine kidney epithelial MDCK cells as model of a differentiated cell type. As model for malignant transformation we used ts-Src-transformed canine kidney MDCK cells which, when cultivated at 40.5 °C, behave as normal epithelial cells, whereas after shift to 35 °C, Src tyrosine kinase is activated and transformation process begins. MDCK cells and Src-MDCK cells were forced to grow in suspension (1D) by adding beta 1 integrin antibody into culture medium. Survivin was not expressed in MDCK cells and consequently, cells went to apoptosis. In contrast, Src-MDCK cells were proliferating and formed large cell clusters, survivin being heavily expressed. In 2D environment, survivin was expressed both in MDCK cells and Src-MDCK cells. There was no apoptosis and