### ੂ**FEBS** Journal





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#### POSTER SESSIONS

high activity in a variety of different conditions, whereas enterobacterial MraY homologues from numerous pathogens were inhibited by detergents and high quality protein samples could only be produced in presence of specific compounds. The complete biosynthetic pathway starting from UDP-N-acetylglucosamine precurosr to lipid II formation could be reconstituted with cell-free expressed proteins and will provide the basis for developing new drug screening platforms in defined environments.

#### P32-016

# Structural investigation into the comprehensive mechanism of concentrative nucleoside transport

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Nucleoside transporters (NTs) are very important in humans, and play vital roles in nucleic acid synthesis, energy metabolism and a host of physiological processes involving regulation of intra- and extra-cellular concentrations of purine and pyrimidine (deoxy) nucleosides. Furthermore, it possesses a wide range of potential applications in the development of drugs, especially for antiviral and anticancer drugs. To date, two main families of membrane nucleoside transporters have been identified in mammalian cells, including the concentrative nucleoside transporter (CNT) and the equilibrative nucleoside transporter (ENT) families. In the former family, concentrative transport of nucleosides is energized by transmembrane sodium and/or proton gradients, whereas in the latter bidirectional nucleoside transport is driven solely by the concentration gradient of the nucleosides across the membrane. However, our understanding of the molecular mechanisms of nucleoside transport remains limited. The only known structure of a CNT is VcCNT from Vibrio cholera in an inwardfacing and partially occluded conformation. At present, we are attempting to resolve the nucleoside transport mechanism by capturing and analyzing the different conformations likely to be involved in the translocation cycles of different bacterial CNTs. Meanwhile, by comparing sodium-driven transporters such as VcCNT and homologous proton-driven transporters such as NupC from Escherichia coli, we aim to illustrate the basis for the differing cation selectivities of CNTs. This work should help to elucidate the molecular mechanisms of concentrative nucleoside transport by a structural approach, not only in the bacterial transporters but also in their physiologically and medically important counterparts in humans.

#### P32-017

# MacAB efflux system of *Serratia marcescens* as a potential protective system against oxidative stress

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Bacterial resistance to antibiotics is one of the major problems in the world. The most important in the appearance of bacterial antibiotic resistance is understanding and investigation of its molecular mechanisms. Bacteria genus of Serratia is opportunistic and antibiotic resistance pathogens with increased clinical significance. Efflux systems of Serratia marcescens involved in an excretion of a wide range of antibiotics. Analysis of genome sequence of S. marcescens allowed discovering a new ABC-type efflux system. This system has a high homology to MacAB system of E. coli. Special characteristic of MacAB efflux system of S. marcescens consists in defending against reactive oxygen species (ROS) addition to participating in antibiotics excretion. Goal of this research was an investigation of resistance of wild type (w.t.) and mutant  $\Delta$  macAB (m.t.) S. marcescens to ROS. Resistance of both strains to hydrogen peroxide (HP) was explored. HP presence in the medium led to m.t. cell death and w.t. viability. Co-cultivation of both strains resulted in the emergence of resistance of m.t. to HP. W.t. supernatant provided a clear protective effect for m.t. in the presence of HP. Thermostability and sensitivity to proteinase K treatment of w.t. supernatant metabolites allowed suggesting that protective compounds have a protein essence. Thus, macAB efflux system of S. marcescens plays a crucial role in a cell defense against ROS and its absence prevent to extracellular protective metabolites formation. This work was funded by the subsidy of the Russian Government to support the Program of Competitive Growth of Kazan Federal University.

#### P32-018

#### Acetazolamide, an inhibitor of carbonic anhydrase, supresses photophosphorylation and stimulates light-induced ATP hydrolysis in isolated spinach chloroplast

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The chloroplast CF1CF0-ATPase/synthase is located in energytransducing thylakoid membranes of chloroplasts where it catalyzes light-induced ATP synthesis and  $\Delta\mu$ H+ generating ATP hydrolysis. It has a membrane sector (CFo) attached to a membrane extrinsic oligomeric complex (CF1), that contains the catalytic sites for ATP synthesis and hydrolysis and noncatalytic (regulatory) sites. The noncatalytic sites can bind some oxyanions (bicarbonate, sulfite, borate etc.), activating CF1-ATPase. We have shown recently (Semenikhin & Zolotarova, 2014) that both  $CF_1CF_0$  complex and its isolated catalytic part, factor  $CF_1$ , are able to accelerate the prosess of interconversion of carbonic acid forms:  $CO_2 + H_2O \Rightarrow H^+ + HCO_3^-$ , ie to exhibit carbonic anhydrase activity. The aim of the present work is studying the effect of acetazolamide, an inhibitor of carbonic anhydrase, on the rate of photophosphorylation and the light-induced ATP hydrolysis in isolated spinach chloroplasts. The rate of ATP synthesis was determined by hexokinase method in chloroplast suspension illuminated for 2 min in the presence of electron acceptors. The amount of ATP was determined enzymatically using glucose-6phosphate dehydrogenase and NADP. Formed in the reaction NADPH was measured by fluorescent method. The amount of Pi released in ATPase reaction of thylakoids was determined by colourimetric method after illumination of the chloroplast suspension for 2-5 min. The data show that acetazolamide inhibits light-induced synthesis and stimulates ATP hydrolysis suggesting participation of carbonic anhydrase activity in transmembrane proton transfer coupled with ATPsynthesis/hydrolysis in thylakoid membrane of chloroplasts.