Pulmonary hypertension (PH) leads to right-ventricular hypertrophy and failure (RVF). RVF involves adverse remodeling of the ventricular extracellular matrix (ECM). Recently we found that estrogen (E2) rescues PH-induced RVF. Here we investigated the effects of E2 on ECM homeostasis. To induce PH, rats were injected with monocrotaline. At day 21, when PH had established, rats either received E2 (E2-group) for 10 days or were left untreated to develop RVF (RVF-group). Some E2-treated rats were sacrificed at day 30, and some were kept 12 days after E2-withdrawal until day 42 (E2-W-group). Echocardiography, immunohistochemistry, Western Blot and RT-PCR were performed. The RVF-group developed severe PH and RV failure with significantly depressed RV ejection fraction (RVF-EF=30.4 ± 1.8% vs. 65.1 ± 1.7% in control). E2-therapy resulted in significant improvements in RVF in E2-group (61.5 ± 0.8% vs. 65.1 ± 0.9% in control). Structural changes in RV were also observed in the RVF-group including fibrosis and re-expression of the ECM proteins osteopontin (OPN) and osteocalcin (OCN). OPN and OCN transcripts were significantly upregulated in RVF(4.5 and 2-fold, respectively). OCN protein was also upregulated 2-fold in RVF. Interestingly, although OPN and OCN transcripts in E2-group were similar to RVF, OPN transcripts and OCN protein levels in E2-W were fully restored to their values in healthy controls. Upregulation of OPN and OCN in RVF and their persistence in E2 suggest their role in mitigating adverse ventricular remodeling. Next, we investigated possible regulation of ECM-degrading enzymes A Disintegrin And Metalloproteinase (ADAM)15 and ADAM17 in PH and by E2 therapy. Both ADAM transcripts were significantly upregulated in RVF (E2>2.5 and 2-fold, respectively) and were restored to control levels in E2-W. These results suggest that a short-term E2-therapy leads to reverse ECM remodeling which could contribute to long-term functional improvements in the RV even after removal of E2.

1872-Plat

Visualisation of the Cytoskeleton in Cardiac Myocytes from a Model of Atrial Fibrillation

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Recently, in a mouse model of atrial fibrillation (RacET mouse) we have shown that co-coupling in ventricular as well as atrial myocytes was majorly disturbed. We identified structural remodeling of T-tubular membranes as a significant underlying mechanisms. In the heart it is well known that increases in Racl-activity (a small GTPase of the Rho family) result in production of reactive oxygen species. Besides this, in other cell systems, Racl can also lead to re-organization of cytoskeletal components. In this study we investigated which components of the cytoskeleton in cardiac myocytes are affected by the overexpression of constitutively active Racl (RacET). For this we used high-resolution confocal microscopy of fixed ventricular and left & right atrial myocytes, that were isolated from age-matched FVBN (wt-control) and RacET mice and probed for the following cytoskeletal and related proteins: actin, alpha-actinin, tubulin, vinculin and desmin. In addition, selected double-labeling of myocytes allowed for the analysis of the relationship between the various components. Confocal stacks (stepsize 100-200 nm) were deconvolved and 3D-reconstructed in IMARIS software (BitPlane, CH). We found the actin cytoskeleton unaltered in ventricular and atrial myocytes while microtubules displayed distinct alterations in RacET ventricular cells. While vinculin showed no differences in its cellular distribution, the important cardiac cytoskeletal protein desmin was distributed away from the z-discs in atrial myocytes from RacET mice and showed a more random and peripheral localization. We suggest that desmin-phosphorylation might lead to this redistribution and might significantly contribute to cellular remodeling in atrial fibrillation.

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1875-Plat

Folding and Self-Assembly of the Pore-Forming Unit Tat-A of the Bacterial Twin-Arginine Translocase

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The bacterial twin-arginine translocation pathway is able to transport fully folded proteins across membranes. In B. subtilis it consists only of two components: TatA, which serves as a receptor for the signal peptide, and the pore forming unit TatAs, which occurs in high stoichiometric excess. According to current models TatA contains a transmembrane segment, an amphiphilic helix, and an unstructured C-terminus. Its detailed molecular structure was resolved by solid-state NMR spectroscopy in oriented bilayers. A striking pattern on the monomeric protein surface allowed us to assemble several units into protomers and into an open oligomeric pore. The stability of these complexes was supported by all-atom MD simulations and using structure-based modeling. The observed interactions suggest that a novel motif for folding and self-assembly motif is present in this membrane-bound transport system, which allows reversible pore formation. Our comprehensive three-dimensional model thus reconciles for the first time TatA transport with a pore size of variable diameter, which can open and close by an energetically feasible mechanism.


1876-Plat

Pre-Insertion Topology of Transmembrane Proteins is Highly Plastic and Can Be Controlled by a Single C-Terminal Residue


The mechanism by which helical membrane proteins are inserted into the cellular membrane remains unclear. It is known that membrane proteins are inserted co-translationally into the lipid bilayer and that positively charged residues in the loop regions of TM proteins are important topological determinants. However, it is unclear whether those charges act strictly locally—affecting only the nearest transmembrane helices—or act globally—affecting the topology of the entire protein. We have found that the topology of an Escherichia coli inner membrane protein with four or five transmembrane helices can be controlled by a single positively charged residue...