distinct structural changes in toposome. The nature of the toposome–bilayer interaction was probed using solid-state NMR. Measurements of quadropole echo decay times indicated that toposome-binding modulated the slow motions of membrane lipids suggesting a peripheral association with the bilayer. Collectively, these results define the structural basis for toposome modulation of membrane–membrane interactions in the sea urchin egg and embryo.

OP-112

Regulation of annexin A1 expression by butyrate in colon adenocarcinoma cells through p53 and NF-YA

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Proliferation and differentiation of intestinal epithelial cells is regulated by butyrate, a product of bacterial fermentation of fibre. An increase in annexin A1 expression correlates with the differentiation of colon adenocarcinoma cells. Overexpression of annexin A1 gene and protein is observed: (a) in BCS-TC2.BR2 cells, a subline resistant to the apoptotic effects of butyrate compared to their parental BCS-TC2 cells and (b) after treatment with butyrate and TSA, an inhibitor of histone deacetylases. The analysis of annexin A1 promoter fragments has allowed the identification of several activating (–318/–172 and –95/–38) and repressing (396/–318 and –172/–95) sequences in BCS-TC2 cells. In BCS-TC2.BR2 cells a similar behaviour is observed except for the region –95/–38. A putative-binding site for p53 is found in the repressor region –172/–95; EMSA supershift assays confirm p53 binding to this region. Both butyrate and TSA decrease p53 expression in both cell lines. Three overlapping oligonucleotides comprising activating region –95/–38 have been used as probes for EMSA. BRE2 (–85/–61) generates a specific band in the EMSA assays identified as NF-YA by supershift assays. Although the binding to this probe seems not to be modified by butyrate or TSA, NF-YA expression is modulated by both agents. In conclusion, butyrate and TSA modulate annexin A1 expression in colon adenocarcinoma cells through distinct sites at the annexin promoter including both p53 and NF-YA sequences.

OP-113

Membrane biogenesis of subunit II of cytochrome bo oxidase

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Membrane insertases such as Oxa1 in mitochondria and its bacterial homolog, YidC are required for insertion of respiratory complexes. Oxa1 is responsible for the insertion of mitochondrial cytochrome c oxidase subunit II (CoxII). Here, we investigate the insertion of bacterial homolog of CoxII, which is subunit II of cytochrome bo oxidase (CyoA). We find that translocation of the short N-terminal domain of CyoA is YidC-dependent. However, the C-terminal domain requires both YidC and SecA/SecYEG complex. An N-terminal truncated mutant of CyoA lacking the C-terminal domain requires only YidC and SRP homolog (Ffh) for insertion. However, a C-terminal truncated mutant lacking the N-terminal domain, is able to insert without YidC but requires the Sec machinery. In addition, the translocation of the N-terminal domain is a prerequisite for the translocation of the C-terminal domain in the full-length CyoA. This study shows that the insertion requirements for the N- and C-terminal domains of CyoA are distinct. Furthermore, it confirms that the membrane biogenesis of subunit II of cytochrome oxidase have conserved features in bacteria and mitochondria.

OP-114

Effects of mutations on conserved residues of cytochrome cbb_3 oxidase in R. capsulatus

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Cytochrome cbb_3 oxidase from R. capsulatus is a member of the heme-copper respiratory oxidase superfamily. This oxidase reduces molecular oxygen to water and pumps the protons across the membrane in the process. Analysis of the amino acid sequence alignment of subunit I from different families of heme-copper oxidases showed that transmembrane helix VIII and the loop between transmembrane helices IX and X contain highly conserved polar residues: Ser333, Thr340, Thr350, Asn390, and Thr394. Conserved amino acids were substituted for alanine using in vitro site-directed mutagenesis to determine the relationships between these amino acids and the activity and assembly of the oxidase. The effects of the mutations on enzyme activity were evaluated by NADI plate assay. Results indicated that among targeted mutations, Ser333Ala substitution decreased the enzyme activity and Asn390Ala mutation led to a complete loss of the enzyme activity. The structural integrity of the mutant oxidases was determined on gels stained with TMBZ which easily indicates polypeptides containing heme c. Heme c containing subunits were expressed at wild-type level but c_o subunit of the enzyme was reduced and c_p subunit was absent in Asn390Ala mutant. Overall, the results indicated that Ser333 and Asn390 are very critical residues for activity or assembly of cbb_3-type oxidase. Acknowledgment: This research was supported by TUBITAK-RIGEB.

OP-115

Multiplex PCR for drug resistance against standard antityphoid drugs in blood samples of typhoid patients

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Objectives: Rapid emergence of MDR strains of Salmonella enterica serovar typhi, is a major health problem. Current methods need nearly a week to give relevant information and have poor sensitivity. This study was aimed at developing a multiplex PCR that could provide diagnosis of typhoid and information about resistance to four common antityphoid drugs, i.e. chloramphenicol, ampicillin, trimethoprim, sulphamethoxazole and ciprofloxacin within 24 h with much better sensitivity when compared with existing methods. Findings: Sixty local MDR isolates of S. typhi were studied for drug resistance pattern by disc diffusion method and relevant genes were analysed. No local strain was found to be resistant to ciprofloxacin, so a target sequence of ciprofloxacin resistance gene (gyrA) from an Escherichia coli strain was introduced into a S. typhi strain naturally resistant to other three drugs. This transformation was confirmed by sequencing. This strain was used for optimization and as a positive control.

Internal primers were designed and a nested multiplex PCR directly applicable on blood samples was evolved. After optimizing the conditions we have successfully applied this procedure on 30 clinical samples. Conclusions: We have been able to develop a multiplex PCR that is much more sensitive when compared with cultivation of bacteria from blood and can give results within 24 h.

OP-116

Consequential catalytic and structural properties for in situ localization of the intestinal lactase phlorizin hydrolase

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Milk lactose is hydrolysed to galactose and glucose in the small intestine of mammals by the lactase/phlorizin hydrolase complex (LPH; EC 3.2.1.23/62). The two enzymatic activities, lactase and phlorizin hydrolase, are located in the same polypeptide chain. We have investigated the use of Triton X-114 phase partitioning to partially purify this hydrolytic enzyme based on its distinctive hydrophobic characteristics relative to its neighbor membrane proteins, which resulted in the enrichment of LPH in detergent poor phase (DPP). Triton X-114 phase partitioning technique was proved to be a rapid and convenient method towards partial purification of desired enzyme activity. In comparison with the membrane-localized LPH (in IBBM), here we are reporting some features on the activity and stability of the solublized lactase (in DPP). Membrane localization provides favoured condition towards lactose hydrolysis by LPH. Meanwhile detergent solublized enzyme represents improved stability, which has been examined through analysis of the thermo-inactivation and stability behaviours of the enzyme. In conclusion phospholipid microenvironment of the LPH can provide efficient lactase phlorizine hydrolase activity that is accompanied with relative destabilization of the enzyme.

OP-117

ERAP1 degrades antigenic peptide precursors with specificity for internal sequence determinants

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The recognition of cytotoxic lymphocytes of antigenic peptides presented by MHC class I molecules play a crucial role in the cellular defenses not only against viruses and cancer but also contributes to autoimmune disease. MHC class I molecules present peptides that are 8–9 residues long and are derived from peptides generated during degradation of proteins by the proteasome. These particles generally release longer precursors to the presented epitopes that are trimmed further by ER-resident aminopeptidases, primarily ERAP1. Overexpression or depletion of ERAP1 has strong effects on the presentation of peptide epitopes in vivo. This enzyme has the novel property of trimming N-extended precursors that have hydrophobic C-termini to peptides of no more than eight or nine residues, the exact size and C-terminus requirements for loading onto most human MHC class I alleles. To further elucidate ERAP1 specificity we have screened a peptide library based on an antigenic epitope sequence template. We have discovered strong internal sequence preferences in peptide precursor degradation by ERAP1. These sequence preferences follow the binding preferences of MHC alleles, indicating a possible bias for antigenic peptide production by ERAP1. This finding suggests that the activity of ERAP1 on the antigenic peptide precursor pool will greatly bias the repertoire of peptides presented toward specific sequences, preferred by specific MHC alleles.

OP-118

Measured prolidase activity versus physiological activity of the enzyme: inhibitory effect of proline

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Prolidase cleaves the bonds of dipeptides containing proline (X-Pro) and an important enzyme in collagen metabolism. Prolidase activity is generally determined by photometric methods based on the measurement of proline levels produced by prolidase. We aimed to investigate the measured prolidase activity is suitable for the assessment of its physiological activity. Effects of manganese (Mn) on the enzyme activation, protein precipitation and reading steps of the photometric method, and inhibitory effect of proline on the enzyme were analysed. The intra- and inter-assay CVs % were higher than 10% for photometric method and turnaround time was 6–8 h per test. The activation reagent containing Mn was not stable and its concentration was not optimal for enzyme activation. Thus, we modified the photometric method by changing Mn concentrations and pH of activating solution, eliminating protein-precipitating step, arranging the pH of colour reagent to the pH optimum for ninhidrin reaction and shortened incubation time with substrate. The inhibitory effect of proline on the prolidase activity even in the physiological and produced proline concentrations during enzymatic analysis may limit the analytical performance of prolidase assays. In conclusion, the modified photometric method presented in this study seems to be more reliable than the classical photometric method and measured prolidase activity may not reflect the true physiological activity of enzyme due to proline inhibition.

OP-119

Protein domains: protein functions and thermal stability

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Elongation factors EF-Tu are three-domain GTPases delivering aminoacyl-tRNAs to the ribosomal A-site and hydrolysing-bound GTP during mRNA translation. The process results in a great reversible shift mainly between domain 1 and domains 2 + 3 cyclically changing the EF-Tu properties. Domain 1 is the site of GDP/GTP binding and of GTPase activity, domains 2 + 3 have no catalytic activity. The reasons for domain arrangement of EF-Tus are not fully understood except for the fact that aminoacyl-tRNA requires all three domains to bind. Here, we studied contributions of individual domains to the stability, GDP/GTP binding and GTPase activity of EF-Tu proteins. We examined, in functional and structural (CD) tests, the domains of mesophilic, Gram–, Escherichia coli (Ec) EF-Tu, and thermophilic, Gram+,