cal value (23453.3 Da). This enables further molecular and structural characterization of Chd64 using a diverse array of biochemical and biophysical methods.

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P20-175
Allosteric communication between subunits of the dimeric phosphofructokinase-2 of E. coli analyzed by hybrid enzymes and molecular simulations
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The activity of phosphofructokinase-2 (Pfk-2) is downregulated by allosteric MgATP binding, avoiding a futile cycle of ATP hydrolysis under gluconeogenic growth conditions. Kinetic studies show that the inhibition is associated with a change in the saturation for the substrate fructose-6-P from a hyperbolic to a sigmoidal behaviour, with a decrease in its apparent affinity. This change suggests allosteric communication between the fructose-6-P binding sites of the monomers according to specific allosteric communication models. The catalytic and allosteric sites in each monomer of the Pfk-2 structure, involve residues from both subunits, indicating that the dimeric structure is closely related with the function and regulation of the activity of the enzyme. In order to determine the allosteric inhibition mechanism of Pfk-2, subunit hybrid enzymes of Pfk-2 mutants were constructed and characterized kinetically. The hybrids dimers that contain only one intact site for fructose-P and one catalytic site in the same subunit, show hyperbolic saturation curves for fructose-6-P and is inhibited by MgATP, since the allosteric sites for the nucleotide are intact in both subunits. However, the hybrid dimer that contains only one intact allosteric site and a catalytic site in same subunit shows MgATP inhibition and sigmoidal response for fructose-6-P. Molecular dynamic simulations in the presence of the allosteric and catalytic ATP, show main chain correlated movements between the fructose-6-P and allosteric binding sites, in agreement with the kinetic experiments. These results indicate that the sigmoidal saturation behaviour for fructose-6-P is due to communication between the fructose-6-P sites. Also, the allosteric communication between subunits would not play an important role in the MgATP inhibition. (Supported by Fondecyt 1090336, Chile).

P20-177
Point mutations preventing post-translational modifications abolish intracellular localization of recombinant Rab7b in the model eukaryote
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Post-translational modifications may affect the function and properties of the proteins. Recombinant Rab7b and its mutagenized variants were used to elucidate factors determining localization of this protein that acquired a new function in unicellular eukaryote Paramecium octaurelia. In the recombinant His-Rab7b constructs Ser140 or Thr200, respectively, were replaced with alanine not undergoing post-translational modifications. The bioinformatic analysis showed that Ser140 and Thr200 in Rab7b are the two unique putative glycosylation and phosphorylation sites as compared to the product of the ohnologous gene Rab7a exhibiting both the different localization and function in the late phagosomal trafficking. Interestingly, besides the divergent C-termini only other difference between the amino acid sequences of these two proteins is the substitution of alanine at position 140 in Rab7a with serine in Rab7b. Incorporation of His-Rab7b or its variants was studied upon in vivo electroporation under the same conditions concerning the ratio between protein amount and the number of cells in each sample. After four hours of recovery cells were fixed and processed for dual fluorescence immunodetection in confocal microscopy using two sets of antibodies enabling the two-colored detection. Only His-Rab7b localized correctly in the cell as the endogenous protein. Two other recombinant Rab7b mutagenized species were abnormally incorporated in the cells indicating that post-translational modifications may affect protein targeting and contribute to neo-functionalization of the products of the duplicated genes.

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P20r-176
Experimental paleogenetics and paleobiochemistry to study the evolutionary history of substrate specificity in archaeal ADP-dependent sugar kinases family
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In several archaea of the Euryarchaeota group the glycolytic flux presents ADP-dependent glucokinase and phosphofructokinase (PFK) activities. These enzymes are homologous and belong to the ADP-dependent sugar kinases family of the ribokinase superfamily. Interestingly, the enzyme from Methanocaldococcus jannaschii is capable to use both sugars, and has been proposed to be an ancestral form of the family. However, recent studies have shown that the ancestral activity in the group is glucokinase and that the bi-functional enzyme belongs to the phosphofructokinase subfamily. Since experimental paleogenetics and paleobiochemistry provide an opportunity to investigate in the laboratory the molecular history of modern organisms and in order to understand the evolutionary history of this family we reconstructed the phylogenetic tree for the archaeal branch using the Bayesian method. Also, we inferred, synthesized and expressed the gene for the last common ancestor of phosphofructokinases from Thermococcales and Methanococcales groups (ancMT) and the PFK ancestor of the Thermococcales group (ancT). From this data we inferred the evolutionary history of substrate specificity and compared it with the experimental evidence obtained for our resurrected ancestral enzymes. We found that the ancMT ancestor is capable to use glucose and fructose-6-P as substrate, while the ancT ancestor is specific for fructose-6-P and that of the Methanococcales group should be bi-functional. The results support the idea that the M. jannaschii enzyme is not an ancestral form of the family. Also indicates that enzymes from the Methanococcales organisms conserved the ancestral trait of bi-functionality whereas the specificity for fructose-6-P is an acquired trait in enzymes of the Thermococcales group (Fondecyt 1110137).

P20-178
Mechanism of L-PK regulation by phosphorylation
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Pyruvate kinase (PK, ATP:pyruvate 2-O-phosphotransferase, EC 2.7.1.40) catalyzes final step of glycolysis transferring phosphoryl
group of phosphoenolpyruvate to ADP producing pyruvate and ATP. In mammals there are four PK isoenzymes: M1, M2, R and L. Only the activity of L-type pyruvate kinase, found in liver, can be regulated by phosphorylation on serine 12 residue of small N-terminal domain: MEGPAGYLRR\(^{0,8,3,5,19}\)VA-QLTEL\(^{2,0,9,2,2,9,7}\)GTAFF. As a result of phosphorylation of this peptide fragment affinity of L-PK to phosphoenolpyruvate decreased. Moreover, the phosphorylation reaction was found to act as a switch of cooperativity of the enzyme in reaction with this substrate. To investigate the mechanism of functioning of this cooperativity switch point mutations were introduced into the phosphorylatable area in positions 9, 10 and 13. It was found that some of these mutations (R9E, R9Q, R9K, R10Q) influenced on L-PK affinity for PEP, while had no effect on the enzyme affinity against its second substrate ADP. Computer modeling of docking of ligands, including fragments of the phosphorylatable N-terminus, to the enzyme molecule was used to characterize the binding sites of substrates and the regulatory peptide fragments that was necessary to develop the general picture of the regulation mechanism.

P20-179
Elucidation of the two-electron transfer process in fumarate reductase from Shewanella
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In the absence of molecular oxygen, some bacteria can use a variety of terminal electron acceptors for respiration, including nitrate, sulfate, insoluble Mn(IV) and Fe(III) oxides and fumarate. Fumarate respiration is one of the most widespread modes of anaerobic respiration, probably because fumarate can be formed from carbohydrates and proteins. The fumarate reductase of Shewanella, is the flavocytochrome Fc\(^{3,3}\), a soluble tetrameric cytochrome of 64 kDa that performs unidirectional fumarate reduction with a FAD group co-factor in the active site. X-ray crystallography showed that this protein folds in three domains: the N-terminal domain that contains four c-type cytochromes, the C-terminal domain with a non-covalently bound FAD-group and a clamp domain that is involved in controlling access of the substrate to the active site of the enzyme. The four hemes are positioned in a quasi-linear arrangement that allow electrons from the redox partner to the buried FAD group located at the catalytic site, for the reduction of fumarate. The thermodynamic properties of Fc\(^{3,3}\) showed that the individual reduction potentials of the hemes are organized in a way that allows a sequential transfer of two electrons to the FAD group. However little is known about the kinetic of electron transfer. Up to date, studies were only performed at a macroscopic level, showing that the electron transfer to the FAD group is slower than within the heme domain. The factors that control the electron transfer process in this multi-centre redox protein and that regulate the two-electron transfer that is essential for the catalytic process remains to be elucidated. Kinetic studies will reveal the reduction and oxidation process performed by Fc\(^{3,3}\) and the contribution of each redox centre will be determined using a kinetic model that allows the discrimination of each individual heme. This information is essential to reveal how a chain of single electron redox co-factors such as hemes is capable of loading a two-electron active site such as the FAD, in flavocytochrome c\(^{3,3}\) is known not to exist in the semiquinone state.

P20-180
Solution structure and biochemical studies on C-terminal domain of Vibrio vulnificus extracellular metalloprotease
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Vibrio extracellular metalloprotease (vEP) secreted by Vibrio vulnificus ATCC29307 exhibits various proteolytic function such as prothrombin activation and fibrinolytic activities. Premature form of vEP is composed of an N-terminal propeptide region (nPP), a catalytic and C-terminal domains. The nPP region is autocleaved for the matured metalloprotease activity. However, there has been no direct evidence that C-terminal domain can modulate vEP’s substrate specificity. To understand how C-terminal domain of vEP modulate its proteolytic activities, NMR studies on C-terminal domain of vEP (vEP\(^{500-610}\)) were performed by heteronuclear NMR spectroscopy. The solution structure revealed that vEP\(^{500-610}\) forms a central β-barrel composed of seven anti-parallel β-strands with two metal ion binding sites. Data from hydrogen/deuterium exchange experiments together with \(^{1\text{H},2\text{H}}\)N heteronuclear NOE (XNOE) suggest that vEP\(^{500-610}\) has a highly compact structure. Through DALI server analysis based on solution structure, vEP\(^{500-610}\) shares a structural similarity with collagen binding domain of collagenase although it has a low sequence homology. Our structure proposes that C-terminal domain of vEP could interact with collagen during the modulating a process of proteolytic activities in the infect pathway systematically. This work was supported by a NRF grant funded by the MEST (J. S. Lee and W. Lee, 20110027675).

P20-181
Structural and dynamical insights on HLA-DRB1: peptide complexes which confer resistance and susceptibility to multiple sclerosis in sardinia: a molecular dynamics simulation study
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Multiple Sclerosis is an autoimmune disease linked to inflammatory and degenerative process in the central nervous system. Human Leukocyte Antigen (HLA) class II system has been identified as the main genetic determinant regions linked to MS [1]. Recent genetic studies [2], have identified and associated five HLA-DRB1 alleles to Multiple Sclerosis (MS) in Sardinia. The basis of adaptive immune response has been associated with recognition of peptides bound to specific membrane glycoprotein, the Major Histocompatibility Complex (MHC) by T-cell antigen receptors (TCR) [3]. Antigen/Peptide presentation by MHC class II is critical component of the adaptive immune response to foreign pathogens. The availability of high resolution x-ray structures of the complexes in some cases have provided structural insights for antigen presentation. Our research is focused on investigation of MHC class II peptide interaction with an emphasis on identifying structural and dynamical differences between the predisposing and protective DRB1 alleles complexed with both self: Myelin Basic protein (MBP) and non-self Epstein Barr Virus (EBV) peptide alternatively at a microscopic level. Our detailed analysis confirm that a functional relation between MS predisposing genetic background and antigen presentation can be investigated by MD simulations.