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Abstracts

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SUBSTRATE LOCKING PROMOTES DIMER-DIMER DOCKING OF AN ENZYME ANTIBIOTIC TARGET

Matthew A Perugini[1,2], Sarah C Atkinson[1,2,3], Con Dogovski[2], Kathleen Wood[4], Michael D W Griffin[2], Michael A Gorman[5], Lilian Hor[1], Cyril F Reboul[3], Ashley M Buckle[3], Joachim Wuttke[6], Michael W Parker[2,5] and Renwick C J Dobson[7]

[1]La Trobe University, Melbourne, Australia
[2]Bio21 Institute, University of Melbourne, Victoria, Australia
[3]Monash University, Clayton, Australia
[4]Australian Nuclear Science & Technology Organisation, New South Wales, Australia
[5]St. Vincent's Institute of Medical Research, Victoria, Australia
[6]JCNS-Heinz Maier-Leibnitz Zentrum (MLZ), Forschungszentrum Juelich GmbH, Garching, Germany
[7]University of Canterbury, Christchurch, New Zealand

Protein dynamics manifested through structural flexibility play a central role in the function of biological molecules. Here we explore the substrate-mediated change in protein flexibility of an enzyme antibiotic target, *Clostridium botulinum* dihydrodipicolinate synthase (DHDPS). We demonstrate that the substrate, pyruvate, stabilizes the more active dimer-of-dimers or tetrameric form of the enzyme. Surprisingly, there is little difference between the crystal structures of apo and substrate-bound DHDPS, suggesting protein dynamics may be important. Neutron and small angle X-ray scattering experiments were used to probe substrate-induced dynamics on the sub-second timescale, but no significant changes were observed. We have therefore developed a simple technique, coined Protein Dynamics-Mass Spectrometry (ProD-MS), which enables measurement of time-dependent alkylation of cysteine residues. ProD-MS together with X-ray crystallography and analytical ultracentrifugation analyses indicates that pyruvate locks the conformation of the dimer that promotes docking to the more active tetrameric form, offering new insight into ligand-mediated stabilization of multimeric enzymes.

M.Perugini@latrobe.edu.au

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BIOMIMICRY AT THE MOLECULAR LEVEL: MOLECULARLY IMPRINTED POLYMERS AS SYNTHETIC ANTIBODIES FOR ENZYME RECOGNITION

Karsten Haupt
Sorbonne Universités, Universite de Technologie de Compiègne, Compiegne, France

Biomimicry is the general term covering any approach aimed at reproducing artificially essential properties of one or more biological systems. At the molecular level, molecularly imprinted polymers (MIPs) are an example mimicking molecular recognition phenomena. MIPs are synthetic antibody mimics that specifically recognize molecular targets. They are highly cross-linked polymers that are synthesized through the polymerization of monomers bearing suitable functional groups, in the presence of the target molecule acting as a molecular template. This templating induces three-dimensional binding sites in the cross-linked polymer network that are complementary to the template in terms of size, shape and chemical functionality. Thus, these so-called 'plastic antibodies' can recognize and bind their targets with an affinity and selectivity similar to biological antibodies. We present different approaches using controlled and localized photopolymerization allowing for the synthesis of MIPs specific for biomolecules. This allows for example to obtain protein-size, soluble MIP nanogels showing specific binding of their targets, small organic molecules or proteins (enzymes), with a good affinity and selectivity. The use of these functional nanomaterials for enzyme detection, inhibition and stabilization, and for bio imaging will be discussed.

karsten.haupt@utc.fr

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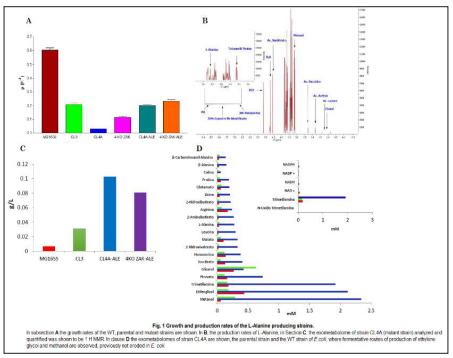
GENERATION OF BACTERIAL STRAINS OF PRODUCTION, WITH A GROWTH-COUPLED FOCUS FOR ITS APPLICATION IN SYNTHETIC BIOLOGY

Cruz Rodriguez Francisco[1], Feist Adam[2] and Utrilla Carreri Jose[3]

[1]Metropolitan Autonomous University, Mexico
[2]University of California San Diego (UCSD), California, USA
[3]Genomics Sciences Center-UNAM, Systems and Synthetic Biology Lab, Mexico

The present project presents a combination of methodologies that manages to turn around the design-construction-test cycle of bacterial strains of metabolic engineering production. We started with an in silico design generated by the genomic scale model of last generation Escherichia coli (ME-iOL1554). From this, the strains were generated using molecular biology tools. The strains generated were characterized in a simple experimental system but with strict micro aerobic conditions and underwent a process of adaptive evolution in the same experimental system, managing to generate strains with fermentative pathways interrupted but that manage to grow under strict micro aerobic conditions. The strains generated produced L-alanine (although not in titles close to that predicted by the metabolic model at genomic scale), the exo-metabolomic analyzes of one of the strains show that it is igniting latent fermentation pathways not previously described. This is why this work constitutes a conceptual advance for several reasons. 1) Test the use of computer models as a design tool, a combination of systems biology and synthetic biology is achieved. Both sciences are of great importance and relevance today. 2) The concept of growth-coupled (Growth-Coupled), a fundamental quality in a production strain, is experimentally validated. 3) A combination of methodologies was implemented: computational design, molecular biology, fermentations, adaptive evolution and exo-metabolomics by H1-NMR. 4) An advance was achieved in the generation of L-Alanine producing strains, however the most important result of the project was the use of computational models as a design tool and the discovery of latent fermentation pathways (ethylene glycol and methanol) in Escherichia coli, which could reinforce what has been said and proposed by other researchers. At the moment, there are two strains whose characteristics make them candidates for strains ¨Chasis¨.

biolex.corp@gmail.com



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THE PROBABLE EFFECT OF MT1A (A>G) AND MT1A (C>G) SNPS OF METALLOTHIONEIN GENE ON WHOLE BLOOD MERCURY LEVELS IN IRANIAN POPULATIONS

Mehdi Abdi
Valiasr Hospital, Tehran, Iran

Polymorphism in metalloproteins may lead to changes in heavy metal levels in the body. The risk factors of polymorphisms in heavy metal concentrations, particularly mercury, may be due to several confounding factors including differences in ethnicity of the analyzes populations, sample size and the type of the studied environment heavy metals to which population are exposed. We study the effect of MT1A (A>G) and MT1A (C>G) polymorphisms on blood mercury level in Iranian population. 300 non exposure people to control group and 150 exposure people to case group were used. DNA extraction and PCR-RFLP (restriction fragment length polymorphism) and DNA sequencing was done and blood mercury level was measured via atomic absorption spectroscopy (AAS) technique by DMA-80. Blood mercury concentration in case group was higher than control group (p value<0.001). There was no significant differences in case and control groups to the effect of MT1A (A>G) and MT1A (C>G) polymorphism on blood mercury levels and P value were 0.69 and 0.44, 0.59 and 0.56 for case and control groups, respectively. MT1A (A>G) and MT1A (C>G) polymorphism were not associated with increased level of mercury concentration in Iranian, which needs further investigations. In conclusion, this study suggest that MT1A (A>G) and MT1A (C>G) polymorphisms are not attractive susceptibility markers for high blood mercury concentration.

drabdim@yahoo.com

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NOVEL ON-OFF KINETICS FOR HUMAN TELOMERASE HOLOENZYME

Qiu-Xing Jiang[1], Mohammed D Sayed[2, 3], Ao Cheng[2, 4], Gaya Yadav[2,1], Andrew T Ludlow[2, 3], Jerry W Shay[2] and Woodring E Wright[2]

[1]University of Florida, Florida, USA
[2]University of Texas Southwestern Medical Center, Texas, USA
[3]University of Michigan, Ann Arbor, Michigan, USA
[4]University of Minnesota, Minnesota, USA

Genome stability in human cells requires proper maintenance of telomere length at both ends of individual linear chromosomes. During cell division, telomerase activity adds telomeric repeats to telomeres. Past studies have revealed profound insights into the cellular functions of the telomerase holoenzyme. However, due to very low abundance of functional telomerase and limited accuracy in quantifying its activity, fundamental thermodynamic and kinetic properties of human telomerase remain uncharacterized. By using newly developed technologies to analyse both endogenous and recombinant telomerase holoenzymes, we demonstrate that each holoenzyme complex contains two different types of active sites. Surprisingly, both types of active sites turn inactive after each round of processive extension reaction, named single-run catalysis. The first type of active sites turns off ~40-fold quicker than the other and exhibits higher affinity to a typical substrate. When the first site is in action, the second site remains unoccupied. The two sites thus act in tandem with the faster site performing before the slower one. The inactive enzyme is reactivated by intracellular telomerase-activating factors (iTAFs) available in multiple different types of cells we have tested. Together, the single-run catalysis and the iTAFs serve as an intrinsic brake to ensure that each fully active dimeric holoenzyme is digitally controlled in the number of telomeres it can work on. Such exquisite kinetic control of telomerase activity is expected to play important roles in normal cell physiology and cancer biology.

qxjiang@ufl.edu

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MULTI-TARGETED INHIBITION OF AN ESSENTIAL BACTERIAL ENZYME

Tatiana P Soares da Costa[1], Chamodi K Gardhi[1], Rebecca Christoff[2], J Mark Sutton[2], Belinda M Abbott[1] and Matthew A Perugini[1]

[1]La Trobe Institute for Molecular Science, La Trobe University, Melbourne, Victoria, Australia
[3]Public Health England, Salisbury, Wiltshire,UK

The cell wall of Gram-negative bacteria consists of peptidoglycan chains linked together by oligopeptidic sequences comprised of the amino acids L-Ala, D-Ala, D-Glu and meso-diaminopimelate (DAP). Meso-DAP is synthesised via the DAP pathway that also yields the basic amino acid, L-lysine. Gene knock-out studies show that enzymes functioning in the DAP pathway are essential to bacteria, including dihydrodipicolinate synthase (DHDPS). DHDPS is an allosteric enzyme that catalyses the first-committed and rate-limiting step in DAP biosynthesis. It forms a homo-tetrameric structure that gives rise to at least two 'druggable' sites, namely (a) the active site and (b) the allosteric site, which binds lysine to mediate a feedback inhibition response. Given its essentiality to bacteria and absence in humans, DHDPS represents a valid but as yet unchartered target for antimicrobial development. Recently, we have developed two classes of small molecule inhibitors that target the DHDPS active site and allosteric site using a contemporary multi-disciplinary workflow spanning biophysics, biochemistry, medicinal chemistry, microbiology and structural biology. Inhibition studies in combination with biophysical techniques have demonstrated that these compounds are broad-spectrum inhibitors of bacterial DHDPS *in vitro*, representing the most potent DHDPS inhibitors discovered to date. Using viability and time-kill assays, these inhibitors have been shown to be bactericidal against both drug-sensitive and drug-resistant strains of Gram-negative bacteria (MIC= 8 – 64 µg/ml), including *Acinetobacter baumannii, Pseudomonas aeruginosa and Escherichia coli*, but are non-toxic to cultured human cells at >1028 µg/ml. Importantly, these compounds have been shown to synergise with FDA-approved classes of antibiotics, including β-lactams, fluoroquinolones, rifampicin and aminoglycosides. This study illustrates the potential for DHDPS inhibitors to be developed into a new class of antimicrobials with excellent potential to be combined with current antibiotics to yield innovative multi-targeted formulations to minimise the emergence of resistance.

T.SoaresdaCosta@latrobe.edu.au

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ROLES OF MICRORNAS IN TH17 AND TREG IN POST STROKE PULMONARY INFECTION

Zhengtian Gu and Fuling Yan

Zhongda Hospital, Southeast University, Nanjing, China

Background: Th17 plays an important role in promoting the inflammatory response in the body, while Treg cells have the function of inhibiting excessive inflammation. Therefore, Th17/Treg ratio plays an important part in regulating the inflammatory response. Th17 and Treg are all differentiated from the naive $CD4^+$T cells, which is mainly in the spleen. In the case of stroke, when infected, the Th17/Treg ratio increases, but the body's immunity is reduced. Studies have shown that there is a specific expression of microRNA in the subsets of T cells. We speculate those microRNAs that are specifically expressed in Th17 and Treg in maintaining their polarity and characteristic cell function.

Objective: We aimed to study the expression of specific expression of microRNA in Th17 and Treg in the case of post stroke pulmonary infection. The effects of these microRNA on the differentiation, function and plasticity of Th17 and Treg were also studied.

Methods: The right MCO rats were injected *Pseudomonas aeruginosa* bacterial suspension into the trachea after 24h (the experimental group) and in the control group, the right external carotid artery was separated and ligated, but the thrombus line was not inserted. The bacterial suspension was injected into the trachea 24h after operation also. After 24h, the two groups of animals were sacrificed and the spleen was taken out. The naive $CD4^+$T cell were isolated by flow cytometry. The expression level of this characteristic microRNA was detected. In *in vitro* experiments, the inhibitors and activators of differential expression of microRNA in Th17 and Treg were applied to Th17 and Treg, respectively. Then, firstly, we use antigen to stimulate the two subsets and measured the cell function by detecting their specific cytokine. Secondly, Treg was cultured under Th17 polarization condition, Th17 was cultured under Treg polarization condition and the proportion of two cells and their specific cytokine were detected by 3 days after.

Results: Some characteristic microRNA in the two subsets expressed differently (miR-519-3P, miR-559, miR-129-3p, miR-542-3p and miR-548). These microRNA affected the level of differentiation (miR-519-3P, miR-559 and miR-129-3p), cell function and plasticity of Th17 (miR-519-3P) and Treg (miR-129-3p, miR-542-3p and miR-548), respectively.

Conclusion: In the presence of stroke, the difference in expression of some microRNA can change differently, function and plasticity of Th17 and Treg and then influence the immune function when there is pulmonary infection.

gztneurology@163.com

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PHOSPHOLIPASE D INHIBITION MITIGATES PULMONARY FIBROSIS BY ATTENUATING BRONCHIAL EPITHELIAL CELL MITOCHONDRIAL DNA DAMAGE AND APOPTOSIS

Vidyani Suryadevara[1], Longshuang Huang[1], Seok-Jo-Kim[2,3], Panfeng Fu[1], Carol Feghali- Bostwick[4], Gilbert Di Paolo[5], David W Kamp[2,3] and Viswanathan Natarajan[1]

[1]University of Illinois at Chicago, Chicago, Illinois
[2]Northwestern University Feinberg School of Medicine, Chicago Illinois
[3]Jesse Brown VA Medical Center, Chicago, Illinois
[4]Medical University of South Carolina, Charleston, South Carolina
[5]Columbia University Medical Center, New York

Idiopathic pulmonary fibrosis (IPF) is a pernicious lung disease characterized by alveolar epithelial apoptosis, dysregulated repair of epithelial injury, scar formation and respiratory failure. Currently there are only two FDA approved drugs for IPF; which do not cure the disease, but just slow the progression of disease, there is a need to identify new therapeutic targets for the disease. Phospholipase D (PLD), an important lipid mediator involved in several pathophysiologies, catalyzes the hydrolysis of phosphatidylcholine, generating phosphatidic acid (PA) and choline. PLD mediated PA generation is involved in regulation of various cellular processes including cell survival, cell migration, cell proliferation, differentiation, cytoskeletal changes, membrane trafficking, and autophagy. In this study, we have identified phospholipase D (PLD) generated phosphatidic acid (PA) signalling in the development of pulmonary fibrosis (PF). Phospholipase D (PLD), an important lipid mediator involved in several pathophysiologies, catalyses the hydrolysis of phosphatidylcholine, generating phosphatidic acid (PA) and choline. Of the PLD isoenzymes, the protein expression of PLD2, but not PLD1, was up-regulated in lung tissues from IPF patients and bleomycin challenged mice. Both PLD2 ($Pld2^{-/-}$) and PLD1 ($Pld1^{-/-}$) deficient mice were protected against bleomycin induced lung inflammation and fibrosis, thereby establishing the role of PLD in fibrogenesis. To further understand how PLD mediates epithelial injury during PF, challenging of bronchial airway epithelial cells (Beas2B) with bleomycin stimulated PLD activity and PLD2 expression in the cells. Further, inhibition of PLD2 with VU0364739 attenuated bleomycin-induced mitochondrial (mt) superoxide production and mtDNA damage that leads to apoptosis in Beas2B cells. These results support a critical role for PLD2 signalling in promoting pulmonary fibrosis in humans and mice. We reason that PLD2 may be a novel therapeutic target in mitigating IPF.

vsurya2@uic.edu