

II.O.1

Genetic evidence of interrelated functions for ppGpp, transcription factors and chaperones

Mike Cashel

Eunice Kennedy Schriver National Institute of Health and Child Development, National Institutes of Health, Bethesda, MD, USA
e-mail: cashelm@mail.nih.gov

Much is known of chaperones and ppGpp, two global bacterial networks responsive to environmental stress. Cells are alerted to stress by ppGpp, which adjusts gene expression to coordinate molecular mechanisms that limit damage and ensure survival. Chaperones also ensure survival but through repair of stress-induced damage by renaturing or recycling denatured proteins and misfolded RNA. Functional interactions between chaperones and ppGpp systems seem largely unexplored. Gene expression changes in *E. coli* require RNA polymerase binding of ppGpp at a junction of omega (ω) and β' subunits. Omega is highly conserved and shown to be required for polymerase maturation only *in vitro*. Yet an ω deletion is not lethal and does not alter regulation of, or by, ppGpp. The reason hypothesized is that some chaperones are functionally redundant with ω . If true, then deleting ω together with the RelA ppGpp synthetase and one or another chaperone might be lethal for growth. This approach could identify a relevant redundant activity. This led us to find: i) even when ω is present, a deletion of *relA* and either *dnaK* or *dnaJ* enhances the intrinsic temperature sensitivity (ts) of a single *dnaJ* or *dnaK* mutant. When ω and *relA* are both absent along with *tig*, *clpB* or *groE* the corresponding triple mutants do display enhanced ts growth phenotypes. This is a clue to shared functions. Another possible clue to chaperone – ppGpp functional intersections is that DksA usually acts as a co-regulator of transcription with ppGpp yet it has another known function: when over expressed, DksA reverses the ts phenotype of *dnaK* mutants. We find that over expression of other transcription accessory proteins (GreA or GreB) share this feature with DksA for *dnaK* as well as for *dnaJ* mutants. These suppressor activities occur even when using mutant GreA, GreB or DksA proteins that are unable to support classical RNA polymerase activities, as if changes in gene expression are not relevant mechanisms. The data do suggest functional interactions exist between stress, chaperones, core transcription machinery and ppGpp. The unexpected chaperone activities of transcription factors could reflect moonlight functions that are present in addition to assigned activities.

II.O.2

Computational and functional analysis of a toxin-antitoxin system of *Staphylococcus pseudintermedius*

Monika Janczak¹, Michał Bukowski¹, Grzegorz Dubin^{2,3}, Adam Dubin¹, Benedykt Władysław^{1,3}

¹Department of Analytical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland; ²Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland; ³Malopolska Centre of Biotechnology, Gronostajowa 7A, 30-387 Kraków, Poland
e-mail: monikarys@gmail.com

Toxin-antitoxin (TA) systems are small genetic modules, widely occurring in bacterial chromosome and mobile genetic elements as well. They may play a significant role in bacterial physiology, particularly as gene expression regulators, thus they are an object of intensive studies. The presented research focuses on computational and functional analysis of chromosomally encoded *pemIK_{sp}* system of *Staphylococcus pseudintermedius*, homologous to the previously characterized, plasmid-located *pemIK_{sa}* system of *Staphylococcus aureus* strain CH91 [1].

Locus of putative *pemIK_{sp}* operon from twenty *S. pseudintermedius* strains were amplified and sequenced. The obtained sequences were analyzed for the presence of *pemK_{sp}* toxin and *pemI_{sp}* antitoxin genes. Gene variants were cloned into an expression vector pETDuet-1 and proteins were produced in *Escherichia coli* BL21 (DE3). Toxins were assayed for endoribonucleolytic activity towards the RNA phage MS2. The cross-interaction with the TA system elements derived from *S. aureus* was analyzed by co-expression and co-purification tests.

Two and five variants of toxins and antitoxins, respectively, were identified among sequenced operones. Endoribonucleolytic activity for toxins was confirmed. Additionally for the full-length toxin the interaction with PemI_{sa} antitoxin was shown. Co-expression and cross-interaction with PemK_{sa} toxin was demonstrated for all the antitoxins except for the shortest variant.

Among chromosome of *S. pseudintermedius* strains, we identified a set of variants of the *pemIK_{sp}* system. The shortening of the toxins did not have an effect on their activity, while the significant truncation of N-terminal part of antitoxins contributed to the loss of inhibitory effect against the toxin, as shown for the shortest variant. The existence of the TA systems consisting of the active toxin and inactive antitoxin may indicate that the jump of the operone from plasmid to the bacterial chromosome may lead to its degradation and the loss of function, as suggest elsewhere [2].

Keywords: PemIK_{sp}, *Staphylococcus pseudintermedius*, toxin-antitoxin system

Acknowledgements: The study was supported in part by the grant UMO-2014/13/B/NZ1/00043 (to B.W.) from National Science Centre, Poland.

References:

1. Bukowski M *et al.* (2012) *Nat Commun* **4**: 2013.
2. Van Melderen L, Saavedra De Bast M (2009) *PLoS Genet* **5**: 3.

II.O.3

ExoY, a nucleotidyl cyclase toxin from *Pseudomonas aeruginosa*

Dorothee Raoux-Barbot¹, Cosmin Saveanu², Louis Renault³, Abdelkader Namane², Vasily Ogryzko⁴, Daniel Ladant¹, Undine Mechold¹

¹Institut Pasteur, CNRS URA3528, Unité de Biochimie des Interactions Macromoléculaires, 25-28 rue du Docteur Roux, 75724 Paris cedex 15, France; ²Institut Pasteur, CNRS UMR3525, Génétique des Interactions Macromoléculaires, 25-28 rue du Docteur Roux, 75724 Paris cedex 15, France; ³Laboratoire d'Enzymologie et Biochimie Structurales, CNRS, Centre de Recherche de Gif, Avenue de la Terrasse, 91190 Gif-sur-Yvette, France; ⁴Institut Gustave Roussy, CNRS UMR 8126, Unité de Signaling, Nuclei and Innovations in Oncology, 94805 Villejuif, France
e-mail: undine.mechold@pasteur.fr

Bacterial adenylate cyclase toxins are potent virulence factors that synthesize cAMP to modulate or disable the function of the host cell. *Pseudomonas aeruginosa*, an opportunistic human pathogen that causes severe acute infections in immuno compromised individuals and is a major cause of chronic infections in cystic fibrosis patients, encodes an adenylate cyclase toxin, called ExoY. Together with 3 other proteins (ExoS, ExoT, and ExoU), ExoY is injected directly into the host cell utilizing the type III secretion system (T3SS) where they contribute to virulence of the pathogen. Recent results show that substrate specificity of ExoY is not restricted to ATP as ExoY was shown to promote the intracellular accumulation of cAMP and cGMP, cCMP as well as cUMP when expressed in cell cultures. The physiological effects of ExoY resulting from the accumulation of these cyclic nucleotides include the hyperphosphorylation of tau and the disruption of microtubules causing the formation of gaps between endothelial cells and increased permeability of the endothelial barrier. Recently discovered long term effects of ExoY on recovery after lung injury from pneumonia suggest ExoY as potential drug target. ExoY is inactive inside the bacterial cell and acquires catalytic activity only after its delivery to the eukaryotic host cell through its interaction with an eukaryotic cofactor. We recently identified this cofactor in our laboratory. The presentation will describe the discovery of said cofactor as well as proteins and conditions that interfere with the activation of ExoY.

II.O.4

Direct and indirect regulation of *kdgM2* gene expression by the OmpR two-component response regulator in *Yersinia enterocolitica*

Marta Niecekarz¹, Adrianna Raczowska¹, Karolina Skorek¹, Ewa Stefańska¹, Iga Milczewska¹, Dorota Stosio¹, Michał Dadlez^{2,3}, Katarzyna Brzostek¹

¹Department of Applied Microbiology, Institute of Microbiology, Faculty of Biology, University of Warsaw, Miecznikowa 1, 02-096 Warsaw, Poland; ²Institute of Biochemistry and Biophysics, PAS, Pawińskiego 5a, 02-106 Warsaw, Poland; ³Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Pawińskiego 5a, 02-106 Warsaw, Poland
e-mail: nieckarmarta@biol.uw.edu.pl

Background: Protein KdgM2 of the human enteropathogen *Yersinia enterocolitica* is a homolog of *Dickeya dadantii* specific oligogalacturonateporin KdgN. *D. dadantii*, a plant pathogen, synthesizes two oligogalacturonateporins, KdgM and KdgN, that overlap functionally and are controlled by the KdgR protein, the general repressor of genes involved in pectin metabolism. Bioinformatics analysis of the *Y. enterocolitica* genome revealed genes involved in pectin degradation and catabolism that are organized similarly to those in the genome of *D. dadantii*.

Objectives: This investigation was initiated following a differential proteome analysis of the *Y. enterocolitica* outer membrane which showed overproduction of KdgM2 in a null *ompR* mutant strain compared to the wild type. Further studies were designed to reveal the role of OmpR in the regulation of *kdgM2* expression.

Methods: Differential proteomic shotgun LC/MS/MS analysis was applied to characterize the overproduction of KdgM2 in the *Y. enterocolitica ompR* mutant. In addition, *kdgM2::lacZ* and *kdgR::lacZ* transcriptional fusions were constructed in strains differing in OmpR content and β -galactosidase activity was measured. This analysis revealed that OmpR modulates the expression of particular genes at the transcriptional level, both negatively (*kdgM2*) and positively (*kdgR*). Furthermore, electrophoretic mobility shift assays showed that OmpR binds to the promoter regions of *kdgM2* and *kdgR*, indicating that this factor directly regulates the transcription of these genes.

Conclusions: Here, we demonstrate for the first time that OmpR, the response regulator of the EnvZ/OmpR system in *Y. enterocolitica*, participates in the negative control of *kdgM2* expression directly, and also indirectly by positively regulating expression of the KdgR repressor.

Key words: *Yersinia enterocolitica*, OmpR, KdgM2, KdgR

II.O.5

Transcriptomic description of a classic model of carbon catabolic repression in *Streptomyces coelicolor*

Alba Romero¹, Romina Rodríguez-Sanoja¹, Daniel Segura², Sergio Sánchez¹

¹Departamento de Biología Molecular y Biotecnología del Instituto de Investigaciones Biomédicas Mexico, D.F. 04510; ²Departamento de Microbiología Molecular del Instituto de Biotecnología, Cuernavaca Mor. 62210, de la Universidad Nacional Autónoma de México
e-mail: albaromero@comunidad.unam.mx

In the genus *Streptomyces*, one of the most remarkable control mechanisms of physiological processes is carbon catabolite repression (CCR). This mechanism regulates the expression of genes involved in uptake and utilization of alternative carbon sources. CCR also affects the synthesis of secondary metabolites and morphological differentiation. Even when the outcome effect of CCR in different bacteria is the same, their essential mechanisms can be quite different. In Gram-negative bacteria CCR relies on the carbohydrate translocation phosphoenolpyruvate-dependent phosphotransferase system (PTS). In low GC Gram-positive bacteria, two control proteins, cAMP-receptor protein (Crp) and the catabolite control protein (CcpA), are responsible for this phenomenon. In *Streptomyces*, neither the PTS system nor Crp orthologous seem to play a significant role in CCR, although an effect has been ascribed to glucose kinase (Glk). Therefore, the aim of this work was to use a high density microarray approach in a *Streptomyces coelicolor glk* deleted mutant, to evaluate the paradigmatic model proposed for CCR in *Streptomyces*. We have described central carbon metabolic pathways dependent and independent of the *glk* gene. Besides the role of *glk* and glucose in central carbon metabolism, we evaluated the effect over the consumption of an alternative source, antibiotics production and differentiation. Additionally, we have gain insight into the transcriptional regulators involved in the CRR in *Streptomyces coelicolor*. The microarray outcome was evaluated by qRT-PCR.

Key words: transcriptome, transcriptional factors, carbon catabolite repression, glucose kinase

Acknowledgements: This work was supported by grants from DGAPA, PAPIIT, UNAM IN201413 and CONACYT CB 219686.

II.O.6

Isothiocyanates reduce toxicity of enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 strains

Dariusz Nowicki, Olga Rodzik, Sylwia Martyn, Wioletta Kobiela, Grzegorz Węgrzyn, Anna Herman-Antosiewicz, Agnieszka Szalewska-Pałasz

Department of Molecular Biology, University of Gdansk, Wita Stwosza 59, 80-308 Gdańsk, Poland
E-mail: dariusz.nowicki@biol.ug.edu.pl

Increasingly these days we need new drugs to fight against bacterial pathogens. Enterohemorrhagic *E. coli* (EHEC) are strains capable of producing Shiga toxin and typically cause bloody diarrhea and severe complications. The heaviest hemolytic-uremic syndrome (HUS) complicates about 10 percent of EHEC infections overall. HUS affects mainly children and elders causing irreversible organ damage.

In this study we focused on phytochemicals, the secondary metabolites known to be involved in plant defense mechanisms, to find antimicrobial compounds useful in EHEC treatment. Comprehensive study shows the mechanism of action of *Brassicaceae* derived isothiocyanates (ITC): a phenethylisothiocyanate (PEITC), sulforaphane (SFN), allylisothiocyanate (AITC), benzyl isothiocyanate (BITC), phenyl isothiocyanate (PITC) against Shiga toxin producing *Escherichia coli*.

First we carried out the antimicrobial characteristics of the compounds, and minimum inhibitory concentrations (MICs) and zone inhibition was determined. Further to elucidate the mechanism of antimicrobial the effect on DNA/RNA synthesis and bacterial stress response (stringent response) was studied in radioactive labeled substrates incorporation assays. We have employed lambdaoid bacteriophages such as 933W and Φ 24_B to test ITCs influence on virus development in bacterial host.

All from 6 tested ITCs exhibit antimicrobial activity, BITC and PEITC were the most effective. We showed that ITCs affect nucleic acid synthesis. Immediate and wide range of reaction of cells to ITCs treatment led us to investigate stress response mechanisms. We noticed in the presence of ITCs significant increase of (p)ppGpp alarm level comparable with that observed during amino acid starvation. Moreover inhibition of bacteriophage development affect also Shiga toxin synthesis and cytotoxicity of EHEC strains.

Key words: EHEC, isothiocyanates, stringent response