

Meeting report

## Bacterial networking

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A report of the ESF-EMBO Symposium Bacterial Networks (BacNet08), Sant Feliu de Guixols, Spain, 13-18 September 2008.

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At a recent symposium on bacterial networks held on the Spanish Costa Brava some 150 participants heard about updates of key developmental and signaling networks in and between bacteria, and contributions that illustrated the direction in which microbiology is evolving. Here we report some highlights of the meeting from a genomic and systems biology perspective.

### Adventures in time and space

Differentiation processes such as spore formation in *Bacillus subtilis* or cell division in *Caulobacter crescentus* require intricate pathways that not only meticulously regulate gene expression, but also locate crucial proteins at the right time and place in the cell. *Caulobacter*, for instance, develops through an asymmetric cell division into two different cell types: a stalked cell and a swarmer cell. Protein degradation by the ClpXP protease plays a crucial role in synchronizing cell differentiation with the cell cycle. ClpXP is therefore dynamically localized at different cellular locations in order to degrade other co-localized proteins. A new example of a co-localized protein involved in asymmetric cell division in *Caulobacter*, KidO, was presented by Patrick Viollier (Case Western Reserve University, Cleveland, USA). KidO, localized near the site of division, has a dual activity: stimulating the kinase activity of DivJ and inhibiting cell division through interference with the FtsZ ring. ClpXP is, in turn, feedback-regulated by KidO through a loop containing DivJ. Another ClpXP target is CtrA, the cell-cycle master regulator that needs to be degraded before chromosome replication can be initiated. This degradation occurs at the cellular pole, after recruitment by the proteins RcdA and

cyclic di-GMP-bound PopA, as reported by Urs Jenal (University of Basel, Switzerland). This illustrates how phospho-signaling, interaction with small molecules, and proteolysis together mediate spatial and temporal control of bacterial development.

Proteins form highly organized complexes in the course of signaling. In *Escherichia coli*, chemotaxis receptors are found in clusters, localized at the cellular poles. These clusters are subdivided into sets of synergistically acting receptor complexes, called 'signaling teams'. Victor Sourjik (University of Heidelberg, Germany) has used *in vivo* FRET analysis to show that cells can, by modifying the sizes of these signaling teams upon receptor modification, dynamically adapt their range of sensitivity to the chemoattractants.

The observation that bacteria are not just simply homogeneous, but highly structured organisms influences the way development and signaling processes should be modeled. This was exemplified by Jeroen van Zon (Imperial College London, UK) who showed by properly modeling the spatio-temporal pattern of *Caulobacter* TipN localization how the polar release of this developmental master regulator is controlled by the cell volume.

### Integrating computational with wet lab approaches

How bacterial cells preclude cross-talk between the sometimes hundreds of two-component regulatory systems they use to sense and respond to environmental stimuli has long remained elusive. Michael Laub (Massachusetts Institute of Technology, Cambridge, USA) reported the use of a simple computational approach combined with the appropriate wet lab validation to solve this enigma. Amino-acid covariation analysis of large sets of cognate histidine kinase-response regulator alignments allowed him to pinpoint the residues that determine the substrate specificity of histidine kinases for their cognate response regulators.

Thorsten Mascher (Georg-August-University, Göttingen, Germany) has comprehensively mapped the phylogenetic distribution of extracytoplasmic function (ECF)  $\sigma$ -factors, the so-called 'third pillar' of bacterial signal transducers. Usually, an ECF  $\sigma$ -factor consists of a transmembrane sensor protein (called the anti- $\sigma$  factor) and a corresponding cytoplasmic transcriptional regulator ( $\sigma$ -factor) that mediates the cellular response through differential gene expression once it is released from its anti- $\sigma$  factor. A member of this family ( $\sigma^E$ /ChrR) was described by Tim Donohue (University of Wisconsin-Madison, USA) in relation to the transcriptional response upon singlet oxygen stress during photosynthesis in *Rhodobacter sphaeroides*. With his genomic analysis, Mascher discovered in  $\alpha$ -proteobacteria a novel class of distantly related ECF  $\sigma$ -factors coupled with a two-component system. The response regulator (RR) of this two-component system has an unusual architecture, with a carboxy-terminal response regulator receiver domain and an amino-terminal ECF  $\sigma$ -factor-like domain, responsible for the interaction with an anti- $\sigma$  factor. The DNA-binding elements found in the classical response regulators are lacking. Julia Frunzke (ETH Zurich, Switzerland) presented the functional analysis of a member of this novel class of ECF  $\sigma$ -factor families (RR, PhyR) in *Methylobacterium extorquens*, which proved to be a central regulator of general stress response.

### A systems view of bacterial networks

Nicholas Luscombe (EMBL-EBI, Hinxton, UK) described an analysis of the *E. coli* transcriptional and metabolic networks. Overlaying the known parts of these networks revealed the presence of important feedback reactions, classified as fast direct reactions in which metabolites target single enzymes (allosteric regulation) and slower indirect reactions in which metabolites trigger transcription factors that amplify the signal by regulating larger sets of genes. Direct feedback seemed to predominantly control anabolic pathways by mainly targeting enzymes located at the branching points of pathways, whereas indirect feedback occurred in both the catabolic and anabolic pathways without specific preference for branching points. Although this static analysis of the network nicely recapitulated information known about the *E. coli* network, Uwe Sauer (ETH Zurich, Switzerland) went a step further by demonstrating that the network's functional behavior only emerges through its dynamic and condition-dependent interactions. Although transcription factors often modulate the expression of many metabolic genes, Sauer showed by  $^{13}\text{C}$ -based metabolic flux analysis that flux distributions in the central metabolism of bacteria and yeast are robust against perturbations of the major global transcription factors.

Dirk Bumann (University of Basel, Switzerland) illustrated that this extreme form of metabolic robustness also plays a role in systemic *Salmonella* infection. By single-cell sorting

and proteomics analysis he discovered that enzymes make up 70% of the proteins induced in the bacterium during infection. Most of these enzymes were non-essential. It seems that the combination of the nutrient-rich host environment and the presence of redundant bacterial biosynthesis and uptake pathways leaves combinatorial therapy as the sole option for effective antimicrobial treatment. Instead of approaching the problem experimentally, Bumann proposes the use of an *in vivo* tuned metabolic flux model to predict which combined perturbations will result in the most severe attenuation of bacterial growth *in vivo*.

### Coping with noise

Michael Elowitz (California Institute of Technology, Pasadena, USA) proposed an intriguing frequency-modulation (FM) model to explain how a cell manages to mediate the transduction of external signals into the highly coordinated expression of hundreds of target genes in an environment where both the signals and responses are inherently noisy. According to this model, external signals do not influence the amplitude of a response, but rather the frequency of the response-triggered states the cell is in. In budding yeast, the calcineurin-responsive zinc finger transcription factor Crz1 is dephosphorylated and translocates into the nucleus in response to extracellular calcium. Elowitz showed that increasing extracellular calcium concentration did influence the frequency, but not the duration of localization bursts. They also showed that this frequency modulation allows cells to maintain coordinated expression of the genes downstream of Crz1 despite differences in promoter characteristics and fluctuations in the input signal.

In many naturally occurring gene networks, random changes in gene expression results in a bistable behavior that allows individual cells within an isogenic population to randomly swap between ON and OFF states of the network, which results in distinct phenotypes. One hypothesis to explain why bacteria maintain such stochastic behavior is 'bet-hedging': random expression of alternative phenotypes would allow a genotype to survive in fluctuating environments. Alexander van Oudenaarden (Massachusetts Institute of Technology, Cambridge, USA) has obtained experimental evidence for this hypothesis by showing that tuning their inter-phenotype switching rates to the frequency of environmental changes provided cells with the most optimal way of blindly anticipating environmental alterations.

Interestingly, Martin Ackermann (ETH Zurich, Switzerland) presented a fundamentally different model based on self-destructive cooperation to explain the benefit of phenotypic noise in bacterial populations. Self-destructive cooperation is an extreme form of division of labor in which, by committing suicide, one of the two phenotypes produces the goods essential for the survival of the other phenotype. Applied to the *Salmonella typhimurium* invasion phenotype, a small

part of the population triggers the host innate immune response by invading the host cell. This suicidal act not only kills most of the invaders but also wipes out many competitor gut commensals, thereby clearing the way for a more successful infection by the remaining *Salmonella* cells. Ackermann indeed found that in this experimental system, gene expression of central invasion-related genes is highly variable within the *Salmonella* population but seems enriched in the subset of the population found in the gut tissues.

### Interactions between bacterial communities and the host

Thanks to modern high-throughput technologies and microscopic techniques, understanding the ecological complexity of bacterial communities in interaction with animals or plants is becoming increasingly feasible. As one example of such intricate interactions, Edward Ruby (University of Wisconsin-Madison, USA) described the symbiosis between the luminous bacterium *Vibrio fischeri* and the light organs of a small squid, *Euprymna scolopes*. The nascent light organ of a newly hatched juvenile is, after being exposed to hundreds of bacterial species living in the sea water, colonized by *V. fischeri* within hours. This highly specific colonization process depends on specific chemoattractants (one of which is chitobiose, as revealed by transcript profiling) produced by the squid and sensed by *Vibrio*.

Another example was presented by Eva Kondorosi (Institut des Sciences du Végétal-CNRS, Gif-sur-Yvette, France), who described the symbiosis between legumes, such as *Medicago*, and rhizobia. During this process both the bacteria and the plant cells undergo a strikingly similar differentiation manifested by endoreduplication-driven cell elongation and an irreversible loss of the capacity for cell division. Kondorosi showed by transcriptome and genome analysis that it is the host plant that controls this irreversible bacterial fate by means of small secreted peptides, homologous to antimicrobial peptides.

The meeting highlighted the importance of time and space in bacterial networks, the power of integrating genomic data with wet lab experiments, the need for a systems-level understanding of an organism in isolation or in interaction with animals and plants, and the importance of single-cell and single-molecule measurements in understanding the role of stochasticity in bacterial communities. Gathering scientists from all these different disciplines allowed for cross-fertilization of ideas thereby setting the horizon for new cutting edge research to be discussed at the next BacNet meeting (Sant Feliu, 2010).