Bacterial regulatory networks—from self-organizing molecules to cell shape and patterns in bacterial communities

Regine Hengge & Victor Sourjik

The ESF–EMBO Conference on ‘Bacterial Networks’ (BacNet13) was held in March 2013, in Pultusk, Poland. It brought together 164 molecular microbiologists, bacterial systems biologists and synthetic biologists to discuss the architecture, function and dynamics of regulatory networks in bacteria.

Over the past 20 years, the biannual Bacterial Networks conference—BacNet for short—has become an important meeting point and showcase for research at the interface between molecular microbiology and bacterial systems biology in Europe. By focusing on the architecture, function and dynamics of bacterial regulatory networks, participants at BacNet presented and discussed the crucial topics required to understand the molecular structure and function of bacteria, their role in pathogenesis and ecology, and their use in biotechnology and synthetic biology.

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Bacterial networks come in many physiological flavours and can be studied at multiple levels. Accordingly, BacNet13 had sessions on stress responses and environmental adaptations, biofilms, motility and chemotaxis, development, intercellular communication and sociality, cell shape, and cell polarity and division. Quantitative network analysis and modelling, as well as rewiring regulatory networks in synthetic biology were also covered. BacNet13 has impressively demonstrated that understanding bacterial regulatory networks requires interdisciplinary approaches that combine molecular biology, biochemistry

and structural biology with the latest microscopy to visualize dynamic cellular processes, single-molecule analysis, whole-cell -omics and high-throughput approaches, quantitative description and mathematical modelling. Moreover, biotechnology—in particular synthetic biology as an engineering science of the future—depends on a thorough understanding of the molecular modules of cellular regulatory networks of bacteria. In this short report, we focus on several newly emerging trends evident in the presentations at BacNet13.

Bacterial second messengers

Although we have known about second-messenger signalling in bacteria—for example, by using cAMP or ppGpp—for decades, the extent of this mode of signalling is only now becoming clear. The star among the more recently identified second messengers is c-di-GMP, but additional players such as c-di-AMP are catching up. The rather ubiquitous c-di-GMP is produced by diguanylate cyclases (DGCs) and degraded by specific phosphodiesterases (PDEs), with most bacterial species having multiples of these enzymes. c-di-GMP is seen as a life-style control signal molecule as, in general, it down-regulates motility and stimulates adhesion and biofilm formation. By acting through a variety of effectors that include several types of protein and riboswitch RNA, c-di-GMP can affect transcription, mRNA turnover and translation, proteolysis and the activities of enzymes, and large cellular structures such as the flagellum and exopolysaccharide secretion systems. This is also a reminder that

regulatory networks can only be understood if our knowledge of transcriptional control is integrated with insight into regulation at the RNA and protein levels.

On the basis of a study with a c-di-GMP-free mutant, in which all DGC genes were deleted, Urs Jenal (Biozentrum Basel, Switzerland) demonstrated that in *Caulobacter crescentus*, c-di-GMP is required for cell-cycle-associated periodic assembly of polar organelles, including the flagellum, pili and the stalk with the adhesive holdfast—in other words, essentially everything associated with asymmetrical division into a swarmer and a stalk cell. In addition, with the flagellar assembly factor TipF, Jenal presented a new example for the tight connection between c-di-GMP signalling and regulated proteolysis.

Regine Hengge (Freie U. Berlin, Germany) described a functional cascade of two c-di-GMP control modules driving transcription of the biofilm regulator CsgD. She highlighted the role of the PDE YciR as a ‘trigger enzyme’ that directly inhibits the DGC YdaM and the transcription factor MlrA. However, this inhibition is relieved when YciR degrades c-di-GMP accumulated in high enough quantities by another DGC. Moreover, this signalling cascade

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is associated with the formation of a two-layer architecture and complex morphology of colony biofilms, in which CsgD—and therefore biofilm matrix components such as amyloid curli fibres and cellulose—are produced by small and starving cells in the upper layer [1].

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Liz Sockett (U. Nottingham, UK) showed that in *Bdellovibrio bacteriovorus*, which invades and kills other Gram-negative bacteria within biofilms, switching between predatory and non-predatory life cycles is under c-di-GMP control [2]. Its four DGCs have specific functions during entry into, growth within and the final destruction of prey bacteria, and with 15 PilZ-like proteins, this formidable bacterium might well be the champion at using this type of c-di-GMP-binding effector protein. The potential of using *Bdellovibrio* as a ‘living antibiotic’ is being assessed.

Spatial organization

Networks seem to ‘go 3D’ at all levels of resolution. This trend reflects the increasing use of high-resolution fluorescence microscopy in molecular microbiology. Over the past years, this kind of imaging has revealed that bacterial cells have a high degree of spatial organization, with many network components being found at specific cellular locations. One of several beautiful examples was presented by Judy Armitage (U. Oxford, UK): *Rhodobacter sphaeroides* expresses two chemosensory pathways, one of which features transmembrane chemoreceptors that localize to membrane patches that can get trapped at the cell poles, whilst the other uses soluble chemoreceptors that localize in the cytoplasm to midcell. By using fluorescent imaging, Armitage’s study followed the ‘choreography’ of these chemosensory systems through the cell cycle, in particular the ‘piggy-backing’ of the soluble clusters on the segregating chromosomes.

In general, we observe a fascination with complex shapes and patterns. At the molecular level, this was illustrated by Martin Loose (Harvard Medical School,

USA), who presented dynamic patterns of the self-organizing cell division protein FtsZ. Cell shape has become an intriguing topic for investigation, with the role of serine/threonine kinases in the control of *Streptococcus* cell wall synthesis demonstrating the complexity of prokaryotic morphogenesis control machineries (Christophe Grangeasse, U. Lyon, France).

Finally, the spatial structure of entire cellular communities—that is, biofilm microanatomy and differentiation into distinct physiological strata and zones—is becoming a hot topic that will contribute to understanding of how complex morphological patterns form at the macroscopic level, such as the elaborate wrinkles and rings observed with bacterial macrocolonies on agar plates [1,3]. Thus, Jörgen Johansson (Umeå U., Sweden) showed the light and dark-induced opaque and translucent ring formation of *Listeria monocytogenes* growing on agar plates. This behaviour is controlled by a LOV-domain (light, oxygen or voltage) blue light receptor that signals into the σ^B -controlled general stress response network, which produces thicker cell walls, matrix components staining with the amyloid dye Congo red and higher stress resistance.

In conclusion, it seems that after many decades of reductive molecular biology, the analysis of complex morphology is back in microbiology, with the former now providing the genetic basis for the latter.

Communication and cooperation

It has become clear that most bacteria in nature do not exist as individual cells, but rather in microbial communities. These communities can be composed of one or multiple species, with examples including surface-attached biofilms or metabolically interdependent species that share a common environmental niche—for example, the human intestine. The development and dynamics of these communities depends on self-generated gradients of nutrients, oxygen, and waste or toxic products, as well as on a sophisticated intra- and inter-species signal exchange. Sine Lo Svenningsen (U. Copenhagen, Denmark) showed that *Escherichia coli* cells lower their susceptibility to infection with at least two bacteriophages, lambda and chi, in response to quorum sensing signals produced by other bacteria. They do so by reducing the amount of phage receptor proteins displayed on the cell surface [4].

The composition of species and evolution within communities is another emerging topic. Kevin Foster (U. Oxford, UK) demonstrated that clonal patches emerge in diverse bacterial communities and that this can promote cooperation among cells of a single genotype, whereas between different strains and species, the evolution of competitive phenotypes is more important: “microbes are nice to clonemates but not much else” [5].

Bacterial systems biology

Another trend observable at BacNet13 was that the quantitative analysis and mathematical modelling of regulatory networks is often no longer separate from the molecular analysis of a system. Rather, these aspects are tackled together during projects, and become integral parts of presentations. Illustrating this tendency was a talk given by James Locke (U. Cambridge, UK), who reported the quantitative analysis of the general stress response in *Bacillus subtilis* using time-lapse microscopy of fluorescent reporter strains. Locke showed that this response occurs in frequency-modulated stochastic or single deterministic pulses, depending on the nature of the input signal [6]. Several other presentations illustrated how quantitative approaches can shed new light on even apparently well-studied systems. Terence Hwa (UCSD, USA) demonstrated that quantitative analysis of the growth rate and *lac* gene expression in *E. coli* reveals an unknown function and mechanism for catabolite repression. Victor Sourjik (U. Heidelberg, Germany) analysed the thermal robustness of the *E. coli* chemotaxis pathway, showing how effects of temperature on individual pathway reactions become compensated to yield a temperature-invariant chemotactic behaviour [7], and emphasizing the overall importance of robustness in the evolutionary design of bacterial networks.

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Interestingly, a consensus seems to have evolved that the collection of large-scale -omics data alone—until recently popularized as ‘non-hypothesis-driven research’—will not lead ‘automatically’ to progress in

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our understanding of the complex regulatory networks that underlie all microbial life functions. After all, it is a long way from data to knowledge and insight. Rather, making progress on this way clearly requires creative hypothesis formation on the basis of the treasure trove of data with which -omics approaches provide us. A fine illustration of this point was presented by Christina Pesavento (EMBL, Heidelberg, Germany). She presented work that used a high-throughput chemical genetics screen [8] to shed light on signal sensing and transduction through the Rcs phosphorelay system, which adjusts cell division in response to cell shape perturbations that can be elicited by different stimuli. Another application of large-scale -omics data on protein and metabolic networks is a retailoring of metabolic pathways in synthetic biology, as demonstrated by Sven Panke (ETH Zürich, Switzerland). Isolating interesting pathways by triggered proteolysis of undesired enzymes allowed Panke to convert cell extracts of appropriately engineered strains into miniature *in vitro* synthetic factories.

The 'Science & Society' session

In addition to the strictly scientific sessions, BacNet13 also provided a new highlight; a 'Science & Society' session designed to reflect the increasing impact of modern bioscience on our society and vice versa. This year, the focus was on 'Creative experiments

at the interface of science and the arts'. A main topic was the generation, use and impact of images both in historic and contemporary science, as seen by the art historian Horst Bredekamp (Humboldt-U., Berlin, Germany). He pointed out the unresolvable conflict between complex images of scientific objects and the epistemological ideal of objectivity: images not only illustrate, they also shape the object they show, as well as our ideas and concepts. Bredekamp also used BacNet13 as an opportunity for 'field research'. His talk resulted in a lively discussion about the unspoken rules—if any—of visualization of data, models and concepts used by the scientists of the BacNet community. Ursula Damm (Bauhaus U., Weimar, Germany) presented her 'artistic research', which aims at generating knowledge by subjective interventions with nature and reveals striking parallels to the convergence of engineering and nature, as is common in synthetic biology. Modern biology as a source for new artistic tools and topics is actually an important trend in contemporary art. Thus, the social impact of contemporary genetics, with its deterministic view focused on DNA as a metaphor for the 'code of life', was addressed by Paul Vanouse (U. Buffalo, USA), who presented examples of his artistic work that uses DNA—literally in electrophoresis devices—as a material and medium. This session turned out to be extremely thought-provoking and generated intense and lasting discussions.

Future perspectives of BacNet

The participants agreed that BacNet13 presented the best research at the forefront of developments in bacterial networks, as outlined in this report. BacNet15, which will probably take place in May 2015, will

further elaborate the state of research along these lines, with an increasing emphasis also on environmental-sensing mechanisms and the design of synthetic intracellular and intercellular networks. With the success of the 'Science & Society' session experiment at BacNet13, a 'Science & Society' session will also be part of future BacNet conferences.

ACKNOWLEDGEMENTS

We thank the mentioned participants for scientific feedback. We apologize to all other participants whose contributions could not be included due to restrictions of scope and space. We also thank the Ernst Schering Foundation and the Society for General Microbiology for supporting BacNet13.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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EMBO reports (2013) **14**, 667–669; published online 12 July 2013; doi:10.1038/embo.2013.93