

Quantitative analysis of microbial networks

Introduction

We are interested in quantitative understanding of the design and functioning of cellular networks. Although specific molecular mechanisms differ, any such system must be able to extract signals from a noisy environment, adapt to large changes in signal and background strengths, and integrate multiple cues. It can thus be expected that biological networks rely on a small number of common evolutionary design features. Elucidating these features requires a combination of quantitative analysis of the network *in vivo* with detailed computer modelling. Ultimately, we want to be able to explain why the observed network designs were evolutionary selected out of a large number of possibilities.

As models, we use several relatively simple and well-studied networks in *Escherichia coli*. Most of our current work is done on the chemotaxis signal transduction pathway, although we recently started investigations of other networks in *E. coli*. We use fluorescence microscopy techniques to analyze network properties *in vivo*, including intracellular protein distributions, protein interactions, and spatio-temporal dynamics of the intracellular signal processing. These data are then combined with the available biochemical information to develop predictive computer models of the networks. We expect the evolutionary design principles that can be derived from such analyses to be general and equally apply them to more complex networks in eukaryotes.

Signal processing in *E. coli* chemotaxis

V. Aliaksiuk, M. Emmerich, D. Kentner, S. Neumann

1. FRET mapping of protein interaction dynamics

In *E. coli* and other bacteria, chemotactic stimuli are detected and processed by sensory complexes that are formed by membrane receptors of five different types, a histidine kinase CheA and an adaptor protein CheW. Output signalling involves stimulus-dependent autophosphorylation of CheA and subsequent phosphorylation of the response regulator CheY. The pathway also includes CheZ, a phosphatase of CheY-P, and an adaptation system that consists of the methyltransferase, CheR, and the methylesterase, CheB. To map protein interactions in the chemotaxis pathway and their stimulation-dependence, we used a customized assay based on fluorescence resonance energy transfer (FRET; Sourjik, Vaknin et al. 2007). Proteins of interest are expressed as fusions to cyan and yellow fluorescent proteins, CFP and YFP, respectively, and their interactions are detected by a distance-dependent quenching of the CFP fluorescence. This approach revealed a complex network of interactions, many of which are dependent on chemotactic stimulation. We also detected a number of interactions between chemotaxis proteins and components of the phosphotransferase system (PTS), which is known to be involved in both glucose uptake and taxis.



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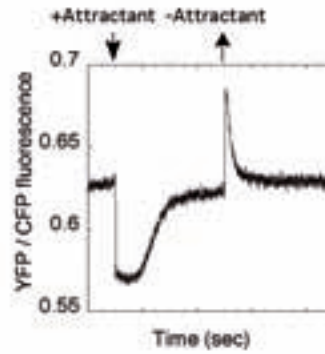
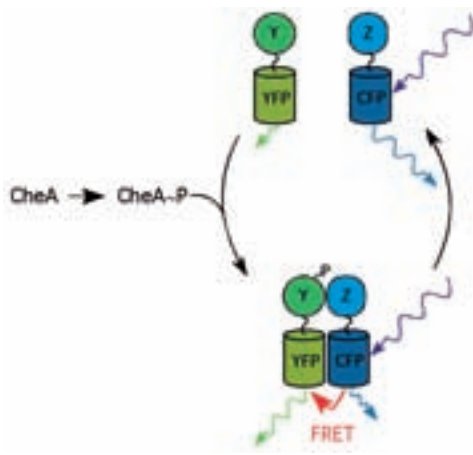


Fig. 1: FRET as a reporter of pathway activity. Stimulation-induced changes in FRET between CheY-YFP and CheZ-CFP, revealed as changes in the ratio between YFP and CFP emission, are shown on the right.

2. Signal processing by sensory complexes

In a second FRET-based project, we use the phosphorylation-dependent interaction between response regulator CheY and its phosphatase CheZ as a pathway activity reporter (Sourjik, Vaknin et al. 2007) to follow changes in kinase activity upon stimulation (Fig. 1) and thereby quantitatively analyze three central aspects of signal processing by the chemosensory complexes *in vivo*: signal amplification, signal integration, and precision of adaptation. Our ultimate goal is to achieve a precise mathematical description of these pathway properties. We showed that amplification and integration of chemotactic signals are mediated by cooperative interactions between receptors in the allosteric oligomers formed by sensory complexes. To derive quantitative parameters that characterize these interactions, we currently measure equilibrium kinase activity and dose-response curves in strains that are engineered to have different compositions of the receptor cluster (Michalodimitrakis, Sourjik et al. 2005). Our ongoing analyses revealed that the size of the complexes dynamically adapts to the background stimulation to allow optimal sensitivity. Additionally, we study chemoeffector spectrum of chemotaxis in *E. coli* and investigate integration of different tactic stimuli, including temperature (Sourjik and Wingreen 2007).

Assembly, dynamics and positioning of chemoreceptor clusters and flagellar motors

D. Kentner, M. Kumar, H. Li, S. Schulmeister, S. Thiem

1. Assembly and positioning of receptor clusters

Sensory complexes are organized in polar and lateral receptor clusters that are essential for signal processing and include thousands of proteins (Kentner and Sourjik 2006). Using receptor, CheW, and CheA fusions to YFP, we demonstrated that receptors possess an intrinsic ability to form larger complexes through the interactions between their cytoplasmic domains, and that CheW and CheA can bind to the clusters independently of each other and further enhance clustering (Kentner, Thiem et al. 2006). We further showed that new clusters in *E. coli* cells apparently self-assemble and then anchor to periodically distributed lateral positions that mark future division sites at every eighth of cell length (Thiem, Kentner et al. 2007). Such positioning enables efficient segregation of protein complexes during bacterial cell division, and ensures that the response time of chemotaxis signalling is not limited by diffusion of response regulator from clusters to the motors. Interestingly, the same structure seems to be responsible for the localization of a DNA replication machinery and chromosomal replication origins.

2. Dynamics of receptor clusters

Fluorescence recovery after photobleaching (FRAP) experiments for YFP fusions to all chemotaxis proteins and receptors revealed multiple levels of cluster stability, which correspond to the physiological functions of the respective proteins. Receptors, CheW, CheA, and CheZ form a core of the cluster that is stable on the time scales of excitation signalling and adaptation; CheR and CheB equilibrate on the time scale of adaptation; and CheY equilibrates on the excitation time scale (Schulmeister, Ruttorf et al. 2008). FRAP experiments also allowed estimation of the diffusion coefficients in *E. coli* cytoplasm and membrane, which is essential for spatially-resolved computer modelling.

3. Assembly and positioning of flagellar motors

Assembly of the extracellular portion of flagellar motors – which are homologous to the type III secretion system used by many pathogenic bacteria – is relatively well understood based on genetics and electron microscopy, but the initial intracellular stages of assembly could not be studied this way. We thus use FRET-based mapping of interactions between cytoplasmic motor components and localization studies of fusion proteins to reconstruct the order of motor assembly. We also investigate positioning of flagellar motors, to understand whether a mechanism of flagella distribution in dividing cells might rely on an attachment to some structure.

Robustness of the pathway

L. Løvdok, A. Müller, G. Schwarz

Chemotaxis pathway, like any biological network, has to produce a defined output under conditions of intra- and extracellular perturbations (Kollmann and Sourjik 2007). We studied robustness of the pathway against stochastic variations in protein levels, or gene expression noise. We quantified gene expression noise and, in collaboration with Markus Kollmann (now at the Humboldt University of Berlin), showed theoretically that the pathway has a simplest possible design that is sufficient to provide robustness against observed level and type of noise (Kollmann, Lovdok et al. 2005). One major robustness determinant is presence of opposing enzymatic activities, which balance each other upon concerted gene overexpression (Løvdok et al., 2007); another determinant is the negative feedback from the kinase activity to the adaptation system (Fig. 2). Similar features are found in many other signalling networks, suggesting that the elucidated strategy to achieve robust output is not restricted to bacterial chemotaxis.

Collaborations

We acknowledge our collaborators H. Berg (Harvard Univ.), B. Bukau (ZMBH), R. Endres (Imperial Coll.), A. Groisman (UCSD), M. Kollmann (Humboldt Univ. Berlin), D. Leibiedz (Univ. Freiburg), M. Mayer (ZMBH), Y. Meir (Ben-Gurion Univ.), W. Ryu (Princeton Univ.), J. Timmer (Univ. Freiburg), A. Vaknin (Hebrew Univ.), N. Wingreen (Princeton Univ.).

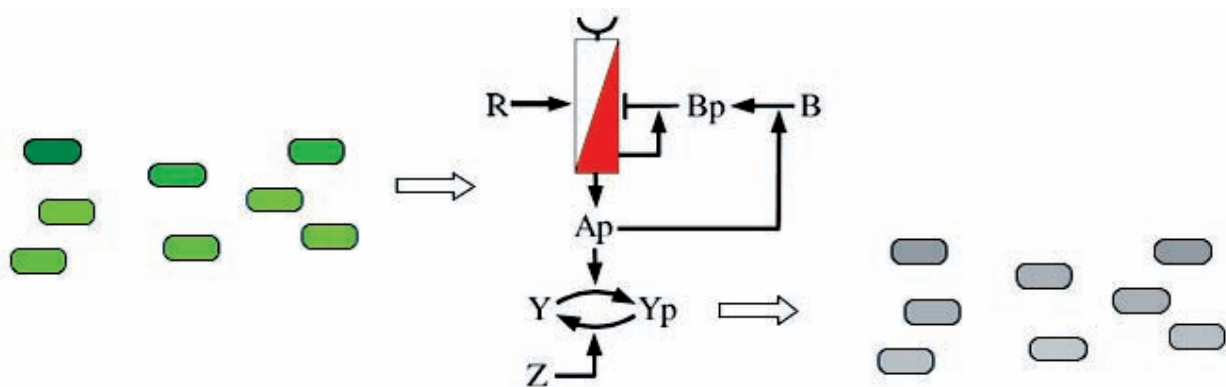


Fig. 2: Pathway robustness to variation in protein levels. Robust design of the chemotaxis pathway, with opposing enzymatic activities and a negative feedback from CheA to CheB, ensures that even large intercellular variations in protein levels (green) result in little variation in the signalling output, the level of phosphorylated CheY-P (gray; adopted from Kollmann, Lovdok et al. 2005).

External funding

Our research was supported by grants and fellowships from the Deutsche Forschungsgemeinschaft (SO 421/3-1, SO 421/3-2, SO 421/6-1), the State of Baden-Wuerttemberg (Promotionskolleg "Molecular machines: mechanisms and functional interconnections"), Human Frontier Science Program RGP0066/2005, EMBO Young Investigator Program.

PUBLICATIONS 2005 - 2008

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THESES

Dissertations

Sebastian Thiem (2008): Chemoreceptor cluster positioning and dynamics in *Escherichia coli*

Masters and Diploma Theses

Silke Neumann (2006): Protein interactions in glucose taxis pathways of *Escherichia coli*

Mohit Kumar (2007): Protein diffusion in the bacterial cytoplasm

Erik Sommer (2007): Effects of protein levels and temperature on the Min-system in *Escherichia coli*

Maren Emmerich (2008): Analysis and modification of specificity of the chemotaxis pathway in *Escherichia coli*

AWARDS

EMBO Young Investigator Award (2006)

Chica and Heinz Schaller Research Award (2007)

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