Design and implementation of a bacterial signaling circuit

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2013

document version
Publisher's PDF, also known as Version of record

Summary

The chemotaxis signaling circuit that enables enteric bacteria to sense and respond to chemicals and physical changes in their environment is one of the best characterized signaling networks in biology. The detailed molecular knowledge about this system has enabled quantitative experimental and theoretical studies that have explained the characteristics of chemotaxis response, such as precise adaptation and high signal amplification. Many of the properties of the chemotactic signaling in bacteria are also observed in the more complex sensory systems of multicellular organisms. Thus, insights from studies of the bacterial chemotaxis system could facilitate the understanding of fundamental properties of sensory systems in biology.

In this thesis, the chemotactic signaling of enteric bacteria was investigated from a functional point of view. Quantitative experiments measuring the chemotactic signaling response of *Escherichia coli* and *Salmonella typhimurium* were used to characterize the properties of the receptor response and adaptation at the systems level. Behavioral strategies were predicted from the transfer functions of the signaling response and confirmed experimentally. The roles of previously uncharacterized chemotaxis proteins were explored using physiological measurements and quantitative imaging.

Physiological studies of sensory responses have highlighted that the threshold of response to a stimulus is proportional to the magnitude of the original stimulus: a property, known as Weber's law. However, Weber's law addresses only the instantaneous response to small step stimuli. In Chapter 2, we provide the first demonstration that an adaptive sensory system rescales the entire dynamics of its response to time-varying inputs. We show for the chemotaxis response of *E. coli* to α-methylaspartate (MeAsp) that the entire shape of the output depends only on the fold changes in the input and not on its absolute levels, a property recently described as fold-change detection (FCD). We used fluorescence resonance energy transfer (FRET) to probe this rescaling of the signaling response in *E. coli*, and we found two ranges of background concentrations, in which

FCD holds, i.e. "FCD regimes". The amplitude of the response differed between the two regimes; however, the adaptation timescale was invariant. We identified three sufficient conditions for FCD in bacterial chemotaxis. FCD was also observed for the distributions of *E. coli* swimming in spatial gradients of MeAsp created in microfluidic platforms.

Input-output relationships of the chemotaxis response have been thoroughly characterized in the model strain *E. coli* K12. FRET-based studies showed high receptor sensitivity and cooperativity and explored the properties of the adaptation system, using time-varying stimuli. However, even within the species *E. coli*, there is a great variety in the chemotactic performance between different strains, as we showed in Appendix B. In Chapter 3 we performed a detailed comparison of the physiological response of *E. coli* K12 and the closely related species *S. typhimurium* LT2 that share homologous chemotactic networks. We revealed that adaptation to MeAsp in *S. typhimurium* is three-fold faster and the apparent cooperativity of the receptor response is three-fold lower than that of *E. coli*. Moreover, the response-rescaling properties differed between the two species: in contrast to *E. coli*, *S. typhimurium* showed a single FCD regime. Using the obtained parameters for the chemotactic signaling transfer functions of both species, we explained the differences in the sensitivity modulation of the response.

In Chapter 4 we study two chemoreceptors of *S. typhimurium*, McpB and McpC, with hitherto unknown functions. The radial migration in soft-agar plates suggested that these receptors sense the amino acid cysteine and its oxidized dimeric form cystine as chemoattractants. However, our FRET measurements of the chemotactic kinase response showed that cells expressing only McpB / C chemoreceptors respond only to the oxidized form, and the response was unexpectedly in the repellent direction. Furthermore, we showed that the reduced form, cysteine, is an attractant sensed by Tsr and Tar. We showed that the adaptation to both cystine and cysteine is methylation dependent, and the adaptation to cystine is incomplete, *i.e.* imperfect adaptation. We discuss that cystine-cysteine interconversion and imperfect adaptation to cystine could explain the attractant-like responses to both components in the soft-agar assays. We explore the dose-response dependence of the opposite responses to

cystine / cysteine redox pair in Appendix A. We observed linear scaling of the magnitude of the response to cystine with the logarithm of cystine concentration. Unexpectedly, we detected McpB / C independent responses to cystine in *S. typhimurium* LT2, which might represent a redox response. In Chapter 6, we present our preliminary results on testing the responses of *S. typhimurium* to redox gradients.

Another chemotaxis protein with previously uncharacterized function in *S. typhimurium* is CheV: a hybrid protein consisting of a scaffolding domain and a phosphorylatable receiver domain. CheV plays a role in receptor-kinase scaffolding and adaptation to chemoeffectors in some bacterial species. The distinctive phenotype of knocking out *cheV* in *S. typhimurium* strains incapable of methylation-dependent adaptation suggested that CheV has a function in *S. typhimurium*'s chemotactic response. Our FRET measurements presented in Chapter 5 revealed methylation-independent partial adaptation to MeAsp, which is CheV-dependent. To understand the mechanistic origin of this partial adaptation, we performed quantitative image analysis of receptor clusters and showed that the number of detectable clusters decreases in *cheV* knockout cells. In particular there are less clusters that are not localized at the poles, *i.e.* lateral clusters, in the *cheV* knockout cells. We speculate that a phosphorylation-dependent feedback on the receptor cluster stability might explain the role of CheV in *S. typhimurium.*

We explored another phosphorylation-dependent feedback mechanism: the negative feedback loop introduced by phosphorylation of the methylesterase CheB in *E. coli* chemotaxis in Appendix C. Using FRET, we characterized the adaptation kinetics of genetically modified cells with disrupted CheB phosphorylation site. We showed that the strong nonlinearity in the transfer function characterizing the rate of change of methylation as a function of the kinase activity could be a consequence of the phosphorylation feedback on CheB activity.

In summary, we have studied the input-output relationships of the chemotaxis response in *E. coli* and *S. typhimurium* using *in vivo* experiments. We have demonstrated the fold-change detection strategy in both species at both the signaling and behavioral levels. We have shown differences in the chemotactic performance of bacteria with homologous chemotaxis

networks, and explained the observed differences in terms of the underlying control physiology. We have used the existing experimental tools to probe the functions of uncharacterized chemotaxis components. Future functional studies of signaling responses could further advance our understanding how biological systems are designed.