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Guidance mechanisms in bacteria and sperm

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Signal transduction in bacterial chemotaxis

We explore signal transduction strategies using chemotaxis of the bacteria *Escherichia coli* and *Salmonella typhimurium* as a model. Bacterial chemotaxis is a sophisticated system that integrates many different signals into a common output — a change in the direction of flagellar rotation. The signal transduction in *E. coli* chemotaxis is between two supramolecular complexes: the receptor supramolecular complex at the poles, and the flagellar-motor supramolecular complex around the cell. The receptor supramolecular complex includes the receptors and the enzymes that modulate the receptor activities as well as the enzymes that are modulated by the receptors. The flagellar-motor supramolecular complex includes the motor and its gearbox, termed a switch. A small protein, the excitatory response regulator CheY, shuttles back and forth between the two supramolecular complexes and transduces sensory information between them (Figure 1). Our research is focused on CheY and the switch.

Bacteria sense stimuli over a wide concentration range and, in spite of the wide range, do so with very high sensitivity, suggesting high amplification of the chemotactic signal. One of the amplification steps probably occurs at the flagellar switch. We took two approaches to determine whether the amplification results from cooperativity of CheY binding to the switch or from a post-binding step within the switch. In one approach, we purified the intact switch complex in quantities sufficient for biochemical work and, using double-labeling centrifugation assays for binding, we found that CheY binds to the isolated, intact switch complex in a phosphorylation-dependent manner, but the binding was not cooperative (Hill coefficient ≈ 1). The other approach, carried out *in vivo*, demonstrated that the dependence of the rotational state of the motor

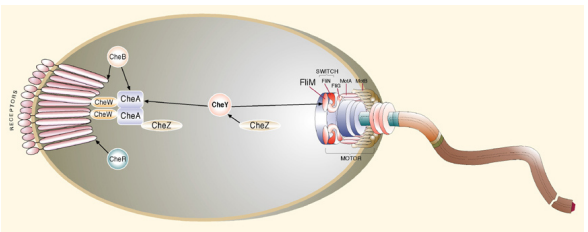

Fig. 1 *Simplified scheme of signal transduction in bacterial chemotaxis of E. coli and S. typhimurium. Black arrows stand for regulated protein-protein interactions. CheY is a response regulator, CheA is a histidine kinase, and CheZ is a phosphatase. The scheme is not drawn to scale.*

on the fraction of occupied sites at the switch is sigmoidal and very steep. These results indicated that the chemotactic signal is amplified within the switch, subsequent to the CheY~P binding.

We found that CheY is activated by both phosphorylation and acetylation. The acetylation occurs at up to 6 lysine residues, all located at the surface of CheY that binds to the switch. While we demonstrated that phosphorylation of CheY increases its affinity for the switch, the role of acetylation is obscure. Furthermore, it is not at all clear why two covalent modifications are needed for regulating the activity and what advantage this dual covalent modification provides over regulation by phosphorylation only. These questions are in the focus of our research. As a first step, we studied the effect that each modification exerts on the other. The phosphodonors of CheY each strongly inhibited both the autoacetylation of the acetylating enzyme, acetyl-CoA synthetase (Acs), and the acetylation of CheY. The enzyme that enhances CheY dephosphorylation had the opposite effect: enhanced Acs autoacetylation and CheY acetylation. Reciprocally, the presence of Acs elevated the phosphorylation levels of CheY, and acetate repressed this stimulation. These

observations suggested that CheY phosphorylation and acetylation are linked and co-regulated. Our studies suggest that CheY acetylation may function at three levels: activation of CheY to modulate the direction of flagellar rotation, linking chemotaxis to the metabolic state of the cell, and serving as a tuning mechanism that compensates for cell-to-cell variations in the concentrations of the proteins that phosphorylate and dephosphorylate CheY.

Sperm guidance in mammals

In recent years it became apparent, mainly due to the work of our group, that sperm guidance to the egg is a crucial step in mammalian reproduction. This guidance is carried out by chemotaxis and, at least in some mammals (humans included), by thermotaxis. Thus, we demonstrated, in contrast to the prevailing dogma, the occurrence of human sperm chemotaxis to follicular fluid; we provided evidence for an extremely strong linkage between the occurrence of sperm chemotaxis to follicular factors secreted from an egg and the successful fertilization of this egg; we showed that only capacitated spermatozoa (spermatozoa in a particular state of readiness for fertilizing the egg) are chemotactically responsive; we found, again in contrast to the dogma, that the capacitated state is transient (1–4 h life span in humans) rather than continuous and static, as had been believed; we demonstrated lack of mammalian species specificity with respect to chemotaxis; and we revealed the occurrence of sperm thermotaxis in mammals and suggested that while this process is a long-range mechanism, which guides capacitated spermatozoa from the cooler sperm storage site in the Fallopian tube to the warmer fertilization site, sperm chemotaxis is a short-range mechanism, which guides spermatozoa through the layers of the egg-surrounding cells into the egg (Figure 2). In spite of this knowledge about the physiology of sperm guidance, close to nothing is known about the molecular mechanism of mammalian sperm chemotaxis and thermotaxis as well as about the way, by which capacitated spermatozoa change their direction of swimming in a chemoattractant gradient. All these questions are currently under intensive investigation in our lab.

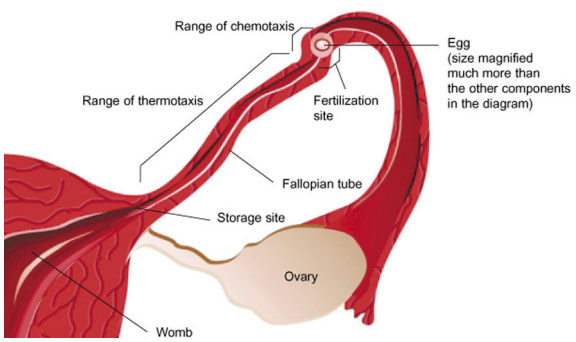

Fig. 2 *A scheme of the female genital tract demonstrating the location of sperm thermotaxis and chemotaxis.*

Selected Publications

Bahat, A., Tur-Kaspa, I., Gakamsky, A., Giojalas, L.C., Breitbart, H. and Eisenbach, M. (2003) Thermotaxis of mammalian sperm cells: A potential navigation mechanism in the female genital tract. *Nature Med*., 9, 149-150.

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