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 complexes 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.

It is well established that the activity of CheY is regulated by its phosphorylation, the level of phosphorylation being determined by the histidine kinase, CheA, and the phosphatase, CheZ. We found that CheY can also be activated by acetylation. CheY acetylation can either be carried out by autocatalysis, with AcCoA serving as an acetyl donor, or by the enzyme acetyl-CoA synthetase (Acs), with acetate as the acetyl donor. In both cases the acetylation sites are six lysine residues (Lys91, 92, 109, 119, 122 and 126), all located on the surface that binds to CheA, CheZ and FliM. Mutational interference with either of these processes results in defective chemotaxis. We even succeeded to detect CheY acetylation *in vivo*, finding that, on average, every molecule of CheY is acetylated by autoacetylation. Our current studies are aimed at identifying the function of CheY acetylation in chemotaxis.

One of the major questions in bacterial chemotaxis is how the switch of the bacterial flagellar motor functions. It is composed of multiple copies of three proteins: FliM, FliG and FliN. Although some information on the structure of the switch proteins is available, there is hardly any information about how the switch functions. Our earlier studies identified fumarate as a switching factor, i.e., a molecule that enables the switch to shift from its default state, counterclockwise, to the clockwise state. We used this finding as an end of a thread to investigate the switch function. Although our studies demonstrated that fumarate affects the switch, we could not detect its binding to the switch complex or to individually isolated switch proteins (and to CheY, too). We, found, however, that the membrane protein fumarate reductase (FRD), known to convert succinate to fumarate and vice versa, mediates fumarate to the switch. We established that FRD forms a 1:1 complex with the switch-protein FliG, and that this interaction is required both for flagellar assembly and switching the direction of flagellar rotation. We further showed that fumarate affects the direction of flagellar rotation via FRD. This unexpected finding of functional interaction between an energy conversion protein and the switch opens major intriguing questions, currently addressed by us. In addition, using sophisticated single-cell imaging, we study the behavior of the switch as a function of the intracellular level of CheY. We also investigate the switching mechanism by quantitatively studying events occurring at the switch, employing bioluminescence resonance energy transfer.

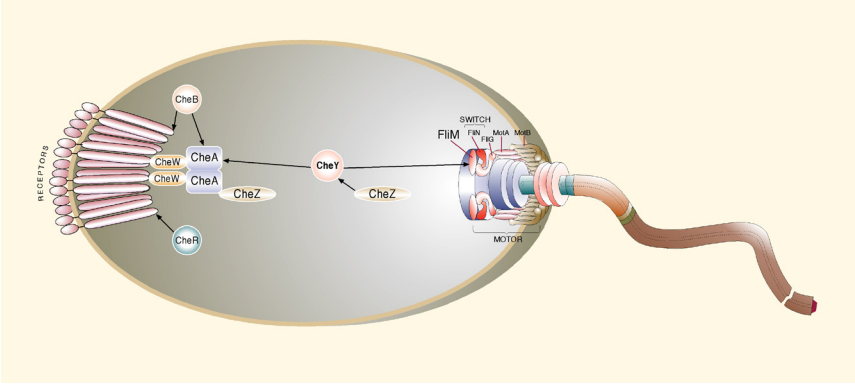

Fig. 1 *Simplified scheme of signal transduction in bacterial chemotaxis of E. coli and S. typhimurium. Black arrows stand for regulated protein-protein interactions. CheY is a response regulator, CheA is a histidine kinase, and CheZ is a phosphatase. The scheme is not drawn to scale.*

Sperm guidance in mammals

Contrary to a prevalent belief, there appears to be no competition in the mammalian female genital tract between large numbers of sperm cells racing towards the egg. Instead, small numbers of the ejaculated sperm cells enter the Fallopian tube and these few must be guided in order to make the remaining long, obstructed way to the egg. We revealed two active guidance (taxis) mechanisms: chemotaxis and thermotaxis. Both mechanisms are restricted to capacitated sperm cells, namely to cells that reached a maturation stage at which they can penetrate the egg and fertilize it. Recently we found that both the egg and its surrounding cumulus cells secrete sperm chemoattractants, and determined that progesterone is the

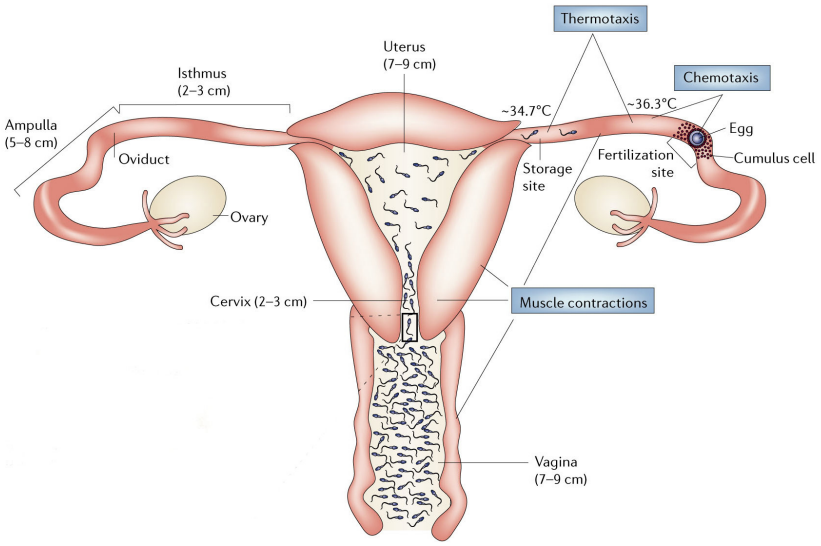

Fig. 2 *A scheme of the female genital tract demonstrating the location of sperm thermotaxis and chemotaxis.*

only chemoattractant secreted from the cumulus cells. In addition we found that a temperature difference is established at ovulation in the female's oviduct as a consequence of a temperature drop at its lower part (isthmus). Our pharmacological studies indicate that the IP_3R Ca^{2+} channel, located on internal Ca^{2+} stores, operates in human sperm thermotaxis and that the response is mediated by PLC and requires intracellular Ca^{2+}. We hypothesize that, *in vivo*, thermotaxis is a long-range mechanism, guiding sperm cells in the Fallopian tube towards the fertilization site, and chemotaxis is a short-range mechanism that is mainly functional at close proximity to the egg (Figure 2). Our current efforts are focused on identification of the chemoattractant secreted by the egg and on revealing the behavioral and molecular mechanisms of mammalian sperm chemotaxis and thermotaxis.

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