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Positioning of bacterial chemoreceptors

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For optimum growth, bacteria must adapt to their environment, and one way that many species do this is by moving towards favourable conditions. To do so requires mechanisms to both physically drive movement and provide directionality to this movement. The pathways that control this directionality comprise chemoreceptors, which, along with an adaptor protein (CheW) and kinase (CheA), form large hexagonal arrays. These arrays can be formed around transmembrane receptors, resulting in arrays embedded in the inner membrane, or they can comprise soluble receptors, forming arrays in the cytoplasm. Across bacterial species, chemoreceptor arrays (both transmembrane and soluble) are localised to a variety of positions within the cell; some species with multiple arrays demonstrate this variety within individual cells. In many cases, the positioning pattern of the arrays is linked to the need for segregation of arrays between daughter cells on division, ensuring the production of chemotactically competent progeny. Multiple mechanisms have evolved to drive this segregation, including stochastic self-assembly, cellular landmarks, and the utilisation of ParA homologues. The variety of mechanisms highlights the importance of chemotaxis to motile species.

Bacterial motility and taxis

Bacteria are subject to large variations in environmental conditions and, to ensure optimum growth across changing environments, they must be able to respond and adapt to these changes. Such responses include altering patterns of gene expression and cellular behaviour. Many bacteria respond to unfavourable conditions by directed movement in a more favourable direction, a process known as taxis. The importance of motility is demonstrated in both the prevalence of motile species and the variety of approaches that bacteria have evolved to achieve movement [1]. These include various strategies for both moving across solid surfaces and for swimming through liquid media. *Flavobacterium johnsoniae* glides rapidly over surfaces, mediated by surface attachment and flow of proteins in the outer membrane causing translocation of the cell body [2]. *Myxococcus xanthus* uses two mechanisms for surface motility, distinct from each other and that of *F. johnsoniae*. S-motility depends on the extension, surface attachment, and retraction of type IV pili [3]. A-motility involves focal adhesion complexes attaching to the surface and moving the cell body forward [4]. *Pseudomonas aeruginosa* has different modes of motility for surface and aqueous movement. Surface movement is mediated by type IV pili [5], while movement through aqueous environments is mediated by rotation of flagella, which are semi-rigid helical filaments projecting from the cell [6]. While there are other mechanisms for swimming through aqueous media, the use of flagella is the most prevalent [1].

Movement is, in general, only useful if there is a mechanism for controlling that movement towards an improving environment. Swimming bacteria are constantly buffeted by their environment and to be able to swim in a positive direction, they need to sense and respond to the environment every second or so. Most bacteria are too small to sense spatial gradients and, therefore, use temporal sensing, comparing current conditions with those a few seconds ago. To allow a comparison, they also need a type of memory in the form of adaptation to current conditions.

The best-studied chemosensory pathway is that of *Escherichia coli* (Figure 1). *Escherichia coli* is propelled through liquid by a bundle of four to six flagella, each powered by a transmembrane motor rotating in a counter-clockwise direction. To reorientate the cell body, one or more of the flagellar motors switch the direction of rotation, causing the flagellar bundle to break apart and the cell to tumble and, thus, stop forward movement [7]. The random tumbling means that, when all the flagella are again rotating counter-clockwise, reforming the bundle, the cell swims off in a new, but random, direction [8]. Modulation of the frequency of these tumbling events in response to changing conditions generates chemotaxis. The switching of flagellar rotation from counter-clockwise to clockwise is controlled by a modified two-component pathway comprising transmembrane receptors (methyl-accepting chemotaxis proteins; MCPs), a histidine protein kinase (CheA), an adaptor protein (CheW), a response regulator (CheY), and two adaptation proteins, CheB and CheR. The MCPs are dimers that form trimers of dimers, which, along with CheW and CheA, pack hexagonally to form large arrays of thousands of proteins [9,10] (Figure 2). The hexagonal arrays of chemoreceptors are formed by the interactions of the CheW and CheA proteins across

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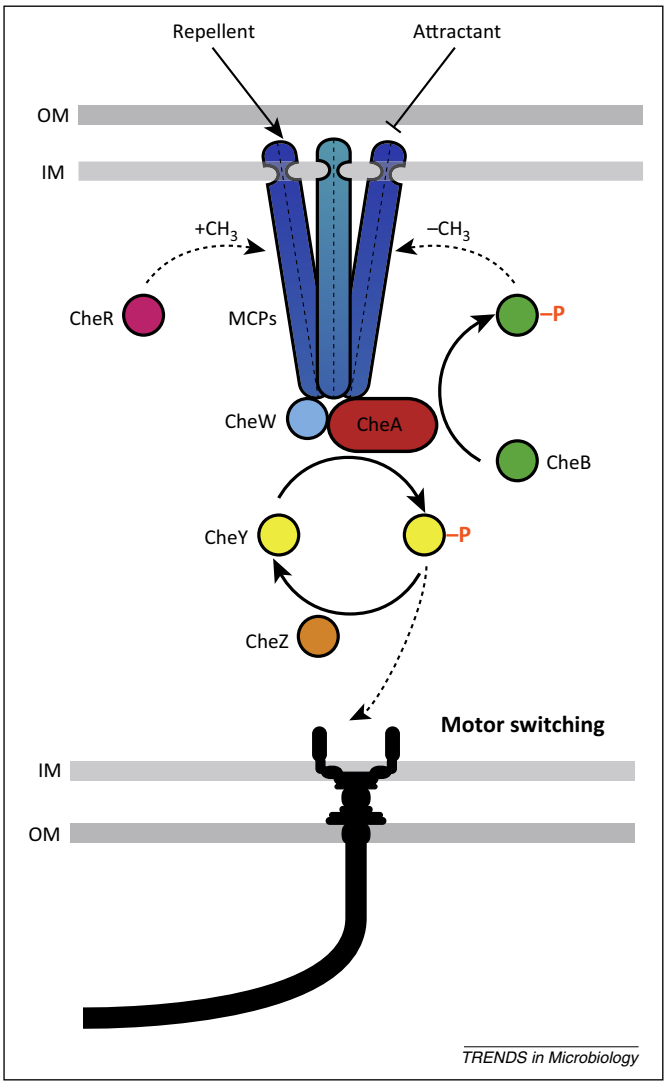

Figure 1. *Escherichia coli* chemotaxis pathway. Transmembrane methyl-accepting chemotaxis proteins (MCPs) activate the kinase CheA in response to repellents or in the absence of attractants. CheA transfers phosphoryl groups to the response regulators CheY and CheB. When phosophorylated, CheY diffuses to the flagellar motor and causes switching of rotational direction. Phosphorylated CheB, along with CheR, changes the methylation state of the MCPs. The demethylation of MCPs in response to CheA activity reduces MCP sensitivity and returns the system to the pre-stimulus level. Abbreviations: IM, inner membrane; OM, outer membrane.

the cytoplasmic tips of the receptor trimers of dimers (Figure 2B). The presence of repellents or absence of attractants result in increased CheA activity and, thus, increased levels of phosphorylated CheY (CheY-P). CheY-P diffuses to, and interacts with, the flagellar motor proteins FliM and FliN, and causes motor switching [11,12]. To stop tumbling, the signal must be terminated. In *E. coli*, this is achieved through the action of CheZ, a CheY-P-specific phosphatase.

CheA also phosphorylates the methylesterase CheB, which, once phosphorylated, removes methyl groups from specific glutamates on the MCPs. The action of CheB is countered by the methyltransferase CheR, which is constitutively active. MCPs with higher methylation have a greater ability to activate CheA, which in turn increases the activity of CheB and reduces the methylation state of the MCPs [13]. Thus, CheB and CheR work in concert to generate adaptation, allowing cells to respond to relative changes in chemical concentrations across a range of background concentrations [14]. In *E. coli*, there are four structurally related MCPs forming mixed trimers of dimers. CheR is anchored by a long tether to the terminal domain of the two high-abundance receptor types (Tsr and Tar), allowing methylation and, thus, adaptation of other MCPs (e.g., Trg) in proximity.

The elegant simplicity of the *E. coli* pathway makes it an attractive paradigm for chemotaxis. However, analysis of the genomes of motile bacterial species has revealed that over 50% contain multiple copies of each of the core chemotaxis genes (*cheA*, *cheB*, *cheR*, *cheW*, and *cheY*) [15]. The diversity of chemotaxis systems is also demonstrated by the proteins themselves; many species contain chemotaxis proteins that are not homologues of any of the *E. coli* proteins, such as the scaffold protein CheV, the deamidase CheD, and phosphatases CheC and CheX [16]. Many species also contain multiple homologues of MCPs [17], often including both transmembrane and cytoplasmic receptors [16]. Recently, it was shown that cytoplasmic receptors from *Vibrio cholerae* and *Rhodobacter sphaeroides* formed trimers of dimers packed hexagonally into large cytoplasmic arrays with the same spacing as transmembrane clusters [18]. This suggests that the packing of the chemoreceptors and the signalling proteins into large arrays is critical for correct signal transduction. Indeed, packing of the MCPs into these large arrays been shown to be important in creating gain in signal transduction through cooperative interactions between MCPs [19]. The methylation state of MCPs has been shown to alter their packing within the array, suggesting that adaptation alters the activity of CheA through changes in receptor packing [20]. The rate of adaptation in response to different chemical stimuli is the same, because of the packing of different receptors into the array and allosteric interactions between these receptors [21], further demonstrating the importance of these large arrays for proper chemotactic function.

The position of flagella varies among species. Many have several randomly positioned flagella (peritrichous), as in *E. coli*, while others, such as *P. aeruginosa*, have a single polar flagellum. To move usefully through the environment, cells need to test the environment every second or so. Data suggest that the time taken for the 15-kDa CheY∼P to diffuse the length of an average cell is only approximately ∼100 ms [22] and, therefore, the positioning of the chemosensory array close to the flagellum is not essential for efficient chemosensory signalling. Electron cryotomography shows that, in most species, including *E. coli*, the membrane arrays are at, or close to, the cell poles, although in some polarly flagellate species, the arrays are found close to the flagellum. Data suggest that being motile and chemotactic is a survival advantage for many bacteria and, therefore, daughter cells need to not only be flagellate, but also inherit a chemosensory array on division to respond to the local environment. Therefore, it is critical that mechanisms exist to ensure that large arrays are inherited on division. If the arrays are localised to only one cell before division, then the daughter cell will