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MicroCommentary

Looking inside the box: bacterial transistor arrays

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Summary

One often compares cells to computers, and signalling proteins to transistors. Location and wiring of those molecular transistors is paramount in defining the function of the subcellular chips. The bacterial chemotactic sensing apparatus is a large, stable assembly consisting of thousands of receptors, signal transducing kinases and linking proteins, and is responsible for the motile response of the bacterium to environmental signals, whether chemical, mechanical, or thermal. Because of its rich functional repertoire despite its relative simplicity, this *chemosome* has attracted much attention from both experimentalists and theoreticians, and the bacterial chemotaxis response becoming a benchmark in Systems Biology. Structural and functional models of the chemotactic device have been developed, often based on particular assumptions regarding the topology of the receptor lattice. In this issue of *Molecular Microbiology,* Briegel *et al.* provide a detailed view of the receptor arrangement, unravelling the wiring of the molecular signal processors.

At the heart of the miracle of modern computing are field-effect transistors (FETs) – three-pronged logic gates that regulate the flow of electrons from one terminal (the source) to another (the drain), in response to a signal voltage supplied by a third (the gate). The contemporary obsession of systems biologists to view cells as computers, and to frame cellular functions as electronic circuits (Bray, 1995; Lok, 2002) then leads quite naturally to ask where in the cell the transistor equivalents are to be found, in what manner they are wired up, and how they function. Receptor molecules seem particularly suited to address these issues because of their location in the plasma membrane, and clearly demarcated input and output functions, as seen in the prominent group of receptors involved in two-component signalling in bacteria (Stock *et al.*, 2000; Szurmant and Ordal, 2004). In these pathways, histidine kinases coupled to sensor domains use cellular energy to phosphorylate a response regulator protein, which in turn interacts with various outputs. In these molecular transistors, the phosphate flux from ATP to response regulator is the analogue of the current between drain and source terminals in electronic FETs. The role of the gate terminal is then provided by whatever environmental cue the sensory receptors respond to, such as chemical ligands, mechanical stress, temperature, or pH.

The article by Briegel *et al.* (2008) in this issue of *Molecular Microbiology* provides the clearest view to date of the organization of a paradigmatic subclass of these microbial signal processors – the architecture of the molecular transistor array that mediates the bacterial chemotactic response. The transmembrane chemotaxis receptors colocalize in large arrays with the histidine kinase CheA and the scaffolding protein CheW (Alley *et al.*, 1992; Maddock and Shapiro, 1993; Maki *et al.*, 2000; Sourjik and Berg, 2000). Although the functional importance of the spatial architecture of this complex has long been recognized, and the individual atomic co-ordinates for most of its components have been available for some time, elucidation of this large, membrane-associated quaternary structure has proved elusive. The new findings from Briegel *et al.* in *Caulobacter crescentus*, together with recent work by Subramaniam and colleagues in *Escherichia coli* (Zhang *et al.*, 2007) provide us with a view of intact chemoreceptor arrays *in situ* under near-native conditions.

The major step forward achieved by Briegel *et al.* depends on a novel method that allows the same biological specimen to be studied first by fluorescence microscopy and then cryo-electron tomography (CET). Applied to wild-type and mutant cells, and correlated with the known structure of the cells, this technique provides an unambiguous demonstration that the large assemblies they observed in their CET images indeed correspond to aggregates of chemotactic signalling proteins. Having

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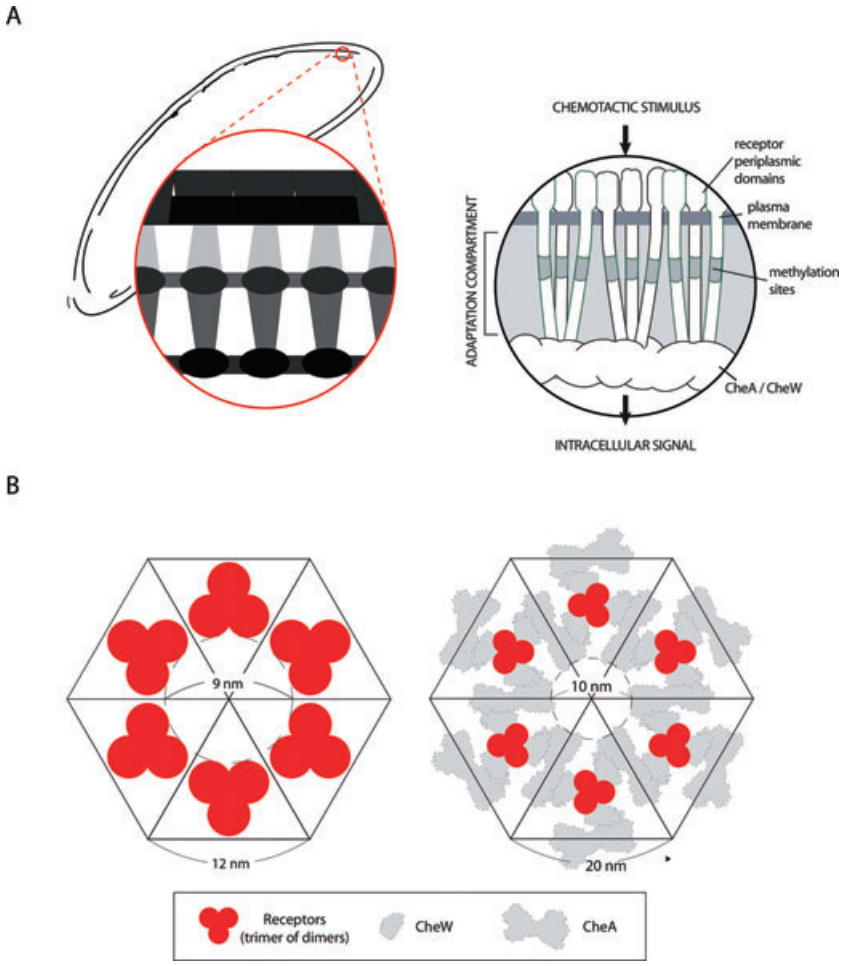

Fig. 1. The new findings of Briegel *et al.* (2008) are consistent with the two principal predictions of Shimizu *et al.* (2000), namely, (A) the presence of an 'adaptation compartment', wherein the negative feedback enzymes CheR and CheB are confined in a small space between the plasma membrane and a 'base-plate' layer composed of CheA/CheW, and (B) a hexagonal arrangement of receptor dimer-trimers that can be indefinitely extended laterally. The zoomed region in (A), encircled in red, schematically illustrates the pattern of electron density observed by Briegel *et al.* on the convex side of the *C. crescentus* cell, with darker shades of grey corresponding to higher density. In addition to the plasma membrane and base plate, a third, fainter layer of electron density was identified within the predicted adaptation compartment, illustrated schematically on the right. In (B), the hexagonal receptor arrangement inferred by Briegel *et al.* (left) by analysis of CET data is compared directly against the original proposal (right) of Shimizu *et al.* (2000). Importantly, the receptor lattice resolved by Briegel *et al.* (2008) is consistent with a higher density of receptors (~12 nm lattice spacing, as opposed to ~20 nm in the earlier proposal) and leaves open the question of how CheA and CheW molecules are arranged in space. In both geometries, the pores in the lattice (~9 and ~10 nm respectively) are large enough to allow the passage of CheB and CheR molecules, the crystal structures of which can be encased in a sphere of 8 nm diameter.

confirmed the identity of the observed assemblies, the authors go on to exploit fully the power of CET to characterize the detailed architecture of chemoreceptor arrays *in situ* in wild-type cells. With striking similarities to structures identified earlier by Subramaniam and colleagues (Zhang *et al.*, 2007) in *E. coli*, the large assemblies found by Briegel *et al.* resemble a dense forest of filamentous electron densities that protrude inward from the plasma membrane in a direction normal to the tangent plane. These were interpreted to be chemoreceptors, based on the similarity in appearance with the previously observed *E. coli* clusters (Zhang *et al.*, 2007), and the fact that their length (31 nm) agrees well with crystallographic evidence (Kim *et al.*, 1999; Park *et al.*, 2006) for the receptor cytoplasmic domain. Additionally, the receptors appear to be supported at their base by a prominent feature that Briegel *et al.* have dubbed the 'base-plate', an extended carpet of high electron density parallel to the inner membrane. This structure is interpreted to correspond to a layer where the CheA and CheW form a two-dimensional scaffold, as predicted in *E. coli* by Shimizu *et al.* (2000) based on molecular modeling (Fig. 1), and supported by immuno-electron microscopy (immuno-EM) results by Zhang *et al.* (2007), who also observed that similar base plate-like densities could be decorated by gold beads coated with anti-CheA antibody. These parallels between the *C. crescentus* and *E. coli* systems highlight the substantial similarity in the physical architecture of chemoreceptor clusters between these two Gram-negative bacterial species, which is also underscored by the observation that 16 of the 18 chemoreceptor species in the *C. crescentus* genome belong to the same phylogenetic group as the *E. coli* receptors, according to categories recently derived by comparative sequence analysis (Alexander and Zhulin, 2007).

There are also differences. Whereas the position and size of the receptor clusters vary greatly from cell to cell in *E. coli* (Zhang *et al.*, 2007), Briegel *et al.* find that the *C. crescentus*, chemoreceptor lattices always appear on the convex side of the cell, providing another example of how dorsal/ventral asymmetry is maintained in the body plan of this bacterium. Furthermore, the authors' use of CET allowed them to measure precisely the distance between the chemoreceptor cluster and the organelle it

controls, namely the flagellar motor, with values between 17 and 130 nm.

Briegel *et al.* also note a fainter, but clearly visible layer of electron density sandwiched between the base plate and inner membrane, about 10 nm below the latter. Given that this maps to the region in the receptor cytoplasmic domain where reversible methylation sites are crucial for adaptation, the authors suggest that the density might correspond to the adaptation enzymes CheR and CheB sequestered to an 'adaptation compartment' (Shimizu *et al.*, 2000; Fig. 1A), and note that the apparent receptor density is also consistent with the 'brachiation' mechanism proposed in an earlier modeling study (Levin *et al.*, 2002).

The most striking discovery of the present paper, however, came when the authors pushed the resolution of the CET technique beyond its usual limit. This was achieved by a clever post-processing of the data, in a scheme similar to those used in single-particle reconstructions (cf. Frank, 2002). Inspired by the regular hexagonal honeycomb patterns that were evident in 'top-view' sections of the tomograms, the authors applied the 'align and average' strategy that is the cornerstone of single-particle reconstruction methods to multiple regions of the tomograms with discernible translational/rotational symmetry. The signal-to-noise ratio was further increased by imposing the sixfold symmetry of the structure that could be separately confirmed in the 2-D power spectra of individual tomogram sections (in which lattice spacing was found to be ~12 nm). The resulting reconstruction yields a compelling network architecture: a tidy hexagonal arrangement near the base plate, which extends half-way up the length of the receptor cytoplasmic domains. Interestingly, this order deteriorates close to the inner membrane, suggesting that the molecular arrangement in this region is more random, and possibly dynamic (Kim *et al.*, 2002; Bray and Williams, 2008). Perhaps the greatest pay-off of the enhanced resolution due to this averaging was the strong constraints obtained for the possible receptor arrangements. The resulting three-dimensional density maps are consistent with a hexagonal lattice consisting of the trimer-of-dimers motif (Fig. 1B).

The association of dimeric chemotaxis receptors into sets of three was one of the important findings made by Kim *et al.* (1999) in their seminal X-ray diffraction analysis. In the following year, a hexagonal lattice composed of these threefold symmetric units was proposed, based on the experimental structures (Bilwes *et al.*, 1999; Griswold *et al.*, 2002) and constraints coming from genetic and biochemical analyses (Liu and Parkinson, 1991; Bass *et al.*, 1999), as a plausible basis for the receptor cluster in *E. coli* (Shimizu *et al.*, 2000). However, the experiments in the ensuing years provided a mixed picture with evidence both in support of and against this original proposal, which was put forward as an atomic-resolution structure (reviewed by Weis, 2006). The most notable and important alternative to the hexagonal geometry proposed so far is that of Park *et al.* (2006) who propose a fundamentally different arrangement based on the 'hedgerow-of-dimers' motif they observed in the solved crystal structure of *Thermotoga maritima*.

The new findings of Briegel *et al.* provide strong support for a hexagonal lattice in *C. crescentus*. It seems very likely that the same is true for *E. coli*. The trimer-of-dimer structure that plays a pivotal role in the hexagonal lattice structure of *C. crescentus* also appears to be fundamental to chemotactic signalling in *E. coli* (Ames *et al.*, 2002; Studdert and Parkinson, 2004; Parkinson *et al.*, 2005; Vaknin and Berg, 2007; Boldog *et al.*, 2006). Furthermore, in view of the congruent sequence types of the *C. crescentus* and *E. coli* receptors (Alexander and Zhulin, 2007), it now seems highly plausible now that an analogous hexagonal arrangement will be uncovered in wild-type *E. coli* cells. Whether the same will be true of more distantly related species such as *T. maritima* remains to be seen. Interestingly, the existence of very similar hexagonal lattices has been proposed for entirely different signalling systems, such as the mammalian glycine receptors (Kneussel and Betz, 2000). The models proposed for those lattices, based on crystallographic evidence and mutational studies (Sola *et al.*, 2004) bear remarkable similarities to the emerging picture for bacterial chemoreceptors, wherein a network of trimeric units enclose hexagonal pores in an extended two-dimensional mesh. It is tempting to ask whether this convergence of topologies in such distantly related systems reflects some common cause or fundamental constraint for receptor-signalling systems.

Returning to our transistor analogy, the physical design of arrays such as the chemotaxis receptors and associated proteins will evidently constrain their performance as signal processors of the cell. This is why studies such as that of Briegel *et al.* will be of interest to such a broad range of disciplines, from biology, to physics and engineering. In the specific context of the bacterial chemotaxis system, much recent work has focused on the question of how thousands of receptor complexes communicate with one another to amplify, integrate and dynamically process input signals (Bray *et al.*, 1998; Kollmann and Sourjik, 2007). Taken together, the results of Briegel *et al.* (2008) and Zhang *et al.* (2007) firmly establish the physical infrastructure required for long–range receptor interactions beyond the trimer-of-dimers structure initially discovered by X-ray crystallography (Kim *et al.*, 1999), and provide a firmer basis on which specifically proposed mechanisms can be interrogated. For example, do receptor–receptor interactions really propagate through extended lattices like atomic spins in a

magnet (Duke and Bray, 1999, Shimizu *et al*., 2003; Mello *et al*., 2005), or are the interactions more short-ranged but rigid, like cooperative haemoglobin molecules (Sourjik and Berg, 2004; Mello and Tu, 2005; Skoge *et al*., 2006)? How many receptors can be affected by a single adaptation enzyme (CheR or CheB) in a given interval of time (Levin *et al*., 2002; Li and Hazelbauer, 2005; Endres and Wingreen, 2006)? While experiments and theories to reverse-engineer these mechanisms are growing cleverer by the day (Hazelbauer *et al*., 2008), some of the most important pieces of these puzzles are still to be found by simply looking inside the box, as Briegel *et al.* have demonstrated so nicely here.

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