Model is as model does

Michael D. Manson and Brian J. Cantwell

Kids love models, and on page 792 of this issue Shimizu et al. give us an early Christmas present. Using plastic replicas of the CheA, CheW, and Tsr protein structures, they assembled the pieces like a jigsaw puzzle. The pleasing result is a scale model of the receptor–kinase signalling complex of bacterial chemotaxis.

Computers outperform people at most tasks, but we still excel at a few. The ability to recognize how objects fit together belongs in this category. The inspiration of Shimizu et al.[1] was to use this human gift to their advantage to produce an intellectually and aesthetically satisfying, as well as testable, model of a chemotaxis signalling complex. This type of scale modelling is used routinely in various fields of engineering, and it is exciting to see its reemergence in structural biology half a century after Pauling constructed his α-helix and Watson and Crick erected their towering spiral of double-stranded DNA.

Using atomic coordinates from the structures of the core region of the CheA dimer[2] and of CheW (provided by F. W. Dahlquist), both from *Thermotoga maritima*, Shimizu and colleagues programmed a three-dimensional printer to manufacture plastic models on a scale of 15 mm per nm, with a resolution of 0.3 mm (or 0.02 nm). They constructed a similar model for the cytoplasmic tip of the trimer of dimers observed in the crystal structure of the intracellular domain of the *Escherichia coli* serine receptor, Tsr[3]. They then manipulated by hand the models of CheW and CheA, and separately of CheW and Tsr, to achieve the best complementary surface fit. They also generated comparable images in modelling applications, and subjected these to energy minimization and other optimization routines to generate the most probable docked structures. In the case of the interction of CheW with Tsr, residues identified in an analysis of allele-specific suppression[4] were located at the predicted contact interface.

One could make the criticism that the proposed molecular lattice incorporates too much imagination and not enough data. Another objection might be that the plastic models are rigid, whereas interacting proteins undergo conformational shifts in order to yield the changes in free energy that drive the assembly process. Conceding these limitations, the deduced model is still an extremely valuable contribution. Like all good heuristic models, it ties together existing information, suggests new paradigms, and is open to experimental refutation or verification. Unlike most scientific models, it also exists in the form of brightly coloured
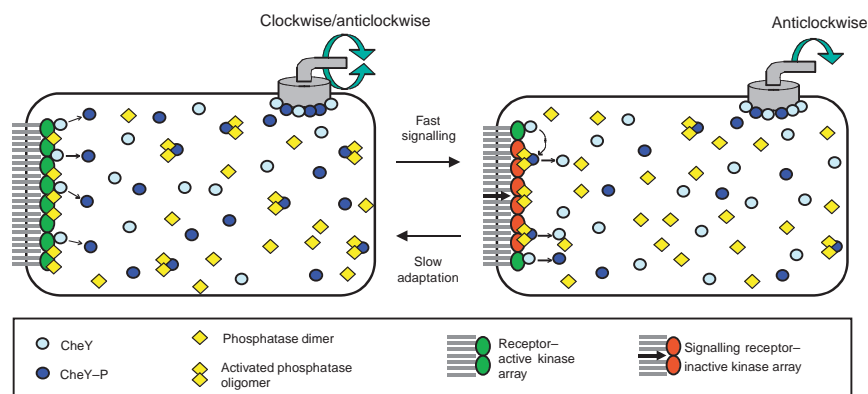

Figure 1 Schematic view of chemotactic signalling in E. coli. Left panel, the cell is in a balanced signalling state that produces alternating smooth swims (anticlockwise flagellar rotation) and tumbles (clockwise flagellar rotation). Cytoplasmic phosphorylated CheY (CheY–P) remains in the narrow range of concentration at which the motor reverses[17] because supply from the receptor patch is balanced by oligomerization and activation of cytoplasmic CheZ phosphatase[18] when CheY–P reaches a threshold level. The concentrations of CheY–P that cause motor reversal and CheZ activation are within the same range (~3 μM). Right panel, an attractant-bound receptor (heavy black arrow) initiates an inhibitory signal that spreads through the patch, shutting off several CheA kinases. CheZ sequestered in the patch, possibly through binding to the short form of CheA[19], also becomes activated in response to the inhibitory signal. Less CheY–P is produced, and CheY–P formed elsewhere in the patch is dephosphorylated by CheZ before it diffuses away into the cytoplasm. CheY–P levels in the cytoplasm fall below the level needed to support clockwise flagellar rotation, and smooth swims are extended. As adaptive methylation restores the receptors to a CheA-activating state, more CheY–P is produced, patch-associated CheZ is deactivated, and CheY–P is again supplied in excess, returning the cell to a balanced signalling state. Lateral spread of the inhibitory signal, activation of CheZ, and the high cooperativity (apparent Hill coefficient of 11; ref. 17) of CheY–P in promoting clockwise rotation may all contribute to signal amplification and integration.

plastic pieces that can be assembled into a structure suitable for public display.

The signal-transduction pathway in bacterial chemotaxis is well characterized[5]. CheA is an autophosphorylating histidine protein kinase that communicates with receptors through the CheW coupling factor. CheA can transfer its phosphoryl group to the response regulator, CheY. Flagellar motors rotate anticlockwise during smooth swimming and clockwise during tumbling; binding of phosphorylated CheY to the motor promotes clockwise rotation.

The intrinsic activity of purified CheA is stimulated 100-fold in a ternary complex of membrane-bound receptors, CheA, and CheW. The response of such a reconstituted system to an attractant ligand establishes the basic paradigm for chemotactic signalling. When an attractant binds to its receptor, CheA activity is inhibited, and phosphotransfer to CheY is correspondingly reduced. As phosphorylated CheY in the cell is rapidly depleted by CheZ phosphatase, the result is a rapid reduction in intracellular levels of phosphorylated CheY

An Arrow straight to the heart of Wingless signalling

Over the last few years the Wingless (Wg)/Wnt patway has been shown to function in cell-fate determination and morphogenesis in both vertebrates and invertebrates. Many members of the signalling cascade downstream of Wg/Wnt have been identified, especially in Drosophila, and include the seven-transmembrane-span receptor Frizzled (Fz). However, the precise mechanism of Wg/Wnt transduction across the membrane of responding cells is still not clearly understood. Three recent papers, by Wherl et al. (Nature 407, 527–520; 2000), Tamai et al. (Nature 407, 530–535; 2000) and Pinson et al. (Nature 407, 535–538; 2000), identify a new member of the pathway in Drosophila, Xenopus and mouse.

Drosophila embryos homozygous for the null allele of arrow, which encodes a member of the low-density lipoprotein (LDL) receptor-related protein family, have severe embryonic defects that mimic the phenotype of wg-null animals (upper-left picture). Arrow is homologous to Xenopus/murine/human LRP6, and mice homozygous for LRP6 exhibit developmental defects, including neural-tube closure, that are very similar to those of homozygous Wnt embryos (upper-right picture). In Xenopus, overexpression of LRP6 leads to duplication of the embryonic dorsal axis, induction of Wnt-responsive genes, and enhanced development of neural-crest cells (lower picture). These phenotypes mimic those of Wnt overexpression. From these phenotypic studies it seems that Arrow/LRP6 acts within the Wg pathway, but where?

Experiments conducted using both Drosophila and Xenopus indicate that Arrow/LRP6 acts in the cells that receive and respond to the Wg/Wnt signal, rather than in those that produce it. Further epistatic

experiments in Drosophila indicate that arrow acts downstream of wg, but upstream of dishevelled (dsh, an intracellular downstream component of the Wg pathway). It was then demonstrated, using Xenopus, that the extracellular domain of LRP6 binds to Wnt-1 and forms a complex with the Fz receptor, but only in a Wnt-1-dependent manner. From this it seems that when Arrow/LRP6 is bound to Wg/Wnt it acts in a complex with Fz to regulate the incoming signal. How Arrow/LRP6 interacts with the proteoglycan molecules that are known to mediate Wg signalling remains to be identified, but yet again the world of Wg/Wnt signalling has become more complicated.

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and an increased probability of anticlockwise flagellar rotation. Thus, smooth swims are extended, and cells migrate up an attractant gradient.

In principle, this situation does not require any interactions beyond those of a receptor dimer with a CheA dimer and two CheW monomers in a 1:1:1 complex. It is adequate to explain responses to large shifts in the concentration of single attractants. Indeed, it has been calculated that the best strategy for an *E. coli* cell with a limited number of receptors (1,500–4,500) to detect chemicals in its environment would be to distribute these receptors uniformly (or randomly) over the cell surface[6].

So compelling was this argument that *E. coli* was selected as a negative control by Maddock and Shapiro[7] in their search for receptor clusters in the asymmetrically dividing species *Caulobacter crescentus*, in which only one daughter cell is flagellated. The result was startling. Receptors in *C. crescentus* indeed cluster at the flagellar pole of the predivisional swarmer cell, but in *E. coli*, which does not sport a polar flagellum, the receptors are also present in polar patches. Furthermore, polar localization of the receptors in *E. coli* diminishes when either CheA or CheW is absent.

These observations beg the question of why *E. coli* chemoreceptors are distributed in a patchy fashion, which is seemingly contrary to sound engineering principles. The

answer presumably lies in the nature of the signalling mechanism, as clustering of receptors could account for several unexplained features of chemotaxis. First, a change of less than 1% in receptor occupancy causes a measurable increase in anticlockwise rotational bias[8]. How can inhibition of the activity of only a few CheA molecules associated with attractant-bound receptors be amplified to give a detectable signal? Second, it is unclear how the low-abundance receptors Tap and Trg mediate strong responses to their attractant ligands when they stimulate CheA activity only weakly[9]. Finally, the means by which responses to different attractants or repellents in a chemically heterogeneous environment are integrated at the levels of signalling and adaptation is unknown[10].

These issues have been dealt with previously by Bray *et al.* in a conceptual model that invokes interconnected arrays within receptor patches[11]. Although there is no experimental basis for such extended networks, they are consistent with the existence of receptor patches. However, the identification of reconstituted aggregates of the soluble cytoplasmic domain of a chemoreceptor with CheW and CheA in a stoichiometry of ~7:1:1 may provide a glimpse of greater structural complexity[12].

The model-building exercise reported by Shimizu and colleagues would have

been useful even if it just predicted how the individual protein partners interact, but its implications are far greater. The geometry of the proposed receptor–CheW–CheA trigonal complex indicates a straightforward way that it can be extended to form a hexagonal array of indefinite expanse, a clear candidate for the receptor patch. Within such an array, conformational perturbations initiated by the binding of ligand to one receptor dimer could spread in order to amplify or integrate signals from different receptors. The mysterious, but crucially important, linker between the second membrane-spanning segment and the extended cytoplasmic domain of the receptors[13] may allow the bending in an otherwise rigid helix that would be required to form the trimer of receptor dimers.

The model also predicts that an 'adaptation compartment' may exist between the cell membrane and the hexagonal lattice. The ability of CheR methyltransferase[14] and CheB methylesterase[15] to bind to the carboxyl-terminal tail of high-abundance receptors, together with their sequestration in this chamber, would restrict their diffusion away from the site at which their activity is needed. Recent studies using proteins fused to green fluorescent protein (GFP) have indicated that CheY and CheZ concentrate at the polar receptor patches as well[16]. Conveniently,

the model predicts that the surface of CheA that interacts with CheY (and probably with CheZ) faces outwards into the bulk cytoplasm, as expected.

The speculative diagram in Fig. 1 represents our attempt to integrate the concept of an extended receptor lattice with other information to present our view of the basic features of chemotactic signalling. This scheme is presented to stimulate thinking and inspire experiments, not as a final explanation. It may also provide a new perspective on how receptor/signalling complexes in eukaryotic cells may be organized. □

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A rough guide to a smooth brain

Ron Morris

Lissencephaly is a devastating genetic disease of children that affects the embryonic development of the brain. It is caused by haploinsufficiency of the Lis1 gene product, which seems to be required for proper functioning of cytoplasmic dynein during neuronal proliferation, migration and morphogenesis.

Lissencephaly, or smooth brain, is caused by a failure of neuronal migration from the paraventricular zone of the developing brain, where neuronal precursors proliferate, to the cerebral cortex[1]. This causes a malformation of the cortex, which consequently lacks the cortical folds seen on the surfaces of normal brains. Not surprisingly, patients with this disease are severely retarded. They develop intractable epilepsy and usually die young. Three exciting papers in this issue, by Faulkner *et al.*[2] (page 784), Liu *et al.*[3] (page 776) and Smith *et al.*[4] (page 767), provide new evidence that Lis1 is required for dynein function. Of particular interest are dynein-related effects of Lis1 in neurons, an effect of Lis1 on mitosis in mammalian cells, the demonstration that there is an actual physical connection between Lis1 and dynein, and some new ideas about how the absence of Lis1 might cause lissencephaly.

The product of the *Lis1* gene resembles the β-subunit of a typical heterotrimeric G protein[5], but it does not interact with α- and γ-subunits. Instead, it purifies from brain as a component of platelet-activating-factor acetyl hydrolase (PAFAH), an enzyme that inactivates the lipid second messenger platelet-activating factor (PAF), indicating that lissencephaly could be caused by a defect in PAF metabolism[6].

However, Lis1 is a conserved protein, and in simpler organisms loss-of-function (LOF) mutations in homologues of *Lis1* phenocopy LOF mutations in cytoplasmic dynein and its activator dynactin[3,7–9]. In fungi these LOF mutations inhibit migration of nuclei and vesicles, and in *Drosophila* they cause defects in germline-cell division, nuclear positioning and oocyte differentiation. Purified Lis1 has also been shown to influence microtubule dynamics *in vitro*[10].

Dynein in higher eukaryotes participates in retrograde axonal transport, transport of microtubules to the cell periphery, vesicular transport on microtubules, and mitosis[11]. Therefore, if Lis1 truly affects dynein function one might expect it to affect some or all of these events. Faulkner and colleagues report that Lis1, like dynein, is found at kinetochores and at the cell cortex in animal cells, and that it affects mitosis. Overexpression of *Lis1* caused a profound mitotic delay with a phenotype indicating a defect in chromosome congression or attachment to microtubules[2]. Reduction in *Lis1* activity by microinjection of anti-Lis1 antibodies, anti-dynein antibodies or treatment with a *Lis1* antisense oligonucleotide caused a very similar phenotype with chromosomes displaced from the spindle. These results are consistent with an effect of Lis1 on dynein at the kinetochore. The authors

comment on the fact that the anti-Lis1 antibodies caused much less delay than anti-dynein antibodies, raising the interesting, albeit far from proven, possibility that Lis1 may be directly involved in mediating the spindle-checkpoint mechanism. Localization of dynein at the cell cortex and alignment of the orientated mitotic spindles in MDCK cells were also perturbed by overexpression of *Lis1*, leading the authors to propose that spindle orientation may be mediated by an interaction between microtubules and cortical dynein. On the basis of their results, Faulkner and colleagues hypothesize that aberrant division of *Lis1*-deficient neuronal progenitor cells may contribute to the deficiency of cortical neurons seen in lissencephaly. As the sequential timing of cell division in developing neurons is known to influence their destination in the

…These three papers leave little room to doubt that Lis1 affects the function of dynein/dynactin in some as yet incompletely defined way… there is a long experimental road to travel before we know exactly how Lis1 does what it does…

brain[12], the authors reason that cell-division abnormalities caused by *Lis1* deficiency could disrupt this timing and thereby affect nuclear migration to the cortex. Inhibition of mitosis could also reduce the number of