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Direct evidence for coupling between bacterial chemoreceptors

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Abstract

Bacterial chemoreceptors form mixed trimers of homodimers that cluster further in the presence of other cytoplasmic components. The physical proximity between receptors is thought to promote conformational coupling that enhances sensitivity, dynamic range, and collaboration between receptors of different types. We investigated conformational coupling between neighboring dimers by co-expressing two types of receptors, only one of which was labeled with yellow fluorescence protein (YFP). The two types of receptors were stimulated independently, and changes in the relative orientation of the labeled receptors were followed by fluorescence anisotropy. Possible coupling via cytoplasmic components of the taxis system was avoided by working with strains lacking those components. We find that binding of ligand to one type of receptor affects the conformation of the other type of receptor, but not in the same way as binding of ligand to that receptor directly. Thus, different receptors are coupled, but not as simply as previously thought.

To follow chemical gradients, cells of *Escherichia coli* utilize several types of transmembrane receptors, including the Tsr receptor that senses serine and the Tar receptor that senses aspartate[1–3]. These sensors form mixed trimers of homodimers, even in the absence of other chemotaxis proteins[4,5], and together with a coupling protein, CheW, and the kinase, CheA, form large receptor arrays[6–8]. These arrays were predicted to facilitate receptor collaborative operation[9,10]. Indeed, synergetic effects between homodimers of different types have been demonstrated both *in-vivo*[11] and *in-vitro*[12]. Here we aimed to test directly whether coupling between homodimers can emerge due to direct physical interactions between neighboring dimers in receptor oligomers. To avoid coupling modes involving cytoplasmic proteins, we made measurements in strains in which CheW and CheA are not expressed. Note that while large clusters appear to be the dominant receptor state in the presence of CheW and CheA, it is the trimer-of-dimers structure that is the dominant state in their absence[4,5,13]. We showed previously that changes in the relative orientation of adjacent homodimers in response to binding of ligand can be detected by labeling receptors with YFP and measuring fluorescence anisotropy[14,15]. Changes in fluorescence anisotropy were traced to changes in energy transfer, i.e., to homo-FRET, that occur upon ligand binding. When serine (aspartate) is added to trimers of homodimers of Tsr-YFP (Tar-YFP), the YFPs move farther apart. These responses are absent in mutants that fail to form trimers and can be diluted out by expression of unlabeled receptors[14,15].

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The rationale of the experiments described here is the following: by forming mixed oligomers composed of homodimers of fluorescently labeled Tar and homodimers of unlabeled Tsr, we can selectively stimulate either type of receptor while reading out changes in the relative orientation of the labeled receptors. Thus, we can directly address the following question: does binding of ligand to the Tsr dimer trigger conformational changes in the Tar dimers? This strategy is illustrated in Fig. 1a. Two extremes are possible: (i) In the absence of coupling, the conformational response of Tsr will not affect Tar and the anisotropy level will not change; (ii) if, however, the trimers form a single allosteric unit (tight coupling) the binding of ligand to Tsr can only toggle the whole structure from the active to the inactive conformation, resulting in a change in anisotropy similar to that observed upon binding of ligand to Tar. The actual behavior turns out to be more complex.

We measured the anisotropy in cells without other chemotaxis proteins (*flhD* cells) expressing different levels of Tsr, together with Tar-YFP, Fig. 1b. At low levels of Tsr expression (lower, blue trace) the addition of serine had little effect, while the anisotropy increased upon addition of aspartate, as observed previously[15]; the YFPs moved farther apart. At higher levels of Tsr expression (upper, red trace), the baseline anisotropy was higher and increased only marginally upon addition of aspartate, as expected from dilution of the YFP label in mixed trimers. However, serine lowered the anisotropy and increased the response to aspartate, as if binding of serine to Tsr in mixed trimers caused the Tar-YFPs to move closer together. Thus, the response of Tar-YFP homodimers to binding of serine to Tsr dimers in a mixed trimer, albeit small, was opposite of what one might expect.

The size of the downward shift in anisotropy (Fig. 1b, red curve) increased with the concentration of serine, as shown in Fig. 2 (inset). The corresponding dose-response curve is shown in the main plot (filled circles) together with a similar dose-response curve measured with a pure population of Tsr-YFP receptors (empty circles) and the curve measured *in vitro*[16] for binding of serine to Tsr (line). The near identity of these curves confirms that the downward shift observed with mixed trimers (Fig. 1b, red curve; and, Fig. 2, inset) is due to the response of the Tsr homodimers to binding of serine, even though it is being read out through a conformational response of the Tar-YFP homodimers. We confirmed that the effect is not unique to Tsr by reversing the roles of Tar and Tsr (data not shown). We also confirmed that the effect appears when Tsr and Tar-YFP are expressed in a strain that is deleted for all other chemotaxis components (UU1581; data not shown). We obtained similar results with constructs where the flexible tail at the C-terminal end of Tar was either present or absent (when YFP was fused to the C-terminus of wildtype Tar or to Tar^{1-527}, missing 27 C-terminal residues[15]), confirming that the effect does not rely on a possibly large effective volume occupied by the YFPs. Thus, we conclude that binding of ligand to one homodimer, e.g., Tsr, in a mixed trimer affects the conformations of the other homodimers, e.g., Tar. However, it does so in a manner that is qualitatively different from that triggered by direct binding of ligand to Tar. Finally, we find that when both the indirect and direct stimuli are present (e.g. both serine and aspartate) the direct stimulus is dominant (Fig. 1b, red curve).

In principle, an alternative interpretation of these results could be that the changes in anisotropy are due to dissociation of receptor oligomers upon ligand binding. Although evidence has been presented elsewhere against such a view (see discussion in ref. [15] and references therein), we further tested this possibility here by varying the location of YFP within the receptor structure. If receptor oligomers dissociate, the efficiency of energy transfer between YFP's on different dimers should markedly decrease, regardless of where the YFPs are placed. However, if the response is due to conformational changes within the trimers, the sign and/or magnitude of the response should depend upon the position and orientation of the YFP, and thus could be different at different locations. We inserted YFP into the cytoplasmic tip of a full-length Tsr receptor at position 390 (for details see the legend of Fig. 3). This construct is termed Tsr-

YFP* and is shown schematically in Fig. 3a. When cells expressing this construct where stimulated with serine, a clear anisotropy response was observed (Fig. 3b) but its sign was opposite of that found with the C-terminal fusion to Tsr. When Tsr-YFP* was co-expressed with increasing levels of unlabeled Tar, the baseline anisotropy increased and the amplitude of the response to addition of serine decreased (Fig. 3c), as expected from dilution of the YFP label within mixed oligomers. When cells expressing Tsr-YFP* with unlabeled Tar at an intermediate level were stimulated by addition of aspartate, an anisotropy response also was observed (Fig. 3d) but again with a sign opposite of that seen with serine (Fig. 3b). Thus, overall, the YFP insertion at the distal cytoplasmic tip of the dimers had a behaviour qualitatively similar to that observed with YFP close to the membrane at the C-terminal end of the receptor, but with smaller amplitude and opposite sign. These findings provide strong evidence for the view that the changes in anisotropy seen in our experiments reflect conformational changes in receptor oligomers rather than dissociation of these oligomers.

A few qualitative characteristics of trimer responses are illustrated in Fig. 4, which shows the trimer of Fig. 1aviewed from the periplasmic side. In the absence of any ligand the trimer is in the active conformation, with the C-terminal ends of the homodimers close to one another (left image). Ignoring possible structural differences between Tar and Tsr, this conformation would be expected to have a three-fold symmetry. When the ligand binding sites are fully occupied, the trimer switches to the inactive conformation, with the C-terminal ends of the homodimers farther apart (right image). This conformation also has a three-fold symmetry. However, when the ligand binding sites are partially occupied, for example when only serine is bound to Tsr, the symmetry of the trimer is broken, and an asymmetric conformation is possible (middle image). To account for the data presented in Fig. 1b, the C-terminal ends of the Tar homodimers should be closer together than they are in the absence of serine. On the other hand, to account for the monotonic change in anisotropy with ligand concentration observed with homogeneous Tar-YFP trimers or with homogeneous Tsr-YFP trimers (Fig. 2, open symbols), the mean distance between dimers should be larger than observed in the absence of ligand. These constraints are met by the middle image of Fig. 4. It is clear that the actual conformational change of trimers is more complex, as indicated by the differences in sign of the anisotropy responses of the Tsr-YFP and Tsr-YFP* constructs (see also ref. [17]); however, the requirement for qualitatively different conformations in the partially or fully bound state should be general. This requirement means, in particular, that trimers on their own do not form tightly coupled ‘two-state’ allosteric units of the sort proposed[18–20] to explain recent measurements of kinase activity[11,15,21–23]. Yet, the conformations of neighbouring dimers are coupled, supporting the view[5,24–26] that dimers act collaboratively in trimers to propagate signals to the associated kinase proteins. Additional contributions to receptor coupling could emerge in fully assembled receptor clusters, from direct trimer-trimer interactions or via their association with CheW and CheA.

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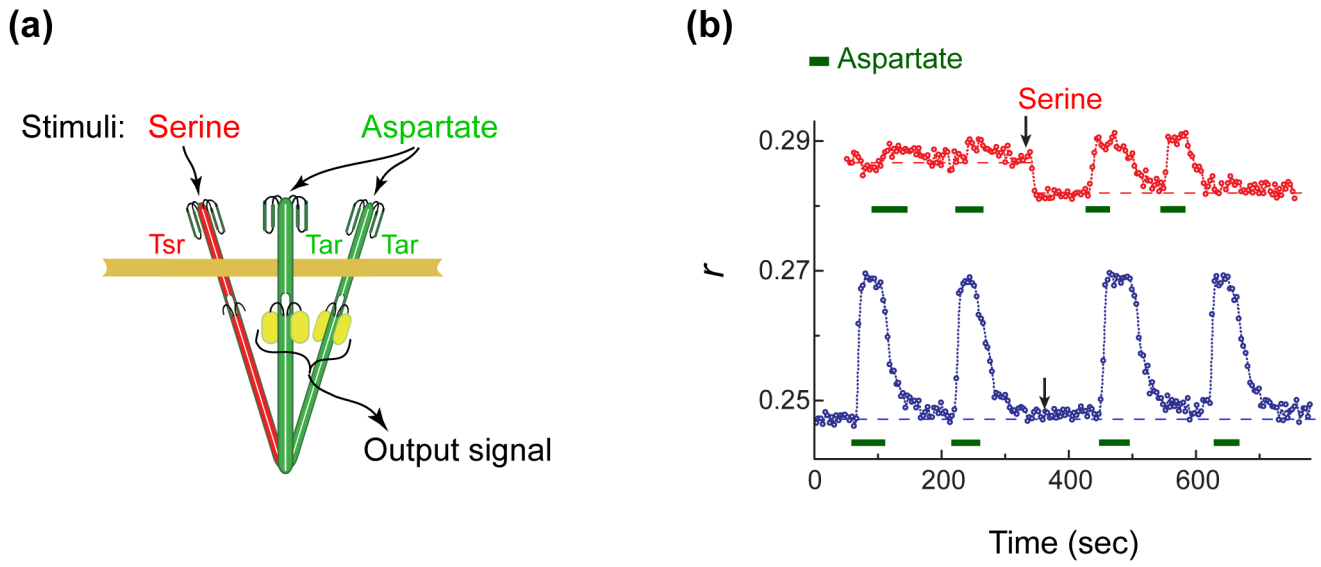

Figure 1.

The effect of direct and indirect stimuli on Tar-YFP. **(a)** The experimental rationale. A mixed trimer is shown containing one Tsr dimer (red) and two Tar-YFP dimers (green). The conformation of the former can be affected directly by binding of serine and the conformation of the latter by binding of aspartate. The relative spacing between the Tar dimers is monitored by measuring the steady-state polarization of the emitted fluorescence, represented here by the fluorescence anisotropy (*r*). Changes in the fluorescence anisotropy are due to homo-FRET between the YFPs at their C-termini; the anisotropy decreases if the YFPs move more closely together and increases if they move farther apart. The experimental setup for measuring fluorescence polarization was described earlier[14]. *r* is defined as $(I_{par} - I_{per}) / (I_{par} + 2I_{per})$, where I_{per} was corrected for imperfections of the optical system. Experiments were done at room temperature (22°C). **(b)** Anisotropy traces measured from *flhD* cells (VS117; a gift of Victor Sourjik) co-expressing Tar-YFP (induced using 17 μM IPTG from plasmid pAV45: pTrc99A vector carrying $tar^{QQQQ}[1\text{-}527]\text{-}yfp^{A206K}$) and different levels of Tsr (induced by different concentrations of sodium salicylate from plasmid pPA114, a gift of Sandy Parkinson). Blue symbols: a low level of Tsr (no sodium salicylate); red symbols: a higher level of Tsr (0.6 μM sodium salicylate). The absolute expression level of Tar was comparable to its native expression level[14]. At its higher induction (red trace) Tsr expression reached levels roughly four times that of Tar-YFP. Aspartate (500 μM) was added for the time intervals indicated by the green bars. Serine (500 μM) was added at the time indicated by the arrows and left in throughout. Dashed lines indicate the anisotropies observed in the absence of ligand and are a guide to the eye.

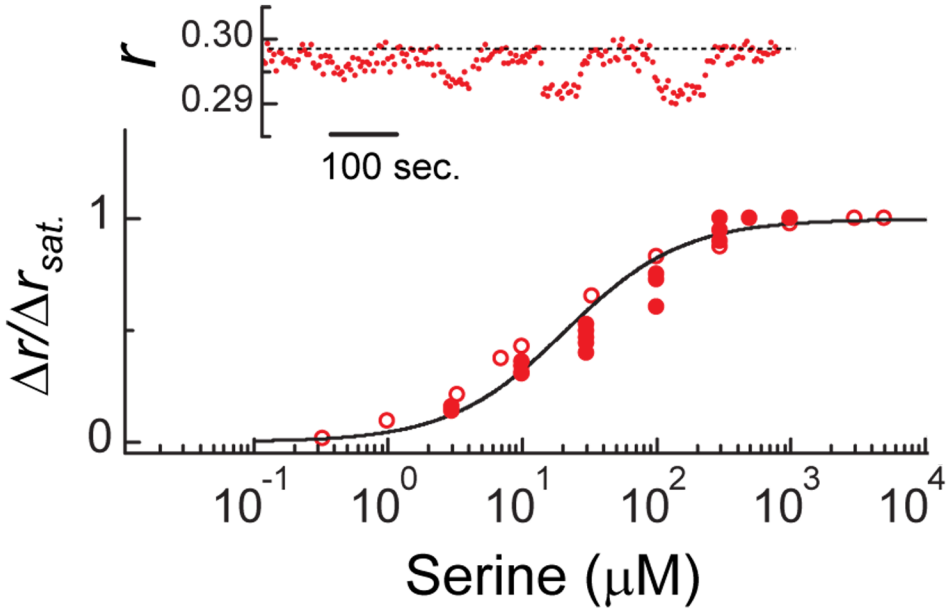

Figure 2.

The dependence of the downward anisotropy shift of Fig. 1b on the concentration of serine. The inset shows the raw data for one sequence with cells similar to those of Fig. 1b in which the concentration of serine was toggled between 0 and 10, 30, 100, and 300 μM, respectively; the dashed line indicates the anisotropy observed in the absence of ligand and is a guide to the eye. The main plot shows dose-response curves: closed symbols, results obtained from experiments of the kind shown in the inset; open symbols, results obtained from the same strain expressing only Tsr-YFP (induced using 5 μM IPTG from plasmid pAV29: pTrc99A vector carrying *tsr–yfp*^{A206K}); line, an *in vitro* binding curve of serine to Tsr (ref. 16: K_d=20 μM, Hill coefficient=1).

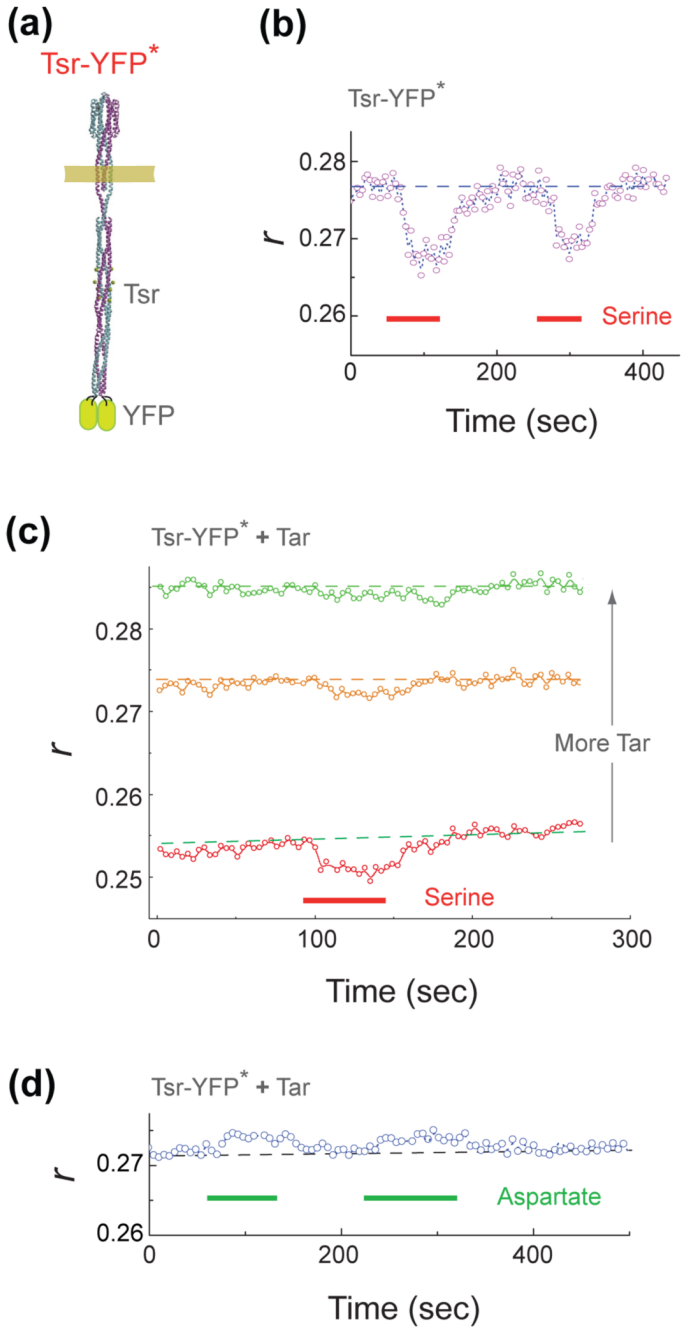

Figure 3.

Anisotropy traces with YFP fused to the cytoplasmic tip of a receptor homodimer. (**a**) The position of YFP in the construct Tsr-YFP*: $tsr^{1\text{-}390}$–yfp^{A206K} –$tsr^{390\text{-}end}$ cloned into pTrc99A vector to form plasmid pAV48. (**b**) An anisotropy trace (see legend of Fig. 1) recorded from *flhD* cells expressing Tsr-YFP* (induced using 5 μM IPTG). Serine was added for the time intervals indicated by the red bars. (**c**) Similar traces recorded from cells co-expressing Tsr-YFP* with increasing levels of Tar (induced using increasing concentration of sodium salicylate from plasmid pLC113, a gift of Sandy Parkinson): reading from bottom to top: 0, 0.5, and 1.2 μM sodium salicylate. Cells were stimulated by addition of serine for the time interval indicated by the red bar. (**d**) Anisotropy traces recorded from cells co-expressing Tsr-

YFP* with an intermediate level of Tar (0.7 μM sodium salicylate) and stimulated by addition of aspartate for the time intervals indicated by the green bars. Dashed lines indicate the anisotropies observed in the absence of ligand and are a guide to the eye.

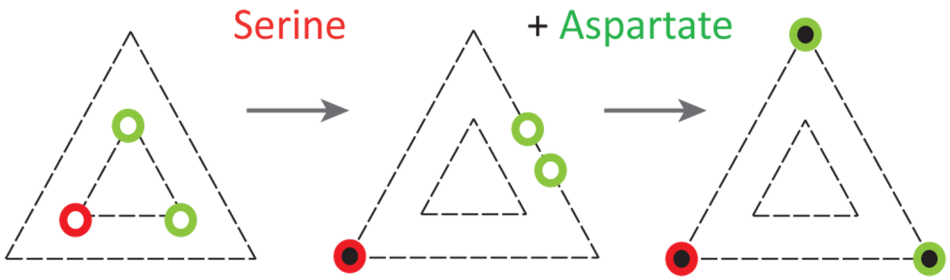

Figure 4.

Characteristics of trimer responses. A trimer containing one Tsr dimer (red) and two Tar dimers (green) is shown viewed from the periplasmic side. In the absence of any ligand, the trimer has three-fold symmetry (left image). Upon binding of serine to Tsr, the three-fold symmetry is broken (middle image). Upon binding of aspartate to Tar, the three-fold symmetry is restored (right image). The results presented in this paper are explained if the Tar dimers in the middle image are closer together than they are in the left image, while the mean distances between dimers are ranked in the order smallest (left), intermediate (middle), and largest (right), as shown.