**(4, 8, 8)**

**1. 1.** Draw a cartoon of the Trg receptor with its bound ligand. Label the N and C-terminal ends of this receptor, as well as the segments that were the focus of the Hughson & Hazelbauer study.

**2.** Fig. 1 in the Hughson-Hazelbauer paper shows a western blot of a crosslinking experiment with Trg, where the extent of crosslinking of particular cysteines in Trg is monitored during a 10 min period, with (+) and without (no symbol) addition of saturating amounts of ribose, in the presence of an oxidizing agent. Why is the banding pattern for 42-42’ so different than that for 42-203 and 38-202 if the Trg receptor is the same? Why is the experiment conducted for 10 min when we know that the cells adapt to added attractant within seconds?

**The 42-42’ crosslink ties the two monomers together, so they will run as a slower moving dimer band in the SDS-PAGE gel. The other two crosslinks are intra- or within each monomer. The crosslinked monomer migrates slightly faster than the un-crosslinked one likely because it has an altered shape. The authors had eliminated the adaptation response by deleting the genes responsible for adaptation - *cheB* and *cheR*. Since they used saturating levels of ribose, and the excitation response takes milliseconds, the signaling conformation change must have already occurred. The experiment was conducted for a long time to optimize the crosslinking reaction with the oxidizing agent.**

**3.** Fig. 4C indicates that crosslinking was strongest between cysteine residues 38 and 202 after ribose addition. Why is this observation used to suggest a downward piston motion? Suggest a hypothetical crosslinking pattern between two residues upon addition of a repellent instead of an attractant; repellents stimulate the CheA kinase.

**The TM1 crosslinking patterns show no change. Therefore, assuming these don’t move, TM2 will have to move down in order for residues 38 and 202 to come close enough to be crosslinked. A repellent causes upward motion of TM2. A hypothetical pattern for upward motion would be increase in crosslinking between 38 and 210.**

**(6, 6, 8)**

**2.** When receptors were first discovered to be clustered, Berg conducted an experiment to see if the receptor cluster serves as a nose. To do so, he marked one pole of the cell with a dye and tracked the bacteria as they ran and tumbled. **1**. The dye did not stain the receptors, but he was still able to conclude that the bacteria were swimming with the receptor cluster at either end (front or behind). Explain how he was able to do this. **2**. Why does this observation lead him to conclude that clustering does not help signal detection, but rather some later step? **3.** When the clusters were first discovered, which two proteins were found to be critical for clustering? How exactly do these proteins assist in clustering?

**1. The dye stains the poles, irrespective of the presence of receptors. It is not important that the dye stain the receptors, only that it allow him to see if the same end (pole) is always in front during swimming. The receptors are fixed at one or the other end. If the dye end orients randomly while running, then the receptor end is also random.**

**He tracked the dye position as the cells ran and tumbled. Half the cells he tracked had started their run with the dye in front, and half with the dye behind. After a tumble, all had effectively randomized the dye position. So, either end could be in front.**

**2. He was testing the notion that the function of clustering is to detect more ligands. If true, cells might always swim with the cluster end in front assuming that while swimming, the fluid flow might decrease the ligand concentration at the rear compared to the front. He did not see this. So, he concluded that the cluster must help some step after ligand detection.**

**3. CheW and CheA. Receptors are organized as trimers of dimers. CheW is a linker protein connecting one receptor dimer in a trimer to one subunit of a CheA dimer. The other subunit of the CheA links to another CheW and a receptor dimer in a different unit of the trimer. Thus, CheW and CheA connect and generate a hexagonal lattice of receptor clusters.**

**(4, 8, 8)**

**3.** **1.** Ames et al. isolated mutants at the Tsr receptor trimer contact sites, which were either defective in clustering or could not regulate kinase activity. Predict and justify the chemotaxis behavior in plate assays for each of these two categories of mutants. **2.** When mixed with wild-type Tsr or Tar, the trimer-contact mutants displayed interesting phenotypes. Explain the molecular basis of the dominant and epistatic phenotypes shown in Fig. 3 i.e. is the mutation affecting homodimer or mixed cluster formation? **3.** Why are some dominant mutants of Tsr rescuable by Tar, but not the epistatic mutants? Which of these mutants would crosslink to wild-type Tar in the experiment shown in Fig. 4? Justify.

**1. They would both be chemotaxis-defective, because clustering is important for regulating CheA.**

**2. The dominant nature of the mutations suggests that the mutation is affecting receptor homodimer formation i.e. a mutant Tsr monomer is pairing with a wild-type Tsr monomer to produce a defective dimer that does not join the cluster. Therefore the mutant Tsr dimers do not interfere with Tar signaling. Epistatic mutants can dimerize and join trimers. They form mixed Tsr-Tar trimeric complexes, thus interfering with both Tsr and Tar signaling.**

**3. The epistatic mutants spoil function the function of Tar, so they cannot be rescued. Homodimers of the these dominant mutants can apparently join the Tar cluster for rescue. The rescuable dominant mutants and the epistatic mutants would crosslink because their ability for rescue and epistatis indicates formation of mixed clusters.**

**(6, 6, 8)**

**4.** **1.** There are major and minor receptors in the chemoreceptor patch. The minor ones do not support chemotaxis when expressed alone, but function well when mixed in with the major ones. Why? Design an experiment that will allow them to function alone. **2.** All components of the chemotaxis pathway are at the cluster. In which of the following mutants would the receptors be expected to maintain their clusters: CheW, CheA, CheY, CheB, CheR, CheZ? Justify. **3.** The function of receptor clustering is now understood to be signal amplification. What exactly is being amplified, by how much, and by what mechanism? Why is signal amplification necessary?

**1. The minor receptors are missing a C-terminal pentapeptide sequence that is the binding site for CheR and CheB, hence they cannot adapt. Major receptors have this sequence, and are able to provide the adaptation function to the minor receptors in trans. If the pentapeptide sequence is engineered on the ends, the minor receptors should be proficient in chemotaxis by themselves.**

**2. Clustering requires only CheW and CheA; these mutants will therefore not support clustering. The receptors would cluster in all other mutants, because although these proteins are present, they are not required for clustering.**

**3. The ligand-binding signal is being amplified to control CheA activity. The signal is amplified 36-fold i.e. one ligand molecule shuts off 36 kinase molecules, by allosteric communication between the CheW-CheA network which links the receptors clusters at the base. Signal amplification is necessary because attractant occupancy inhibits kinase activity; therefore there must be a way to quickly drop the CheY~P already present, which is taken care of by CheZ.**

**(6, 6, 8)**

**5. 1.** In the nanodisc experiments of Boldog et al (see Signal Amplification Ppt, slides 32-34), only two assays were employed: kinase stimulation was measured by monitoring CheY~P levels upon adding aspartate, and adaptation was measured by monitoring altered migration of methylated Tar. What then was the basis of the conclusion by Boldog et al that both monomers and trimers are proficient in ‘transmembrane signaling’? **2**. In the Nanodisc experiments, which step do Tar trimers favor that monomers don’t, and how do you think this step influences signal amplification? **3**. Imagine eight nanodiscs linked by CheW/CheA. Six of these discs contain Tar trimers and two have Tsr trimers. Which ligand – serine or aspartate – will inhibit the kinase more?

**1. Both monomers and trimers showed methylation after aspartate addition. For this to happen, the ligand signal must have passed through the transmembrane, HAMP and MH domains and changed MH conformation to promote methylation.**

**2. Both monomers and trimers supported ligand binding, transmembrane signaling and adaptational modification, but only the trimers supported kinase activation. Amplification is dependent on the kinase network.**

**3. One ligand stimulates 36 kinases. Eight nanodiscs will likely have a maximus of 24 kinase dimers. Because CheA-CheW link all the receptors, irrespective of receptor type, the signal will likely be amplified to the same degree with either ligand.**

**(6, 5, 9)**

**6. 1.** Pioneering experiments with Vibrio parahaemolyticus concluded that the polar flagellum is a surface sensor. What does sensing allow this bacterium to do? On slide 10 of the Flagellum as a Sensor Ppt, why is it taking nearly 50 min after addition of PVP or Antibody to see a light response from the Laf-lux reporter? **2.** Of the many conditions that induce Laf expression (slide 20), which ones directly implicate the motor and why? **3.** You studied three flagella-mediated surface sensing responses, where slowing/stopping the motor was a common sensory theme. Yet each case had a different consequence, product, and lifestyle promoted. Briefly summarize each case (one sentence for each).

**1. The sensory pathway promotes the synthesis of hundreds of lateral flagella, which allow the bacterial to swarm on a surface. The induction of Laf is at the transcriptional level, monitored by the Laf-lux reporter. It takes time to transcribe and translate luciferase.**

**2. Mot mutations and Na+ channel blockers because they inactivate or impair stator function; the other reagents could also affect osmolarity or exert a force on the cell body.**

**3. V. parahaemolyticus: transcriptional output, lateral flagella synthesis, promote swarming life style**

**B. subtilis: transcriptional output, PGA synthesis, promote biofilms**

**C. crescentus: post-translational output, holdfast synthesis, promote attachment.**

**(7, 6, 7)**

**7. 1.** You have watched movies of flagella and pili working. What differences in form, function and spatial positioning between pili and flagella were important for arrest of flagella rotation by pili in the Li et al paper? **2.** Fig. 3A and B in Li et al. show fluorescent holdfast production (y-axis) with time in three wild-type *C. crescentus* cells (different colors). In A, why is the green cell (around 25 min) making more holdfast initially? InB, why are all three cells making more holdfast compared to A? **3.** Predict the behavior of a *C. crescentus* mutant missing the flagellar filament but having a short hook – would it resemble any of patterns in panels A-C in Fig. 3 or will it have a different pattern entirely?

**1. Pili are straight and retractile. In C. crescentus, pili are placed in close proximity to flagella. They sense the surface to attach and retract, pulling the bacterium to the surface and pinning the flagellum there as well, thus stopping its rotation.**

**2. In A, the green cell is making more holdfast because it attaches at 25 min; holdfast synthesis increases in a developmentally programmed manner with time i.e. even in the absence of attachment. In B, the cells were treated with the polymer crowding agent Ficoll, which restricts flagellar rotation and induces holdfast secretion even before cells attach.**

**3. A flagella-less mutant can still attach using pili, but it is hard to say if the motor with a short hook will be stopped by pili retraction. If it is, then the pattern will resemble A. If not, then the pattern will be like the green cell in A, where holdfast is made as part of the developmental cycle, not in response to attachment.**