

Chemotaxis

Bacteria can detect a concentration gradient of attractant

- Pfeffer (1880s)

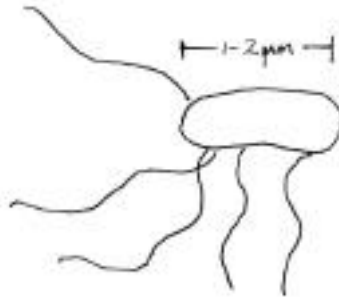


What is being detected? (Adler, 1969)

- Attractant?
- Metabolite?
- chemicals that are extensively metabolized are not chemoattractants
 - aspartate vs. fumarate, oxaloacetate, succinate, pyruvate
- some nonmetabolizable chemicals are attractants
 - mutants that cannot metabolize chemical are still attracted to it
 - essentially nonmetabolizable analogs attract bacteria (D-fucose)
- chemicals attract even in the presence of metabolizable chemicals
- attractants related in structure to each other compete
- mutants that fail to chemotax to attractant but can still metabolize it

CONCLUSION: There are specific receptors for chemoattractants

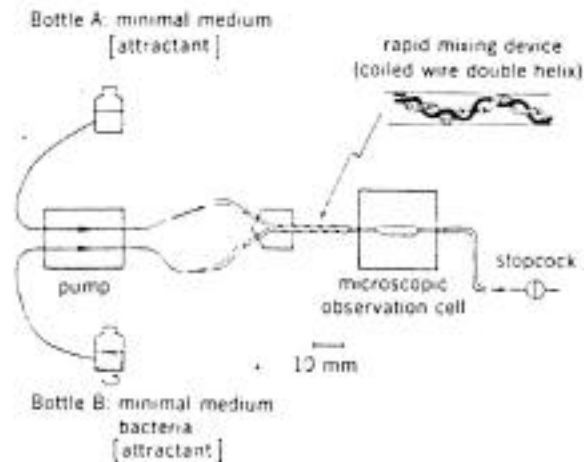
How do bacteria measure a concentration gradient?



PROBLEM 1: 1 in 10^4 difference in concentration between front and back of bacterium

PROBLEM 2: noise

Macnab and Koshland (1972); stopped flow "temporal gradient" apparatus



- decrease leads to tumbling; increase leads to supercoordinated swimming (straight lines)



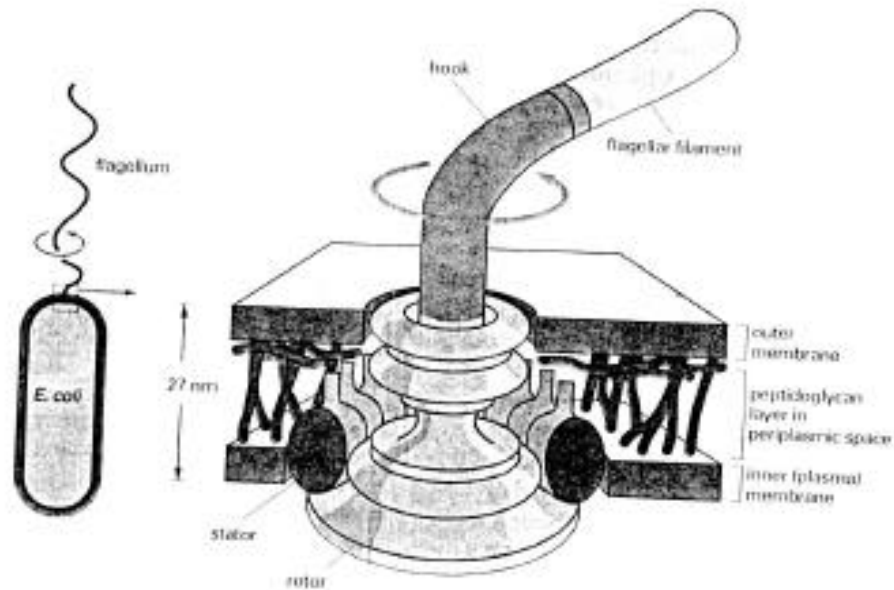
- responses eventually decay to steady-state motility

By integrating over time it solves both problems
Berg (1988)

- if memory is 1 min and bacterium is moving at $30 \mu\text{m sec}^{-1}$, concentration comparison is being made over ~ 2 mm, or 1000-body-lengths – lessens accuracy needed to 1 in 10
- memory is actually only ~ 1 -10 sec
- Why not have longer memory? - information might be processed after a change in direction; cannot simply increase run length - Brownian motion

How do bacteria move?

- was known that they have a flagellum, a hook, and then disks and rods to attach to membrane



Silverman and Simon (1974)

- tether by flagella with Ab
- cells move CCW mostly, but also stop, go CW

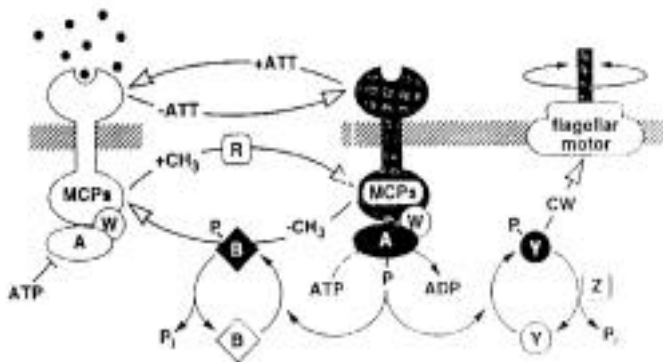
PROPOSE THAT FLAGELLAR ROTATION MIGHT BE BASIS FOR MOVEMENT

Is cessation or reversal of flagellar movement involved in chemotaxis?

Larsen et al. (1974)

- attractants - CCW rotation; repellents - CW rotation
- chemotaxis mutants - tumbling occurs from CW rotation and smooth swimming from CCW rotation

Identify genes defective in chemotaxis mutants and isolate proteins - define signaling pathway



MCP –receptor
CheW – adaptor
CheA – kinase
CheY – phospho-acceptor
CheZ – phosphatase

Ninfa et al. (1991) – Tar + CheW + CheA phosphorylate CheY

Receptor	-	+	-	+	-	+	-	+
CheW	-	-	+	+	-	-	+	+
CheA	-	-	-	-	+	+	+	+
P-CheY								

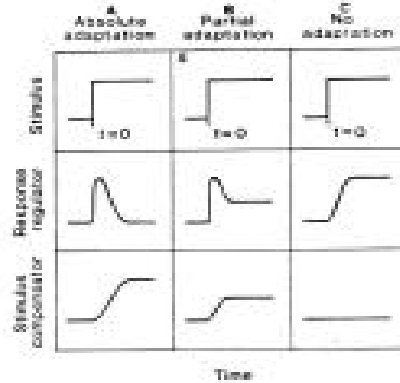
Gegner et al. (1992)

- CheW binds receptor but CheA does not
- CheA binds receptor via CheW (1:1:1)
- complex unaffected by ligand

Schuster et al. (1993) - Biacore

- Tar:CheW:CheA complex binds CheY
- addition of ATP causes phosphorylation and dissociation of CheY

Chemotaxis (and other sensory pathways) exhibit adaptation

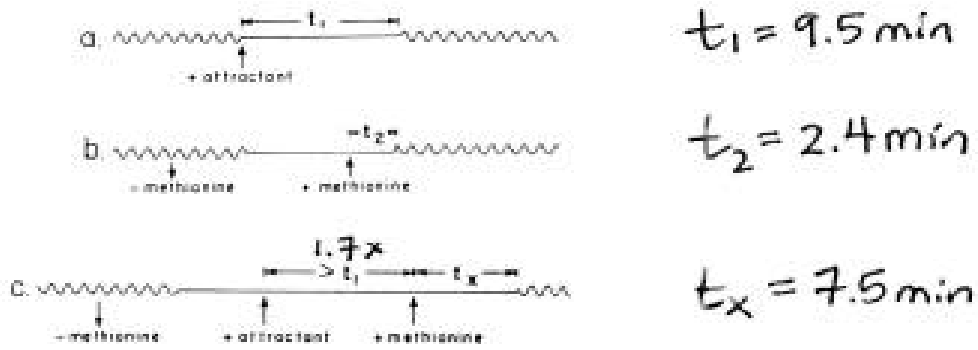


How does adaptation work?

- chemotaxis requires methionine; cells cannot tumble in the absence of methionine

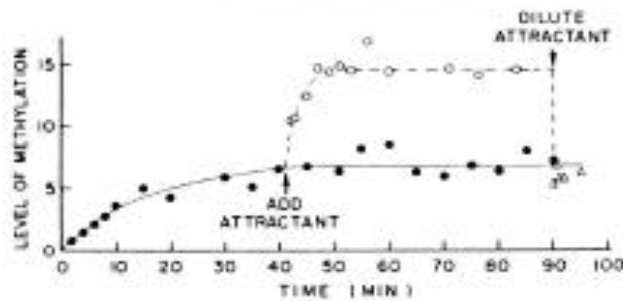
Springer et al. (1977)

- adaptation requires methionine (actually see some weak adaptation in absence of methionine)

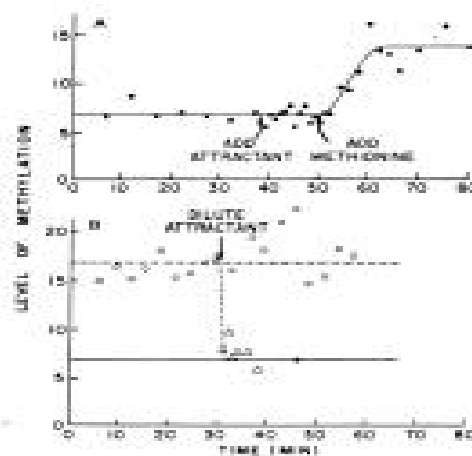


Goy et al. (1977)

- attractants stimulate methylation of receptors



- methylation of MCPs requires methionine

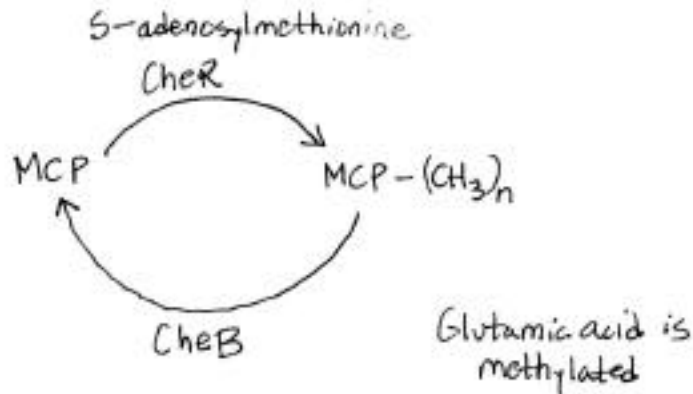


Conclude that methylation of MCPs is involved in adaptation

What proteins control methylation?

- cheR and cheB mutants do not incorporate radioactive methyl groups into MCPs

- cheR mutants - smooth swimming; cheB mutants - tumbling; cheR cheB are tumbling too



Is methylation regulated?

Multiple levels of regulation:

Lupas and Stock (1989)

- CheA activates CheB by phosphorylation (~10-fold); CheR is unregulated

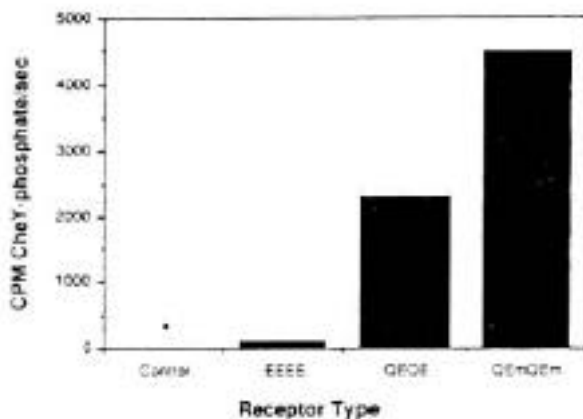
Borczuk et al. (1986)

- demethylation of MCPs by constitutively active CheBc is inhibited by attractant
- esterase only acts on MCPs that are activating CheA

How does methylation affect MCP function and CheY phosphorylation?

Borkovich et al. (1992) - prepare membranes from cells with specifically modified forms of the receptor and measure ability of these forms to regulate CheA kinase in reconstituted system with CheW, CheY, ATP, and CheA

- analyze three forms - EEEE, QEQE, QEmQEm – ABSENCE OF ATTRACTANT



- methylation stimulates kinase activity

- measure inhibition of kinase activity - use tar mutant that causes constitutive activation of kinase

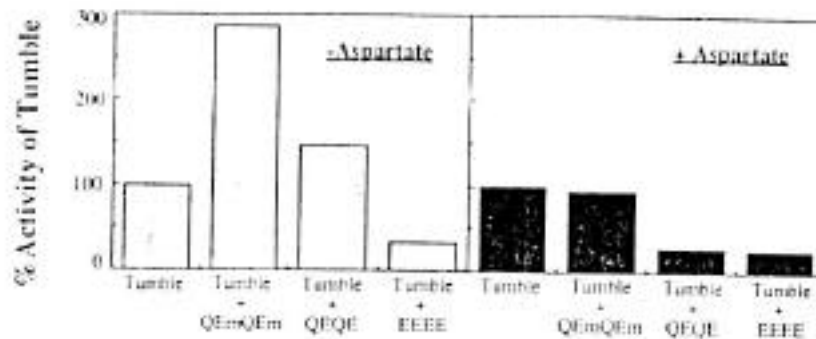


FIG. 4. Inhibition of a tumble mutant Tar receptor by each of the modified forms in the presence or absence of aspartate.

SUMMARY:

- EEEE is poorest activator, most potent inhibitor, even in absence of ligand
- QEmQEm cannot inhibit but is the most potent activator, even when saturated with attractant
- QEQE activates in absence of attractant and inhibits in its presence

Another way of looking at this phenomenon:

- in vitro system - measure inhibition of CheY~P production by attractant in system containing MCP, CheA, CheW, CheY

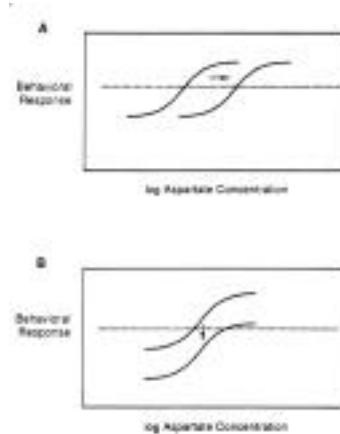
IC₅₀ for inhibition of CheY~P formation:

EEEE	0.65 μ M
QEQE	0.70 μ M
QEmQEm	120 μ M
EmEmEmEm	>50 x 10 ³ μ M

CONCLUSION: Receptors that are highly methylated are less sensitive to inhibition by attractant

How might this work?

Two possibilities (Dunten and Koshland, 1991)



- (A) Affinity of MCP for attractant decreases as methylation state increases
- test by measuring K_d
 - done many times by people in partially purified systems lacking CheA, CheW (which are normally associated with MCP)
 - find little difference in K_d between methylated and unmethylated MCPs
- (B) Signaling strength of receptor changes in response to methylation state

2000 - Li and Weis

Repeat titration of attractant serine and measure inhibition of CheY~P production

- MCPs are in complex with CheA, CheW (show by sedimentation)

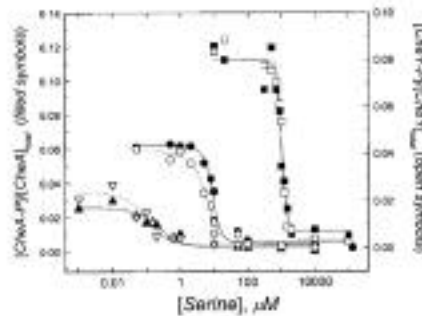


Figure 3. Steady-State Phosphoprotein Levels as a Function of the Serine Concentration

Observe what has been seen before:

- in absence of attractant unmethylated receptors less efficient at promoting formation of CheY~P, methylated receptors most efficient
- unmethylated receptors more sensitive to inhibition by attractant

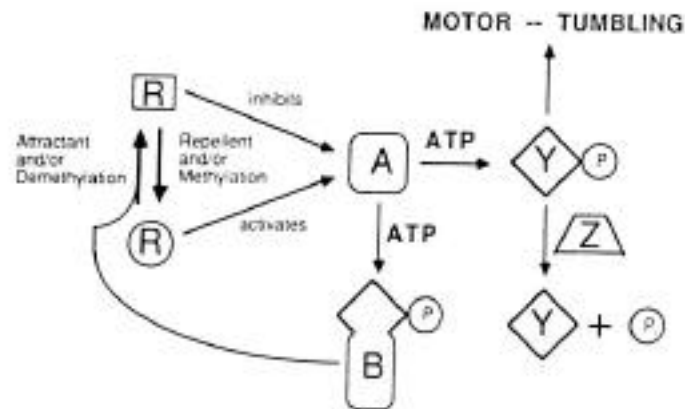
New observations:

- high degree of cooperativity, especially with methylated receptors ($n > 10$)
- fit inhibition to two-state model for kinase regulation - ligand-bound complex is inactive

- measure K_i from curve fit of data - find 10,000-fold difference between methylated and unmethylated!

CONCLUSIONS: Methylation mediates adaptation by decreasing affinity of MCP for attractant

MODEL:



Is methylation the only mechanism of adaptation?

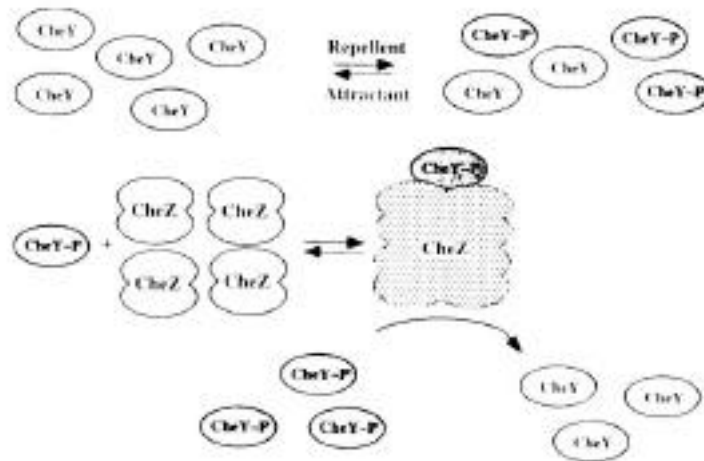
Stock et al. (1981)

NO - CheR mutants adapt differently from wild-type, but they do still adapt; implies the existence of another mode of adaptation

What is the other mechanism of adaptation?

Blat & Eisenbach (1996)

- cheZ mutants tumble constitutively and adapt more slowly
- binds to phosphorylated CheY 100-1000-fold better than to unphosphorylated form
- CheY~P binding induces oligomerization of CheY and activates its phosphatase activity



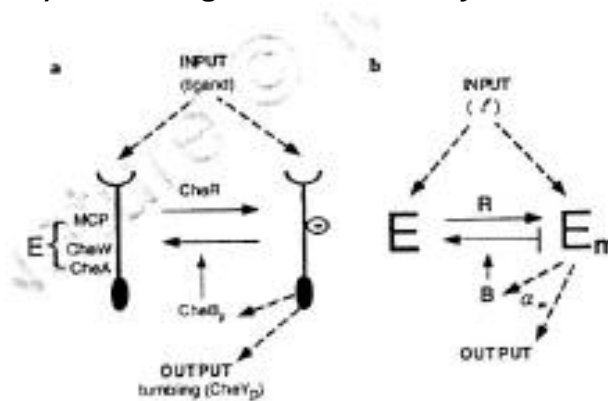
- thought that oligomerization of CheZ is rate-limiting, thereby providing a delay
- part of adaptive response - helps to reset levels of CheY~P

How finely-tuned is the adaptation process?

Are all of the kinetic constants and protein levels adjusted just so to produce adaptation (a theoretical model produced adaptation by this fine-tuning mechanism), or is adaptation robust and resistant to changes in the system?

- adaptation is a robust property of the bacterial chemotactic system

Barkai & Leibler (1997) - modeling of chemotaxis system



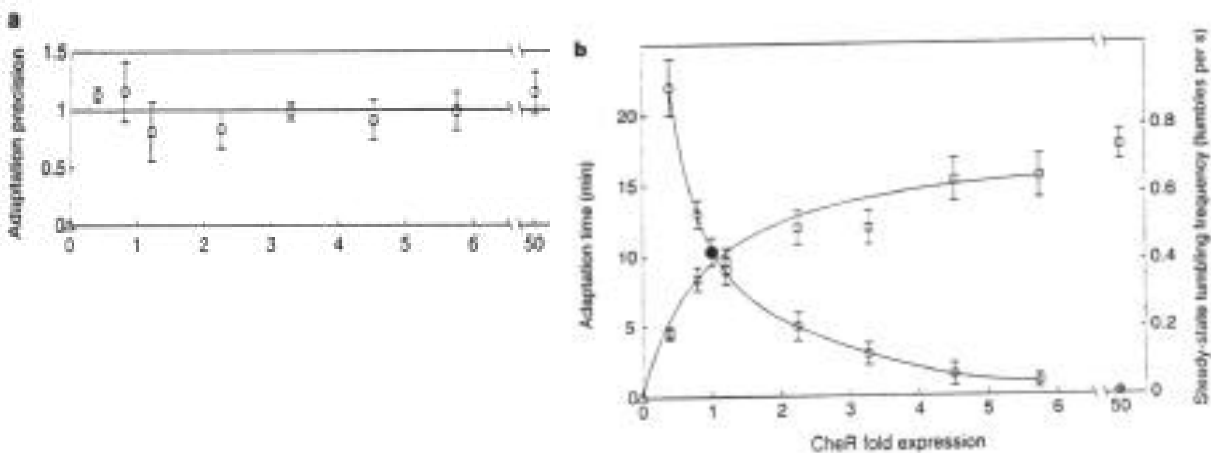
- two-state model with feed-back system
- enzyme, E, sensitive to ligand (I)
- E can be modified by CheR to methylated form

- only methylated form of can promote phosphorylation of CheY; methylated E also activates esterase CheB, which removes methyl groups from E, thereby returning it to inactive form
- assume methylation and demethylation occur on slower timescale than activation and CheB acts only on active form of E

CONCLUSION: adaptation is due to a feedback loop that is dependent on the activity of the signal transduction output; the precision of adaptation is insensitive to changes in concentrations of the components, although the timescale of adaptation is affected by these changes

Is this model really correct?

Alon et al. (1999) - tested model experimentally by varying concentration of signaling components



- precision of adaptation is robust, but other properties (such as adaptation time and steady-state behavior) are not
- robustness is a consequence of the architecture of the signaling network

Sensitivity and dynamic range

Segall et al. (1986)

- change in occupancy of one receptor produces a significant response; with a K_d of $1\mu\text{M}$, this corresponds to a minimum detectable concentration of 2 nM aspartate
- upper limit of detection is 1 mM

How does the system achieve such exquisite sensitivity and dynamic range?

(1) Part of the answer is *adaptation* – important in response to higher concentrations of attractants and repellents

(2) Bray et al. (1998) – sensitivity and range may result in part from clustering of receptors on surface of bacterium

- postulate that change in receptor induced by ligand binding is propagated to neighboring receptors
- postulate: (i) cell has both clusters and single receptors in equilibrium and (ii) spread is controlled in response to external conditions
- spread will be lowest when cell has adapted to high concentrations of attractant; highest when not adapted
- model can account for observed dynamic range

Is there any data to support this clustering?

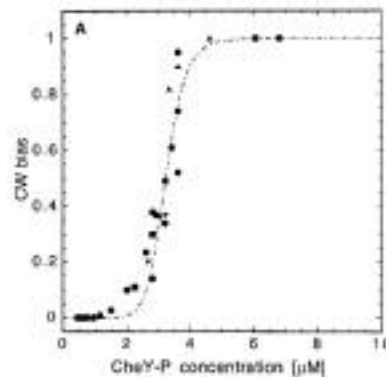
Maddock and Shapiro (1993)

- immunofluorescence and immuno-EM studies of receptors, CheA, CheW



- why? How?
- not clear clustering is activity-dependent (does not need to be – could just be spread/communication)

(3) Cluzel et al. (2000) - Sensitivity might also arise from interactions downstream



in pathway

- measure dependence of motor output on CheY~P concentration in living cells
- find steep input-output relationship, with Hill coefficient >10!!!

CONCLUSION: Motor acts as amplifier; high gain of chemotactic system may result from properties of motor

Chemotaxis Bibliography

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