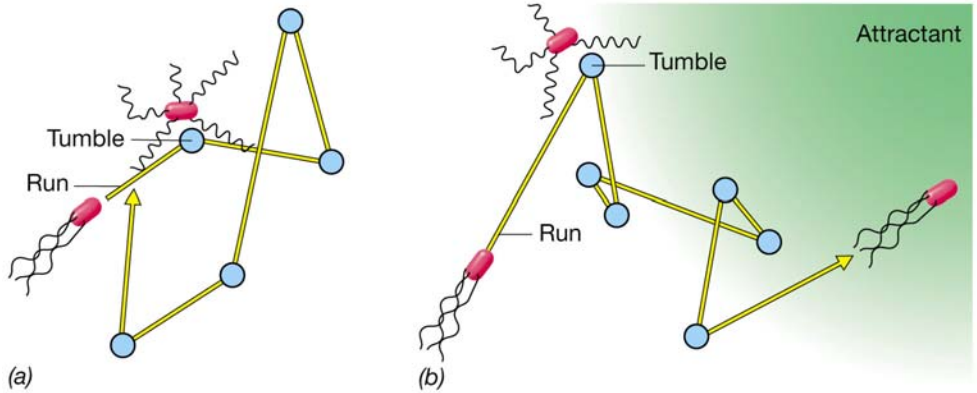
Behavioral Responses

- Taxes: directional movement
- Chemotaxis
  - Prokaryotes: too small to sense gradient along body length
  - While moving must compare physical and chemical state of environment with that sensed few seconds before
  - Respond temporally rather than spatially: comparison of external state with what they received seconds ago
- Phototaxis
  - Movement toward light
  - Phototrophic organism orients itself in a manner favorable for photosynthesis

Mechanism of Chemotaxis

- No gradient:
  - Runs: cell swims forward in smooth fashion (several minutes)
  - Tumbles: when cell stops and jiggles about (0.1 second)
  - Direction of next run is random
  - Cell moves randomly, but does not go anywhere -- It is casually HUNTING
- Chemical gradient:
  - Random movements become biased BIASED RANDOM WALK - Has detected PREY
  - Sense higher conc of gradient
  - Runs longer, tumbles less frequent – Hunt is on
  - Organism moves up gradient
  - Repellent: decrease in conc gradient promotes run



Chemotaxis

Image removed due to copyright considerations.

Please see:
Grebe TW, Stock J. "Bacterial chemotaxis: the five sensors of a bacterium.“ *Curr Biol*. 1998 Feb 26;8(5):R154-7.

Immunolocalization of the Receptor Cluster

Image removed due to copyright considerations.

Please see:
Figure 5 in Weis RM, Hirai T, Chalah A, Kessel M, Peters PJ, Subramaniam S. "Electron microscopic analysis of membrane assemblies formed by the bacterial chemotaxis receptor Tsr." *J Bacteriol*. 2003 Jun;185(12):3636-43.

Image removed due to copyright considerations.

Please see:
Figure 1b in Lybarger SR, and Maddock JR. "Clustering of the chemoreceptor complex in Escherichia coli is independent of the methyltransferase CheR and the methylesterase CheB." *J Bacteriol*. 1999 Sep;181(17):5527-9.

E. coli was stained with antibody to Tsr. At top, shows receptor clustering. To right, shows a detailed view of two intracellular domains intercalating with each other. The intercalation is artificial but you can see all of the signaling elements on each leaflet.

Chemotaxis: Five Sensory Apparatuses

Image removed due to copyright considerations.

Please see:
Figure 1 in Mikhail N. Levit, Yi Liu, and Jeffry B. Stock. "Stimulus response coupling in bacterial chemotaxis: receptor dimers in signalling arrays." *Molecular Microbiology*, Volume 30 Issue 3 Page 459, November 1998.

The Front End of the Signal Transduction System

Image removed due to copyright considerations.

Please see:
Figure 5 in Mikhail N. Levit, Thorsten W. Grebe, and Jeffry B. Stock "Organization of the Receptor-Kinase Signaling Array That Regulates Escherichia coli Chemotaxis." *J. Biol. Chem*., Sep 2002; 277: 36748 - 36754.

The Chemotactic Receptor

Image removed due to copyright considerations.

Please see:
Figure 1 in Weis RM, Hirai T, Chalah A, Kessel M, Peters PJ, Subramaniam S. "Electron microscopic analysis of membrane assemblies formed by the bacterial chemotaxis receptor Tsr." *J Bacteriol*. 2003 Jun;185(12):3636-43.

Image removed due to copyright considerations.

Please see:
Figure 2 in Mikhail N. Levit, Yi Liu, and Jeffry B. Stock. "Stimulus response coupling in bacterial chemotaxis: receptor dimers in signalling arrays." *Molecular Microbiology*, Volume 30 Issue 3 Page 459, November 1998.

Image removed due to copyright considerations.

Please see:
Figure 2 in Mikhail N. Levit, Yi Liu, and Jeffry B. Stock. "Stimulus response coupling in bacterial chemotaxis: receptor dimers in signalling arrays." *Molecular Microbiology*, Volume 30 Issue 3 Page 459, November 1998.

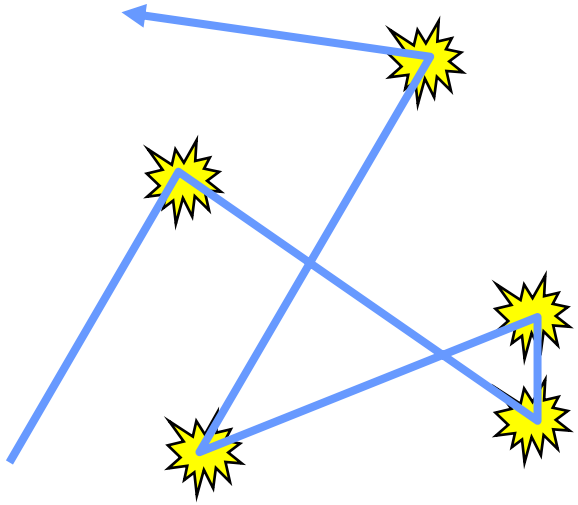
- This apparatus traverses the cell membrane
- The periplasmic space is above
- The response regulator, CheY, picks up the "signal" from CheA

Linking CheY-PO_4 to Motion (To Tumble or Not to Tumble)

Image removed due to copyright considerations.

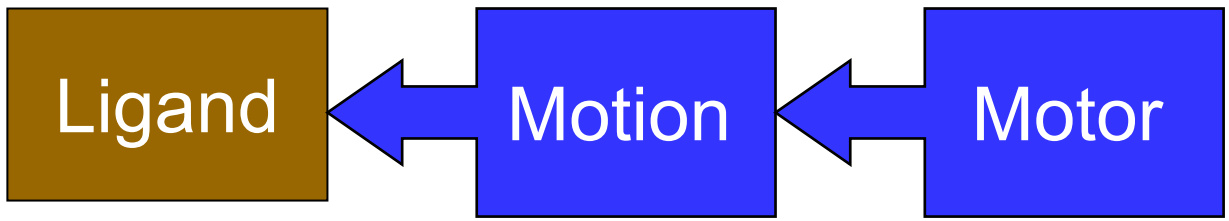
Please see:
Figure 1 in Bren A, Eisenbach M. "How signals are heard during bacterial chemotaxis: protein-protein interactions in sensory signal propagation." *J Bacteriol*. 2000 Dec;182(24):6865-73.

Random Walk



- No gradient of ligand
- Motor may be on
- But no DIRECTED motion

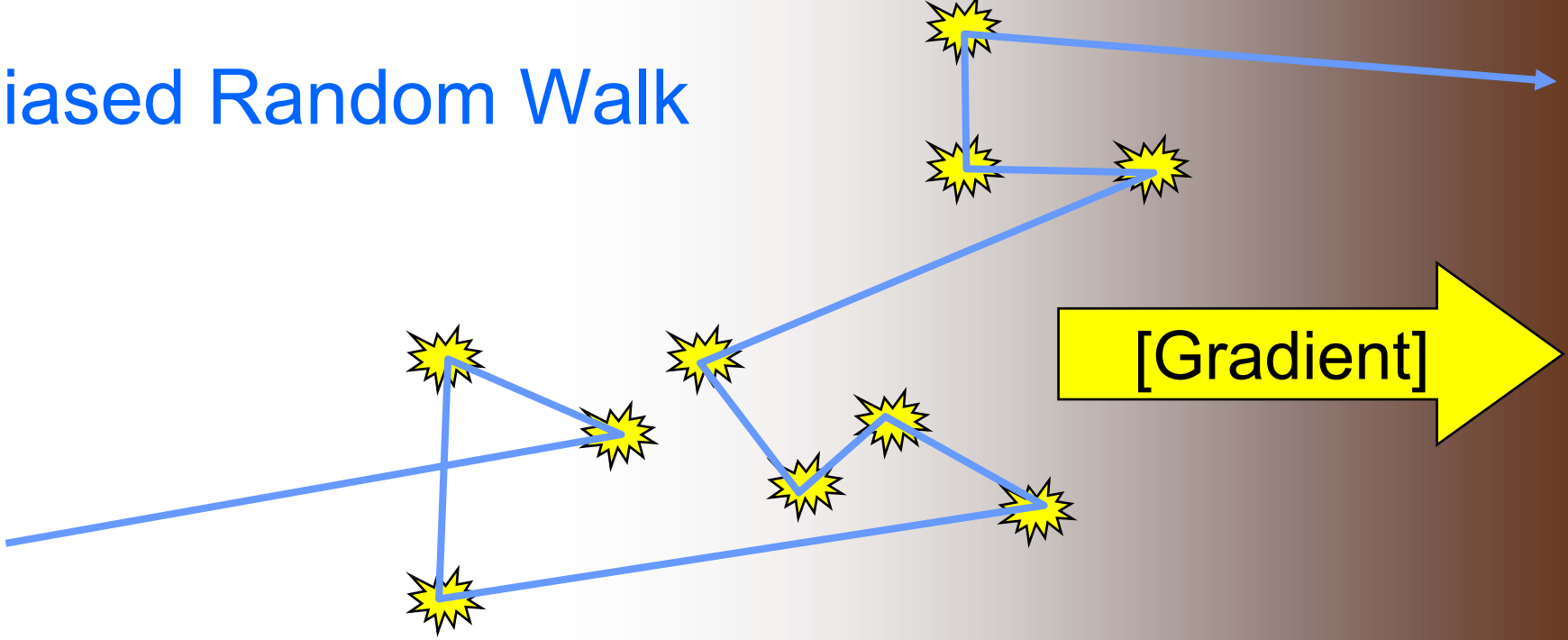
Absent

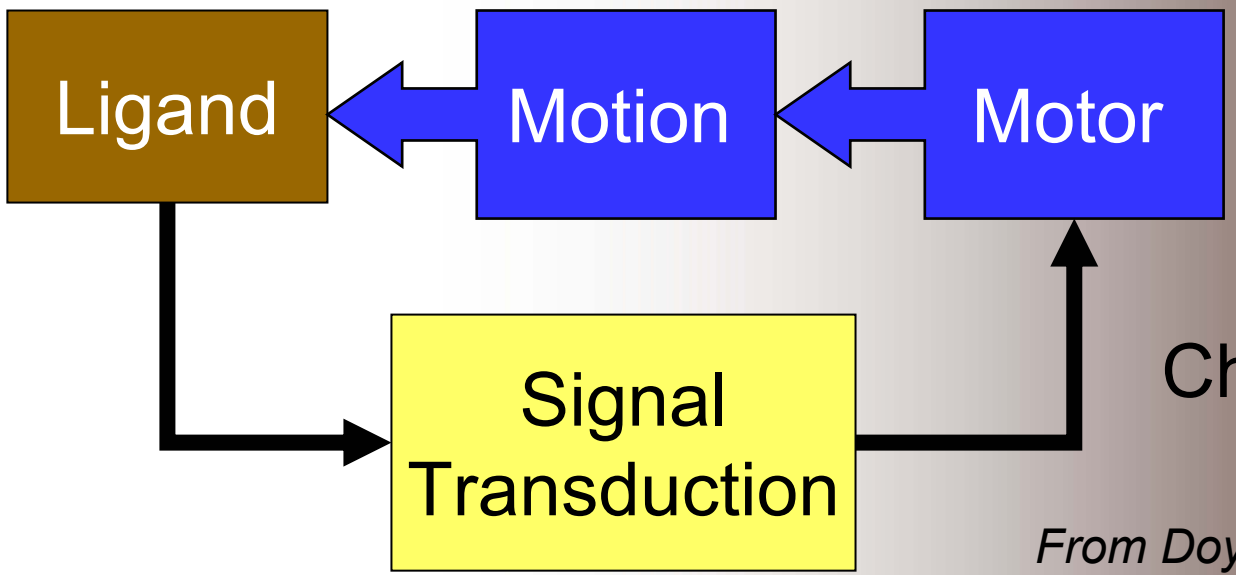


Bacterial chemotaxis (Yi, Huang, Simon, Doyle)

From Doyle, Simon ...

Biased Random Walk



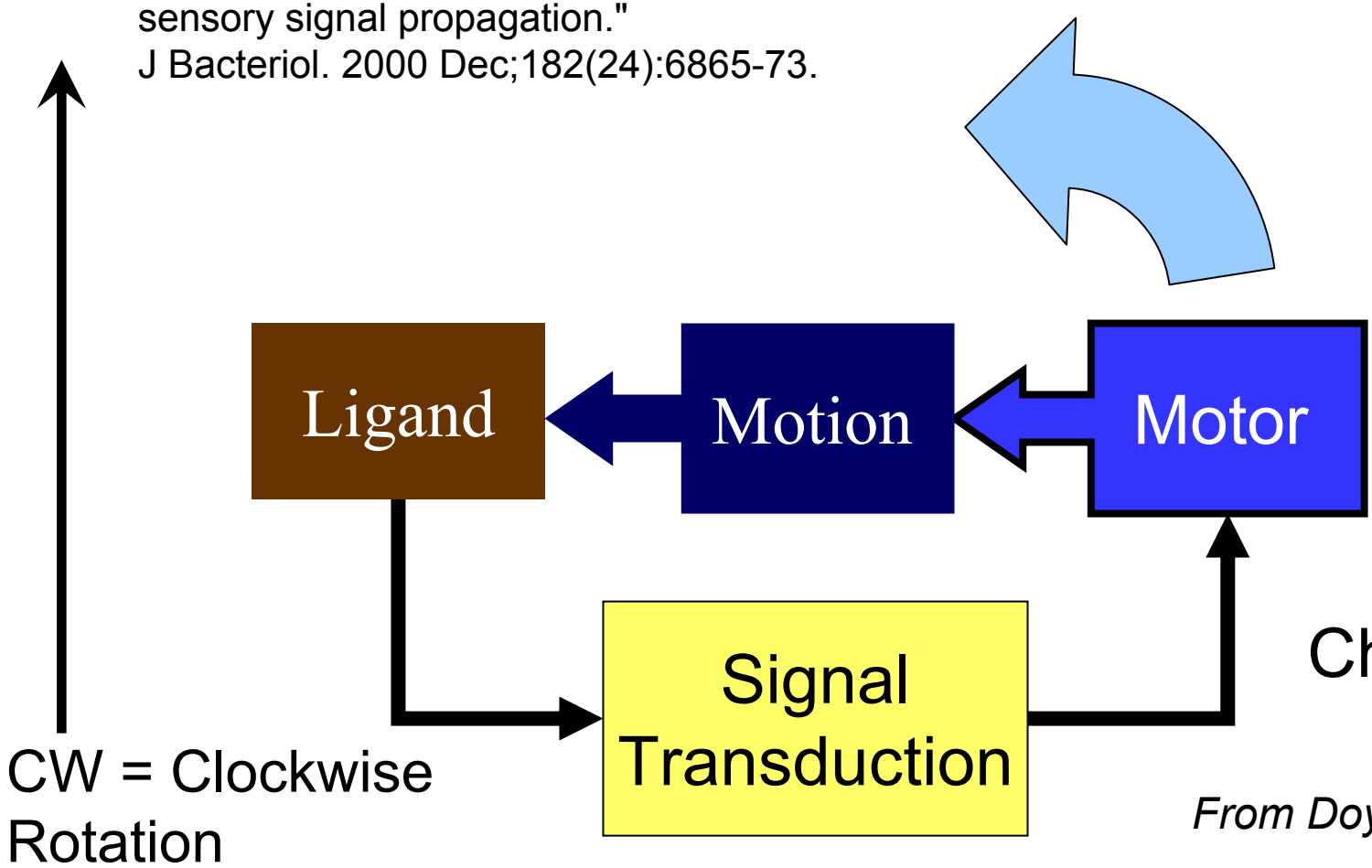


From Doyle, Simon ...

Image removed due to copyright considerations.

Please see:
Figure 2a in Bren A, Eisenbach M. "How signals are heard during bacterial chemotaxis: protein-protein interactions in sensory signal propagation."
J Bacteriol. 2000 Dec;182(24):6865-73.

High gain (cooperativity)



From Doyle, Simon ...

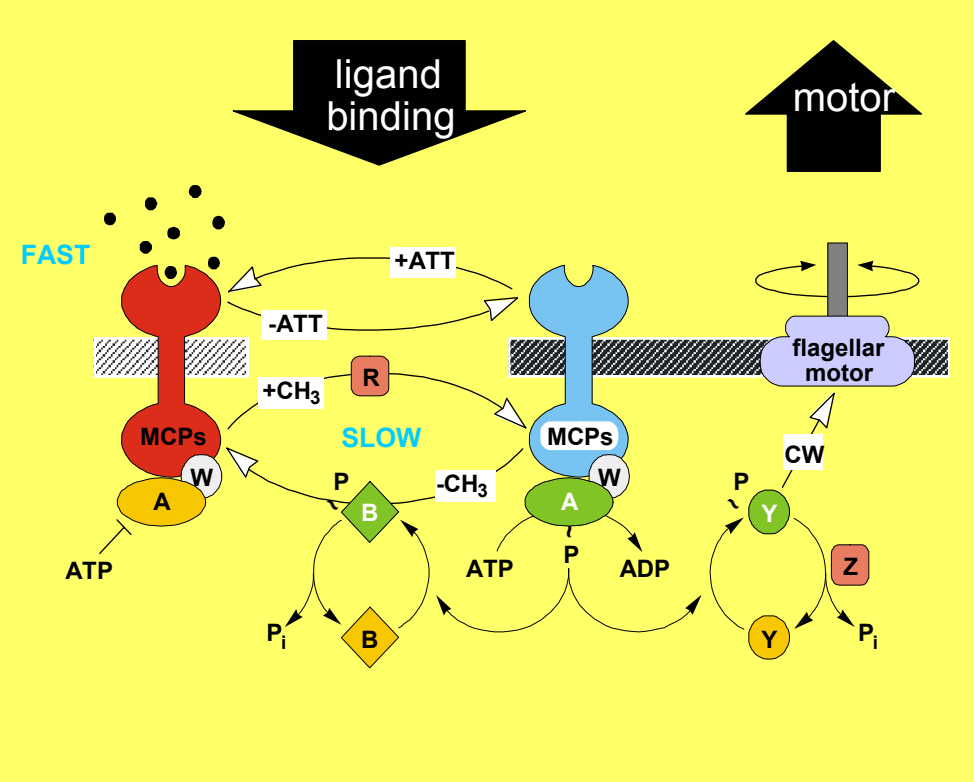
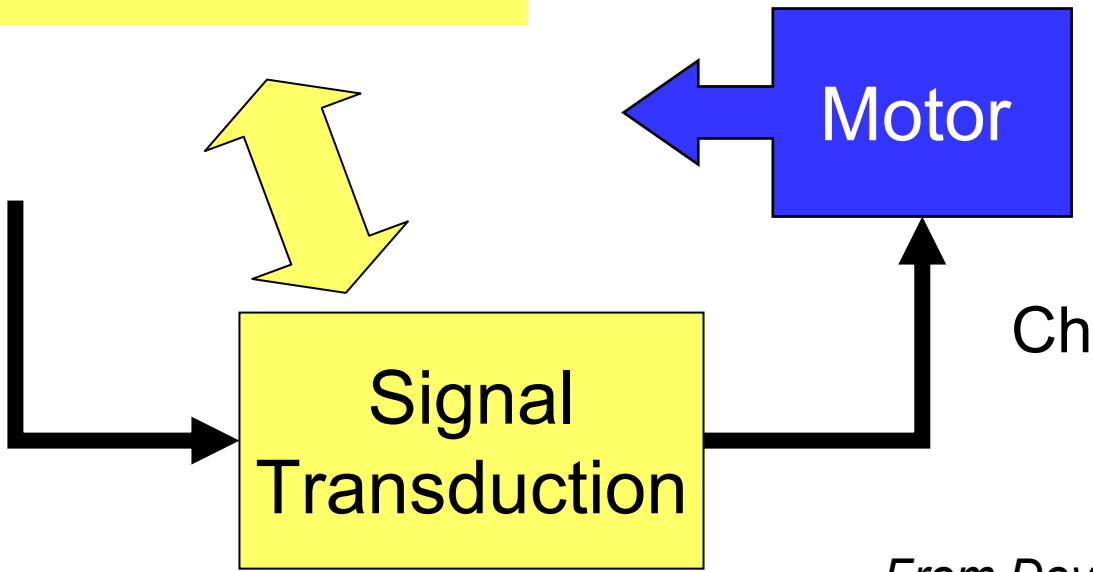


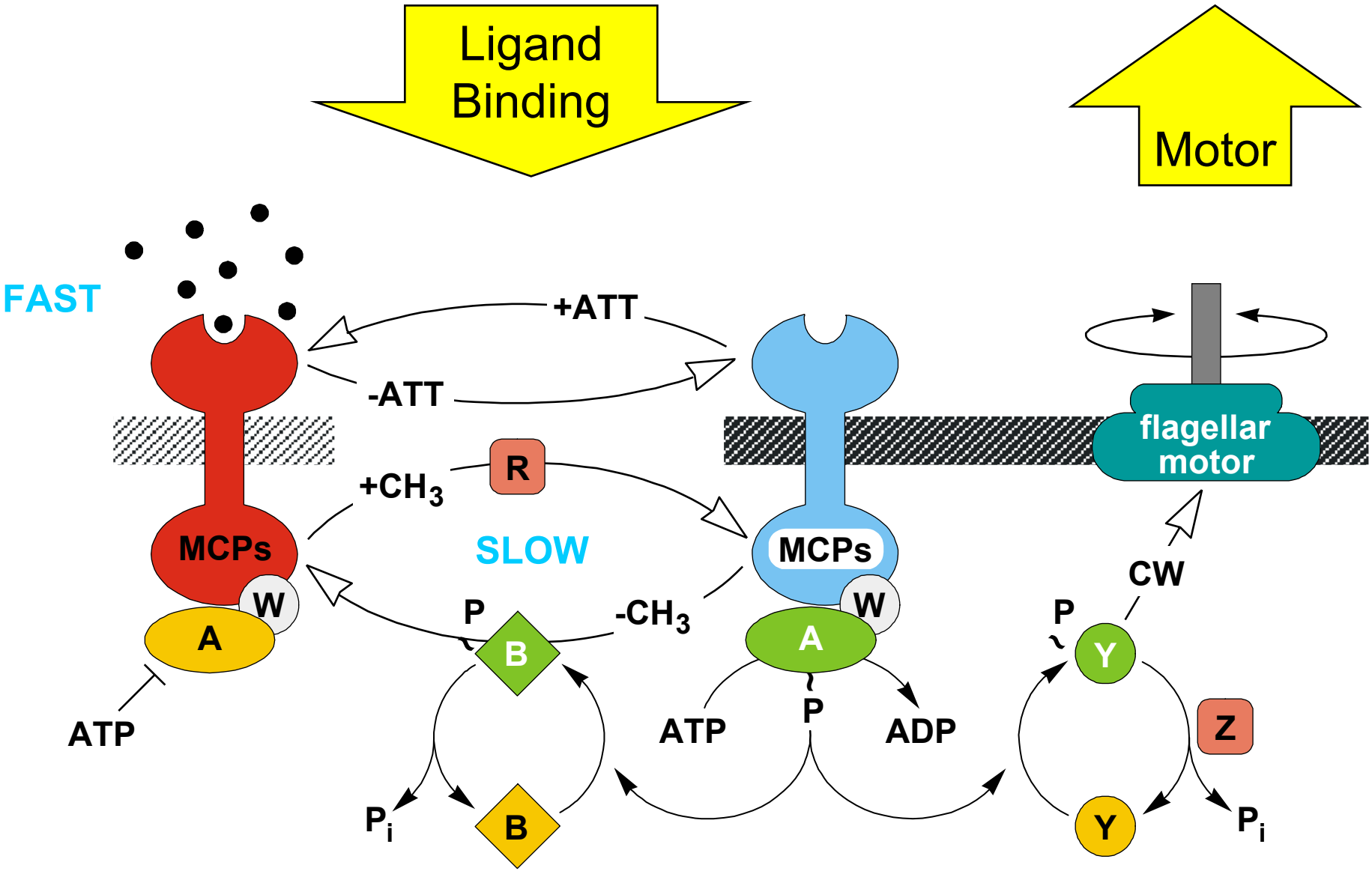
Image removed due to copyright considerations.

Please see:
Figure 2a in Bren A, Eisenbach M. "How signals are heard during bacterial chemotaxis: protein-protein interactions in sensory signal propagation." J Bacteriol. 2000 Dec;182(24):6865-73.

References:
Cluzel, Surette, Leibler +
Alon, Barkai, Bray, Simon, Spiro, Stock, Berg, …



From Doyle, Simon …



ATT = Attractant

From Doyle, Simon ...

Linking CheY-PO_4 to Motion (To Tumble or Not to Tumble)

Image removed due to copyright considerations.

Please see:
Figure 1 in Bren A, Eisenbach M. "How signals are heard during bacterial chemotaxis: protein-protein interactions in sensory signal propagation." *J Bacteriol*. 2000 Dec;182(24):6865-73.

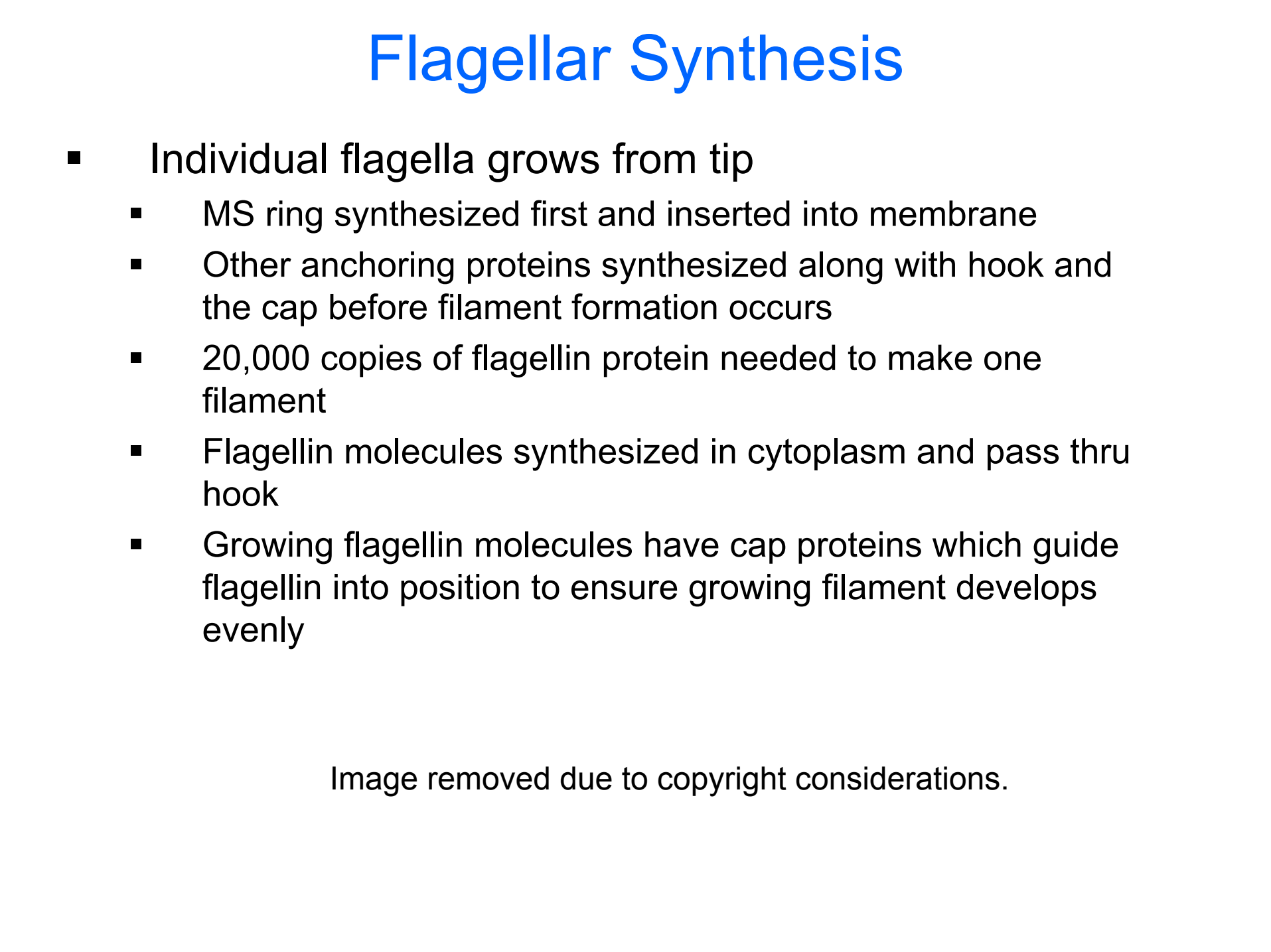
Bacterial Flagellar Motor

Image removed due to copyright considerations.

Please see:
Figure 15-61 in Alberts, Bruce et al. *Art of Molecular Biology of the Cell*. Philadelphia: Taylor & Francis, Inc., 2002 (CD-ROM). ISBN: 0-8153-4083-4.

Flagellar Synthesis

- Individual flagella grows from tip
  - MS ring synthesized first and inserted into membrane
  - Other anchoring proteins synthesized along with hook and the cap before filament formation occurs
  - 20,000 copies of flagellin protein needed to make one filament
  - Flagellin molecules synthesized in cytoplasm and pass thru hook
  - Growing flagellin molecules have cap proteins which guide flagellin into position to ensure growing filament develops evenly



Structure of the Flagellum

Next Slide Has More Detail

Image removed due to copyright considerations.

More Detail
Flagellar Motor

Image removed due to copyright considerations.

Please see:
Figure 1 in Berg HC. "The rotary motor of bacterial flagella." *Annu Rev Biochem*. 2003;72:19-54. Epub 2002 Dec 11.

Flagellar Movement

1. Moves by rotation like a propeller
2. Rotary motion from motor
3. Energy required comes from proton motive force
    - H+ movement across membrane through Mot complex drives rotation of flagellum
    - 1000 H+ must be translocated per single rotation of flagellum

Where Do the Protons Come From?

Image removed due to copyright considerations.

- NADH donates electrons to the electron transport chain, with O_2 as the ultimate acceptor of electrons.
- Protons are pumped into the periplasmic space.
- The proton gradient thus generated drives the flagellum, as well as lactose and proline symports, sodium and calcium ion antiports, and the F0F1 ATPase.

Hinkle and McCarthy, 1978

Image removed due to copyright considerations.

Putting Things Into Perspective

- Oxidation of one NADH at its dehydrogenase yields 10 protons pumped across the membrane
- Ten protons returned to the cytoplasm (on left in figure) yields 3 ATPs
- One flagellar rotation is 100 NADHs or the energy equivalent of 300 ATPs

Flagellar Movement

- Flagella do not rotate at constant speed
  - Increase or decrease speed in relation to strength of proton motive force
- Flagella can move bacteria 60 cell lengths/sec
  - Only 0.00017 km/hr, but extremely fast when compared to higher organisms in terms of number of lengths moved per second
- Cheetah: 110 km/hr, only 25 body lengths/sec
- When size accounted for, prokaryotic cells swimming at 50-60 lengths/sec move much faster than higher organisms!!

Negative Feedback Loop

Role of CheB

Image removed due to copyright considerations.

Please see:
Blair, DF. "How Bacteria Sense and Swim." *Annual Review of Microbiology*. Volume 49, Page 489-520, October 1995

Note: As of 2002, It is not thought that CheB phosphorylation Plays a direct role in Adaptation (it may play Some role, however).

Rather, the methylation Status of the receptor seems to be the signal. Things Are vague …..

David Blair, 1995

Other Taxes

1. Aerotaxis: movement toward or away from air
2. Osmotaxis: movement toward or away from conditions of high ionic strength
3. Cells sample environment and process information via signal transduction pathway
    - Leads to control of direction of flagellation
    - Motile prokaryotes aware of chemical and physical state of environment
    - Can move toward or away from various stimuli to remain competitively successful

Ideas for a Grant Proposal

- There are at least five different receptor types (Tsr, Aer, etc.). What if a cell received conflicting signals? That is, O2 decreased in one direction and Ser increased in the other. Would the cell go for the O2 or the Ser? How are such decisions made?
- Has anyone tried to exploit an understanding of chemotaxis to design an antibiotic?

Ideas for a Grant Proposal

- Could we design a unique chemotactic system? See Elowitz and Leibler, Nature 403: 335 (2000).
- CheW seems to be a mystery. Can we clarify how it works?
- How does Aer sense the redox state of the cell (via its flavin)? How does it interface wth CheY? Where is it spatially as compared to the location of the respiratory apparatus (is Aer only at the cell poles, whereas the respiratory apparatus is distributed more uniformly?)

Ideas for a Grant Proposal

- The Kiessling work offers interesting opportunities. Could we design a custom ligand to trigger a specific periplasmic domain (e.g., Tsr) -- and then tether that domain to the CheY signaling system. Instead of FliM, perhaps link FliM to some other response mediator -- so the custom ligand (unique to the cell) triggers a unique programmable response; maybe a proton pump fueled molecular machine designed to do a task we define, like open a gate).
- Are genes for enzymes that introduce Asp, ribose, etc. into metabolism induced if chemotaxis is triggered? If so, is it a global response to all attractants or just to the subset to which a receptor responds?
- Is CheR regulated (go back and look at its slide)?