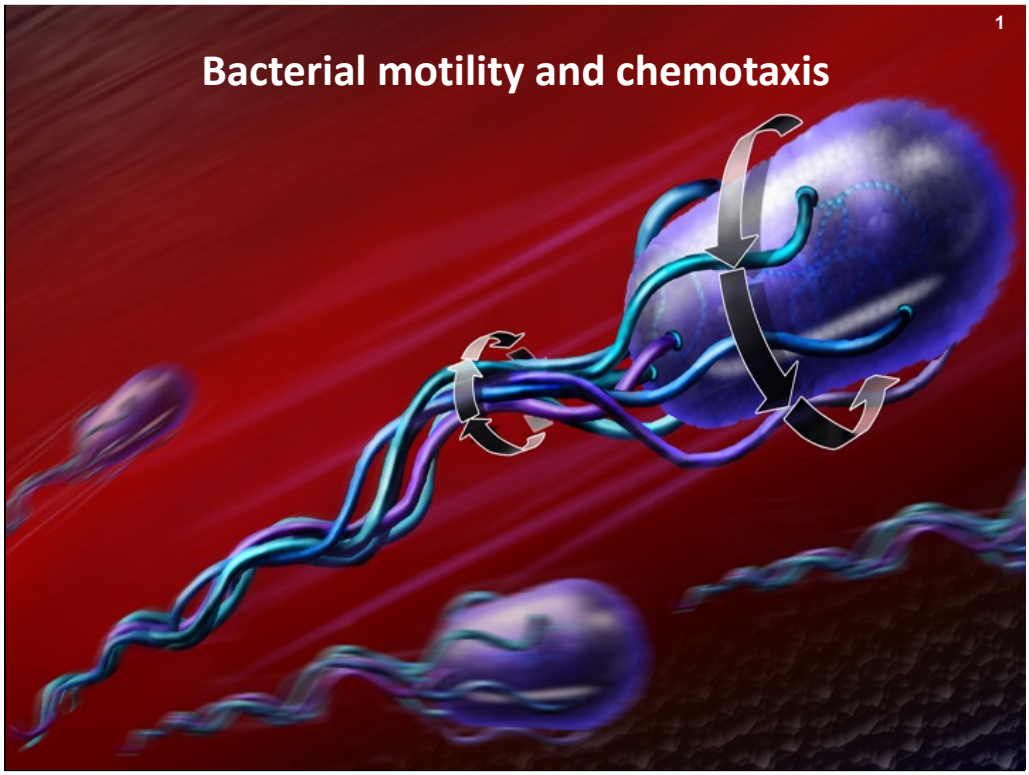
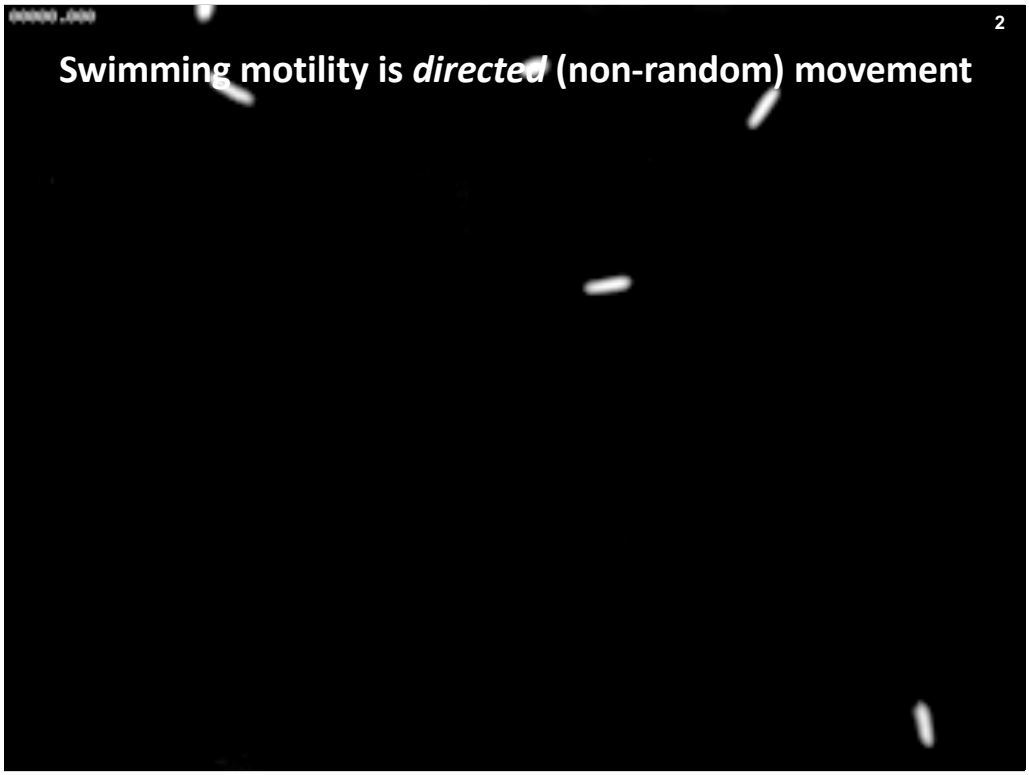

1
Bacterial motility and chemotaxis

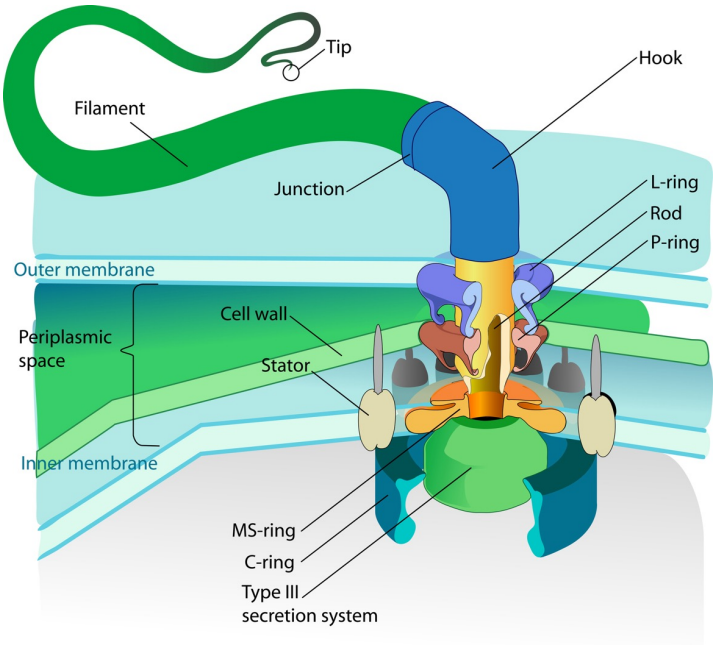


See: Movie_Slide02 posted on Moodle.

SOURCE: http://www.rowland.harvard.edu/labs/bacteria/movies_smarc.html

Movie 1. Swimming *Serratia marcescens.* When grown on soft agar and a rich medium, cells of *Serratia marcescens* (and other Gram-negative bacteria) elongate and produce more flagella; these "swarmer cells" move over the surface of the agar in a coordinated manner. Serrawettin, a lipopeptide, appears to be important as a wetting agent. Swarming is more vigorous in *Serratia* than in *Salmonella*, and cells at the very edge of the swarm are more active. The first, darkfield video (Movie 1, this slide) shows that cells removed from a swarm swim vigorously when placed in liquid media. The second, phase-contrast video (Movie 2, next slide) shows spreading near the edge of a swarm on solid media.

Flagella are cell-surface appendages that mediate motility



SOURCE (IMAGE): http://en.wikipedia.org/wiki/Flagellum

SOURCE (TEXT): *Brock Biology of Microorganisms [15th Edition]*, Chapter 2: Microbial Cell Structure and Function (pp. 70-108), published by Benjamin Cummings © 2018.

Figure 2.37. Structure of the flagellum and rotary motor in Gram-negative bacteria. The L ring is embedded in the LPS. The P ring is embedded in the peptidoglycan. The MS ring is embedded in the cytoplasmic membrane. The C ring is located in the cytoplasm. A narrow channel exists in the rod and filament through which flagellin molecules diffuse to reach the site of flagellar synthesis at the distal tip of the flagellum. The Mot proteins function as the flagellar motor. The Fli proteins function as the motor switch. The flagellar motor rotates the filament to propel the cell through the medium.

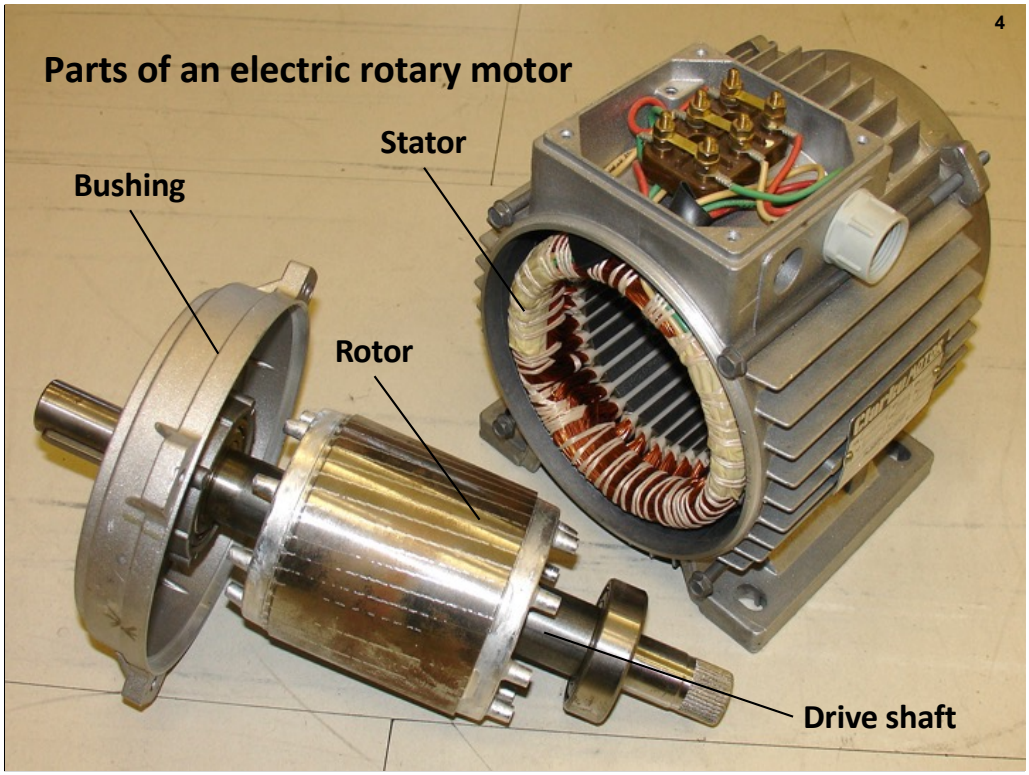
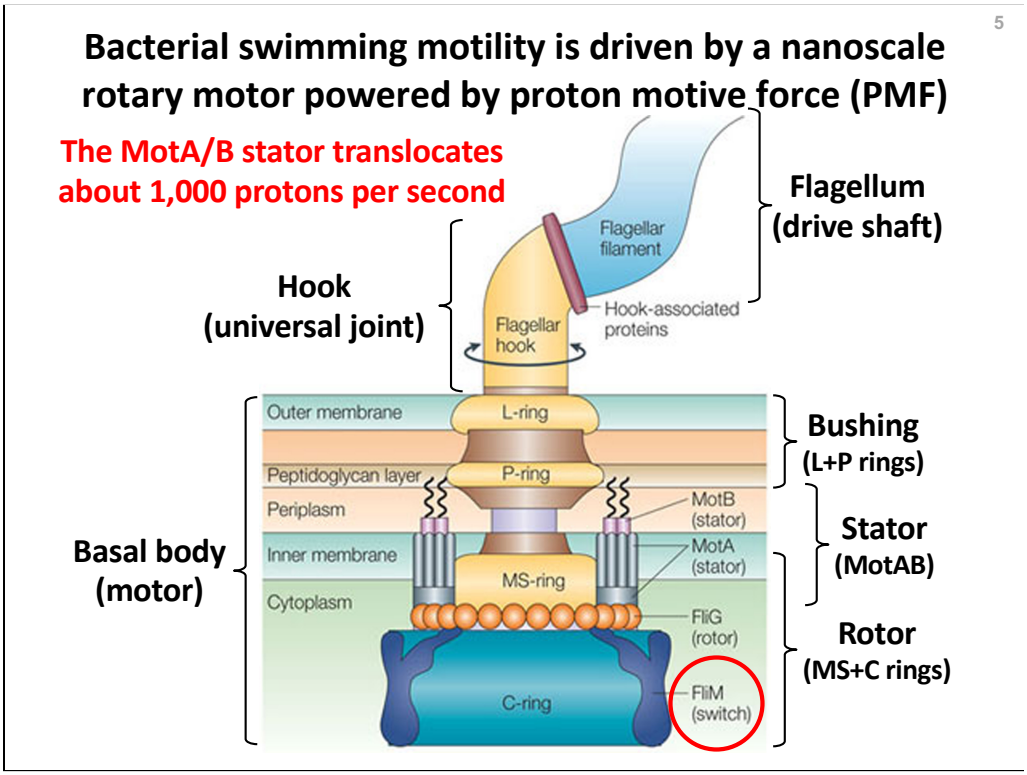


PHOTO SOURCE: http://en.wikipedia.org/wiki/Stator

Basic components of a rotary motor: stator, rotor, drive shaft, bushing.



SOURCE: Xing J, Bai F, Berry R, Oster G (2006) Torque-speed relationship of the bacterial flagellar motor. *Proc Natl Acad Sci USA* 103(5): 1260-1265 PMID:16432218.

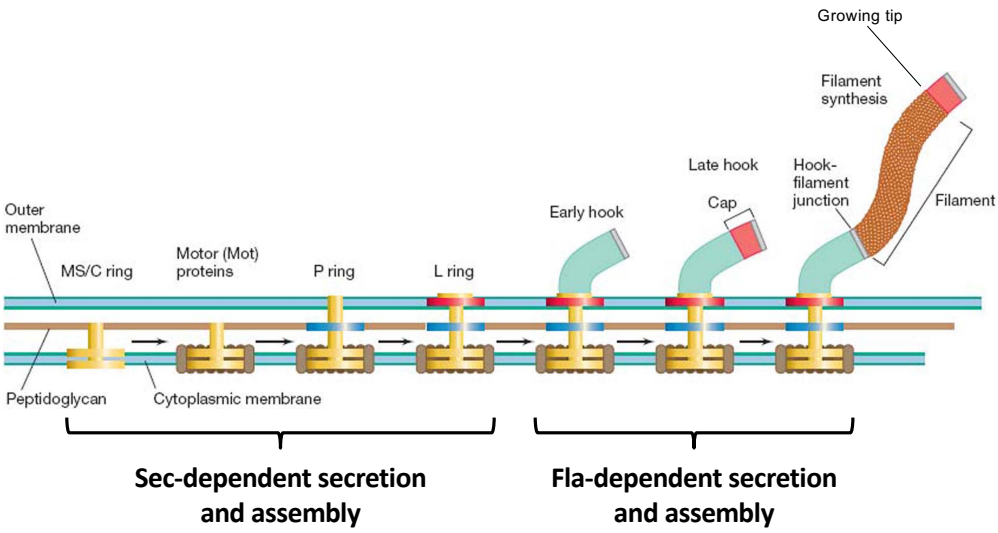
SOURCE: Minamino T, Imada K, Namba K (2008) Molecular motors of the bacterial flagella. *Curr Opin Struct Biol* 18(6): 693-701 PMID:18848888.

SOURCE: Jarrell KF, McBride MJ (2008) The surprisingly diverse ways that prokaryotes move. *Nat Rev Microbiol* 6(6): 466-476 PMID:18461074.

SOURCE: Wadhams GH, Armitage JP (2004) Making sense of it all: bacterial chemotaxis. *Nat Rev Mol Cell Biol* 5(12): 1024-1037 PMID:15573139.

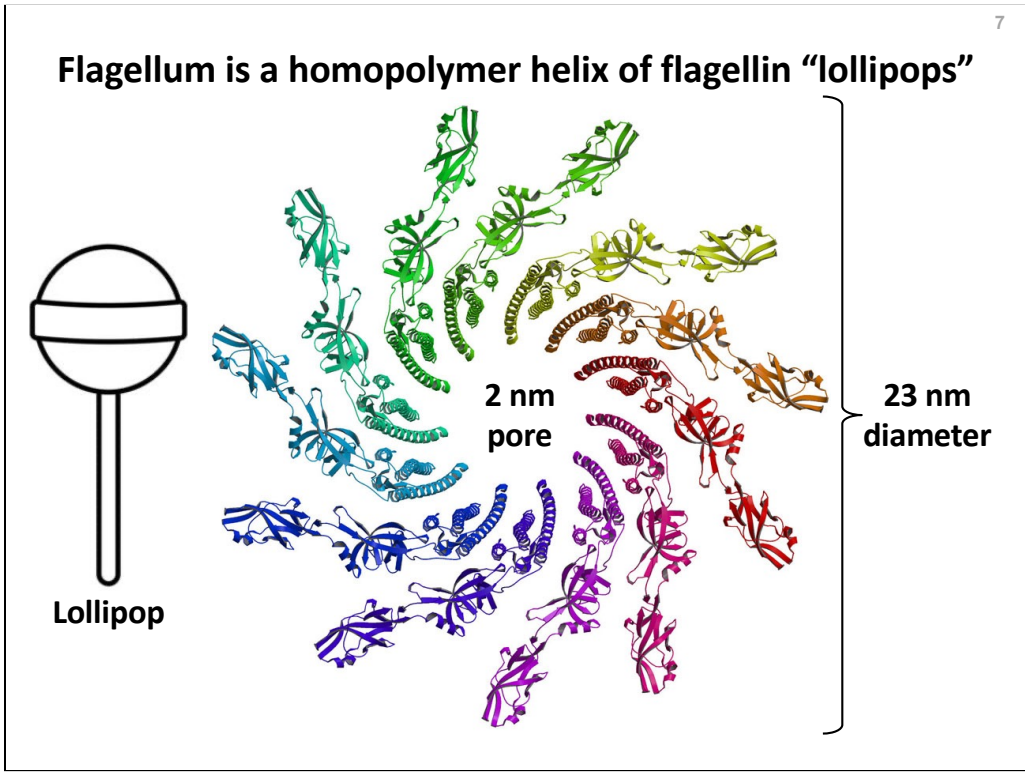
Box 3. A cartoon of the key structural components involved in torque generation. The bacterial flagellar motor is the most complex structure in a bacterial cell, with protein components in the cytoplasm, across the cytoplasmic membrane, the periplasmic space, the outer membrane and in the external environment (the figure shows the structure of the *Escherichia coli* motor complex). It is the product of the controlled expression of about 50 genes. Expression of the genes is tightly regulated to assure ordered protein assembly. Whereas the early transmembrane proteins are transported through the classic Sec secretion pathway, the later proteins, including those of the hook and filament, use a flagellar-specific export pathway (Fla pathway) that is closely related to that used for the type III secretion of toxins by pathogenic species.

Assembly of the bacterial motor and flagellum



SOURCE: *Brock Biology of Microorganisms [15th Edition]*, Chapter 2: Microbial Cell Structure and Function (pp. 70-108), published by Benjamin Cummings © 2018.

Figure 2.38. Flagella biosynthesis. Synthesis begins with assembly of MS and C rings in the cytoplasmic membrane. Then the other rings, the hook and the cap, form. At this point, flagellin protein flows through the hook to form the filament. Flagellin molecules are guided into position by cap proteins to ensure that the growing filament develops evenly.



SOURCE: http://www.nanonet.go.jp/english/mailmag/2004/011a.html

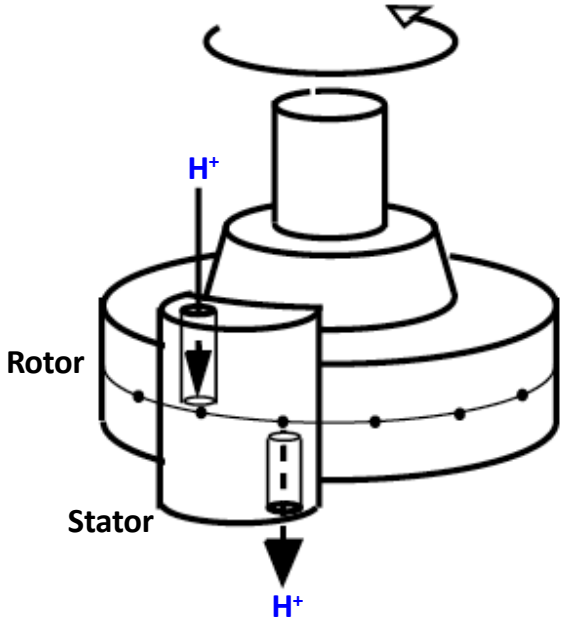
Figure 6. Partial atomic model of the *Escherichia coli* flagellar filament in the end-on view (like looking down the barrel of a rifle). The diameter is 23 nm. The central channel appears large because the inner core domain formed by both terminal regions is missing in this model. The actual internal diameter of the channel is only about 2 nm.

See: Movie_Slide08 posted on Moodle.

MOVIE SOURCE: http://www.fbs.osaka-u.ac.jp/labs/namba/npn/movie5.html (website of Keiichi Namba's laboratory).

Figure 7. Self-assembly process of the bacterial flagellum. The process goes from the top left to the bottom right corner. Once the FliF ring (in brown) has formed in the cytoplasmic membrane, other protein molecules are self-assembled on this structural base one after another in a well-defined order. All the axial component proteins of the flagellum are synthesized in the cytoplasm and transported by the type III flagellar protein export apparatus (Fli pathway) through the long narrow central channel to the distal end of growing structure, onto which they self-assemble. The assembly process requires three different types of caps at different stages, and these caps are always attached at the distal end of the growing structure to promote efficient self-assembly of component molecules.

"Thermal ratchet model" of rotary motor function



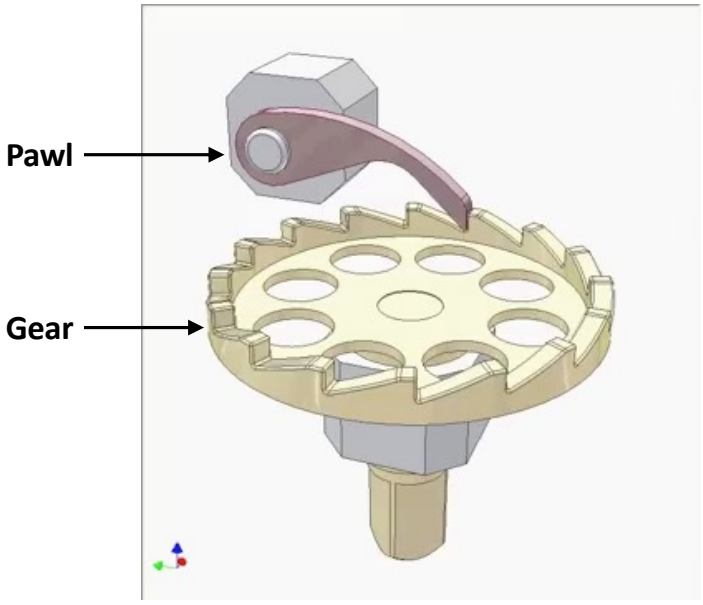
SOURCE: Elston TC, Oster G (1997) Protein turbines. I: The bacterial flagellar motor. *Biophys J* 73(2): 703-721 PMID:9251788.

SOURCE: Berry RM (2000) Theories of rotary motors. *Philos Trans R Soc Lond B Biol Sci* 355(1396): 503-509 PMID:10836503.

Figure 2B. In the "thermal ratchet" model of rotary motor function (a.k.a. the "turnstile model"), ions are deposited onto the rotor by channels that extend to the outside of the cell (top), and are removed by separate channels that extend into the cytoplasm (bottom). To pass into the cell, ions must be carried from one type of channel to the other by rotation of the rotor. The model relies upon thermal fluctuations to carry the rotor and the stator unit past each other to the point where the proton can pass into the cell. The model is a "thermal ratchet", in that the role of the free energy supplied by the influx of protons is to "save" thermal fluctuations in the "correct" direction rather than to create directly a torque-generating state.

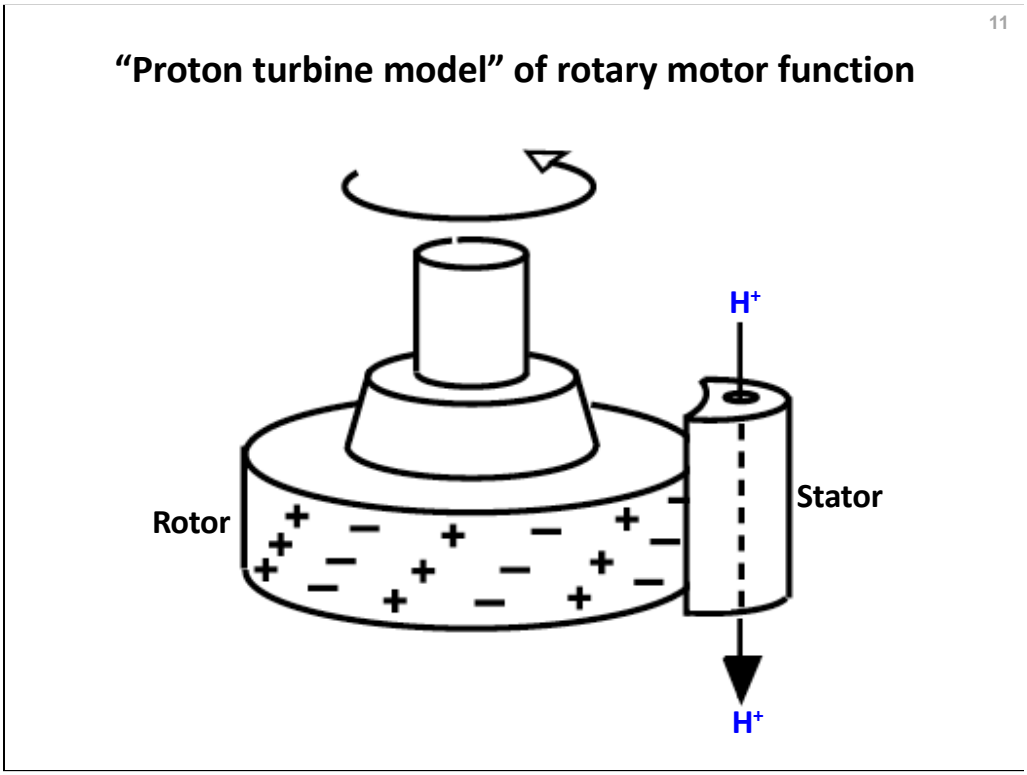
10

Ratchets (cliquets) restrict movement to one direction



See: Movie_Slide10 posted on Moodle

A **ratchet** consists of a round **gear** (or a linear rack with teeth) and a pivoting, spring-loaded finger called a **pawl** that engages the teeth.

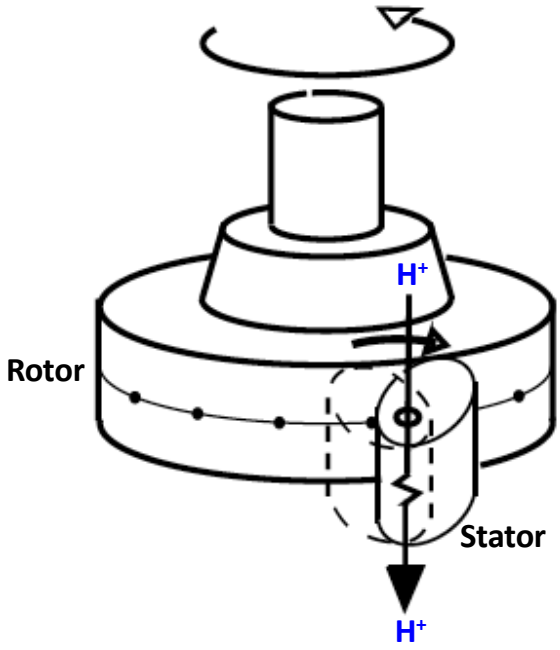


SOURCE: Elston TC, Oster G (1997) Protein turbines. I: The bacterial flagellar motor. *Biophys J* 73(2): 703-721 PMID:9251788.

SOURCE: Berry RM (2000) Theories of rotary motors. *Philos Trans R Soc Lond B Biol Sci* 355(1396): 503-509 PMID:10836503.

Figure 2C. A "proton turbine" model has been proposed to explain rotation of the flagellum. Positively charged ions flowing through the stator attract lines of negative charge and/or repel lines of positive charge on the rotor. These electrostatic forces keep the line of negative charges close to the ion as it passes into the cell, which leads to rotation if the lines of charge on the rotor are tilted relative to the ion channel in the stator.

"Mechanochemical model" of rotary motor function

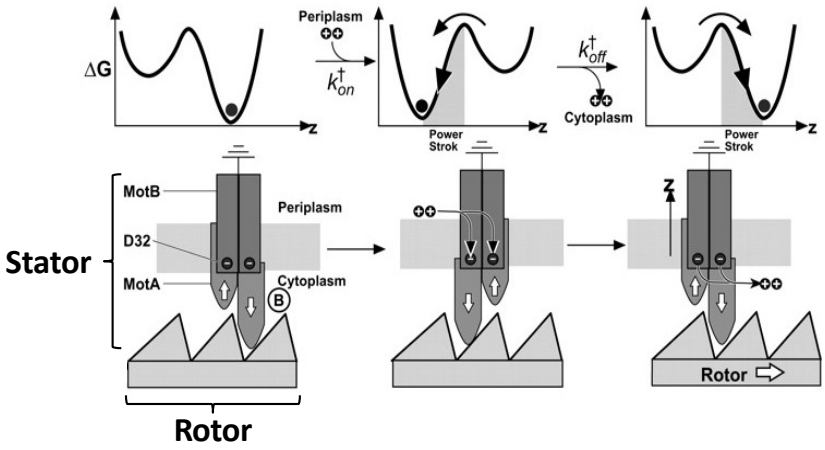


SOURCE: Elston TC, Oster G (1997) Protein turbines. I: The bacterial flagellar motor. *Biophys J* 73(2): 703-721 PMID:9251788.

SOURCE: Berry RM (2000) Theories of rotary motors. *Philos Trans R Soc Lond B Biol Sci* 355(1396): 503-509 PMID:10836503.

Figure 2A. In the "mechanochemical" model of rotary motor function, torque is generated by the following mechanical cycle. (i) The stator unit binds the rotor in the conformation indicated by the dashed lines. (ii) A conformational change to the conformation indicated by the solid lines occurs, generating torque via the attachment between the rotor and stator. (iii) The stator unbinds from the rotor. (iv) The stator returns to its original conformation, completing the cycle. This mechanical cycle is coupled to the influx of one or more protons through the stator unit.

"Mechanochemical model" of rotary motor function



SOURCE: Xing J, Bai F, Berry R, Oster G (2006) Torque-speed relationship of the bacterial flagellar motor. *Proc Natl Acad Sci USA* 103(5): 1260-1265 PMID:16432218.

Figure 4. The "mechanochemical model" of the rotor-stator interaction. The stator assembly $MotA_4/MotB_2$ is a bistable system: two conformations are separated by an energy barrier. The figure shows a schematic illustration of one motor cycle.

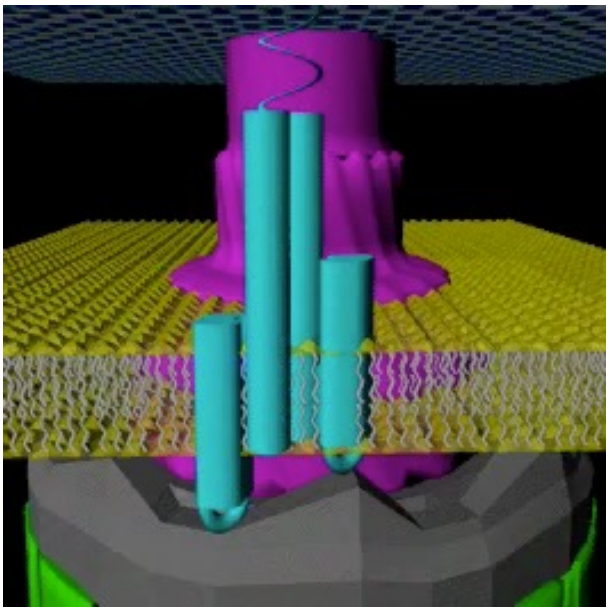
Step 1. At the end of the previous cycle, Aspartate-32 (D32) residues on the stator are unprotonated and the stable conformation is as shown on the left; the cytoplasmic loop of one MotA (the right one in the figure) is down, engaging the rotor. Binding of two protons to the MotB D32 residues neutralizes them, allowing a thermally activated transition to the alternate conformational equilibrium to perform the first power stroke with the other MotA loop engaging the rotor. This process is characterized by the **transition rate**, which is a composite of **ion hopping on rates** and the **thermally activated conformational transition rate**.

Step 2. At the end of the first power stroke, the two binding protons are released to the cytoplasm. This transition triggers another conformational change of the stator so the (right) MotA loop engages to the rotor to perform the second power stroke. This process is characterized by the **transition rate**, which is a composite of **ion-hopping off rates** and the **thermally activated conformational transition rate**. At the end of the cycle, the stator has returned to its conformation at the beginning of the cycle, with the rotor advancing one step to the right.

On finishing one cycle, the cytoplasmic loop should traverse a closed cycle with nonzero area. During the entire two-step cycle, the rotor is almost always engaged, so that the duty ratio is close to 1. The stator loops interact sterically with 26 copies of FliG arrayed circumferentially around the rotor. The asymmetry in the steric interaction determines the direction of rotation; reversals are triggered by reversing this asymmetry in response to CheY binding to the rotor. The motion of the rotor is tracked by means of a large load (with drag coefficient $_L$) attached to the motor via a compliant elastic linkage.

14

“Mechanochemical model” of rotary motor function



See: Movie_Slide14 posted on Moodle.

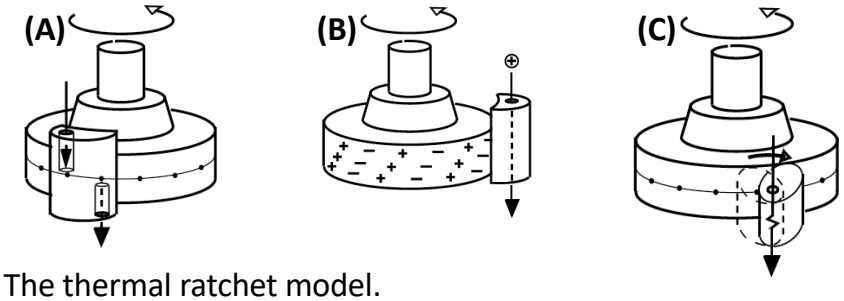
SOURCE: Xing J, Bai F, Berry R, Oster G (2006) Torque-speed relationship of the bacterial flagellar motor. *Proc Natl Acad Sci USA* 103(5): 1260-1265 PMID:16432218.

SOURCE: http://www.pnas.org/content/103/5/1260/suppl/DC1

Supporting Movie 1 (online). The bacterial flagellar rotary motor power stroke. The movie schematically illustrates motor cycles. The stator complex can exist in two conformations, and the transitions between these conformations are triggered by successive binding and releasing of two protons to and from the two negatively charged Aspartate-32 (D32) residues on the two MotB helices in the stator. The torque thus generated is transmitted to the rotor when the MotA loops are in contact with the FliG molecules. Actual motion of the MotA/MotB complex may be rotational as well as vertical.

15

Which model of the bacterial rotary motor involves conformational changes to the stator complex?



A. The thermal ratchet model.

B. The proton turbine model.

C. The mechanochemical model.

Answer: (C)

Question: How fast can bacteria swim?

H^+-driven motor rotates at 300 Hz (18k rpm)
Na^+-driven motor rotates at 1,667 Hz (100k rpm)

***Escherichia coli* swims at 25-35 µm s^{-1}**

If I swam that fast in relation to my body length,
I would be clocking in at 65 m s^{-1} (234 km h^{-1})

***Bdellovibrio bacteriovorus* swims at 160 µm s^{-1}**

If I swam that fast in relation to my body length,
I would be clocking in at 300 m s^{-1} (1,100 km h^{-1})

Answer: Very fast (relative to their size)!

SOURCE: Jarrell KF, McBride MJ (2008) The surprisingly diverse ways that prokaryotes move. *Nat Rev Microbiol* 6(6): 466-476 PMID:18461074.

17

Question: What is the *specific power* (power output per kg) of the bacterial rotary motor?

A H^+-driven motor rotates at about 300 s^{-1} (18k rpm)

Motor torque (work) is about 1.3 x 10^{-18} N * m

Motor power is about 2.4 x 10^{-15} watts (in N * m * s^{-1})

Motor size is about 50 nm x 50 nm (cylinder)

Motor volume is about 10^{-22} m^3

Motor mass is about 1.3 x 10^{-19} kg

Specific power is about 18,460 watts per kg

SOURCE (Torque): Shrivastava A, Lele PP, Berg HC (2015) A rotary motor drives *Flavobacterium* gliding. *Curr Biol* 25(3): 338-341 PMID: 25619763.

SOURCE: Peid SW, Leake MC, Chandler JH, Lo CJ, Armitage JP, Berry RM (2006) The maximum number of torque-bearing units in the flagellar motor of *Escherichia coli* is at least 11. *Proc Natl Acad Sci USA* 103(21): 8066-8071 PMID: 16698936.

SOURCE: Xue R, Ma Q, Baker MA, Bai F (2015) A delicate nanoscale motor made by nature – the bacterial flagellar motor. *Adv Sci (Weinh)* 2(9): 1500129 PMID: 27980978.

SOURCE: Marden JH (2005) Scaling of maximum net force output by motors used for locomotion. *J Exp Bio.* 208(9): 1653-166 PMID: 15855397.

Power per unit mass, also known as **specific power**, is the power output for a given mass of a substance or machine. In the case of an engine or vehicle this would be referred to as the power-to-mass ratio.

For a "typical" bacterial rotary motor:

Rotation frequency = 300 s^{-1}

Angular velocity = 2π * rotation frequency = 6.28 * 300 s^{-1} = 1,884 s^{-1}

Torque = Force * Distance = 1,300 pN * nm = 1.3 x 10^{-18} N * m

Power Output = Angular Velocity * Torque = (1884 * s^{-1}) * (1.3 x 10^{-18} N * m) = 2.4 x 10^{-15} N * m * s^{-1} = 2.4 x 10^{-15} watts

Motor size is about 50 nm x 50 nm (cylinder)

Motor volume is about 10^5 nm^3 = 10^{-22} m^3

Protein density is about 1.3 g cm^{-3} = 1.3 x 10^6 g m^{-3}

Motor mass is about (10^{-22} m^3) * (1.3 x 10^6 g m^{-3}) = 1.3 x 10^{-16} g = 1.3 x 10^{-19} kg

Power output per kg = (2.4 x 10^{-15} watts) * (1.3 x 10^{-19} kg)$^{-1}$ = 18,460 watts * kg^{-1}

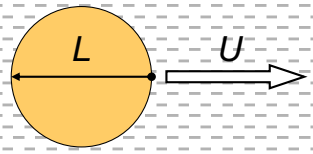
ENGINE/MOTOR	SPECIFIC POWER (watts * kg^{-1})
Steam pump (18th century)	10
Eukaryotic cilia	**30**
Electric motor	200
Skeletal muscle	**200**
Automobile engine	400
Motorcycle engine	1,000
Aircraft engine, piston	1,500
Aircraft engine, turbine	6,000
Bacterial flagellar motor	**18,000**

SOURCE: Vogel S, *Comparative Biomechanics: Life's Physical World [2nd Edition]*, Princeton University Press © 2013.

Power output per unit mass, also known as **specific power**, is the power output for a given mass of a substance or machine. In the case of an engine or vehicle this would be referred to as the power-to-mass ratio.

19

Swimming at low Reynolds number (*Re*)



ρ = fluid density = 10^3 kg * m^{-3} for water
η = fluid viscosity = 10^{-3} kg * m^{-1} * s^{-1} for water
S = cross-section area across the flow (in m^2)
U = fluid velocity (in m * s^{-1})
L = length with the flow (in m)

$S = \pi(L/2)^2$

$$\text{Reynolds number } (Re) = \frac{F_i \text{ (inertial forces)}}{F_v \text{ (viscous forces)}} = \frac{\rho S U^2}{\eta S U L^{-1}} = \frac{\rho U L}{\eta}$$

At microbial scales, fluid dynamics are dominated by viscous forces and inertial forces are negligible

Source: *Comparative Biomechanics: Life's Physical World [2nd Edition for Kindle], Chapter 6: Viscosity and the Patterns of Flow* (pp. 87-109), by Vogel S, published by Princeton University Press © 2013.

U is the fluid velocity in m * s^{-1}

L is the characteristic length in (m), by convention this is the maximum length of the object in the direction of flow across the object

η is the (absolute) dynamic fluid viscosity in Pa * s = N * s * m^{-2} = kg * m^{-1} * s^{-1}

η = 10^{-3} kg m^{-1} s^{-1} for water (you should memorize this value with the correct units!)

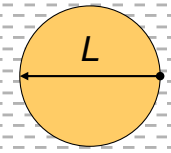
ρ is the density of the fluid in kg * m^{-3}

ρ = 10^3 kg * m^{-3} for water (you should memorize this value with the correct units!)

The equation for Reynolds number is a "primary concept" – you should memorize it and you should feel comfortable manipulating it. Indeed, you should already be familiar with this equation from your physics courses. You should also memorize the values (with units!) for the density and the dynamic viscosity of water.

A note to avoid potential confusion: You may already be familiar with kinematic fluid viscosity (ν), defined as $\nu = \eta * \rho^{-1}$ = m^2 * s^{-1}. Note that this is not the same thing as dynamic fluid viscosity (η), defined as η = Pa * s = N * s * m^{-2} = kg * m^{-1} * s^{-1}. Be careful not to confuse them with each other!

Viscous forces dominate swimming at low *Re*



$S = \pi(L/2)^2$

$\rho = 10^3$ kg m^{-3} for water
$\eta = 10^{-3}$ kg m^{-1} s^{-1} for water
$S \approx 10^{-6}$ m^2 (bacterium)
$U \approx 10^{-5}$ m s^{-1} (bacterium)
$L \approx 10^{-6}$ m (bacterium)

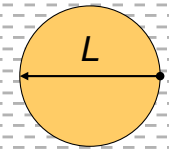
$$F_i = \rho S U^2 = (10^3 \text{ kg m}^{-3})(10^{-6} \text{ m}^2)(10^{-5} \text{ m s}^{-1})^2 = 10^{-13} \text{ kg m s}^{-2}$$

$$F_v = \eta S U L^{-1} = \frac{(10^{-3} \text{ kg m}^{-1} \text{ s}^{-1})(10^{-6} \text{ m}^2)(10^{-5} \text{ m s}^{-1})}{(10^{-6} \text{ m})} = 10^{-8} \text{ kg m s}^{-2}$$

$$\text{Reynolds number } (Re) = \frac{F_i \text{ (inertial forces)}}{F_v \text{ (viscous forces)}} = \frac{10^{-13} \text{ kg m s}^{-2}}{10^{-8} \text{ kg m s}^{-2}} = 0.00001$$

Source: *Comparative Biomechanics: Life's Physical World [2nd Edition for Kindle], Chapter 6: Viscosity and the Patterns of Flow* (pp. 87-109), by Vogel S, published by Princeton University Press © 2013.

Inertial forces dominate swimming at high *Re*



$S = \pi(L/2)^2$

$\rho = 10^3$ kg m^{-3} for water
$\eta = 10^{-3}$ kg m^{-1} s^{-1} for water
$S \approx 10^0$ m^2 (tuna fish)
$U \approx 10^1$ m s^{-1} (tuna fish)
$L \approx 10^0$ m (tuna fish)

$$F_i = \rho S U^2 = (10^3 \text{ kg m}^{-3})(10^0 \text{ m}^2)(10^1 \text{ m s}^{-1})^2 = 10^5 \text{ kg m s}^{-2}$$

$$F_v = \eta S U L^{-1} = \frac{(10^{-3} \text{ kg m}^{-1} \text{ s}^{-1})(10^0 \text{ m}^2)(10^1 \text{ m s}^{-1})}{(10^0 \text{ m})} = 10^{-2} \text{ kg m s}^{-2}$$

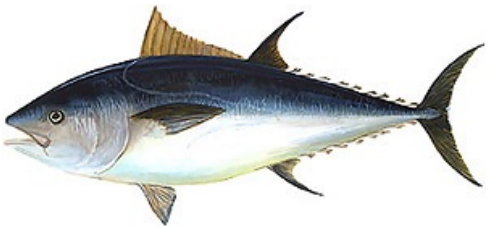
$$\text{Reynolds number } (Re) = \frac{F_i \text{ (inertial forces)}}{F_v \text{ (viscous forces)}} = \frac{10^5 \text{ kg m s}^{-2}}{10^{-2} \text{ kg m s}^{-2}} = 10{,}000{,}000$$

Source: *Comparative Biomechanics: Life's Physical World [2nd Edition for Kindle], Chapter 6: Viscosity and the Patterns of Flow* (pp. 87-109), by Vogel S, published by Princeton University Press © 2013.

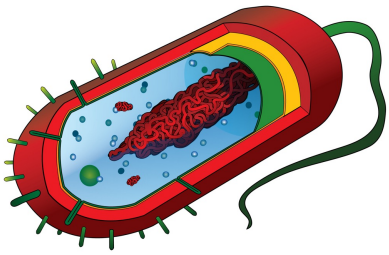
Source: https://www.fisheries.noaa.gov/feature-story/fun-facts-about-atlantic-tunas.

Yellowfin tunafish can swim faster than 80 kilometers per hour = 22 meters per second.

Question: Why aren't bacteria streamlined?



Tuna fish are streamlined (*Re* is about 10,000,000)

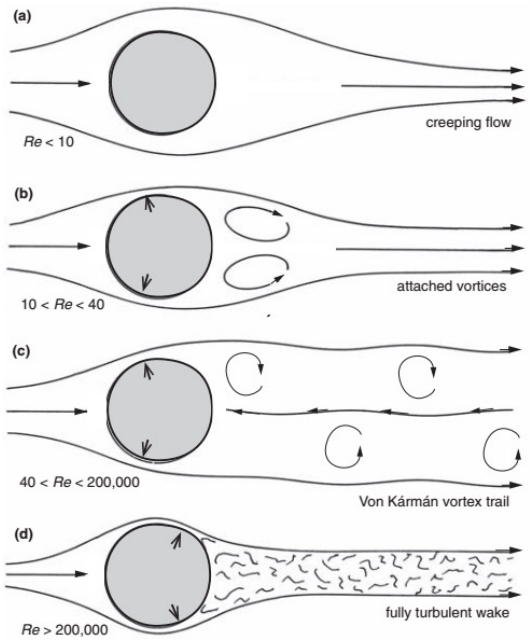


Bacteria are not streamlined (*Re* is about 0.00001)

Tuna fish have streamlined bodies built for speed and endurance. They can even retract their dorsal and pectoral fins into slots to reduce drag.

Bacterial cells are not streamlined. Why not?

Laminar flow (low *Re*) vs. turbulent flow (high *Re*)



Re (bacteria) = 0.00001

$$Re = \frac{\text{inertial forces}}{\text{viscous forces}} = \frac{\rho U L}{\eta}$$

Re (tuna fish) = 10,000,000

Source: *Comparative Biomechanics: Life's Physical World [2nd Edition for Kindle], Chapter 6: Viscosity and the Patterns of Flow* (pp. 87-109), by Vogel S, published by Princeton University Press © 2013.

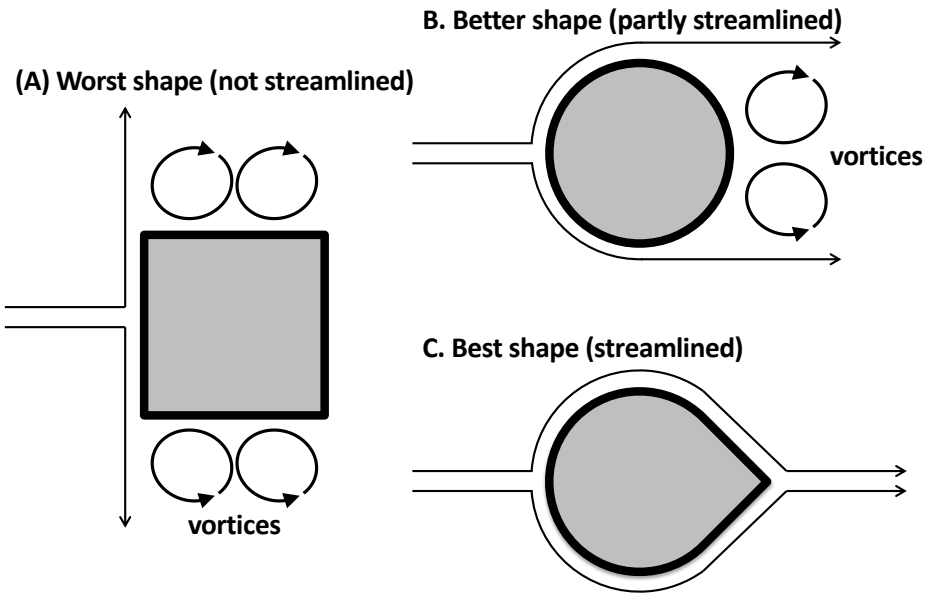
Figure 6.8. The character of flow around a circular cylinder (shown in cross section) depends strongly on the Reynolds number. Orderly flows (a) break into attached vortices (b), which are then periodically shed (c), and finally become disorganized in a wake that's narrower overall (d). Arrows mark the separation points.

At Reynolds numbers < 10 flow is "boring" (laminar and predictable).

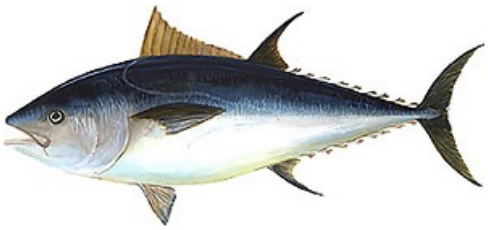
At Reynolds numbers > 200,000 flow is turbulent.

At Reynolds numbers > 10 but < 200,000 flow is "interesting" (chaotic and unpredictable)...

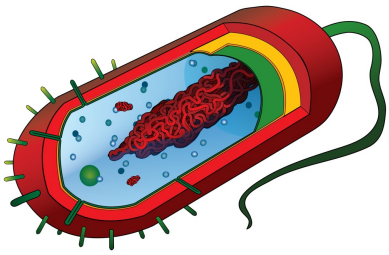
"Streamlining" prevents premature detachment of streamlines from an object moving through a fluid



The optimal shape for swimming depends on the scale!



Tuna fish are streamlined (minimizes turbulence)



Bacteria are not streamlined (minimizes skin friction)

Tuna fish have streamlined bodies built for speed and endurance. They can even retract their dorsal and pectoral fins into slots to reduce drag. At high Reynolds Number, streamlining increases swimming speed by decreasing turbulence. However, this comes at the price of increasing skin frication, which reduces swimming speed.

Bacterial cells are not streamlined. At low Reynolds number, turbulence isn't an issue so there is no advantage to streamlining. However, skin friction decreases swimming speed, even at low Reynolds Number, so streamlining would actually be disadvantageous at low Reynolds number.

26

Bacteria live in a world of low Reynolds number (*Re*) because:

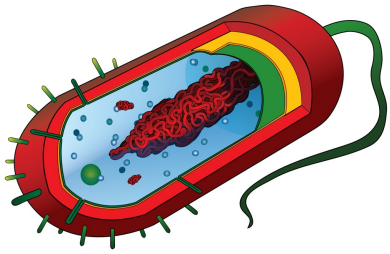
A. Bacteria move slowly (in absolute terms).

B. Bacteria are small.

C. Viscous forces are strong.

D. Inertial forces are weak.

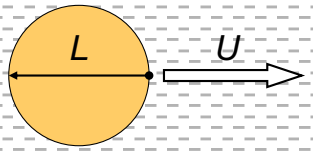
E. All of the above.



Answer: (E)

27

Consider a bacterial cell swimming in water…what will happen if the flagellar motor stops rotating?



$S = \pi(L/2)^2$

$\rho = 10^3$ kg * m^{-3} for water
$\eta = 10^{-3}$ kg * m^{-1} * s^{-1} for water
$S \approx 10^{-6}$ m^2 (bacterium)
$U \approx 10^{-5}$ m * s^{-1} (bacterium)
$L \approx 10^{-6}$ m (bacterium)
$Re = 0.00001 = 10^{-5}$

Coasting time ~ 0.6 microseconds
Coasting distance ~ 0.01 nm

The diameter of a hydrogen atom is ~ 0.1 nm

Conclusion: bacteria do not "coast along"!

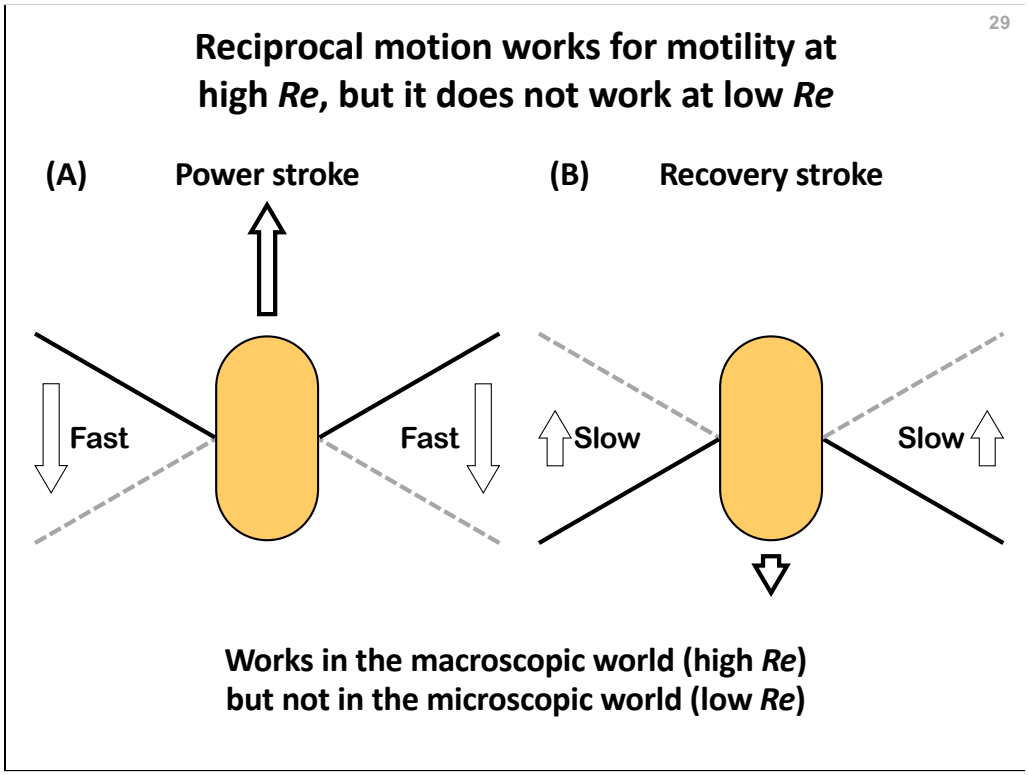
SOURCE: Lauga E, Powers TR (2009) The hydrodynamics of swimming microorganisms. *Rep Prog Phys* 72(9): 096601 doi: 10.1088/0034-4885/72/9/096601.

Note that a hydrogen atom has a diameter of about 0.11 nm (a proton has a diameter of about 0.000001 nm).

Fluid flows are reversible at low Reynolds number (*Re*)

See: Movie_Slide28 posted on Moodle (make sure you turn on the sound).

SOURCE: https://www.youtube.com/watch?v=51-6QCJTAjU

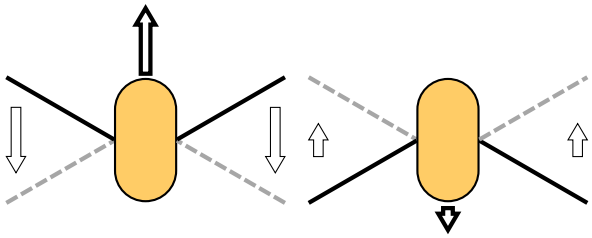


SOURCE: Berg HC (1996) Symmetries in bacterial motility. *Proc Natl Acad Sci USA* 93(25): 14225-14228 PMID: 8962029.

Figure 1. An organism propelled by two rigid oars. **(A)** If the organism strokes reciprocally, pulling both oars rapidly downwards, then returning them slowly upwards, and repeating this motion, it could swim upwards if macroscopic but not if microscopic. The position of the oars at the beginning of the power stroke is shown by the dotted lines. Their position at the end of the power stroke is shown by the solid lines. **(B)** If, instead, the organism stroked cyclically by moving its oars one by one, in the sequence right down, left down, right up, left up, and repeating this motion, it could swim, even if microscopic. The position of the oars at the end of the first step of the cycle is shown by the solid lines.

30

Bacteria cannot move by making reciprocal motions because:



A. Inertial forces are too strong at low Reynolds number.

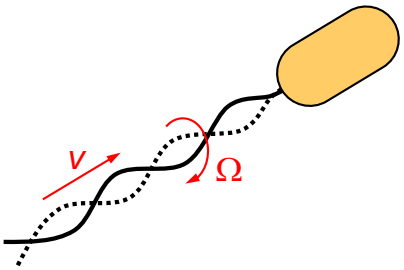
B. Viscous forces are too weak at low Reynolds number.

C. Flows are reversible at low Reynolds number.

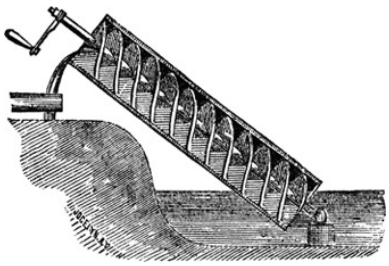
Answer: (C)

Propulsion at low *Re*: a rotating corkscrew can propel bacteria forward by translocating fluid

Bacterial cell



Archimedes screw



SOURCE: Purcell EM (1997) The efficiency of propulsion by a rotating flagellum. *Proc Natl Acad Sci USA* 94(21): 11307-11311 PMID: 9326605.

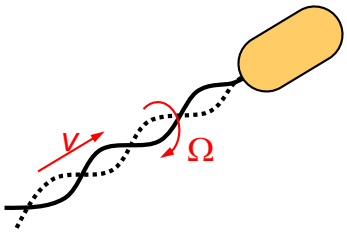
Propulsion at low *Re*: a rotating corkscrew can propel bacteria forward by translocating fluid

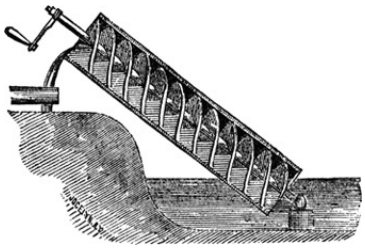
See: Movie_Slide32 posted on Moodle (make sure you turn on the sound).

SOURCE: http://www.youtube.com/watch?v=51-6QCJTAjU

33

Bacteria can move using a rotating propeller (the flagellum) because:





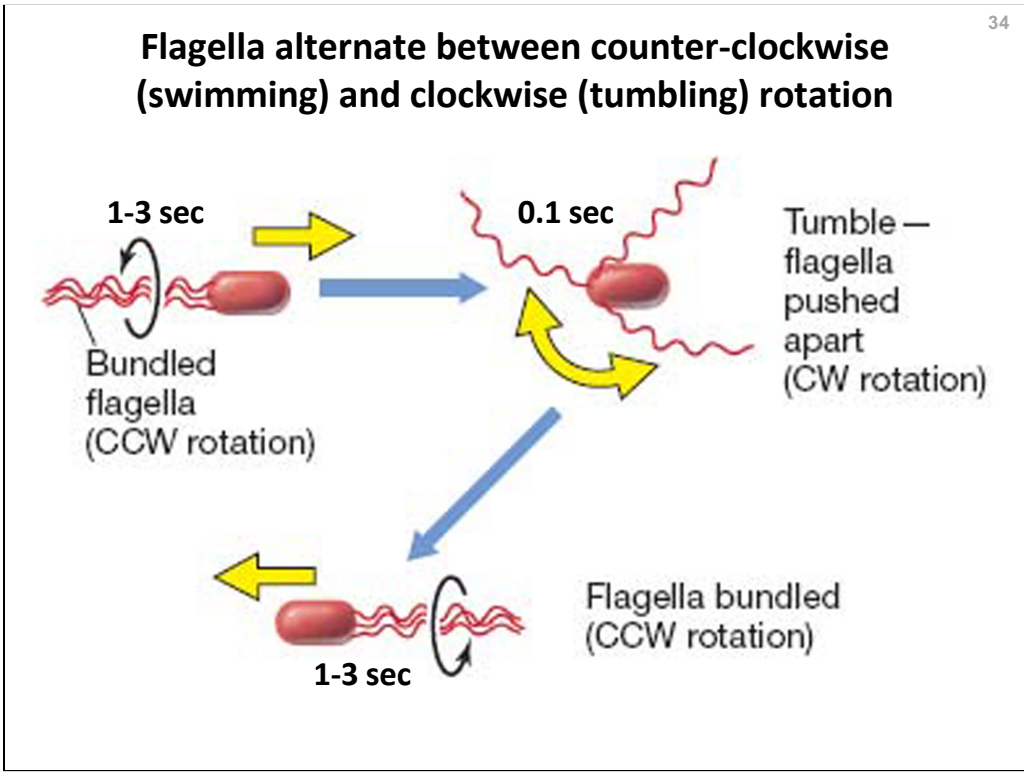
A. A rotating propeller is not subject to viscous forces.

B. A rotating propeller is not subject to inertial forces.

C. A rotating propeller creates a turbulent wake.

D. A rotating propeller does not undergo reciprocal motion.

Answer: (D)



SOURCE: *Brock Biology of Microorganisms [15th Edition]*, Chapter 2: Microbial Cell Structure and Function, Section IV: Cell Locomotion (pp. 56-64), published by Benjamin Cummings © 2018.

Figure 2.36. Movement in peritrichously flagellated prokaryotes, such as *Escherichia coli*. Forward motion is imparted by all flagella rotating counterclockwise (CCW) in a bundle. Clockwise (CW) rotation causes the cell to tumble, and then a return to counterclockwise rotation leads the cell off in a new direction. The yellow arrows show the direction the cell is traveling.

Much research on chemotaxis has been done with the peritrichously flagellated bacterium *E. coli*. To understand how chemotaxis affects the behavior of *E. coli*, consider the situation in which a cell encounters a gradient of some chemical in its environment. In the absence of the gradient, cells move in a random fashion that includes **runs**, in which the cell is swimming forward in a smooth fashion, and **tumbles**, when the cell stops and jiggles about. During forward movement in a run, the flagellar motor rotates **counterclockwise**. When flagella rotate **clockwise**, the bundle of flagella pushes apart, forward motion ceases, and the cells tumble.

Following a tumble, the direction of the next run is **random**. Thus, by means of runs and tumbles, the cell moves about its environment in a random fashion. However, if a gradient of a chemical attractant is present, these random movements become **biased**. If the organism senses that it is moving toward higher concentrations of the **attractant**, runs become longer and tumbles are less frequent. The result of this behavioral response is that the organism moves up the concentration gradient of the attractant. If the organism senses a **repellent**, the same mechanism applies, although in this case it is the decrease in concentration of the repellent (rather than the increase in concentration of an attractant) that promotes runs.

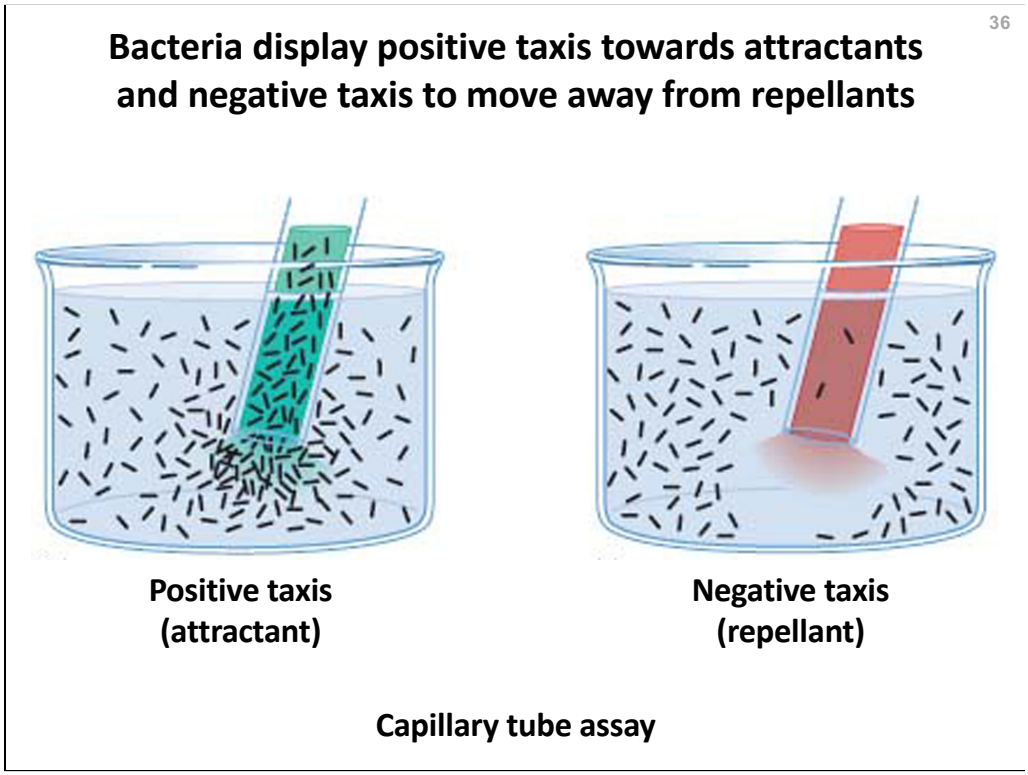
How are chemical **gradients** sensed? Prokaryotic cells are too small to sense a gradient of a chemical along the length of a single cell. Instead, while moving, bacteria "monitor" their environment by sampling chemicals periodically and comparing the concentration of a particular chemical with that sensed a few moments before. Bacterial cells thus respond to **temporal** rather than **spatial** differences in the concentration of a chemical as they swim. Sensory information is fed through an elaborate cascade of proteins that eventually affect the direction of rotation of the flagellar motor. The attractants and repellents are sensed by a series of membrane proteins called **chemoreceptors**. These sensory proteins bind the chemicals and begin the process of sensory transduction to the flagellum. Chemotaxis can thus be considered a type of sensory response system, analogous to sensory responses in the nervous system of animals.

Chemotaxis
Phototaxis
Aerotaxis
Magnetotaxis
Osmotaxis
Hydrotaxis
Reotaxis
Others…

SOURCE: *Brock Biology of Microorganisms [15th Edition]*, Chapter 2: Microbial Cell Structure and Function, Section IV: Cell Locomotion (pp. 56-64), published by Benjamin Cummings © 2018.

Cells of *Bacteria* and *Archaea* often encounter gradients of physical or chemical agents in nature and have evolved means to respond to these gradients by moving either toward or away from the agent. Such a directed movement is called a **taxis** (plural, **taxes**). **Chemotaxis**, a response to chemicals, and **phototaxis**, a response to light, are two well-studied taxes. The ability of a cell to move toward or away from various stimuli has ecological significance in that the directed movement may enhance a cell's access to resources or allow it to avoid harmful substances that could damage or kill it. Chemotaxis has been well studied in swimming bacteria, and much is known at the genetic level about how the chemical state of a cell's environment is communicated to the flagellum. Many swimming species of *Archaea* are also chemotactic, and several of the proteins that control chemotaxis in *Bacteria* have homologs in these *Archaea*.

Many phototrophic microorganisms can move toward light, a process called **phototaxis**. Phototaxis allows a phototrophic organism to position itself most efficiently to receive light for photosynthesis. Two different light-mediated taxes are observed in phototrophic bacteria. One, called **scotophobotaxis**, can be observed only microscopically and occurs when a phototrophic bacterium happens to swim outside the illuminated field of view of the microscope into darkness. Entering darkness negatively affects photosynthesis and thus the energy state of the cell and signals the cell to tumble, reverse direction, and once again swim in a run, thus reentering the light. Scotophobotaxis is presumably a mechanism by which phototrophic bacteria avoid entering darkened habitats when they are moving about in illuminated ones, and this likely improves their competitive success. **Phototaxis** differs from scotophobotaxis in that cells move up a light gradient from lower to higher intensities. Phototaxis is analogous to chemotaxis except that the attractant is light instead of a chemical. Several components of the regulatory system that govern chemotaxis also control phototaxis. A photoreceptor, a protein that functions similarly to a chemoreceptor but senses a gradient of light instead of chemicals, is the initial sensor in the phototaxis response. The photoreceptor then interacts with the same cytoplasmic proteins that control flagellar rotation in chemotaxis, maintaining the cell in a run if it is swimming toward an increasing intensity of light. Other bacterial taxes, such as movement toward or away from oxygen (**aerotaxis**) or toward or away from conditions of high ionic strength (**osmotaxis**), are known among various swimming bacteria. In some gliding cyanobacteria, **hydrotaxis** (movement toward water), has also been observed. Hydrotaxis allows gliding cyanobacteria that inhabit dry environments, such as desert soils, to glide toward a gradient of increasing hydration.



SOURCE: *Brock Biology of Microorganisms [15th Edition]*, Chapter 2: Microbial Cell Structure and Function, Section IV: Cell Locomotion (pp. 56-64), published by Benjamin Cummings © 2018.

Figure 2.43. Measuring chemotaxis using a capillary tube assay - the first quantitative microbial chemotaxis assay, developed by Julius Adler in the 1960s. **(A, not shown on this slide)** Insertion of the capillary into a bacterial suspension. As the capillary is inserted, a gradient of the chemical begins to form. **(B, not shown on this slide)** A control capillary contains a salt solution that is neither an attractant nor a repellent. Over time, the cell concentration inside the capillary becomes the same as the cell concentration outside the capillary. **(C, left panel)** Accumulation of bacteria in and around a capillary containing an attractant. **(D, right panel)** Repulsion of bacteria by a repellent.

Diffusion times for small molecules and small cells

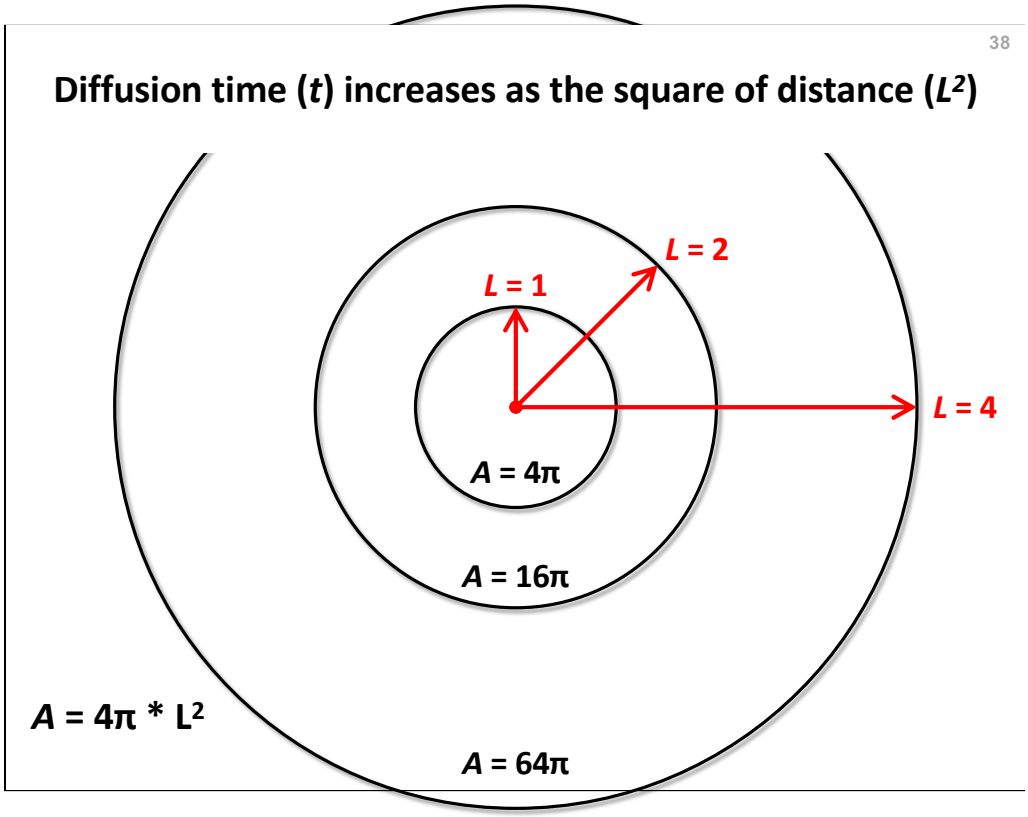
Diffusion time $t = L^2 * D^{-1}$

t = time required for diffusive transport
L = distance traveled by diffusion
D (diffusion coefficient) = 100 $\mu m^2 * s^{-1}$ for an amino acid
D (diffusion coefficient) = 0.01 $\mu m^2 * s^{-1}$ for a bacterial cell

Time for an amino acid to diffuse 100 µm
$= L^2 * D^{-1} = (10^4\ \mu m^2) * (100\ \mu m^2 * s^{-1})^{-1}$ = **100 seconds**

Time for a bacterial cell to diffuse 100 µm
$= L^2 * D^{-1} = (10^4\ \mu m^2) * (0.1\ \mu m^2 * s^{-1})^{-1}$ = **1,000,000 seconds (about 12 days)**

The equation for diffusion time is a "primary concept" – you should memorize it and you should feel comfortable manipulating it. Indeed, you should already be familiar with this equation from your physics courses.



The surface area (A) of a sphere is given by $A = 4\pi * r^2$. In the example shown here we make r = *L*, the distance of diffusion, so $A = 4\pi * L^2$. Imagine a set of nested spheres, as depicted on the slide. Imagine that the spheres are filled with water or some other incompressible fluid, and a "spot" of some diffusible molecule is placed at the center of the spheres. As the molecules diffuse outwards they must pass through an "infinite" number of nested spheres with ever-increasing radii. I have drawn only a few of these spheres, as I do not have an "infinite" amount of time to draw them all... ;-)

Bernoulli's Principle of Continuity (based on the law of conservation of mass) states that, for an incompressible fluid (for example, an aqueous solution of the aforementioned molecule) flowing in a tube of varying cross-section, the mass flow rate is the same everywhere in the tube. The mass flow rate is simply the rate at which mass flows through a given cross-sectional area of the tube, so it's the total mass flowing through the cross-sectional area divided by the time interval. The equation of continuity can be reduced to:

$$\rho_1 * A_1 * U_1 = \rho_2 * A_2 * U_2 = \rho_3 * A_3 * U_3 \ldots$$

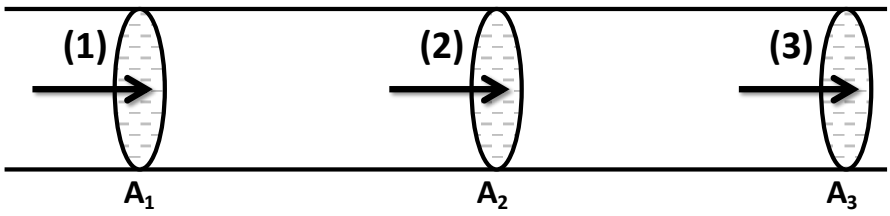
where ρ = density, A = cross-sectional area, U = velocity. Generally, the density (ρ) stays the same and so the flow rate (A * U) must also be the same at all points (1, 2, 3...).

By analogy, as the "spot" of molecules moves outwards from the center (referring again to the slide), each sphere (or "shell") that it must pass through is larger than the last: specifically, the surface area of these spherical shells increases as the square of the radius. Thus, the area that the molecules must pass through as they move outwards from the center also increases as the square of the radius. As stated in Bernoulli's Principle of Continuity, if the area that the fluid moves through *increases* then the velocity of movement must *decrease*. Since the area of the spherical shells increases as the square of *L* moving outwards from the center, so the velocity of movement of the fluid decreases as the square *L*.

I am not a physicist and I don't know what a physicist would think of this analogy (not much, probably...). But for me, at least, this analogy is a useful way of thinking about the diffusion problem in a "geometric" or "intuitive" way.

39

Bernoulli's Principle of Continuity



$$(\rho_1 * A_1 * U_1) = (\rho_2 * A_2 * U_2) = (\rho_3 * A_3 * U_3)\ldots$$

Where ρ = density, A = cross-sectional area, U = velocity.

Generally, the density (ρ) stays the same so the flow rate (A * U) must also be the same at all points (1, 2, 3).

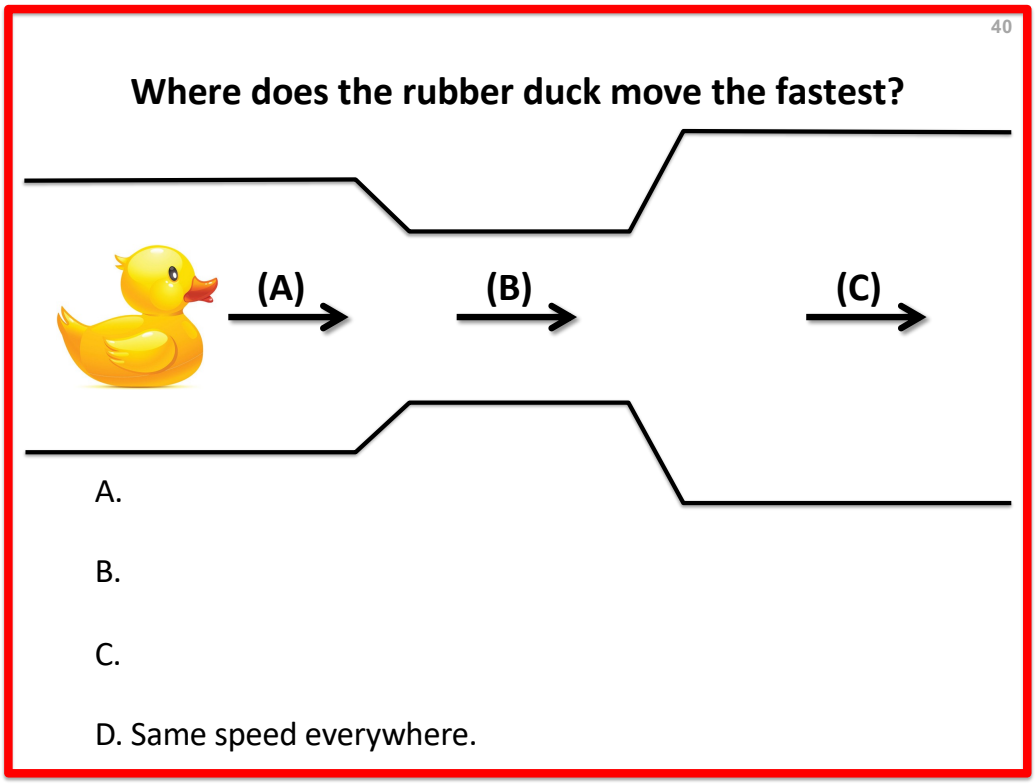
SOURCE: http://physics.bu.edu/~duffy/py105/Bernoulli.html

Bernoulli's Principle of Continuity (based on the law of conservation of mass) states that, for an incompressible fluid (like water) flowing in a tube of varying cross-section, the mass flow rate is the same everywhere in the tube. The mass flow rate is simply the rate at which mass flows through a given cross-sectional area of the tube, so it's the total mass flowing through the cross-sectional area divided by the time interval. The equation of continuity can be reduced to:

$$(\rho_1 * A_1 * U_1) = (\rho_2 * A_2 * U_2) = (\rho_3 * A_3 * U_3)\ldots$$

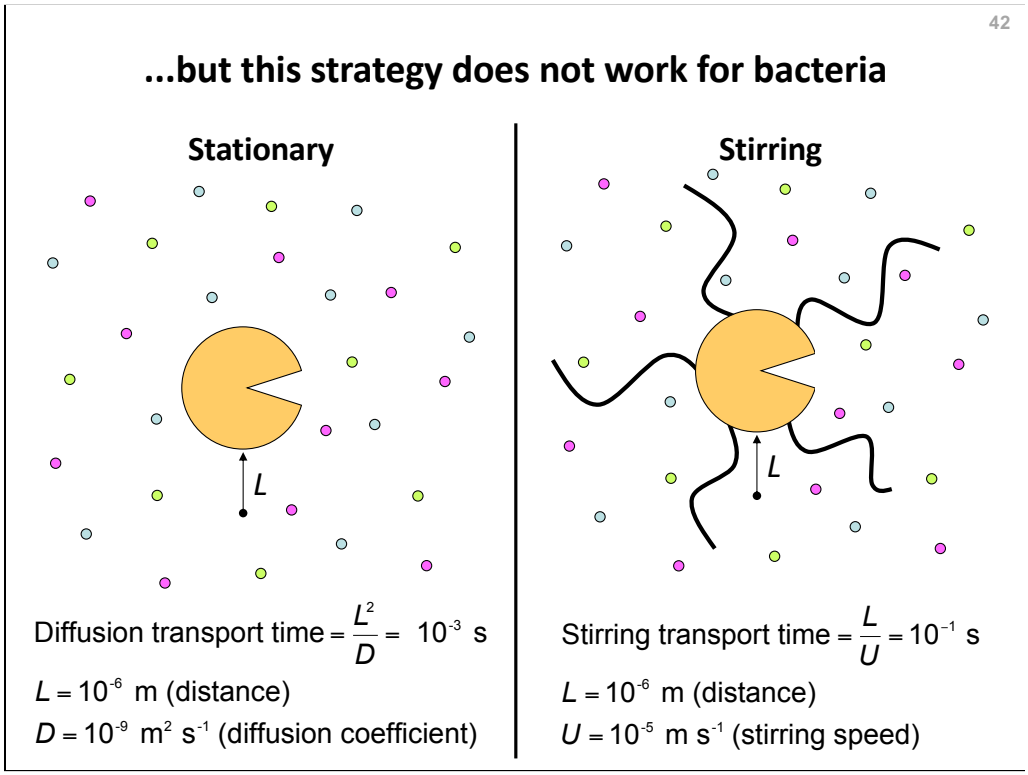
where ρ = density, A = cross-sectional area, U = velocity. Generally, the density (ρ) stays the same and so the flow rate (A * U) must also be the same at all points (1, 2, 3...).

Bernoulli's Principle of Continuity is a "primary concept" – you should memorize it and you should feel comfortable manipulating it. Indeed, you should already be familiar with this equation from your physics courses.


40
Where does the rubber duck move the fastest?
(A)
(B)
(C)
A.
B.
C.
D. Same speed everywhere.

Answer: (B)

Stirring speeds up mixing milk into our coffee…



SOURCE: Purcell EM (1977) Life at low Reynolds number. *Am J Phys* 45: 3-11. doi: 10.1119/1.10903.

Figure 18. "A better way to say it is that the bug can collect, by diffusion through the surrounding medium, enough energetic molecules to keep moving when the concentration of those molecules is 10^{-9} M. I've now introduced the word diffusion. Diffusion is important because of another very peculiar feature of the world at low Reynolds number, and that is, stirring isn't any good. The bug's problem is not its energy supply; its problem is its environment. At low Reynolds number you can't shake off your environment. If you move, you take it along; it only gradually falls behind. We can use elementary physics to look at this in a very simple way. The time for transporting anything a distance *L* by stirring, is about *L* divided by the stirring speed *U*. Whereas, for transport by diffusion, it's L^2 divided by *D*, the diffusion coefficient. The ratio of those two times is a measure of the effectiveness of stirring versus that of diffusion for any given distance and diffusion constant. I'm sure this ratio has someone's name but I don't know the literature and I don't know whose number that's called. Call it *S* for *stirring number,* it's just $L * U * D^{-1}$. You'll notice by the way that the Reynolds number was $L * U * \nu^{1}$, where ν is the kinematic viscosity in $cm^2 * sec^{-1}$, and *D* is the diffusion constant in $cm^2 * sec^{-1}$, for whatever it is that we are interested in following, let us say a nutrient molecule in water. Now, in every reasonably sized molecule, something like 10^{-5} cm^2 sec^{-1}. In the size domain that we're interested in, of micron distances, we find that the stirring number *S* is 10^{-2}, for the velocities that we are talking about. In other words, this bug can't do anything by stirring its local surroundings. It might as well wait for things to diffuse, either in or out. The transport of wastes away from the animal and food to the animal is entirely controlled locally by diffusion. You can thrash around a lot, but the fellow who just sits there quietly waiting for stuff to diffuse will collect just as much."

Incidentally, stationary feeding and stirring feeding become equally effective when the two terms achieve an equivalency, i.e., when $L^2 * D^{-1} = L * U$. Keeping the values of *D* and *U* constant, we can solve for *L*, which equals 10^{-4} m = 100 µm. This is the characteristic length (given the listed values of *D* and *U*) at which diffusion and stirring are equally effective. This length will decrease if *D* increases (because diffusion will be slower) or if *U* increases (because stirring will be more effective).

Abbreviations:

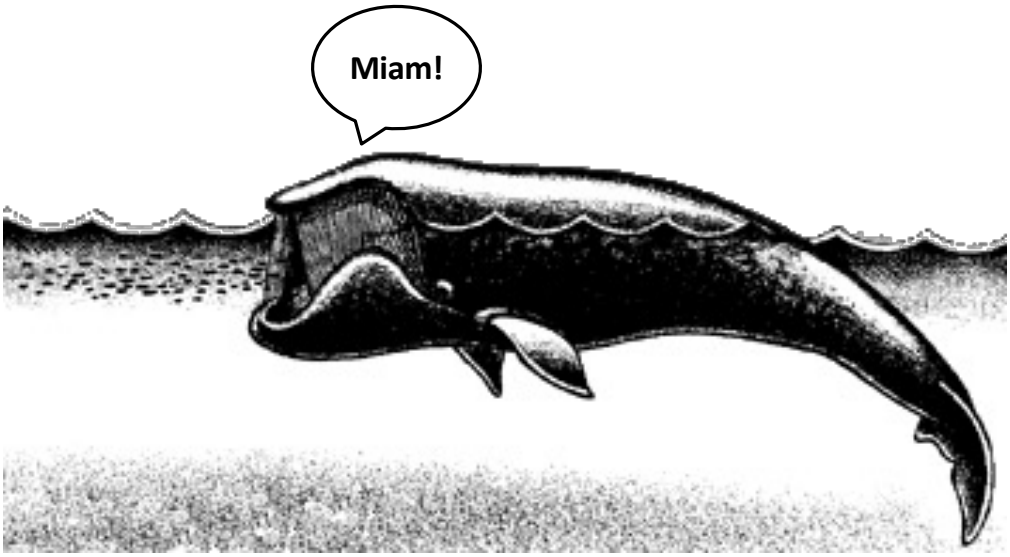
D, Diffusion coefficient

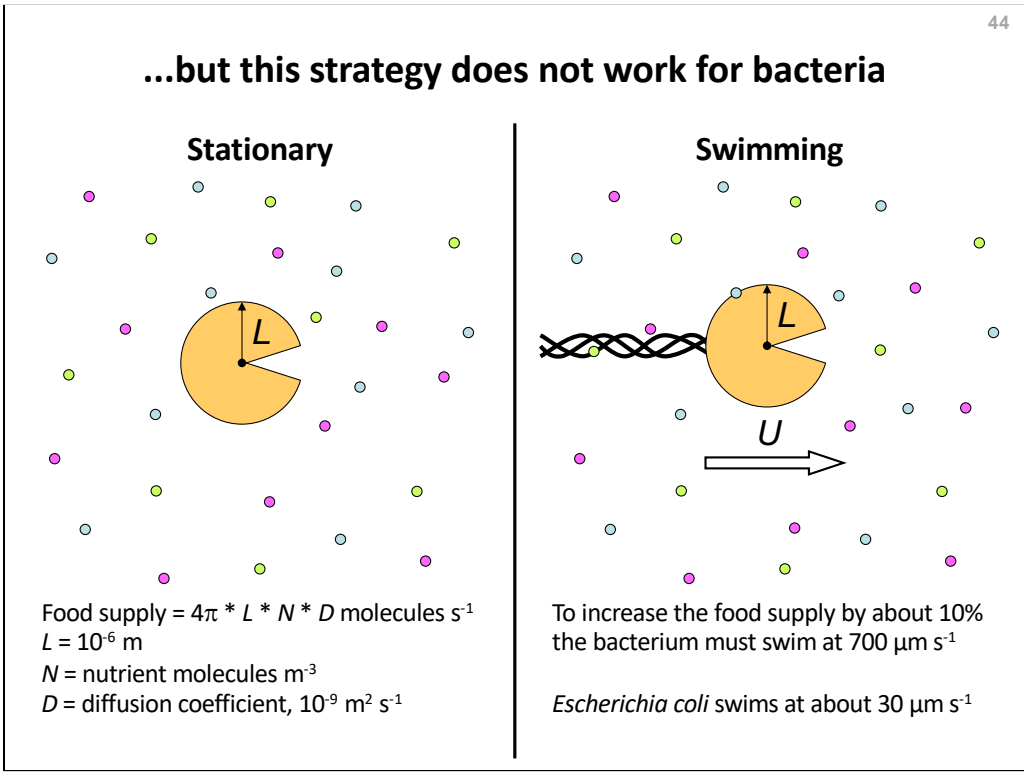
U, velocity

L, cell radius

ν, kinematic velocity

Swimming helps whales “scoop up” more stuff...





SOURCE: Purcell EM (1977) Life at low Reynolds number. *Am J Phys* 45: 3-11. doi: 10.1119/1.10903.

Figure 19. "At one time I thought that the reason the thing swims is that if it swims it can get more stuff, because the medium is full of molecules the bug would like to have. All my instincts as a physicist say you should move if you want to scoop that stuff up. You can easily solve the problem of diffusing in the velocity field represented by the Stokes flow around a sphere – for instance, by a relaxation method. I did so and found out how fast the cell would have to go to increase its food supply. The food supply if it just sits there is $4\pi * L * N * D$, where L is the cell's radius (Fig. 19) and N is the concentration of nutrient molecules. To increase its food supply by 10% it would have to move at a speed of 700 microns sec^{-1}, which is 20 times as fast as it can swim. The increased intake varies like the square root of the bug's velocity so the swimming does no good at all in that respect. But what it can do is find places where the food is better or more abundant. That is, it does not move like a cow that is grazing a pasture – it moves to find *greener pastures.* And how far does it have to move? Well, it has to move far enough to outrun diffusion. We said before that stirring wouldn't do any good locally, compared to diffusion. But suppose it wants to run over there to see whether there is more over there. Then it must outrun diffusion, and how do you do that? Well, you go that magic distance, $D * U^{-1}$. So the rule is then, to out swim diffusion you have to go a distance which is equal to or greater than this number we had in our S constant. For typical D and U, you have to go about 30 µm and that's just about what the swimming bacteria were doing. If you don't swim that far, you haven't gone anywhere, because it's only on that scale that you could find a difference in your environment with respect to molecules of diffusion constant D (Fig. 20)"

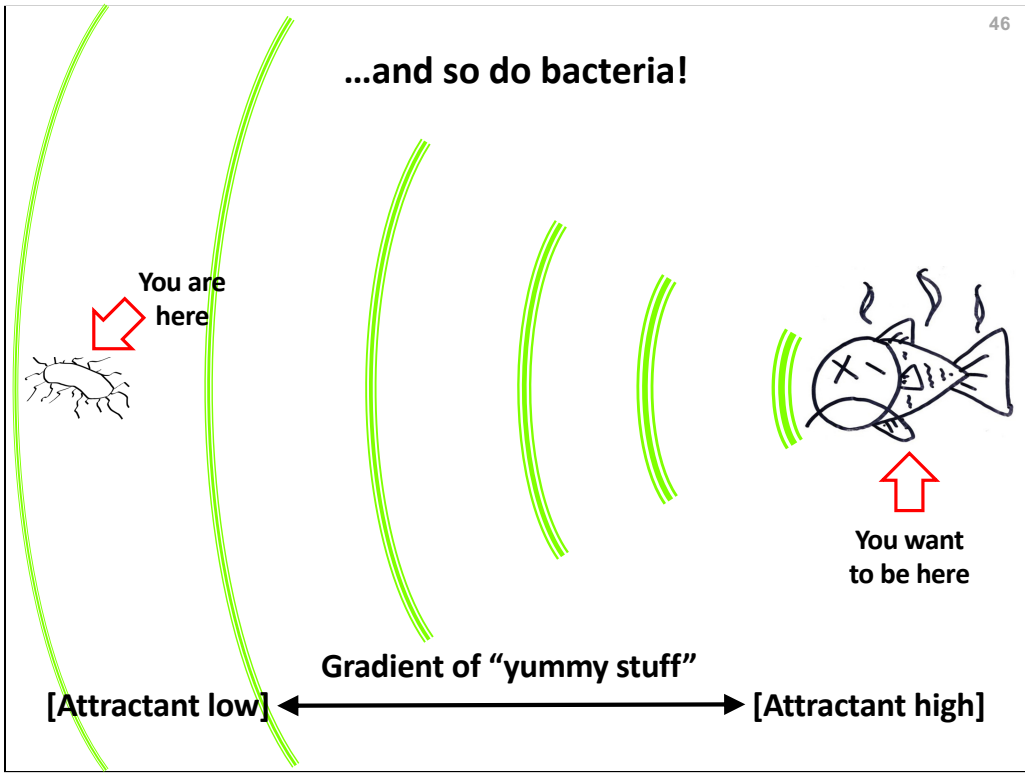
Abbreviations:

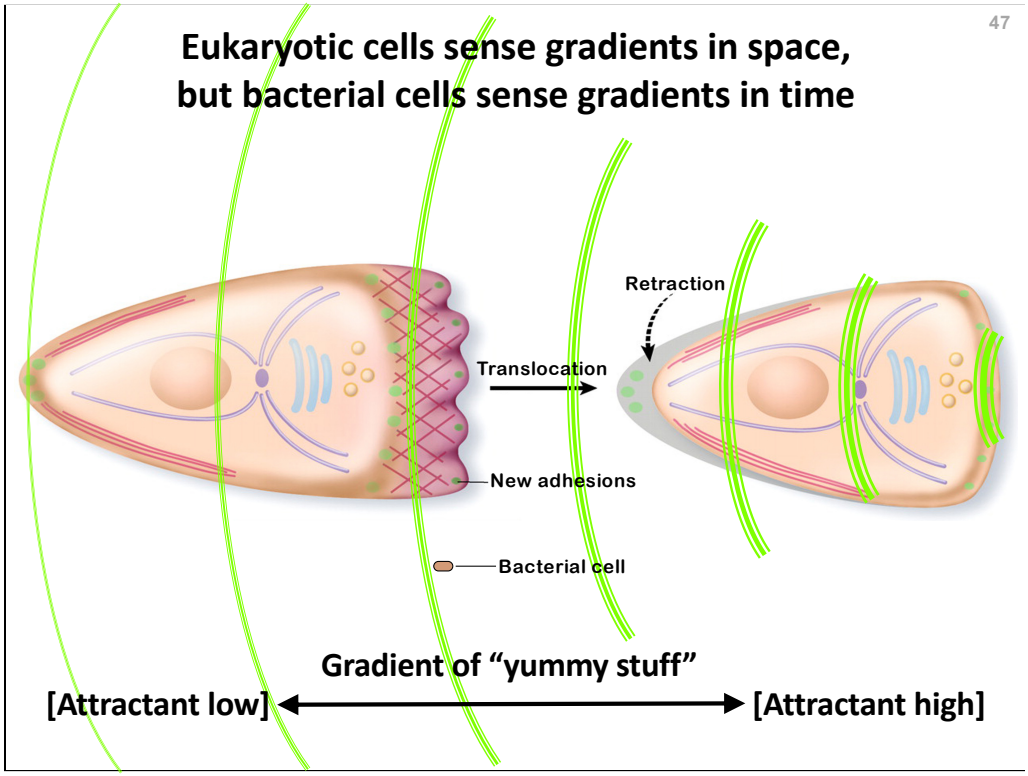
D, Diffusion coefficient

U, velocity

L, cell radius

Cows move to find “greener pastures”...


46
…and so do bacteria!
You are here
You want to be here
Gradient of "yummy stuff"
[Attractant low]
[Attractant high]



SOURCE: Ridley AJ, Schwartz MA, Burridge K, Firtel RA, Ginsberg MH, Borisy G, Parsons JT, Horwitz AR (2003) Cell migration: integrating signals from front to back. *Science* 302(5651): 1704-1709 PMID: 14657486.

Figure 2. Steps in eukaryotic cell migration.

(A) Polarity is intrinsic to a migrating eukaryotic cell. Many proteins (Cdc42, Par, aPKC, …) are implicated in polarity, which results in directed vesicle trafficking toward the leading edge, organization of microtubules, and localization of the microtubule organizing center and Golgi apparatus in front of the nucleus. In the presence of an attractant gradient, PIP3 is produced at the leading edge through the localized action of PI3K, which resides at the leading edge, and PTEN, a PIP3 phosphatase that resides at the cell margins and rear. PTEN and myosin II are implicated in restricting protrusions to the cell front. **(B)** The migration cycle begins with the formation of a protrusion at the cell front facing up the attractant gradient. WASP/WAVE proteins are targets of various signaling pathways (Rac, Cdc342, …) and regulate the formation of actin branches on existing actin filaments by their action on the Arp2/3 complex. Actin polymerization is regulated by proteins that control the availability of activated actin monomers (profilin) and debranching and depolymerizing proteins (ADF/cofilin), as well as capping and severing proteins. Protrusions are stabilized by the formation of adhesions. This process requires integrin activation, clustering, and the recruitment of structural and signaling components to nascent adhesions. Integrins are activated by talin binding and various signaling pathways (PKC, Rap1, PI3K, …). Integrin clustering results from binding to multivalent ligands and is regulated by Rac. **(C)** At the cell rear, adhesions disassemble as the rear retracts. This process is mediated by several possibly related signaling pathways (Src/FAK/ERK, Rho, myosin II, calcium, calcineurin, calpain, …) and the delivery of components by microtubules. Some of these molecules may also regulate the disassembly of adhesions at the cell front, behind the leading edge.

You do not need to memorize the specific information (protein players, etc.) about chemotaxis in eukaryotic cells. The "take home message" is that eukaryotic cells, being large in size, can detect chemical gradients in **space** because the attractant concentrations at the **cell front** and **cell rear** are significantly different. Eukaryotic cells move up gradients of attractant by steering their movement in the correct direction. In contrast, bacterial cells are too small to sense gradients in space because the attractant concentrations at the **cell front** and **cell rear** are nearly the same. Instead, bacteria move up gradients of attractant by detecting changes in attractant concentration in the local environment as a function of **time** while they move. If the local concentration of attractant increases over time the bacteria tend to keep moving in the same direction (**run**). If the local concentration of attractant decreases over time the bacteria tend to stop running and **tumble** in order to change direction before initiating another run in a randomly selected direction. Thus, on average, runs in the "good" direction are longer and runs in the "bad" direction are shorter.

The speed of diffusive transport decreases rapidly with increasing transport distance

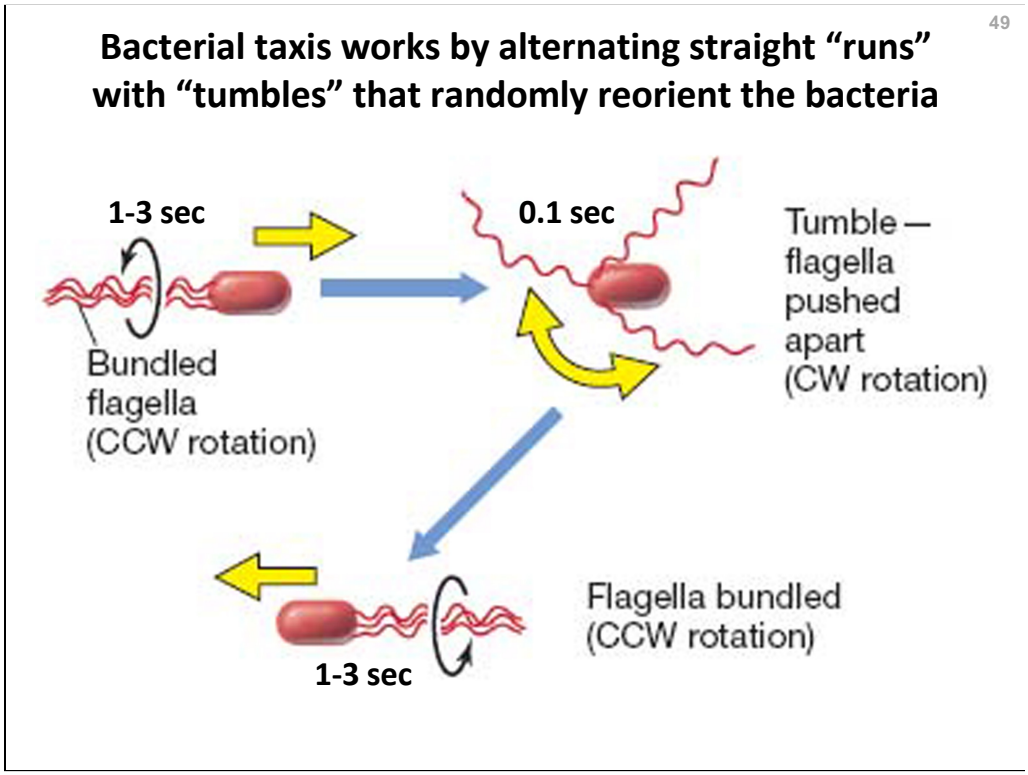
Diffusion time $t = L^2 * D^{-1}$

t = time required for diffusive transport
L = distance traveled by diffusion
D (diffusion coefficient) = 100 $\mu m^2 * s^{-1}$ for a small molecule

Time to diffuse the length of a prokaryotic cell ($L \approx 2$ µm)
= $L^2 * D^{-1}$ = $(2\ \mu m)^2 * (100\ \mu m^2 * s^{-1})^{-1}$ = **0.04 seconds**

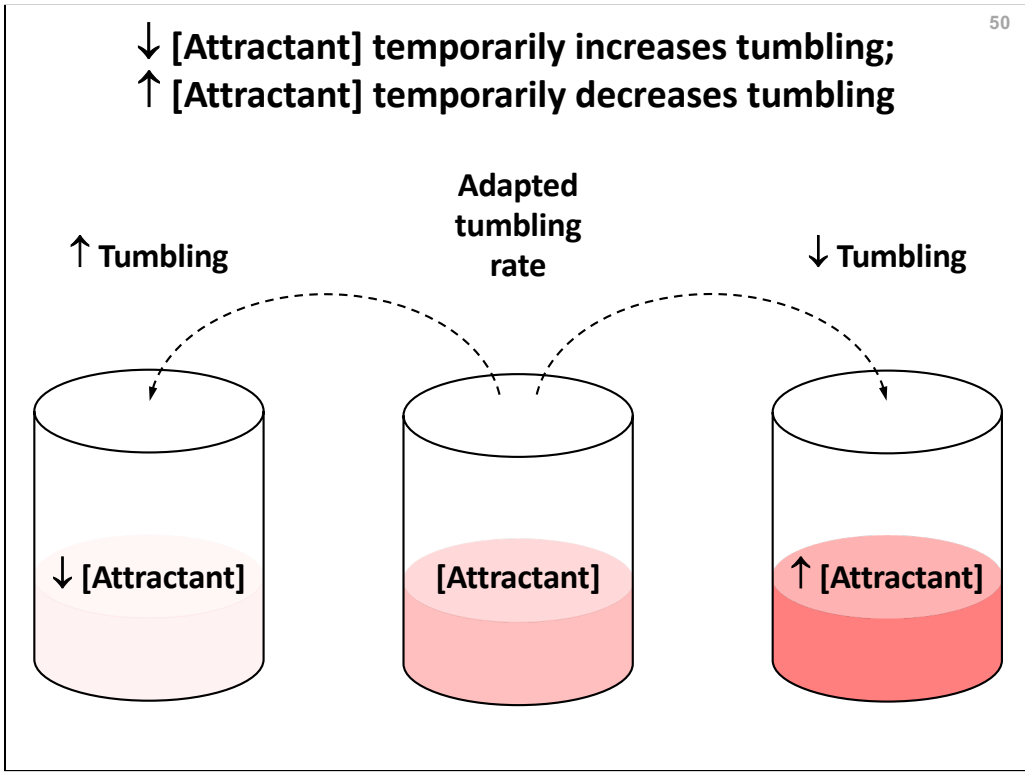
Time to diffuse the length of a eukaryotic cell ($L \approx 20$ µm)
= $L^2 * D^{-1}$ = $(20\ \mu m)^2 * (100\ \mu m^2 * s^{-1})^{-1}$ = **4 seconds**

Time to diffuse the length of a human body ($L \approx 185$ cm)
= $L^2 * D^{-1}$ = $(1.85 * 10^6\ \mu m)^2 * (100\ \mu m^2 * s^{-1})^{-1}$ > **1,000 years!!!**

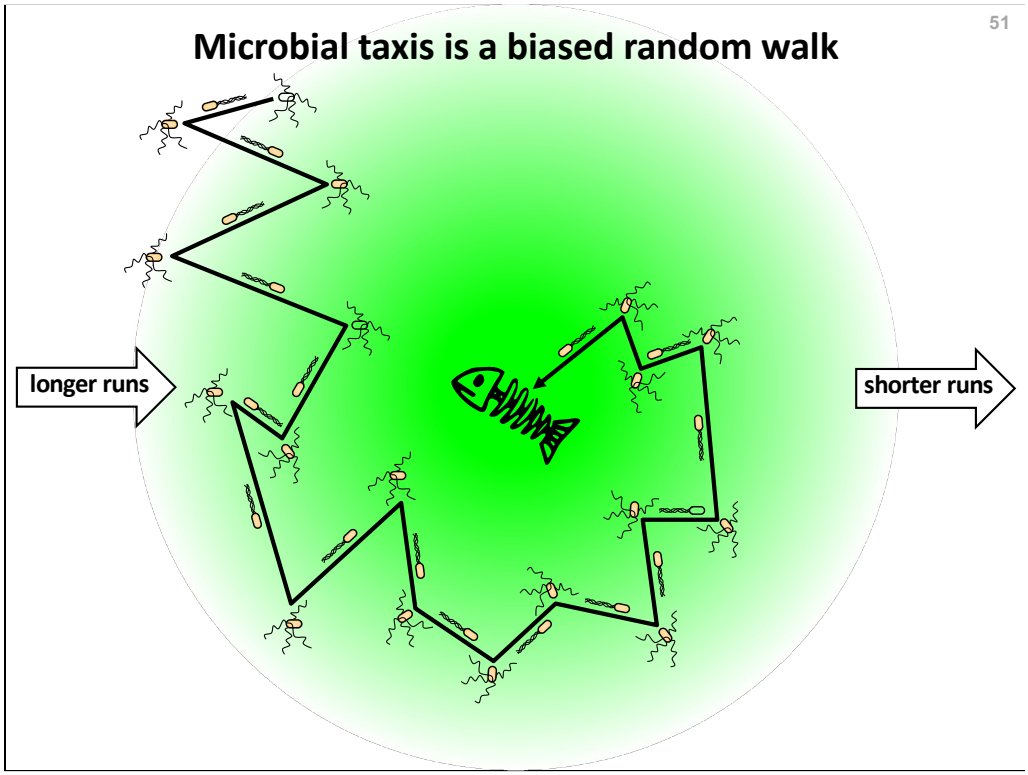


SOURCE: *Brock Biology of Microorganisms [15th Edition]*, Chapter 2: Microbial Cell Structure and Function (pp. 70-108), published by Benjamin Cummings © 2018.

Figure 2.36. Movement in peritrichously flagellated prokaryotes. Forward motion is imparted by all flagella rotating counterclockwise (CCW) in a bundle. Clockwise (CW) rotation causes the cell to tumble, and then a return to counterclockwise rotation leads the cell off in a new direction. The yellow arrows show the direction the cell is traveling.



Bacteria that are well-adapted to a spatially and temporally homogeneous concentration of an attractant (or a repellant) run and tumble at a 'default' rate. For *Escherichia coli*, this means runs averaging 1 sec and tumbles averaging 0.1 sec. Bacteria that are shifted to a lower concentration of attractant rapidly increase their tumbling frequency, resulting in shorter runs (on average). Bacteria that are shifted to a higher concentration of attractant rapidly decrease their tumbling frequency, resulting in longer runs (on average). However, on a somewhat slower (but still rapid, i.e., seconds) time scale, bacteria shifted to a new environment undergo adaptation, which is mediated by increased or decreased chemoreceptor methylation, and the tumbling frequency returns to the default rate.

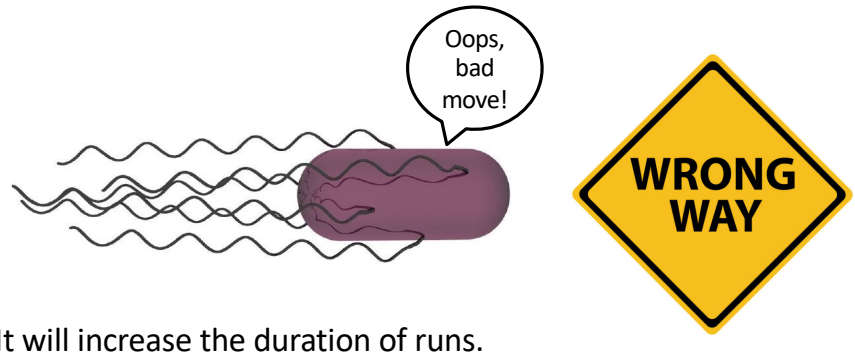


SOURCE: Webre DJ, Wolanin PM, Stock JB (2003) Bacterial chemotaxis. *Curr Biol* 19(2): R47-R49 PMID: 12546801.

Figure 1. Chemotaxis is migration towards attractants and away from repellents. Bacteria such as *Escherichia coli* exhibit two modes of swimming: runs and tumbles. Cells tend to continue on course when running towards attractants; when swimming away from attractants they tend to tumble and change direction.

52

If a bacterium swims in the wrong direction (away from an attractant or towards a repellent):



A. It will increase the duration of runs.

B. It will increase the duration of tumbles.

C. It will increase the frequency of tumbling.

D. It will decrease the frequency of tumbling.

Answer: (C)

53

Four "modules" of chemotaxis proteins

(1) Signal reception/transduction module:
MCPs (transmembrane chemoreceptors)

(2) Excitation module:
CheW (adaptor protein): links MCPs to CheA
CheA (histidine kinase): phosphorylates CheY and CheB
CheY (switch protein): activated by phosphorylation

(3) Signal termination module:
CheZ (phosphatase): always active

(4) Adaptation module:
CheR (methyltransferase): always active
CheB (methylesterase): activated by phosphorylation

SOURCE: Wadhams GH, Armitage JP (2004) Making sense of it all: bacterial chemotaxis. *Nat Rev Mol Cell Biol* 5(12): 1024-1037 PMID: 15573139.

SOURCE: Parkinson JS, Ames P, Studdert CA (2005) Collaborative signaling by bacterial chemoreceptors. *Curr Opin Microbiol* 8(2): 116-121 PMID: 15802240.

SOURCE: Eisenbach M (2007) A hitchhiker's guide through advances and conceptual changes in chemotaxis. *J Cell Physiol* 213(3): 574-580 PMID: 17708539.

MCPs are "methyl-accepting chemotaxis proteins". They are transmembrane chemoreceptors that bind directly to the substrate.

CheW is an adaptor protein that couples CheA to the MCPs and enhances activation of CheA by MCPs upon substrate binding.

CheA is a histidine protein kinase that phosphorylates CheY and CheB.

CheY is the key "response regulator" in bacterial chemotaxis. Phosphorylated CheY interacts with the flagellar motor complex and switches the direction of rotation from counter-clockwise (running) to clockwise (tumbling). CheY is activated by phosphorylation by CheA.

CheZ is a phosphatase that dephosphorylates CheY, which accelerates chemotactic responses to environmental changes. This is important because swimming bacteria must sense and respond to changes in attractant or repellant concentrations on millisecond timescales. CheZ is always active.

CheR is a methyltransferase that methylates MCPs, which increases their ability to activate CheA kinase. CheR is always active.

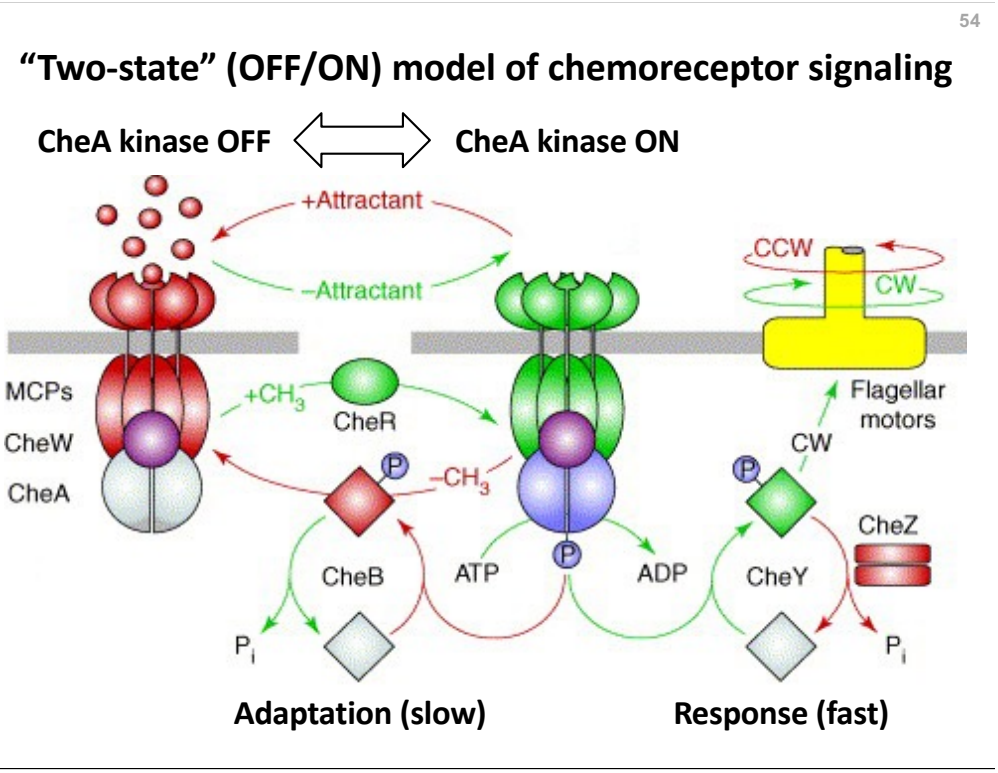
CheB is a methylesterase that demethylates MCPs, which decreases their ability to activate CheA kinase. CheB is activated by phosphorylation by CheA.

Abbreviations:

+CH_3, adds a methyl group (CheR adds a methyl group to MCPs)

–CH_3, removes a methyl group (CheB removes a methyl group from MCPs)

+Pi, phosphate group (CheA adds a phosphate group to CheY and CheB; CheZ removes a phosphate group from CheY)



SOURCE: Eisenbach M (2007) A hitchhiker's guide through advances and conceptual changes in chemotaxis. *J Cell Physiol* 213(3): 574-580 PMID: 17708539.

SOURCE: Parkinson J, Ames P, Studdert CA (2005) Collaborative signaling by bacterial chemoreceptors. *Curr Opin Microbiol* 8(2): 116-121 PMID: 15802240.

Schematic of protein-protein interactions that transduce the sensory signal from the chemotaxis receptor supramolecular complex to the flagellar-motor supramolecular complex in *Escherichia coli* chemotaxis.

Abbreviations:

+CH_3, adds a methyl group (CheR adds a methyl group to MCPs)

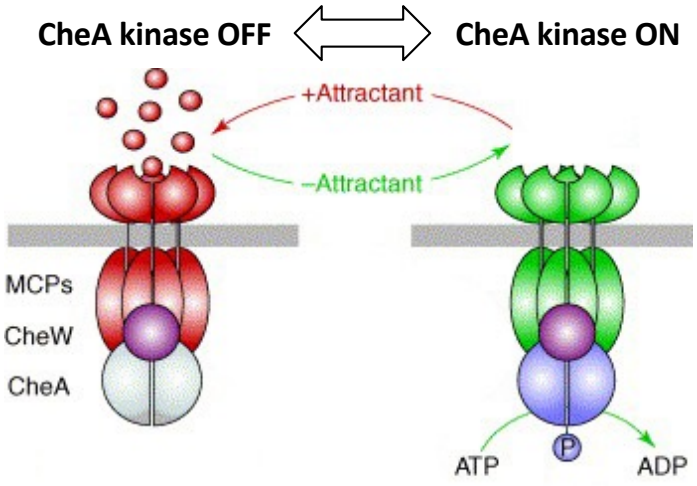
–CH_3, removes a methyl group (CheB removes a methyl group from MCPs)

+Pi, phosphate group (CheA adds a phosphate group to CheY and CheB; CheZ removes a phosphate group from CheY)

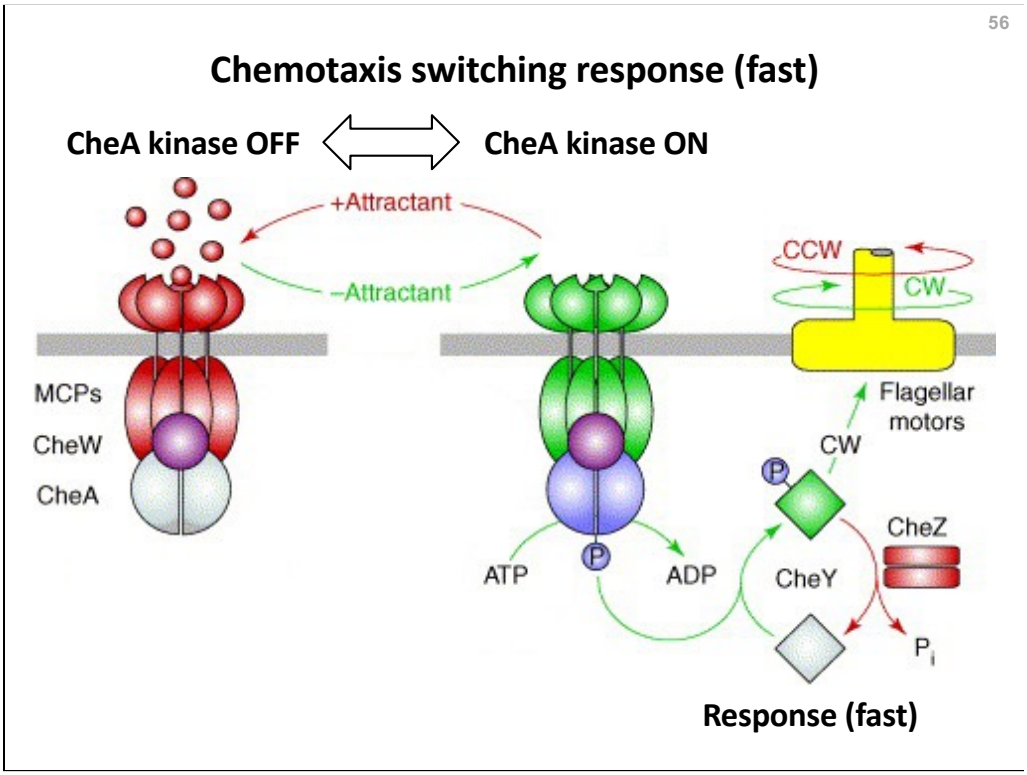
Figure 1. Two-state model of receptor signaling and the chemotaxis phosphorelay pathway in *Escherichia coli*. The chemoreceptors a.k.a. "methyl-accepting chemotaxis proteins" (**MCPs**) and cytosolic signaling proteins (**CheA**, **CheB**, **CheR**, **CheW**, **CheY**, **CheZ**) are depicted in their native subunit organizations. The receptor dimers are further arranged in trimers, which comprises the active unit for receptor signaling. Colored components represent functionally active states; gray components represent inactive signaling forms. Green components and reaction arrows represent signaling states that enhance clockwise (**CW**) flagellar rotation, which causes tumbling. Red components and reaction arrows represent signaling states that augment counter-clockwise (**CCW**) flagellar rotation, which causes smooth swimming (the default condition). Binding of an attractant ligand or removal of methyl groups shifts chemoreceptor signaling complexes from the kinase-on (green) to the kinase-off (red) signaling state. Release of attractant and addition of methyl groups shift chemoreceptor signaling complexes from the inactive CheA state (gray) to the active CheA state (blue). Conversely, binding of attractant and removal of methyl groups shift chemoreceptor signaling complexes from the active CheA state (blue) to the inactive CheA state (gray).

55

"Two-state" (OFF/ON) model of chemoreceptor signaling



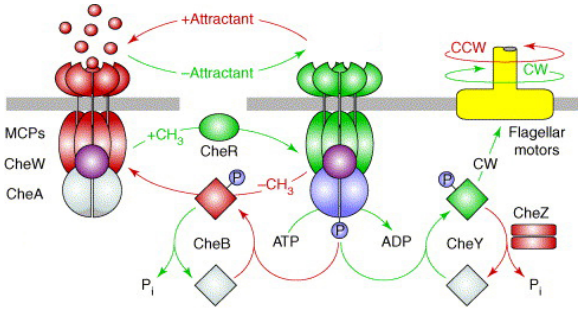
SOURCE: Parkinson J, Ames P, Studdert CA (2005) Collaborative signaling by bacterial chemoreceptors. *Curr Opin Microbiol* 8(2): 116-121 PMID: 15802240.



SOURCE: Parkinson J, Ames P, Studdert CA (2005) Collaborative signaling by bacterial chemoreceptors. *Curr Opin Microbiol* 8(2): 116-121 PMID: 15802240.

57

A mutant cell that lacks the CheA kinase will:



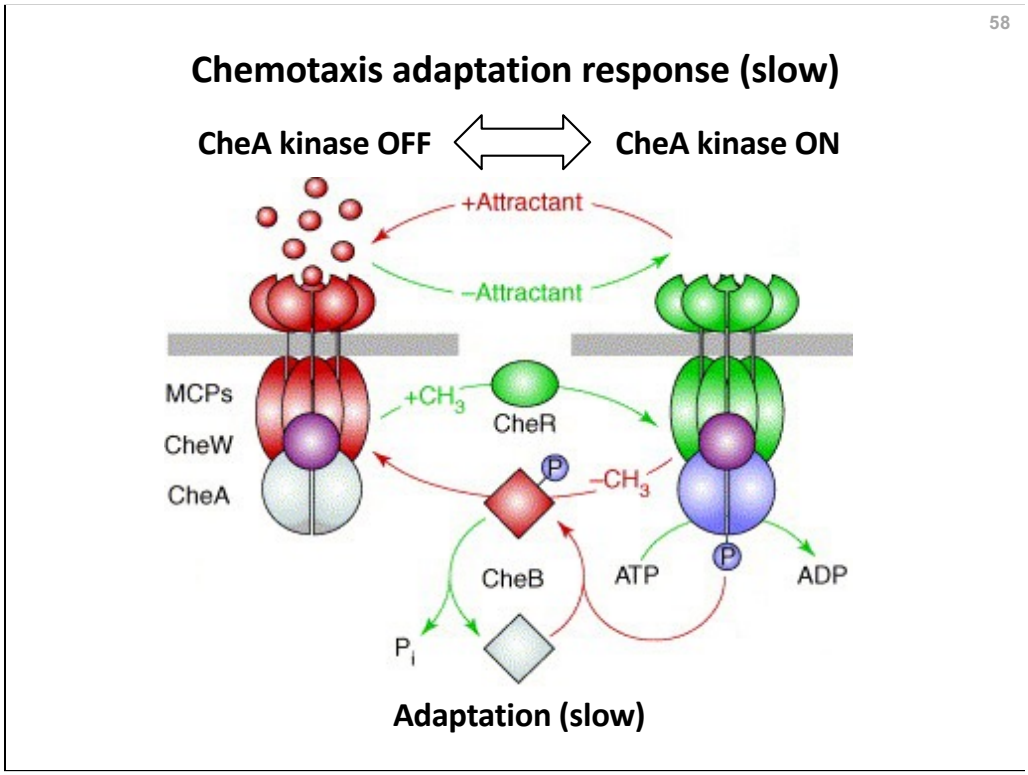
A. Run all the time.

B. Tumble all the time.

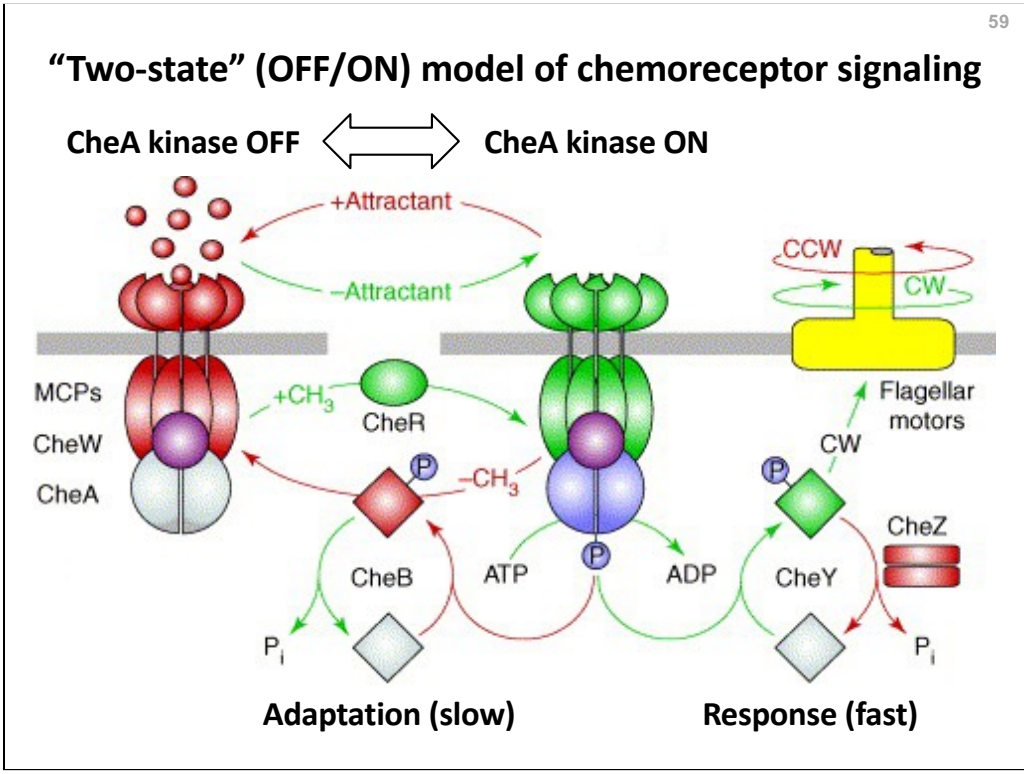
C. Alternate between running and tumbling but with slower kinetics than wild-type cells.

D. Adapt more slowly than wild-type cells to changes in the attractant concentration.

Answer: (A)



SOURCE: Parkinson J, Ames P, Studdert CA (2005) Collaborative signaling by bacterial chemoreceptors. *Curr Opin Microbiol* 8(2): 116-121 PMID: 15802240.



SOURCE: Eisenbach M (2007) A hitchhiker's guide through advances and conceptual changes in chemotaxis. *J Cell Physiol* 213(3): 574-580 PMID: 17708539.

SOURCE: Parkinson J, Ames P, Studdert CA (2005) Collaborative signaling by bacterial chemoreceptors. *Curr Opin Microbiol* 8(2): 116-121 PMID: 15802240.

Schematic of protein-protein interactions that transduce the sensory signal from the chemotaxis receptor supramolecular complex to the flagellar-motor supramolecular complex in *Escherichia coli* chemotaxis.

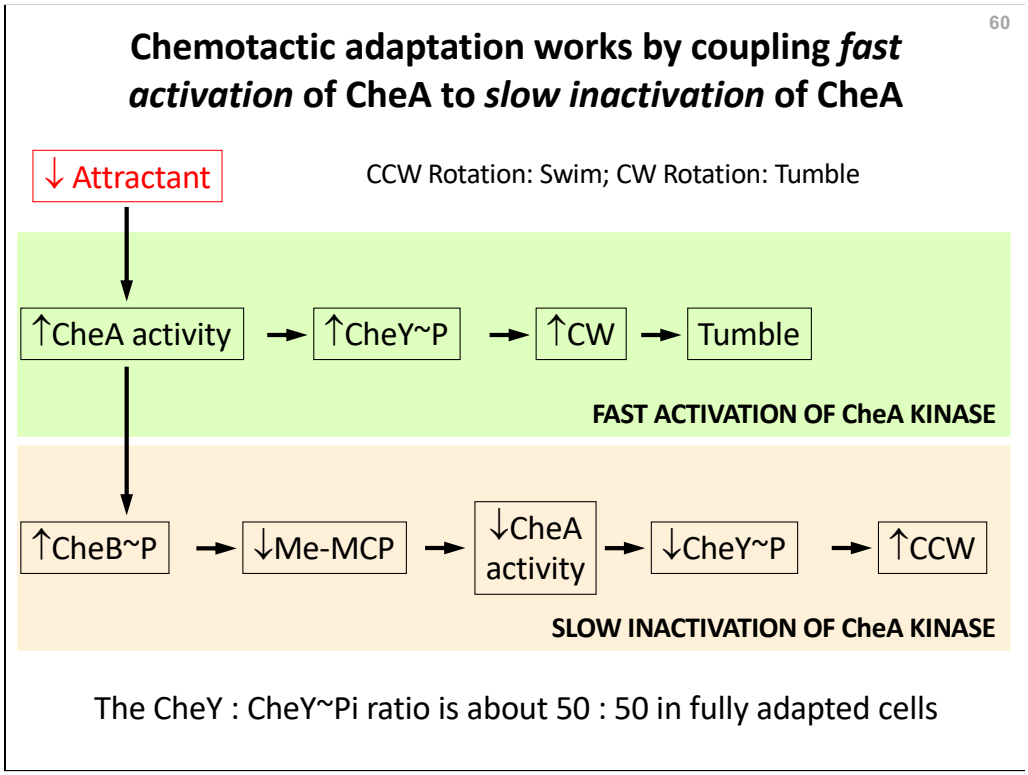
Abbreviations:

+CH_3, adds a methyl group (CheR adds a methyl group to MCPs)

–CH_3, removes a methyl group (CheB removes a methyl group from MCPs)

+Pi, phosphate group (CheA adds a phosphate group to CheY and CheB; CheZ removes a phosphate group from CheY)

Figure 1. Two-state model of receptor signaling and the chemotaxis phosphorelay pathway in *Escherichia coli*. The chemoreceptors a.k.a. "methyl-accepting chemotaxis proteins" (**MCPs**) and cytosolic signaling proteins (**CheA**, **CheB**, **CheR**, **CheW**, **CheY**, **CheZ**) are depicted in their native subunit organizations. The receptor dimers are further arranged in trimers, which comprises the active unit for receptor signaling. Colored components represent functionally active states; gray components represent inactive signaling forms. Green components and reaction arrows represent signaling states that enhance clockwise (**CW**) flagellar rotation, which causes tumbling. Red components and reaction arrows represent signaling states that augment counter-clockwise (**CCW**) flagellar rotation, which causes smooth swimming (the default condition). Binding of an attractant ligand or removal of methyl groups shifts chemoreceptor signaling complexes from the kinase-on (green) to the kinase-off (red) signaling state. Release of attractant and addition of methyl groups shift chemoreceptor signaling complexes from the inactive CheA state (gray) to the active CheA state (blue). Conversely, binding of attractant and removal of methyl groups shift chemoreceptor signaling complexes from the active CheA state (blue) to the inactive CheA state (gray).



SOURCE: Wadhams GH, Armitage JP (2004) Making sense of it all: bacterial chemotaxis. *Nature Rev Mol Cell Biol* 5(12): 1024-1037 PMID: 15573139.

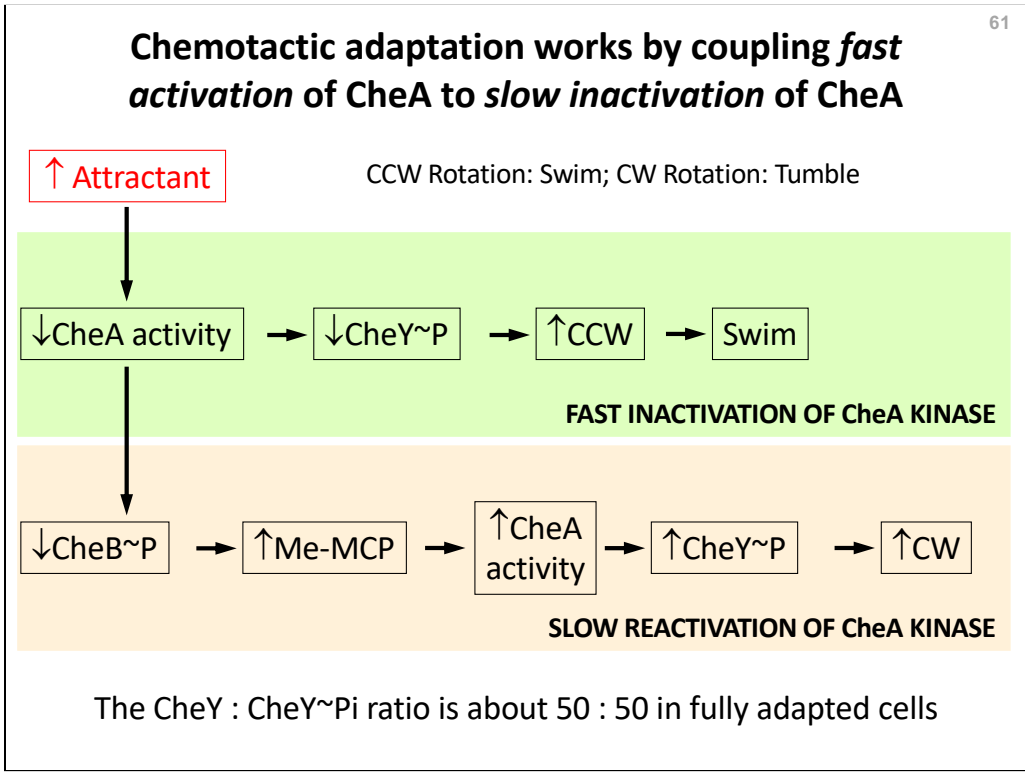
SOURCE: Parkinson JS, Ames P, Studdert CA (2005) Collaborative signaling by bacterial chemoreceptors. *Curr Opin Microbiol* 8(2): 116-121 PMID: 15802240.

In the "two-state model" chemoreceptors switch back and forth between "OFF" and "ON" states. In the "ON" state the chemoreceptor activates CheA kinase. In the "OFF" state the chemoreceptor deactivates CheA kinase. Binding of an attractant to the chemoreceptor favors the "OFF" state. Methylation of the chemoreceptor favors the "ON" state.

Imagine a bacterial cell swimming in a "bad" direction so the concentration of attractant is decreasing over time. Alternatively, imagine a bacterial cell that is transferred from a "high" concentration of attractant to a "low" concentration of attractant. Two things will happen on different time scales: *fast response* and *slow adaptation*.

Fast Response: When the [attractant] decreases, decreased binding of attractant to chemoreceptors favors the "ON" state of the chemoreceptors. Consequently CheA kinase activity increases. Consequently the concentration of phosphorylated CheY switch protein [CheY~P] increases (remember: CheY~P is constantly being dephosphorylated by CheZ phosphatase, which is always active). Consequently the probability increases that CheY~P will bind to the motor complex and trigger a switch from counter-clockwise (CCW) rotation to clockwise (CW) rotation. Consequently the probability of a tumble (CW rotation) increases and the probability of continuing to run (CCW rotation) decreases. This makes sense: if the [attractant] decreases over time, it means that the cell is moving in a "bad" direction so why keep going in that direction?

Slow Adaptation: When the [attractant] decreases, decreased binding of attractant to chemoreceptors favors the "ON" state of the chemoreceptors. Consequently CheA kinase activity increases. Consequently the concentration of phosphorylated CheB methylesterase [CheB~P] increases. Consequently methylation of the chemoreceptors decreases (remember: methyl groups are constantly being added to chemoreceptors by CheR methylase , which is always active). Demethylation of the chemoreceptors favors the "OFF" state of the chemoreceptors. Consequently the concentration of phosphorylated CheY switch protein [CheY~P] decreases. Consequently the probability decreases that CheY~P will bind to the motor complex and trigger a switch from counter-clockwise (CCW) to clockwise (CW) rotation. Consequently the probability of a tumble (CW rotation) decreases and the probability of continuing to run (CCW rotation) increases. When the system has fully adapted, 50% of CheY is phosphorylated and 50% of CheY is dephosphorylated. Thus, the adapted system is "poised" to move in either direction: increased tumbling (shorter runs) or decreased tumbling (longer runs).



SOURCE: Wadhams GH, Armitage JP (2004) Making sense of it all: bacterial chemotaxis. *Nature Rev Mol Cell Biol* 5(12): 1024-1037 PMID: 15573139.

SOURCE: Parkinson JS, Ames P, Studdert CA (2005) Collaborative signaling by bacterial chemoreceptors. *Curr Opin Microbiol* 8(2): 116-121 PMID: 15802240.

In the "two-state model" chemoreceptors switch back and forth between "OFF" and "ON" states. In the "ON" state the chemoreceptor activates CheA kinase. In the "OFF" state the chemoreceptor deactivates CheA kinase. Binding of an attractant to the chemoreceptor favors the "OFF" state. Methylation of the chemoreceptor favors the "ON" state.

Imagine a bacterial cell swimming in a "good" direction so the concentration of attractant is increasing over time. Alternatively, imagine a bacterial cell that is transferred from a "low" concentration of attractant to a "high" concentration of attractant. Two things will happen on different time scales: *fast response* and *slow adaptation*.

Fast Response: When the [attractant] increases, increased binding of attractant to chemoreceptors favors the "OFF" state of the chemoreceptors. Consequently CheA kinase activity decreases. Consequently the concentration of phosphorylated CheY switch protein [CheY~P] decreases (remember: CheY~P is constantly being dephosphorylated by CheZ phosphatase, which is always active). Consequently the probability decreases that CheY~P will bind to the motor complex and trigger a switch from counter-clockwise (CCW) rotation to clockwise (CW) rotation. Consequently the probability of a tumble (CW rotation) decreases and the probability of continuing to run (CCW rotation) increases. This makes sense: if the [attractant] increases over time, it means that the cell is moving in a "good" direction so why change directions?

Slow Adaptation: When the [attractant] increases, increased binding of attractant to chemoreceptors favors the "OFF" state of the chemoreceptors. Consequently CheA kinase activity decreases. Consequently the concentration of phosphorylated CheB methylesterase [CheB~P] decreases. Consequently methylation of the chemoreceptors increases (remember: methyl groups are constantly being added to chemoreceptors by CheR methylase , which is always active). Methylation of the chemoreceptors favors the "ON" state of the chemoreceptors. Consequently the concentration of phosphorylated CheY switch protein [CheY~P] increases. Consequently the probability increases that CheY~P will bind to the motor complex and trigger a switch from counter-clockwise (CCW) to clockwise (CW) rotation. Consequently the probability of a tumble (CW rotation) increases and the probability of continuing to run (CCW rotation) decreases. When the system has fully adapted, 50% of CheY is phosphorylated and 50% of CheY is dephosphorylated. Thus, the adapted system is "poised" to move in either direction: increased tumbling (shorter runs) or decreased tumbling (longer runs).