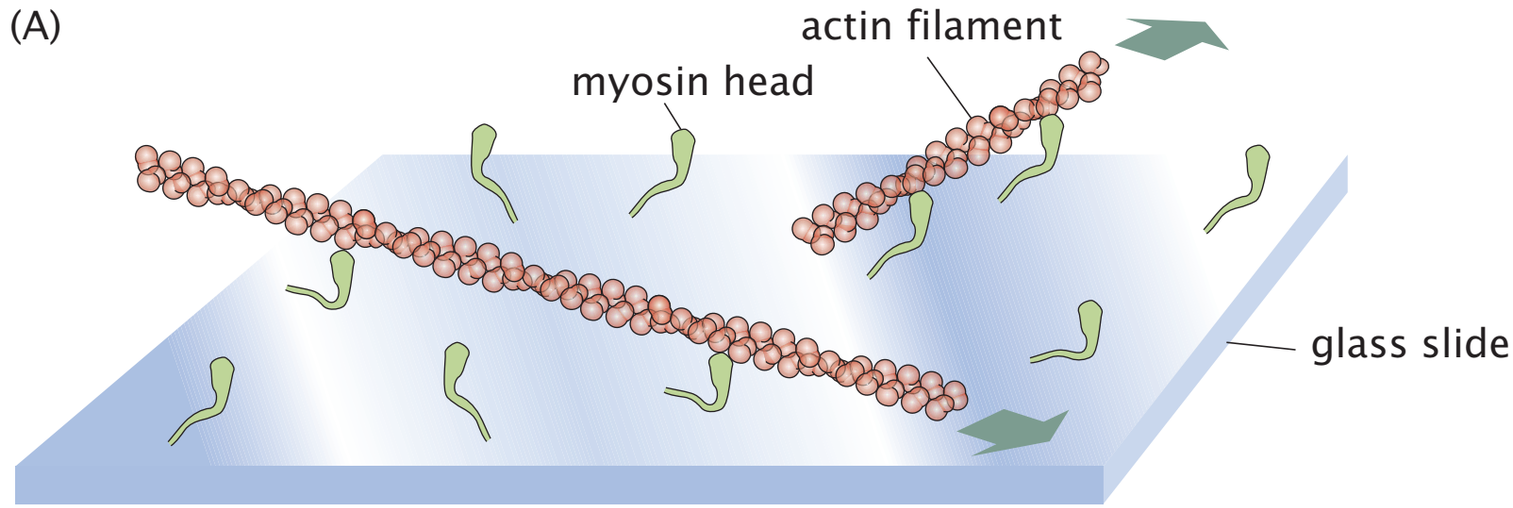
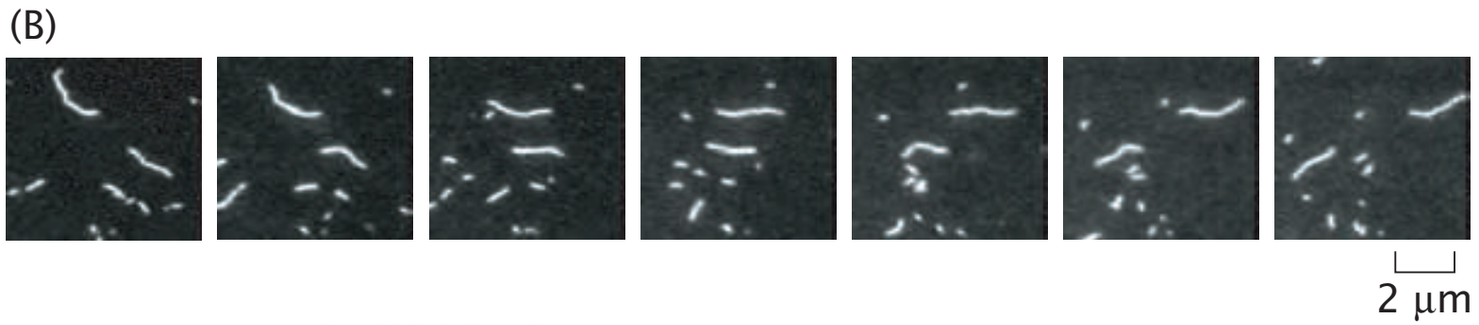
Today’s class:

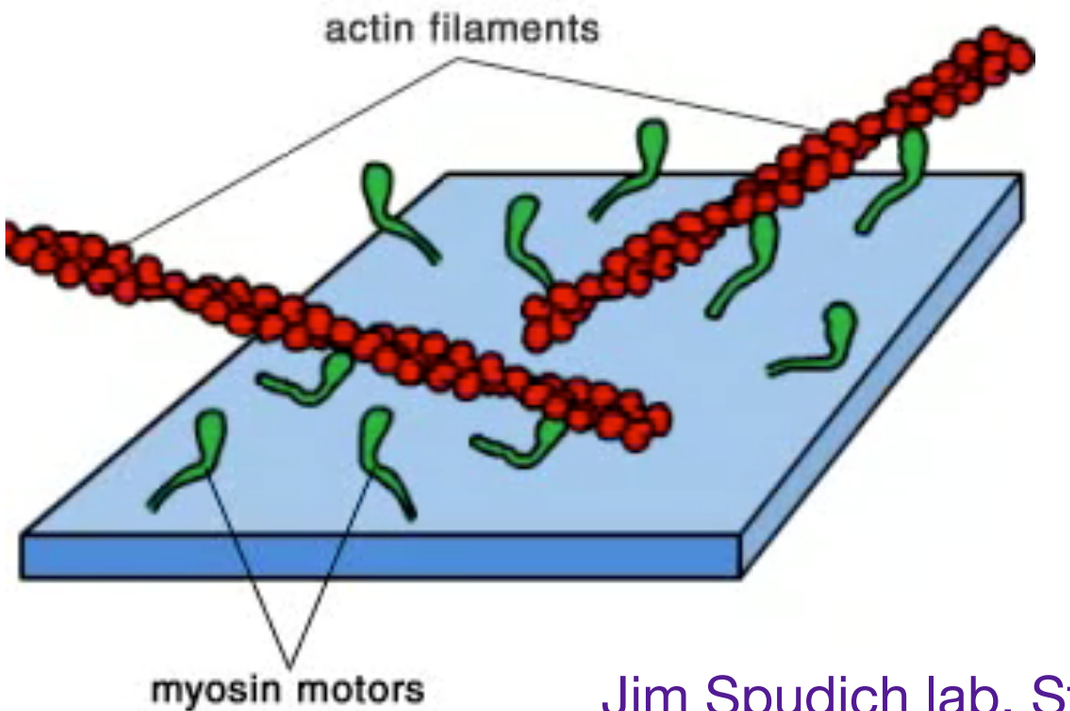
Active transport in the cell part 2

This lecture follows the Chapter 16 in the book ‘Physical Biology of the Cell’ by Philips et al. and parts of chapter 14 & 17 in the book ‘The Molecules of Life’ by Kuriyan et al.

Measuring motor action: gliding motility assay

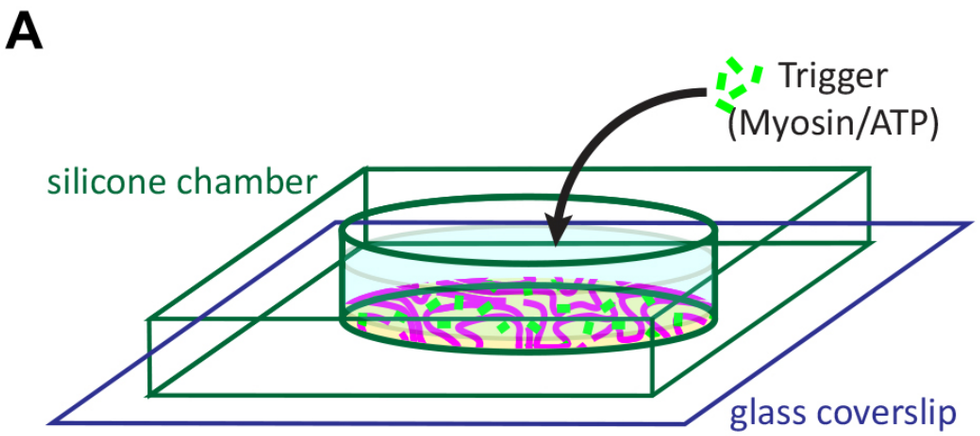


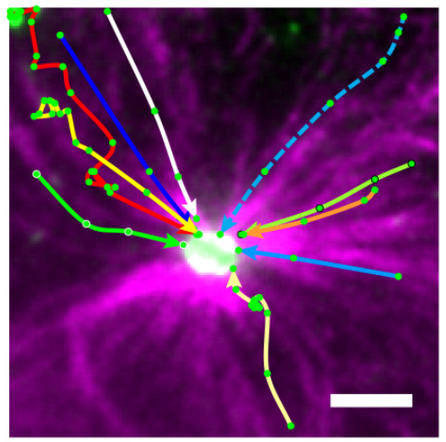


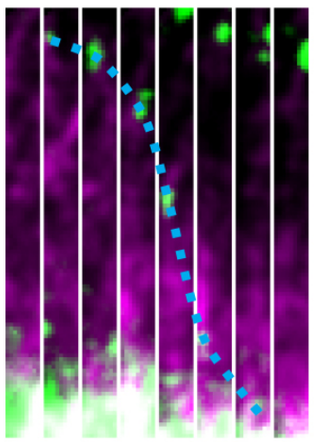


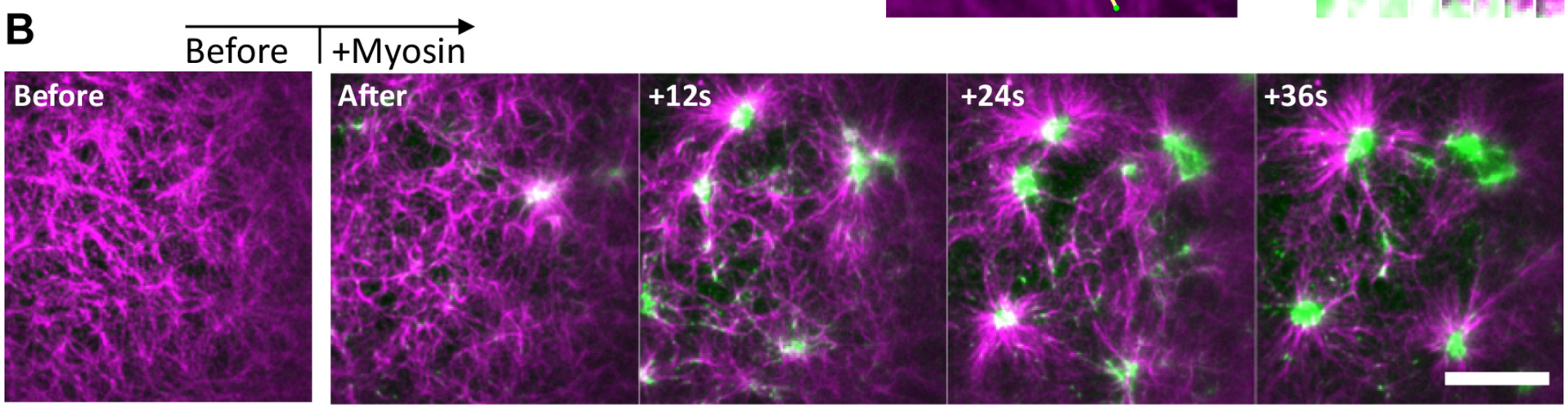
Jim Spudich lab, Stanford

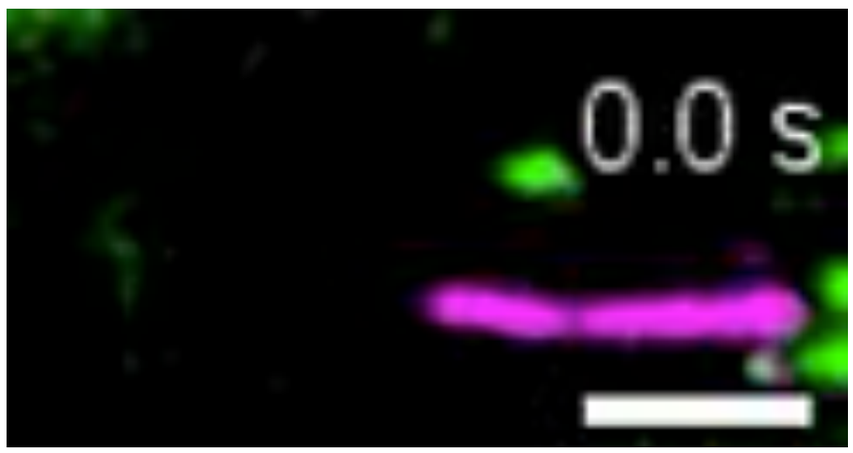
Measuring motor action: TIRF microscopy











Polarity sorting of actin filaments by myosin clusters

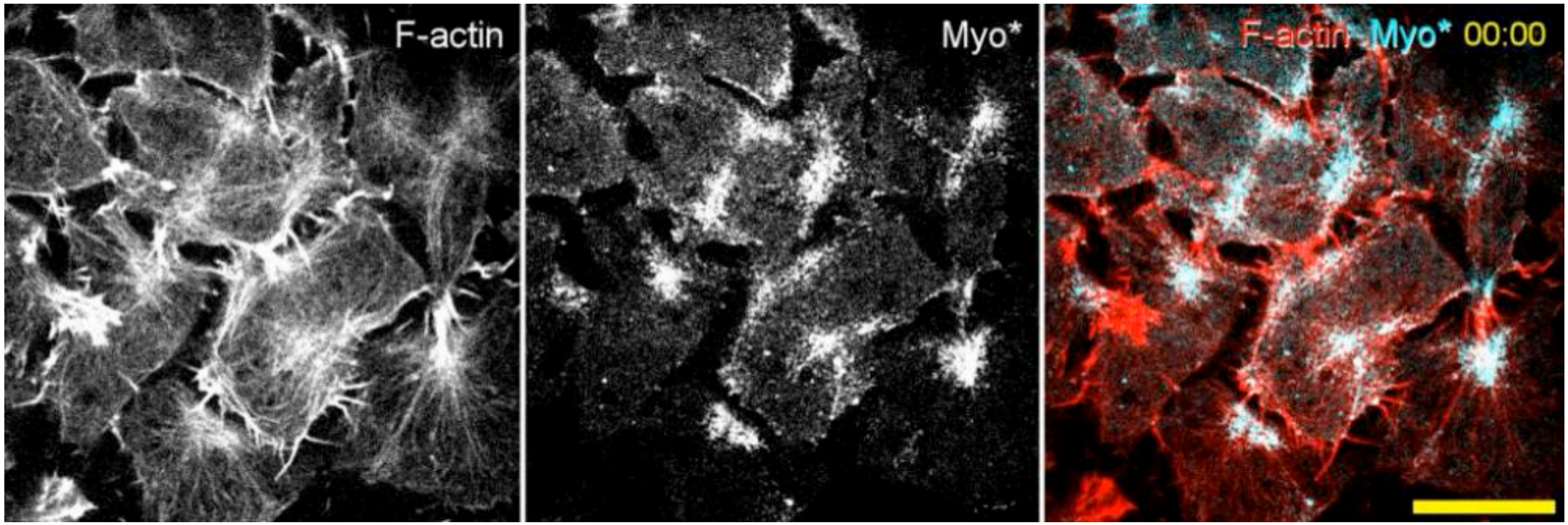
Wollrab et al J Cell Sci 2019

Watching myosin in action *in vivo* elsewhere from muscles

Migration of a mouse flibreoblast cell
Actin in purple
Myosin in green

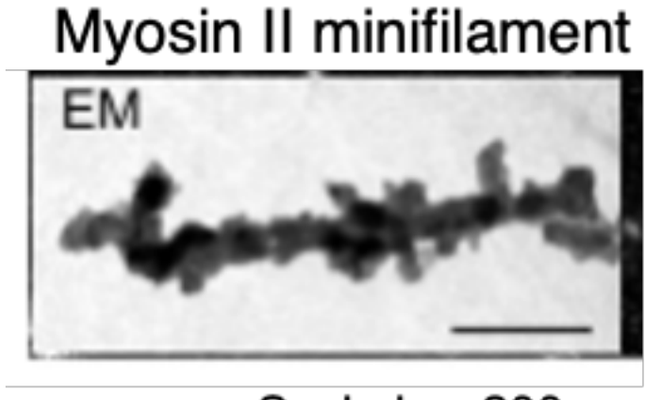
D. Li et al., Science 349, aab3500 (2015).

Myosin in action *in vivo* elsewhere from muscles…*contd*



Actomyosin contraction in Xenopus embryo

Miller et al Plos Comp Bio 2018



Scale bar 200 nm

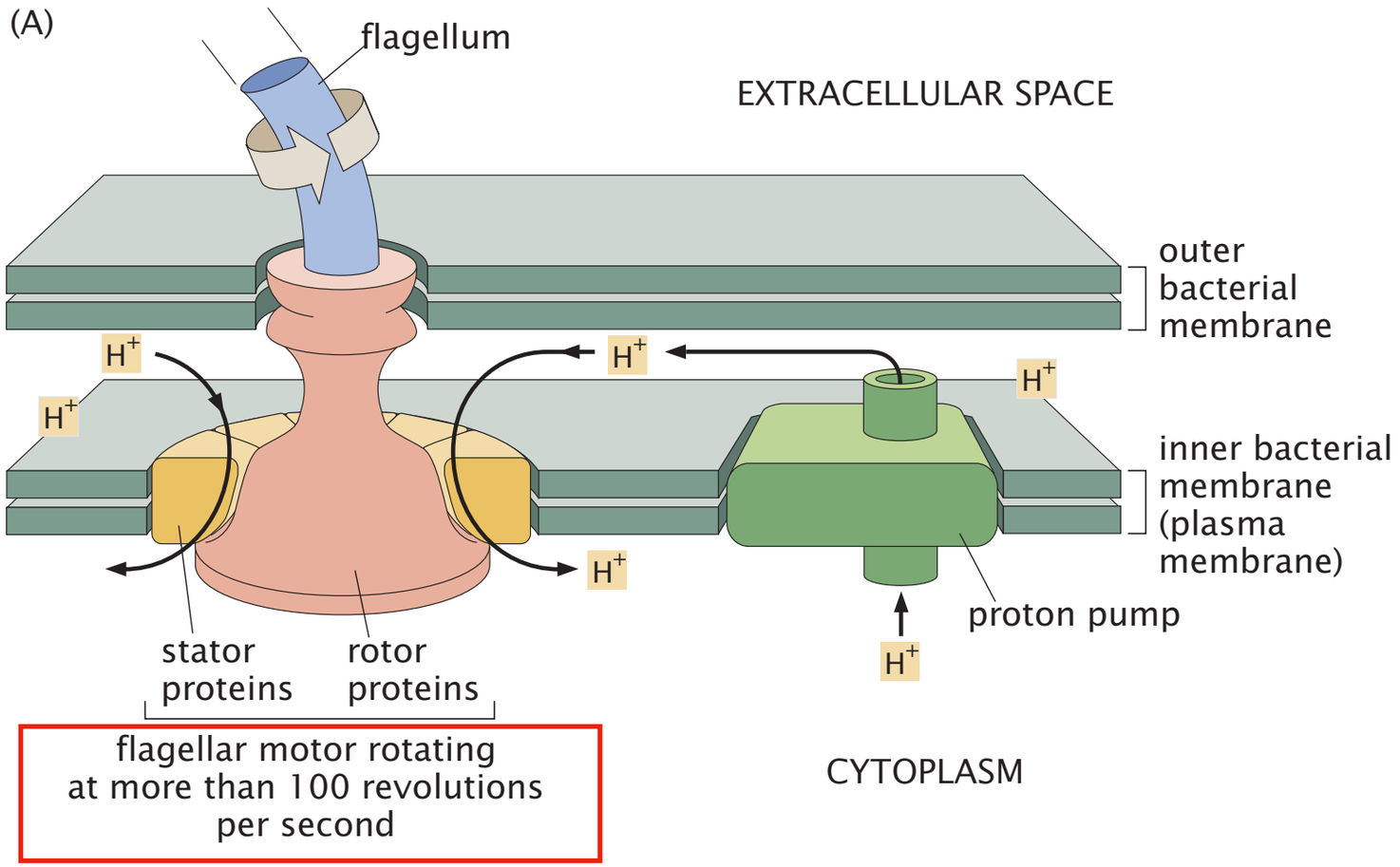
Koester et al PNAS (2016)

Rotatory motors

Molecular motors that generate rotational motion

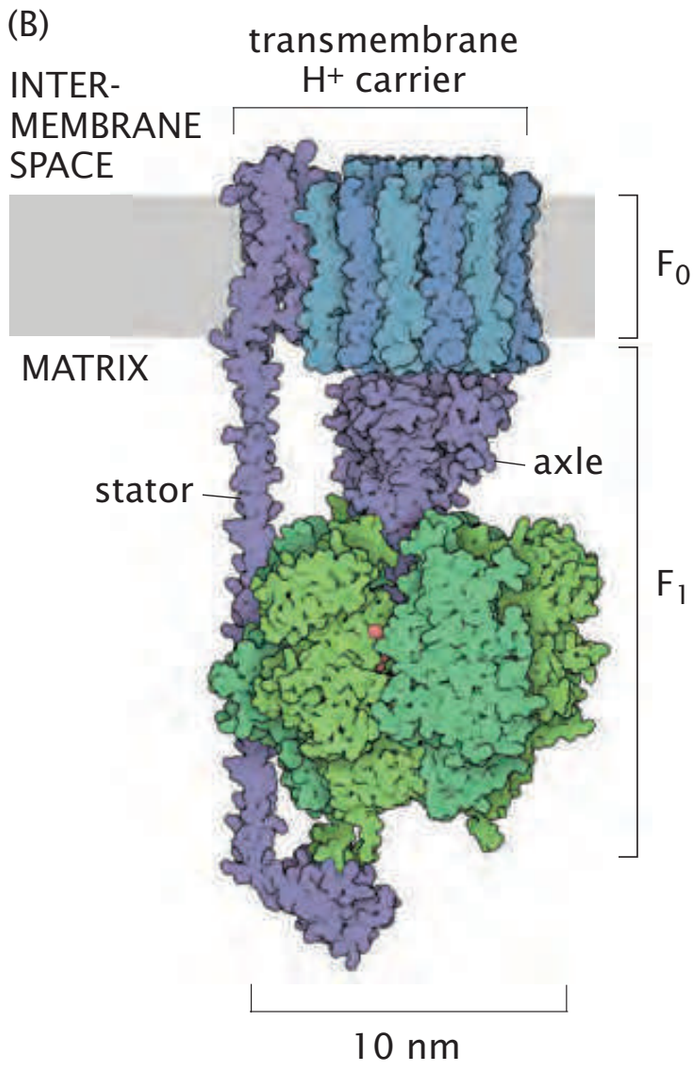
Two most studied rotatory motors are

Bacterial flagellar motor

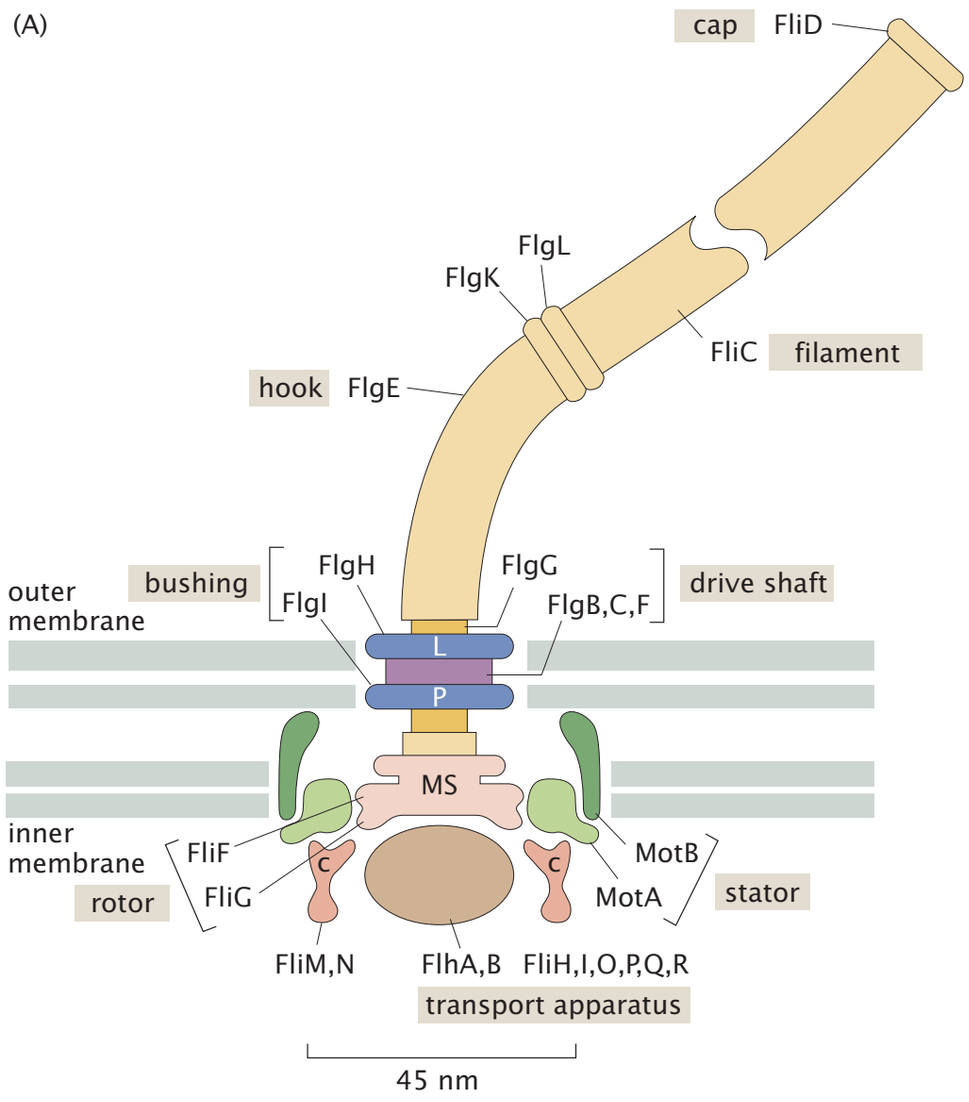


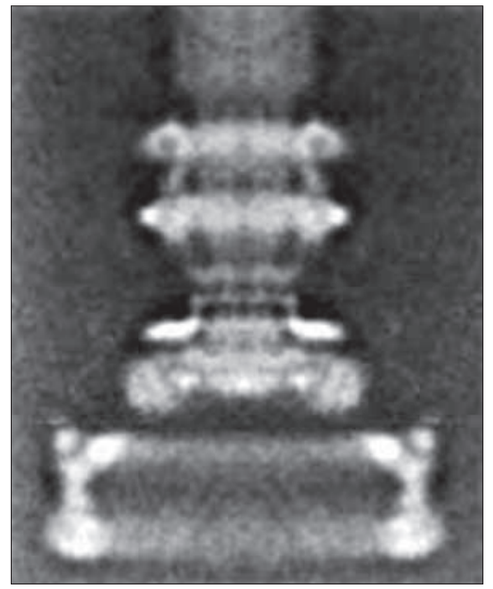
flagellar motor rotating at more than 100 revolutions per second

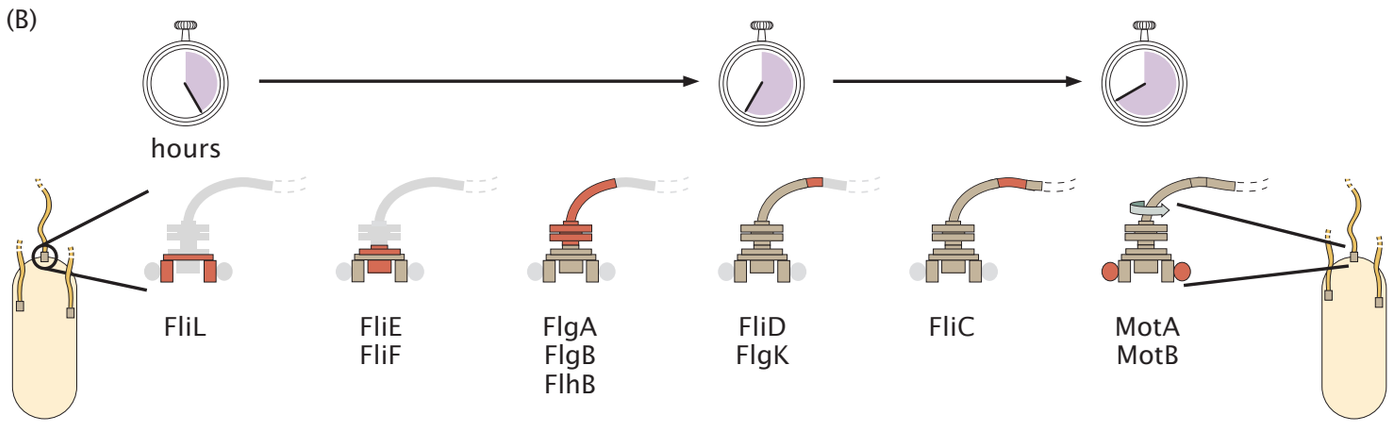
ATP synthase

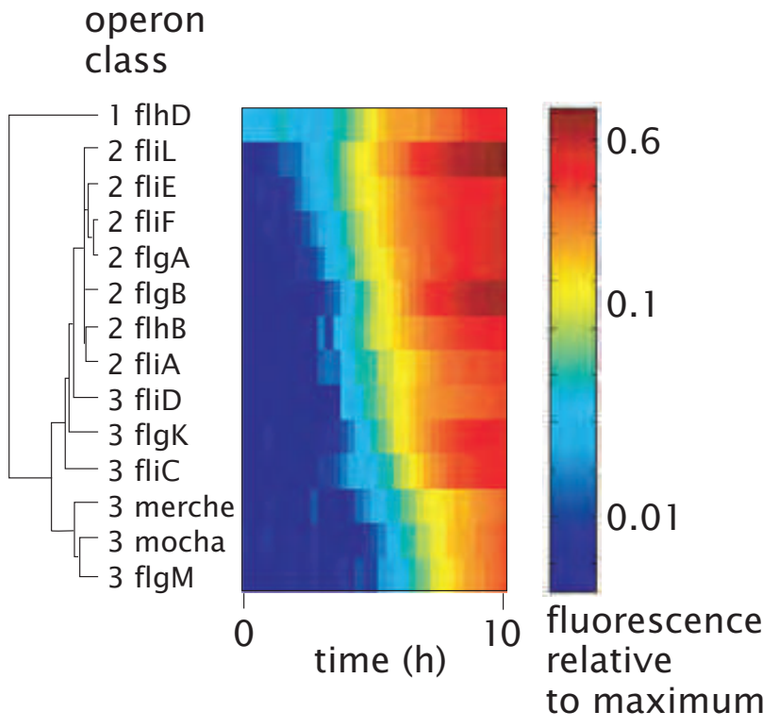


Bacterial flagellum and motor is made by subunits assembled step by step

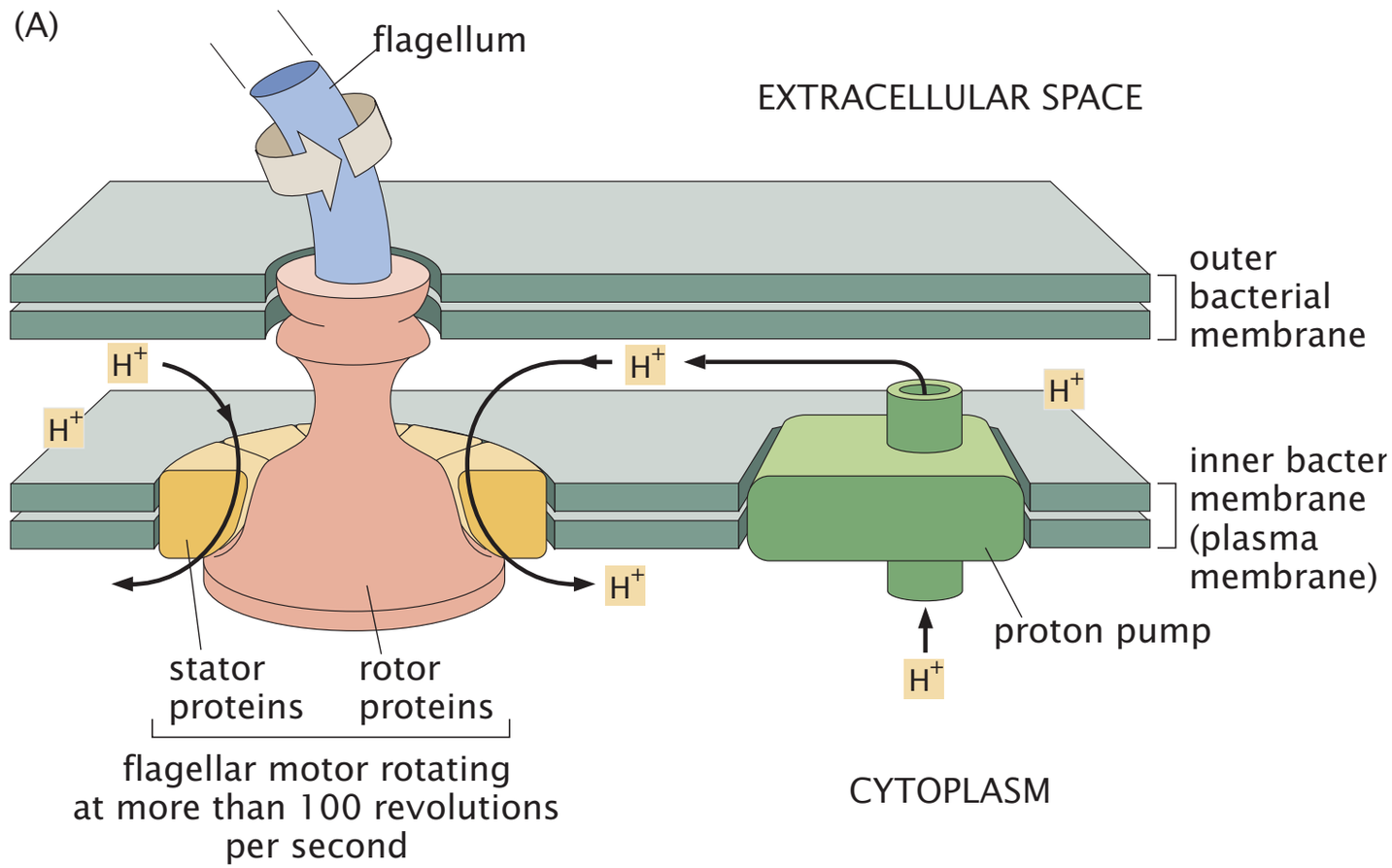








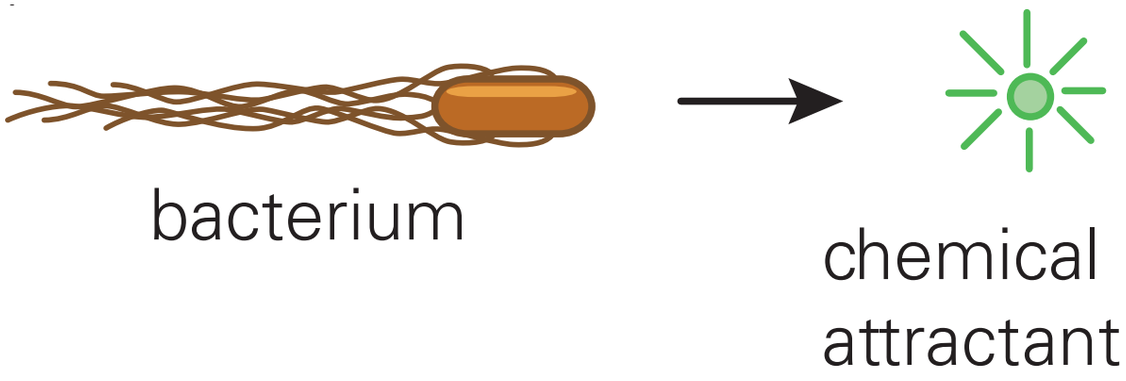
Properties of the bacterial flagellar motor



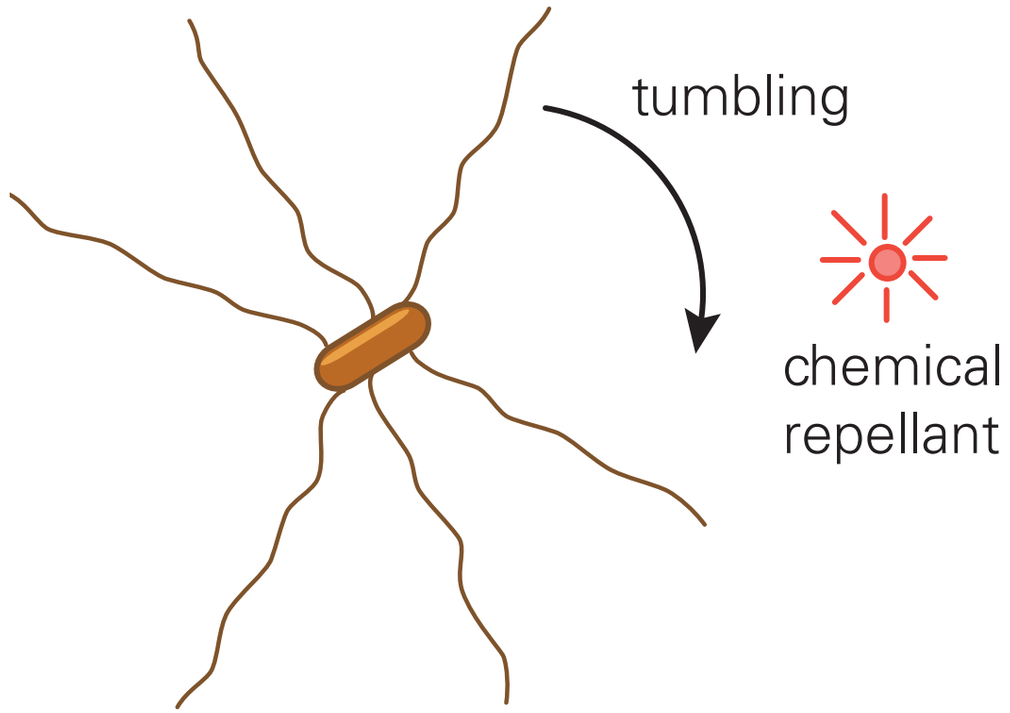
- The bacterial flagellum is like a tiny propeller driven by the gradient of H^+ ions across the bacterial inner membrane.
- Continuous motor operation is driven by the gradient maintained by ATP-consuming proton pumps.
- The flagellum is an extremely long (10 μm) helical filament attached at its base to the motor.
- The motor is embedded in the bacterial inner membrane and anchored to the cell wall with a shaft passing through the outer membrane.
- The motor is capable of rotating in either direction at speeds up to 100 Hz.
- Astonishingly, the motor can reverse its direction of rotation without reversing the direction of ion flow

Rotational modes of the bacterial flagellar motor

Anti-clock wise rotation

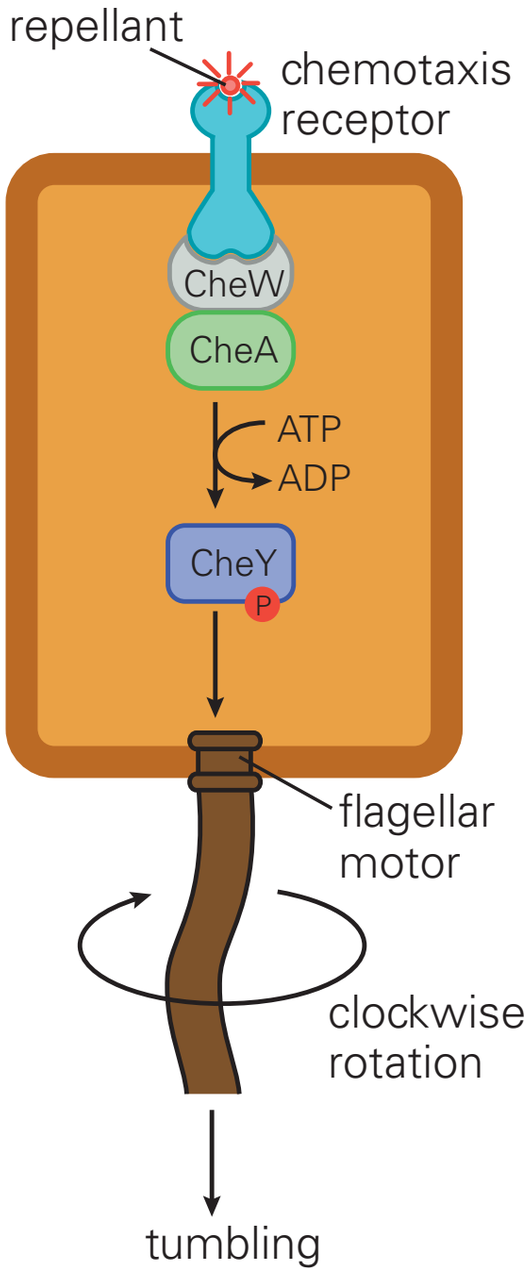


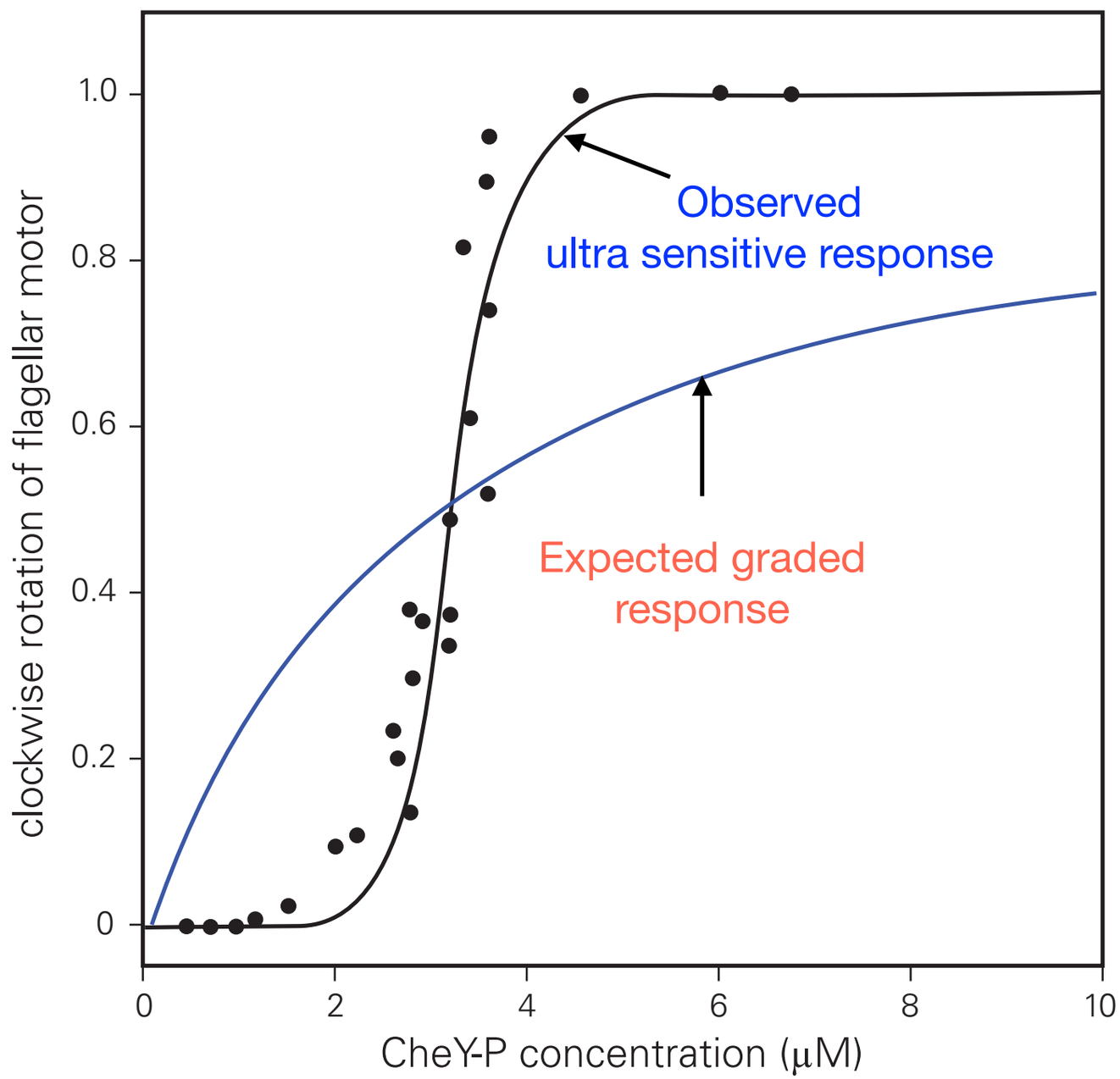
Clock wise rotation



This direction *switching* is controlled by a protein CheY that binds to the flagellar motor and activates a tumbling response

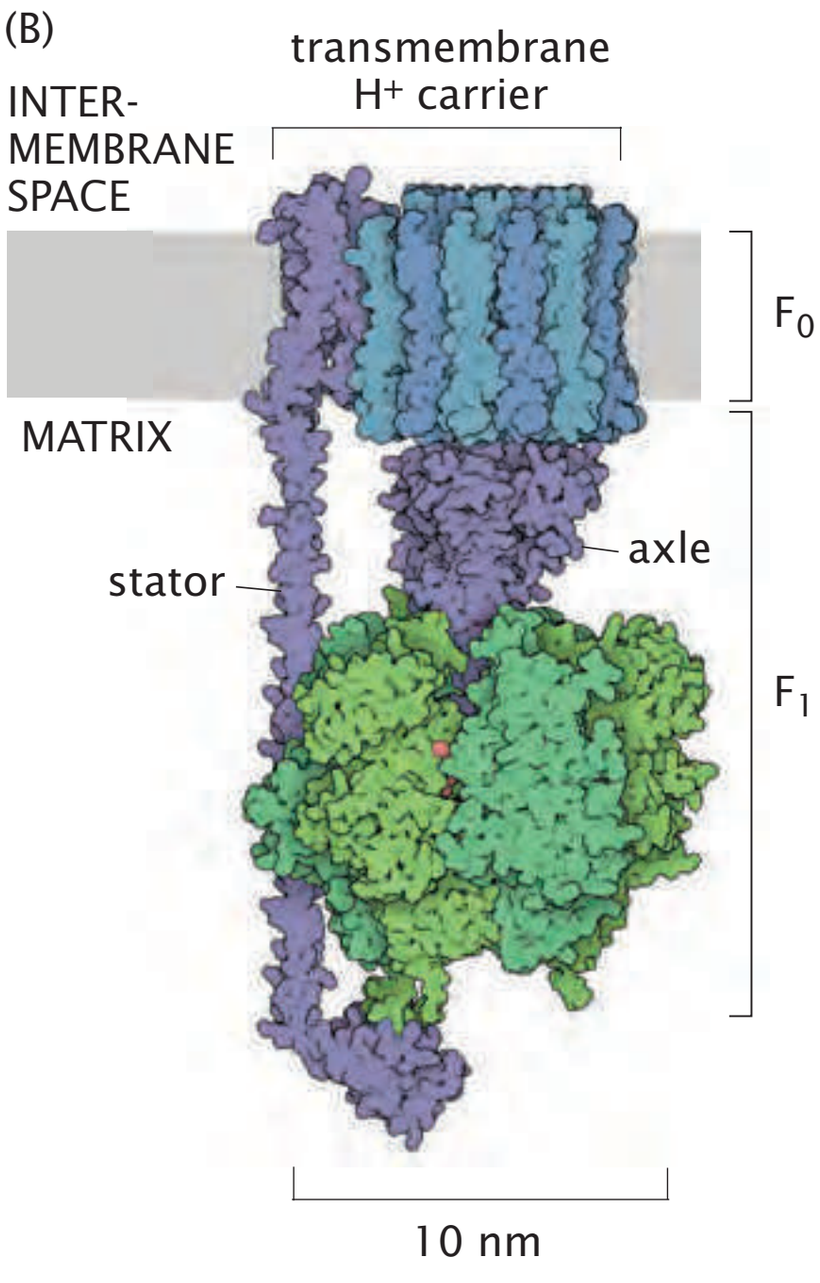
Tumbling response generation in the bacterial flagellar motor is allosteric





$$\text{motor} + \text{CheY} - \text{P} \rightleftharpoons [\text{motor} \cdot \text{CheY} - \text{P}] \longrightarrow \text{Activation of clock-wise rotation}$$

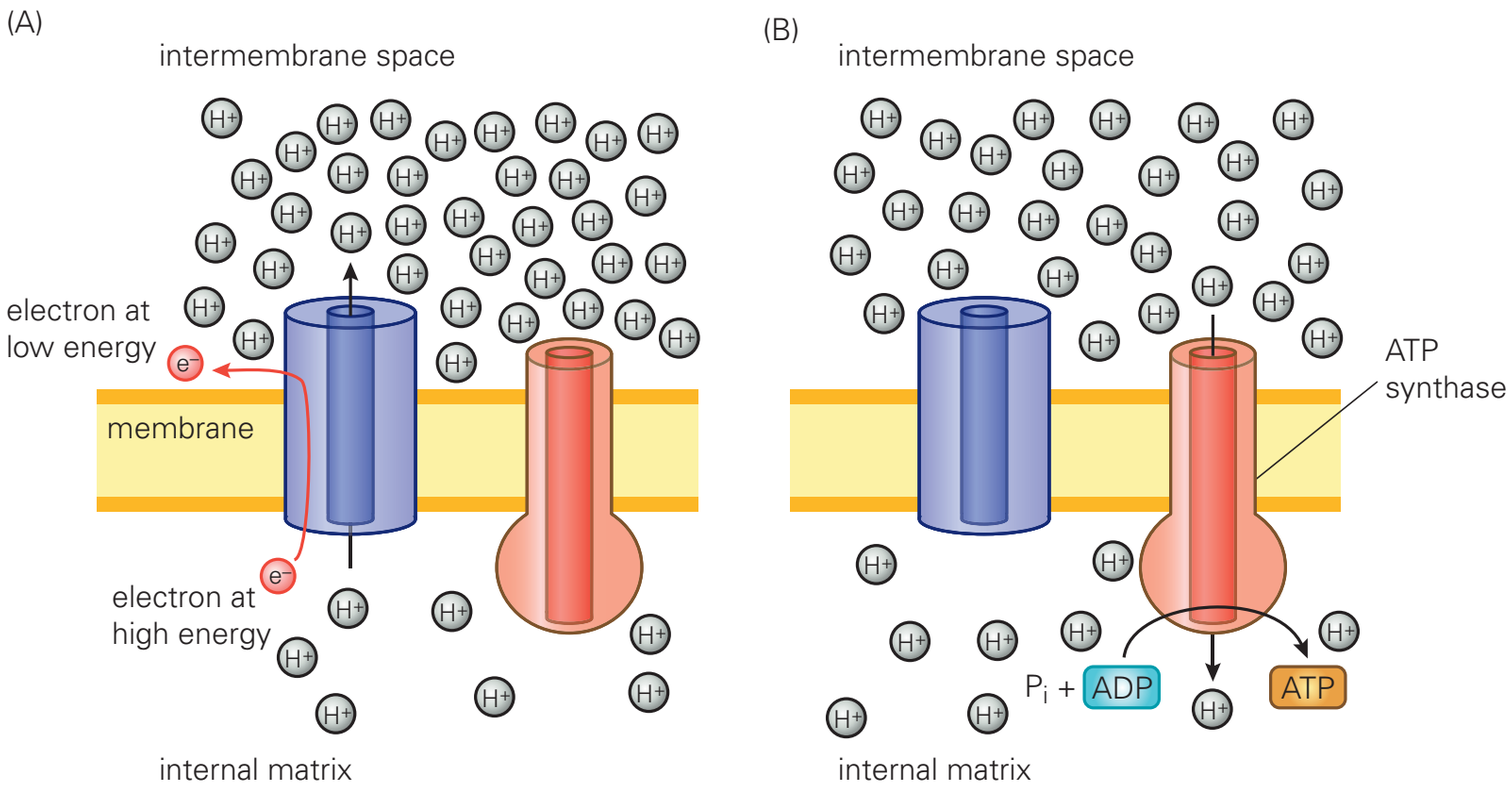
ATP synthase is the central powerhouse of the cell



Key features

- found in the inner membrane of bacterial cells and also in the mitochondria of eukaryotic cells.
- It is made of two different rotary motors - F_0 and F_1 - connected to a common drive shaft.
- The F_0 motor is similar to the flagellar rotary motor, uses the energy stored in the transmembrane gradient of H^+ ions to rotate.
- The F_1 motor uses energy of ATP hydrolysis to rotate in the opposite direction.

Functioning of ATP synthase depends on the transmembrane electrochemical gradient



- When the transmembrane H^+ gradient is strong - this is the normal condition

  $\Longrightarrow$ torque (F_0) >> torque (F_1)

  $\Longrightarrow$ F_0 forces F_1 to rotate in reverse and the motor synthesize ATP

- When the gradient is weak - the balance tilts in opposite direction

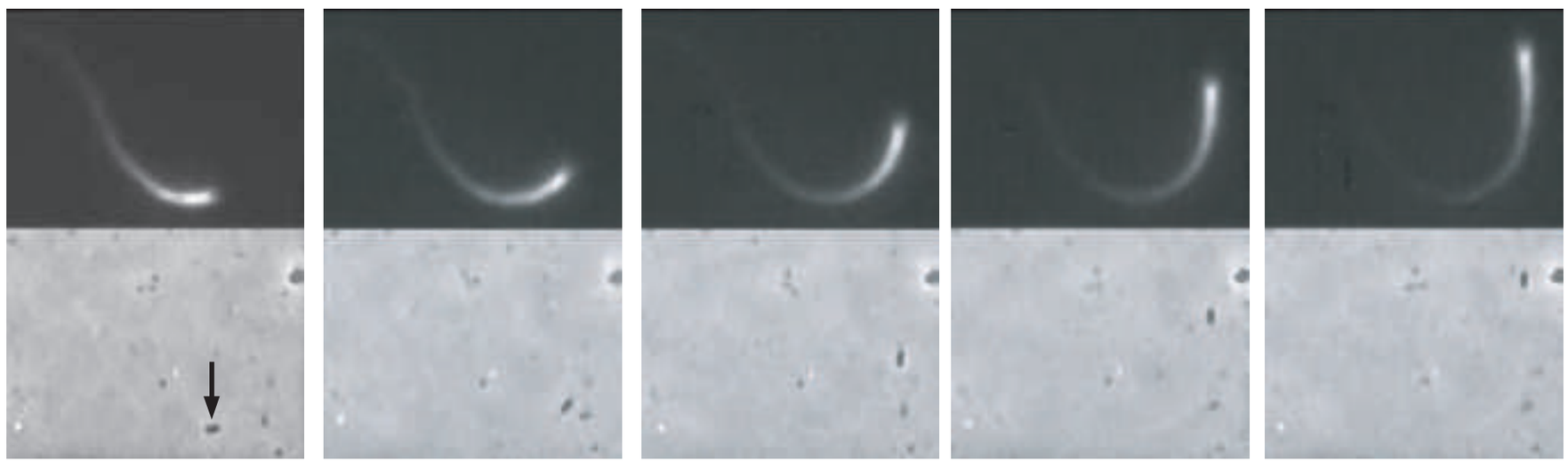
  $\Longrightarrow$ torque (F_1) >> torque (F_0)

  $\Longrightarrow$ ATP is hydrolyzed and the motor pumps H^+ ions out of the cell.

Polymerization motors: Force generation by growth

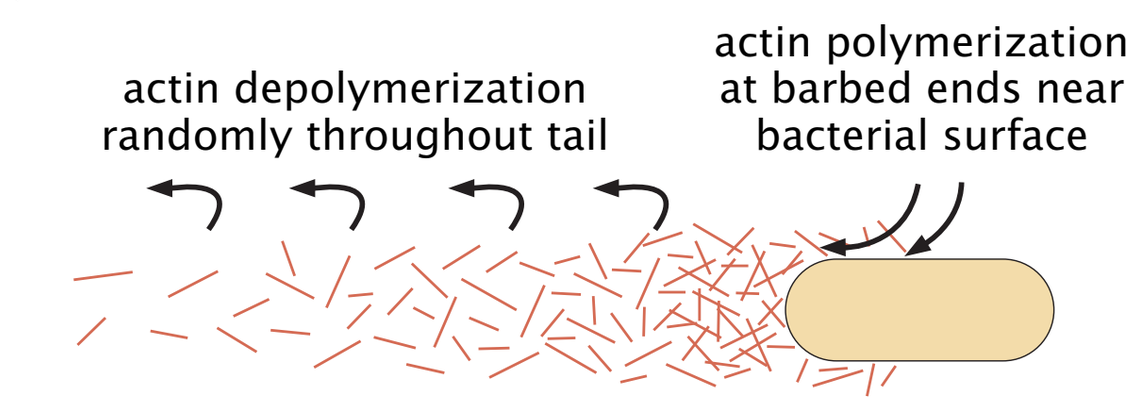
(A)

Actin polymerization-driven movement of *Listeria monocytogenes*

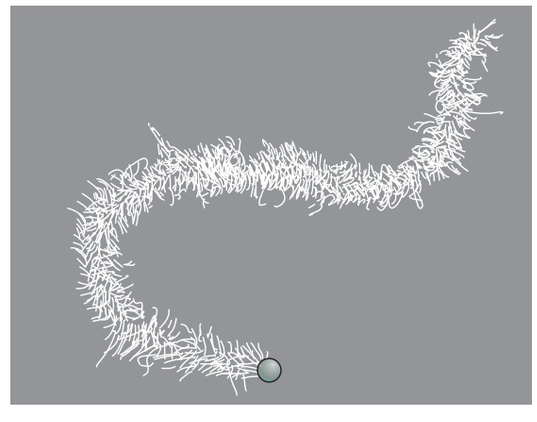


5 μm

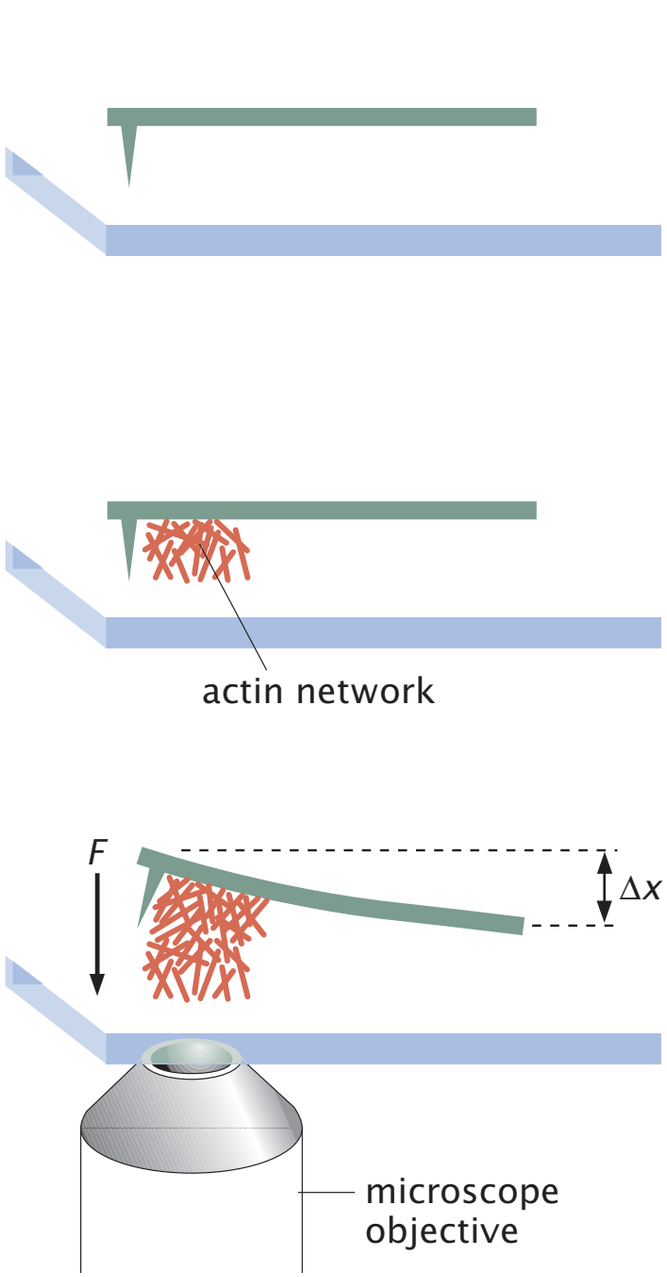
(B)

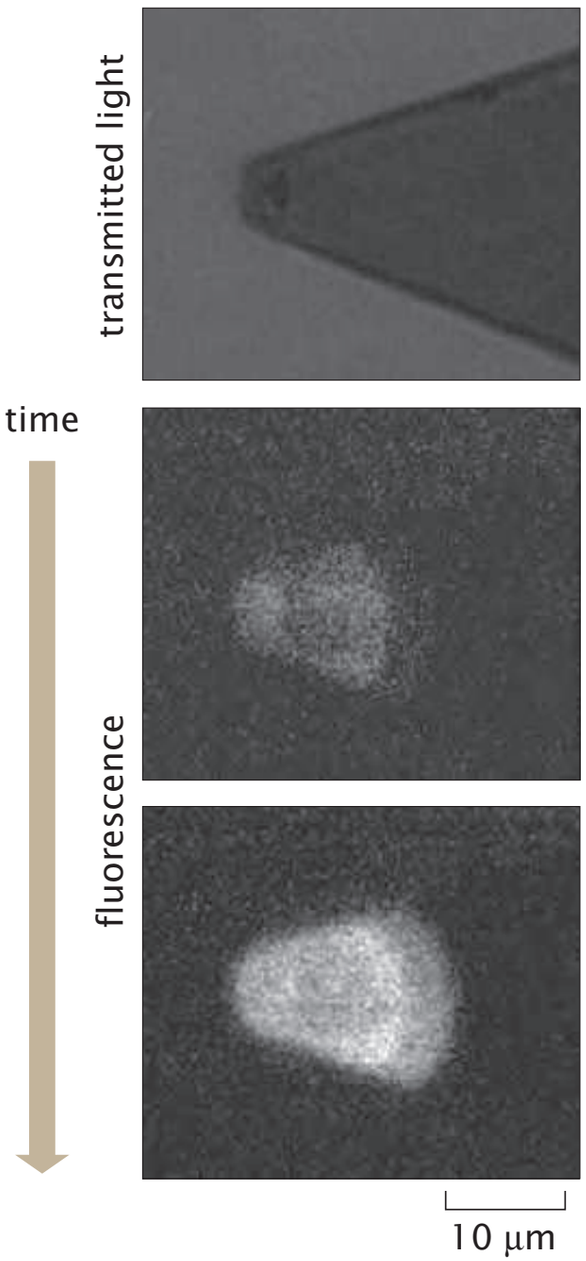


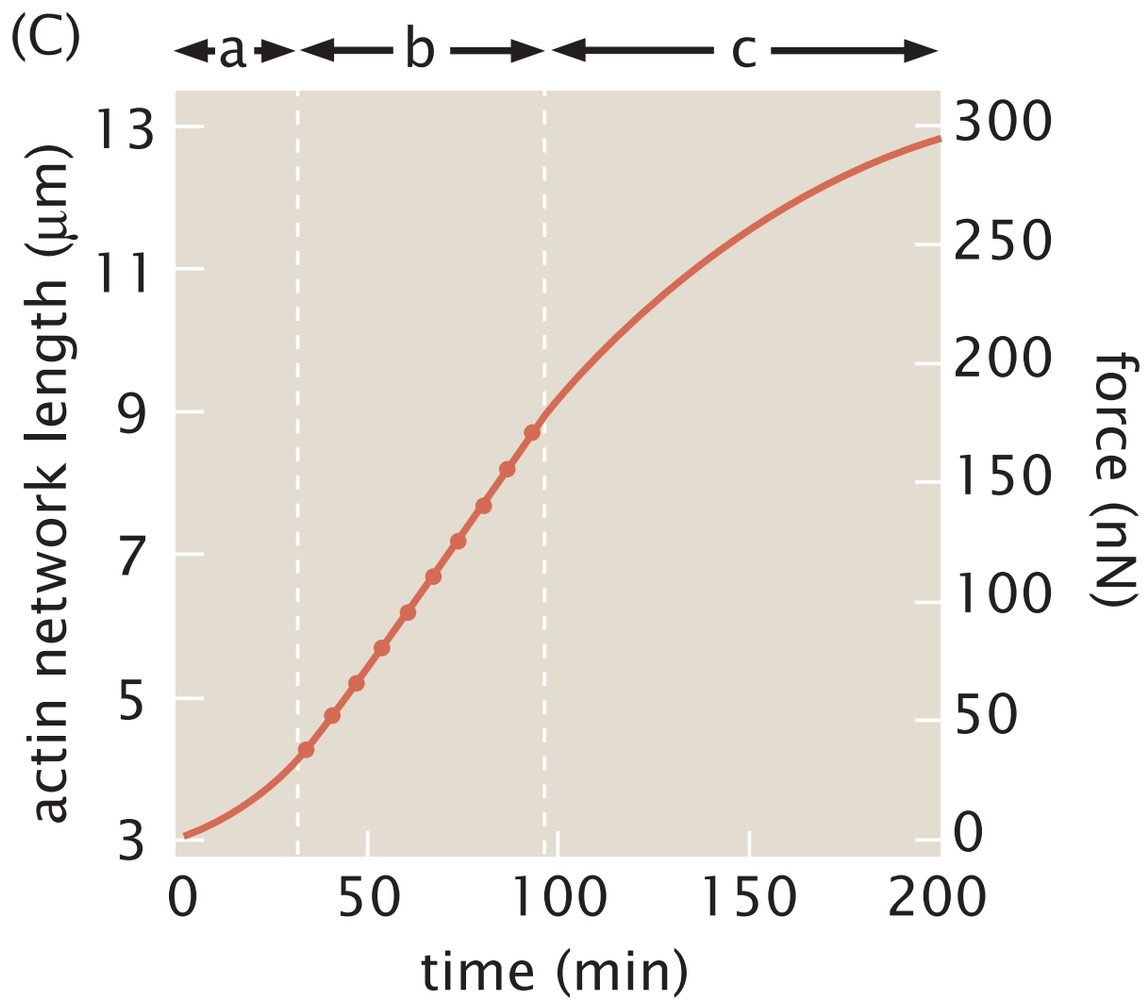
(C)



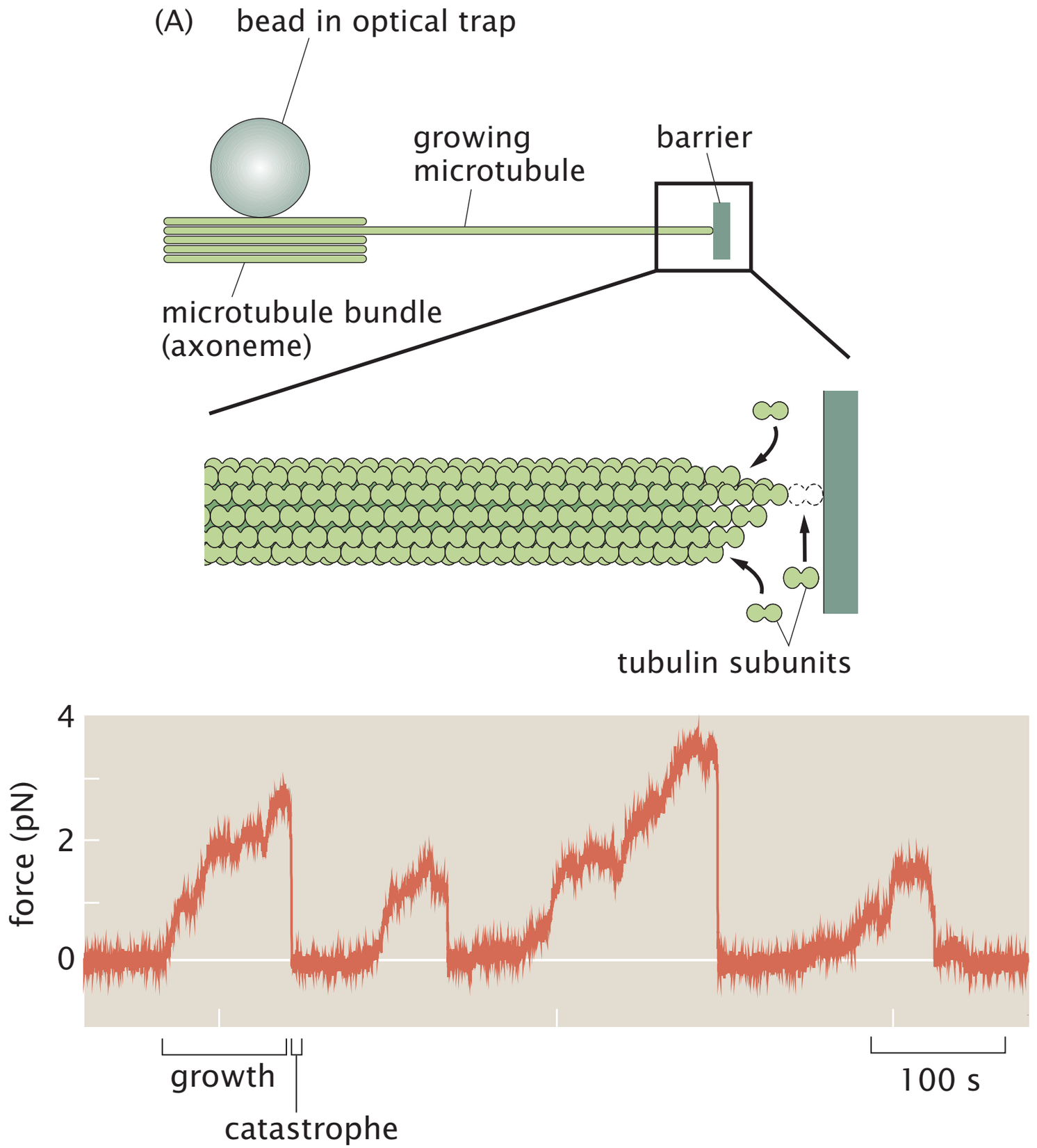
Measurement of the force of actin polymerization







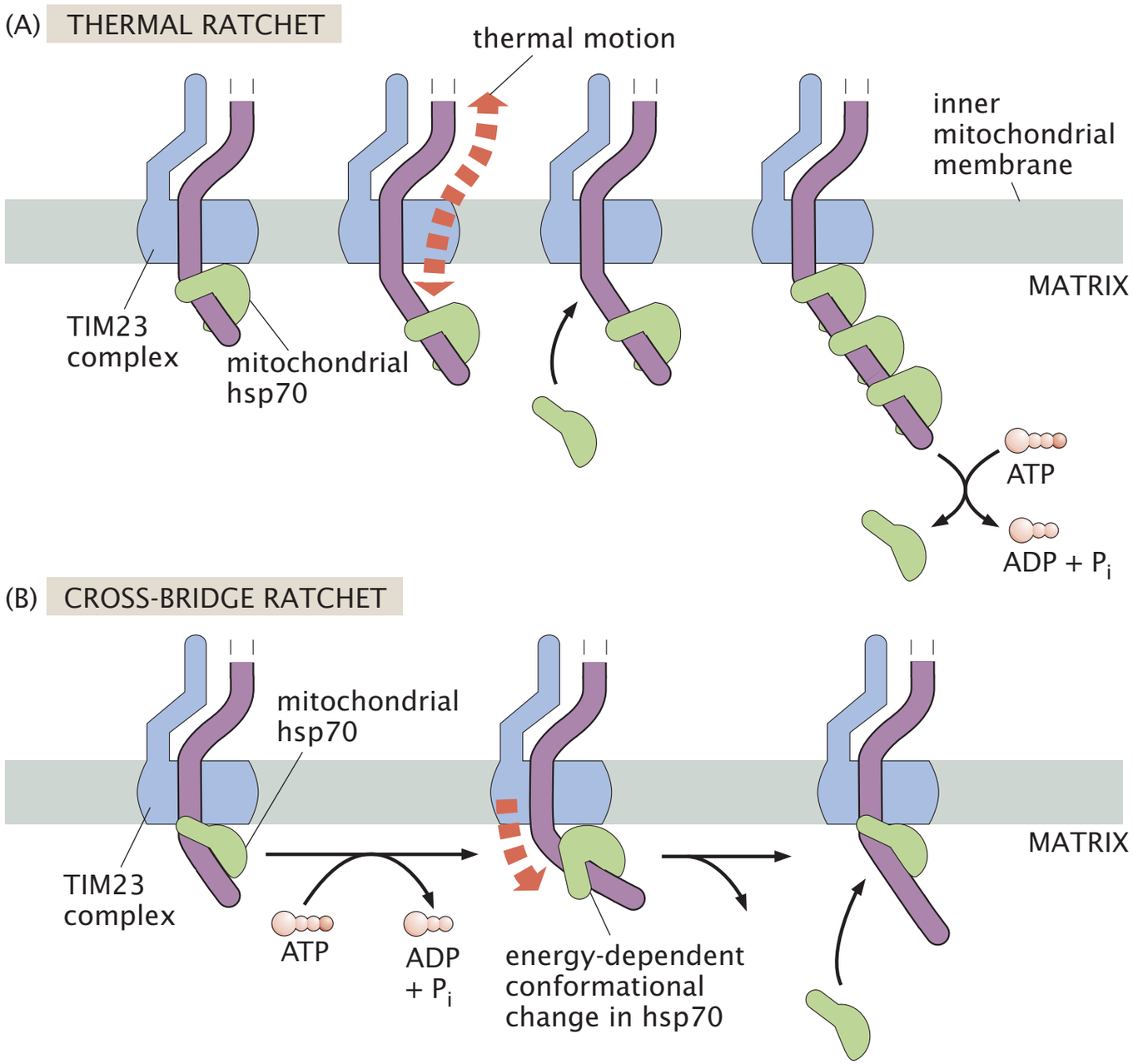
Measurement of the force of microtubule polymerization



Translocation motors

Motors that mediate the transport of biomolecules, like protein, DNA, RNA, sugars from one membrane bound organelle to another

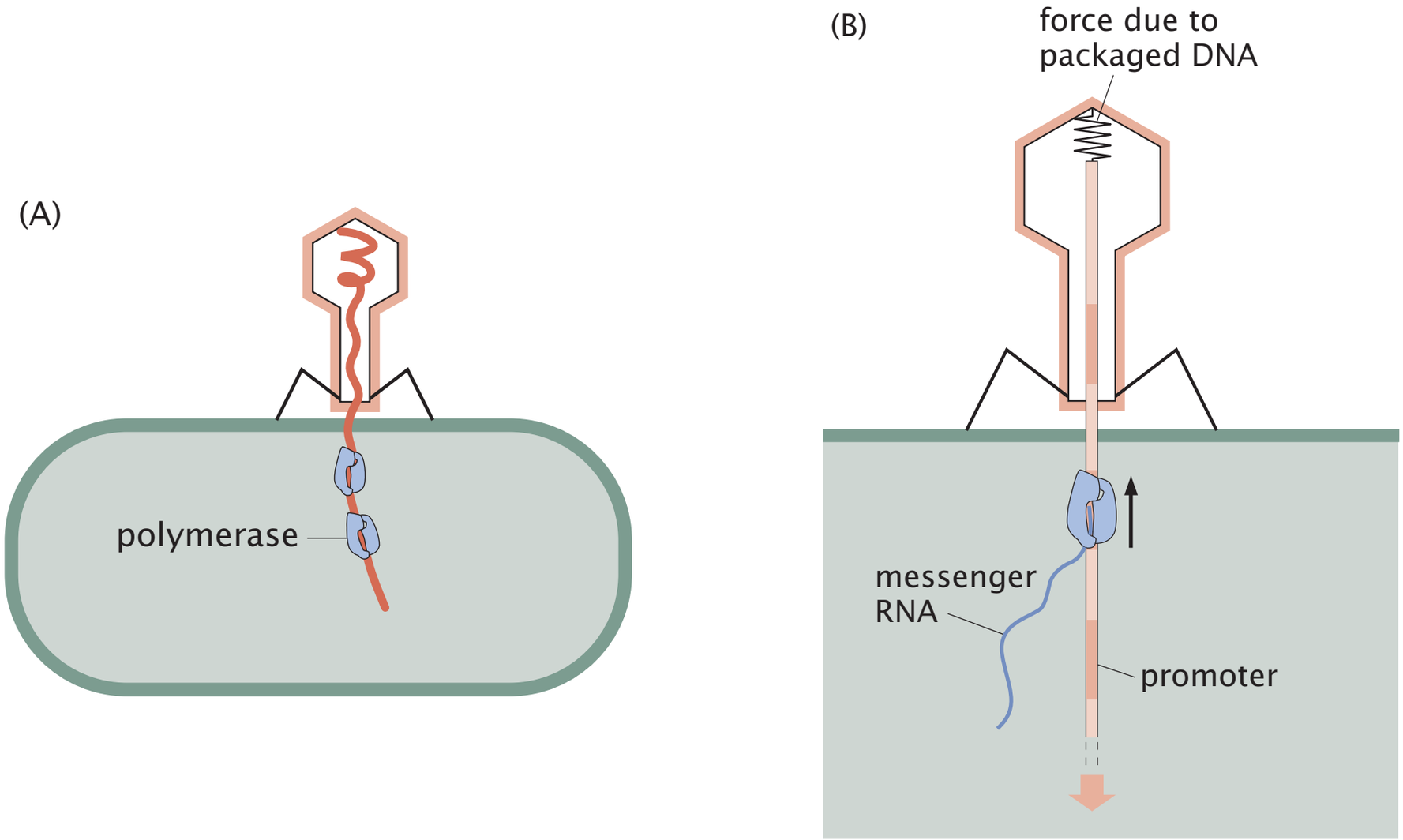
Two possible mechanisms of translocation by motors



Thermal motion of the polymer in and out of the pore is biased by the presence of binding proteins on only one side of the barrier.

Binding proteins on one side of the barrier may also use energy-dependent conformational changes to further insure that the cargo polymer moves in only one direction.

Translocation of phage DNA into host cell



Here two driving forces are in play

- The DNA packing pressure inside virus produces a driving force.
- In addition, the RNA polymerase can begin transcription and help to pull the DNA out of the viral capsid.