

BE/APH161 – PHYSICAL BIOLOGY OF THE CELL

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LIFE AT THE CELL SURFACE

- We now undertake a real case study, that of cellular signaling. This will take us a few weeks.
- The stars of the show will be membrane proteins.

CELL-SURFACE RECEPTORS

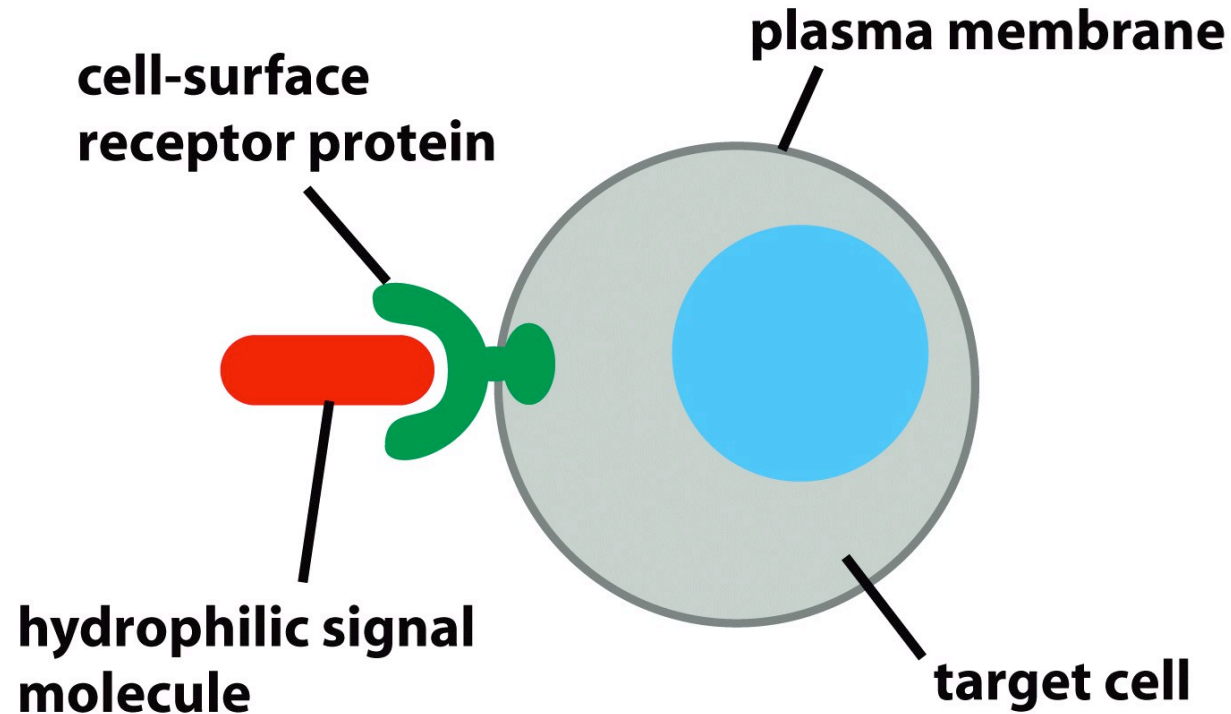
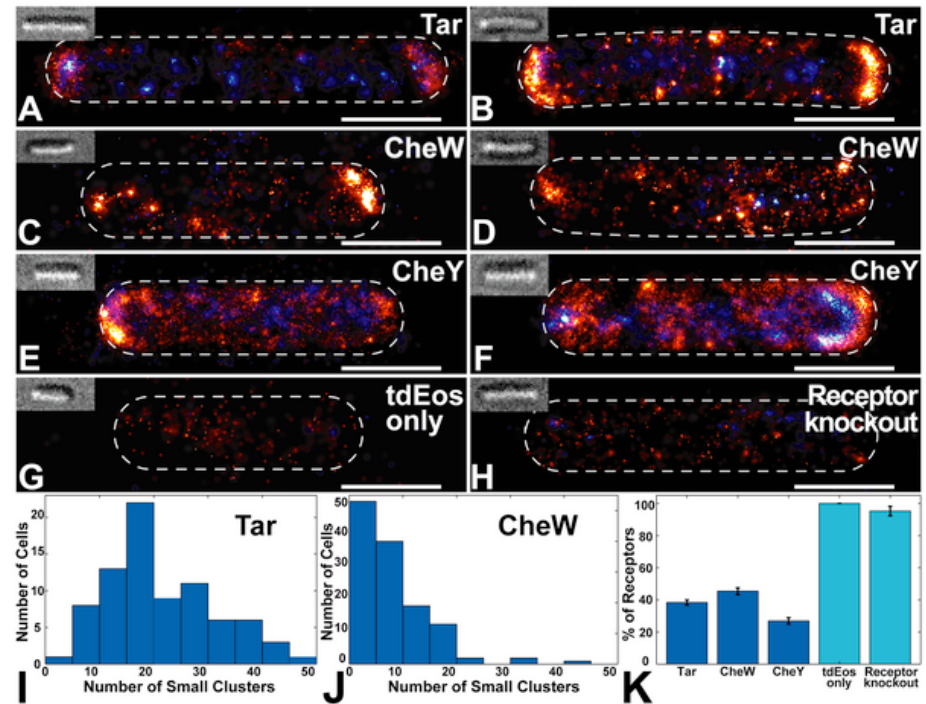
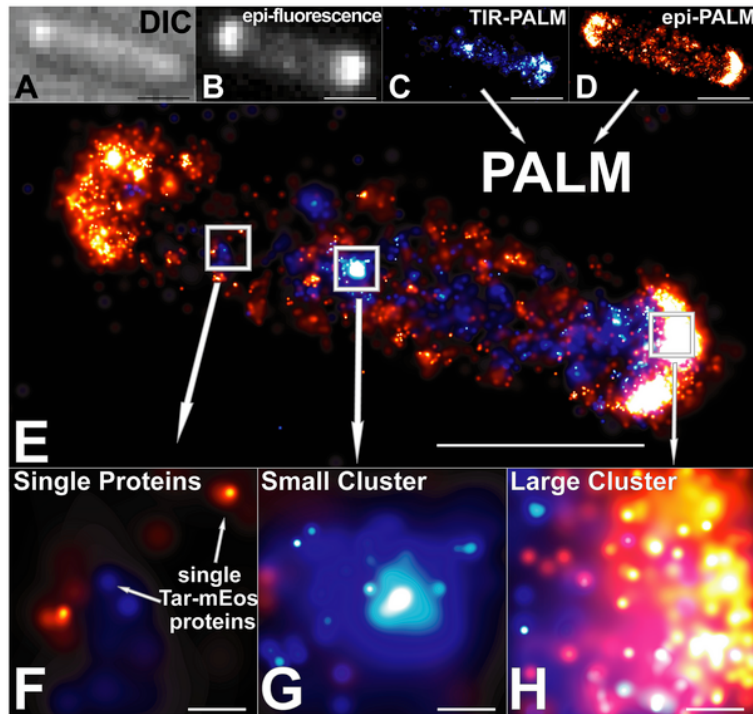


Figure 15-3a Molecular Biology of the Cell 5/e (© Garland Science 2008)

HIGH RESOLUTION IMAGING OF CHEMOTAXIS PROTEINS

- PALM is a high-resolution technique that permits beating the diffraction limit.
- In this case, they are looking at chemotaxis proteins in *E. coli*.

(Greenfield, Liphardt et al, PLoS Biology, 2009.)



HOW DO CELLS TALK TO EACH OTHER?

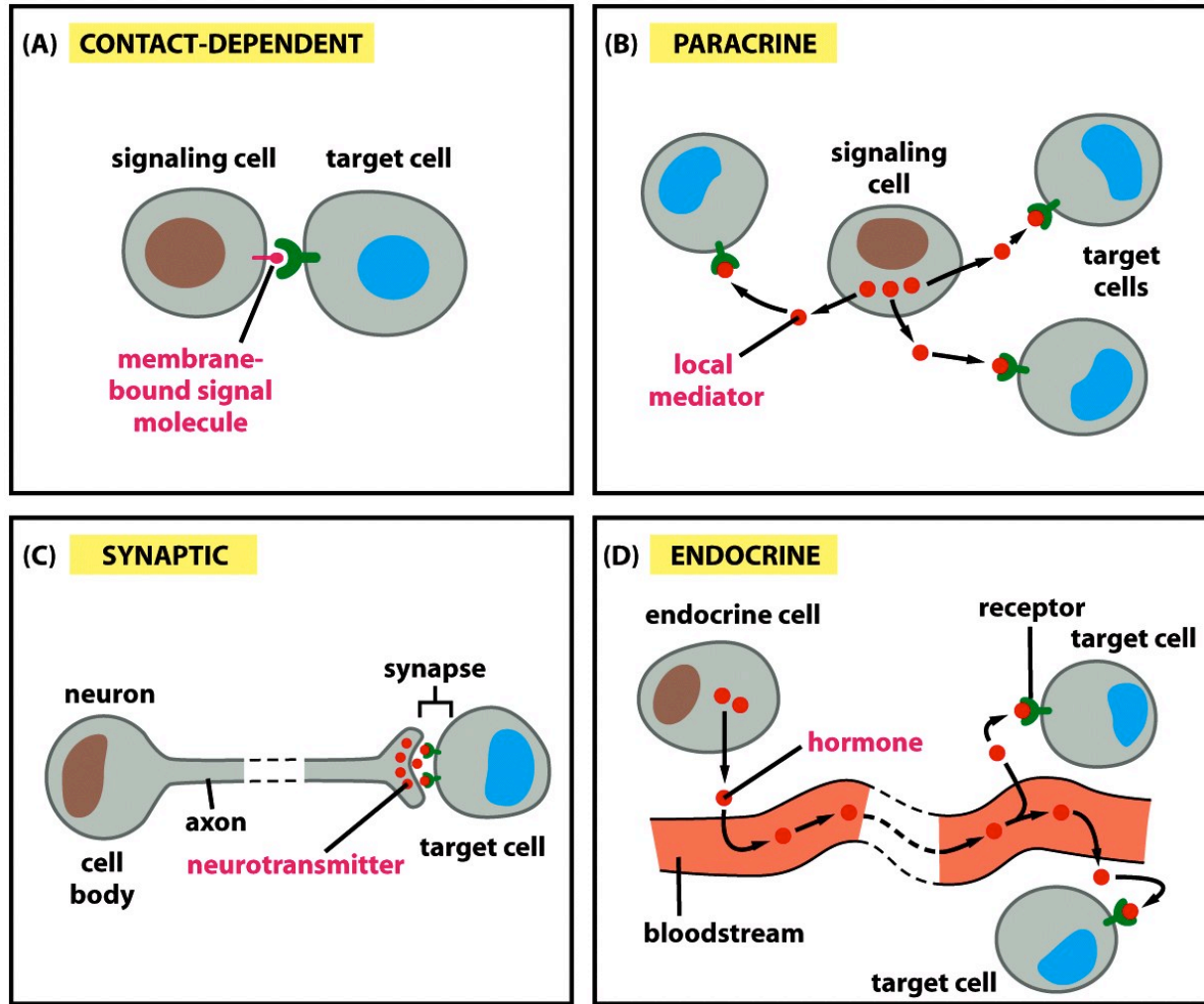


Figure 15-4 Molecular Biology of the Cell 5/e (© Garland Science 2008)

THE SIGNALING CONCEPT

- Our goal will be to find out what kinds of measurements people can do about such systems and then how we can turn the cartoon into a mathematical description.

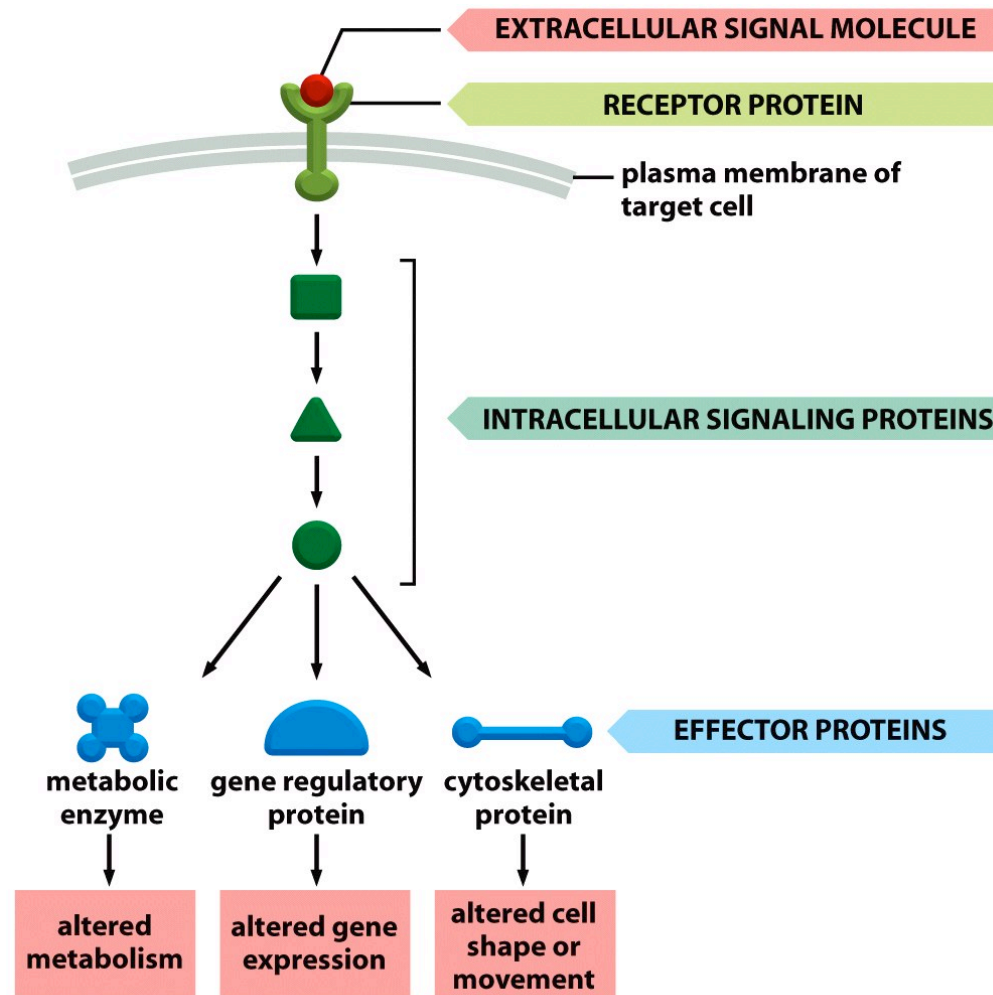
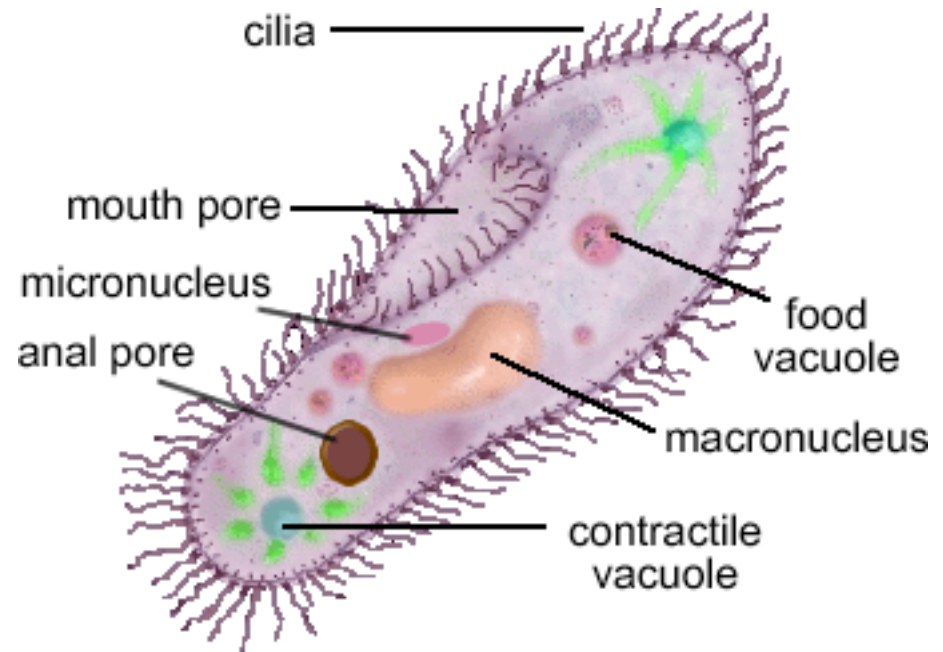


Figure 15-1 Molecular Biology of the Cell 5/e (© Garland Science 2008)

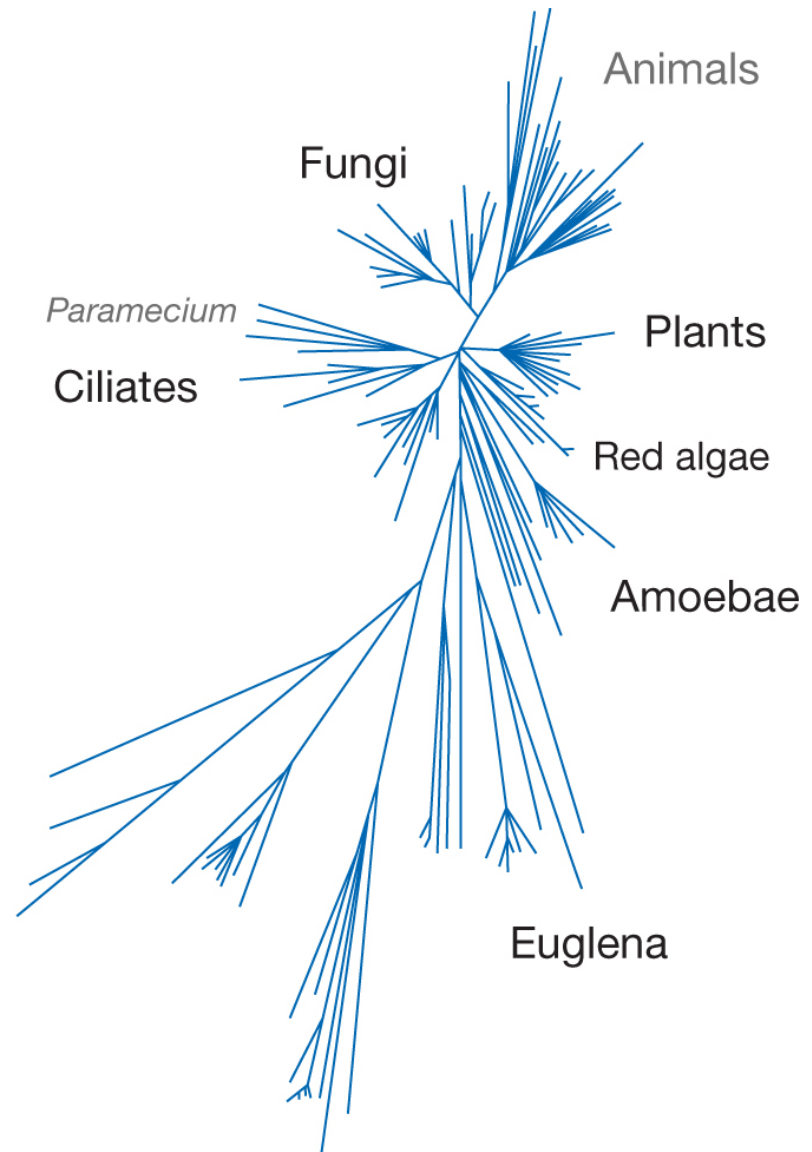
PARAMECIUM AND THE AVOIDANCE RESPONSE

- We begin our story of signaling with the BEHAVIOR of *Paramecium* as manifested in its avoidance response.
- H. S. Jennings, “The behavior of the lower organisms”, 1906.
- Most of the figures on *Paramecium* are from “An Introduction to Nervous Systems” by Ralph Greenspan.



PARAMECIUM AND THE TREE OF LIFE

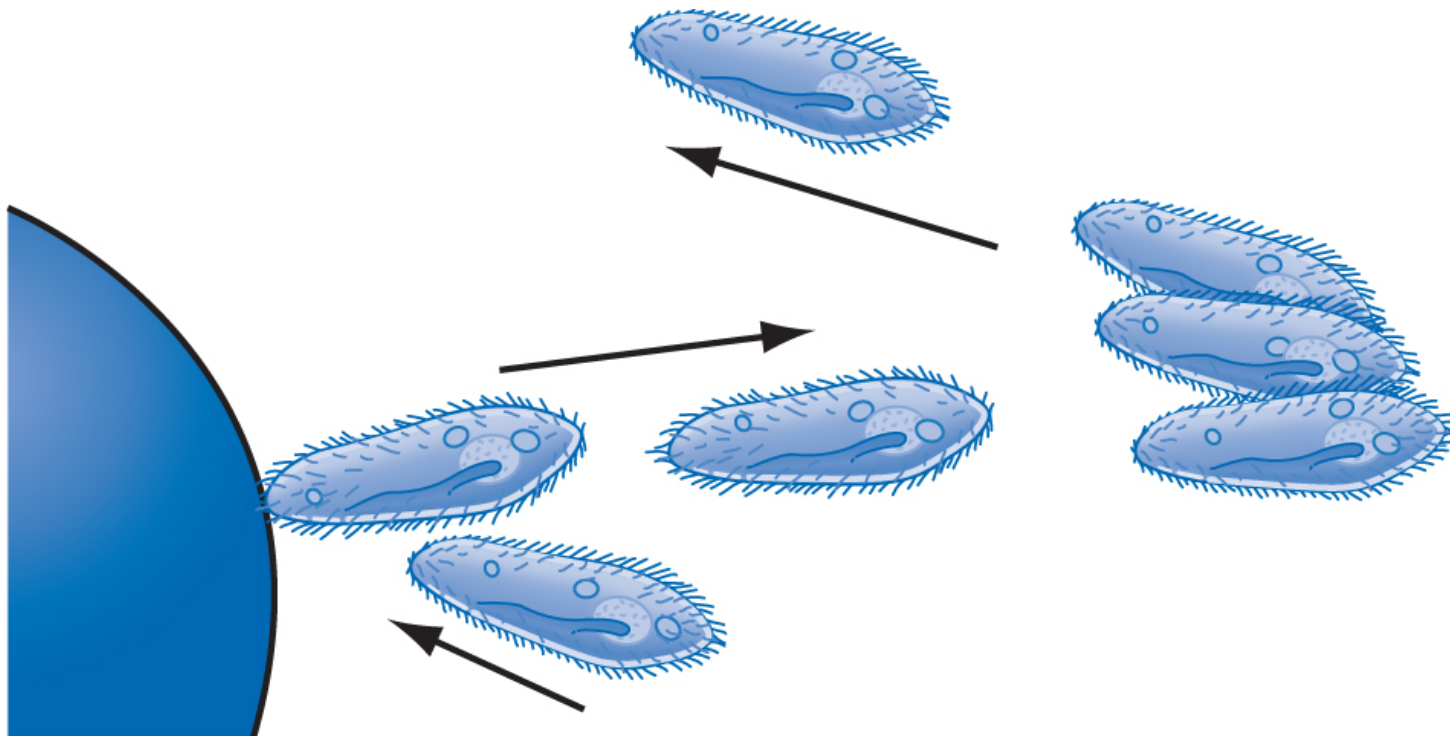
- This diagram reiterates my earlier statements about the amazing diversity of life.
- Note that in this case our tree only reflects on the relatedness of eukaryotes.
- Question: how does one construct such a tree?



(Ralph Greenspan, An Introduction to Nervous Systems)

THE AVOIDANCE RESPONSE

- These single-celled eukaryotes manage a sophisticated response to their environment.
- One of the things I am most excited to tell you is that this response is mediated by similar tricks to those we are used to in the context of nervous systems.
- We need to take a little detour and talk about ion channels. The first of the membrane proteins that we will study in some detail.



(Ralph Greenspan, An Introduction to Nervous Systems)

REMINDER ON ION DISTRIBUTION AND TRANSPORT IN CELLS

- ◆ Cells (eukaryotes) divided into a number of membrane-bound compartments.
- ◆ Concentrations in different compartments can be orders of magnitude different.
- ◆ Proteins (ion channels, transporters) mediate these concentration gradients.
- ◆ Membrane proteins central to huge range of processes – cell signaling, nerve impulses, nutrient transport, etc.

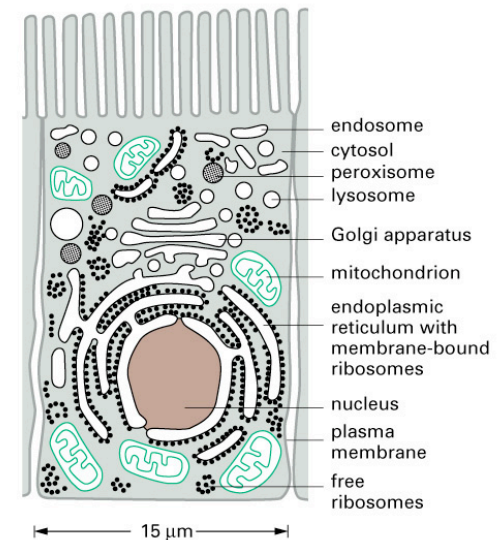


Figure 15-2 Essential Cell Biology, 2/e. (© 2004 Garland Science)

$$Ca_{in}^{2+} \approx 10^{-4}mM \quad Ca_{out}^{2+} \approx 1mM$$

$$K_{in}^{+} \approx 140mM \quad K_{out}^{+} \approx 5mM$$

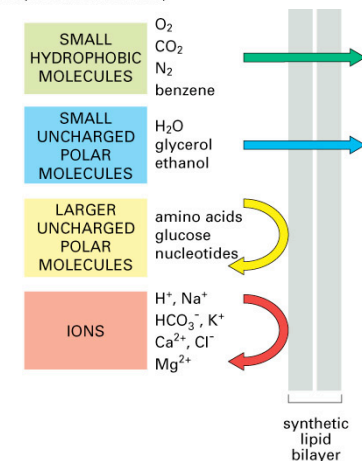


Figure 12-2 Essential Cell Biology, 2/e. (© 2004 Garland Science)

MEMBRANE PERMEABILITY

- Membrane susceptibility to mass transport characterized by a “material parameter” known as the permeability.
- Notice that the permeability ranges over 16 orders of magnitude.

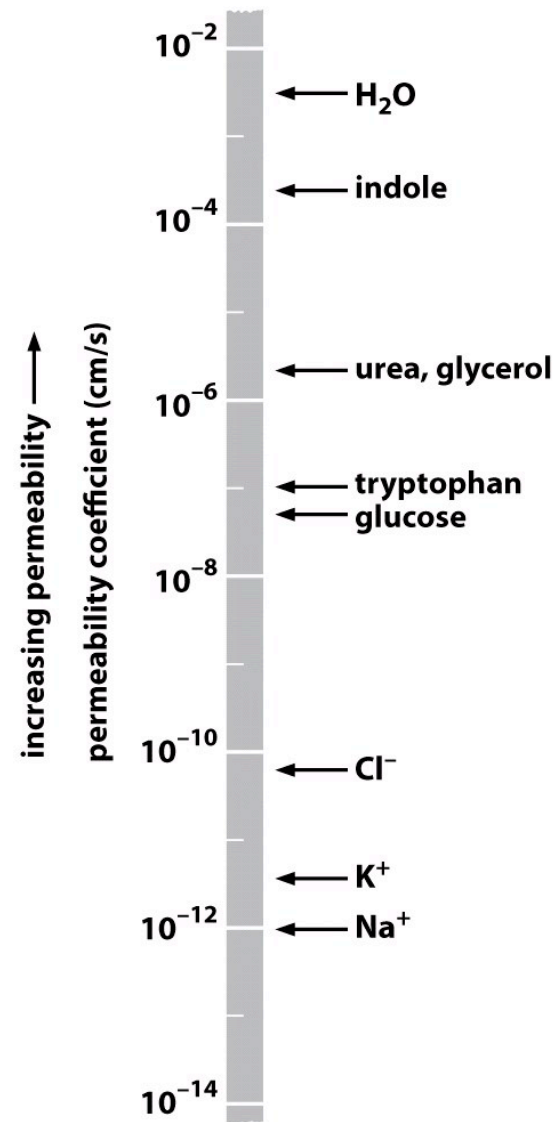
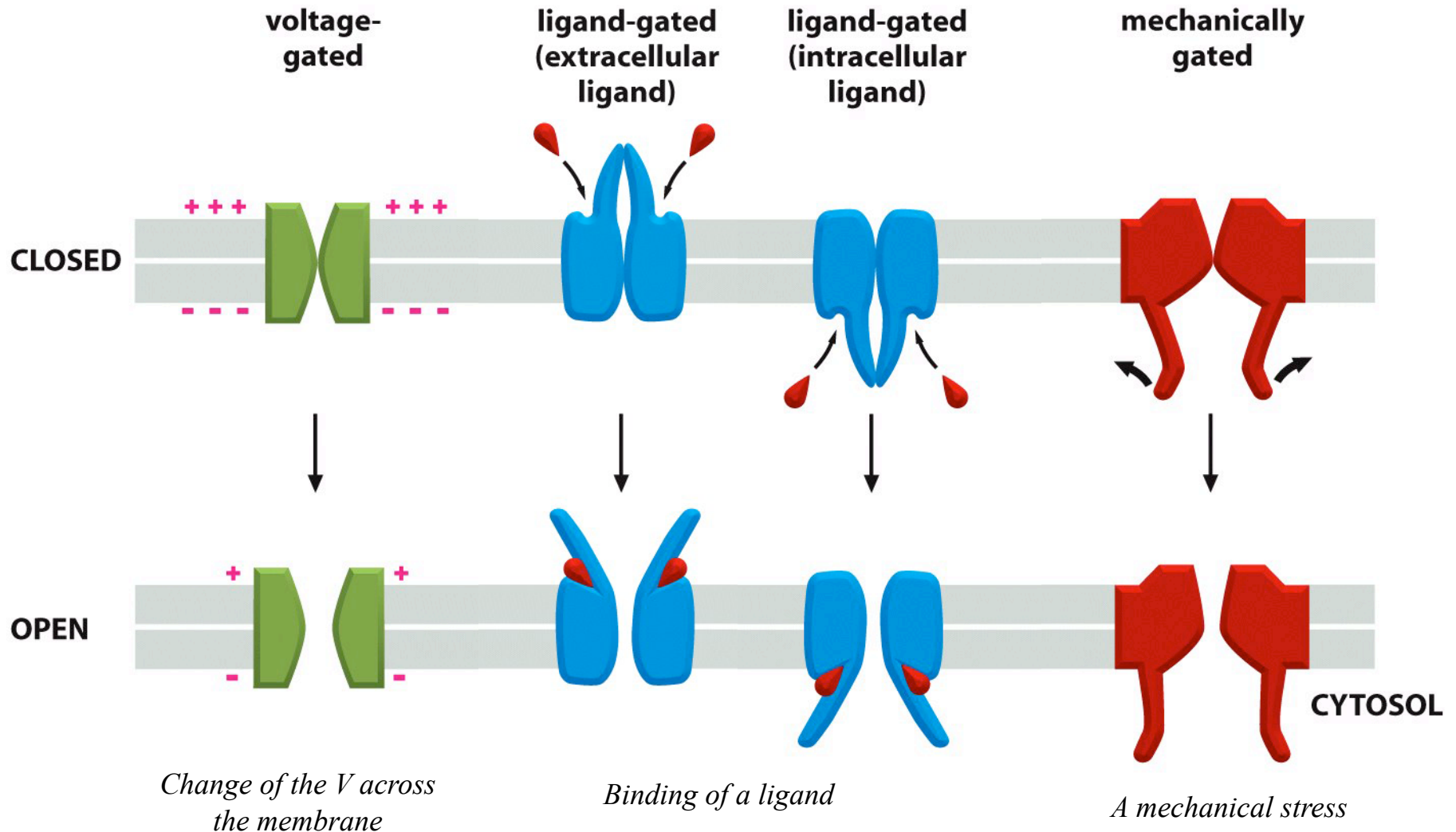


Figure 11.11 Physical Biology of the Cell (© Garland Science 2009)

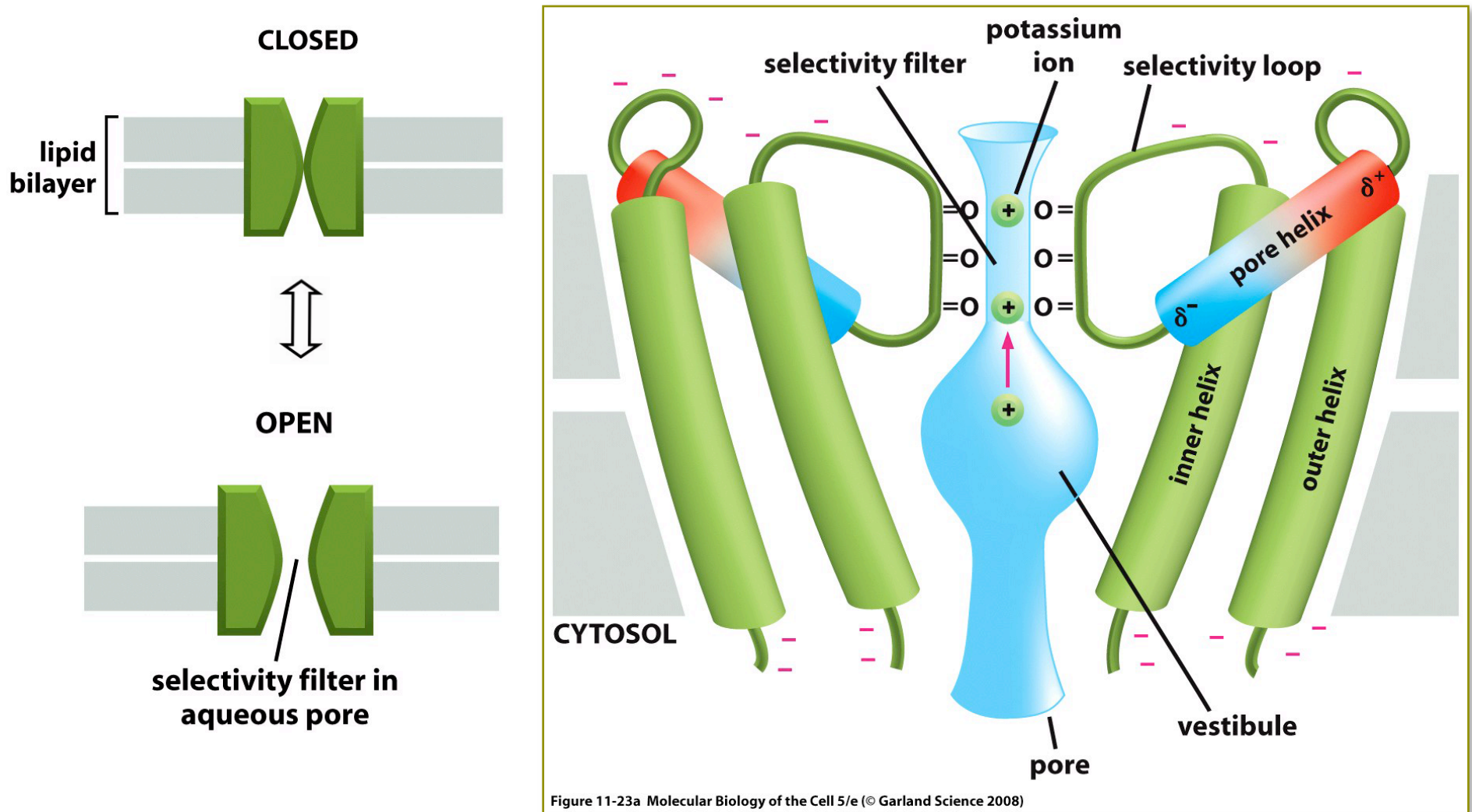
ION CHANNELS UNDER EXTERNAL DRIVING FORCE

- The probability for ion channels of being in open or closed states can be tuned by various external agents:



ION CHANNELS

- Ion channels - transmembrane proteins that mediate the transport of ions in and out of cells



Structure of a bacterial K⁺ channel. The channel is made from 4 identical transmembrane subunits (only 2 are shown) which together form a central pore through the membrane

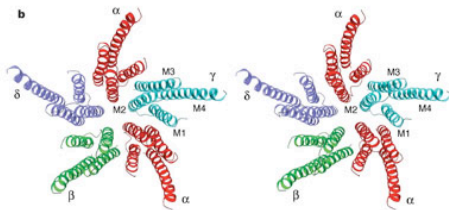
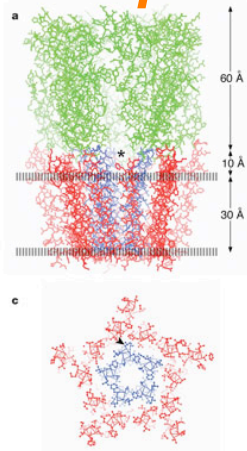
HOW WE KNOW: STRUCTURE

- Knowledge of the structures of different membrane proteins gives us a substrate to reason on about how they work.

Nicotinic acetylcholine receptor



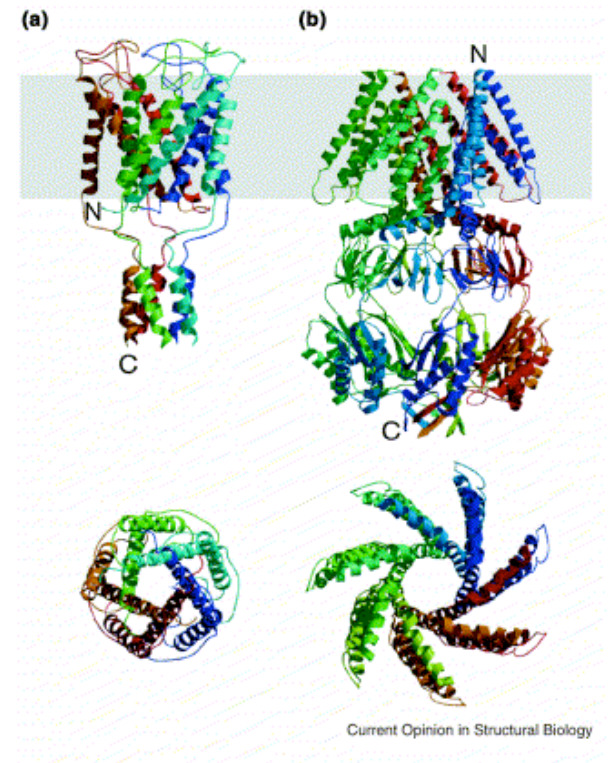
EM & X Ray structures



(Unwin et al.)



(Rees et al.)



MscL and MscS

HOW WE KNOW: CURRENT-PRESSURE RELATIONSHIP

- ◆ The idea: grab a patch of membrane and apply a potential difference to measure the currents.
- ◆ Fraction of time spent open depends upon magnitude of driving force.

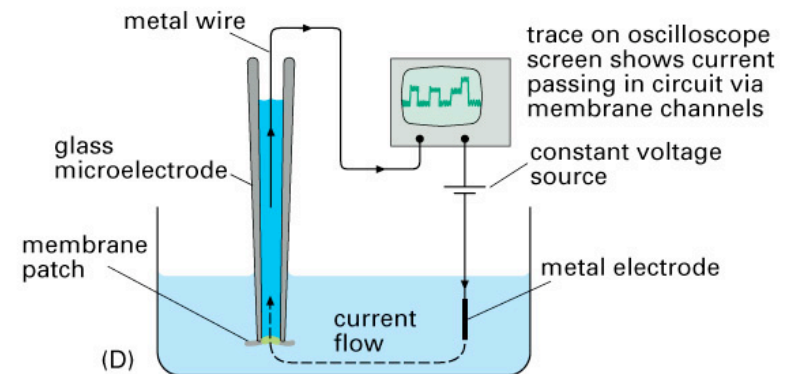
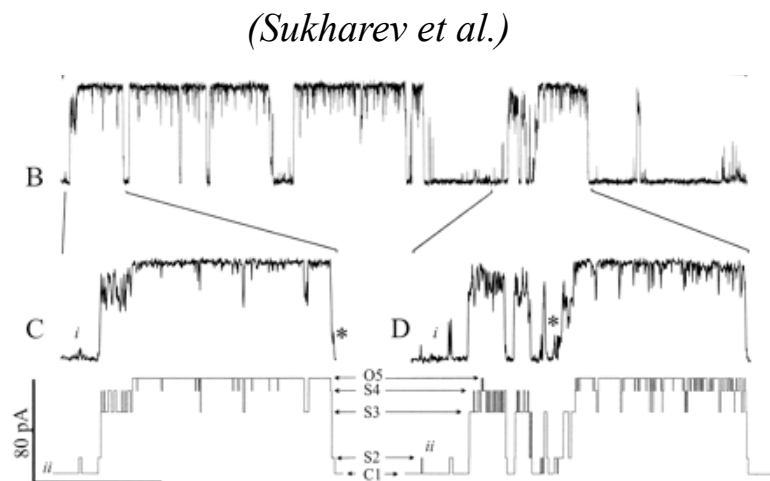
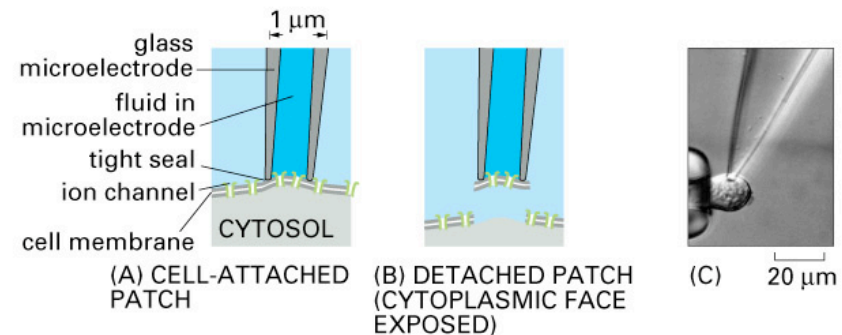
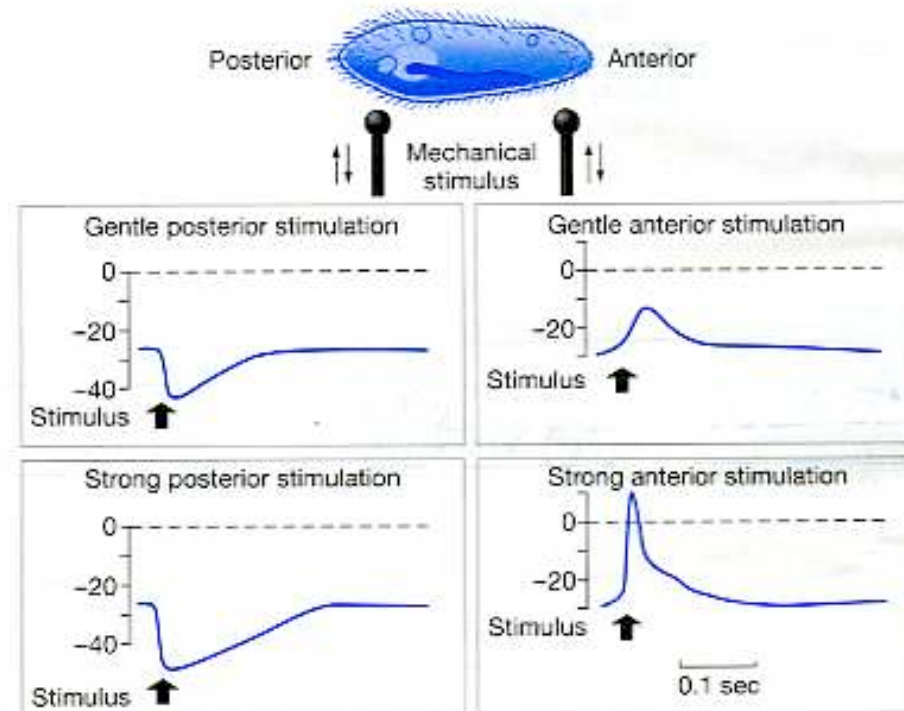


Figure 12-22 Essential Cell Biology, 2/e. (© 2004 Garland Science)

pA currents lasting several milliseconds.

THE AVOIDANCE RESPONSE REVISITED

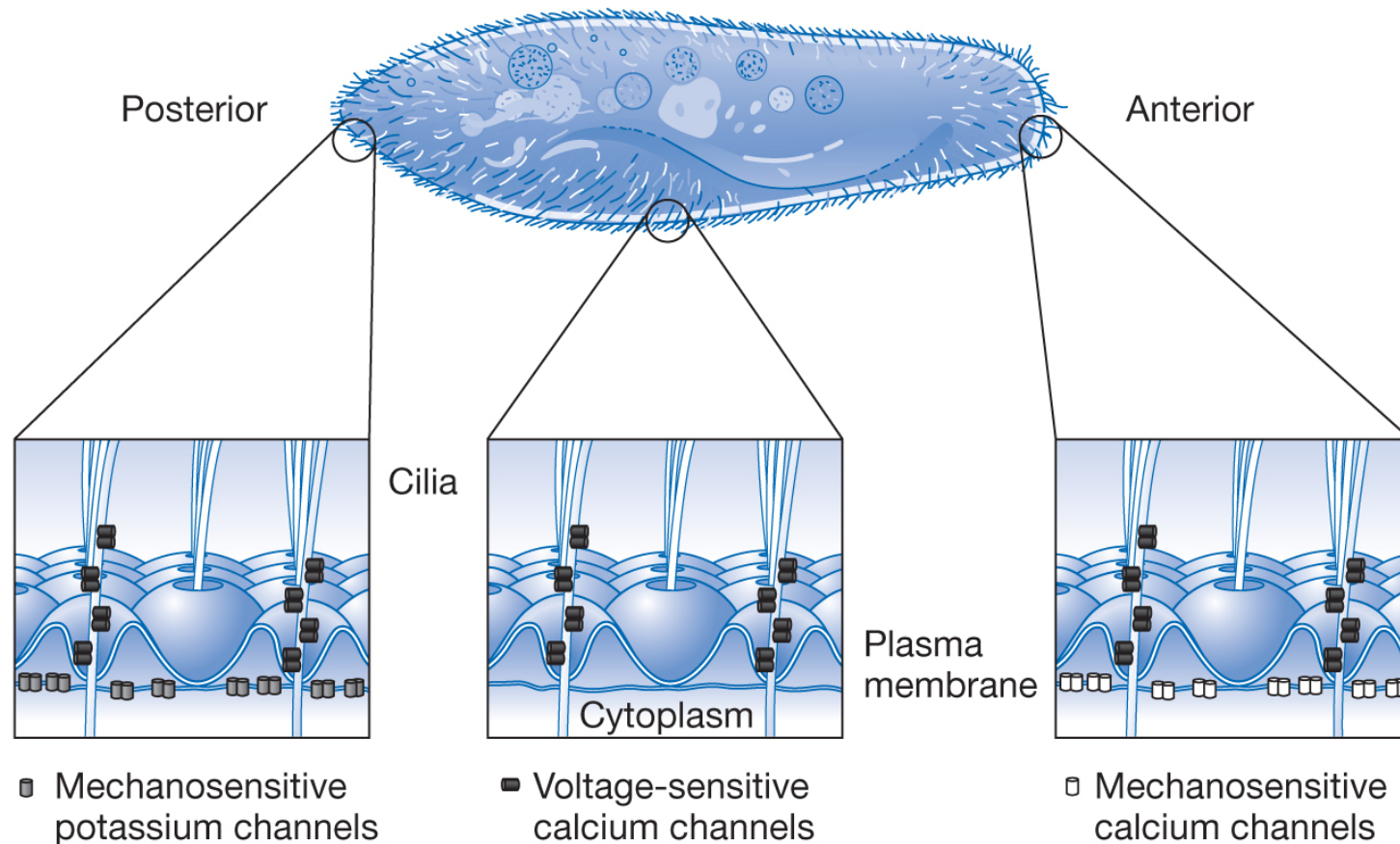
- This schematic traces the series of processes that occur when a *Paramecium* is subjected to a mechanical stimulus.



(Ralph Greenspan, *An Introduction to Nervous Systems*)

ION CHANNELS AND THE IMPLEMENTATION OF DECISIONS

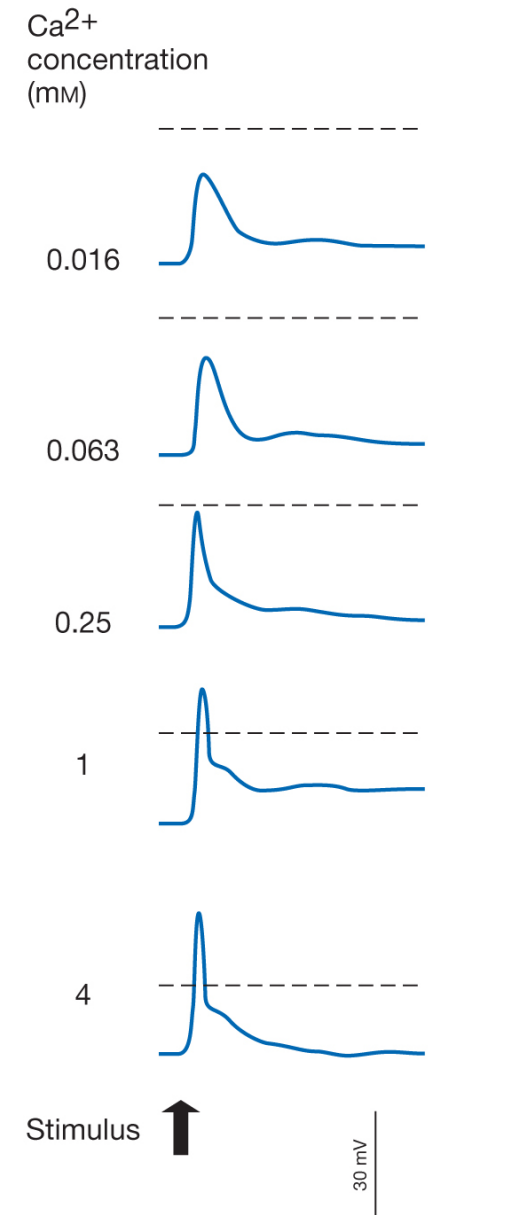
- The particular scheme exploited by *Paramecium* has several facets involving both mechanosensitive and voltage-gated ion channels.



(Ralph Greenspan, *An Introduction to Nervous Systems*)

WHICH ION SPECIES MEDIATES THE PROCESS?

- The clever experiment involves tuning the extracellular concentrations of the different ion species in turn and then examining the resulting membrane potential.



(Ralph Greenspan, *An Introduction to Nervous Systems*)

THE ACTION POTENTIAL

- Action potentials are a stereotyped response once a critical threshold has been crossed.
- The shape and duration of the action potential are key variables.
- NOTE: Helmholtz measured the velocity of propagation of nerve impulses.

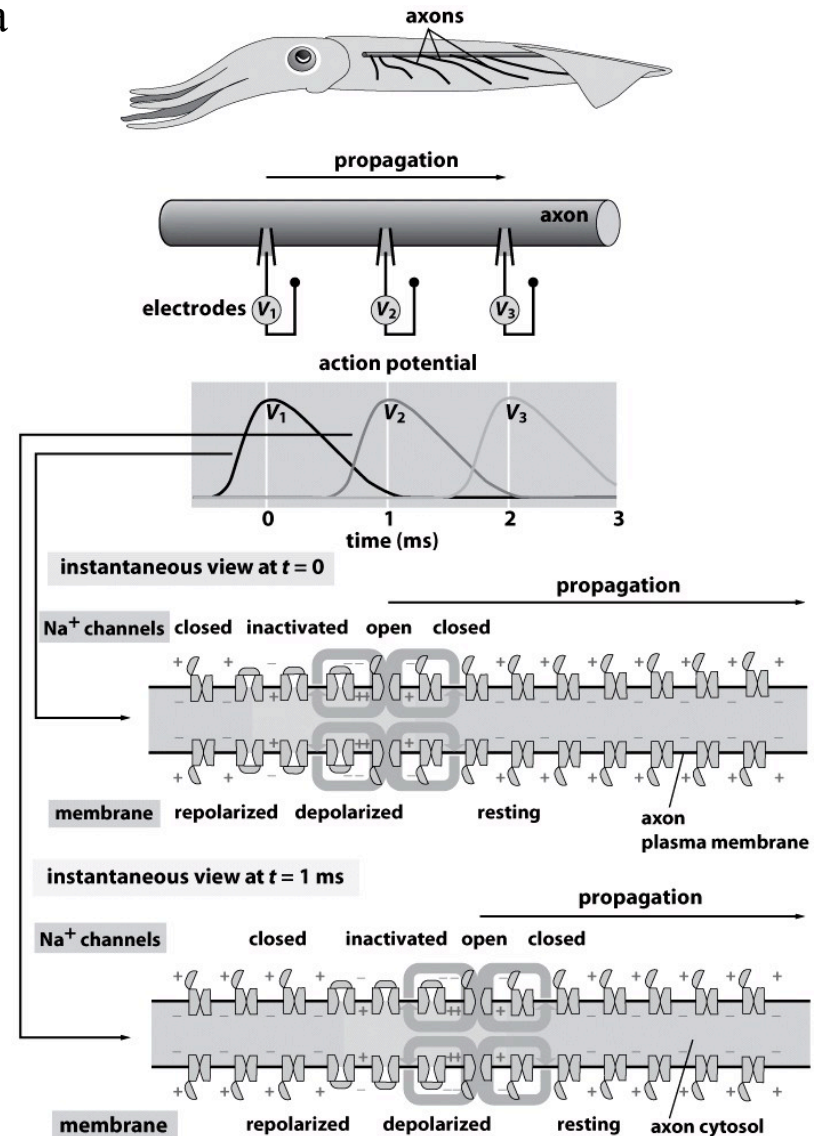
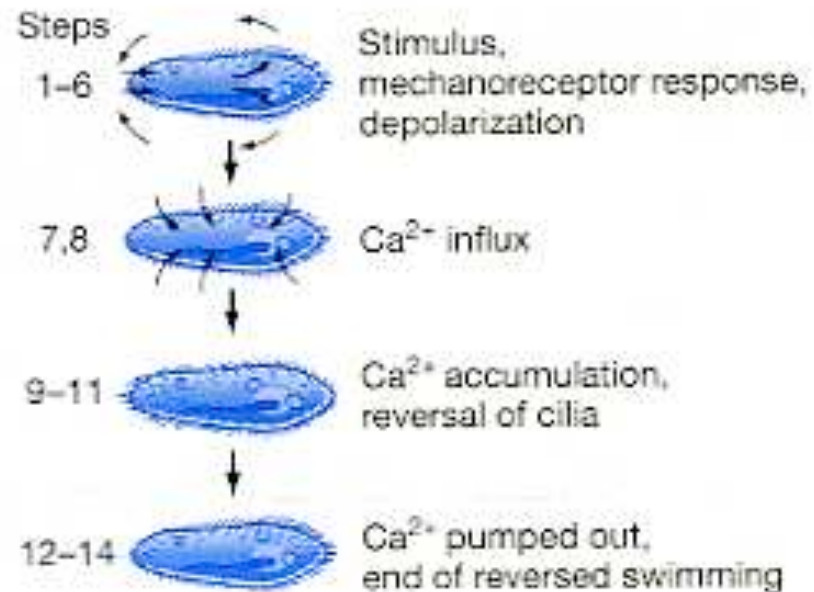
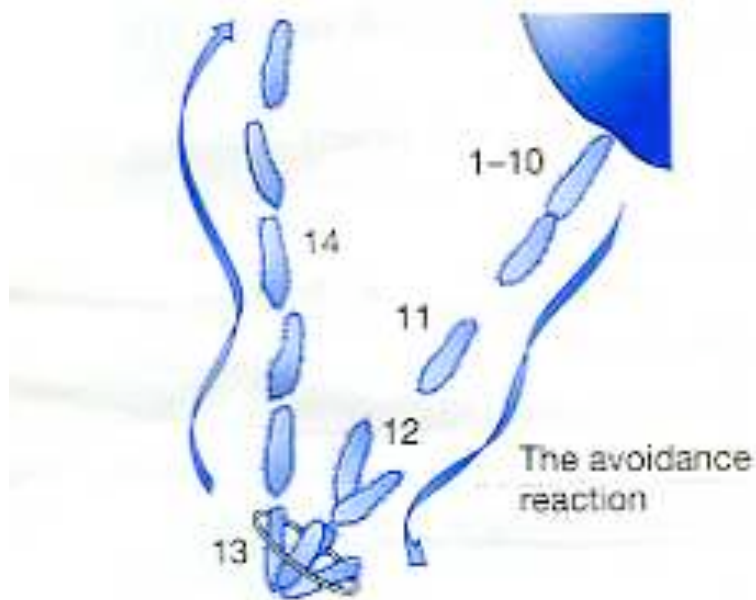


Figure 4.25 Physical Biology of the Cell (© Garland Science 2009)

THE AVOIDANCE RESPONSE: DETAILS

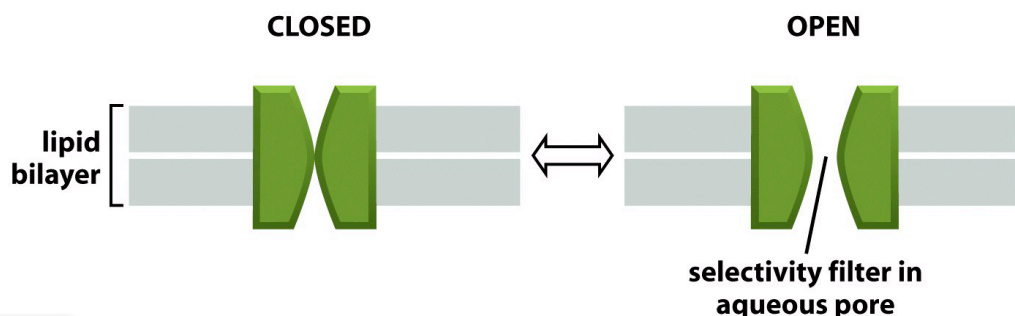
- These single-celled eukaryotes manage a sophisticated response to their environment.
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(Ralph Greenspan, *An Introduction to Nervous Systems*)

ION TRANSPORT RATES IN ION CHANNELS

- Another way in which cells manipulate transport rates: selectively and transiently altering the permeability of cell membranes through **protein channels and pumps**



A typical ion channel, which fluctuates btw closed and open conformation



20 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Estimate the flux of ions through the channel, assuming purely diffusive motion

- Ion channel = 5 nm (width of lipid bilayer)
0.5 nm (size of hydrated ion)

- $D_{\text{small ions}} \approx 2000 \mu\text{m}^2/\text{s}$ (e.g., Na^+)

$$J_{\text{ion}} \approx 2 \times 10^9 \frac{\text{nm}^2}{\text{s}} \times \frac{6 \times 10^{-2} \text{ molecules}/\text{nm}^3}{5 \text{ nm}}$$

$$\approx 2 \times 10^7 \text{ nm}^{-2} \text{ s}^{-1}$$

- Fick's law (details - later): $J_{\text{ion}} \approx D \frac{\Delta c}{l}$

where Δc = difference in ion c across the cell membrane, l = distance btw the two "reservoirs"

- mammal.cell: $\Delta c \approx 100 \text{ mM}$ ($\approx 6 \times 10^{-2} \text{ molecules}/\text{nm}^3$); $l \approx 5 \text{ nm}$
- $A_{\text{channel}} = d^2 \pi/4 \approx 0.2 \text{ nm}^2 \Rightarrow N$ of ions traversing the membrane per second:

$$\frac{dN_{\text{ion}}}{dt} \approx J_{\text{ion}} A_{\text{channel}}$$


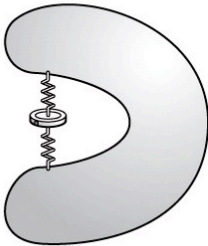
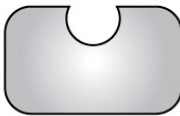

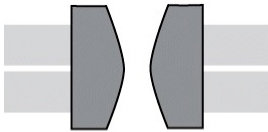
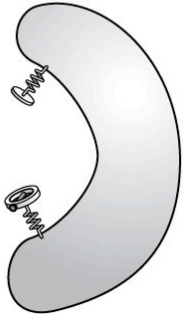

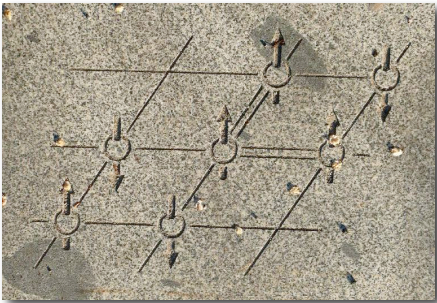
$$\approx 2 \times 10^7 \text{ nm}^{-2} \text{ s}^{-1} \times 0.2 \text{ nm}^2 = 4 \times 10^6 \text{ s}^{-1}$$

TWO-STATE SYSTEMS

*There are two models that a statistical mechanic is interested in, the one they are working on and the Ising model.
A Statistical Mechanician*

• In many bio problems, the *state* of the system is described by one or several simple two-state variables (σ), each of which can only take the values of 0 or 1

• Examples of the internal state variable description of macromolecules:

STATE	STATE	STATE	STATE
 <p>$\sigma = 0$</p>	 <p>$\sigma = 0$</p>	 <p>$\sigma = 0$</p>	<p>$\sigma_i =$</p>  <p>Overall state of the DNA is described by the vector $(\sigma_1, \sigma_2, \dots, \sigma_N) = \{\sigma_i\}$ where σ_i refers to the state of hybridization of the i^{th} base pair</p>
 <p>$\sigma = 1$</p> <p>ion channel gating</p>	 <p>$\sigma = 1$</p> <p>protein conformational change</p>	 <p>$\sigma = 1$</p> <p>ligand receptor interaction</p>	<p>base pairing</p> 

A paving stone at UBC: Ising model of spins on a regular lattice

ION CHANNELS

- Aim: compute the probability of finding the channel open, p_{open}
- Boltzmann distr.: the probability of finding a state with energy E is

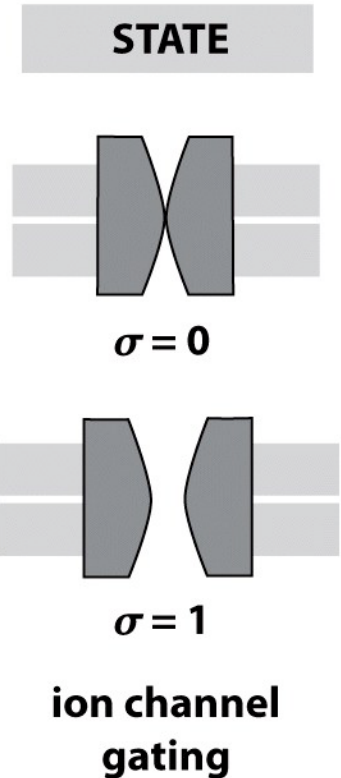
$$p(E) = e^{-\beta E} / Z$$

$E = E(\sigma) = ?$

- If there is no external driving force, the energy as a function of σ is

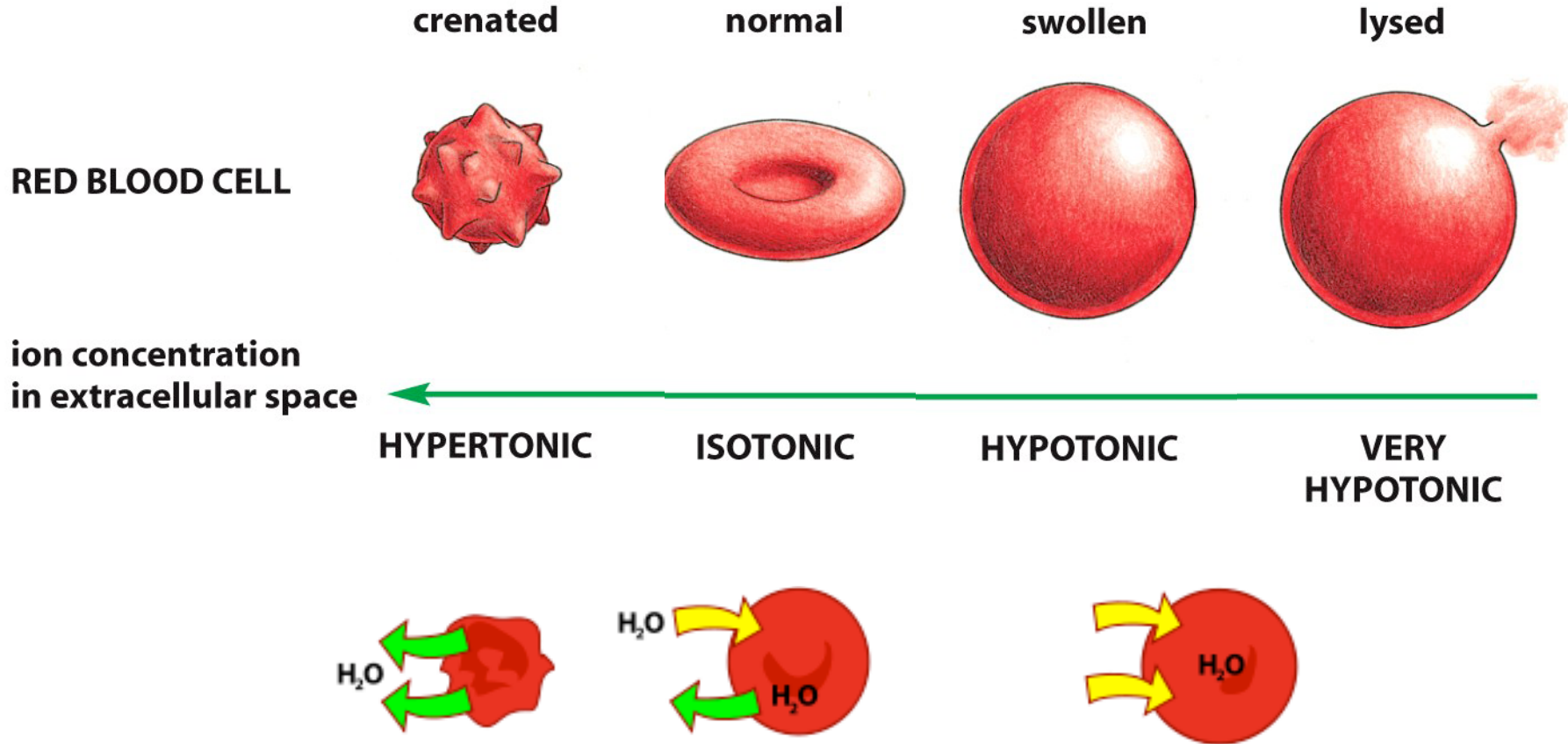
$$E(\sigma) = \sigma \varepsilon_{open} + (1 - \sigma) \varepsilon_{closed}$$

where ε_{open} (ε_{closed}) is the energy of the open (closed) state



ION CHANNELS UNDER APPLIED TENSION

- For concreteness, we begin by considering the case of mechanosensitive channels. Driving force: the tension in the membrane
- Mechanosensitive channels have been hypothesized to serve as safety valves to protect cells against membrane rupture due to osmotic imbalance.



ENERGY OF ION CHANNEL UNDER APPLIED TENSION

- In open state, the energy of the loading device is reduced
- => energy as a function of the applied tension τ

$$E(\sigma) = \sigma \epsilon_{open} + (1 - \sigma) \epsilon_{closed} - \underbrace{\sigma \tau \Delta A}_{\text{favors the open state}}$$

where ΔA is the area change upon gating.

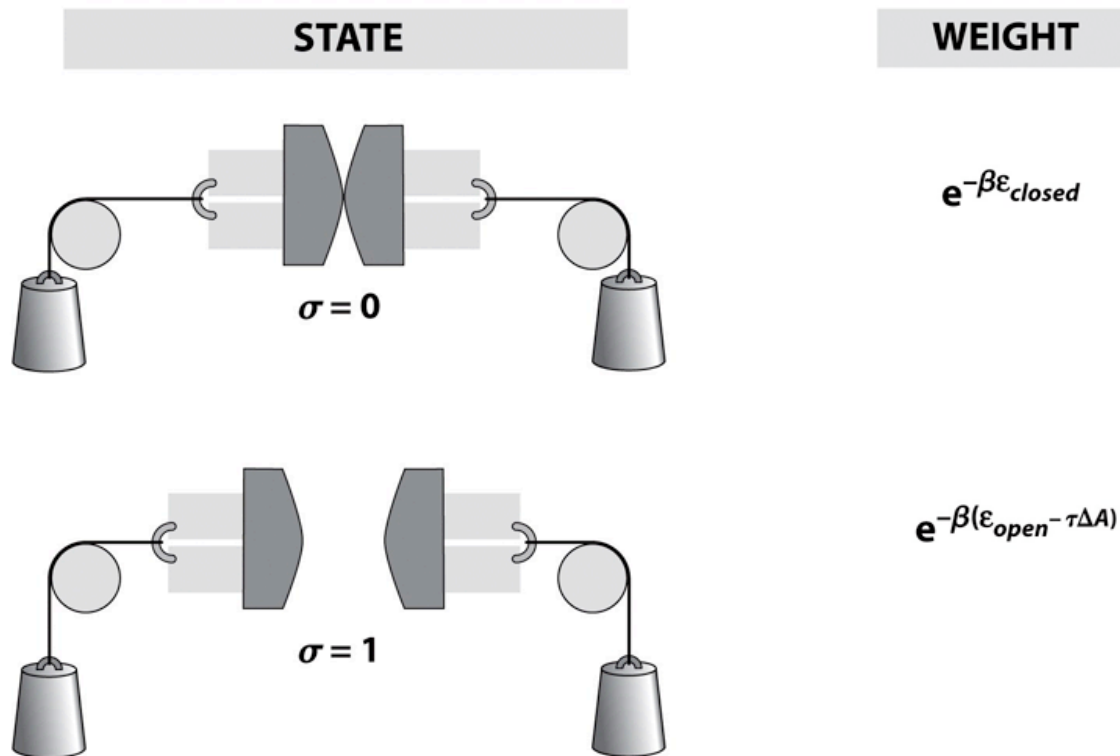
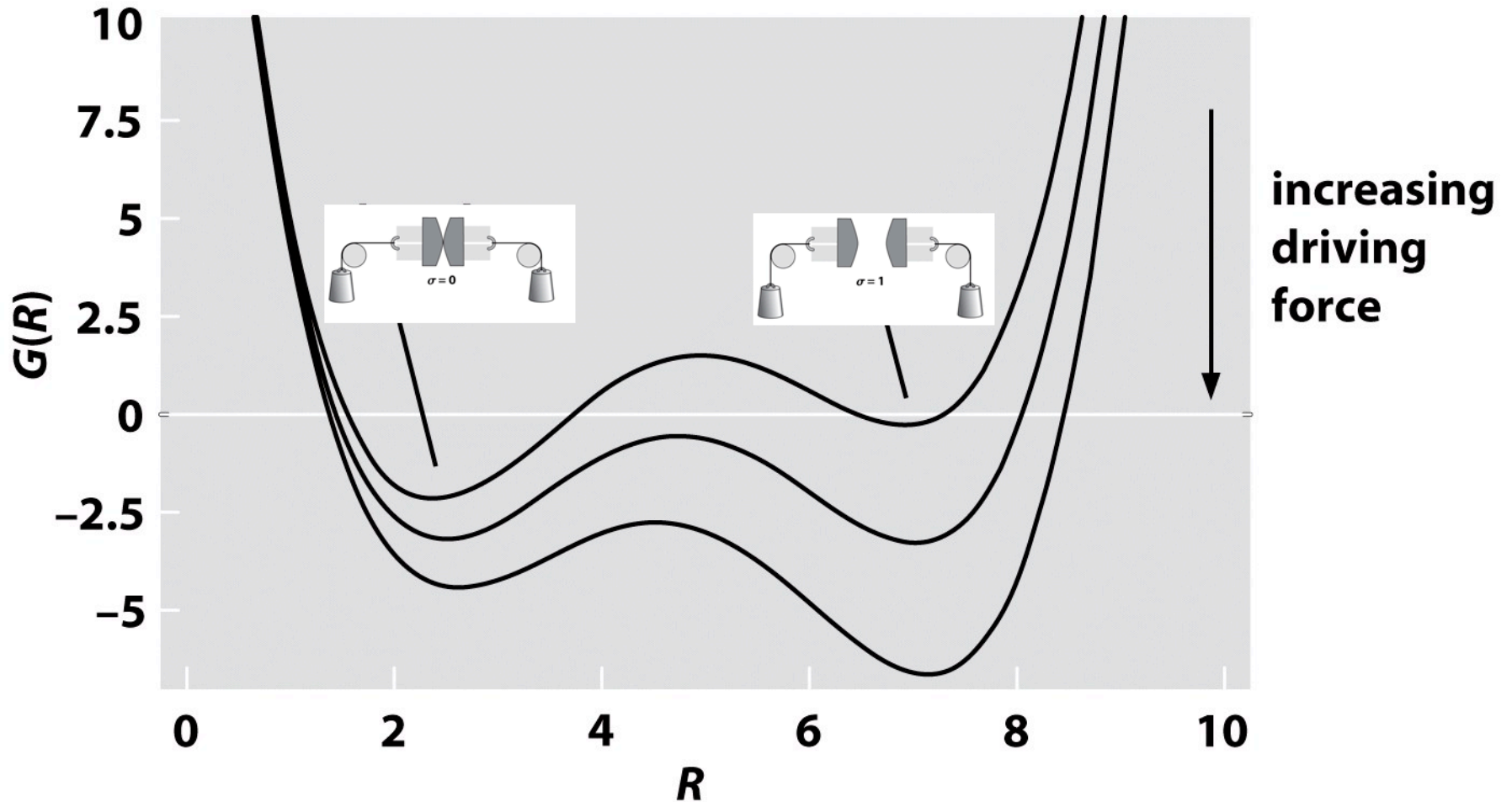


Figure 7.3 Physical Biology of the Cell (© Garland Science 2009)

ENERGY LANDSCAPE FOR AN ION CHANNEL

- The entire energy landscape for a channel as a function of the radius for different choices of external gating parameter (membrane tension, ligand concentration, or applied voltage):



- Presence of the driving force shifts the balance between the energies of the closed and open states.

OPEN PROBABILITY OF THE ION CHANNEL

- Compute the open probability of the channel. The energy is

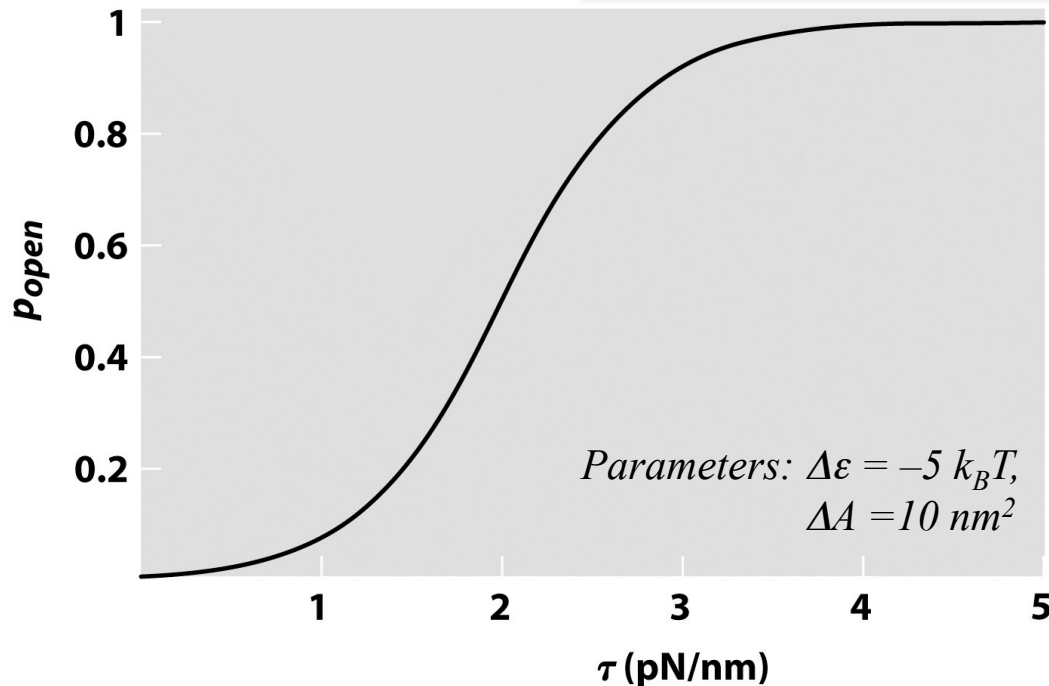
$$E(\sigma) = \sigma \varepsilon_{open} + (1 - \sigma) \varepsilon_{closed} - \sigma \tau \Delta A$$

- The partition function:

$$Z = \sum_{\sigma=0}^1 e^{-\beta E(\sigma)}$$

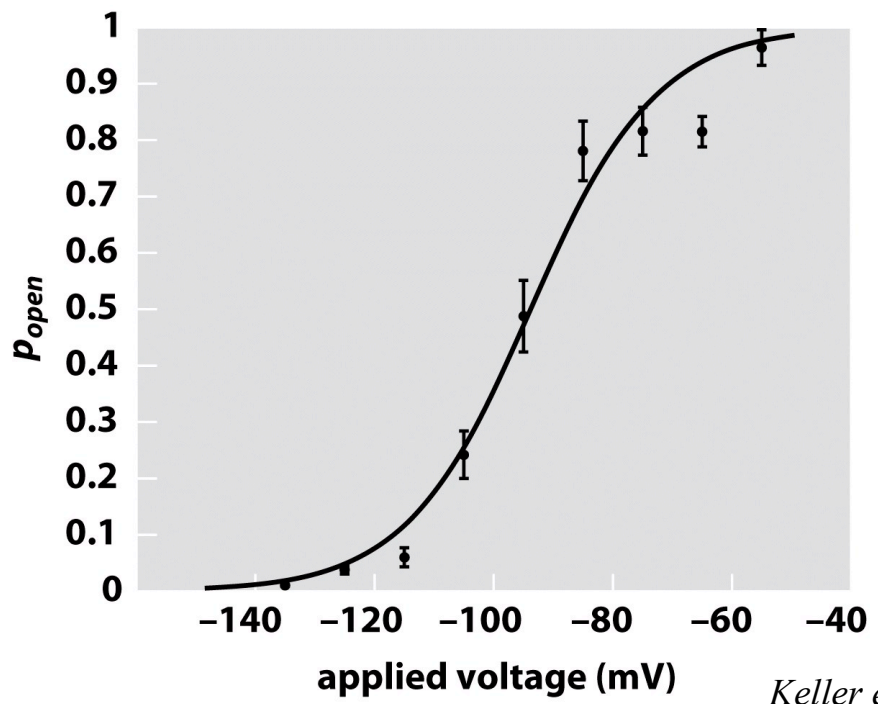
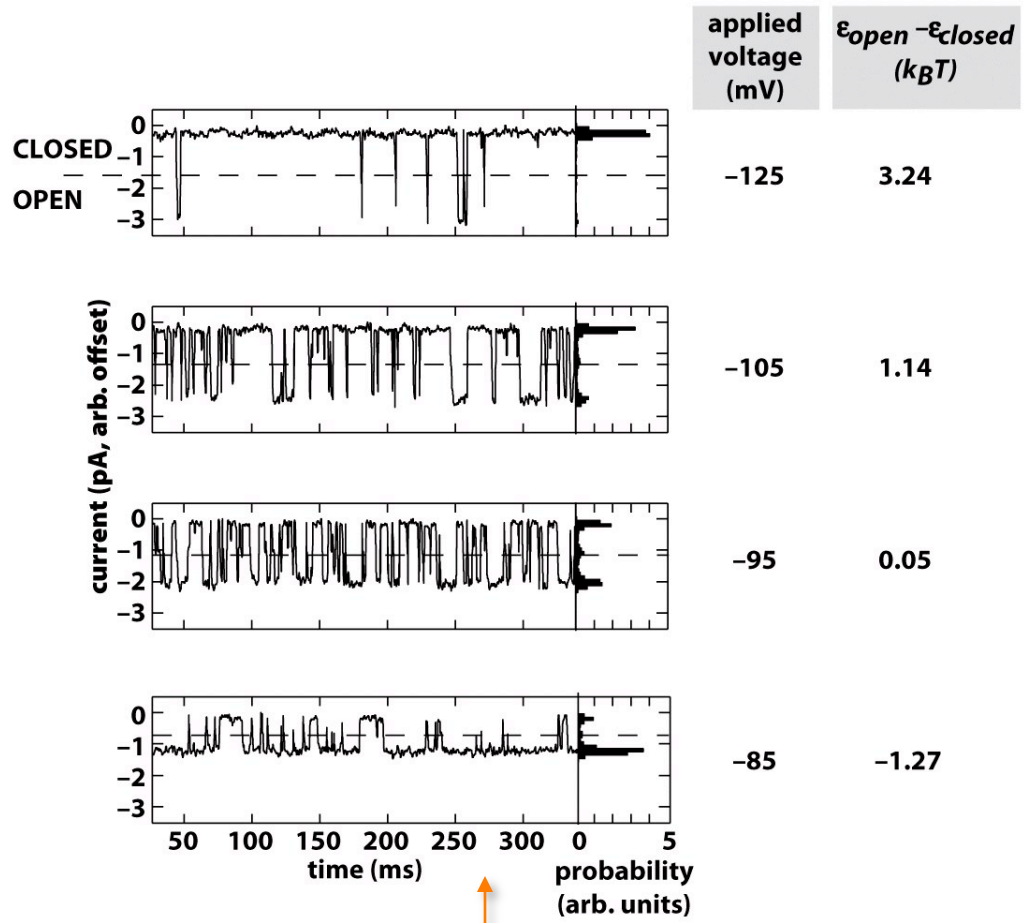
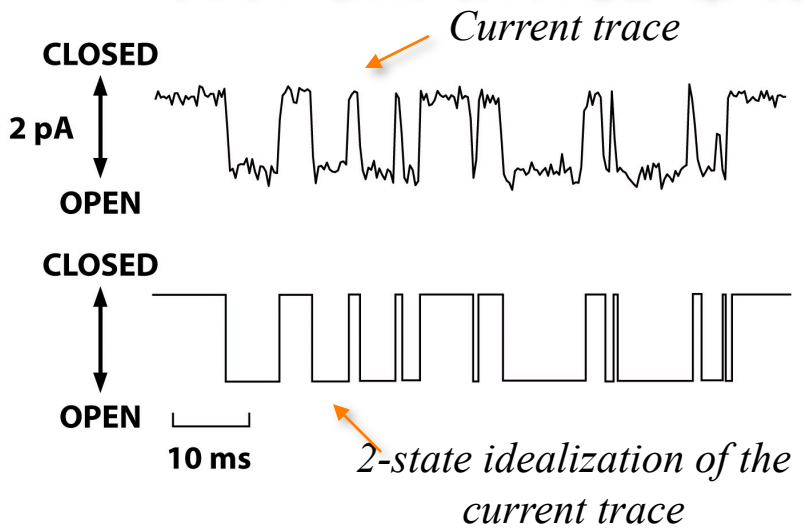
- \Rightarrow the open probability:

$$p_{open} = \frac{e^{-\beta(\varepsilon_{open} - \tau \Delta A)}}{e^{-\beta(\varepsilon_{open} - \tau \Delta A)} + e^{-\beta \varepsilon_{closed}}}$$



- To understand how a channel will behave under a driving force we need to know 2 things:
 - (1) The intrinsic preference for each of its 2 states
 - (2) how the external driving force alters the relative energies of these 2 states \Rightarrow stat. mech. allows us to compute the behavior of the channel under a range of driving forces

ION CHANNEL GATING: EXPERIMENTAL DATA

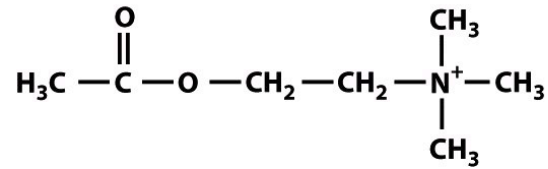


A single channel incorporated into a pure lipid bilayer is subjected to different voltages. The resulting current is measured. Tuning the driving force for gating only changes the relative dwell time and not the existence of the states themselves.

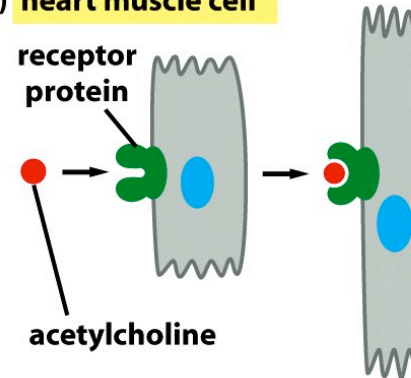
Keller et al., J.Gen.Physiol. 1986

ION GATED CHANNELS: ACETYLCHOLINE

(A) acetylcholine

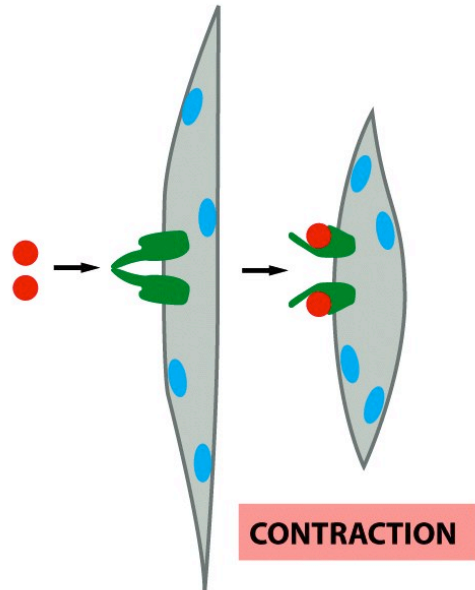


(B) heart muscle cell



DECREASED RATE AND FORCE OF CONTRACTION

(C) skeletal muscle cell



(D) salivary gland cell

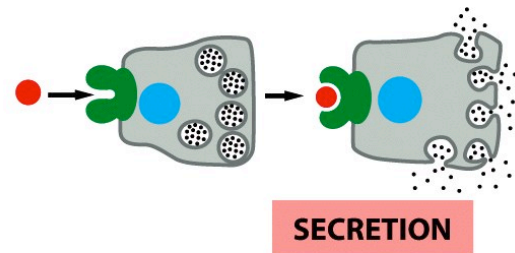


Figure 15-9 Molecular Biology of the Cell 5/e (© Garland Science 2008)