PRINCIPLE: TO EXIST, LIFE MUST SENSE AND RESPOND TO THE EXTERNAL ENVIRONMENT

DKN
- How do microbes sense and respond to their environment?
- What type of things do they sense?
- How does signal transduction work?

Skype with Prof. Ned Wingreen, Caltech Class of 1984, Physics

MMN
- How do multicellular organisms integrate sensory information across tissues, organs and organ systems?
- What is the basis of activity in the animal nervous system?

Clicker quiz round
How do microbes sense their environment?

Overview cartoon:

sense (input) \(\rightarrow\) integrate \(\rightarrow\) respond (output)
If you were a microbe, what information from the outside would you care about?

- **Nutrients** (attractants, *e.g.* light, organics, inorganics -- can be both substrates & products)

- **Toxins** (repellents)

- **Stress** (*e.g.* cell envelope stress)

- **Time** (circadian rhythms)

- **Other cells** (relatives)

- **Other cells** (competitors)
How do microbes sense, integrate and respond to external cues?

Answer: 2-component systems

There are many different types of inputs, integration & outputs. Highly modular!
Domain architecture of sensor histidine kinases (HK) (INPUT domain):

Dimerization/phosphotranser, catalytic

CA = ATP binding, catalyzes auto-phosphotransfer to DhP domain, Which then transfers It to RR

Extracellular signal  Transduce signal

Examples of how a domain senses light:
LOV (light-oxygen-voltage) domain, belong to PAS superfamily (Per-Arnt-Sim)
Non covalently bound flavin cofactor, makes a covalent bond when absorbs blue light, results in conformational rearrangement of the protein:

LOV domain of C. reinhardtii
Domain architecture of response regulators (RR) (OUTPUT domain):

 Receiver domain | Effector domain | Function
---|---|---
 D | DBD | DNA-binding 60%
 D | AAA+ | Cyclic-di-GMP
 D | GGDEF | methyltransferase
 D | EAL | CheY, chemotaxis example 15%
 D | CH₄ |
Caltech iGEM 2011 Team – Designed bacteria to degrade endocrine-disruptors (compounds that disrupt development & reproduction of wild organisms)
A Sampling of Two-Component Signal Transduction Systems

<table>
<thead>
<tr>
<th>Organism</th>
<th>Histidine Kinases</th>
<th>Response Regulators</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Caulobacter crescentus</strong></td>
<td>62</td>
<td>44</td>
<td>106</td>
</tr>
<tr>
<td><strong>Sinorhizobium meliloti</strong></td>
<td>36</td>
<td>50</td>
<td>86</td>
</tr>
<tr>
<td><strong>Bacillus subtilis</strong></td>
<td>21</td>
<td>31</td>
<td>52</td>
</tr>
<tr>
<td><strong>Escherichia coli</strong></td>
<td>29</td>
<td>32</td>
<td>61</td>
</tr>
<tr>
<td><strong>Geobacter sulfurreducens</strong></td>
<td>86</td>
<td>75</td>
<td>161</td>
</tr>
<tr>
<td><strong>Nostoc punctiforme</strong></td>
<td>120</td>
<td>131</td>
<td>251</td>
</tr>
<tr>
<td><strong>Streptomyces coelicolor</strong></td>
<td>84</td>
<td>80</td>
<td>181</td>
</tr>
<tr>
<td><strong>Pseudomonas aeruginosa</strong></td>
<td>60</td>
<td>73</td>
<td>133</td>
</tr>
<tr>
<td><strong>Streptococcus pneumoniae</strong></td>
<td>7</td>
<td>12</td>
<td>19</td>
</tr>
<tr>
<td><strong>Staphylococcus aureus</strong></td>
<td>9</td>
<td>13</td>
<td>22</td>
</tr>
<tr>
<td><strong>Saccharomyces cerevisiae</strong></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td><strong>Dictyostelium discoideum</strong></td>
<td>13</td>
<td>2</td>
<td>23</td>
</tr>
<tr>
<td><strong>Arabidopsis thaliana</strong></td>
<td>8</td>
<td>23</td>
<td>31</td>
</tr>
</tbody>
</table>

1.) A lot in bacteria but absent in metazoans: antibiotic target! 2.) What challenge might this abundance of 2-component systems pose?
Specificity?
Solving the problem of specificity....

Control over production of the sensor (regulation)

Control over spatial localization of the sensor/response regulator

Fine-tuned molecular specificity***, achieved by co-evolution
Specificity determining residues identified by coevolution analysis

Figure from M. Laub, MIT

structure: PDB 3DGE
Two examples of signal transduction:

A.) Chemotaxis

<table>
<thead>
<tr>
<th>input</th>
<th>output</th>
</tr>
</thead>
<tbody>
<tr>
<td>attractant</td>
<td>tumbling frequency</td>
</tr>
</tbody>
</table>

B.) Quorum Sensing (bacteria can count!)

<table>
<thead>
<tr>
<th>input</th>
<th>output</th>
</tr>
</thead>
<tbody>
<tr>
<td>counts cell #</td>
<td>makes light (other things)</td>
</tr>
</tbody>
</table>
Chemotaxis: Biased random walk

CW rotation: tumble; CCW rotation: run

http://emonet.biology.yale.edu/agentcell/
Movie of chemotaxis – run and tumble

Howard Berg (Caltech class of 1956, Chemistry)
Professor of Physics, Harvard
How do cells adapt to new conditions yet preserve their ability to respond?

Concept of robust exact adaptation – lecture reading assignment!

Sketch out Figure 7.5, 7.10 from reading! Moral of the story: ability to return to the steady state activity does not depend on ligand levels (robustness) But the ligand levels fine tune the exact steady state level (data in Fig. 7.11)

*Extra credit question (worth one iClicker question): under what conditions does robustness break down? Solve mathematically, using specific components of the chemotaxis network. Due in class next Tuesday at beginning of lecture.*
Quorom sensing: sensing oneself (mechanism for “social behavior”)

K. Nealson and W. Hastings discovered in 1970 (Nealson Thursday!)

Auto-induction, critical threshold

This is another two component system

Signal Receptor Protein

Gene transcription (results in making light)
Dramatic examples of quorum sensing…

*Hawaiian bobtail squid*
*Euprymna scolopes*

*Vibrio fisherii, bioluminescence*

Ruby and Mcfall-Ngai

Egland and Leadbetter
...responsible for the “milky seas” described by ancient mariners

(Vibrio harveyi associated with microalgae)

Jan. 25, 1995

~ size of Connecticut
Quorum sensing: sensing oneself AND OTHERS!

Signal Producing Protein

Intra-species

Intra-genus

Inter-species

Rich chemical lexicon!

http://www.ted.com/talks/bonnie_bassler_on_how_bacteria_communicate.html

virulence, biofilm formation, etc...
Movie of traveling waves in *Myxobacteria*

Dale Kaiser (Ph.D. Caltech, 1955)
Professor of Developmental Biology, Stanford
Accessible application of blocking QS: prevent refrigerator rot!

How could you do this?

Erwinia carotovora
Application of blocking QS: prevent rotten lettuce in your refrigerator!

*How could you do this?*
PRINCIPLE: TO EXIST, LIFE MUST SENSE AND RESPOND TO THE EXTERNAL ENVIRONMENT

DKN
- How do microbes sense and respond to their environment? 
  *Using highly modular 2-component systems*

- What type of things do they sense? 
  *Attractants, repellents, cell number, time, stress…*

- How does signal transduction work? 
  *Sensor histidine kinas detects signal, phosphorelay → activates response regulator*
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Clicker quiz round
Sensing/integrating/responding to the environment

Unicellular – receptor on the cell/ integration across the cell/ effector mediates response

Multicellular – 2 interacting tasks:
   1) responding to the abiotic environment
   2) coordinating cells

Nature of the:
   - Sensors
   - Integrators
   - Effectors

A. Animals - nervous and endocrine systems in animals – typical short vs. sustained
B. Plant hormones
Environmental cue (e.g., shed bacterial surface molecules) leads to the activation of a sensor, which in turn activates a phosphorelay system. The phosphorelay integrates transcriptional regulation, leading to the activation of a transcription factor and effectors. This results in a response (e.g., cell death) through the NF-kappaB pathway. The diagram is attributed to David Baltimore.
Coordination of a multicellular organism

cells – tissues – organs – organ systems

FASTER RESPONSE

nerve cell - e.g., myelin – e.g. brain - nervous system

SECRETORY RESPONSE

secretory cell - e.g., islet cells – pancreas – endocrine system (make insulin)

SLOWER, SUSTAINED RESPONSE

plant growth

progression of insect development
Trends in Nervous System Evolution and Correlations with Body Plan

No well coordinated NS, no symmetry

Bidirectional nervous conduction
Radial symmetry

Unidirectional nervous conduction
Bilateral symmetry

Increasing cephalization

~100 billion neurons in brain
Spinal cord 1 billion

~300 million neurons in brain

~300 million neurons in brain
ANIMAL NERVOUS SYSTEMS

FROM: Simple reflex arc

BRAIN OR SPINAL CORD

environmental cue

sensor

transmitters

integrators

effector

receptor

afferent

relay

efferent

effector
Example of a reflex arc

Z-fish movie
Complexity of wiring for complex human behavior:

Human brain ~100 billion neurons –

10,000 neuronal classes

...each nerve cell or neuron communicates with 1000 others.

HAR1
To: More complex behaviors

Neural circuitry and the genetics of aggression

Movie

David Anderson
Div Biology, Caltech
Multiple exposure (infrared) of owl hunting in the dark

Locates prey by using time over which sound reaches the two ears, which are asymmetric
Interaural time difference
Sound-source localization

Complex neural computation that translate auditory location cues into representations in space.

The system is integrated with the owls visual system; learned.

Owl chicks – with prisms.
Orienting responses to auditory or visual targets

Before Prisms

Day 1 (R23° Prisms)

Eight weeks

Prisms Removed

auditory
visual
Prism experience shifts auditory receptive fields

Normal

Immediate Effect of Prisms

After 8 Weeks of Prism Experience

After 8 Weeks of Prism Experience
What are these cells and how do they function?

Nerve cell = neuron

3 principal kinds:

- Sensory neuron
- Interneuron (relay)
- Motor neuron
Structure of a neuron

Direction of neural transmission

Dendrite (receiving)  Axon (sending)

uninsulated node

insulation (myelin) +/-

stimulus

nucleus

Synapse (electrical or chemical)

Speed:
nervous conduction – myelinated (vertebrate nerves, with an exception) – up to 100 m/sec
unmyelinated (invertebrate) – typical up to 1 m/sec
giant axon (1 mm diameter max) aver 0.5 mm - 25 m/sec
Physiology of a neuron – selection on a pre-existing character of all cells – membrane potential - difference in charge across the membrane

K⁺ 150 mM
Na⁺ 15 mM
Cl⁻ 10 mM

K⁺ 5 mM
Na⁺ 150 mM
Cl⁻ 120 mM

60 mV
Amount of membrane voltage at equilibrium = equilibrium potential ($E_{ion}$) is given by Nernst equation, applies to single ion type.

$$E_{ion} = 62 \text{ mV} \left( \frac{\log \text{[ion] outside}}{\log \text{[ion] inside}} \right)$$

$$\log 5 \text{ mM}$$

Thus, for K+ $E_k = 62 \text{ mV} \log 150 = -92 \text{ mV}$ (at body temp)

$Na^+ + 62 \text{ mV}$

Membrane potential always described as inside relative to outside

**Typical membrane potentials:**

- bacterial cell = -140 to -105 mV
- mammalian neuron = -60-70 mV

MEMBRANE POTENTIAL ACTUALLY SET BY K+, as more K+ channels open at rest

MAINTAINED BY PUMPS IN THE MEMBRANE
**Action potential** a depolarization of the membrane - nerve fires

- **stimulus**
- **Direction of neural transmission**
- **Dendrite (receiving)**
- **Axon (sending)**
- **uninsulated node**
- **Synapse** (electrical or chemical)
- **insulation (myelin)** +/-
- **nucleus**

ACTION POTENTIAL BEGINS HERE

SUM OF EXCITATORY & INHIBITORY INPUT
Action potential - a depolarization of the membrane
Action potential - a depolarization of the membrane

1. Stimulus
2. Depolarization
3. Action potential
4. Repolarization
5. Refractory period
6. Resting state

Na+ channels open

Na+ channels close/K+ channels open

Potassium channels close
Axon terminal of motor neuron interfaces with effectors:

- e.g.,
  - muscles
  - chromatophores
receptor

afferent

Sensory neuron

Motor neuron

Interneuron (relay)

effector

efferent

Neuromuscular Junction

Axon of motor junction

Neuromuscular junction

Presynaptic terminal

Synaptic vesicles

Sarcolemma

Synaptic cleft

Mitochondrion

Postsynaptic membrane

Muscle fiber

Capillary

Myofibrils