Persistence – Multicellularity and Development

DKN
- Some examples of differentiation in the bacterial world
- A research story about biofilms and metabolic differentiation

MMN
- How does an organism progress from an egg to an adult?
- What is the basis for variation in development?

Exam1 update – some good news
iClicker quizzes
From iron oxides to infections: roles for redox-active “antibiotics” in microbial survival and development
Shewanella oneidensis strain MR-1
Pathway of respiration for a single cell

OM

CM

H+

H+

H+

H+

Fe(OH)₃

Electron Acceptor

ADP + Pᵢ

ATP
Pathway of respiration for a single cell

OM

CM

MQ

CymA

MtrA

OmcB

Fe(II)

Fe(III)

H+

e-

ADP + P_i

ATP

H+
But what about at a distance?
Could redox-active “antibiotics” be endogenous e\textsuperscript{-} shuttles?
Fe(OH)₃

Fe(III)-mineral surface

H₂O

O₂

H₂O

O₂

reduced oxidized shuttle

Hernandez and Newman (2001) CMLS

Fe(III)-mineral surface

[Legend: reduced, oxidized shuttle, outer membrane protein]
Reoxidation shuttle between Fe(III) hydroxide and membrane-bound NO reduction.

Hypoxic mucus

Periciliary liquid layer

Infected surface

Reduced oxidized shuttle

$2O_2 \rightarrow 2O_2^-$

NADH

NAD$^+$
Metabolic activity persists long after growth ceases

Growth activity \((rrnB\ P1)\)

Metabolism \((mtrB)\)

Unstable reporter \((t_{1/2} \sim 60\text{min})\)

Teal et al. (2006) AEM
Various species produce electron shuttles when in biofilms

\[ \text{MnO}_2 + 2e^- \rightarrow \text{Mn(II)} \] (dissolved)
Pseudomonas aeruginosa PA14 produces phenazine “antibiotics” (electron shuttles)

http://www.pseudomonas.com
Fun (and important) redox properties

- Colorless to blue with 2e-

2e- + \text{colorless} \rightarrow 2\text{O}_2 \rightarrow 2\text{O}_2^- + \text{blue}
Conventional wisdom: phenazines are toxic, “secondary” metabolites

The toxicity of phenazines is attributed mainly to superoxide generation.

Corollary: under anaerobic conditions, toxicity is diminished.
Conventional wisdom: phenazines are toxic, "secondary" metabolites

The toxicity of phenazines is attributed mainly to superoxide generation.

**Corollary: under anaerobic conditions, toxicity is diminished.**

Fact: phenazines are produced under hypoxic conditions, at later stages of community development
Phenazines play a role in development at high cell density

Phenazines play a role in development at high cell density

5 mm

\(\Delta \text{phz}\)

WT

\(\uparrow \text{phz}\)

day 3

Phenazines play a role in development at high cell density

The molecular basis of this phenotype is complex

**HYPOTHESIS: THE REDOX-STATE OF THE CELL IS LINKED TO COLONY MORPHOLOGY**

**LET’S CONNECT WHAT YOU’VE LEARNED ABOUT METABOLISM TO BACTERIAL MUTICELLULARITY**
Phenazines are versatile redox-active molecules

\[
\begin{align*}
E_0' (mV) & \\
-320 & \text{NAD}^+ / \text{NADH} \\
-174 & \text{1-OHPHZ}_{\text{ox}} / \text{1-OHPHZ}_{\text{red}} \\
-140 & \text{PCN}_{\text{ox}} / \text{PCN}_{\text{red}} \\
-114 & \text{PCA}_{\text{ox}} / \text{PCA}_{\text{red}} \\
-40 & \text{PYO}_{\text{ox}} / \text{PYO}_{\text{red}} \\
+59 & \text{Fe(OH)}_3(\text{am,s}) / \text{Fe}^{2+}(\text{aq}) \\
+620 & \text{MnO}_2(\text{s}) / \text{Mn}^{2+}(\text{aq}) \\
+820 & \text{O}_2 / \text{H}_2\text{O}
\end{align*}
\]
Phenazine cycling promotes survival

Surviving cells have more oxidized NAD$^+/\text{NADH}$ than dying cells.

$2\text{O}_2 \rightarrow 2\text{O}_2^-$

$kern et al. (2012), in prep$
Is wrinkling consistent with an oxidant access strategy?
Microelectrode profiling of colonies can define oxic zones

Dietrich et al. (2012) in prep
Oxygen is reproducibly consumed at 60µm depth in WT and Δphz mutant biofilms
Phenazines promote $O_2$-independent survival

- $\Delta phz$ mutant only inhabits zone where $O_2$ is present
- Increasing ambient $O_2$ increases habitable zone
Redox balancing appears key to this phenotype

\[ \text{Δphz} \quad \text{wt} \quad \text{ΔnapA} \]

+ PYO or NO$_3^-$

red, NO$_2^-$

ox, NO$_3^-$

NO$_2^-$

NO$_3^-$
How conserved is this role for redox active “antibiotics”?

Streptomyces coelicolor

actinorhodin
Actinorhodin also affects colony morphology.
Key concepts

• What is a bacterial biofilm? A collection of bacterial cells attached to a surface.
• How are *P. aeruginosa* biofilms multicellular? Different cells differentiate their metabolic programs according to their local microenvironment.
• This type of multicellularity is different from differentiation that is committed (*i.e.*, no going back: differentiation into different cell types with different structures and functions, such as the stalks in fruiting bodies of *Myxobacteria* whose cells never go on to reproduce.). The metabolic program in *P. aeruginosa* biofilms can change as environmental conditions change.
• Sporulation by *Bacillus* is an example of differentiation that isn’t terminal (while spores are very different than vegetative cells, they can germinate and become vegetative cells again).
• This lecture integrated topics you were exposed to earlier in the course: quorum sensing, metabolism (redox chemistry), chemical gradients maintained by living systems and bacterial genetics.
Animal Development

*Two principal questions:*

1) How does an organism progress from an egg to an adult?
2) What is the basis for variation in developmental programs?
MODELS IN DEVELOPMENTAL BIOLOGY
1) How does an organism progress from an egg to an adult?

- What happens at fertilization?
- What determines cell fate? (how do the cells know what to do and when?)
- What are the genes doing? What are the cells doing?

At the basis is REGULATION!
One e.g., - What determines cell fate? (what to do and when?)

*Caenorhabditis elegans*

*C. elegans*

‘the worm’

A great model for the student of cell fate

Small, transparent, easy to grow in the lab and…. only and always 959 cells in adult hermaphrodite!

MOVIE

Cell Lineage in *C. elegans*
e.g., - what are the genes doing?

One of the gene regulatory networks operating in sea urchin development

Eric Davidson
Caltech
What is the basis for variation in developmental programs?
Take home:
Animals have between 30 and 40 different major body plans. [Just a few in this tree.]
Evolution of Developmental Mechanisms = ‘Evo Devo’

http://www.cbsnews.com/video/watch/?id=5658057n

Sean Carroll
University of Wisconsin-Madison
Regulatory Cassettes –
Hox genes are a group of related genes that determine basic structure and orientation of an organism.

Hox genes contain a 60 amino-acid long ‘homeodomain’ = a transcription factor – binds DNA and influences transcription
**Euprymna scolopes**

**Vibrio fischeri**
Evolutionary Tinkering – using the same tool kit to make generate different structures

Similar anatomy
Similar biochemistry
Homeodomain proteins specifying eye development

- **pax6**
- **six**
- **eya**
- **dac**

Initiation
Feedback
All of these genes are expressed in both eyes and light organs.
Punctuated equilibrium – Steven J Gould
Long periods of stability and short episodes of change

KEY INNOVATION – in biology, trait that enables a group of organisms to diversify – often results in a change during development
DEVELOPMENTAL CHANGES LEADING TO KEY INNOVATIONS

baleen whales

toothed whales (incl porpoises/dolphins)

millions of years ago
Toothed Whales

BALEEN WHALE KEY INNOVATION

baleen
DEVELOPMENTAL CHANGES LEADING TO KEY INNOVATIONS

INSERTION OF A TRANSPOSON IN ‘MMP20’ GENE – critical for normal tooth development

baleen whales

toothed whales (incl porpoises/dolphins)

millions of years ago
What about the formation of features that occur exclusively within one group?

e.g., jellyfish and their relatives – stinging cells
clams and their relatives – tissues that secrete the shell

ORPHAN GENES –
The genes that seem to be unique to a lineage; likely involved in lineage specific traits - likely arise from duplication and alternative splicing and then very fast divergence.
MMN – Developmental Biology
- How does an organism progress from an egg to an adult?
- What is the basis for variation in development?

In both cases, it’s all about gene regulation. Animals have many of the same genes, but use them in different ways.