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Experimental Methods in Systems Biology

Part of the Coursera Certificate in Systems Biology

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Icahn School of Medicine at Mount Sinai

Important Features of Any Experiment

- Given a specific question, one can then come up with answers to the following three key properties of the experiment:
  1. What biological system?
     - E.g. Do I look at human cell lines, a mouse, yeast, etc.?
  2. What perturbation/treatment conditions?
     - E.g. What compounds should I apply to the system to elicit a relevant response?
  3. What measurements?
     - E.g. What transcripts do I need to look at, and/or do I need to look at protein levels instead?
- Often (but not always), if you can't design an experiment that only has a handful of conditions and measurements, results may be difficult to interpret
  - Usually the question is too complex or not significant
  - Exceptions are screening based studies, but those also typically have a specific question of interest

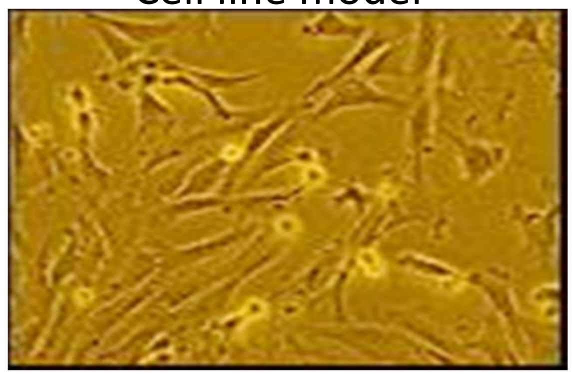
Outline

- What is a model?
- Single-cell organisms
  - *E. coli* (bacteria) and *S. cerevisiae* (yeast)
- Multi-cellular organisms
  - *C. elegans* (nematode), *D. melanogaster* (fruit fly), *D. rerio* (zebrafish), *A. thaliana* (mustard plant)
- Mammalian systems
  - Cell lines, primary cells and tissues, *M. musculus* (mouse), *R. norvegicus* (rat)
- One of the promises of systems biology: computational models

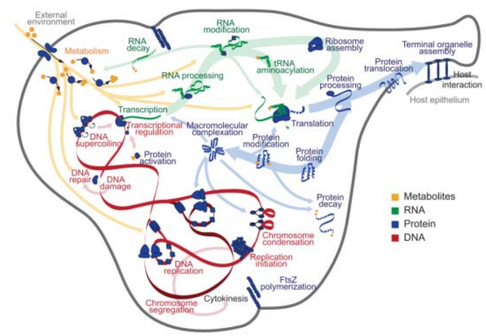
What is a model?

Mouse model

Cell line model



Computational model



Karr et al., 2012 Cell 150 389-401

A simplified/convenient version of a complex/inconvenient entity. The model captures the key characteristics of the entity needed for addressing the task at hand.

Why do we use models for biological research?

We usually can't study what we want (e.g. human disease) directly.

Whither Model Organism Research?

SCIENCE VOL 307 25 MARCH 2005

Stanley Fields and Mark Johnston

1. "Over the coming few decades, model organisms will continue to provide insights into replication, transcription, … and many other aspects of cell biology, biochemistry, and physiology, because they offer the keenest methods of analysis."
   - E.g. new disease gene in a conserved cellular process
2. "Model organisms will increasingly be used for the direct investigation of medical problems that seemingly have little to do with them."
   - E.g. neurodegeneration disorders (e.g. Alzheimer's) involving protein folding/aggregation
3. "Model organisms will remain at the forefront for the forseeable future in efforts to sort out biological complexity and achieve a more quantitative understanding of life processes…"
   - Thus particularly relevant for systems biology
4. "Model organisms offer the best hope for coming to grips with the breadth of genetic diversity and the depth of its consequences"
   - Needed for tailored and personalized medicine
5. "Model organisms will remain the proving ground for developing new technologies, which typically spread quickly"
   - E.g. yeast two hybrid screens, genetic manipulation

Single-cell Organisms—*E. coli*

SCIENTIFIC AMERICAN™

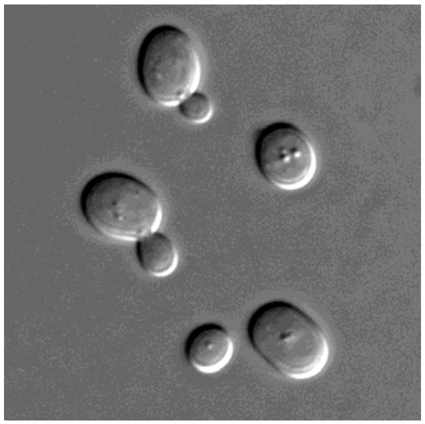
Everything You Ever Wanted to Know about *E. Coli*, Part 1

Author and journalist Carl Zimmer talks about *E. coli*, the bacteria that are the subject of his new book *Microcosm:* E. Coli *and the New Science of Life*. Web sites mentioned in this episode include www.carlzimmer.com

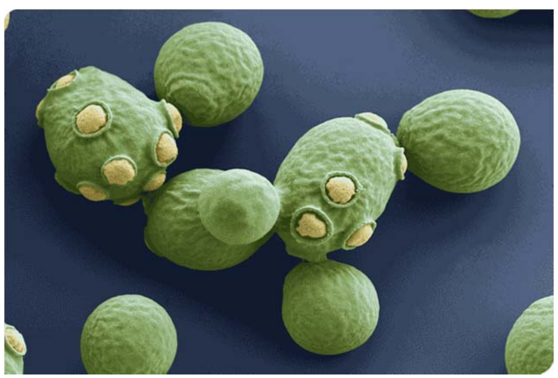
Oct 8, 2008 | By Steve Mirsky

- There are many laboratory strains, a common strain is K12.
  - Non-pathogenic bacterium
- Fast doubling time and simple growth requirements, only ~4,000 genes which can be manipulated with relative ease
- Arguably "the best understood species on earth"
  - Allows us to ask very deep questions about life, and stand on a mountain of prior research
    - understanding how molecular networks and circuits give rise to biological function (e.g. Shen-Orr et al., 2002, Nat Genetics)
    - Understanding evolutionary processes (e.g. Barrick et al., 2009, Nature)
    - Molecular motors such as flagellum and proton pumps
- Also used as a tool
  - E.g. production of DNA plasmids or proteins of interest in molecular biology research
- Disadvantages—doesn't have a nucleus/chromatin, not all protein production mechanisms such as glycosylation

Single-cell Organisms—*S. cerevisiae*



http://en.wikipedia.org/wiki/Saccharomyces_cerevisiae#mediaviewer/File:S_cerevisiae_under_DIC_microscopy.jpg

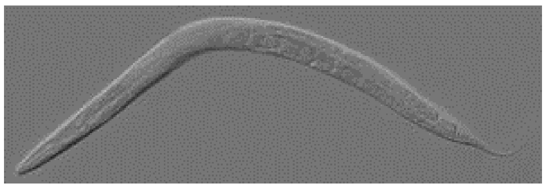


http://www.microbiologyonline.org.uk/about-microbiology/introducing-microbes/fungi
©Eye of Science/Science Photo Library

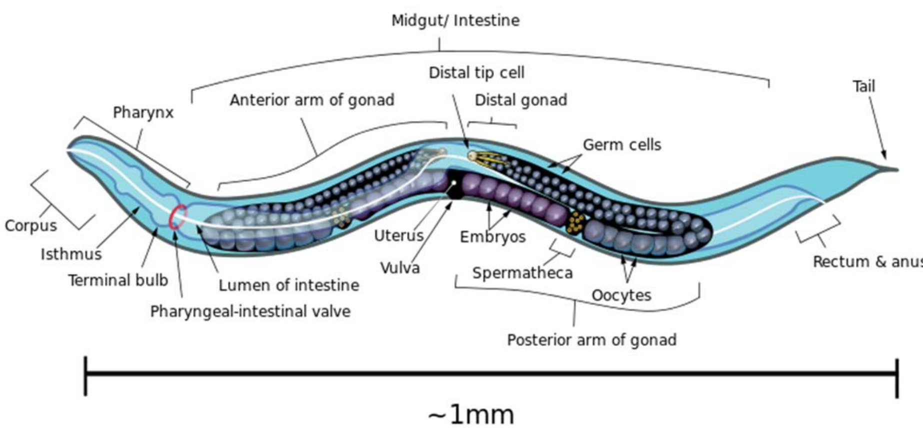
Yellow scars are from previous budding events which give rise to new cells.

- Baker's yeast or budding yeast—same used to brew beer
- As opposed to E. coli, they are eukaryotes and therefore have a nucleus and many other basic cellular processes that human cells have.
  - E.g. mitochondria, cell cycle, trafficking through golgi and endoplasmic reticulum, meiosis
  - Much of our understanding of the cell cycle comes from studies on S. cerevisiae (Pray, 2008, Nat. Education 1(1):183), in fact the Nobel prize in 2001 to Hartwell is based on such work (Nurse and Hunt used other model organisms)
- Easy to genetically manipulate through homologous recombination
- Grow quickly and easily

Multi-cellular Organisms—*C. elegans*



http://en.wikipedia.org/wiki/Caenorhabditis_elegans



- One of the simplest organisms with a nervous system
  - Nervous system is completely mapped
- Can be used to study reproduction and development
  - Developmental fate of every single cell (1031 in adult males) is known
  - 2002 Nobel Prize to Brenner, Horvitz and Sulston on organ development and apoptosis
  - 2006 Nobel Prize to Fire and Mello for RNA interference (will be discussed in Lecture 3)

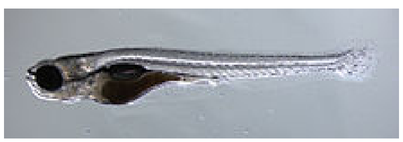
Multi-cellular Organisms—*D. melanogaster*

http://en.wikipedia.org/wiki/Drosophila_melanogaster

- Sexual reproduction and rapid generation time facilitates genetic studies
  - Many human disease genes have homologs in drosophila
- Also has
  - Vision
  - More complex features and organs than *C. elegans*

Multi-cellular Organisms—*D. rerio*

http://en.wikipedia.org/wiki/Zebrafish



Juveniles are transparent, facilitating observation of development

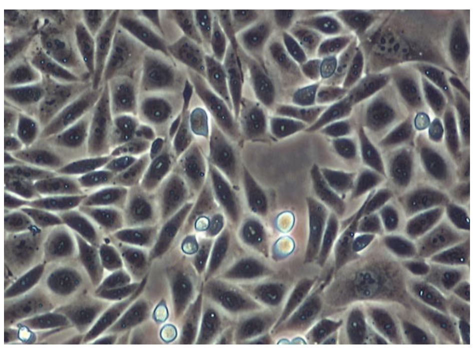
- It is transparent during development and therefore amenable to study of development by light microscopy
  – Also used as a model for developmental toxicity
- Like the others, it grows quickly and is easy to manipulate genetically.

Multi-cellular Organisms—*A. thaliana*

http://en.wikipedia.org/wiki/Arabidopsis_thaliana

- Like other model organisms is robust and has a short life cycle
- Used for genetics, has a small genome for plants
  - Light sensing and circadian clocks
  - flowering

Mammalian Systems—Cell Culture



http://www.microscopyu.com/staticgallery/phasecontrast/chocellspositive.html

Phase contrast image of CHO cells in culture

- Cells from various tissue sources, most notably humans, can sometimes be cultured in a dish or flask
  - American Type Culture Collection (ATCC) has thousands of human cell lines
- It is much easier to study cells in culture than whole organisms
  - Can do more perturbation and measurement techniques
  - Quicker
  - Cheaper
- Most cell lines are "transformed" meaning they grow indefinitely in culture, and there are some artifacts due to that.
  - A large majority are derived from tumors

Mammalian Systems—*M. musculus*

http://en.wikipedia.org/wiki/House_mouse

http://en.wikipedia.org/wiki/Laboratory_mouse

- Quickly reproducing and amenable to genetic manipulation
  - preferred genetic model for mammalian systems
  - A variety of strains with gene knock out, knock-in and conditional expression exist
- Strains exist without an immune system to facilitate studies involving human cell line xenografts
  - E.g. tumor growth and drug response

Mammalian Systems—*R. norvegicus*

http://en.wikipedia.org/wiki/Laboratory_rat

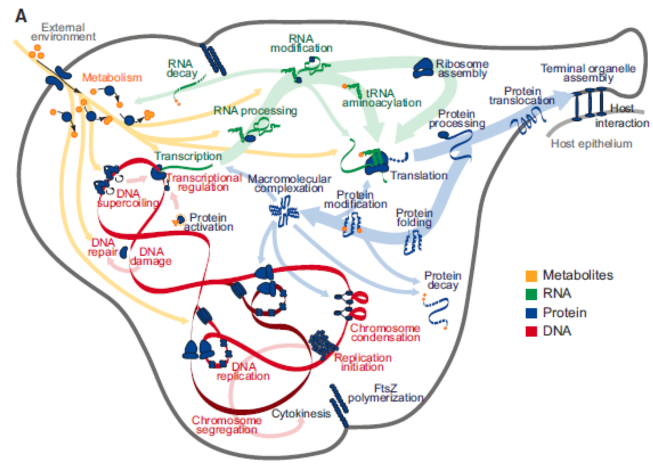
- Similar to mice, but
  - are not as amenable to genetic manipulation as mice
  - Are regarded as more intelligent than mice, so are preferred in neuroscience and behavioral experiments

Computational Models as a Biological Model System

- One of the main motivations for systems biology is to build computational models that are good enough to be considered another type of model system.
  - We are a long way off
  - The most relevant effort:

A Whole-Cell Computational Model Predicts Phenotype from Genotype

Jonathan R. Karr,[1,4] Jayodita C. Sanghvi,[2,4] Derek N. Macklin,[2] Miriam V. Gutschow,[2] Jared M. Jacobs, Benjamin Bolival, Jr.,[2] Nacyra Assad-Garcia,[3] John I. Glass,[3] and Markus W. Covert[2,*]



Computational Models as a Biological Model System

- Potential applications
  - Design microorganisms that
    - Do a better job a remediation of waste water
    - Produce a certain complement of small organic molecules from raw materials
  - Predict efficacy and toxicity of potential drugs prior to expensive clinical trials
    - Get rid of potential problem drugs earlier in the development pipeline
  - Identify patient populations that are more or less likely to respond to a drug
    - “pharmacogenomics”
    - Personalized medicine, e.g. in cancer
  - Predict new uses for already approved drugs—repurposing
    - New drug combinations to improve efficacy or reduce toxicity
    - Using a cancer drug for arthritis--methotrexate