Experimental systems for answering Biological questions
Viral systems

• Proteins involved in DNA, RNA and protein synthesis
• Cancer genes
• Human infectious disease and immunity
• Gene therapy vectors
• Evolution at a speed that permits experimental study
Bacterial systems

- Regulation of DNA, RNA and protein synthesis, regulation metabolism, and systems analysis of these functions
- Animal and plant infectious disease
- Host-pathogen interactions
- Cell-cell signaling
- Evolution, adaptation to extreme environments
Dictyostelium discoideum

- Fast and easy genetics
- Genome sequenced recently
- Good model for animal cellular behaviors such as directed cell migration, aggregation/adhesion

Limited cellular diversity
Saccharomyces cerevisiae (yeast)

- Fast and easy genetics
- Powerful molecular genetics and systems biol.
- Good model for animal cellular behaviors such as secretion, protein degradation, membrane biogenesis, cytoskeletal function, aging, cell cycle, RNA localization

Limited cellular diversity
Caenorhabditis elegans, nematode

- Fast, extremely powerful and easy genetics
- Good model for studies in cell lineage, cell death, aging, RNA interference, microRNA function, host-pathogen interactions, behavior, nervous system structure and function
- Direct analysis of gene expression patterns difficult
- Biochemistry difficult, although not impossible, no cell culture
- Limited morphological diversity - 959 cells
C. elegans showing a mutation in the let-7 gene that causes the worm to rupture
Drosophila melanogaster, fruit fly

- Very rich and powerful genetics, genomics, and mutant phenotypes, transgenesis easy
- Model for gene regulation, development and evolution of body patterning, innate immunity, simple behaviors, cell death, cancer, drug abuse, wound repair, cell polarity, cell signaling, cell shape control
- Easy and inexpensive whole animal biochemistry
  biochemistry of individual tissue difficult (cell sorting)
  targeted knock-out possible only recently
  limited repertoire of cultured cells
**Danio rerio, zebrafish**

- Simplest vertebrate with good forward genetics, rich collection of phenotypes
- Organ systems very similar to other vertebrates (eyes, heart, blood, GI tract)
- Regenerates some adult organs and structures

Cloning mutants easier recently with genomic structural mapping
targeted knock-out technology still under development
duplicated genes
Xenopus laevis, amphibian, frog

- Excellent source of biochemical material for cell biological studies, cell cycle, DNA replication, intracellular localization of protein and RNA
- Large oocytes, nuclei and cells, easily injected, easily manipulated experimentally

Genetics poor, although

Xenopus tropicalis is coming along
Mus musculus, mouse

- Mammals - brains very similar to humans in nearly all areas, cell types
- Targeted gene knockouts routine, although duplicate genes occasionally complicate things
- Forward genetics always improving, although still expensive, slow and difficult compared to other model systems, very large mutant collection, if you can get them
- Large enough that relatively homogeneous tissue can be harvested and studied biochemically

Animal care and experimental restrictions can be onerous
• Phenomenal similarity of some developmental genetic mutant phenotypes
  (piebaldism -- genetic defects in melanocyte migration, differentiation, survival --
  Kit transmembrane receptor tyrosine kinase)
Mammalian (and other animal) cultured cells

- Cells can take up DNA easily, and many lines exist whose properties resemble their cell type/tissue of origin - often used for studies of gene regulation
- Molecular visualization in studies of transport, cytoskeleton, cell division, intracellular transport is exceptionally good

Until recently, genetics was terrible, but with advent of siRNA methods, can now do genomic level studies of biological phenomena that can be assayed in such cells
Arabidopsis thaliana

- Very rich and powerful genetics, genomics, and mutant phenotypes
- Model for plant development, physiology, cell polarity, cell signaling, cell shape control, immunity, infectious disease, environmental stress, evolution, and every other biological phenomenon that plants can accomplish
- Real-world agricultural impact of many research findings

Not a crop plant, so other models such as corn, rice better for making some agricultural discoveries
Guidelines to answering questions with experiments

1. Convince *yourself* that a question is worth answering with background reading in books, review articles, research articles. Talk to your colleagues and explain the background and question to them. Write your own summary of the previous findings and how your experiments would fit in. Try to read a paper or two every day as a habit. Email authors if you have thoughtful questions about their papers; they are often eager to be asked.

2. Once you have decided on an approach/method, carefully read (and outline in your own words) a few of the original papers on the method. Go over the protocol(s), and write them out in your own words. Draw little pictures of what is happening in your experiment. Inevitably, you will now have some questions, so talk to other people who have done the protocol before (successfully!) Talk to your advisor every day if possible, tell him/her what you are reading, thinking, and planning.

3. Amaze your advisor and your friends by planning positive and negative control experiments to be done the *first* time you do your experiment.

4. Before you do the experiment, draw a picture and write down what you expect/hope the results will be. Order and get the materials together well before you plan to use them.

5. Attain *mastery* of the techniques you use, try to be the best in the world. Before you use a kit or a bacterial strain; have a clear mental image of how it works.

6. Keep a notebook for personal reflections on your experiments, and what you read. Is there a different or novel method I could use? Is there another way I could interpret my results? What will I be doing in the lab six months from now?
Analyzing research papers: Outline of an approach

A. Introduction, context at the time the paper was published.

Brief statement of the research field in which the paper is embedded. What was the conventional wisdom at the time? What previous results did the authors state had influenced their thinking? How had previous researchers attacked the problem? Why had they failed to solve it? Was it even recognized as a problem? What experience did the authors bring to the problem, were they new to the field or lifelong devotees? Had previous papers actually solved the problem, but were unappreciated . . if so, why?

B. Analysis of the experiments and conclusions paper(s).
Plan of attack – How did the authors justify their series of experiments as a progressive dissection of their problem? Did they use novel or standard approaches?

The results – one experiment at a time . . what method was used, what are the advantages and disadvantages of that method, was it appropriate for the question? What other methods might have been employed? what conclusions were drawn from each result? Were such conclusions justified? Be analytical! Question every assumption! Be critical!

C. Overall message – How have these results and conclusions changed the scientific thinking in this research area? Or did it have any effect? Did the authors synthesize information from widely different fields? Did they apply new techniques to old problems? Did they actually solve a new, important problem of their own creation? Were there reasonable extrapolations could have been made from the results that the authors failed to propose?