Molecular Cell Biology

Logic and Approaches to Research

Reductionism Science

1. Define a biological problem	Genetics, physiology, medicine
2. Inventory of parts	Biochemistry, genetics, genomics
3. Concentrations	Biochemistry, microscopy
4. Molecular structures	X-ray crystallography, NMR
5. Partners	Biochemistry, genetics
6. Rate & equilibrium constants	Biophysics, microscopy
7. Biochemical reconstitution	Biochemistry, microscopy
8. Mathematical model	Analytical or numerical
9. Physiological tests	Drugs, genetics, RNAi

Approaches to Cell Biology Research

Genetics

- Screen for mutants with a phenotype.
- Crosses to define complementation groups.
- Details of the phenotypes. Divide into classes.
- Order the classes by epistasis.
- Clone the genes.

Approaches to Cell Biology Research

Anatomy

- Structure of cells and tissues.
- Ultrastructure (EM), to detect fine structures, such as filaments or membranes.
- Correlate structures with function.
- Identify molecules if possible.

Approaches to Cell Biology Research

Biochemistry

- Purify molecules, such as metabolites, proteins, or even membranes.
- Study their chemical properties in vitro.
- Attempt to re-create in vitro a phenomenon observed in vivo.
- Reconstitution as an ultimate test for "sufficiency."

Approaches to Cell Biology Research

Physiology

- Observe the phenomena exhibited by living cells or organisms, such as movement.
- Quantify parameters such as rate of movement and ask how they correlate with each other factors.
- Decrease or increase the activity of a component.

Approaches to Cell Biology Research

Pharmacology

- Find drugs (chemicals) that inhibit or enhance a phenomenon, such as movement.
- Identify their molecular targets, such as proteins.
- Use in physiology studies to inhibit a process acutely.

Example of How the Techniques Interact

- Find a cell that moves, like Dictyostelium. Study its movement up a chemotactic gradient, and quantify various parameters. Find drugs that inhibit this movement.
- Study the fine structure of the cell, especially the areas that seem to be moving. Are there small structures, such as filaments and crosslinkers, and are they in an arrangement that suggests how movement can be generated?

Example of How the Techniques Interact

- Purify proteins that make up those fine structures, such as filaments. Purify proteins that bind to those proteins. Look for how the different proteins regulate the relevant activity (which you have to guess at). Determine whether the drugs above affect this in vitro activity.
- Localize those proteins with Ab staining of cells to show that the proteins really are associated with those fine structures. Microinject Abs or fragments of proteins looking for an effect on cell movement (inhibition or enhancement).

Example of How the Techniques Interact

- Reverse genetics. Use the protein (Abs or sequence) to clone cDNA's and/or genes encoding it. Correlate expression with cell movement. More important, use the DNA to inhibit the protein (antisense or knockout), or overexpress the protein (transfection expression) or express fragments or mutated versions of the protein (dominant effects), and look for effects on cell movement.

Example of How the Techniques Interact

- Forward genetics. Instead of all this, start by making mutants. Study phenotype and classify, to give information about different steps at which one can stall the process. Use the physiology and anatomy to classify. Do epistasis to order the genes. Clone and sequence the genes. Hope you find some of the proteins identified above. If not, make the protein, make Abs, and do the experiments above.

Example of How the Techniques Interact

- Reconstitution as an ultimate goal. Genetics defines a set of genes / proteins important for movement. Those proteins constitute structures or regulate them. Reconstitution with pure proteins creates the movement.

Experiment Design: Necessary vs Sufficient

- Necessary
  - Process with multiple parts. Remove one and observe loss of function.
  - Distinguish Necessary from Regulatory.
- Sufficient
  - Synthetic mixture of pure components.
  - Add a new component to a mixture.
    - Recognize the context.

Hypothesis-Driven Experiments

- State the hypothesis
  - Not a "straw man" or trivial
- State the experiment
- Possible outcomes
- Interpretation of each outcome
- Controls - positive, negative
- Limitations and Alternative Interpretations

"Proof" of a Hypothesis or Model

- Observed Results as Predicted
- What Alternatives are Excluded?
  - How strong is the evidence against the alternatives?
  - Obligation to raise and test credible alternatives
  - Or the ones that others find compelling

Mathematical Modeling

- What features of the model are verified by experiments?
- How many parameters are varied, to fit the data?
- What models are excluded?