MCDB 153 – Spring 2013

"Molecular and Cellular Approaches to Neural Development"

Professor: Dr. Stuart Feinstein

TAs: Julianna Erickson
Sarah Benbow

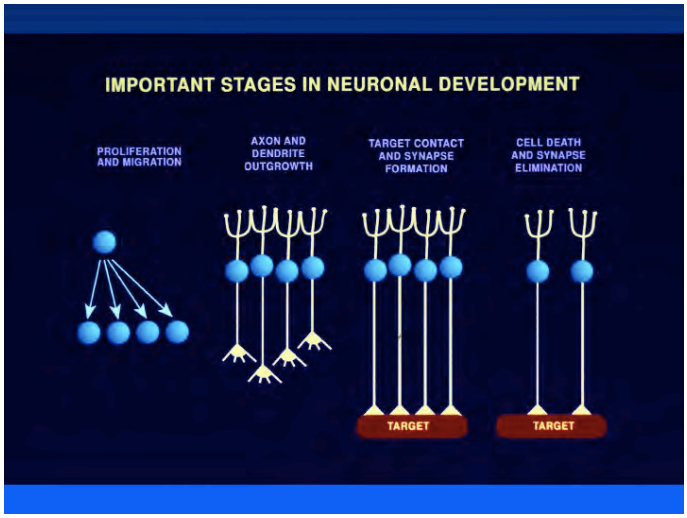
Lecture Set 1

A Photographic View of Neural Development

A Neurocentric Perspective of Development

Fertilization --> Blastula --> Gastrula --> Neurula

- •Neuroblast Proliferation and Differentiation
- •Neuroblast Migration
- •Axon Outgrowth
- •Target Contact/Programmed Cell Death
- •Target Contact/Synaptogenesis
- •Synaptic Rearrangements
- •Active Cell Maintenance
- •Neurodegeneration



Tentative Lecture Schedule for MCDB 153
"Molecular and Cellular Approaches to Neural Development"
Spring Quarter, 2013
Tues/Thurs 9:30-10:45 Rathmann Auditorium
Professor: Dr. Stuart Feinstein

Date	Lecture Topic(s)
1. April 2	Course logistics; Signal Transduction; Experimental Strategies
2. April 4	Early Development; Early Neural Development (descriptive presentation) (Chap. 1)
3. April 9	Early Neural Development - Neural Induction; Polarity and Segmentation (Chap. 2)
4. April 11	Early Neural Development - Polarity and Segmentation; Neurogenesis and Migration (Chap. 3)
5. April 16	Early Neural Development - Neurogenesis and Migration; Determination and Differentiation (Chap. 4)
6. April 18	Early Neural Development - Determination and Differentiation; tie up loose ends……(Chap. 4)
7. April 23	Axon Outgrowth and Guidance (Chap 5)
8. April 25	Midterm Examination 1
9. April 30	Axon Outgrowth and Guidance (Chap. 5)
10. May 2	Neuron-Target Interaction – Recruitment Model to Programmed Cell Death; Nerve Growth Factor (Chap.7)
11. May 7	Neuron-Target Interaction – Nerve Growth Factor (Chap. 7)
12. May 9	Neuron-Target Interaction – Nerve Growth Factor; Molecular Basis of Programmed Cell Death (Chap. 7)
13. May 14	Target Selection; Synapse Formation and Function (Chap. 6)
14. May 16	Synapse Formation and Function; Synapse Refinement (Chapters 8,9)
15. May 21	Midterm Examination 2
16. May 23	Stem Cells
17. May 28	Neurodegeneration - Alzheimer's Disease and Related Dementias
18. May 30	Neurodegeneration - Alzheimer's Disease and Related Dementias
19. June 5	Special Topic
20. June 7	Special Topic

MCDB 153 *Molecular and Cellular Approaches to Neural Development*
Spring Quarter, 2013

Lectures: Tuesday/Thursday; 9:30 - 10:45; Rathmann Auditorium, LSB

Teaching Assistants: Juliana Erickson
Sarah Benbow

Office Hours: Stu Feinstein *TBA* 5123 BioSci2 feinstei@lifesci.ucsb.edu
Sarah Benbow *TBA* 5119 BioSci2 benbow@lifesci.ucsb.edu
Julianna Erickson *TBA* 5119 BioSci2 erickson@lifesci.ucsb.edu

Website: Gauchospace

Discussion Sections:

- All discussion sections are on Fridays in 1228 BSIF. All sections are "full". Unless you have spoken/written with me ahead of time, you must attend the first meeting of your assigned section in order to reserve your place in the class! If you wish to try to change to a different section, see Julianna and Sarah after the first lecture. We will try to accommodate people to the extent possible, but we cannot make any promises.

Textbook: *Development of the Nervous System*, by Sanes, Reh and Harris (**3rd Edition**)

Supplemental resources:

"*Developmental Biology*" by Scott Gilbert (Don't buy the 9th edition; 10th edition is coming out soon)

"*Molecular Biology of the Cell*" by Bruce Alberts et al., (5th Edition)

We will also make extensive use of the primary literature and review articles that we will place on the course website.

MCDB 153 Honors: Monday 12-1 4164 BioSci2
Organizational meeting: Wednesday, April 3; 12-1; 4164 BioSci2

Grading:

- •Discussion section will represent 10% of the course grade, which will be based upon the quality of your weekly summaries and your general participation in the section.
- •Midterm Examinations: There will be two midterm examinations, each accounting for 25% of your grade. The midterms will also generally include one question based upon the papers presented in Discussion Section. (This is in addition to the 10% of the course grade for the discussion section.)
- •Final Examination: The final exam will be cumulative and will account for 40% of the total grade.
- •"Make-up" exams or alternative exam times are not possible except in extremely dire situations. See Dr. Feinstein immediately if such a situation is anticipated or arises.

Discussion Section Schedule

April 5	Experimental Techniques
April 12	Research Paper 1
April 19	Midterm 1 Review
April 26	Research Paper 2
May 3	Research Paper 3
May 10	Research Paper 4
May 17	Midterm 2 Review
May 24	Research Paper 5
May 31	Research Paper 6
June 7	Final Exam Review

Two important perspectives

It is a fundamental tenet of modern biological research that all phenomena can be understood at the molecular and cellular levels

"It is a truism of modern biomedical science that the development of therapies expected to slow or arrest the progression of a disease requires as detailed an understanding of its molecular and cellular pathogenesis as possible."

Dennis Selkoe
Harvard University

Things To Think About…….

1. *What is/are the question(s) being asked,or what is the hypothesis being tested?*
2. *What are some possible and plausible answers?*
3. *What experiments could be done to assess the questions or hypothesis?*
4. *What are the outcomes of those experiments? How reliable are the experiments and their outcomes?*
5. *What are the implications of the answers? Are they consistent with, or inconsistent with, the hypothesis?*
6. *Given all that, what is the next question or hypothesis to be tested?*

Three Review Topics

1. A brief overview of the nervous system, because that is what we are building;
2. A brief overview of signal transduction, because signalling is what drives much of development;
3. A brief overview of experimental strategies commonly used in neural development reserach

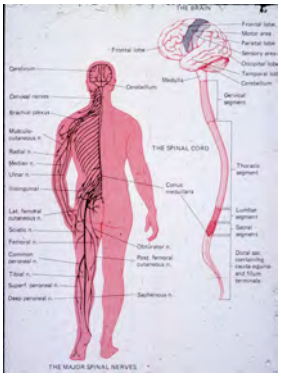
The Nervous System:

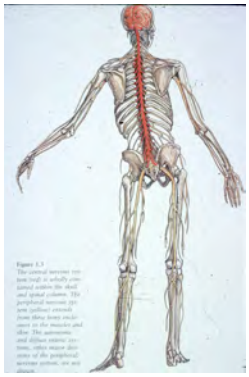
•DETECTS changes in the external and internal environment (light, touch, sound, pain, taste, muscle stretch, etc);

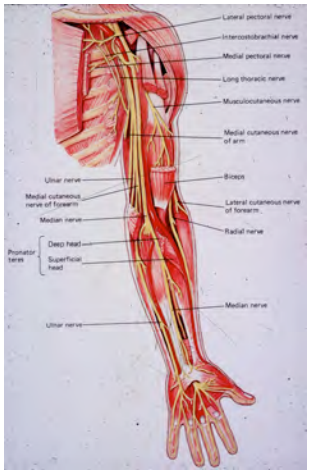
•TRANSMITS this information to other cells for processing and storage;

•INSTRUCTS other cells how to respond to the changes originally detected

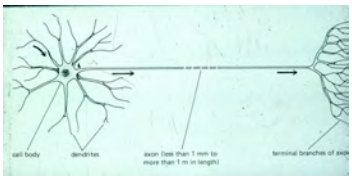
What are we building?
An adult nervous system with macroscopic "nerves".

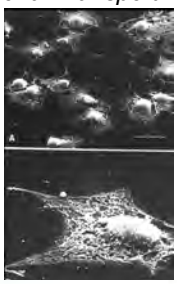


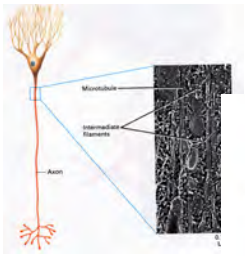


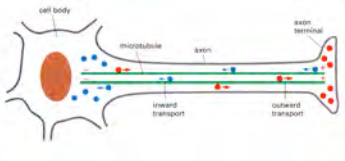


Cells of the Nervous System: "Neurons"
Morphology, Cytoskeleton and Axonal Transport

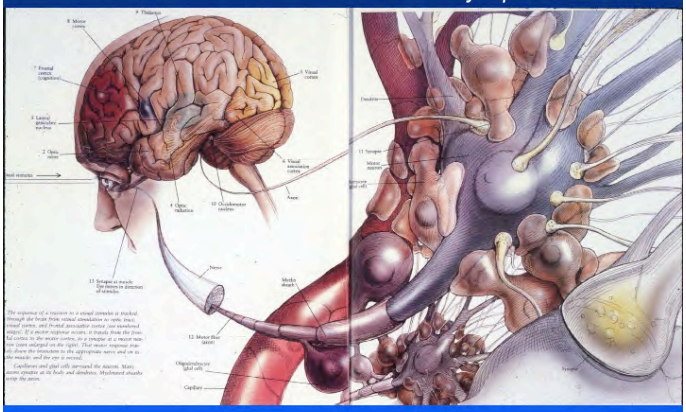








Intercellular Communication Via Synapses



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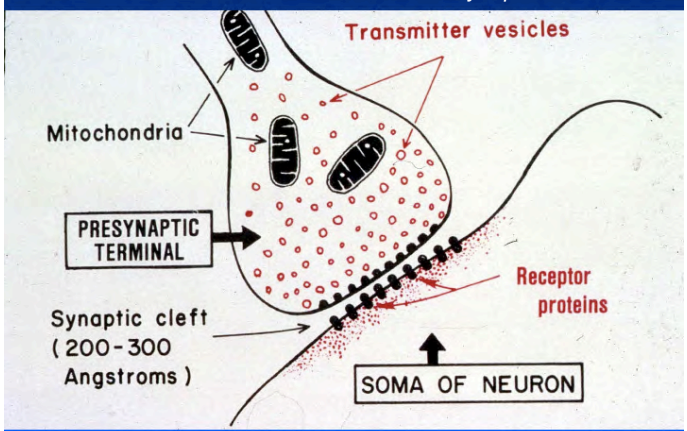

Signal Transduction is Key to all of Development

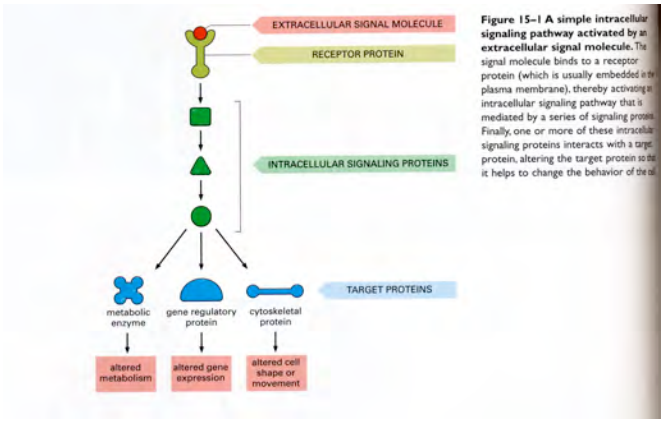

Figure 15–1 A simple intracellular signaling pathway activated by an extracellular signal molecule. The signal molecule binds to a receptor protein (which is usually embedded in the plasma membrane), thereby activating an intracellular signaling pathway that is mediated by a series of signaling proteins. Finally, one or more of these intracellular signaling proteins interacts with a target protein, altering the target protein so that it helps to change the behavior of the cell.

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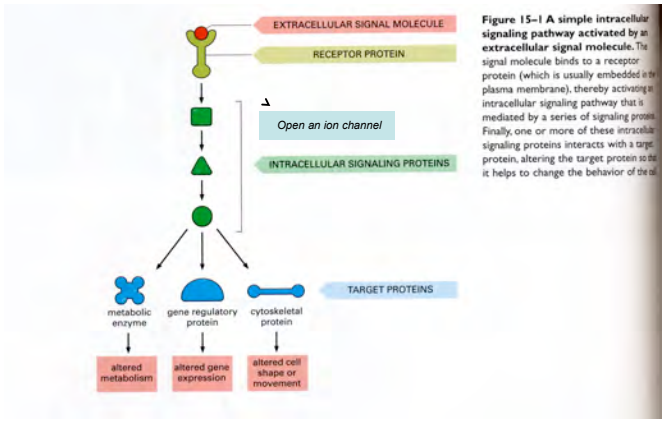

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Four General Types of Intercellular Signalling

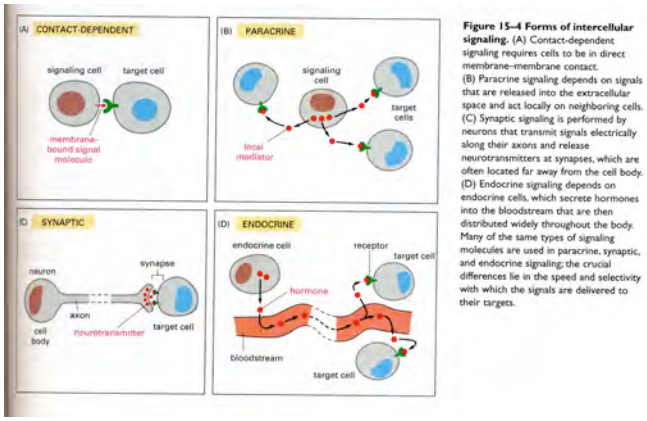

Figure 15–4 Forms of intercellular signaling. (A) Contact-dependent signaling requires cells to be in direct membrane–membrane contact. (B) Paracrine signaling depends on signals that are released into the extracellular space and act locally on neighboring cells. (C) Synaptic signaling is performed by neurons that transmit signals electrically along their axons and release neurotransmitters at synapses, which are often located far away from the cell body. (D) Endocrine signaling depends on endocrine cells, which secrete hormones into the bloodstream that are then distributed widely throughout the body. Many of the same types of signaling molecules are used in paracrine, synaptic, and endocrine signaling; the crucial differences lie in the speed and selectivity with which the signals are delivered to their targets.

Focusing on Receptors : Three Classes of Ligand-Activated Cell Surface Receptors

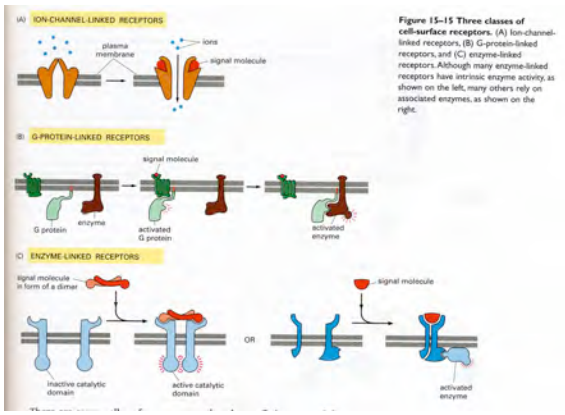

Figure 15–15 Three classes of cell-surface receptors. (A) Ion-channel-linked receptors, (B) G-protein-linked receptors, and (C) enzyme-linked receptors. Although many enzyme-linked receptors have intrinsic enzyme activity, as shown on the left, many others rely on associated enzymes, as shown on the right.

Combinatorial Signalling

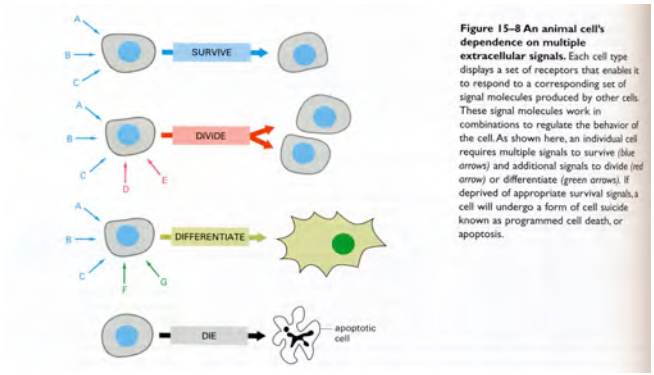

Figure 15–8 An animal cell's dependence on multiple extracellular signals. Each cell type displays a set of receptors that enables it to respond to a corresponding set of signal molecules produced by other cells. These signal molecules work in combinations to regulate the behavior of the cell. As shown here, an individual cell requires multiple signals to survive (*blue arrows*) and additional signals to divide (*red arrow*) or differentiate (*green arrows*). If deprived of appropriate survival signals, a cell will undergo a form of cell suicide known as programmed cell death, or apoptosis.

Experimental Strategies:
How do we study the development of the nervous system?

- Whole Animals
  most commonly rodents (rat, mouse), chick, flies, worms, others
- Cultured Tissues from animals
  ("explants")
- Cultured Cells from animals
  "transformed" cells
  "primary" cells

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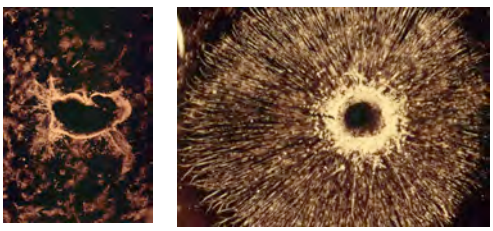
- Cultured Tissues from animals
  ("explants")

Dorsal Root Ganglia Explants in Culture
Question: Is NGF a neuronal survival and/or differentiation factor?

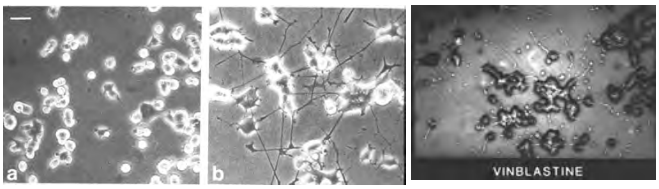


-NGF +NGF

How do we study the development of the nervous system?

•Cultured Cells from animals
"primary" cells
"transformed" cells (PC12 cells below)

Question: Is NGF a neuronal differentiation factor?
Question: Are microtubules important for axonal structure?



How about trying to understand the molecular basis of developmental phenomena?

•Localizing Proteins of Interest

Immunofluorescence Microscopy on tissues or cultured cells
Visualize a protein of interest with "GFP" (or one of its relatives)

•Determining the Functions of Proteins of Interest

Manipulation	Animal	Cultured Cells
Express a gene of interest	"Transgenic" Animal	"Transfection"
Express a protein of interest		"Microinjection"
Delete a protein of interest	"Knockout"	"Knockout"
"Delete" a protein of interest	"Dom. Negative"	"Dom. Negative"
"Delete" a protein of interest	"RNAi"	"RNAi"
"Delete" a protein of interest	Function Blocking Antibody"	

Immunofluorescence Microscopy: Using Antibodies to Identify Specific Proteins in "Fixed" Cells

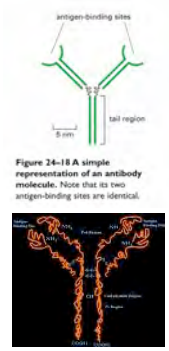
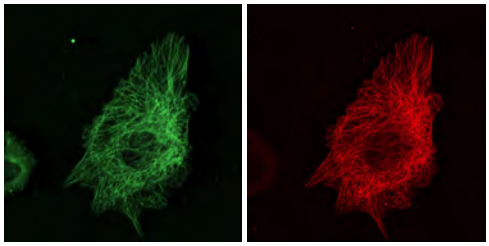


Figure 24–18 A simple representation of an antibody molecule. Note that its two antigen-binding sites are identical.



anti-tubulin (MTs) anti-tau

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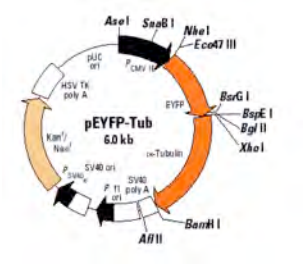
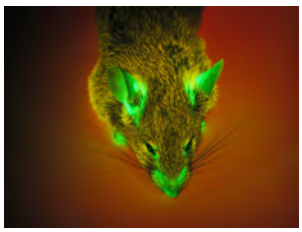
Visualizing a protein of interest in living cells
(NOT the gene, only the protein!)

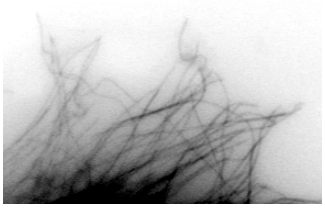
A Common Strategy - Live cell imaging via GFP-fusion proteins (GFP = green fluorescent protein); images viewed via fluorescence microscopy as images or videos

Step 1 - Introduce a plasmid encoding fused GFP-(protein of interest) into animal or cells of interest



Imaging proteins of interest in real time





GFP-skin protein GFP-tubulin

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Transgenic Animals (Adding a gene; GOF)

Question: What is the effect of newly discovered factor X?

Over-expressing Factor X

Control, WT

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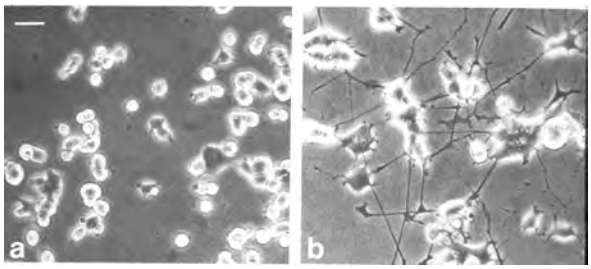
Transfection: Adding a gene (GOF)

Does protein X promote neurite outgrowth?
Transfect in the gene encoding X, and observe a new property (in this case, neurite outgrowth).



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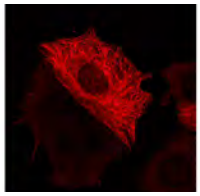
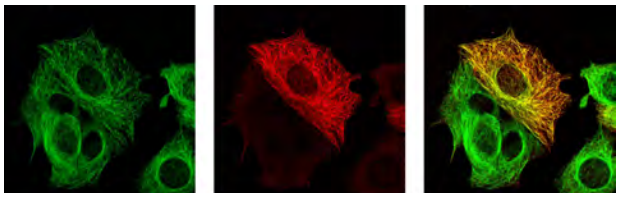
Microinjection of a protein of interest

Tau



Mammalian Cells in Culture: Transfection of GFP-tubulin and Microinjection of Tau (look for activities, GOF, other things)

Tubulin | Tau | Merge



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Delete a Protein of Interest: Knockouts(LOF)

Question: What is the effect of a newly discovered factor?

WT | BMP7-/- | WT | BMP7-/-

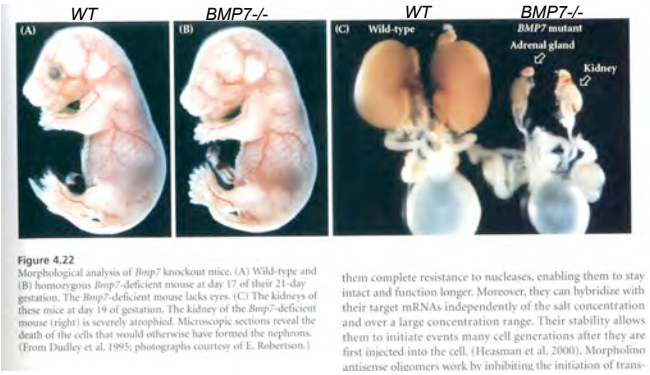

Figure 4.22
Morphological analysis of *Bmp7* knockout mice. (A) Wild-type and (B) homozygous *Bmp7*-deficient mouse at day 17 of their 21-day gestation. The *Bmp7*-deficient mouse lacks eyes. (C) The kidneys of these mice at day 19 of gestation. The kidney of the *Bmp7*-deficient mouse (right) is severely atrophied. Microscopic sections reveal the death of the cells that would otherwise have formed the nephrons. (From Dudley et al. 1995; photographs courtesy of E. Robertson.)

them complete resistance to nucleases, enabling them to stay intact and function longer. Moreover, they can hybridize with their target mRNAs independently of the salt concentration and over a large concentration range. Their stability allows them to initiate events many cell generations after they are first injected into the cell. (Heasman et al. 2000). Morpholino antisense oligomers work by inhibiting the initiation of trans-

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Delete a protein of interest	"Knockout"	"Knockout"
"Delete" a protein of interest	"Dom. Negative"	"Dom. Negative"
"Delete" a protein of interest	"RNAi"	"RNAi"
"Delete" a protein of interest	Function Blocking Antibody"	

"Delete" a Protein of Interest: Dominant Negatives (LOF)

Example 1: "poisoning" of a multimeric complex

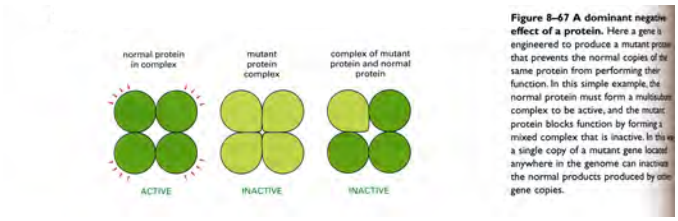
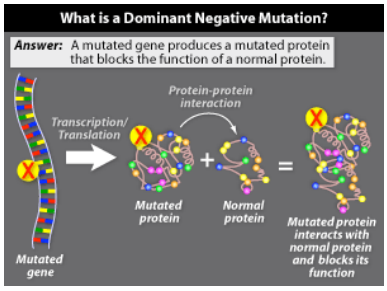


Figure 8–67 A dominant negative effect of a protein. Here a gene is engineered to produce a mutant protein that prevents the normal copies of the same protein from performing their function. In this simple example, the normal protein must form a multisubunit complex to be active, and the mutant protein blocks function by forming a mixed complex that is inactive. In this way a single copy of a mutant gene located anywhere in the genome can inactivate the normal products produced by other gene copies.

"Delete" a Protein of Interest: Dominant Negatives (LOF)

Example 2: Competing for an essential site



How about trying to understand the molecular basis of developmental phenomena?

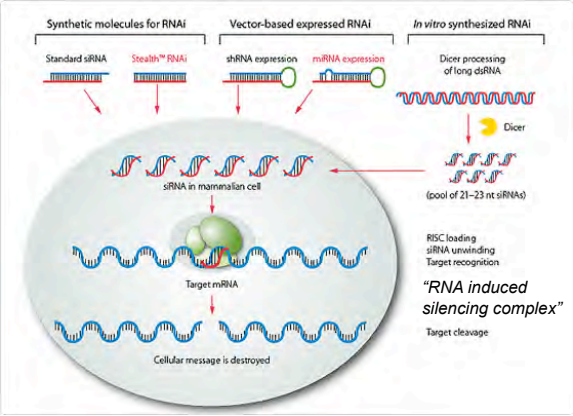
•Localizing Proteins of Interest

Immunofluorescence Microscopy on tissues or cultured cells
Visualize a protein of interest with "GFP" (or one of its relatives)

•Determining the Functions of Proteins of Interest

Manipulation	Animal	Cultured Cells
Express a gene of interest	"Transgenic" Animal	"Transfection"
Express a protein of interest		"Microinjection"
Delete a protein of interest	"Knockout"	"Knockout"
"Delete" a protein of interest	"Dom. Negative"	"Dom. Negative"
"Delete" a protein of interest	"RNAi"	"RNAi"
"Delete" a protein of interest	Function Blocking Antibody"	

Delete a Protein of Interest: RNAi (LOF)



Delete a Protein of Interest: RNAi (LOF)

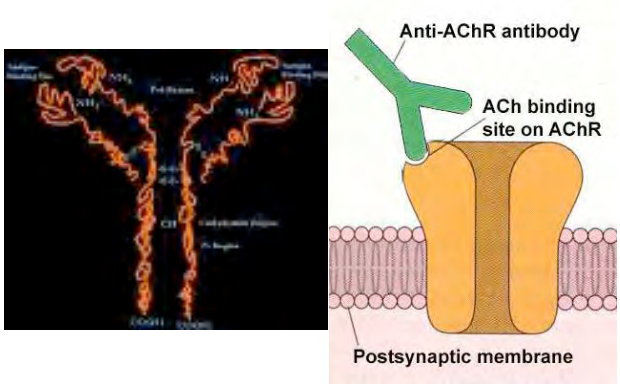
How about trying to understand the molecular basis of developmental phenomena?

•Immunofluorescence Microscopy on tissues or cultured cells

•Molecular Genetics: Manipulating the genome to ask questions

Manipulation	Animal	Cultured Cells
Express a gene of interest	"Transgenic" Animal	"Transfection"
Express a protein of interest		"Microinjection"
Visualize a protein of interest	"GFP"	"GFP"
Delete a protein of interest	"knockout"	"knockout"
"Delete" a protein of interest	"Dom. Negative"	"Dom. Negative"
"Delete" a protein of interest	"RNAi"	"RNAi"
"Delete" a protein of interest	Function Blocking Antibody"	

Function Blocking Antibody Against ACh Receptor



How about biochemical investigations? Radioactivity

Allows you to ask questions about a particular molecule of interest;

- Basic premise is that cells can't tell the difference between a radioactive version of a particular molecule compared to a non-radioactive version of the same molecule, but we can easily "detect" and "follow" the radioactive ones.

More Biochemistry: Molecular Analysis of Proteins: Fractionation of Proteins by Electrophoresis

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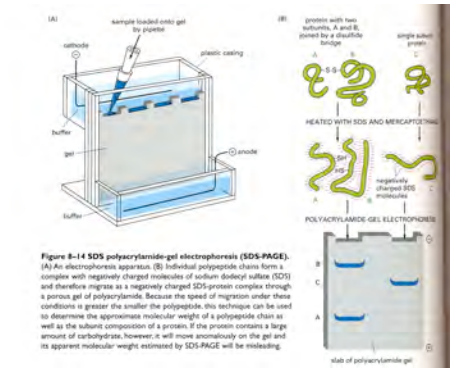
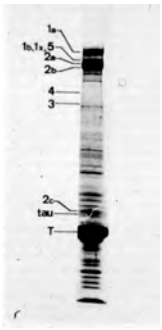


Figure 8–14 SDS polyacrylamide-gel electrophoresis (SDS-PAGE). (A) An electrophoresis apparatus. (B) Individual polypeptide chains form a complex with negatively charged molecules of sodium dodecyl sulfate (SDS) and therefore migrate as a negatively charged SDS-protein complex through a porous gel of polyacrylamide. Because the speed of migration under these conditions is greater the smaller the polypeptide, this technique can be used to determine the approximate molecular weight of a polypeptide chain as well as the subunit composition of a protein. If the protein contains a large amount of carbohydrate, however, it will move anomalously on the gel and its apparent molecular weight estimated by SDS-PAGE will be misleading.



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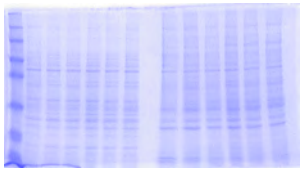
Immunoblotting: Using Antibodies to Identify Specific Proteins on Gels



Coomassie Blue: Visualizes All Proteins

Anti-Tubulin: Visualizes only tubulin

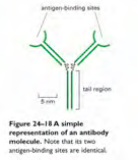


Figure 24–18 A simple representation of an antibody molecule. Note that its two antigen-binding sites are identical.

Antibody directed against tubulin
"Anti-tubulin"