Signaling Pathways

Properties and approaches to intracellular signaling

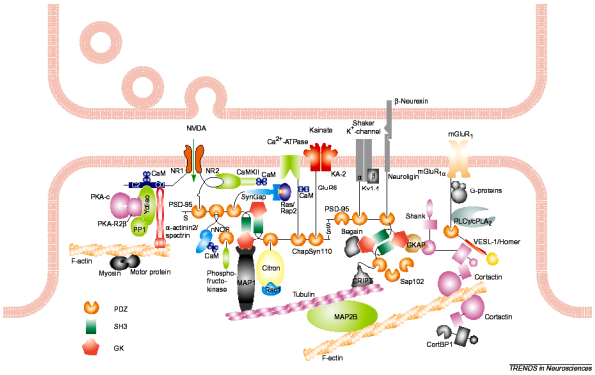
- Properties of signaling enzymes & molecules
- Structure of G proteins, kinases
- Example of analysis using long-term potentiation and MAP kinase
- Example of analysis using Leptin receptor and Jak-Stat signaling

Approaches to molecular signaling

Example of CREB and c-Fos as molecular signals

1

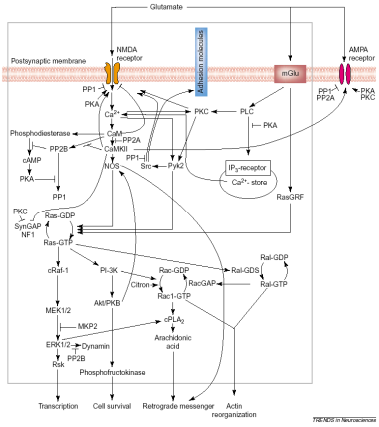
Complexity of the postsynaptic density



Interactome: the defined interactions between all proteins in the genome/proteome

Grant 2001

2



Grant 2001

Table 1 Summary of PSD protein classifications

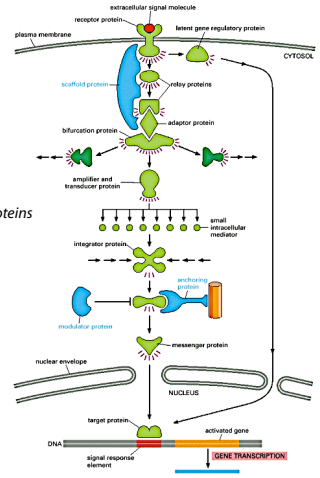
	Total PSD
Channels and receptors	80
MAGUKs/adaptors/scaffolders	54
Serine/threonine kinases	46
Tyrosine kinase	3
Protein phosphatases	18
G proteins and modulators	77
Signalling molecules and enzymes	278
Transcription and translation	119
Cytoskeletal and cell adhesion molecules	153
Synaptic vesicles and protein transport	159
Novel	107
Other	30
Summary	1124

Collins 2006

3

4

1. *Relay proteins*
2. *Messenger proteins*
3. *Adaptor proteins*
4. *Amplifier proteins*
5. *Transducer proteins*
6. *Bifurcation proteins*
7. *Integrator proteins*
8. *Latent gene regulatory proteins*



General Principles of Cell Communication

http://www.ncbi.nlm.nih.gov/books/NBK26813/

5

1. *Relay proteins* simply pass the message to the next signaling component in the chain.

2. *Messenger proteins* carry the signal from one part of the cell to another, such as from the cytosol to the nucleus.

3. *Adaptor proteins* link one signaling protein to another, without themselves conveying a signal.

4. *Amplifier proteins,* which are usually either enzymes or ion channels, greatly increase the signal they receive, either by producing large amounts of small intracellular mediators or by activating large numbers of downstream intracellular signaling proteins. When there are multiple amplification steps in a relay chain, the chain is often referred to as a **signaling cascade**.

5. *Transducer proteins* convert the signal into a different form. The enzyme that makes cyclic AMP is an example: it both converts the signal and amplifies it, thus acting as both a transducer and an amplifier.

6. *Bifurcation proteins* spread the signal from one signaling pathway to another.

7. *Integrator proteins* receive signals from two or more signaling pathways and integrate them before relaying a signal onward.

8. *Latent gene regulatory proteins* are activated at the cell surface by activated receptors and then migrate to the nucleus to stimulate gene transcription.

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Common Intracellular Signaling Pathways

cAMP pathway

AC → **cAMP** → **PKA** → **pCREB** → **Gene Expression (~5,000 genes)**
PDE ← **PP1**

AC = adenyl cyclase
PDE = phosphodiesterase
PKA = protein kinase A
pCREB = phospho cAMP Response Element Binding protein
PP1 = protein phosphatase 1

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Common Intracellular Signaling Pathways

cAMP pathway

AC → cAMP → PKA → pCREB → Gene Expression
PDE ← PP1

Ca++/Calmodulin

PLC/DAG/IP3 pathway

Receptor Tyrosine Kinases
e.g. Neurotrophins, JAK, STAT

MAPK (mitogen activating protein) pathway

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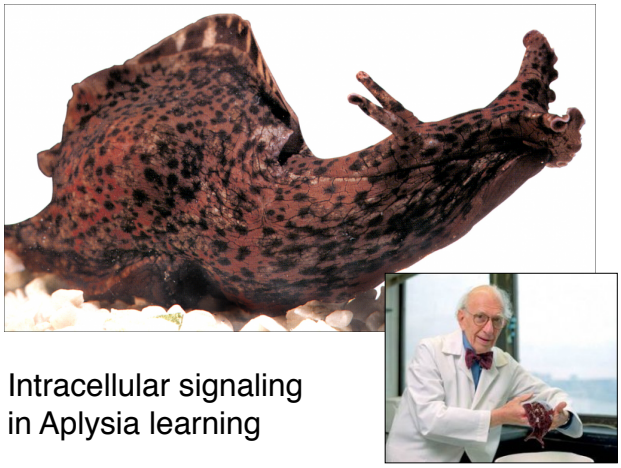

Intracellular signaling
in Aplysia learning

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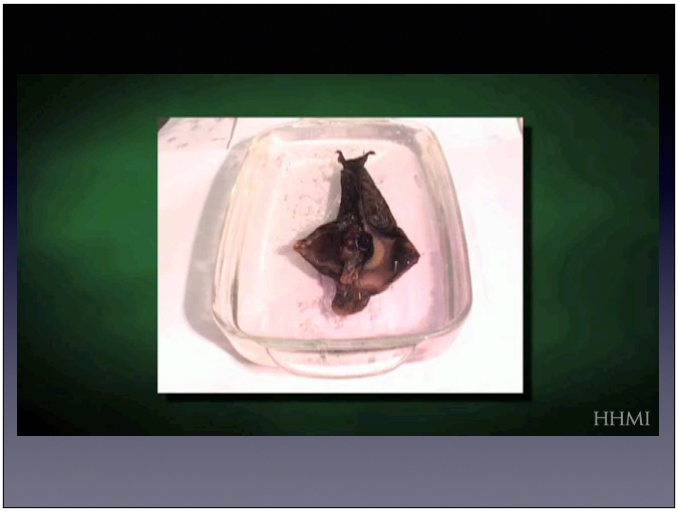

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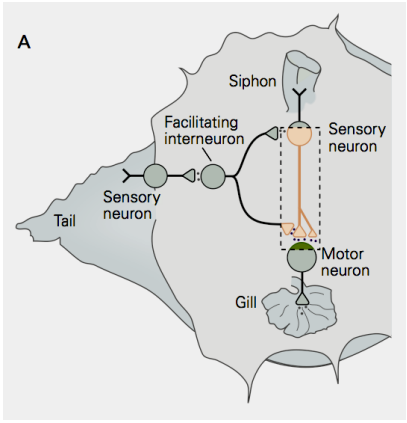
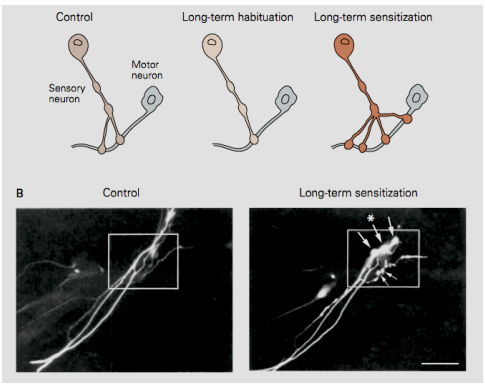
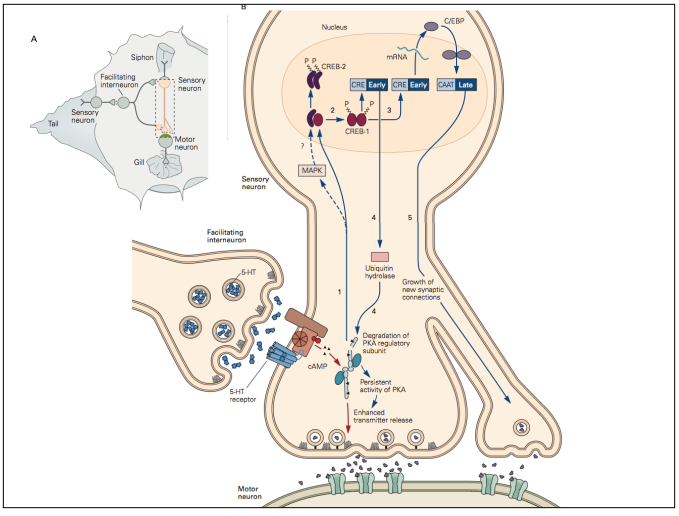

A
Siphon
Facilitating interneuron
Sensory neuron
Sensory neuron
Tail
Motor neuron
Gill

11


Control
Long-term habituation
Long-term sensitization
Sensory neuron
Motor neuron
B
Control
Long-term sensitization

12


A
Siphon
Facilitating interneuron
Sensory neuron
Sensory neuron
Tail
Motor neuron
Gill
B
Nucleus
C/EBP
mRNA
CREB-2
CRE
Early
CRE
Early
CAAT
Late
CREB-1
MAPK
Sensory neuron
Facilitating interneuron
5-HT
Ubiquitin hydrolase
Growth of new synaptic connections
Degradation of PKA regulatory subunit
cAMP
Persistent activity of PKA
5-HT receptor
Enhanced transmitter release
Motor neuron

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Principles of Intracellular Signaling

Mediated by factors and enzymes (not ions)

Diffusion of factors and enzymes takes milliseconds to seconds

Enzymes make covalent modifications

e.g. phosphorylation

Enzymatic reactions take milliseconds

Enzymatic reactions are substrate-specific

Reaction products can persist from milliseconds to weeks

Factors, Enzymes, and their subtrastes and compartmentalized

Members of a signaling pathways are often regulated by each other

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Components of Signaling Pathway are Compartmentalized within the Neuron

membrane bound/associated

localized to PSD or dendrite

sequestered in membrane compartment
e.g. Ca^{++} in sarcoplasmic reticulum

localized to nucleus

translocation to other compartments
e.g. dendrite to nucleus (PKA, MAPK)
e.g. axon terminal to nucleus (Trks)

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Intracellular Signaling mediated by Messengers & Enzymes

Messengers:
- a generated/released small factor
- a phosphorylated protein

Enzymes:
- generate/release small factors
- phosphorylate proteins (kinases)
- dephosphorylate proteins (phosphatases)
- modify cellular targets (usually by phosphorylation)

Examples of Signaling Messengers

Generated/released small factors, e.g.

DAG
membrane-bound factor derived from phospholipids.
can activate membrane-associated enzymes

cAMP
cytoplasmic factor derived from ATP by adenylate cyclase.
can activate cytoplasmic enzymes such as protein kinase A (PKA)

NO
a gaseous factor generated from amino acids by NO synthase.
can diffuse to surrounding cells and that can activate enzymes,
esp. activate guanylate cyclase -> cGMP

Endocannabinoids
derived from membrane phospholipids
bind retrogradely to presynaptic cannabinoid receptors

Phosphorylated proteins, e.g.

Inhibitor I
blocks protein phosphatase I

Signaling Enzymes can:

Generate Small Factors
Adenylate Cyclase
generates a small factor, cAMP

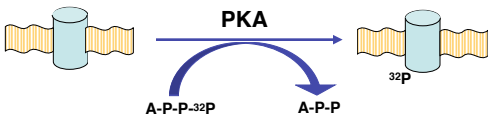
Phosphorylate
Protein Kinase A
when activated by cAMP, adds PO_4^- to serine/threonine residues of proteins

Dephosphorylate
Protein Phosphatase 1
removes the PO_4^- from ser/thr residues

Modify Cellular Targets
Substrates of PKA and PP1 include
NMDA glutamate channel (regulates excitability)
CREB transcription factor (regulates gene expression)

Detection of second messengers & signaling pathways

1. **Chemical assay**
   e.g. HPLC to detect small amounts of a simple compound
2. **Visualization**
   e.g. Ca^{++} sensitive dyes that fluoresce in high Ca^{++}
   e.g antibodies, such as anti-cAMP
3. **Incorporation of radio-tracer**
   e.g. GTP with ^{32}P for G-Proteins
   ATP with ^{32}P for kinases



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Detection of second messengers & signaling pathways

Immunohistochemistry for protein or phosphorylated protein

Western blot of protein

e.g. with ^{32}P labeling of substrate
size shift from protein (smaller) to phosphoprotein (bigger)

Western blot for phosphorylated protein

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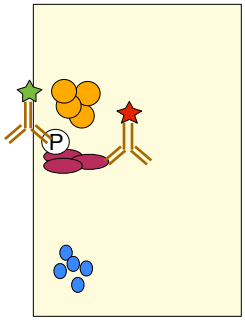
Western Blot

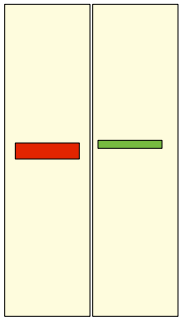
5. Blot proteins onto nylon membrane



6. Visualize proteins using labeled antibodies

7. Visualize phosphoylated proteins using phospho-specific antibodies

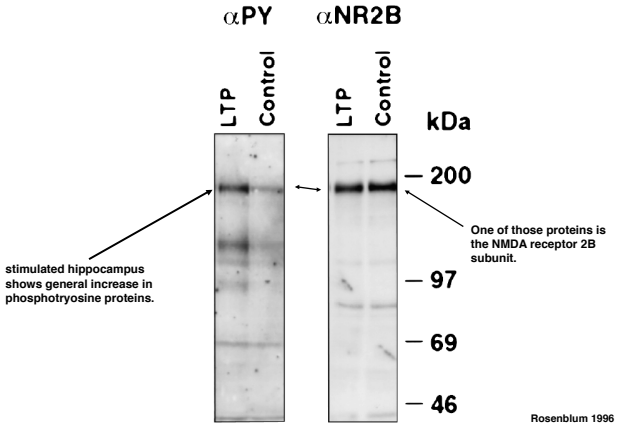
Developed Blot



protein phospho protein

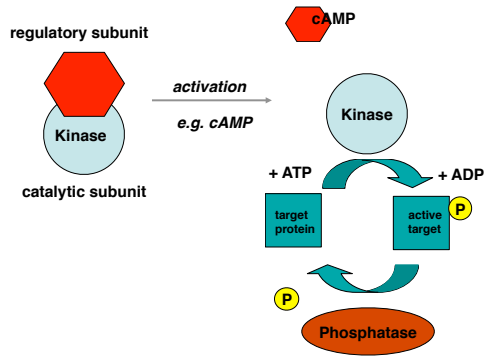
21

αPY = antibody against phosphotyrosine residue



Kinases and Phosphatases

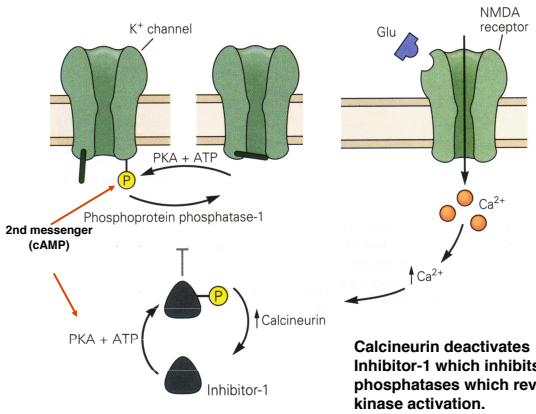
22



Squire 10.10

Don't forget to regulate the phosphatases...

23



Calcineurin deactivates Inhibitor-1 which inhibits phosphatases which reverse kinase activation.

24

Implications of Signaling Properties

Slower
time course of seconds to hours

Persistant
subtrates remain modified for minutes to weeks

Spatial Resolution
can localize to specific compartments

Visualization
easier to detect activity and modifications of specific large molecules and proteins

Protein Domains:

e.g., G-Protein coupled receptors:

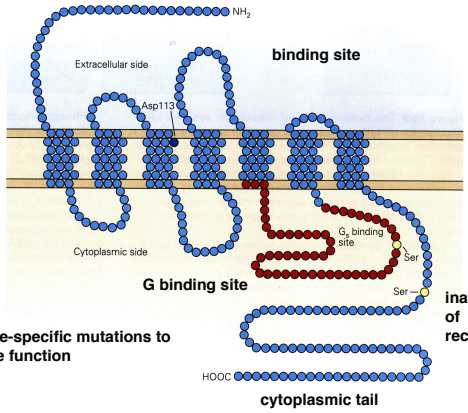
- **7-transmembrane domains**
- **ligand-binding domains**
- **G-protein binding domains**

Kinases

Phosphatases

25

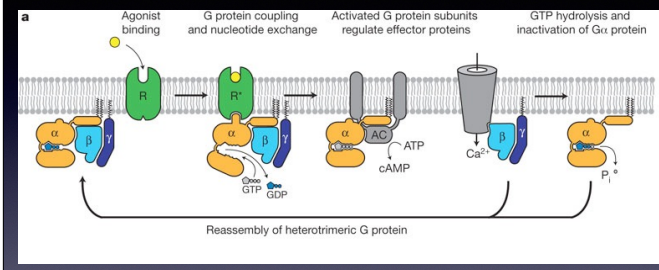
G-protein coupled receptors



Use site-specific mutations to analyze function

inactivation of receptor

26



Nature **477** (2011) 549–555

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Useful toxins to block Signaling Pathways

G-Protein Toxins:

1. Cholera Toxin

allows binding of GTP, but prevents hydrolysis.

Causes overproduction of cAMP, leading to loss of electrolytes and water from intestinal cells.

2. Pertussis Toxin

blocks release of GDP from alpha subunit, so G-protein locked in the inactive state.

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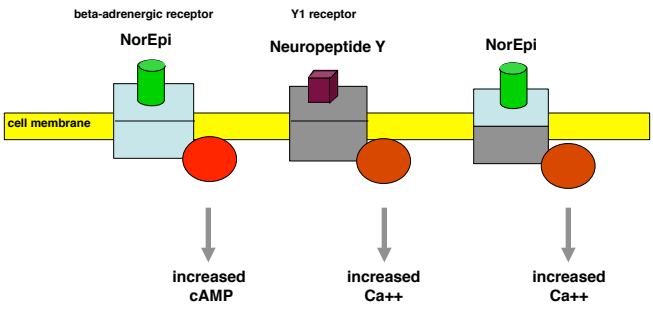
Chimeric receptors



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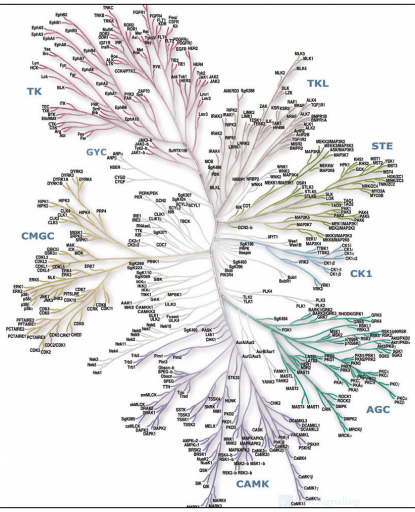
Complexity of the kinase families

Human Protein Kinases



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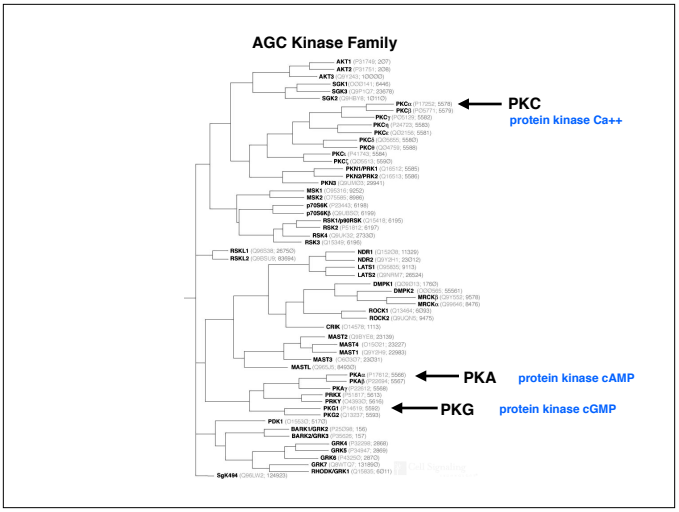

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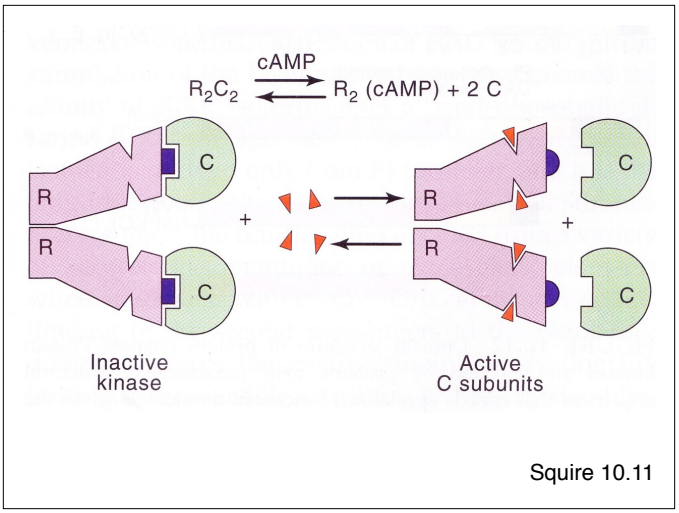

Squire 10.11

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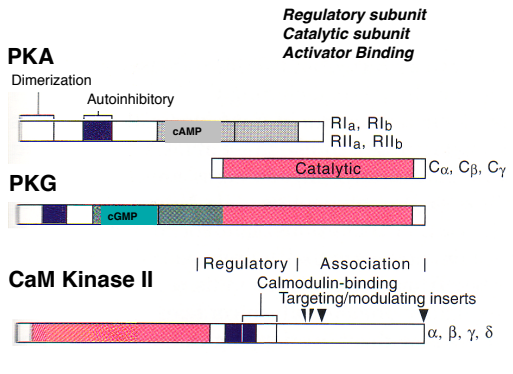
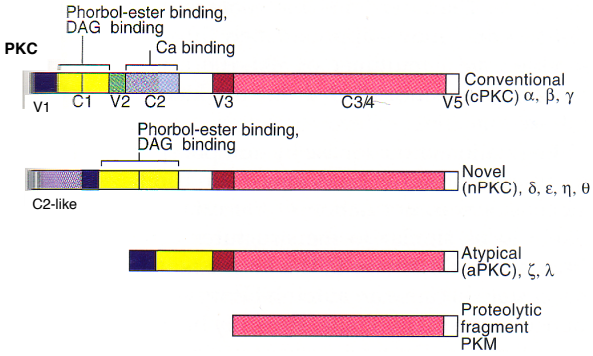
Kinase proteins have functional domains



Squire 10.12

Kinases have domains that can be mixed and matched



Alternative splicing/processing of PKC enzyme

Squire 10.12

34

Kinases phosphorylate specific sites in targets

TABLE 10.4 Consensus Phosphorylation Sites of Some Protein Kinases

Protein kinase	Consensus phosphorylation site
PKA	R-R/K-X-S*/T*
PKG	$R/K_{2\text{-}3}$-X-S*/T*
cPKC	$(R/K_{1\text{-}3}\text{-}X_{2\text{-}0})$-S*/T*-$(X_{2\text{-}0}\text{-}R/K_{1\text{-}3})$
CaM kinase II	R-X-X-S*/T*
MLCK (smooth muscle)	$(K/R_2\text{-}X)\text{-}X_{1\text{-}2}\text{-}K/R_3\text{-}X_{2\text{-}3}\text{-}R\text{-}X_2$-S*-N-V-F

Source. Adapted from Kennelly and Krebs.[54]
R, Arg; K, Lys; S*, phospho-Ser; T*, phospho-Thr; X, polar amino acid; N, Asn; V, Val; F, Phe.

1) can use sequence to predict phosphorylation site
2) can raise antibody against phosphorylated consensus site

35

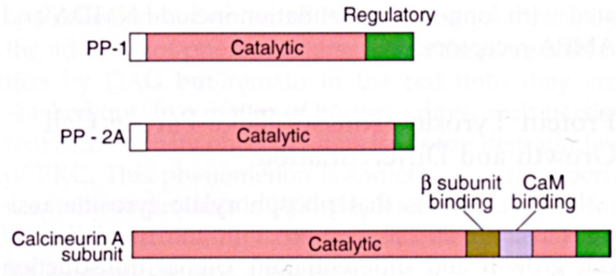


FIGURE 10.14 Domain structure of the catalytic subunits of some Ser/Thr phosphatases. The three major phosphoprotein phosphatases, PP-1, PP-2A, and calcineurin, have homologous catalytic domains but differ in their regulatory properties.

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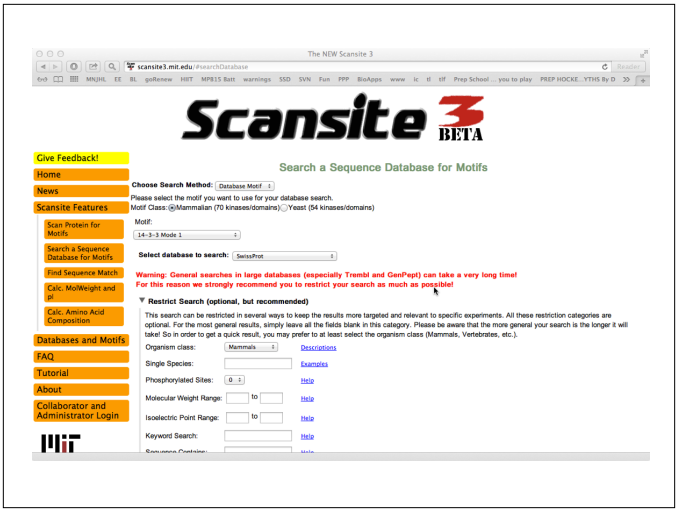
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Please select the motif you want to use for your database search.
Motif Class: Mammalian (70 kinases/domains) Yeast (54 kinases/domains)
Motif:
14-3-3 Mode 1
Select database to search: SwissProt
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For this reason we strongly recommend you to restrict your search as much as possible!
Restrict Search (optional, but recommended)
This search can be restricted in several ways to keep the results more targeted and relevant to specific experiments. All these restriction categories are optional. For the most general results, simply leave all the fields blank in this category. Please be aware that the more general your search is the longer it will take! So in order to get a quick result, you may prefer to at least select the organism class (Mammals, Vertebrates, etc.).
Organism class: Mammals Descriptions
Single Species: Examples
Phosphorylated Sites: 0 Help
Molecular Weight Range: to Help
Isoelectric Point Range: to Help
Keyword Search: Help

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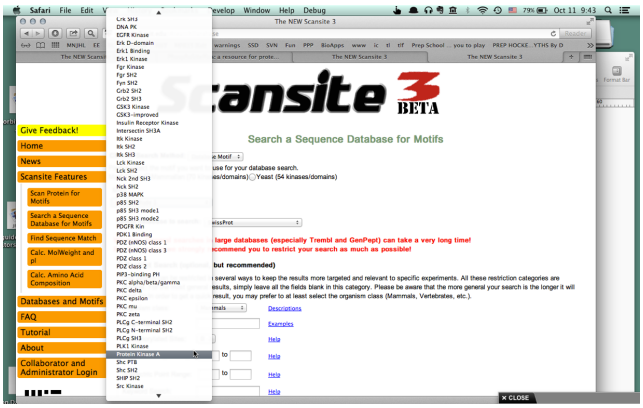
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DNA PK
EGFR Kinase
Erk D-domain
Erk1 Binding
Erk1 Kinase
Fgr Kinase
Fgr SH2
Fyn SH2
Grb2 SH2
Grb2 SH3
GSK3 Kinase
GSK3--improved
Insulin Receptor Kinase
Intersectin SH3A
Itk Kinase
Itk SH2
Itk SH3
Lck Kinase
Lck SH2
Nck 2nd SH3
Nck SH2
p38 MAPK
p85 SH2
p85 SH3 mode1
p85 SH3 mode2
PDGFR Kin
PDK1 Binding
PDZ (nNOS)-class 1
PDZ (nNOS)-class 3
PDZ class 1
PDZ class 2
PIP3-binding PH
PKC alpha/beta/gamma
PKC delta
PKC epsilon
PKC mu
PKC zeta
PLCg C-terminal SH2
PLCg N-terminal SH2
PLCg SH3
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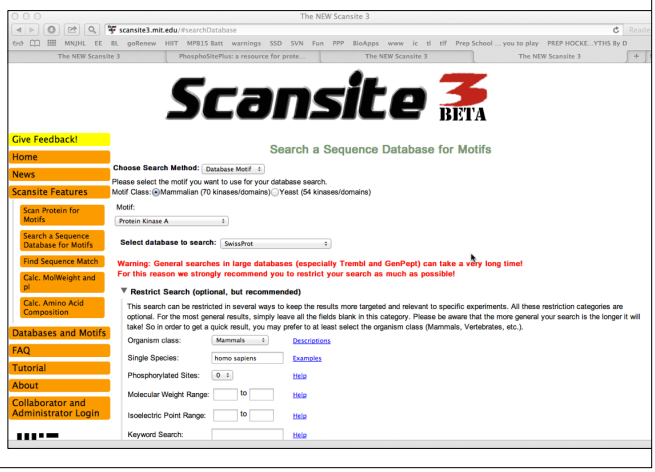
Protein Kinase A
Shc PTB
Shc SH2
SHIP SH2
Src Kinase
CLOSE

39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Organism class: Mammals Descriptions
Single Species: homo sapiens Examples
Phosphorylated Sites: 0 Help
Molecular Weight Range: to Help
Isoelectric Point Range: to Help
Keyword Search: Help

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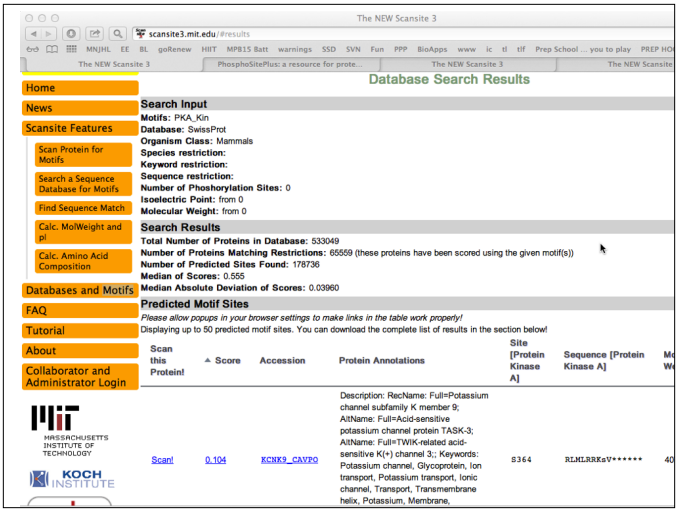
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Database: SwissProt
Organism Class: Mammals
Species restriction:
Keyword restriction:
Sequence restriction:
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Isoelectric Point: from 0
Molecular Weight: from 0
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Number of Proteins Matching Restrictions: 65559 (these proteins have been scored using the given motif(s))
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Median of Scores: 0.555
Median Absolute Deviation of Scores: 0.03960
Predicted Motif Sites
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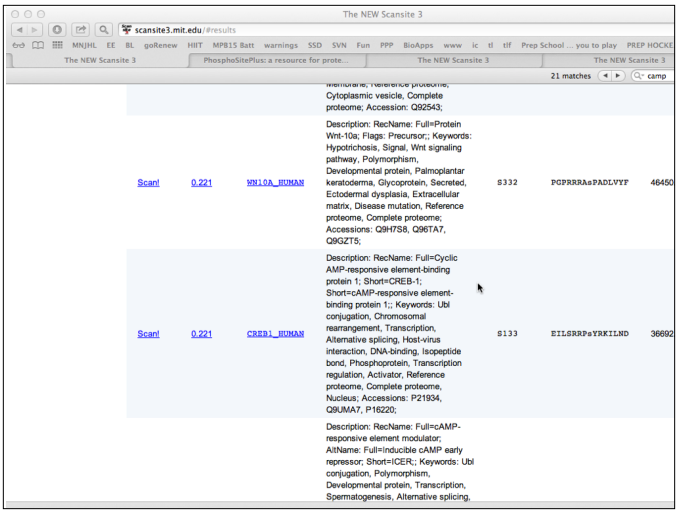
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0.221
WN10A_HUMAN
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S332
PGPRRRAsPADLVYF
Scan!
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S133
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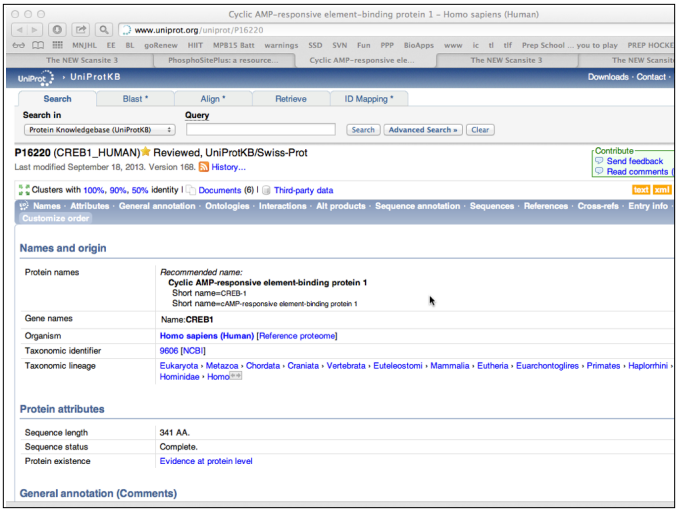
42


Cyclic AMP-responsive element-binding protein 1 - Homo sapiens (Human)
www.uniprot.org/uniprot/P16220
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Blast *
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Names and origin
Protein names
Recommended name:
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Short name=CREB-1
Short name=cAMP-responsive element-binding protein 1
Gene names
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Organism
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Taxonomic identifier
9606 [NCBI]
Taxonomic lineage
Eukaryota · Metazoa · Chordata · Craniata · Vertebrata · Euteleostomi · Mammalia · Eutheria · Euarchontoglires · Primates · Haplorrhini · Hominidae · Homo
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Sequence status
Complete.
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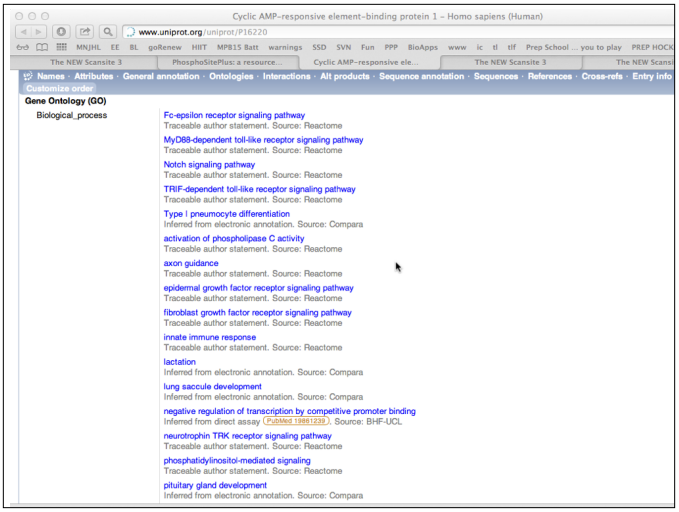
Evidence at protein level
General annotation (Comments)

43


Cyclic AMP-responsive element-binding protein 1 – Homo sapiens (Human)
www.uniprot.org/uniprot/P16220
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Traceable author statement. Source: Reactome
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TRIF-dependent toll-like receptor signaling pathway
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activation of phospholipase C activity
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axon guidance
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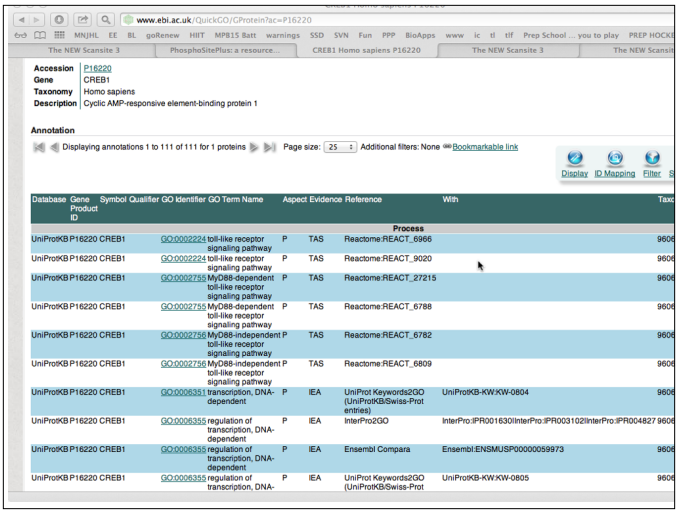
44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Taxonomy Homo sapiens
Description Cyclic AMP-responsive element-binding protein 1
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Displaying annotations 1 to 111 of 111 for 1 proteins
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Additional filters: None
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ID Mapping
Filter
Database | Gene Product ID | Symbol | Qualifier | GO Identifier | GO Term Name | Aspect | Evidence | Reference | With | Taxo
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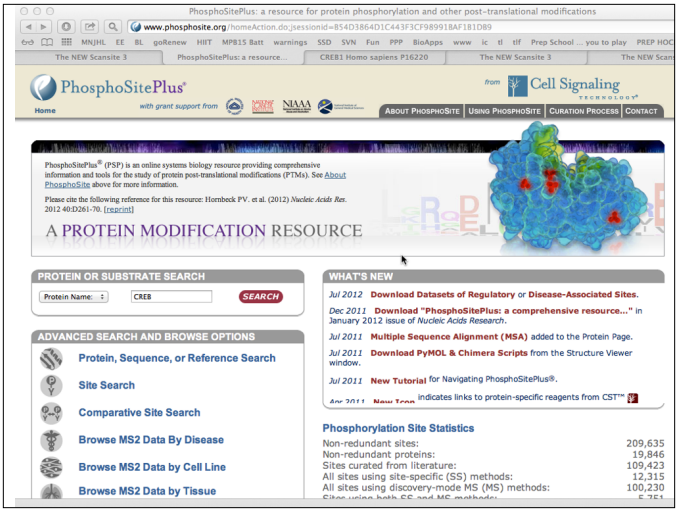
PhosphoSitePlus: a resource for protein phosphorylation and other post-translational modifications
www.phosphosite.org/homeAction.do;jsessionid=B54D3864D1C443F3CF98991BAF1B1DB9
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Protein, Sequence, or Reference Search
Site Search
Comparative Site Search
Browse MS2 Data By Disease
Browse MS2 Data by Cell Line
Browse MS2 Data by Tissue
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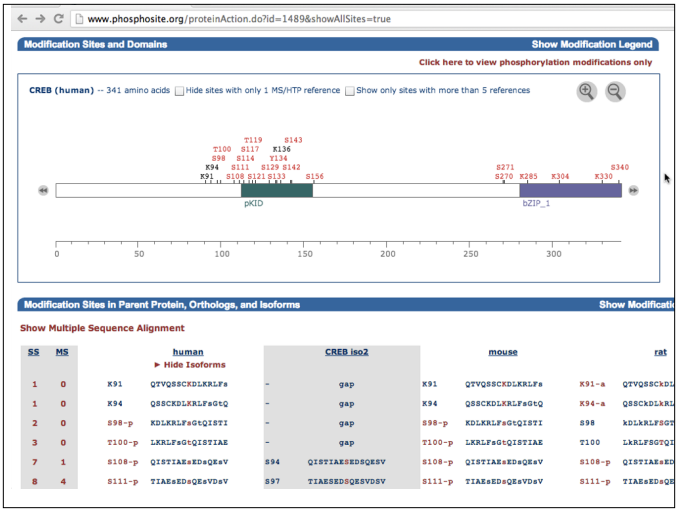
Dec 2011 Download "PhosphoSitePlus: a comprehensive resource..." in January 2012 issue of Nucleic Acids Research.
Jul 2011 Multiple Sequence Alignment (MSA) added to the Protein Page.
Jul 2011 Download PyMOL & Chimera Scripts from the Structure Viewer window.
Jul 2011 New Tutorial for Navigating PhosphoSitePlus®.
indicates links to protein-specific reagents from CST™
Phosphorylation Site Statistics
Non-redundant sites: 209,635
Non-redundant proteins: 19,846
Sites curated from literature: 109,423
All sites using site-specific (SS) methods: 12,315
All sites using discovery-mode MS (MS) methods: 100,230

46



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1	0	K94	QSSCKDLKRLFsGtQ	-	gap	K94	QSSCKDLKRLFsGtQ	K94-a	QSSCkDLkRL
2	0	S98-p	KDLKRLFsGtQISTI	-	gap	S98-p	KDLKRLFsGtQISTI	S98	kDLkRLFSGT
3	0	T100-p	LKRLFsGtQISTIAE	-	gap	T100-p	LKRLFsGtQISTIAE	T100	LkRLFSGTQI
7	1	S108-p	QISTIAEsEDsQEsV	S94	QISTIAESEDSQESV	S108-p	QISTIAEsEDsQEsV	S108-p	QISTIAEsED
8	4	S111-p	TIAEsEDsQEsVDsV	S97	TIAESEDSQESVDSV	S111-p	TIAEsEDsQEsVDsV	S111-p	TIAEsEDsQE

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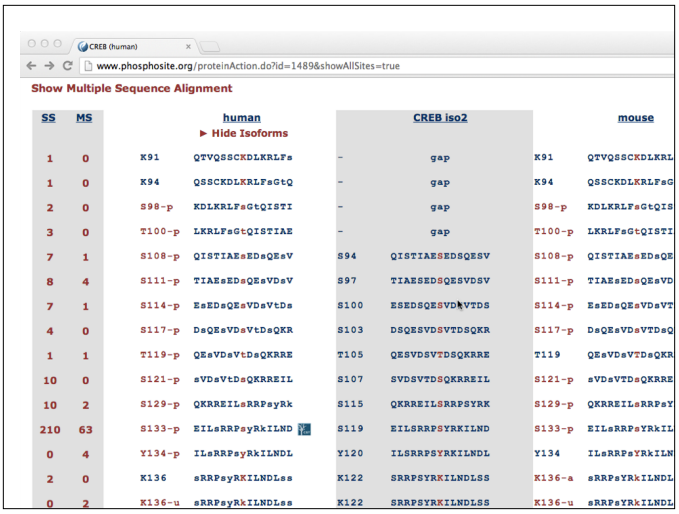

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2	0	S98-p	KDLKRLFsGtQISTI	-	gap	S98-p	KDLKRLFsGtQIS
3	0	T100-p	LKRLFsGtQISTIAE	-	gap	T100-p	LKRLFsGtQISTI
7	1	S108-p	QISTIAEsEDsQEsV	S94	QISTIAESEDSQESV	S108-p	QISTIAEsEDsQE
8	4	S111-p	TIAEsEDsQEsVDsV	S97	TIAESEDSQESVDSV	S111-p	TIAEsEDsQEsVD
7	1	S114-p	EsEDsQEsVDsVtDs	S100	ESEDSQESVDSVTDS	S114-p	EsEDsQEsVDsVT
4	0	S117-p	DsQEsVDsVtDsQKR	S103	DSQESVDSVTDSQKR	S117-p	DsQEsVDsVTDsQ
1	1	T119-p	QEsVDsVtDsQKRRE	T105	QESVDSVTDSQKRRE	T119	QEsVDsVTDsQKR
10	0	S121-p	sVDsVtDsQKRREIL	S107	SVDSVTDSQKRREIL	S121-p	sVDsVTDsQKRRE
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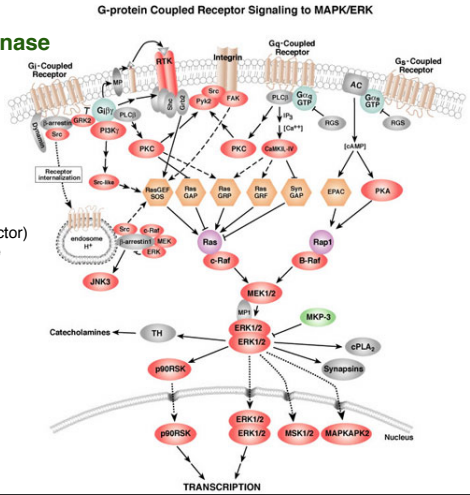
2	0	K136	sRRPsyRKILNDLss	K122	SRRPSYRKILNDLSS	K136-a	sRRPsYRkILNDL
0	2	K136-u	sRRPsyRkILNDLss	K122	SRRPSYRKILNDLSS	K136-u	sRRPsYRkILNDL

48

Example: cAMP & MAP kinase



MAP Kinase
mitogen (e.g. growth factor) activated protein kinase

aka

ERKs
extracellular signal-regulated kinases

cellsignaling.com

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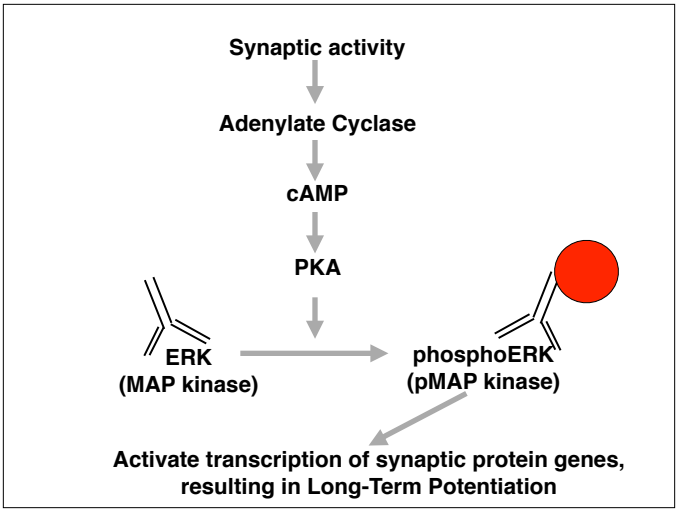

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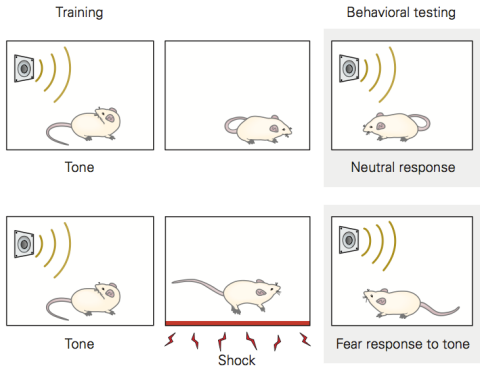
Fear Conditioning and Long-Term Potentiation



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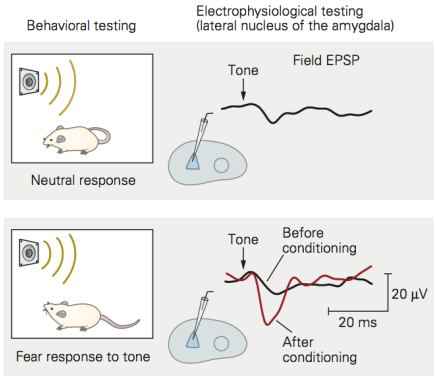
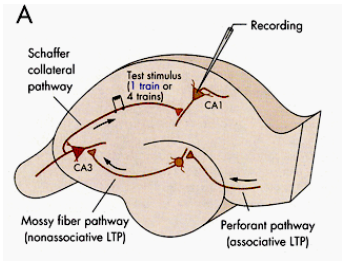
Fear Conditioning and Long-Term Potentiation



Long-Term Potentiation (LTP) in Hippocampus

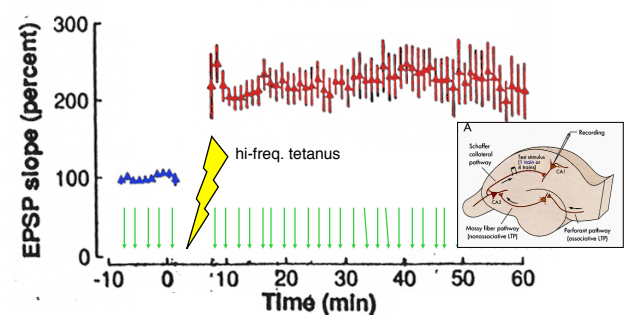
52



Long-Term Potentiation (LTP) in Hippocampus

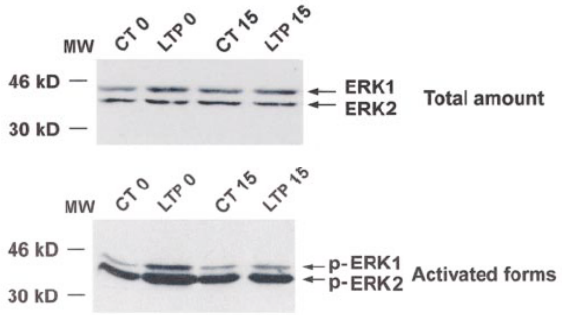
53

High frequency stimulus strengthens transmission at a synapse, by upregulating future transmitter release, receptor number, second messengers, etc.



Western Blot to identify phosphoprotein

54



LTP 0 = immediately after stimulation
LTP 15 = 15 min after stimulation

Davis 2000

Immunohistochemistry for phosphoMAP Kinase

CT 0

LTP 0

LTP 15

Davis 2000

55

Some useful analogs to stimulate 2nd Messenger systems

Forskolin
activates Adenyl Cyclase

permeable analogs of cAMP/cGMP
activate PKA/PKG

phorbol esters
(e.g. TPA)
activate PKC by simulating DAG

A variety of new drugs…

Note: most of these drugs cannot be used systemically.

56

Useful toxins to block Signaling Pathways

G-Protein Toxins:

1. Cholera Toxin
allows binding of GTP, but prevents hydrolysis.

Causes overproduction of cAMP, leading to loss of electrolytes and water from intestinal cells.

2. Pertussis Toxin
blocks release of GDP from alpha subunit, so G-protein locked in the inactive state.

57

58

Phosphatase Inhibitors

should enhance kinase effects by blocking removal of phosphate from substrate

Okadaic acid

blocks PP1 and PP2a

diarrhetic shellfish toxin

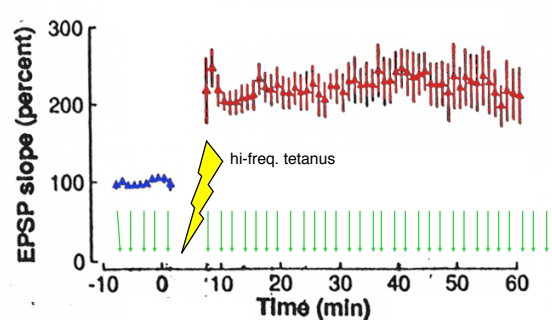
Vanadate (VO_4^{-})

blocks tyrosine phosphatases

mimics phosphate group...

59

Long-Term Potentiation (LTP) in Hippocampus



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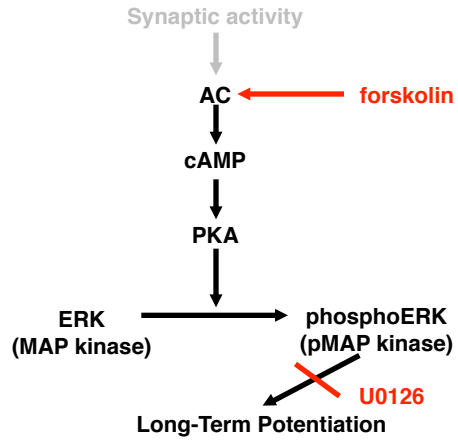

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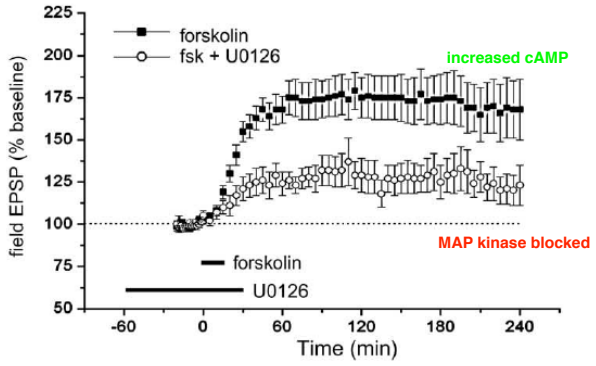
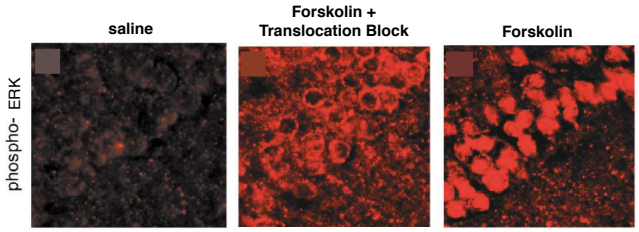


note time scale of minutes to hours

62

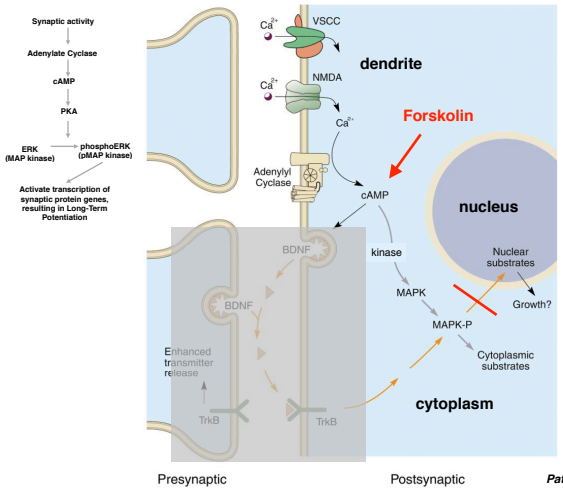
Kinases distribute signals throughout the cell



Stimulation causes ERK to be phosphorylated then translocated from dendrites into cell body and nucleus

Patterson 2001

63



Patterson 2001

Criteria for Establishing a Signaling Pathway

64

Receptor should have appropriate domains

Kinase should be activated by the physiological stimulus

Downstream targets of pathway should be activated by stimulus.

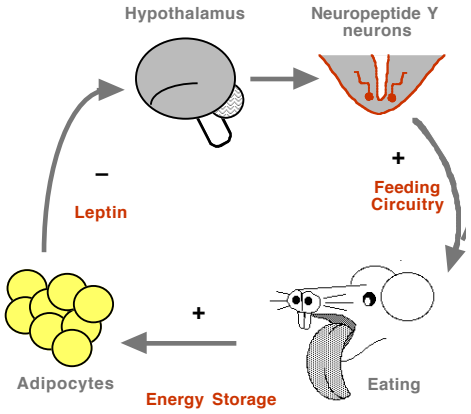
Drugs that alter kinase/phosphatase activity in the pathway should alter physiological response

Genetic manipulations should alter signaling and physiological response

Example: Leptin / Jak-Stat / SOCS pathway

Feedback Loop: Leptin -> NPY

65



Obese Mutant Mice

66

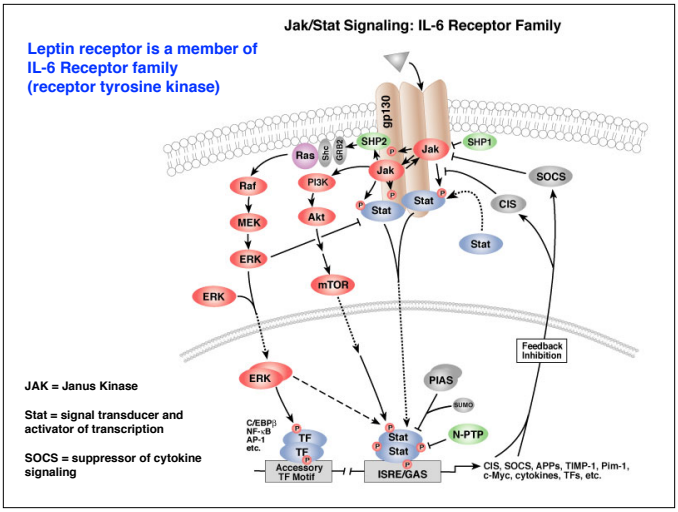
ob/ob obese mouse
db/db diabetic mouse
fa/fa fatty (Zucker) rat

have impaired leptin signaling

db/db mice lack functional leptin receptors

	Body mass	Adiposity
wildtype (+/+):	18 g	12 %
obese (ob/ob)	64 g	60 %

67



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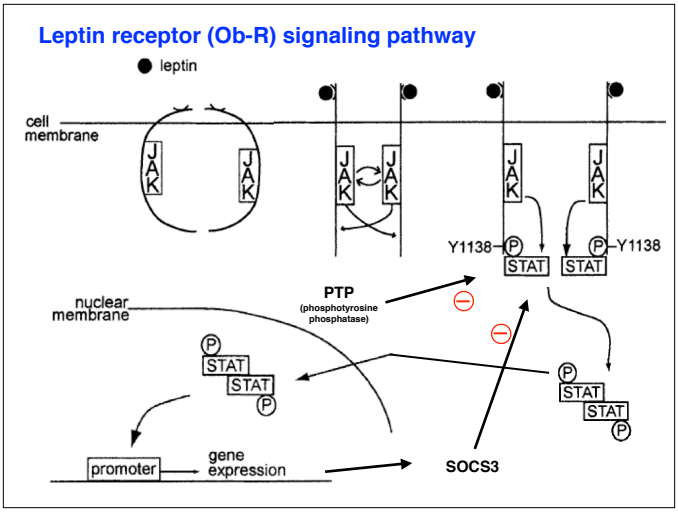


69

Which Stat (Stats 1-6) is coupled to Leptin Receptor?

Are JAK & Stat phosphorylated?

Does Stat translocate to the nucleus?

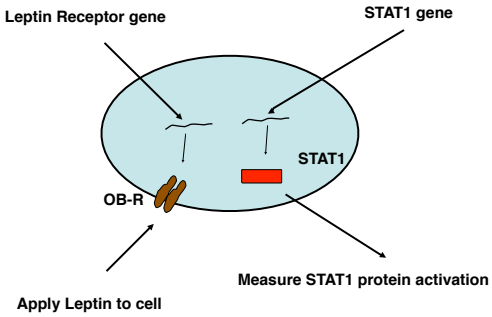
Is SOCS induced? Which SOCS (SOCS1-3)?

Does tyrosine phosphatase downregulate Leptin Receptor activity?

Is this signaling pathway absent in receptor KO mutant?

70

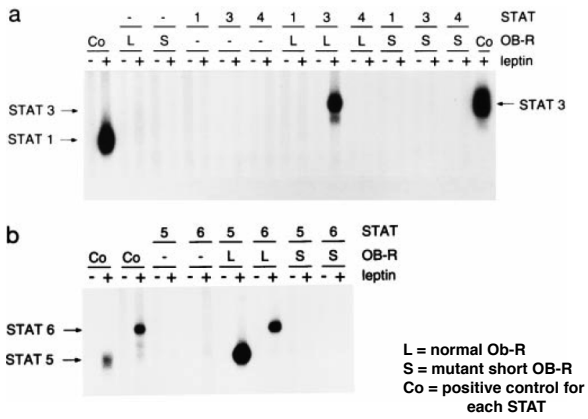
Activation of STAT in transfected Cell



Repeat for STAT3,4,5, and 6

71

Measure phosphoStat levels in presence of leptin and leptin receptor



L = normal Ob-R
S = mutant short OB-R
Co = positive control for each STAT

72

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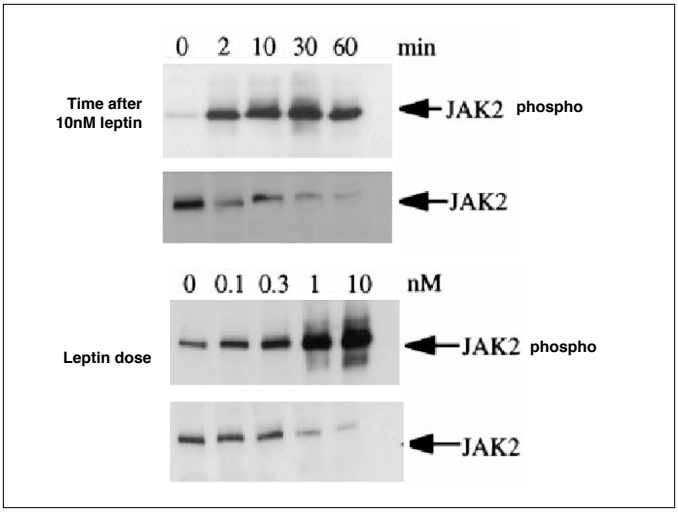

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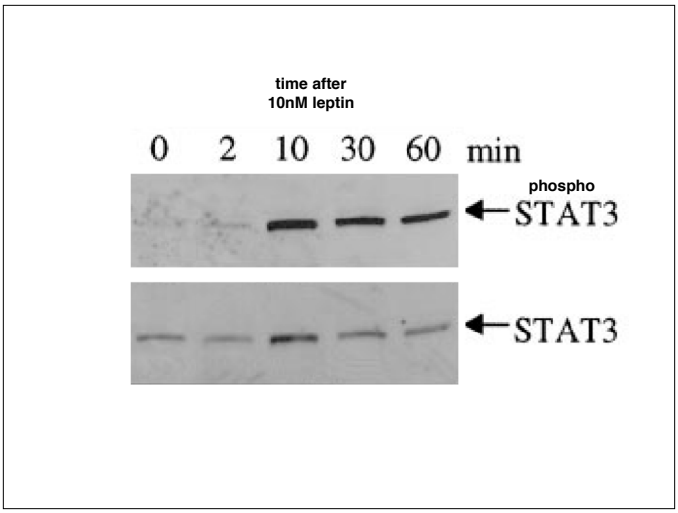

74

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Does Stat translocate to the nucleus?

Is SOCS induced? Which SOCS (SOCS1-3)?

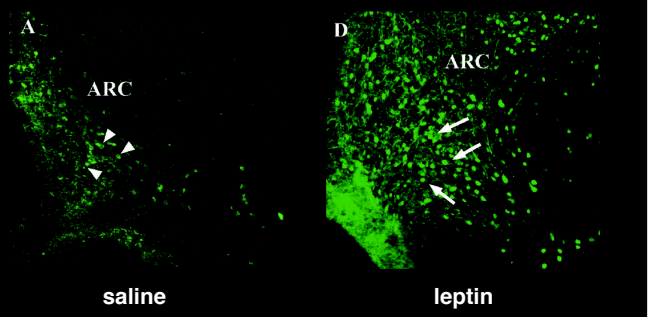
Does tyrosine phosphatase downregulate Leptin Receptor activity?

Is this signaling pathway absent in receptor KO mutant?

75

76

Translocation of STAT from cytoplasm to nucleus



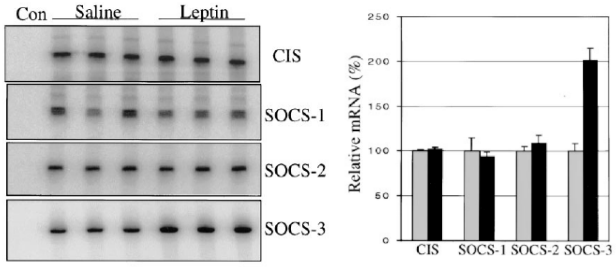
saline leptin

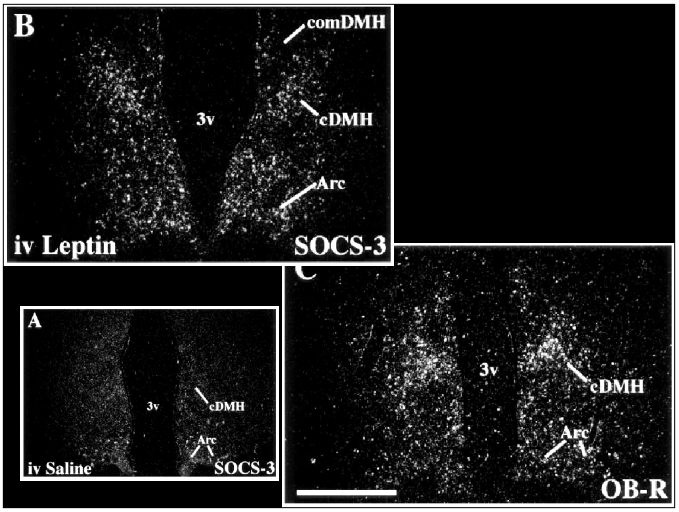
77

- Which Stat (Stats 1-6) is coupled to Leptin Receptor?
- Are JAK & Stat phosphorylated?
- Does Stat translocate to the nucleus?
- Is SOCS induced? Which SOCS (SOCS1-3)?
- Does tyrosine phosphatase downregulate Leptin Receptor activity?
- Is this signaling pathway absent in receptor KO mutant?

78

Leptin and SOCS3 signaling: RT-PCR from hypothalamus after Leptin





79

80

Which Stat (Stats 1-6) is coupled to Leptin Receptor?

Are JAK & Stat phosphorylated?

Does Stat translocate to the nucleus?

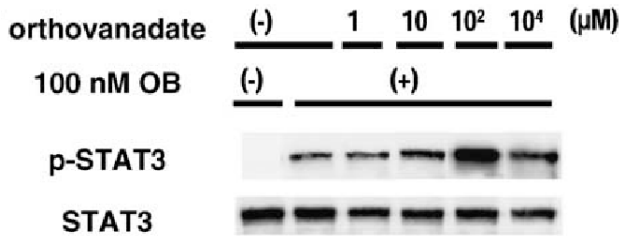
Is SOCS induced? Which SOCS (SOCS1-3)?

Does tyrosine phosphatase downregulate Leptin Receptor activity?

Is this signaling pathway absent in receptor KO mutant?

81

Blockade of Tyrosine Phosphatases with Vanadate enhances leptin effect



82

Which Stat (Stats 1-6) is coupled to Leptin Receptor?

Are JAK & Stat phosphorylated?

Does Stat translocate to the nucleus?

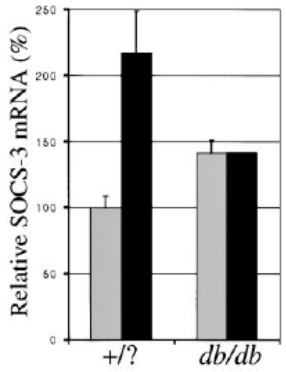
Is SOCS induced? Which SOCS (SOCS1-3)?

Does tyrosine phosphatase downregulate Leptin Receptor activity?

Is this signaling pathway absent in receptor KO mutant?

83

SOCS3 not induced in Receptor KO mutant.



84

Which Stat (Stats 1-6) is coupled to Leptin Receptor?

Are JAK & Stat phosphorylated?

Does Stat translocate to the nucleus?

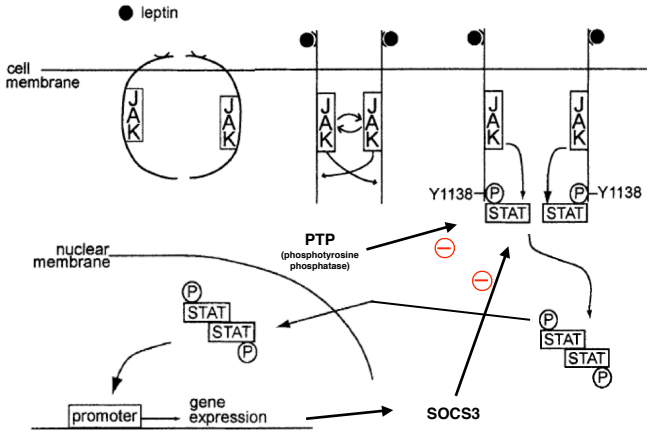
Is SOCS induced? Which SOCS (SOCS1-3)?

Does tyrosine phosphatase downregulate Leptin Receptor activity?

Is this signaling pathway absent in receptor KO mutant?

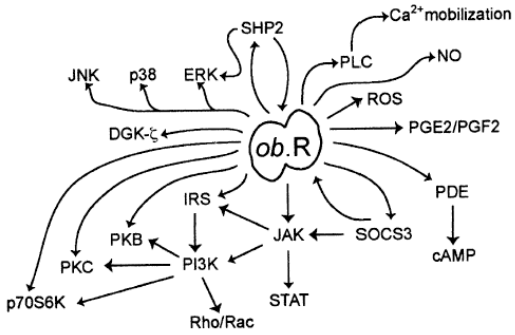
Leptin receptor (Ob-R) signaling pathway

85



Signaling pathways regulated by leptin.

86

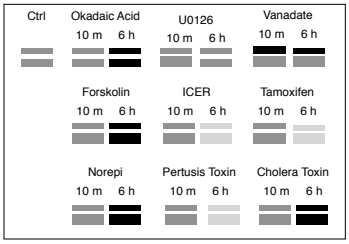


"Note that arrows (a) are intended to depict regulation rather than imply activation and (b) do not imply a direct association as in some cases multiple potential intermediates are as yet uncharacterized or excluded."

87

Figure 3. shows a series of Western Blots for a specific protein extracted from treated cells. The protein is visualized with a specific antibody that yields 2 bands: a major band and a slightly larger molecular weight band that contains a subset of the total protein. For each blot, the cells were treated for either 10 min or 6 h with the indicated compound. The compounds decreased or increased the intensity of one or both bands as indicted.

Outline the regulation of the protein's gene expression and phosphorylation (i.e. arrows connecting receptors, enzymes, transcription factors.



88

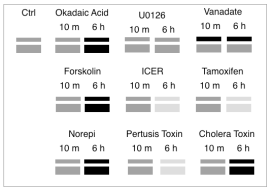
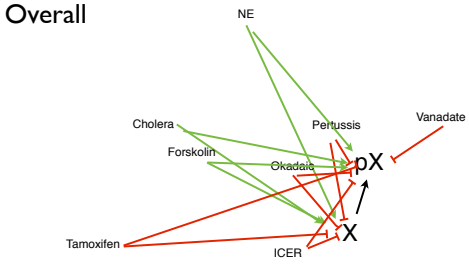


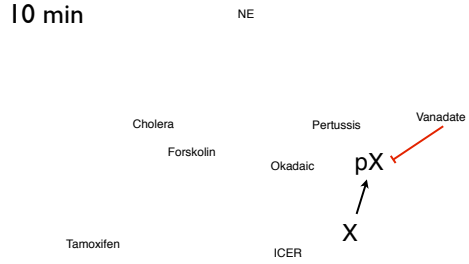
Figure 3: For each Western blot, the same antibody was used to stain a specific protein. Two bands were detected, a major band and a minor band of slightly greater molecular weight. Note that each band may appear at 3 different intensities relative to control (no treatment): lighter than control, same as control, or darker than control.

Drug	Target	10 m	6 h	Conclusion
Okadaic Acid	Ser/Thr PPs	none	increase	
U0126	MAPK	none	none	
Vanadate	pTyr PPs	increase pX	increase pX	
Forskolin	cAMP	none	increase X and pX	
ICER	CRE	none	decrease X and pX	
Tamoxifen	Estrogen Receptor	none	decrease X and pX	
Norepi	Adrenergic receptors	none	increase X and pX	
Pertussis	block G proteins	none	decrease X and pX	
Cholera Toxin	activate G proteins	none	increase X and pX	

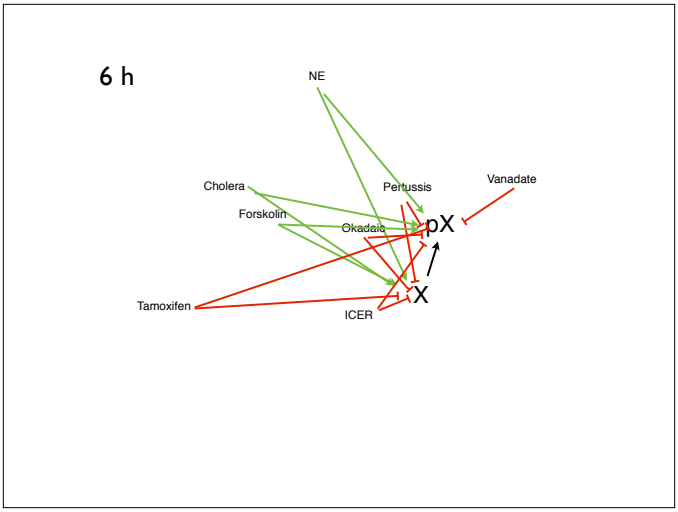
89



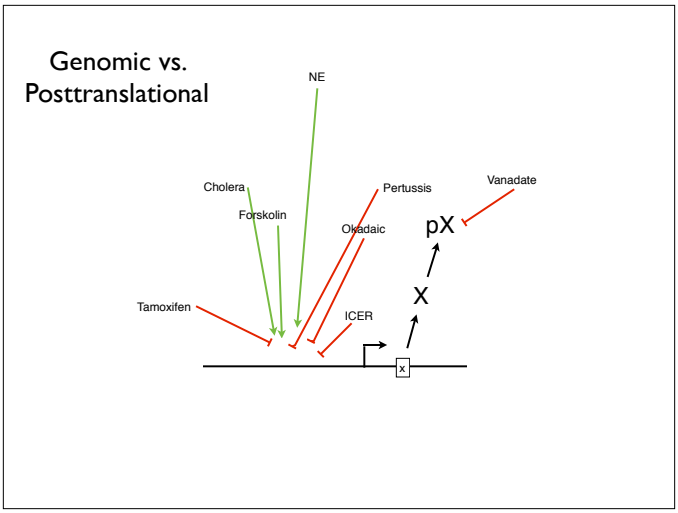
90



91



92



93

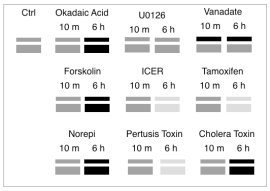


Figure 3: For each Western blot, the same antibody was used to stain a specific protein. Two bands were detected, a major band and a minor band of slightly greater molecular weight. Note that each band may appear at 3 different intensities relative to control (no treatment): lighter than control, same as control, or darker than control.

Drug	Target	10 m	6 h	Conclusion
Okadaic Acid	Ser/Thr PPs	none	increase	PPs constrain gene X
U0126	MAPK	none	none	MAPK not involved
Vanadate	pTyr PPs	increase pX	increase pX	pTyr PPs constrain phosphorylation but not gene X expression
Forskolin	cAMP	none	increase X and pX	cAMP stimulates gene X expression but not phosphorylation
ICER	CRE	none	decrease X and pX	CREB stimulates gene X expression (but not phosphorylation)
Tamoxifen	Estrogen Receptor	none	decrease X and pX	estrogen stimulates gene X expression (but not phosphorylation)
Norepi	Adrenergic receptors	none	increase X and pX	Norepi stimulates gene X expression but not phosphorylation (consistent with B-adrenergic receptors -> cAMP)
Pertussis	block G proteins	none	decrease X and pX	G-proteins stimulate gene X expression but not phosphorylation
Cholera Toxin	activate G proteins	none	increase X and pX	G-proteins stimulate gene X expression but not phosphorylation

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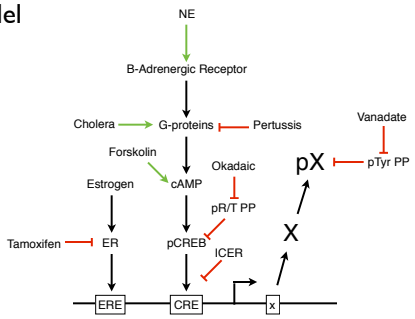
Model

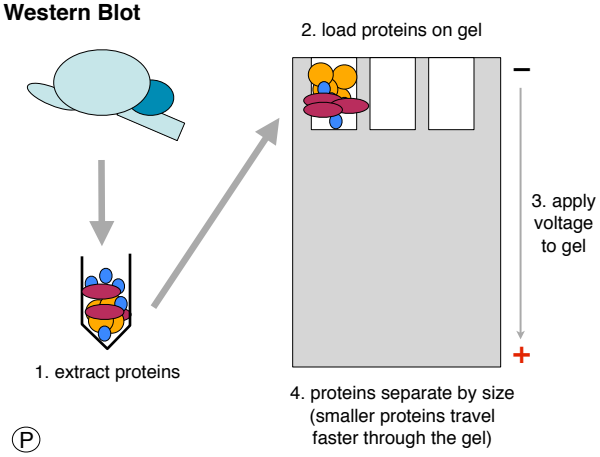


95

end

96

Western Blot



Ⓟ

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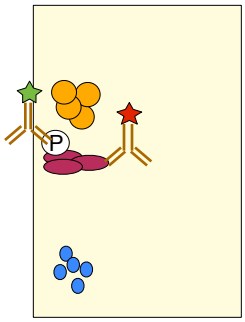
Western Blot

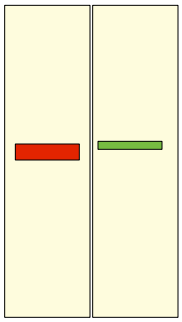
5. Blot proteins onto nylon membrane



6. Visualize proteins using labeled antibodies

7. Visualize phosphoylated proteins using phospho-specific antibodies

Developed Blot



protein phospho protein

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2D Gel of Postsynaptic Density

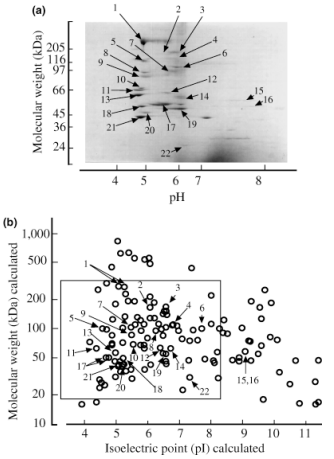

Figure 1. Proteins in the PSD fraction revealed by two-dimensional (2D)-gel electrophoresis and 2D-display. (a) 2D-gel electrophoresis of the PSD fraction. PSD protein (200 μg) was subjected to 2D-gel electrophoresis and stained with Coomassie blue. Some of the major Coomassie-stained protein spots were identified; 1, α and β fodrin; 2, NMDA-receptor 2B subunit; 3, densin 180; 4, SAPAP; 5, neurofilament M protein; 6, GluR1; 7, SAP−97; 8, α actinin; 9, PSD-95/SAP-90; 10, HSP70; 11, neurofilament L protein; 12, TOAD-64; 13, α internexin; 14, β CaM kinase II; 15, IRSp58; 16, IRSp53; 17 α and β tubulin; 18, GFAP; 19, α CaM kinase II; 20, homer 1b; 21, actin; 22, VDAC. (b) Theoretical 2D-gel representation of data assuming that identified peptides correspond to full-length proteins. PSD proteins with an identification number greater than 5 are shown. Their predicted molecular weight and pI values are indicated. The region in the box indicates the region measured in 2D-gel electrophoresis. Proteins are numbered the same as in (a).

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