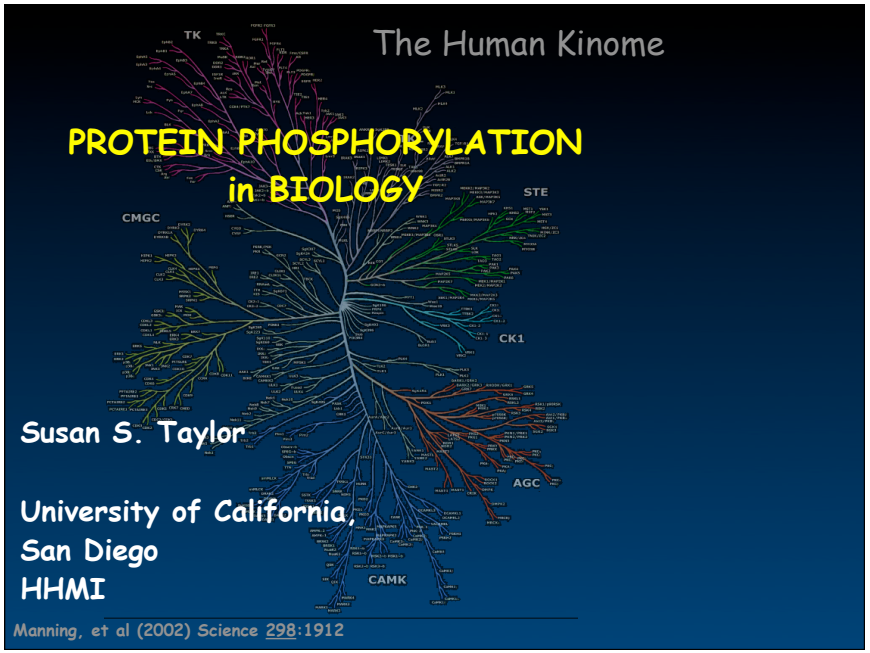
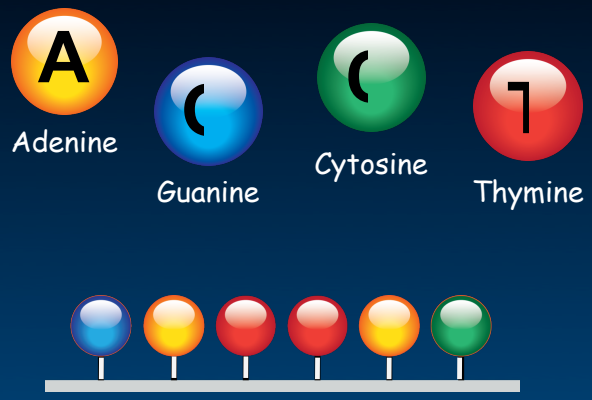


Central Dogma of Biology

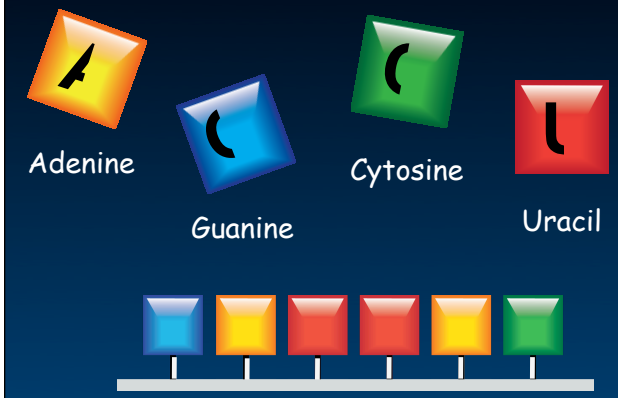
DNA → RNA → Proteins

Deoxyribonucleic Acid (DNA)



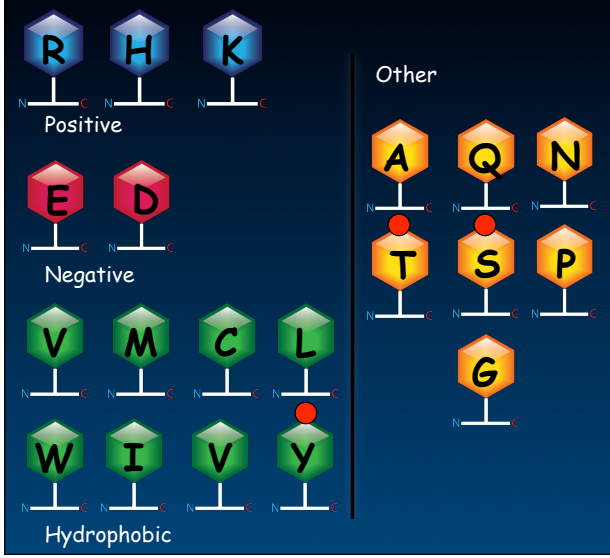
graphics A. Doo

Ribonucleic Acid (RNA)

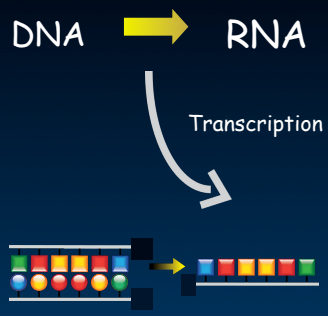


graphics A. Doo

Proteins – Amino Acids



Transcription



Genomic Era of Science

Transcription

DNA → RNA

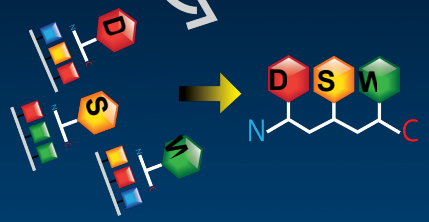
Transcription

Genomic Era of Science

Translation

RNA → Proteins

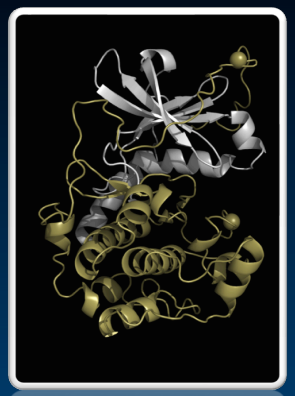
Translation



graphics A. Doo

Sequence → Structure

GNAAAAKKGSEQESVKEFLAKAKEDFLKKW
ETPSQNTAQLDQFDRIKRLGTGSFGRVMLV
KHKESGNHYAMKILDKQKVVKLKQIEHTLN
EKRILQAVNFPFLVKLEFSFKDNSNLYMVM
EYVAGGEMFSHLRRIGRFSEPHARFYAAQI
VLTFEYLHSLDLIYRDLKPENLLIDQQGYI
QVTDFGFAKRVKGRRWTLCGTPEYLAPEII
LSKGYNKAVDWWALGVLIYEMAAGYPPFFA
DQPIQIYEKIVSGKVRFPSHFSSDLKDLLR
NLLQVDLTKRFGNLKNGVNDIKNHKWFATT
DWIAIYQRKVEAPFIPKFKGPGDTSNFDDY
EEEEIRVSINEKCGKEFTEF



Central Dogma of Biology

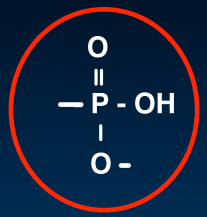
DNA → RNA → Proteins

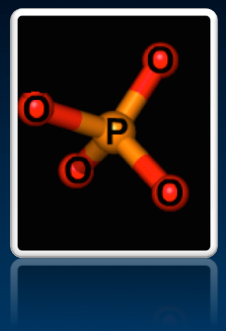
"PROTEOGENOMICS"

What atoms are in these building blocks?

CARBONS
NITROGENS
OXYGENS
HYDROGENS
SULFUR
PHOSPHATES

PHOSPHATE



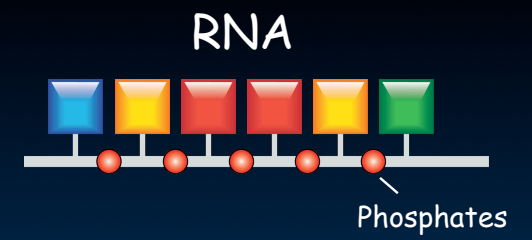


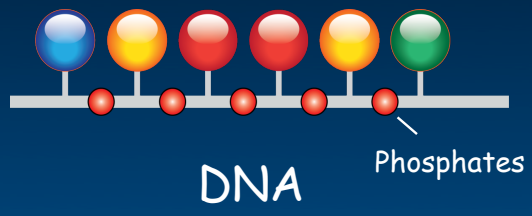
Mol Wt 80 daltons

Why Nature Chose Phosphates

F.H. Westheimer - (1988)Science 235: 1173

1. DNA and RNA –
   Genetic Information





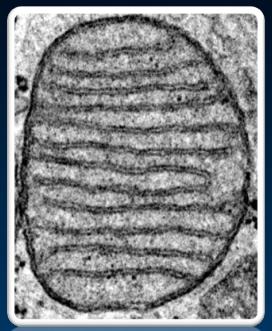
Why Nature Chose Phosphates

F.H. Westheimer - (1988)Science 235: 1173

1. DNA and RNA –
   Genetic Information

2. Intermediary Metabolism -
   Energy

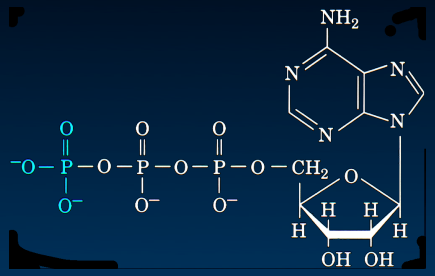
Mitochondria



Courtesy of Guy Perkins
UCSD

ATP

Adenosine Triphosphate (ATP)



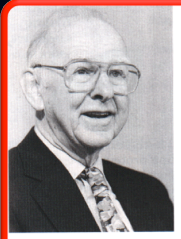
The average 70 kg person turns over 40 kg of ATP per day

Why Nature Chose Phosphates

F.H. Westheimer - (1988)Science 235: 1173

1. DNA and RNA – Genetic Information
2. Intermediary Metabolism – Energy
3. **Protein Phosphorylation – Regulation**

Krebs and Fischer - 1950's

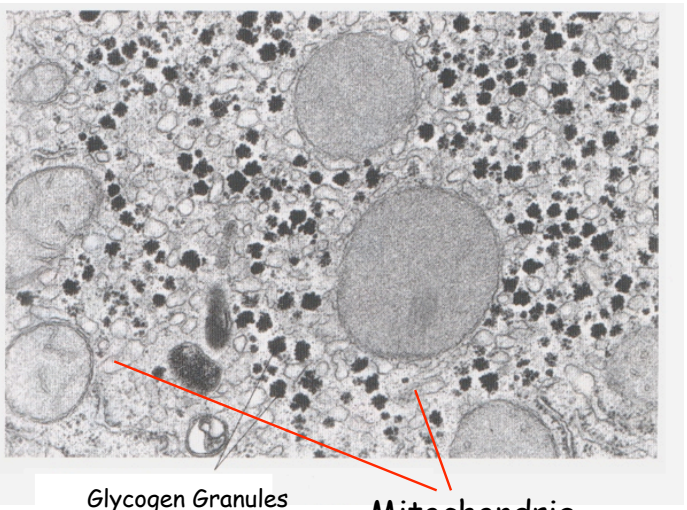


Edwin G. Krebs

Edmond H. Fischer

Nobel Prize
1992

Liver Cell

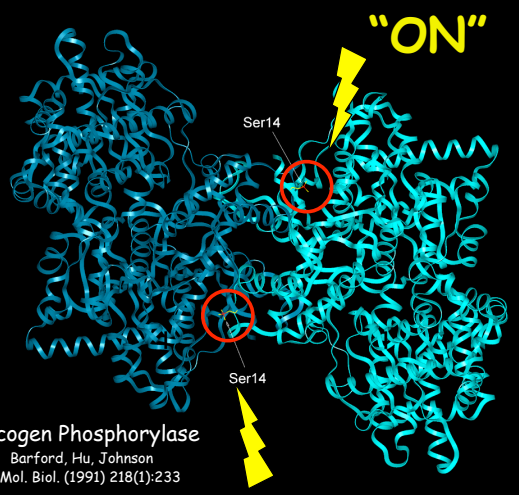


Glycogen ⟶ GLUCOSE

Glycogen Phosphorylase

Glycogen Phosphorylase catalyzes the breakdown of Glycogen

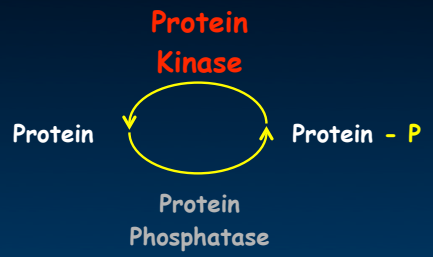
Krebs & Fisher - Nobel Prize 1992



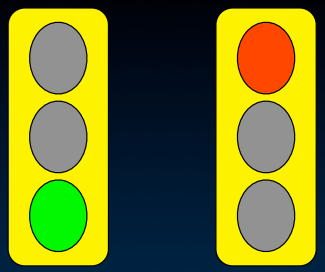
Glycogen Phosphorylase
Barford, Hu, Johnson
J. Mol. Biol. (1991) 218(1):233

PROTEIN KINASES

Molecular switches that regulate biology



Fischer & Krebs, JBC (1955) 216:121



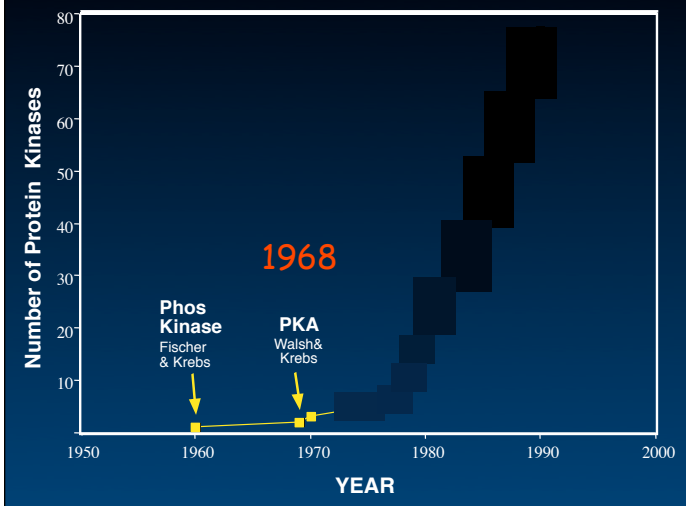
"GO" "STOP"

Protein Kinases are
Molecular Switches that
Regulate Biological Function

Kinases in Cell Division

Protein kinases serve as essential switches that allow a cell to navigate through the cell cycle.

PROTEIN KINASES



Glycogen Phosphorylase

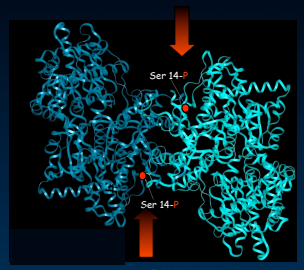
OFF

GLYCOGEN

Glucose

High Glucose
High Insulin
Low Glucagon

Glycogen Phosphorylase

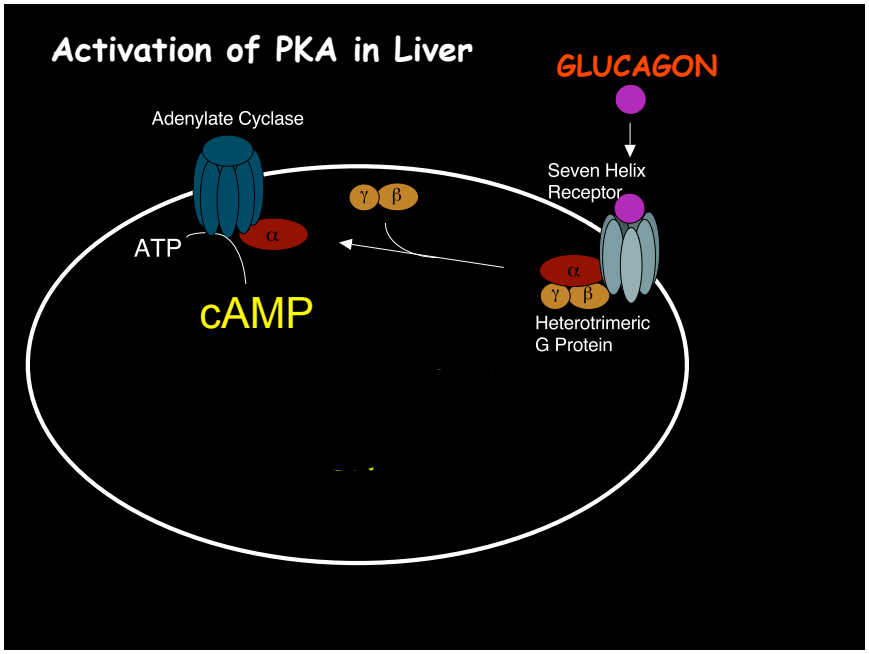


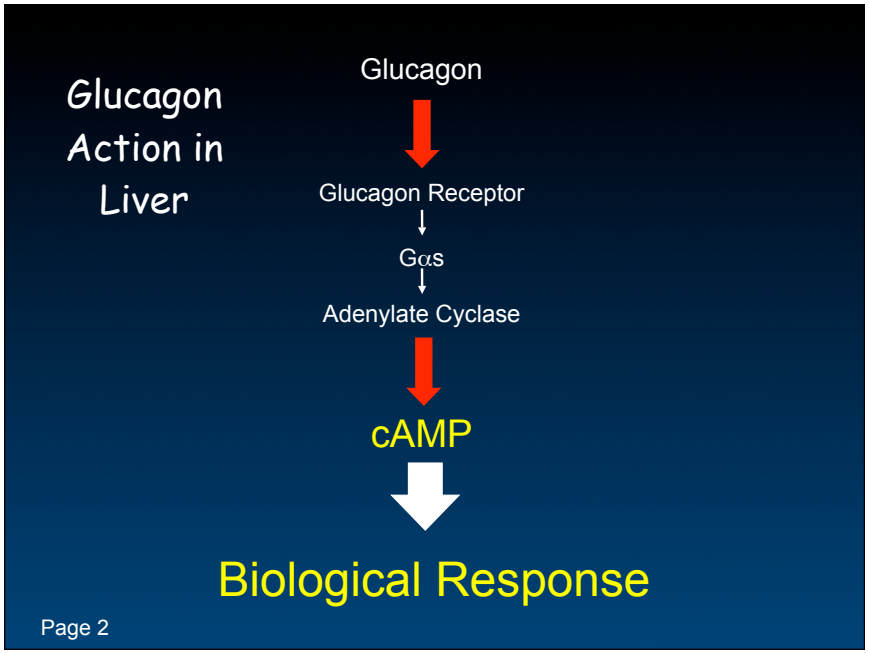
ON

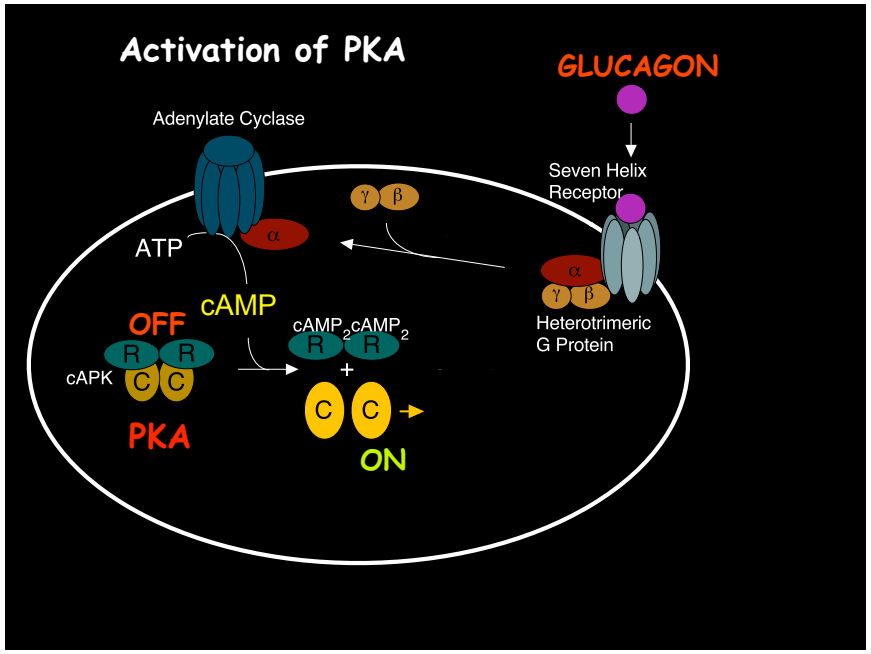
GLYCOGEN

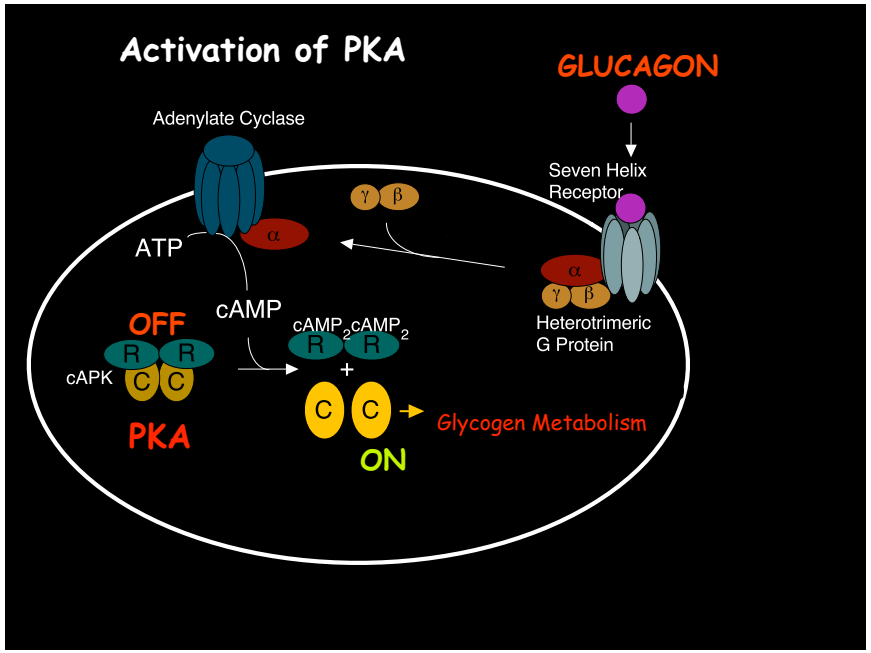
Glucose

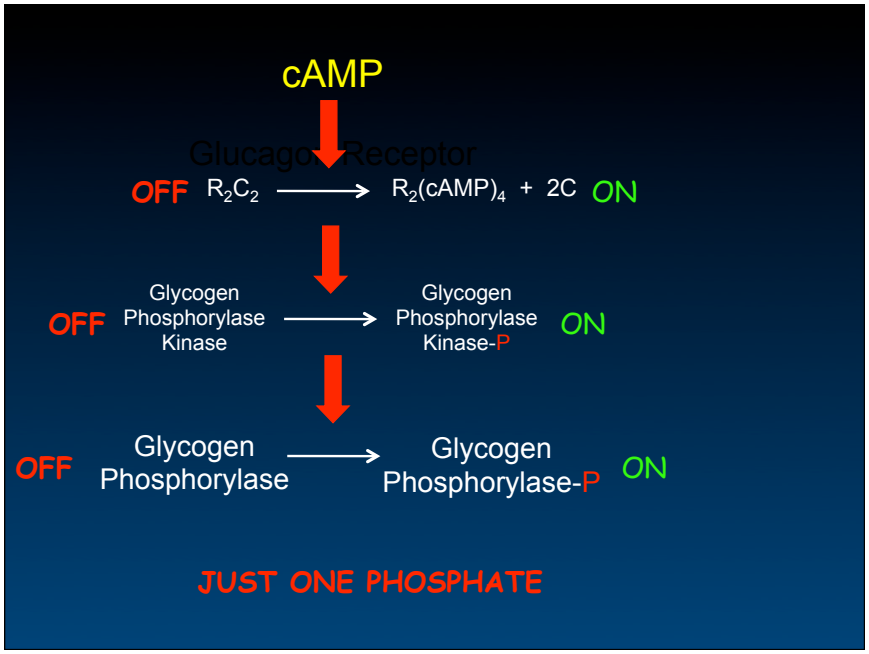
Low Glucose
High Glucagon
Low Insulin

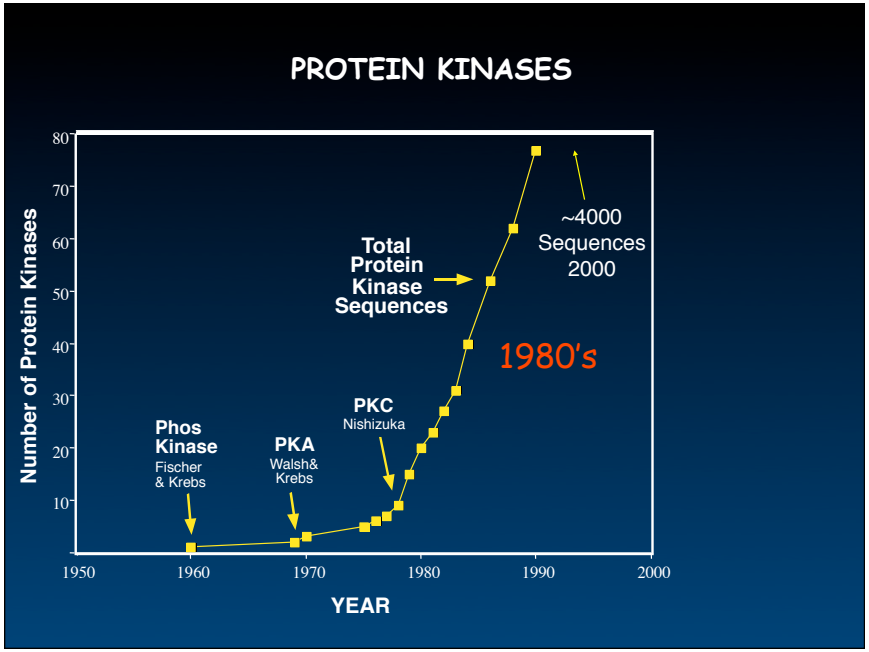

Activation of PKA in Liver
GLUCAGON
Adenylate Cyclase
Seven Helix Receptor
γ β
α
ATP
cAMP
α
γ β
Heterotrimeric G Protein

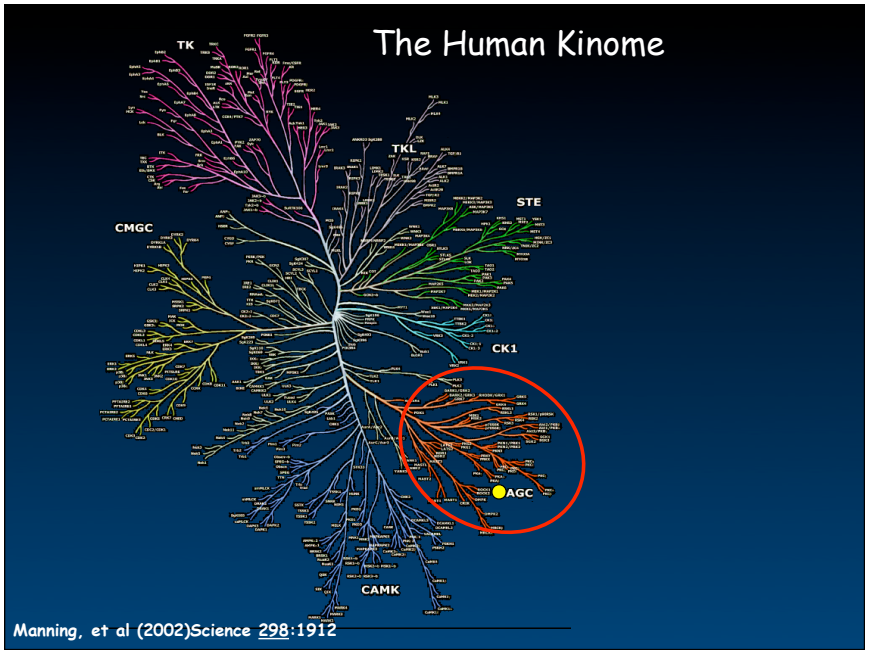

Glucagon Action in Liver
Glucagon
Glucagon Receptor
Gαs
Adenylate Cyclase
cAMP
Biological Response

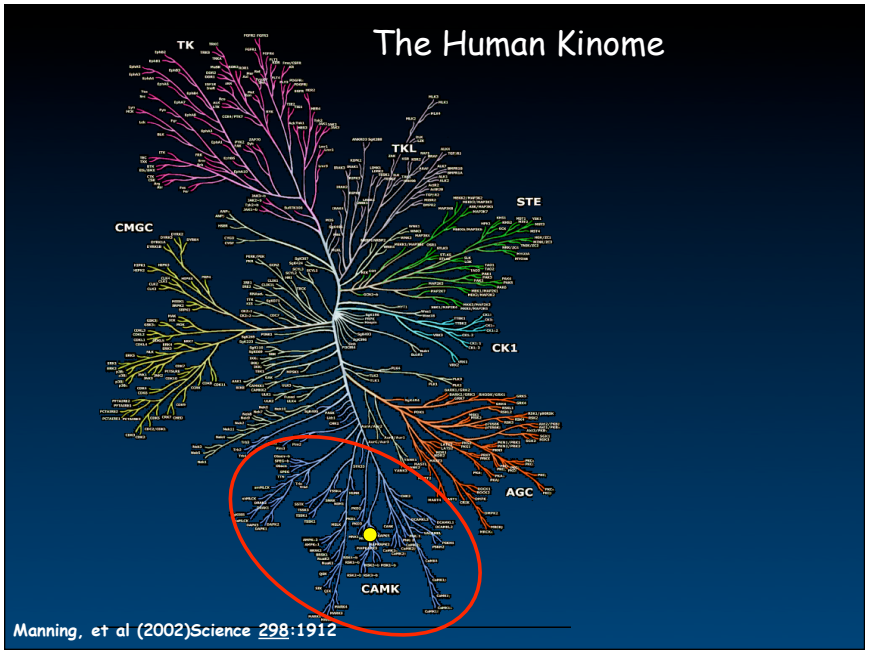

Activation of PKA
GLUCAGON
Adenylate Cyclase
Seven Helix Receptor
γ β
α
ATP
cAMP
α
γ β
Heterotrimeric G Protein
OFF
cAMP2cAMP2
R R
cAPK
C C
+
PKA
C C
ON


Activation of PKA
GLUCAGON
Adenylate Cyclase
Seven Helix Receptor
γ β
α
ATP
cAMP
α
γ β
Heterotrimeric G Protein
OFF
cAMP2cAMP2
R R
cAPK
C C
+
PKA
C C
Glycogen Metabolism
ON

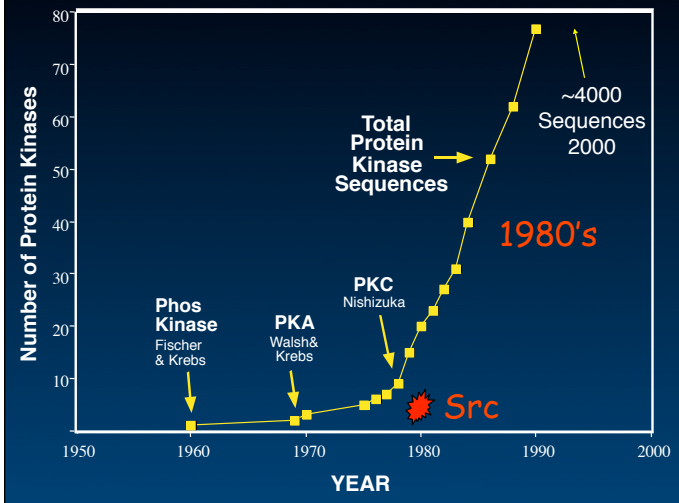

cAMP
Glucagon Receptor
OFF
R_2C_2
$R_2(cAMP)_4$ + 2C
ON
Glycogen Phosphorylase Kinase
OFF
Glycogen Phosphorylase Kinase-P
ON
Glycogen Phosphorylase
OFF
Glycogen Phosphorylase-P
ON
JUST ONE PHOSPHATE


PROTEIN KINASES
Number of Protein Kinases
80
70
60
50
40
30
20
10
1950
1960
1970
1980
1990
2000
YEAR
Phos Kinase
Fischer & Krebs
PKA
Walsh& Krebs
PKC
Nishizuka
Total Protein Kinase Sequences
1980's
~4000 Sequences 2000

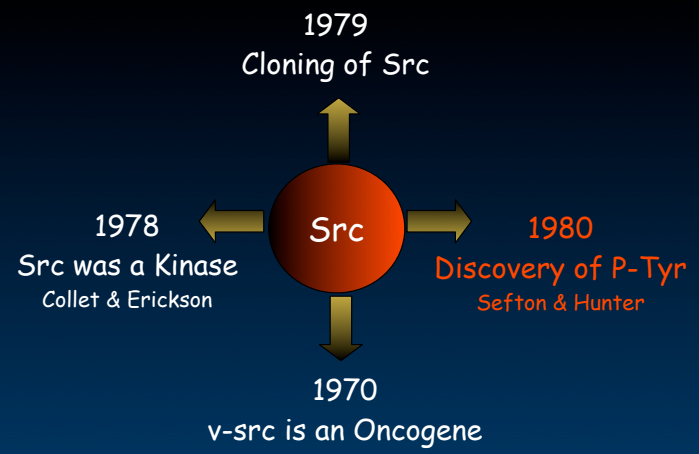

The Human Kinome
TK
TKL
STE
CMGC
CK1
AGC
CAMK
Manning, et al (2002)Science 298:1912


The Human Kinome
TK
TKL
STE
CMGC
CK1
AGC
CAMK
Manning, et al (2002)Science 298:1912

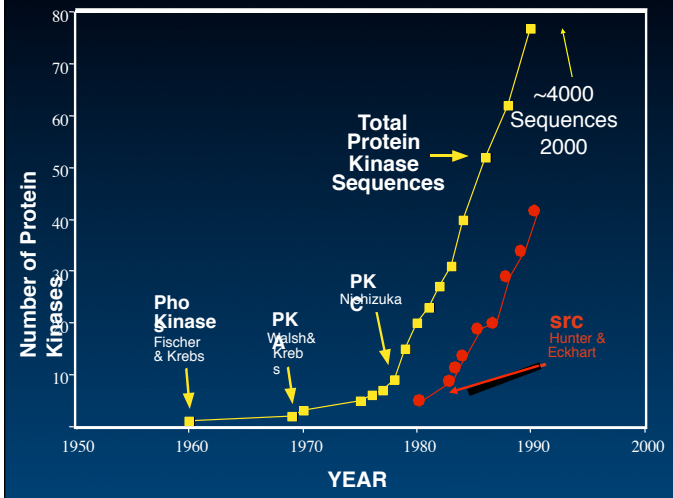
PROTEIN KINASES



History of Src

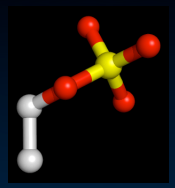


PROTEIN KINASE FAMILY

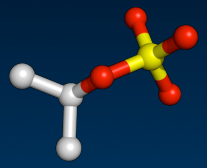


What Residues are Phosphorylated?

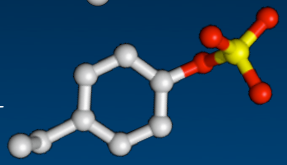
SERINE-O-PO_3^-

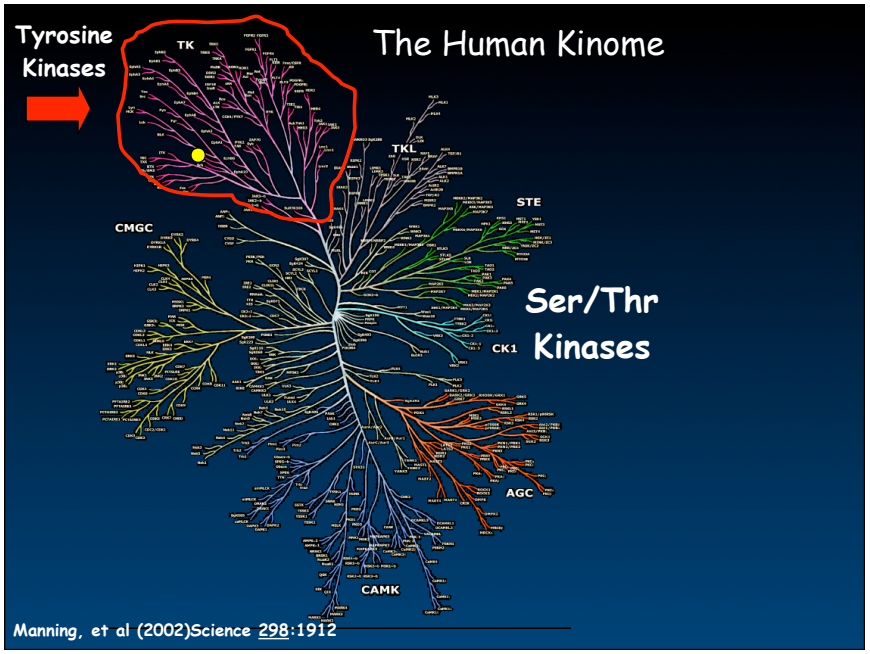


THREONINE-O-PO_3^-



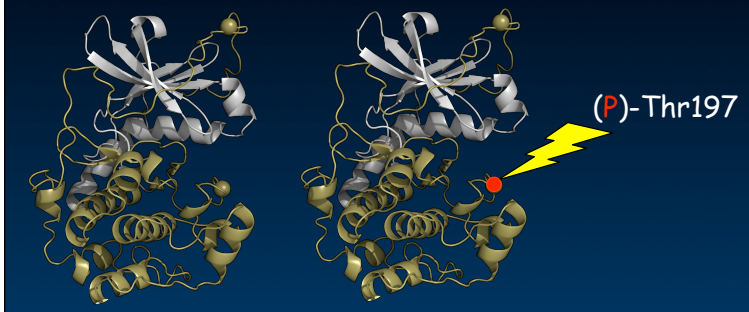
TYROSINE-O-PO_3^-



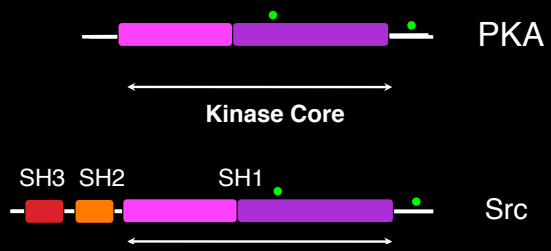


CONSEQUENCES OF ADDING OR REMOVING ONE PHOSPHATE

Kinases are also regulated by phosphorylation.



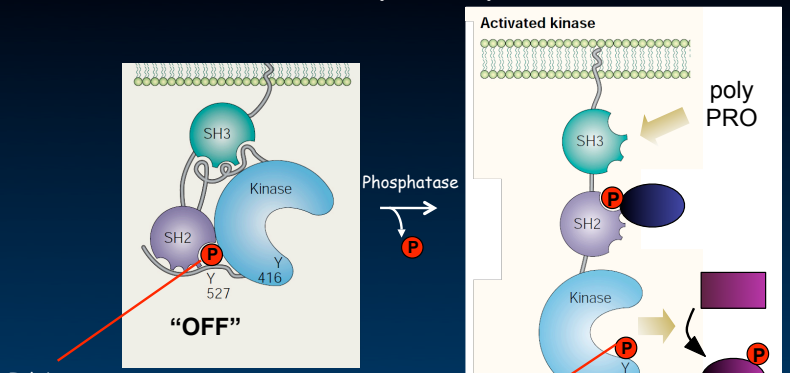
PKA and Src



1990 Pawson showed that SH2 Domains bind to P-Tyrosine

ADAPTOR MODULES MEDIATE PROTEIN INTERACTIONS

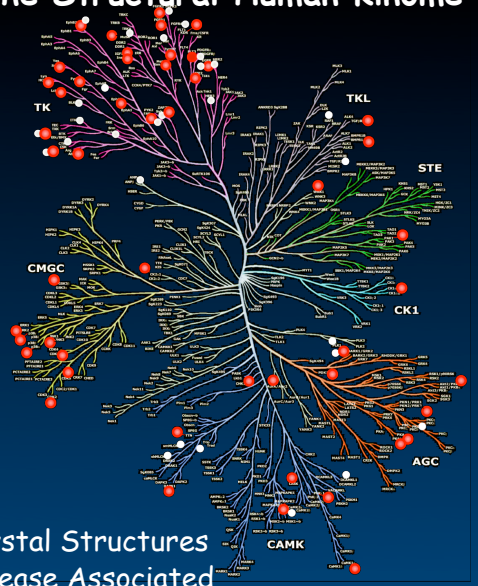
SRC is a Non-Receptor Tyrosine Kinase



Inhibitory Phosphate

ADAPTOR MECHANISM

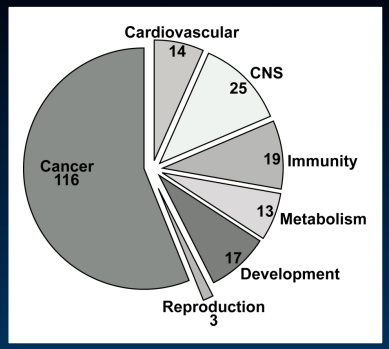
The Structural Human Kinome



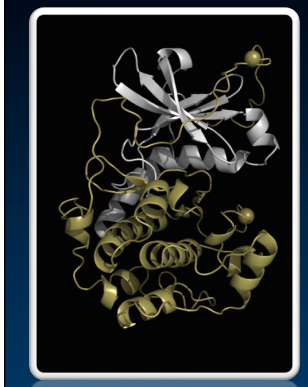
- Crystal Structures
- Disease Associated

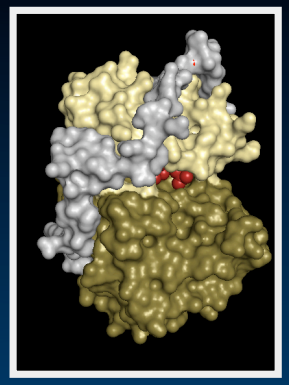
Kinases and Diseases

- 30% of protein kinases published are implicated in various diseases.
- Many more are likely to follow, from expression, SNP analyses, genetics and functional genomics.
- Kinases are tractable drug targets with several approved drugs and huge development efforts



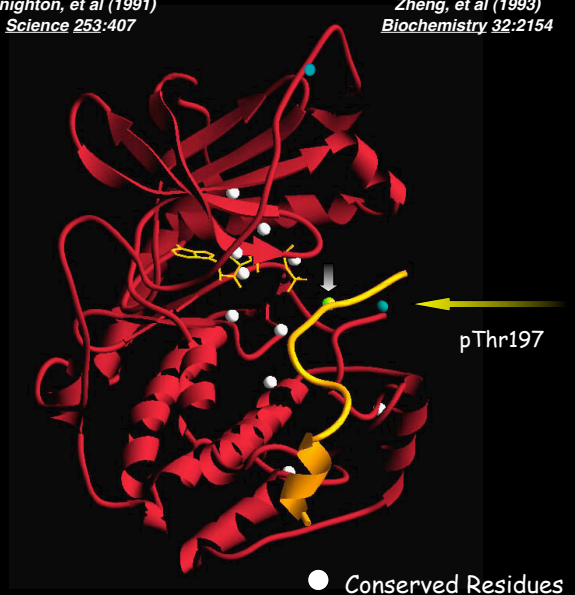
Structure → Function



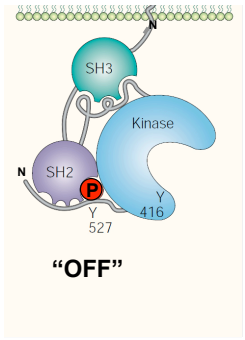


Knighton, et al (1991)
Science 253:407

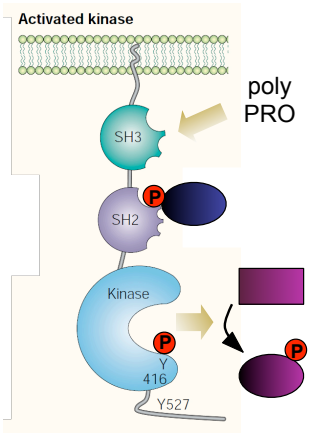
Zheng, et al (1993)
Biochemistry 32:2154



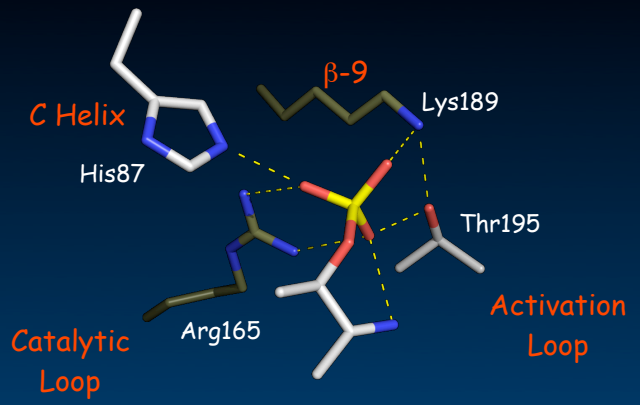
- Conserved Residues



ADAPTOR MECHANISM

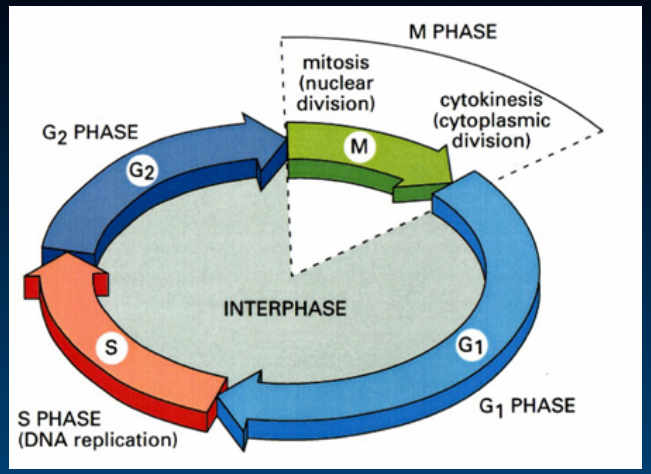


Phosphorylation of P-Thr197

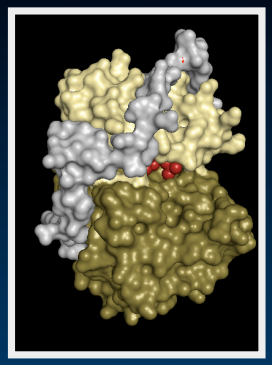


Phosphorylation causes conformational changes that activate PKA

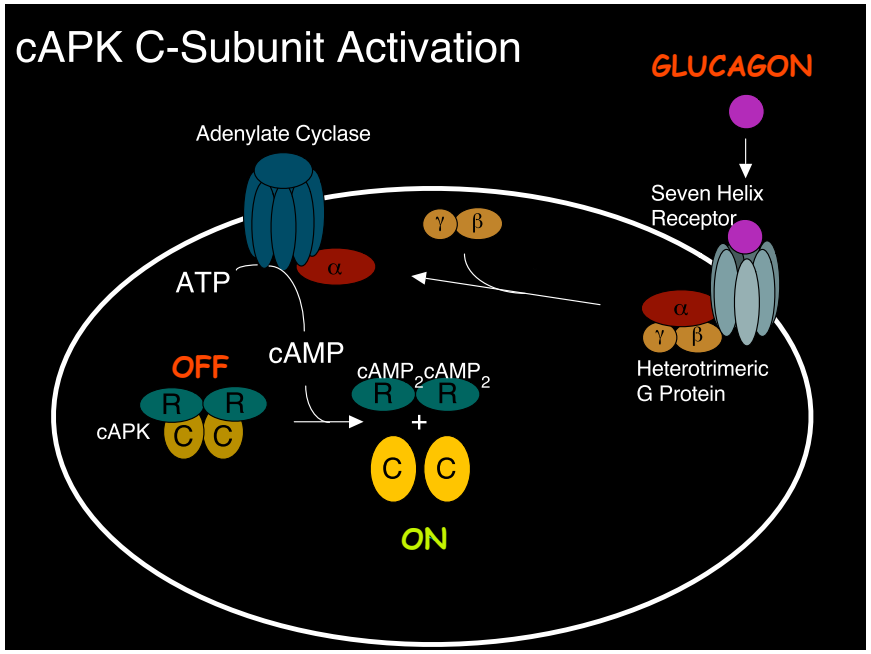
Kinases and Phosphatases Orchestrate Cell Division

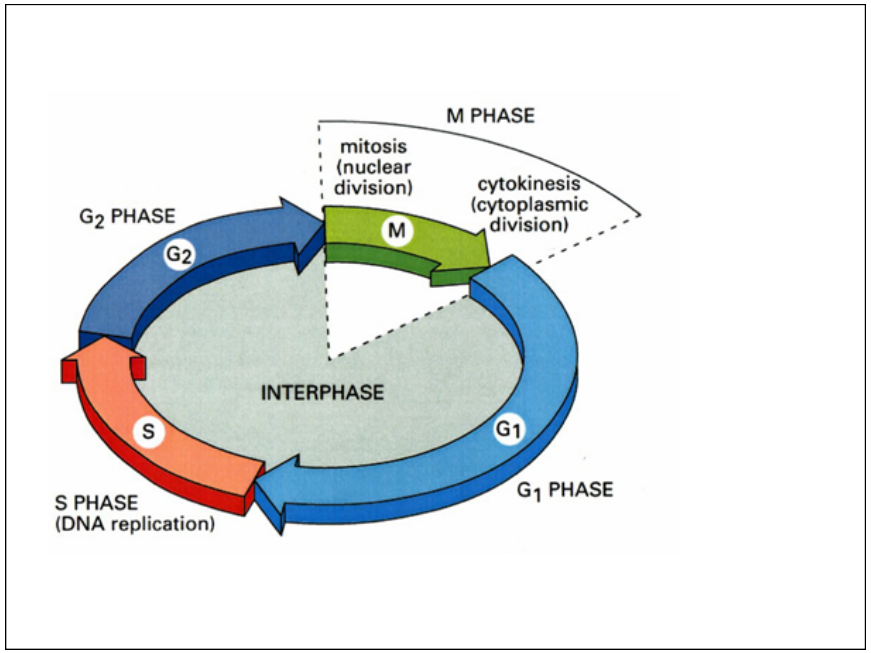


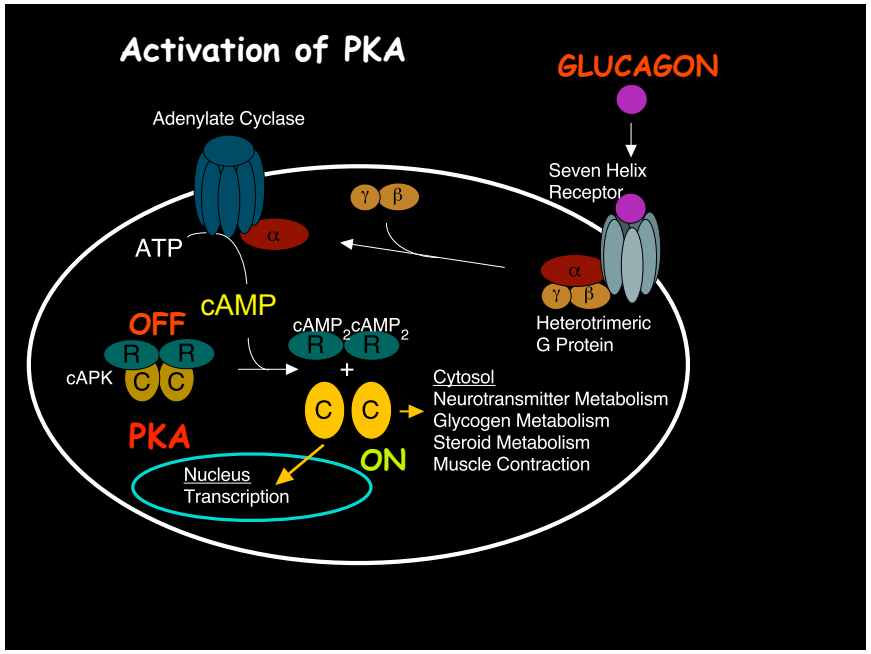
Protein Kinase A (PKA)

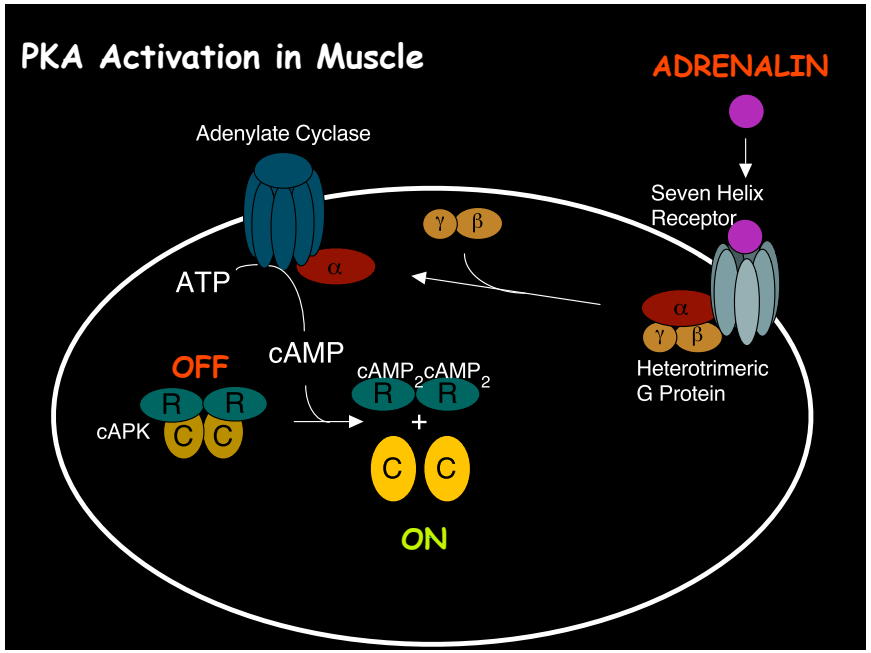


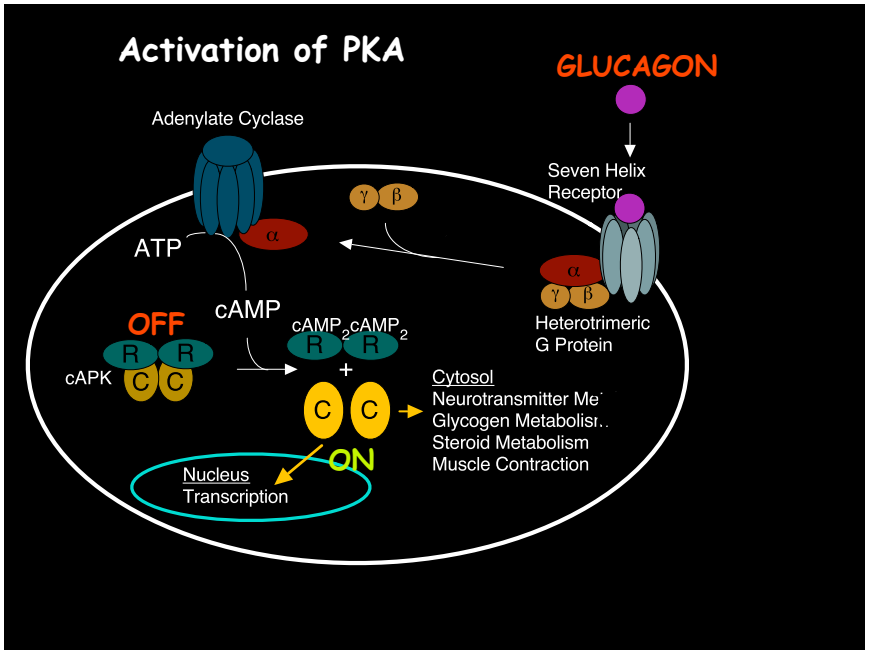
- Two regulatory, two catalytic subunits
- Activated by secondary messenger cAMP
- Plays important role in every cell:
  - Adipocyte
  - Hepatocyte
  - Neurons in the NAcc
  - Etc.

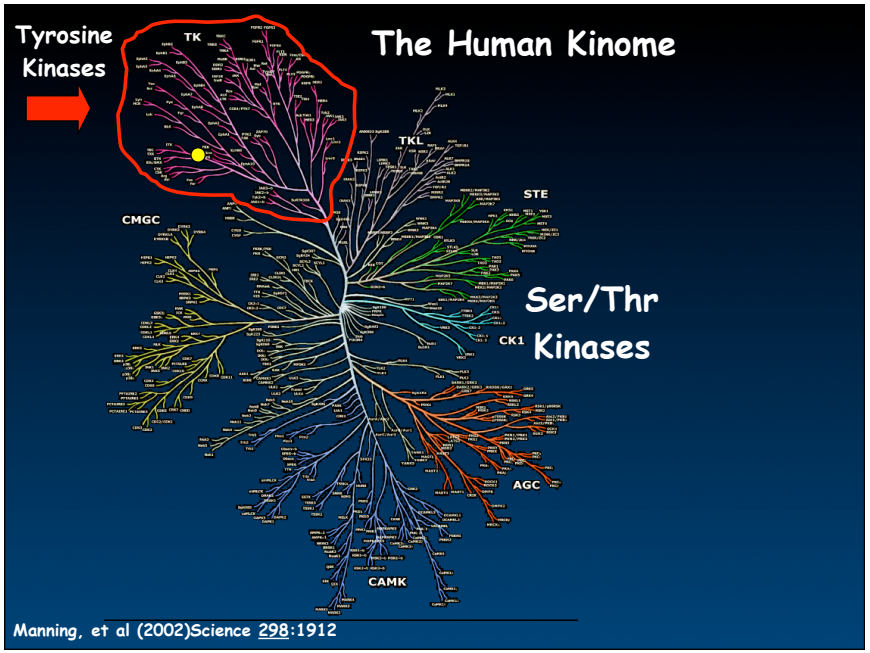

cAPK C-Subunit Activation
GLUCAGON
Adenylate Cyclase
Seven Helix
Receptor
γ β
α
ATP
cAMP
α
γ β
Heterotrimeric
G Protein
OFF
cAMP2 cAMP2
R R
cAPK
C C
R R
+
C C
ON


M PHASE
mitosis
(nuclear
division)
cytokinesis
(cytoplasmic
division)
G2 PHASE
G2
M
INTERPHASE
S
G1
S PHASE
(DNA replication)
G1 PHASE


Activation of PKA
GLUCAGON
Adenylate Cyclase
Seven Helix Receptor
ATP
cAMP
OFF
cAMP2cAMP2
cAPK
Heterotrimeric G Protein
Cytosol
Neurotransmitter Metabolism
Glycogen Metabolism
Steroid Metabolism
Muscle Contraction
PKA
ON
Nucleus
Transcription


PKA Activation in Muscle
ADRENALIN
Adenylate Cyclase
Seven Helix Receptor
ATP
cAMP
OFF
cAMP2cAMP2
cAPK
Heterotrimeric G Protein
ON


Activation of PKA
GLUCAGON
Adenylate Cyclase
Seven Helix Receptor
ATP
cAMP
OFF
cAMP2cAMP2
cAPK
Heterotrimeric G Protein
Cytosol
Neurotransmitter Me
Glycogen Metabolisr..
Steroid Metabolism
Muscle Contraction
ON
Nucleus
Transcription


Tyrosine Kinases
The Human Kinome
TK
TKL
STE
CMGC
Ser/Thr Kinases
CK1
AGC
CAMK
Manning, et al (2002)Science 298:1912

Src Family Members

Discovered New "Domain"

Named it SH2 Domain