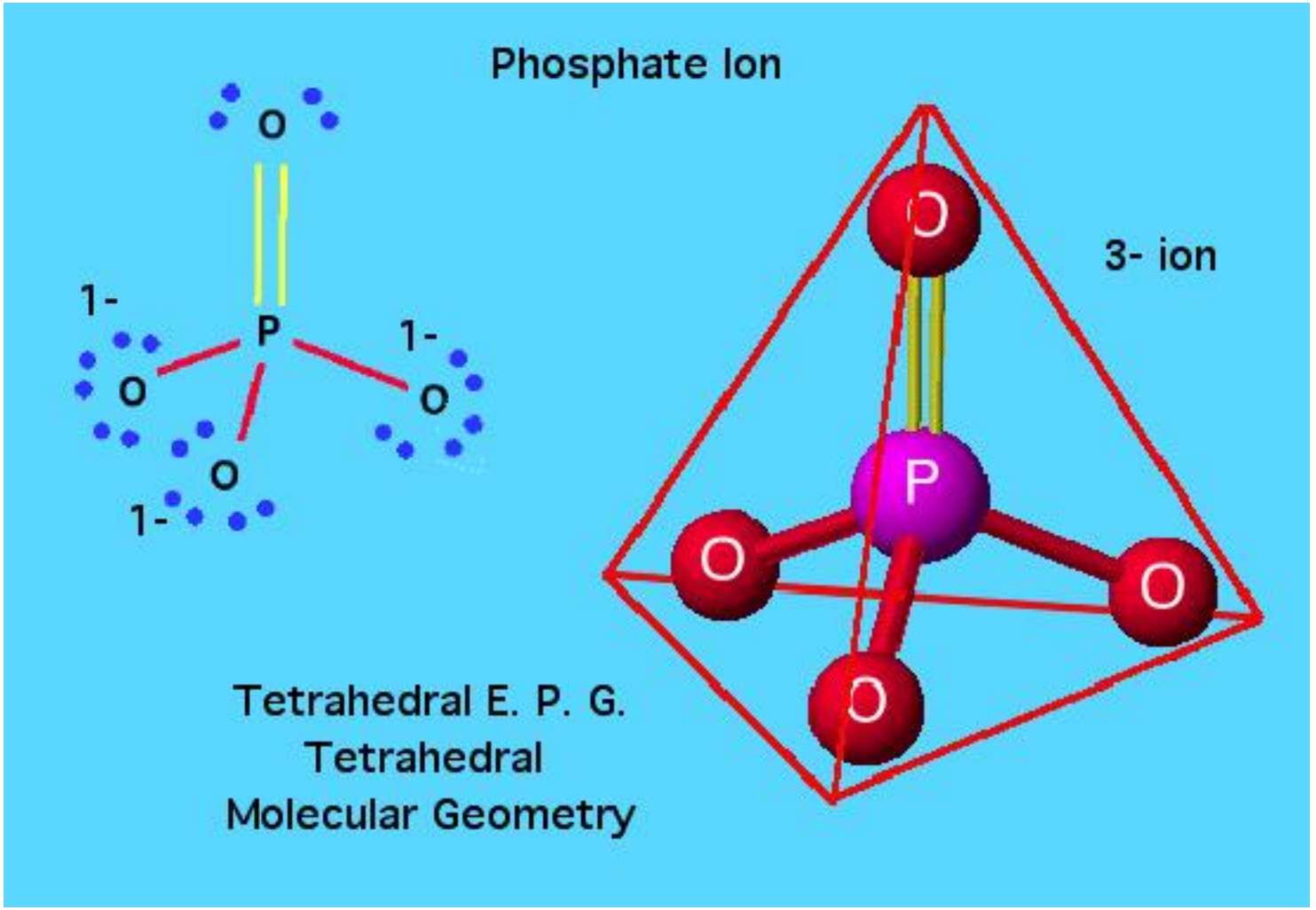
KINASE AND PHOSPHORYLATION REGULATION

Dr. Zhiyi Wei
SUSTC

Phosphate


Phosphate Ion
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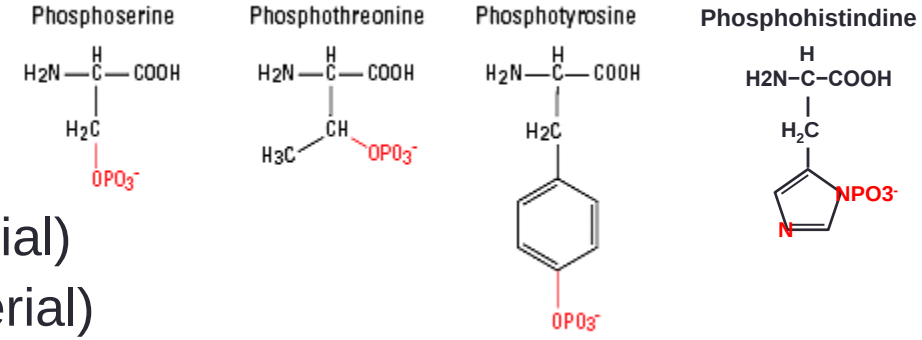
Tetrahedral E. P. G.
Tetrahedral
Molecular Geometry

Reversible phosphorylation

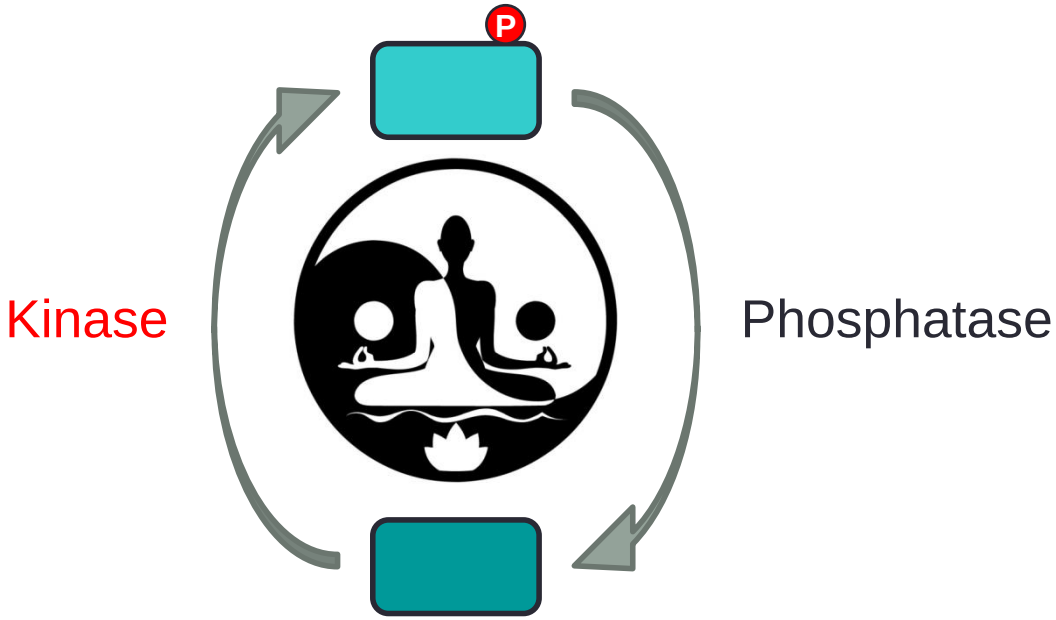
- The commonest form of covalent modification on proteins
  - Almost every protein in human can be modified by phosphorylation
- Phosphorylation in amino acids:
  - **Serine**
  - **Threonine**
  - **Tyrosine**
  - Histidine (bacterial)
  - Aspartate (bacterial)



- Phosphorylation is reversible
  - A widely used regulatory mechanism for intracellular signaling pathways
  - Responses can be turned off rapidly as well as turned on
- Kinase signaling cascade

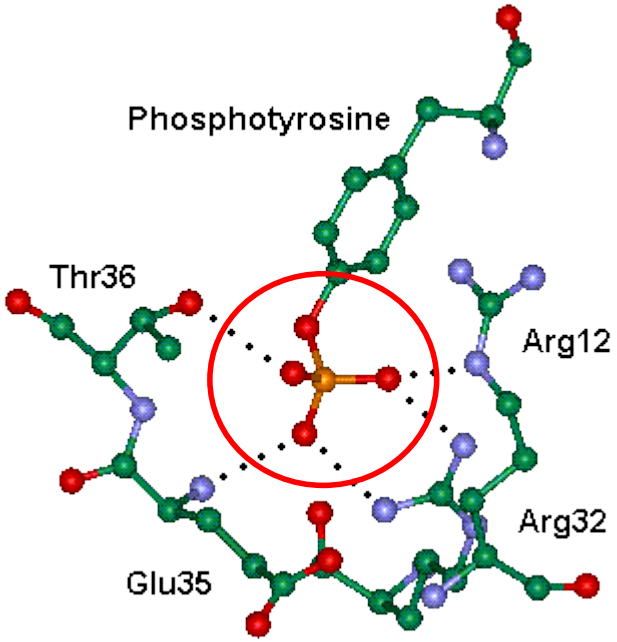
Phosphorylation switch

- Phosphorylation is the most important covalent switch mechanism for the control of protein function
  - Protein kinase: phosphorylation
  - Protein phosphatase: dephosphorylation
- Phosphorylation switch controls many diverse cellular processes including metabolic pathways, signaling cascades, intracellular membrane traffic, gene transcription, and movement



Biochemical effects by phosphorylation

- Covalent addition of a phosphoryl group to the side chain of serine, threonine, tyrosine, histidine or aspartic acid
- Phosphorylated residues have acquired a group that carries a double negative charge and is capable of multiple hydrogen-bonding interactions
  - Hydrogen bonding to main-chain amide groups at the positively polarized amino-terminal end of an alpha helix
  - Salt-bridging to one or more arginine residues



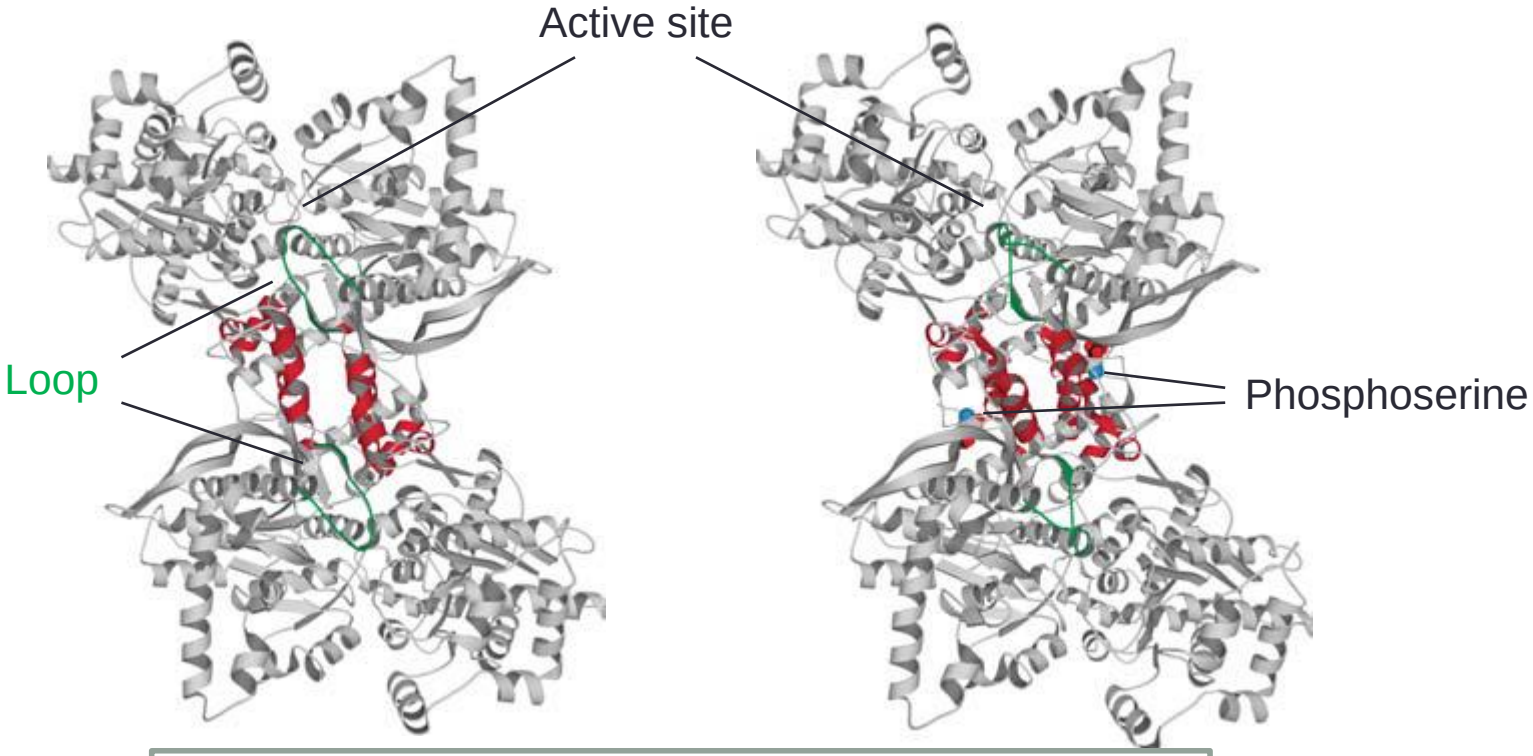
The phosphate group of phosphotyrosine forms several hydrogen bonds with the SH2 domain

Functional effects by phosphorylation

- Phosphorylation can affect the target protein in two ways

1. To change the activity of the target protein, either considerably or subtly
   - may come about solely from the added bulk and charge properties of the phosphoryl group, or may result from a large conformational change in the protein, or both
2. To provide a new recognition site for another protein to bind
   - Such protein–protein interactions usually involve specialized interaction domains on the second protein that recognize the phosphorylated peptide segment
   - pThr/pSer recognition domain: WW, WD40, FHA
   - pTyr recognition domain: SH2, PTB, GK

Phosphorylation activating glycogen phosphorylase

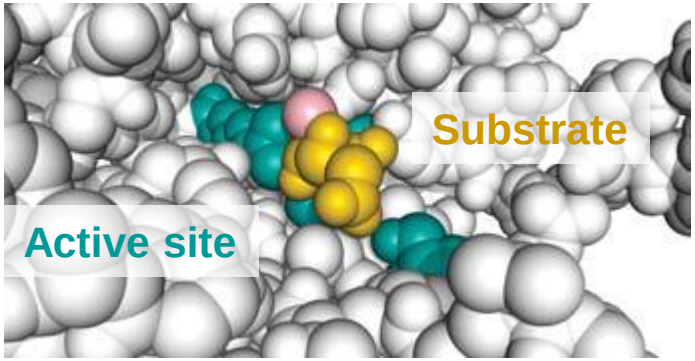


Phosphorylation can modulate protein activities by inducing large conformational changes

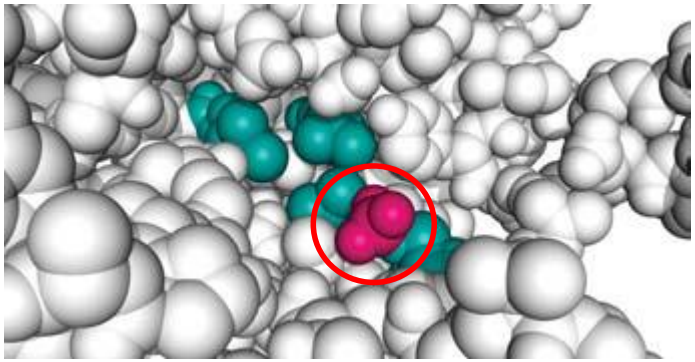
- Muscle glycogen phosphorylase is a homodimer
- A loop that sterically restricts access to the active site
- The enzyme is phosphorylated in a serine and activated in response to hormonal
- The phosphorylation results in a rearrangement of interface residues
- The loop moves out, making the substrate-binding site more accessible

Inactivation of isocitrate dehydrogenase by phosphorylation

Substrate bound state



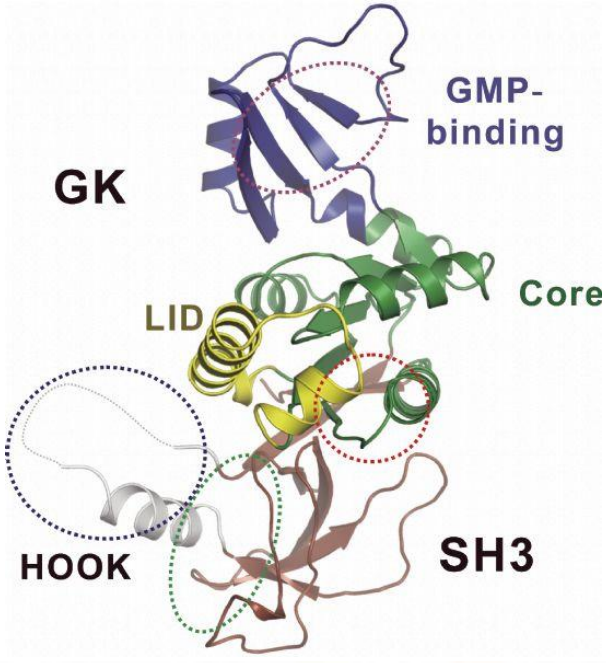
Phosphorylated state



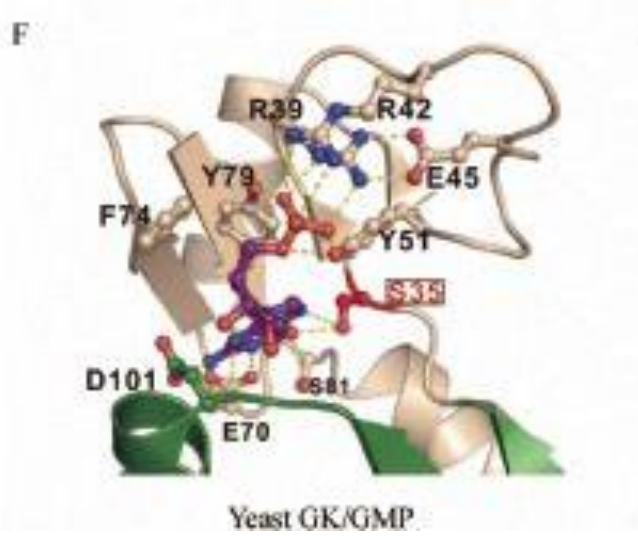
- Isocitrate dehydrogenase is a TCA cycle enzyme
- The phosphorylation of the enzyme involves no conformational changes
- Serine 113, the residue phosphorylated, is located in the active site
- Attachment of a phosphoryl group inhibits binding of the negatively charged substrate isocitrate by steric exclusion and electrostatic repulsion
- The phosphoserine is stabilized by helix dipole and main-chain hydrogen-bond interactions

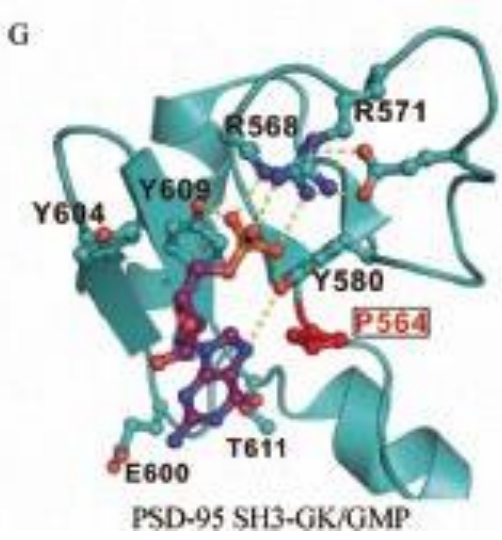
Phosphorylation in active site directly blocks the enzyme activity, needing no conformational changes

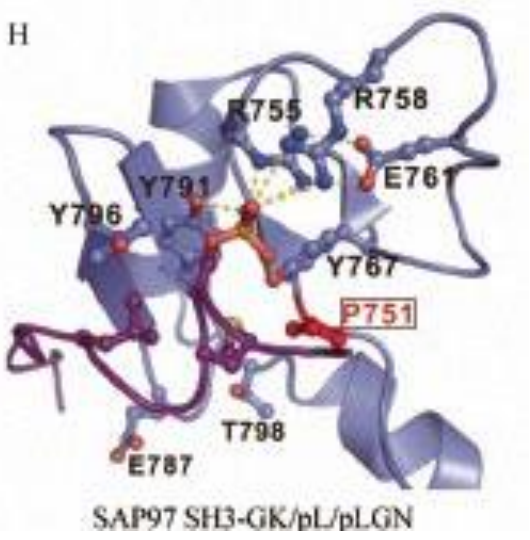
GK domain, a specific phospho-protein interaction module



- Membrane associated guanylate kinases (MAGUKs) are a family of scaffold proteins
- Every member of the MAGUK family contains a guanylate kinase-like (GK) domain
- GK domains in MAGUKs contain no enzymatic activity (pseudo-kinase)
- Evolving from the enzyme catalyzing GMP to GDP conversion to become a protein–protein interaction module
- Recognizing pSer/pThr sequences



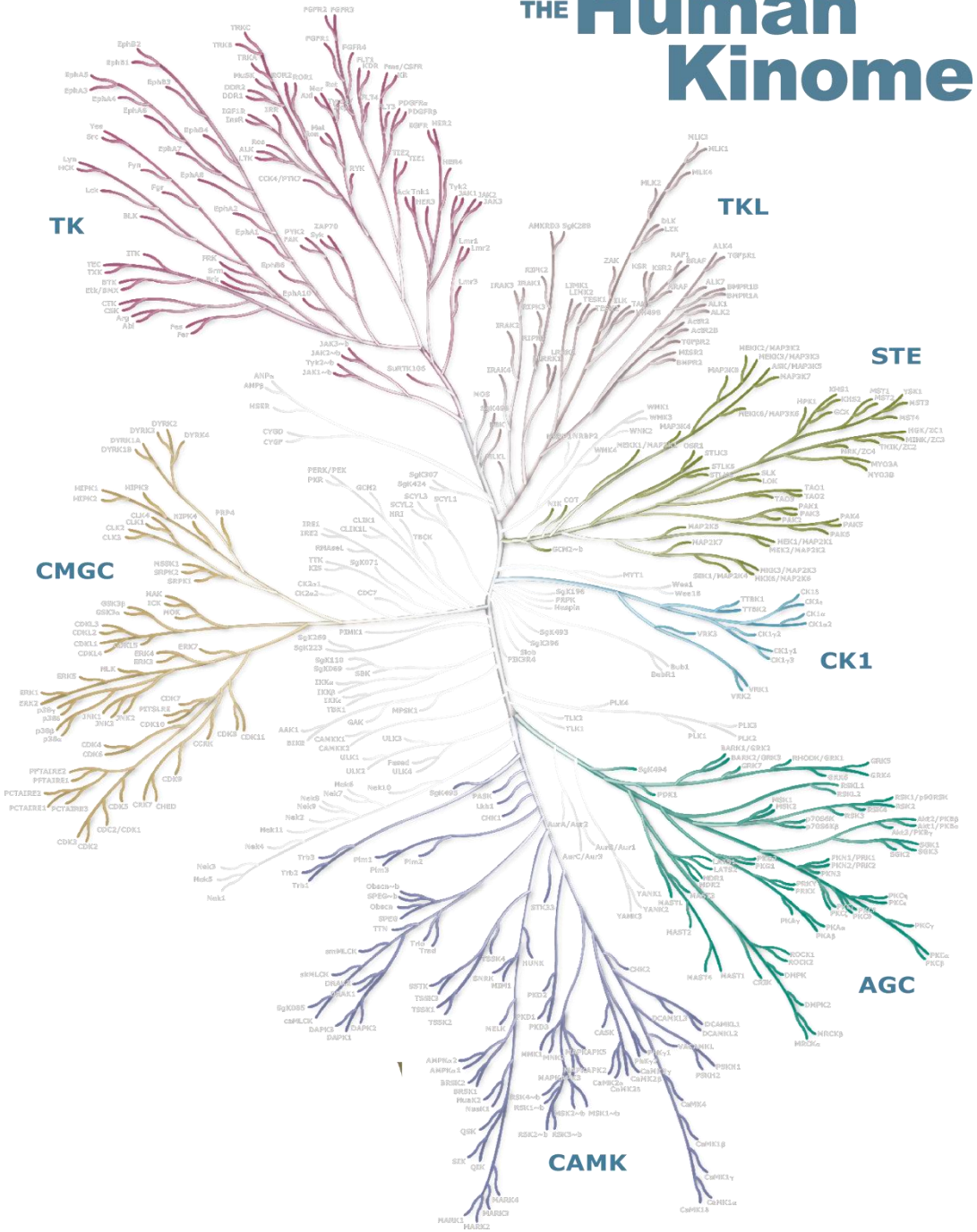




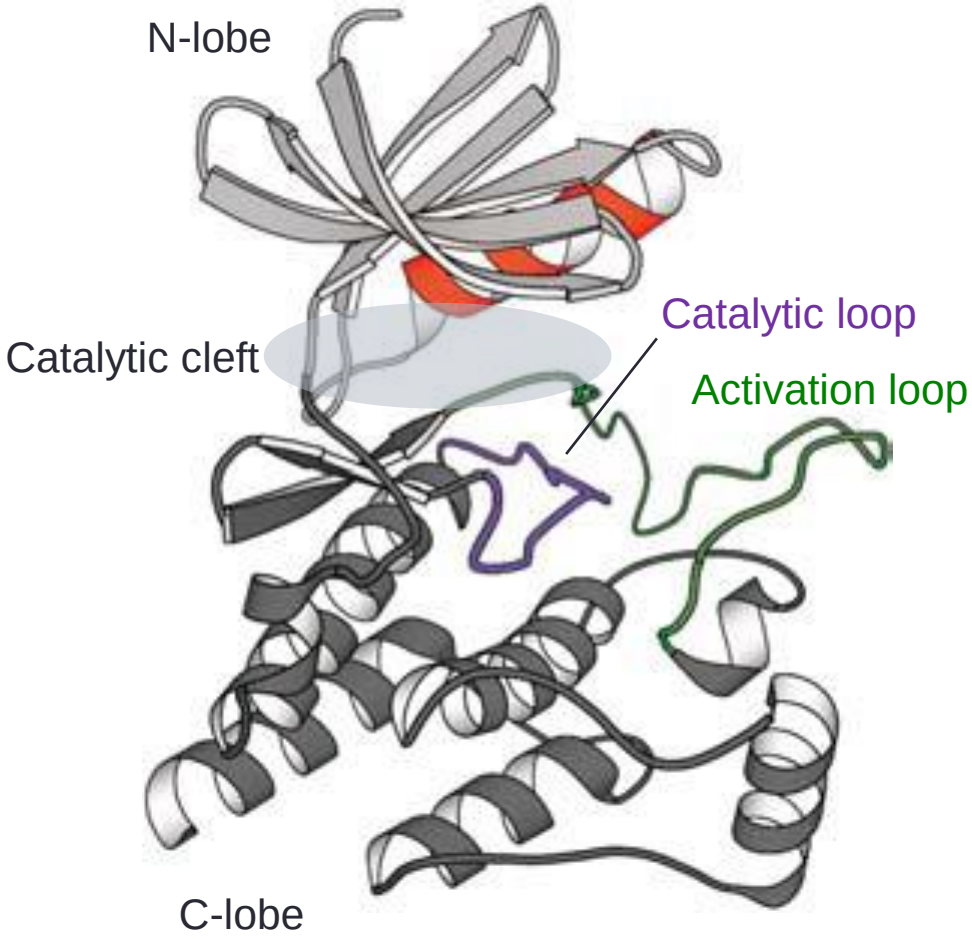
Kinase

- Kinases constitute the third most common domain encoded in the human genome sequence
  - Kinase domain
  - ~600 kinases (2% of genome)
- Kinases have low substrate specificity
  - The number of identified human protein phosphorylation site is more than 100,000
- Kinome
  - The kinome of an organism is the set of protein kinases in its genome
- The protein kinases responsible for phosphorylating proteins on serine, threonine and tyrosine residues all have the same fold for the catalytic domain
  - Containing several conserved segments that are required for kinase activity

THE Human Kinome



The structure features of kinase domain



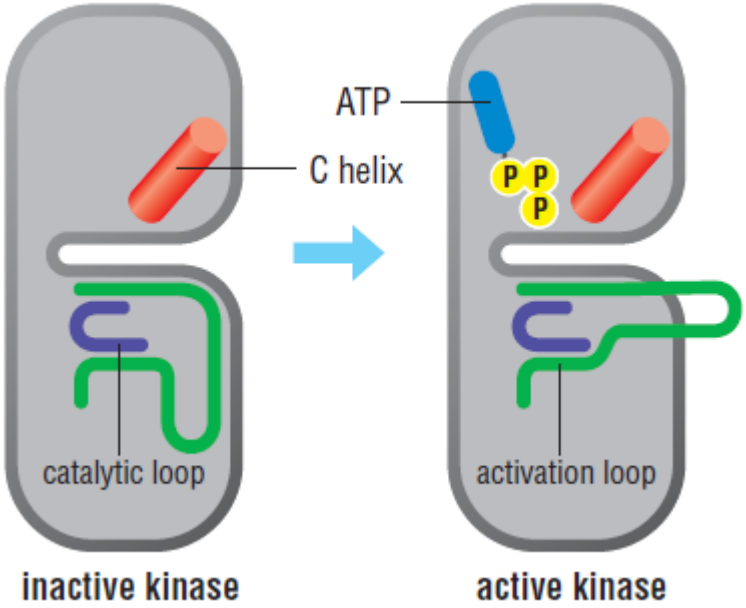
The kinase domain of Lck

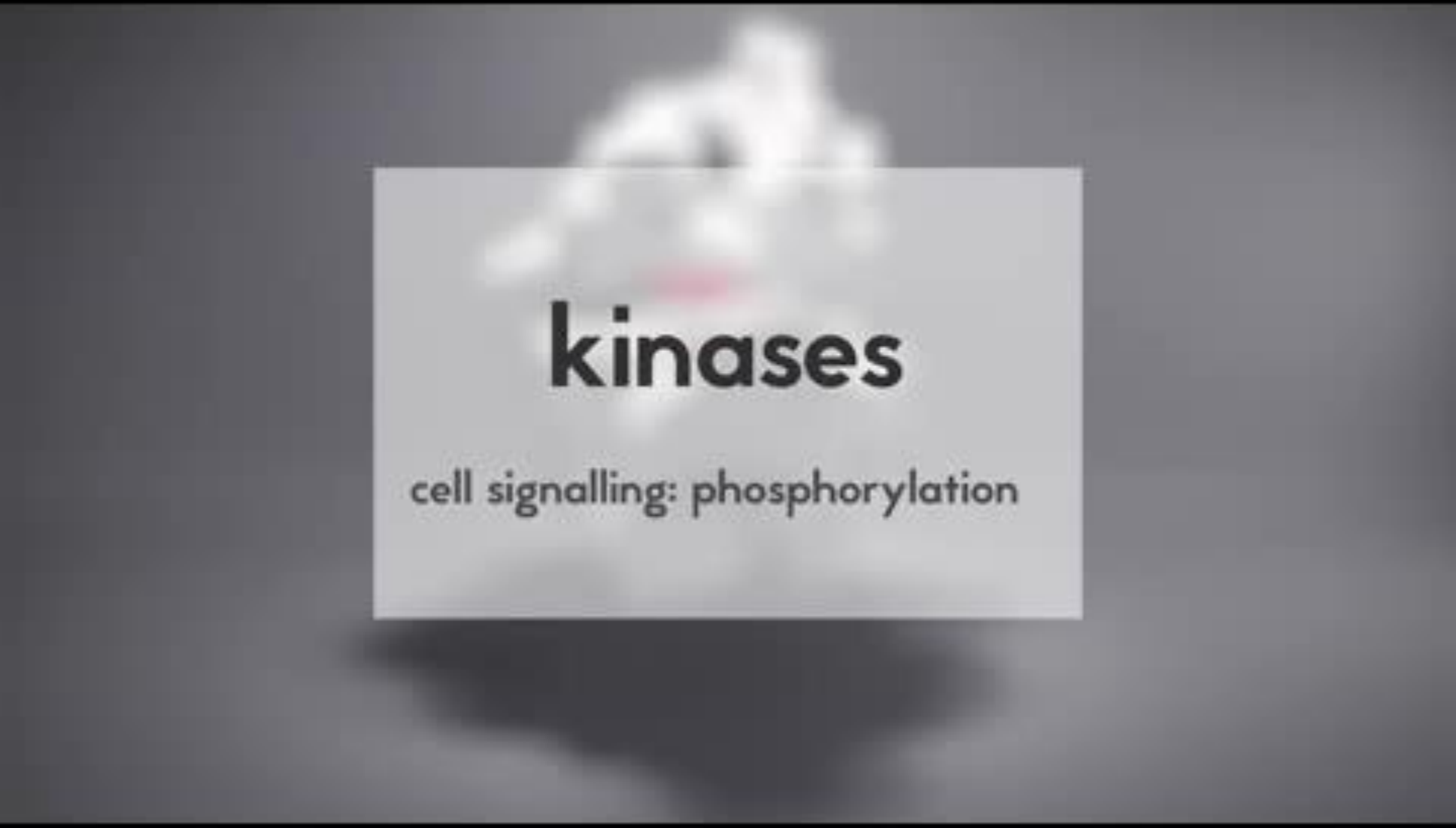
cAMP dependent protein kinase (Protein kinase A)

Mariko Hayashi Ph.D. 2012.3.16.

Activation mechanism of kinase

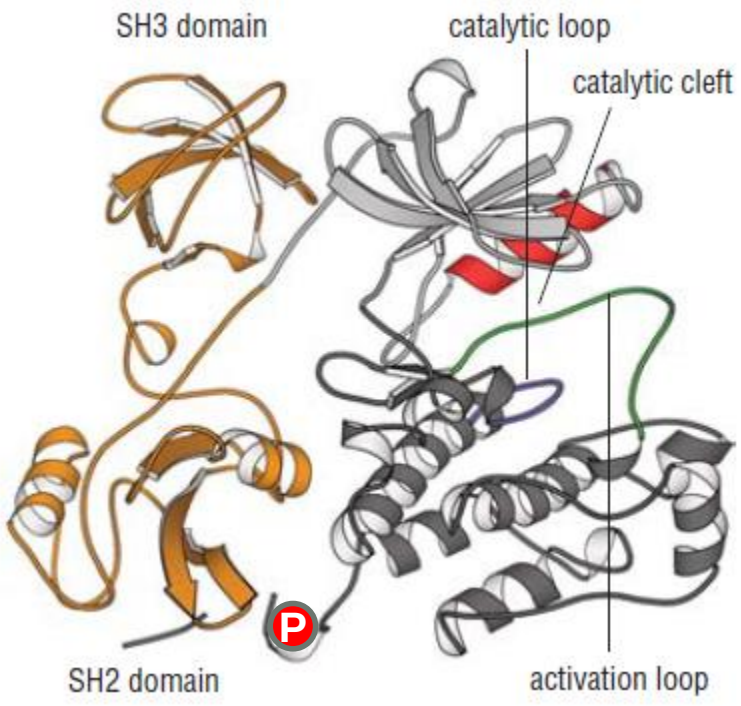
- Most kinases are normally inactive
- Protein kinases are themselves controlled by phosphorylation
  - Activation requires phosphorylation of a Thr or Tyr residue that is located in a region termed the activation segment or activation loop
  - Usually, in the inactive state, the activation loop blocks access to the active site
  - The formation of signal transduction cascades




kinases
cell signalling: phosphorylation

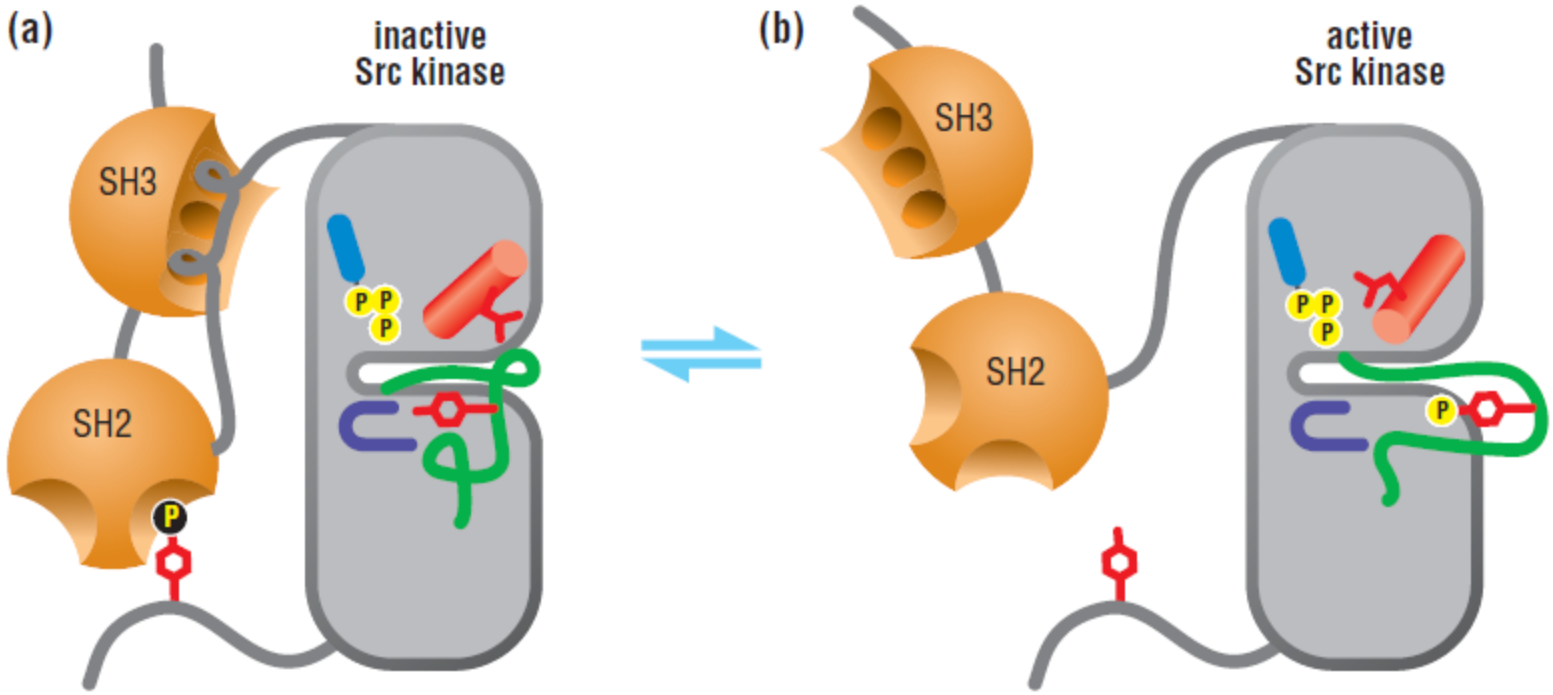
Src kinases

- Src-family kinases are activated early in many signaling pathways via autophosphorylation, providing for a large amplification of the signal
- Unregulated autophosphorylation leads to a sustained growth signal, resulting in tumorigenesis
- In the absence of activating signals, the SH2 and SH3 domains bind to the kinase domain, holding it in an inactive conformation
- The SH2 domain binds to an inhibitory phosphate on a tyrosine close to the protein carboxyl terminus
- The linker region joining the SH2 domain to the catalytic domain forms a polyproline helix to which the SH3 domain can bind, which clamps the catalytic domain in an inactive state



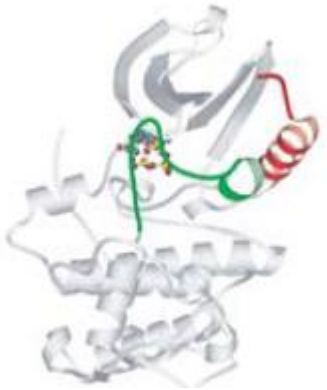
Kinases can both activate and inhibit themselves

Activation mechanism of Src

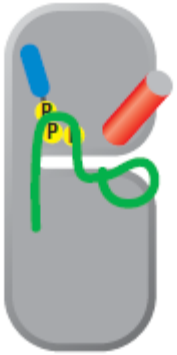


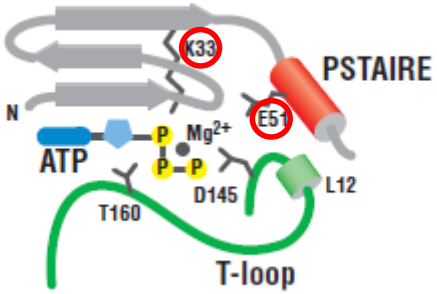
Dephosphorylation of the tyrosine on the carboxyl tail of the protein, or binding of the SH2 domain by a phosphotyrosine on the cytoplasmic tail of an activated receptor tyrosine kinase

Cyclin-dependent kinases (Cdks)



- Cdks are the enzymes that drive the cell cycle
- Cdks are activated periodically during the cycle
- In the inactive (unphosphorylated) state, Cdk2 is autoinhibited by the activation loop (T-loop), which partially blocks the ATP-binding site





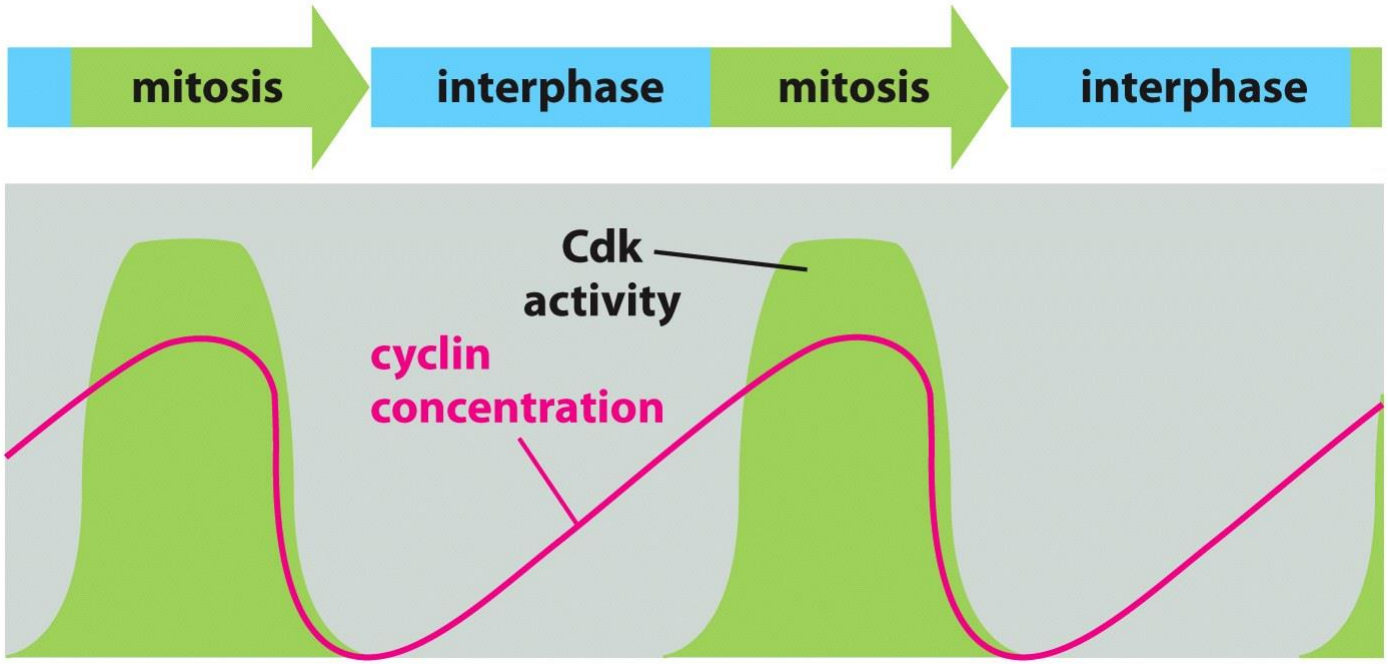
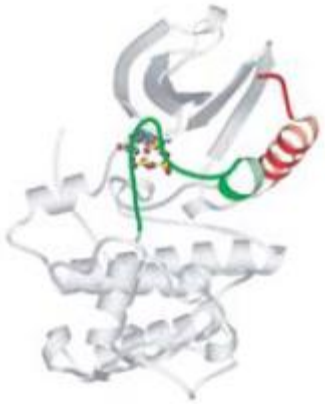


Figure 18-5 Essential Cell Biology 3/e (© Garland Science 2010)



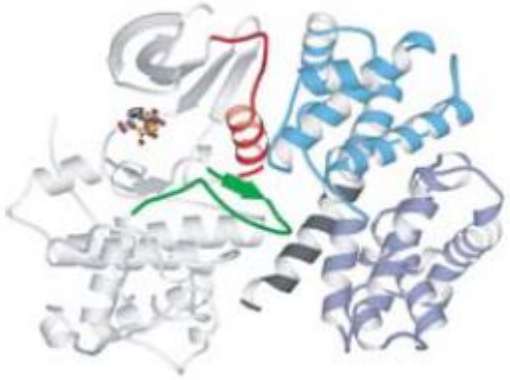
Activation mechanism of Cdks

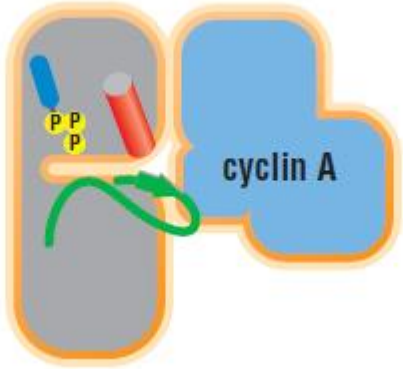
The kinase activities are highly controlled by multiple factors, providing exquisite control of protein function

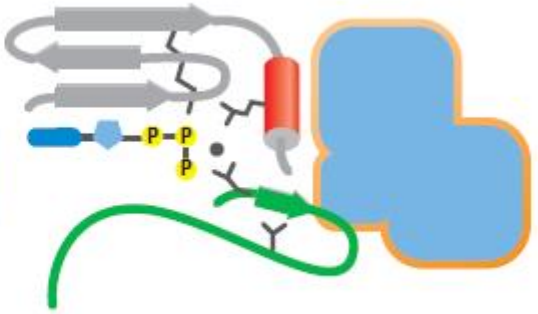
Phosphorylated Cdk2: 0.3% active

Cyclin bound, unphosphorylated Cdk2: 0.3% active

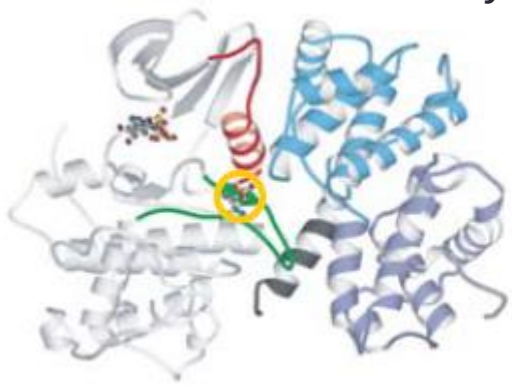
Cdk2 + cyclin A

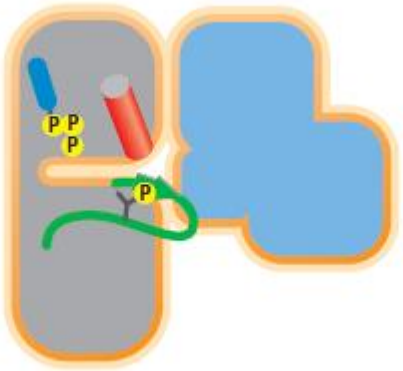


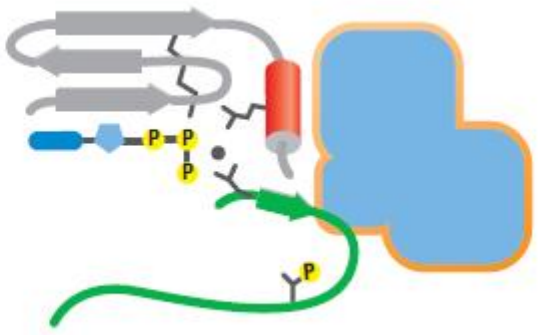




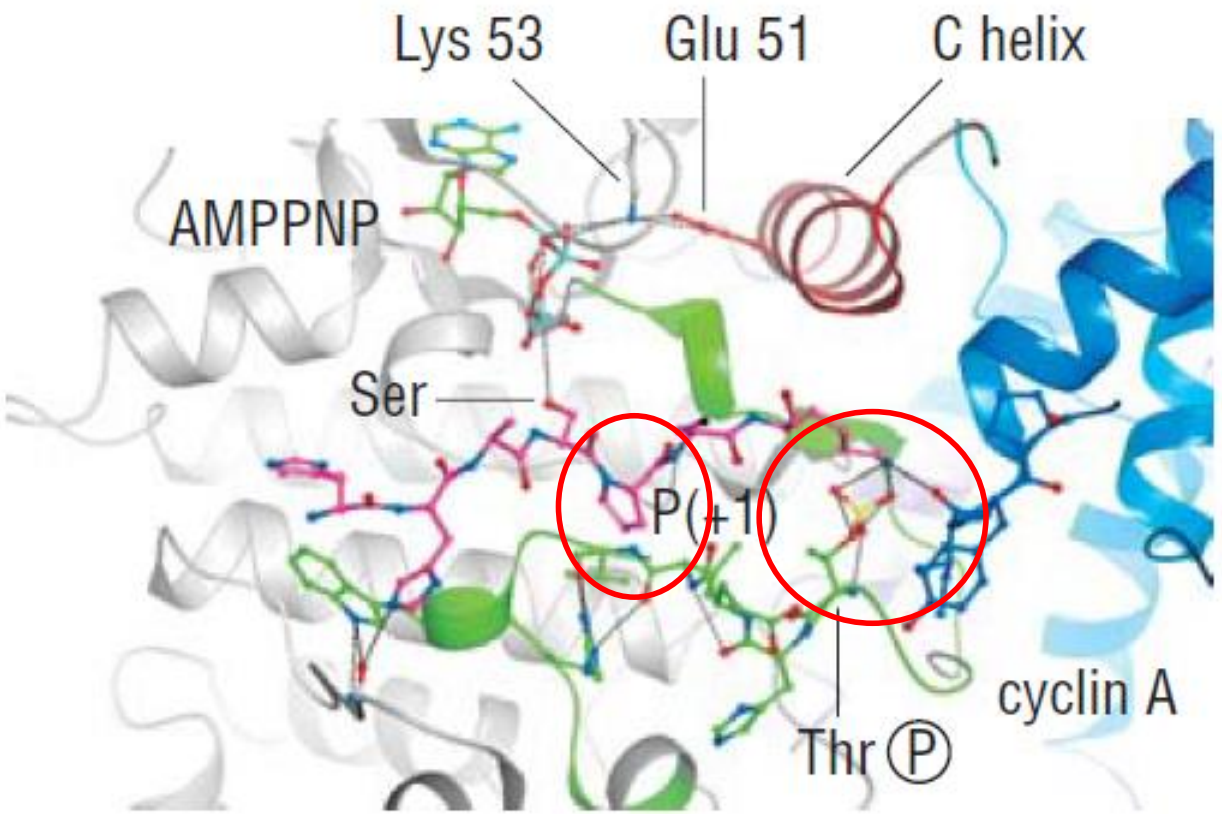
Cdk2 + cyclin A + Thr160 phosphorylation







Substrate specificity of Cdks



- Cdk2 recognizes the sequence **S**PX**R/K**
- This peptide segment binds in an extended conformation across the catalytic cleft and primarily makes contact with the activation loop
- In the unphosphorylated Cdk2–cyclin complex, the conformation of the activation segment does not allow the proline residue to fit properly into the active site
- Any other amino acid other than Pro would have its –N–H to be an unsatisfied hydrogen bond donor