Protein Kinase Structure, Activation, and Inhibition

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I. Background  
What protein kinases do  
On a molecular level  
On a cellular level  
Classification and phylogeny  
Regulation of protein kinases

II. Protein kinase structure

III. Protein kinase activation

IV. Inhibition of protein kinases
What protein kinases do on a molecular level

Transfer of ATP’s γ-phosphate to hydroxyl group

Unidirectional

State of p’n depends on kinase ↔ phosphatase

P’n affects proteins:
1. Phosphorylation-dependent conformational transition
2. Phosphorylation-dependent epitopes for protein-protein interactions

Signal transduction through phosphorylation-dependent protein-protein interactions

Phospho-epitope binding domains:

- SH2 binds PTyr (tyrosine kinase signaling)
- PTB binds PTyr (tyrosine kinase signaling)
- 14-3-3 binds PSer/PThr (various pathways)
- BRCT binds PSer (DNA repair)
- FHA binds PThr (various pathways)
- F-box binds PSer/PThr (poly-ubiquitination)
Role of protein kinases in cellular function

Reversible phosphorylation controls the activity, structure, and cellular localization of many proteins

More than 1/3 of the 10,000 or so proteins in a typical mammalian cell are thought to be phosphorylated at any given time

Important in cell cycle and signal transduction from plasma membrane to nucleus

Protein kinase classification and phylogeny

The human kinome: 518 protein kinases
- 1.7% of genome
- 244 map to disease loci
- 164 map to cancer amplicons

Phylogeny
- Primarily by sequence comparison of catalytic domains (also noncatalytic sequence/domain structure, biological functions, conservation)
- Gain insight into kinase evolution, function
- Infer functions of human kinases from family members in model organisms

Non-catalytic domains
- 83 domains in 258 of 518 kinases
- Regulate kinase activity, link to other signaling modules, subcellularly localize
- Receptor kinases
Regulation of protein kinase activity

- **Allostery**: non-covalent binding at site other than active site

- **Non-competitive inhibition**: covalent changes (often addition or removal of a phosphate group) to protein that make kinase inactive

- **Competitive inhibition**: when a non-substrate binds at active site and blocks access of substrate there

- **Not mutually exclusive**

Regulation of protein kinase activity: PKC
Protein kinase structure

3-D structure of a typical eukaryotic protein kinase catalytic domain

How kinases differ:
- Short sequences inserted in loops
- Sequences on either side of kinase domain
- Regulatory sequences

Conserved kinase core:
- Two lobes
  - N: smaller, β sheet, C helix,
  - C: larger, helical
- Hinge, ATP-binding site

Catalytic site:
- Gly-rich ATP-binding loop
- Catalytic loop HRD motif
- Activation segment DFG motif

Protein kinase activation:
insights from structure

Surface comparison of active and inactive protein kinases identifies a conserved activation mechanism

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Mechanism of Activation: Act 584 (2008) 258–263
Surface comparison method based on graph theory

Protein represented by graph:
- Vertices = AAs
- Residue type
- Orientation of Cα-Cβ vectors

Comparison of two graphs:
1. Each graph → set of subgraphs of pairs of vertices (an edge) for all pairs whose Cα atoms are within a predefined distance (1 nm), considering only water-accessible residues
2. Two edges similar if residue pairs match chemically & spatially
3. Matching pairs of edges combined into connected graphs representing similarity of protein surfaces

- Residues known to play important roles in catalysis have numerous connections on the graph

Surface comparison of two active kinases (PKA and Cdk2)

Able to detect most conserved, functionally important residues:
- Yellow = catalytically important residues known to be in contact with ATP/substrate
- Gray = secondary structures and residues known to be involved in regulation of catalytic activity
- Black arrows = hydrophobic interactions between HRD, DFG, C helix
- Dashed lines = important polar contacts
Surface comparisons of active and inactive kinases

Reason for conservation of DFG Gly

DFG Motif: Active Inactive
Conserved Mg-binding loops of 23 active kinases
Surface comparisons of active and inactive kinases

A hydrophobic spine is assembled in active kinases

- Present in all 23 active kinase structures examined
- Can coordinate rocking movements of N & C lobes in active conformation during catalysis
- Not a 1st sequence or particular 2nd structure, so could only have been detected by a structural analysis method
General model of protein kinase activation

1. Phosphorylation primary driving force for activation segment movement.
2. Cascade of conserved H-bonds connect to catalytically important DFG D.
3. DFG G acts as bipositional switch to induce correct orientation of D.
4. Activated configuration of DGF F facilitates inward movement of αC-helix, locking spine in place. K72-E91 salt bridge also secures spine.
5. Motions of two lobes thus become coordinated for precisely positioning ATP bound to the N lobe with respect to the catalytic site on the C lobe.

Protein Kinase Inhibitors: Insights into Drug Design from Structure

I. rTKs, non-rTKs, Ser/ThrKs

II. Structural insights for targeting active vs inactive form, entire ATP site vs other less conserved pockets or single residues, noncatalytic domains

- Diseases including cancer, diabetes, inflammation linked to PK signaling
- ATP-binding pocket has been focus
- Selectivity by exploiting differences in kinase structure & pliability
- All stages of signal transduction have been targeted
Kinases as drug targets: rTKs

Herceptin
- mAb
- receptor internalization
- effective alone for 15% of HER2-overexpressing patients
- small molecule inhibitors
- Bind TK domain
- NSCLC

Kinases as drug targets: non-rTKs

Abl kinase missing N-term, important for autophosphorylation
- approved 2001
- Remission in nearly 100% of patients in early stages
Kinases as drug targets: Ser/ThrKs

Signal-transducing serine-threonine kinases

Kinases as drug targets: CDKs
Principles for structure-based lead optimization

- Targeting a unique inactive conformation
- Targeting the global constellation of residues w/in the ATP site
- Targeting less conserved additional pockets
- Targeting single residues
- Targeting noncatalytic domains

Targeting a unique inactive conformation

Active kinases are all alike, but inactive kinases are different

- Gatekeeper residue
- Hinge region
- DFG's F
- CDK2/ATP
- Abl/Gleevec

Reference:
ATP binding to fully active CDK2. Gleevec binds inactive Abl

- PD173955
  - more potent than Gleevec
  - perhaps binds both conformations
  - but also less selective

Tarceva binds active EGFR
Targeting the global constellation of residues within the ATP site

Selectivity of RhoK inhibitor H-1152P likely due to its interaction with a combination of residues at the ligand-binding site
→ Sequence alignment of 491 kinases showed that only 6 kinases have the same combination of residues at the ATP site as RhoK
→ Individual residues show considerable conservation.

Targeting less conserved additional pockets

Reference: ATP binding to fully active CDK2
Phenyl ring extends into C-lobe pocket below ATP pocket exploited by several CDK-selective inhibitors
Acetylene directed into pocket guarded by gatekeeper Thr766
Targeting single residues

UCN-01’s 7-hydroxy group H-bonds with protein side chain

SU5402’s carboxyl H-bonds with side chain of Asn568 at end of hinge

Targeting noncatalytic domains

Herceptin binds HER2 extracellular domain
- close to the juxtamembrane region
- may allow engagement with the endocytic machinery while avoiding kinase activation
Future of structural studies in anti-kinase drug design

- Most important consideration in drug design: target selection
- Kinome-scale structure determination
  - Target selection
  - Inhibitor specificity
- Target kinases from Mycobacterium tuberculosis, Plasmodium falciparum
- Methods to shortcut lead compound ID
- Target less conserved noncatalytic domains
- Mitigate disease resistance to anti-kinase therapy

Future challenges in protein kinase field

- Prediction of regulatory mechanisms for ePKs based on their primary amino acid sequences
- Kinome-wide analyses of cellular responses:
  - How many kinases are expressed, depressed, activated, inactivated, in a given biological condition?
  - How many kinases are involved in a given biological response?
- Live cell assays for kinase functions: specificity, subcellular location and translocation, real-time measurements.