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POST-TRANSLATIONAL MODIFICATIONS AT THE ATP-POSITIONING G-LOOP THAT REGULATE PROTEIN KINASE ACTIVITY

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Abstract

Protein kinases are a superfamily of enzymes that control a wide range of cellular functions. These enzymes share a highly conserved catalytic core that folds into a similar bilobar three-dimensional structure. One highly conserved region in the protein kinase core is the glycine-rich loop (or G-loop), a highly flexible loop that is characterized by a consensus GxGxxG sequence. The G-loop points toward the catalytic cleft and functions to bind and position ATP for phosphotransfer. Of note, in many protein kinases, the second and third glycine residues in the G-loop triad flank residues that can be targets for phosphorylation (Ser, Thr, or Tyr) or other post-translational modifications (ubiquitination, acetylation, O-GlcNAcylation, oxidation). There is considerable evidence that cyclin-dependent kinases are held inactive through inhibitory phosphorylation of the conserved Thr/Tyr residues in this position of the G-loop and that dephosphorylation by cellular phosphatases is required for CDK activation and progression through the cell cycle. This review summarizes literature that identifies residues in or adjacent to the G-loop in other protein kinases that are targets for functionally important post-translational modifications.

Keywords

protein kinase; phosphorylation; ATP-positioning G-loop; post-translational modifications; oxidative stress

1. INTRODUCTION

Protein kinases play a central role in a large number of physiologic processes and they are implicated in the pathogenesis of many diseases. These factors have fueled interest in (and considerable investment by) both academia and the pharmaceutical industry toward solving the three-dimensional structures of many of these enzymes¹. We now recognize that eukaryotic protein kinases fold into a highly conserved bilobar structure, with a smaller mainly β -stranded N-lobe connected by a short flexible hinge region to a larger mainly α -helical C-lobe (Figure 1A). ATP is sandwiched in a deep cleft between the N- and C-lobes of this kinase domain structure; the substrate-binding site also is located in the cleft between the two lobes (Figure 1B).

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The structural and functional properties of many protein kinases are fine-tuned by phosphorylations at key sites within the kinase domain². For example, members of the AGC superfamily of eukaryotic protein kinases undergo a series of ordered ‘priming’ or regulatory phosphorylations at conserved motifs in the activation loop and C-terminus. Phosphorylations at these sites play a critical role to stabilize the catalytically competent conformation of the enzyme and regulate kinase activity. The notion that post-translational modifications at other positions strategically located in the structure can influence protein kinase function is not generally considered. This review focuses on recent studies indicating that the Gly-rich loop (or G-loop) constitutes an additional target for regulatory phosphorylation (or other regulatory post-translational modifications) on certain protein kinase enzymes.

The G-loop motif (characterized by the consensus GxGxxG sequence) connects the β 1 and β 2 strands of the N-lobe. The G-loop functions as a nucleotide-positioning motif to anchor ATP in an orientation that is optimal for catalysis and to shield bound nucleotide from solvent³. The three glycine residues in the G-loop (residues that impose minimal steric interference) make the G-loop one of the most flexible elements in the catalytic core. These glycine residues are highly conserved across protein kinases, with the first glycine present in ~95% of kinases, the second in more than 99% of kinases, and the third conserved in ~85% of kinases (with replacement of this least conserved third position glycine largely restricted to small amino acids such as alanine or serine³). Mutations localized to glycine residues in this triad typically disrupt G-loop conformation and/or sterically interfere with ATP binding and are not – or are only poorly – tolerated⁴. Mutations at these sites that disrupt kinase activity have been implicated in certain human diseases. For example, a G75V substitution at the first position in the triad of RSK2 has been implicated in the pathogenesis of Coffin-Lowry syndrome (an X-linked disorder characterized by severe psychomotor retardation, facial and digital dysmorphisms, and progressive skeletal deformations⁵) and Gly-Glu or Gly-Arg substitutions at this position in tropomyosin receptor kinase A (TRKA, the receptor tyrosine kinase for nerve growth factor) is implicated in congenital insensitivity to pain with anhidrosis (CIPA) syndrome^{6,7}. A Gly-Arg substitution in protein kinase D1 has been implicated in syndromic-congenital heart disease (a disorder that presents with atrioventricular septal defects, developmental delay and limb abnormalities⁸). Finally, a Gly-Val substitution at the third glycine in the triad in InsR disrupts activity and causes diabetes⁹, a Gly-Arg substitution at this position in c-Kit inhibits activity and causes piebaldism (an autosomal dominant disorder caused by defective proliferation or migration of melanocytes from the neural crest during early development¹⁰), and a Gly-Arg substitution at this position in the *VEGFR3* (*FLT4*) gene that encodes vascular endothelial growth factor receptor 3 (VEGFR3) is implicated in Milroy disease (an autosomal dominant inherited form of primary lymphedema¹¹).

Residues between the second and third glycines in the GxGxxG triad extend outward toward the catalytic cleft, sit in close proximity to the substrate peptide recognition regions of the protein kinase, and also can be critical (Figure 1B). There is growing evidence that these G-loop residues (which have been characterized as hot spots for cancer-driving or drug resistance mutations) are targets for post-translational modifications (PTMs) that control the binding, positioning and/or recognition of substrate (Figure 1C). This review summarizes

recent literature that identifies PTMs at the G loop as an underappreciated mechanism to regulate many protein kinase activities.

2. Cyclin-dependent kinases (CDKs)

CDKs are a family of serine/threonine kinases whose activity is influenced by a cyclin – a protein regulatory subunit named for its oscillatory pattern during the cell cycle. CDKs play a critical role in eukaryotic cell division and transcription. Detailed descriptions of the classification and function of the many CDK family members as regulators of cell-cycle progression have been published¹² and go beyond the scope of this review, which focuses on one specific aspect of CDK regulation involving G-loop phosphorylation.

CDKs were the first enzymes shown to be regulated through G-loop phosphorylation. CDKs are held inactive by phosphorylation at Tyr¹⁵ and to a lesser extent the adjacent Thr¹⁴ in the G-loop GEGTYG motif^{13, 14}. Thr¹⁴/Tyr¹⁵ phosphorylation (by the related Wee1 and Myt1 kinases) does not lead to major changes in CDK structure, but rather reduces the affinity of CDK for substrates and produces an unproductive binding mode for ATP¹⁵. Progression through the cell cycle requires activation of dual specificity cell division cycle 25 (Cdc25) phosphatases that dephosphorylate these two residues and thereby activate CDK-cyclin complexes.

3. Protein kinase C (PKC)

3.1 PKC δ

PKC δ is a serine/threonine kinase that plays a key role in signal transduction pathways that control a wide range of normal cellular responses and also contribute to the pathogenesis of ischemia reperfusion injury^{16, 17}.

Like other PKC isoforms, PKC δ contains a highly conserved C-terminal catalytic domain and an N-terminal regulatory domain consisting of a lipid-binding C1 domain and a C2 domain. While most C2 domains function as membrane-targeting modules (being calcium-sensitive in the case of conventional PKCs, or calcium-insensitive in the case of novel PKCs), the PKC δ C2 domain is a topological variant that does not bind lipids, but rather functions as a phosphotyrosine (pY) binding motif that binds proteins with a consensus sequence (Y/F)-(S/A)-(V/I)-pY-(Q/R)-X-(Y/F)¹⁸.

The conventional model for PKC δ activation by growth factor receptor pathways involves the generation of diacylglycerol, a second messenger that interacts with the lipid-binding C1 domain and anchors FL-PKC δ in an active conformation to membranes. This PKC δ activation mechanism accounts for the enzyme's membrane-delimited actions, but it does not adequately explain the full repertoire of PKC δ 's actions in other cellular compartments. Recent studies address this dilemma by showing that PKC δ is activated via a distinct lipid-independent mechanism during oxidative stress¹⁹. Oxidative stress leads to the activation of Src and Src-dependent phosphorylation of PKC δ at Tyr³¹³ a residue in the V3 hinge region of the enzyme that is flanked by sequence that conforms to a C2 domain consensus-binding motif (VGI-Y³¹³-QGF)²⁰. This results in an intramolecular interaction between the Tyr³¹³-

phosphorylated hinge region and the phosphotyrosine-binding C2 domain that controls PKC δ 's enzymology indirectly by inducing a long-range change in the phosphorylation status of Ser³⁵⁹, a site at the tip of the ATP-positioning G-loop in the kinase domain (GKGS³⁵⁹FG) ²¹. Further studies show that PKC δ is recovered from resting cells as a Ser³⁵⁹-phosphorylated enzyme that (when activated by lipid-cofactors) translocates to lipid membranes and acts as a serine kinase (i.e., shows a strong preference for substrates with a serine residue at the phosphoacceptor site). Oxidative stress triggers a redox-induced C2 domain-pTyr³¹³ docking interaction that results in a long-range conformational change that facilitates PKC δ -Ser³⁵⁹ dephosphorylation and converts PKC δ into a lipid-independent Ser/Thr kinase; the redox-activated PKC δ enzyme is poised to phosphorylate substrates with either serine or threonine residues at the phosphoacceptor site throughout the cell – not just on lipid membranes. These results implicate G-loop phosphorylation at Ser³⁵⁹ as a dynamically-regulated mechanism that regulates PKC δ 's lipid-requirement for activation, changes its phosphoacceptor site specificity, and calibrates its activation of signaling pathways that contribute to cellular responses. This alternate mode for PKC δ activation during oxidative stress (that is presumed to contribute to the pathogenesis of ischemic injury) could be specifically targeted for therapeutic advantage.

3.2 Other PKCs and PKA.

The G-loop Ser phosphorylation site in PKC δ is highly conserved in other PKCs and in PKA; there is evidence that this site is phosphorylated in PKA ²² and tentative evidence that this site is O-GlycNAcylated (serves as an acceptor for a single O-linked β -N-acetyl glucosamine – or O-GlcNAc - sugar molecule) in PKC α , PKC β , and/or PKC ϵ ²³ (Table 1). Studies to date, that examined the *in vitro* kinase activity of enzymes bearing single residue substitutions at the G-loop serine residue in PKA and PKC α , provide rather consistent evidence that G-loop phosphorylation plays a general role to inhibit PKA and PKC α activity ^{21, 24}. Effects on substrate specificity remain uncertain, since the early evidence that a G-loop modification can also function to alter the substrate specificity of PKA was not subsequently substantiated ^{21, 24}. The physiologic controls and *in vivo* functional consequences of G loop phosphorylation on these other enzymes have not been examined.

4. RAF

Oncogenic mutations in B-RAF are identified in ~60% of malignant melanomas and they occur with moderate to high frequency in certain other cancers (papillary thyroid carcinomas, colorectal cancer, non-small cell lung cancer, and hairy cell leukemia ²⁵). The V600E substitution in the activation loop (the 15–30 amino acid sequence flanked by the almost invariant DFG and APE motifs – a flexible loop that contributes to substrate recognition and functions to structure the enzyme for catalysis) accounts for ~80% of the cancer-associated B-Raf mutations, with other B-Raf mutations clustering in or adjacent to the activation loop or in the G-loop ^{26, 27}. The V600E substitution increases B-Raf kinase activity and leads to constitutive activation of the mitogen-activated protein kinase (MAPK) pathway in cells ²⁷. Structural studies suggest a mechanism for B-Raf activation by the V600E substitution. These studies identify a hydrophobic interaction between the DFG motif in the activation loop and the G-loop that stabilizes B-Raf in an inactive conformation.

This model predicts that a V600E substitution (or other V600D, V600K, or V600R charge substitutions commonly found in tumors) disrupts the activation loop-G-loop interaction and lead to B-Raf activation – in a manner that mimics the effect of growth factor stimuli that activate B-Raf at least in part by promoting B-Raf autophosphorylation at adjacent residues in the highly conserved activation loop T⁵⁹⁹VKS⁶⁰² motif ²⁸.

Certain mutations in the G-loop also increase B-Raf activity, but the mechanism appears to be less straightforward, since Ala, Val or Ser substitutions at the 3rd glycine residue in the G-loop increase activity, but a Glu substitution at the identical site decreases catalytic activity ^{25, 27, 29}. Hints that these phenotypes reflect an additional independent role of the G-loop to control B-Raf activity come from studies by Holderfield et al. showing that the two serine residues (GSGSFG) in the B-Raf G-loop are targets for inhibitory autophosphorylation. These authors proposed that under basal conditions B-Raf exists in an autoinhibited state as a result of constitutive G-loop autophosphorylation and that phosphatases reverse this phosphorylation during B-Raf activation ³⁰. This model predicts that certain oncogenic mutations in the G-loop (adjacent to the autophosphorylation sites) activate B-Raf by preventing inhibitory G-loop autophosphorylations – effectively bypassing the built-in autoinhibitory brake on enzyme activity ³⁰.

Finally, while B-Raf G-loop substitutions have been studied most intensively in models of clinically important cancers, it is worth noting that B-Raf G-loop mutations (S467A, F468S, and G469E) also have been identified in cardio-facio-cutaneous syndrome (a developmental disorder that has features similar to other Rasopathies – genetic syndromes such as Noonan's syndrome or Costello syndrome that are due to mutations in genes that alter signaling through the Ras pathway ³¹).

5. Abelson murine leukemia virus (Abl) proto-oncogene

The c-Abl proto-oncogene is a ubiquitous non-receptor tyrosine kinase that is activated by extrinsic ligands such as growth factor receptors or intrinsic signals such as DNA damage or oxidative stress. c-Abl shuttles between the cytosolic and nuclear compartments, phosphorylates a diverse set of cellular substrates (including adaptor proteins, other kinases, cytoskeletal proteins, transcription factors, and chromatin modifiers) and controls signaling pathways that influence actin polymerization and cytoskeletal remodeling, cell adhesion and cell motility, transcriptional regulation, the DNA damage response, and cellular apoptosis.

The c-Abl G-loop contains a tyrosine residue (Tyr²⁵³) that can be phosphorylated ^{32, 33}. While a single early study used a mutagenesis approach to show that Tyr²⁵³ phosphorylation functions to limit c-Abl activity (i.e., a Y253F substitution is sufficient to increase c-Abl activity ³⁴), subsequent literature has focused primarily on the role of this tyrosine residue - and a second tyrosine residue adjacent to the G-loop (Tyr²⁵⁷) - in the context of the BCR-Abl oncoprotein, a fusion protein that results from a translocation between the BCR (breakpoint cluster region) on chromosome 22 and the ABL1 gene on chromosome 9. c-Abl tyrosine kinase activity is tightly regulated in normal cells, but the BCR-Abl fusion protein is a constitutively active kinase that plays a central role in the pathogenesis of essentially all cases of Philadelphia (Ph) chromosome-positive (Ph⁺) chronic myeloid leukemia (CML) as

well as ~3–5% of pediatric acute lymphoblastic leukemia and 25% of adult acute lymphoblastic leukemias. The observation that the transforming ability of BCR-Abl is closely tied to its tyrosine kinase activity lead to the development of imatinib mesylate (also known as STI571 or Gleevec), a 2-phenylamino pyrimidine that targets the ATP binding site and stabilizes the Abl kinase-domain in an inactive conformation. Imatinib typically produces durable remissions in patients in the early more chronic phase of CML³⁵. However, advanced phase CML or blast crisis typically is characterized by the appearance of additional mutations in the BCR-Abl kinase domain that impair imatinib binding (i.e., mutations that result in escape from effective inhibition of BCR-Abl kinase activity³⁶). G-loop Y253F or Y253H mutations have been identified in a subset of patients with advanced CML and acquired imatinib resistance; these G-loop mutations (which eliminate the inhibitory break on enzyme activity) carry a dire prognosis as they typically are associated with a more aggressive phenotype and a shortened survival compared with other mutations^{37, 38}. A T315I substitution (at the gatekeeper residue in the kinase domain) is the more common mutation that confers resistance to imatinib as well as other 2nd generation Abl inhibitor compounds such as dasatinib³⁶. Of note, the T315I drug-resistant mutation has been detected in imatinib-naïve CML patients in blast crisis, suggesting that this mutation confers an oncogenic fitness advantage over the wild-type BCR-Abl allele. The precise underlying mechanism remains somewhat uncertain. While there is evidence that the T315I mutant displays very high transformation potency but little-to-no kinase activity when tested against a panel of typical Abl substrates, other studies suggest that a T315I substitution may alter the enzyme's substrate specificity, raising questions regarding the interpretation of studies that sample kinase activity with only a limited set of substrates³⁹. Of relevance to this review on G-loop modifications, there is evidence that the highly oncogenic T315I substitution leads to a high level of kinase domain autophosphorylation at Tyr²⁵⁷ (a site just N-terminal to the G-loop); c-Abl-T315I phosphorylation at Tyr²⁵³ is not detected, a finding that has been taken as tentative evidence that these phosphorylation events are mutually exclusive. These results have fueled speculation that an increase in Tyr²⁵⁷ phosphorylation forces an unfavorable G-loop conformation that limits access to Tyr²⁵³ kinases and prevents G-loop phosphorylation at Tyr²⁵³ (a PTM that inhibits kinase activity) This model predicts that strategies to enhance Tyr²⁵³ phosphorylation (by inhibiting the relevant phosphatase) or abrogate Tyr²⁵⁷ phosphorylation might be used to therapeutic advantage, particularly in patients harboring the drug-resistant T315I allele³⁹.

Finally, there is evidence that BCR-Abl contains several nuclear-localization signals but nevertheless is localized exclusively to the cytosol - in fact its oncogenicity requires its exclusion from the nucleus. Studies of mechanism indicate that BCR-Abl is retained in the cytosolic compartment through a mechanism that requires its kinase activity and specifically G-loop phosphorylation at Tyr²⁵³ and Tyr²⁵⁷ (i.e., that these post-translational modifications inhibit nuclear-localization signal function⁴⁰).

6. Redox inactivation of Src and other protein tyrosine kinases with G-loop cysteine residues.

Oxidative stress leads to changes in the activity of a large number of signaling responses. Most studies have focused on redox regulation of protein tyrosine phosphatases, enzymes that contain redox-sensitive catalytic cysteine residues in their activation site; oxidation of the free sulfhydryl groups on these cysteine residues disrupts protein tyrosine phosphatase activity. However, there also is evidence that reactive oxygen species (ROS) can alter protein tyrosine phosphorylation by directly regulating Src and certain other protein tyrosine kinase activities. Specifically, Src contains a cysteine residue at the tip of its G-loop (Cys²⁷⁷) that is prone to reversible oxidation. Kemble et al. used mutagenesis and biochemical approaches to show that this site within the G-loop is architecturally positioned to act as a redox-sensor and that oxidation of this strategically placed cysteine residue leads to a loss of Src activity⁴¹. While the precise mechanism for oxidative inactivation of Src remains uncertain, there is evidence that Cys²⁷⁷ oxidation leads to formation of inactive Src homodimers or oxidized Src heterodimerization with Csk. This observation resonates with structural studies that identify a disulfide bond between Cys²⁷⁷ in c-Src and Cys²⁹⁰ in Csk⁴², but the biological consequence of this disulfide linkage (between a Src molecule from one Csk-Src kinase substrate complex to a Csk in a different Csk-Src substrate complex) remains uncertain. It is worth noting that this redox-sensing mechanism is built into the G-loops of two other Src family kinase members (Yes and Fgr) and four members of the FGFR family of receptor tyrosine kinases, whereas it is not conserved in other protein tyrosine kinases (including other Src family kinases –that contain a Gln at the cognate position - or Csk).

7. Insulin Receptor (IR).

The insulin receptor (IR) is a tetrameric protein comprised of two extracellular insulin-binding α -subunits linked by disulfide bonds to two transmembrane β -subunits that display tyrosine kinase activity. Insulin binding to the IR- α -subunit leads to a conformational change that increases β -subunit tyrosine kinase activity and leads to β -subunit tyrosine transphosphorylation; this serves to further increase kinase activity toward exogenous substrates and it also provides docking sites for receptor binding-partners and substrates.

While tyrosine phosphorylation is essential for IR activation, serine/threonine phosphorylation of the IR in response to certain stimuli (including phorbol 12-myristate 13-acetate, cAMP, and insulin itself) provides an inhibitory brake that serves to prevent sustained/uncontrolled activation of downstream signaling pathways, severe perturbations of cellular metabolism, and excessive cellular growth responses that can contribute to tumorigenesis. Of note, Ser/Thr phosphorylation of the IR can become excessively increased, and lead to impaired IR activation, in various animal models of insulin resistance as well as certain insulin-resistant states in humans. While the IR β -subunit contains multiple serine phosphorylation sites, Ser⁹⁹⁴ in the G-loop has emerged as a functionally important inhibitory PTM. There is evidence that IR-Ser⁹⁹⁴ phosphorylation is altered in various models of insulin resistance⁴³ and mutagenesis studies support the conclusion that Ser⁹⁹⁴ phosphorylation is sufficient to disrupt basal and insulin-stimulated IR tyrosine

kinase activity^{44, 45}. Efforts to identify the pathophysiologically relevant IR-Ser⁹⁹⁴ kinases have generally focused on PKC isoforms, which are increased in many insulin-resistant states and display *in vivo* IR-Ser⁹⁹⁴ kinase activity⁴³. However, there is limited evidence that TANK-binding kinase 1 (TBK1, an I κ B kinase-related serine/threonine kinase that plays a role in certain inflammatory/immune responses) also can function as a IR-Ser⁹⁹⁴ kinase and thereby link inflammation to the pathogenesis of insulin resistance⁴⁶.

8. PTMs near the G-loop.

While the primary focus of this review is on PTMs within the G-loop, the notion that a PTM adjacent to the G-loop can force an unfavorable G-loop conformation and thereby impact on catalytic function has been evoked to explain phosphorylation-dependent regulation of BCR-Abl (see section 5). Of note, a review of the PTM data curated by PhosphositePlus provides many examples of PTMs (phosphorylation as well as lysine ubiquitination or acetylation) at residues adjacent to the G-loop of many protein kinases (Table 1). In fact, there is evidence that ERK2 phosphorylation by serum and glucocorticoid-inducible protein kinase1 (SGK1) at Ser²⁹ (a residue that sits just C-terminal to the G-loop) leads to increased MEK/ERK2 complex formation and enhanced ERK2 signaling; this mechanism is specific for ERK2, since this serine residue is replaced by a non-phosphorylatable Q in ERK1⁴⁷. The functional role of PTMs adjacent to the G-loop in other protein kinases remains to be determined.

9. CONCLUSION

This review summarizes literature that identifies an important role for the G-loop as a target of PTMs that influence the catalytic properties of many protein kinase enzymes. It is worth noting that with the exception of CDKs, the kinases and phosphatases that dynamically regulate G-loop phosphorylation – and could be targeted to prevent the development of clinical phenotypes – have not unambiguously been identified. This is relevant, since with the more widespread application of phosphoproteomic techniques to studies designed to identify molecular signatures for various cancer and other clinical disorders, G-loop phosphorylation is increasingly identified on many protein kinases. This suggests that PTMs localized to the G-loop may play a more general role in the regulation of protein kinase function and that strategies to manipulate G-loop phosphorylation might be harnessed for therapeutic advantage.

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ABBREVIATIONS:

cAbl	Abelson murine leukemia virus proto-oncogene
BCR	breakpoint cluster region
CDK	cyclin-dependent kinase

CML	chronic myeloid leukemia
IR	Insulin Receptor
O-GlcNAc	O-linked β - <i>N</i> -acetyl glucosamine
PKA	protein kinase A
PKC	protein kinase C
PTM	post-translational modification

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Figure 1: Ser/Thr Kinase Catalytic Domain Structure.

Figures are based upon the crystal structure of the mouse PKA catalytic domain complexed with ATP and the inhibitor peptide PKI (Protein Data Bank [PDB] accession number 1ATP). *Panel A* depicts a ribbon structure representation of the PKA N-lobe (residues 40–125, in blue) and C-lobe (residues 126–280, in red). The N- and C-terminal tails of PKA - that wrap around the catalytic core and serve as tethers to structure determinant in the catalytic core - are not depicted in this structure. *Panel B*: ATP (in blue) and the PKI inhibitor protein (in green, with an Ala-Ser substitution at the phosphoacceptor site [P-site] in red sticks) are introduced into the catalytic domain structure (in grey). The catalytic domain G-loop is highlighted in orange; G-loop glycine residues are depicted as orange spheres and the G-loop serine residue that is a target for phosphorylation is depicted as red sticks. The figure serves to emphasize the close proximity of the ATP- γ -P, the G-loop serine phosphorylation site, and the P-site on peptide substrate in the catalytic pocket. *Panel C*: An alignment of G-loop sequences that are targets for PTMs in PKA, PKC, CDK, c-Abl, Src, and B-Raf.

TABLE 1.
KINASES WITH KNOWN G-LOOP POST-TRANSLATIONAL MODIFICATIONS

PROTEIN KINASE	PTM at or adjacent to the G-loop	Frequency with which the PTM has been identified	References	Comments	
AGC Kinases					
PKA	TL GTGSFG	6	22, 48–51		
	TL GTGSFG	2	52		
	TL GTGSFG	5	22, 48, 49, 53, 54		
PKCs					
PKCα	GKGSFG K	2	55		
	GKGSFG K	1		O-GlcNAcylation	
	GKGSFG K	1	56	Ubiquitination	
PKCβ	GKGSFG K	1	23	O-GlcNAcylation	
PKCγ	GKGSFG K	1		Acetylation	
	GKGSFG K	1	57	ubiquitination	
PKCδ	GKGSFG K	3	21, 5855		
	GKGSFG K	1	57	ubiquitination	
PKCε	GKGSFG K	1	23	O-GlcNAcylation	
	GKGSFG K	1		Acetylation	
PKCθ	GKGSFG K	1	56	ubiquitination	
PKCη	GKGSFG K	2	55		
RSK Subfamily					
MSK1	GTGAYG	1			
MSK2	KVL GTGAYG	3	56, 59, 60	ubiquitination	
	KVL GTGAYG	1	61		
	KVL GTGAYG	2	61		
p70S6K	GKGGYG K	2	56, 62	ubiquitination	
	GKGGYG K	2	56, 57	ubiquitination	
RSK1	GQGSFG K	3	56, 59	ubiquitination	All RSK PTMs are located in the N-terminal kinase domain. PTMs have not been identified in RSK3.
	GQGSFG K	1		Acetylation	
RSK2	GQGSFG K	3	48, 63, 64		
	KVL GQGSFG	2	59	ubiquitination	
RSK4	GQGSFG K	1		Acetylation	
	GQGSFG K	3	56, 59	ubiquitination	
CAMK Group					
CaMK2					
CaMK2α	GKGAFS	11	57	ubiquitination	

PROTEIN KINASE	PTM at or adjacent to the G-loop	Frequency with which the PTM has been identified	References	Comments
	GKGAFS	3	65–67	
CaMK2 β	GKGAFS	8	57	ubiquitination
	GKGAFS	2	66, 67	
CAMK2 δ	GKGAFS	1	68	Acetylation
	GKGAFS	2	66, 67	
CAMK2 γ	GKGAFS	1	68	Acetylation
	GKGAFS	6		ubiquitination
	GKGAFS	2	66, 67	
AMPK α 1	TL GVGTFG	3	54, 64, 69	
PIM1	GSGGFG	1		
PIM2	GKGGFG	25	56, 60, 70	ubiquitination
CMGC Group				
CDK1	GEGTYG	1846	13, 71–73	
	GEGTYG	4125	13, 71–77	
ERK2	SYI GEGAYG	1	47	Not in ERK1 where S is replaced by Q
	SYI GEGAYG	3		Sequence conserved in ERK1, but phosphorylation not detected
GSK3 β	GNGSFG	2	48, 55	not in GSK3 α
STE Group				
MEK1	SEL GAGNGG	4	78	
MEK2	SEL GAGNGG	1	79	
Tyrosine Kinases				
EGFRs				
EGFR	GSGAFG TVY	8	48, 50, 51, 78, 80–82	
	GSGAFG TVY	150	78, 80, 83–95	
ErbB2	GSGAFG TVY	9	48, 50, 51, 78, 80, 81	
	GSGAFG TVY	148	78, 80, 83–88, 90, 92, 93, 95, 96	
ErbB3	GSGVFG	2	48, 53	
ErbB4	GSGAFG TVY	7	48, 50, 51, 78, 80, 81	
	GSGAFG TVY	147	78, 80, 83–88, 90, 92, 93, 95, 96	
InsR	GQGSFG	5	43, 44, 46, 97, 98	
IGF1R	GQGSFG MVY	1	48	
	GQGSFG MVY	3	83	
c-Abl	GGGQYG EVY	288	33, 39, 40, 86, 99	
	GGGQYG EVY	339	32, 33, 39, 40, 54, 83, 86, 88, 92	
Alk	GHGAFG EVY	22	100, 101	

The data summarizes records curated by PhosphoSitePlus, a comprehensive online resource provided by Cell Signaling Technology. PhosphoSitePlus provides comprehensive information on post-translational modifications identified in studies that use both traditional low-throughput methods (i.e., studies that focus on few modification sites that are experimentally validated using robust techniques such as amino acid sequencing, phospho-specific antibodies, site-directed mutagenesis, dominant-negative constructs, etc.) as well as studies that use high throughput discovery mass spectrometry methods ¹⁰². Since the significance of a protein kinase G-loop PTM identified in only a single study using high-throughput MS methods is uncertain, these have generally not been included in the table, unless PTMs (phosphorylation, ubiquitination, acetylation, O-GlcNAcylation) are identified at multiple sites in or adjacent to the G-loop (or on the G-loops of multiple members of a single protein kinase subfamily).

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