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Review

AGC protein kinases: From structural mechanism of regulation to allosteric drug development for the treatment of human diseases ☆

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ABSTRACT

The group of AGC protein kinases includes more than 60 protein kinases in the human genome, classified into 14 families: PDK1, AKT/PKB, SGK, PKA, PKG, PKC, PKN/PRK, RSK, NDR, MAST, YANK, DMPK, GRK and SGK494. This group is also widely represented in other eukaryotes, including causative organisms of human infectious diseases. AGC kinases are involved in diverse cellular functions and are potential targets for the treatment of human diseases such as cancer, diabetes, obesity, neurological disorders, inflammation and viral infections. Small molecule inhibitors of AGC kinases may also have potential as novel therapeutic approaches against infectious organisms. Fundamental in the regulation of many AGC kinases is a regulatory site termed the "PIF-pocket" that serves as a docking site for substrates of PDK1. This site is also essential to the mechanism of activation of AGC kinases by phosphorylation and is involved in the allosteric regulation of N-terminal domains of several AGC kinases, such as PKN/PRKs and atypical PKCs. In addition, the C-terminal tail and its interaction with the PIF-pocket are involved in the dimerization of the DMPK family of kinases and may explain the molecular mechanism of allosteric activation of GRKs by GPCR substrates. In this review, we briefly introduce the AGC kinases and their known roles in physiology and disease and the discovery of the PIF-pocket as a regulatory site in AGC kinases. Finally, we summarize the current status and future therapeutic potential of small molecules directed to the PIF-pocket; these molecules can allosterically activate or inhibit the kinase as well as act as substrate-selective inhibitors. This article is part of a Special Issue entitled: Inhibitors of Protein Kinases (2012).

1. Protein kinases and AGC protein kinases

1.1. Protein kinases

Protein phosphorylation has evolved as a widely employed "molecular language" to transfer information within a cell, to regulate cellular and organism-wide functions, and to adequately respond to cellular signals. It is estimated that one third of the total proteins in a cell may be phosphorylated at any one time, affecting a very large set of cellular pathways by turning activities ON or OFF. The importance of protein kinases in eukaryotic organisms is reflected by the abundance of genes that code for protein kinases. These genes represent 1.7% of the human genome (coding for 518 protein kinases) [1], 4% of the genome of the plant *Arabidopsis thaliana* (coding for 369 protein kinases) [2], 2% of the genome of the budding yeast *Saccharomyces cerevisiae* (coding for 112 protein kinases) [3], 1.6% of the genome of the protist parasite *Plasmodium falsiparum* (coding for 65 protein kinases) [4] and 2.0% of the genome of *Phytophthora infestans* (coding for 354 protein kinases) [5]. Given the number of protein kinases and the very large number of cellular proteins that must be phosphorylated at specific sites in response to certain stimuli, complex mechanisms have evolved in signal transduction through protein phosphorylation to achieve the specific and timely phosphorylation of substrates. In spite of their importance in the regulation of cells and organisms, more than 50 years after their discovery, the detailed mechanisms of protein kinase regulation and substrate selectivity remain vastly uncharacterized.

It is widely accepted that an imbalance in the regulation of protein kinases can be a major cause of human disease. Given this important link and the suitability of the ATP-binding site for drug development, the pharmaceutical industry has dedicated approximately one third of new drug development programs over the last decade to the development of protein kinase inhibitors. The ATP-binding site is highly conserved across more than 500 human protein kinases, and thus the challenge has been the identification of "selective" compounds that will inhibit one target kinase without affecting multiple other kinases. It is anticipated that the development of more selective agents that target protein kinases with fewer side effects will increase their use in the treatment of chronic diseases and enable their use in personalized therapies for the treatment of cancers, whether alone or

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in combination regimens. Similarly, selective protein kinase drugs may also permit the targeting of a protein kinase from an infectious organism without affecting the activity of the human counterpart, permitting the treatment of infectious diseases.

Over the years, our research has been led by the vision that an understanding of the molecular mechanisms of protein kinase regulation will support and guide the rational development of the next generation of protein kinase inhibitors. This review summarizes the current knowledge of the mechanisms of regulation of AGC protein kinases, highlighting the importance of the PIF-pocket, a regulatory ON–OFF switch that can be targeted with low-molecular-weight compounds and therefore used for the development of innovative medicines with a greater degree of selectivity.

1.2. AGC kinases

Based on the evolutionary relationships between their catalytic domains, the human protein kinases are classified into 8 groups [1]. The AGC kinases are one of the most evolutionary conserved groups and are represented widely within eukaryotes, including in all vertebrates, invertebrates, fungi, plants, unicellular algae, and protists[1]. The AGC kinase group comprises 12% of the human kinome. Similarly, this group represents 15% and 20% of the kinomes of yeast-like and filamentous fungi, respectively, 4% of the plant kinome, 6–8% of the trypanosome, leishmania and amoeba kinomes, and 6% of the giardia kinome [2,7–10].

The AGC kinase group was named after 3 representative families, the cAMP-dependent protein kinase (PKA), the cGMP-dependent protein kinase (PKG) and the protein kinase C (PKC) families. In the human genome, 63 genes encode 61 AGC kinases and 2 pseudokinases, which are classified into 14 families and 21 subfamilies [1] (Tables 1 and 2). There are also 6 pseudogenes (MRCKps, GPRK6ps, PRKXps, PKCips, p70S6Kps1 and p70S6Kps2), which are included in 5 different subfamilies [1]. The 2 pseudokinases, RSKL1 and RSKL2, are predicted to be proteins that lack essential features for activity [11]. In more distant eukaryotic organisms, the number of families within the AGC group varies, with PKA/PKG, PDK1, RSK, MAST and NDR family members widely present throughout the eukaryotic kingdoms (Table 1). The Aurora kinases are closely related to AGC kinases; the Aurora kinases share many aspects of the molecular mechanisms of regulation of AGC kinases and are also widely present in eukaryotes (Table 1).

2. AGC kinases in physiology and disease

We briefly summarize here the physiological roles of diverse AGC kinases and their involvement in human diseases and discuss their potential as drug targets.

2.1. Human AGC kinases

2.1.1. PDK1

PDK1 is a master kinase, responsible for the phosphorylation of the activation-loop site and therefore activating at least 23 other AGC kinases, including AKT/PKB, SGK and PKC isoforms as well as the p70 and p90 ribosomal S6 kinases (S6K and RSK). PDK1 has been widely studied because of its regulation of AKT/PKB and AKT/PKB-dependent cellular functions. It plays an important role in cancer where alterations of the PI3K-PTEN pathway are commonly found as well as in diabetes [11–16]. Targeting PDK1 pharmacologically is considered a potential approach for the treatment of cancer (reviewed in [12]).

Table 1
AGC kinase families in the different biological kingdoms. AGC kinase families in Fungi (represented by *Saccharomyces cerevisiae*) [3], Plantae, represented by the *Arabidopsis thaliana* kinome [2], Protists, represented by the kinome of *Plasmodium falsiparum*, *Dictyostelium discoideum* or *Toxoplasma gondii* [4,9] (Toxoplasma database) and Chromista represented by *Phytophthora infestans* [5], in comparison to the human kinome [1] (Animalia), complemented by Phototropin and the AGC-related Aurora kinase. AGC kinases are not found in the remaining kingdom, Prokaryotes. MAST, YANK and Phototropin were identified by the Conserved Domains and Protein Classification resource from the National Center for Biotechnology Information (NCBI).

Domain / Kingdom	Eukaryota				
	Animalia	Fungi	Plantae	Protist	Chromista
Representative organism / AGC kinases	*H. sapiens*	*S. cerevisiae*	*A. thaliana*	*P. falciparum* *D. discoideum* *T. gondii*	*P. infestans*
PDK1	●	●	●	●	●
AKT/PKB	●	●			●
SGK	●	●		●	●
RSK	●	●	●	●	●
PKA	●	●	●	●	
PKG	●		●	●	●
PKC	●	●		●	
PKN /PRK	●				
NDR	●	●	●	●	●
MAST	●	●	●	●	●
YANK	●	●		●	●
DMPK	●	●		●	
GRK	●				●
SGK494	●				
Aurora[a]	●	●		●	●
Phototropin[b]		●	●	●	

[a]Closely related to AGC kinase group and sharing aspects of the molecular mechanism of regulation.
[b]AGC kinase with LOV domain that allows stimulation by light. Not present in humans.

2.1.2. AKT/PKB family

AKT/PKB is involved in numerous cellular processes downstream of PI3-kinase and its deregulation leads to cancer, diabetes, cardiovascular and neurological diseases [11,17]. There are 3 isoforms of AKT/PKB. The knockout mice of AKT1/PKBα and AKT2/PKBβ provided evidence that these isoforms have a major role in cell survival and insulin signaling, respectively [18,19]. Since all three isoforms are overexpressed in cancer, there are intense efforts to develop small molecule inhibitors targeting AKT/PKB for cancer treatment (reviewed in [17]). On the other hand, due to the specific role of AKT2/PKBβ in insulin signaling, it could be speculated that transient activators of AKT2/PKBβ will mimic the intracellular effects of insulin and therefore be useful for the treatment of diabetes without having a major impact in cell survival.

2.1.3. SGK family

There are three SGK isoforms that share similar biochemical properties and comparable roles in the regulation of a wide range of physiological and pathophysiological functions. SGK1 is the most studied member of the family and is involved in cell proliferation and survival as well as tumor growth [20], aldosterone and insulin release, glucose metabolism, gastric acid secretion, regulation of ion transporters and channels and blood pressure, among others [21]. There is increasing evidence that SGK1 is involved in the development and complications of diabetes and neurological disorders [21,22] and the use of SGK inhibitors may be useful for the treatment of different diseases as has recently been demonstrated in diabetic-associated hypertension [23].

[1] Prokaryotic protein kinases include a large number of eukaryotic-like protein kinases that are distantly related and do not belong to the eukaryotic protein kinase groups ([6] N. Kannan, S.S. Taylor, Y. Zhai, J.C. Venter, G. Manning, Structural and functional diversity of the microbial kinome, PLoS Biol 5 (2007) 5(3):e17.).