● **PERSPECTIVE**

The roles of atypical protein kinase Cs (aPKCs) in the nervous system: targets for neuroregeneration?

The protein kinase C (PKC) family: PKC family is a subgroup of the AGC serine-threonine kinases that consists of 10 distinct members in mammals (Spitaler and Cantrell, 2004). They all contain a C-terminal catalytic domain that is connected to the N-terminal regulatory domains. PKCs are subdivided into three classes according to their structures and activators. The classical/conventional PKC isoforms possess tandem C1 domains that bind to their well-known activators diacylglycerol and phorbol esters and also contain a C2 domain that mediates the binding of Ca^{2+}-sensitive anionic phospholipids. Novel PKC isoforms also contain C1 domains that bind diacylglycerol and a novel C2 domain that does not coordinate Ca^{2+}. Unlike the classical/conventional and novel PKC classes, atypical PKC isoforms (aPKCs) which include PKCζ and PKCι (also known as PKC λ in rodents), contain an atypical C1 domain that has no affinity to diacylglycerol or phorbol esters. Instead of a C2 domain, a protein binding Phox/Bem domain 1 is located at the N-terminal of the regulatory region. This domain has been shown to control aPKC activity through interactions with protein adaptors at specific locations. In addition, a truncated isoform, namely protein kinase Mζ (PKMζ), is generated in the nervous system through a neuronal-tissue specific alternative transcription start site in PRKCZ gene. Since all the N-terminal regulatory domains are omitted in the alternative transcription, PKMζ functions as a constitutively active form of PKCζ (**Figure 1**).

Neuronal roles of aPKCs: Increasing evidence suggests that aPKCs participate in various neuronal processes. In addition to PKCι and PKCζ, PKMζ is expressed abundantly in the mammalian nervous system. PKMζ is a constitutively active form of PKCζ which is generated from an alternative transcript from the PKCζ gene that lacks the coding region of the regulatory domains. PKMζ is essential for the maintenance of long-term potentiation. Treatment with the zeta-inhibitory peptide that resembles the pseudosubstrate sequence of PKCζ prevents long-term memory formation (Glanzman, 2013). Moreover, a dominant-negative PKMζ disrupts memory. Although the exact mechanisms of the role of PKMζ in memory formation remain elusive, PKMζ and PKCι have been shown to participate in various stages of long-term potentiation and therefore function distinctively in memory maintenance. Notably, several studies have revealed that PKMζ regulates the levels of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor on the postsynaptic membrane. Such increases in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors by PKMζ leads to the enhancement of synaptic strength (**Figure 2b**).

There is increasing evidence suggesting that aPKCs participate in signaling pathways that regulate neuronal cell survival and differentiation. aPKCs are essential in nerve growth factor (NGF)-mediated neuronal differentiation. The overexpression of aPKCs promotes the NGF-induced differentiation and neurite outgrowth of PC12 cells (**Figure 2a**). PKCι is also involved in cell survival responses through the NGF-mediated activation of nuclear factor-κB (Wooten et al., 1999). In addition to NGF signaling, evidence shows that PKCζ could also enhance the survival of PC12 cells downstream of the A(2A) adenosine receptors-PKA pathway.

Neurogenesis is a crucial process of producing functional neurons from adult neural precursors. Recently findings underscore the roles of aPKCs in this process. PKCι and PKCζ have been shown to promote the differentiation of radial glial and cortical precursor cells, respectively. To note, PKCζ has been identified to play a role in regulating neuronal differentiation by the phosphorylation of histone acetylase cAMP-response element binding protein-binding protein, which functions in the activation of several neural differentiation genes (Wang et al., 2012). The phosphorylation of cAMP-response element binding protein-binding protein by aPKCs also acts as a compensatory mechanism for the reduced cAMP-response element binding protein activity in adult animals (**Figure 2f**). This event is essential for maintaining hippocampal neurogenesis and forming hippocampal-dependent memories.

aPKCs are also essential in neuronal development. They have been identified as components of the polarity complex together with the partition defective (Par) proteins Par3 and Par6. The polarized distribution of the Par3-Par6-aPKC complex is critical to the establishment of neuronal polarity, and disruption of the complex impairs axon specification (**Figure 2d**). Notably,

PKCι and PKCζ are found spatially segregated during this process. The two aPKCs are reported to differentially regulate axon specification. PKCι promotes axon formation whilst PKMζ inhibits the process (Parker et al., 2013). Furthermore, several reports have revealed the roles of aPKCs in the noncanonical Wnt signaling, showing that they participate in Wnt-mediated neuronal development. PKCζ is required for Wnt4-mediated axon guidance, acting downstream of Wnt5a-mediated axon differentiation through interactions with dishevelled-1 (**Figure 2c**). Likewise, Wnt3A has been shown to enhance the interaction of PKCι and dishevelled-2 to stimulate neurite extension (Greer et al., 2013). PKCζ also modulates the activity of Aurora A at the neurite hillock (**Figure 2e**). Perturbations of the PKCζ-Aurora A pathway disrupt microtubule emanation and therefore decreases the neurite length of dorsal root ganglion neurons (Mori et al., 2009).

aPKCs in neurodegeneration: Abnormalities in protein kinases have been implicated in various neurodegenerative diseases as many of these kinases participate in various neuronal signaling pathways. In fact, aPKC mRNA expression levels are reduced in the affected brain regions of Alzheimer's disease, and an age-associated decrease in PKCζ has also been observed in the brain. Intriguingly, aPKCs are the only PKC isoforms that are found in abnormal protein aggregates including neurofibrillary tangles, α-synuclein-positive Lewy bodies and superoxide dismutase aggregates (Shao et al., 2006). It is also noteworthy that aPKCs have been shown to negatively regulate glycogen kinase synthase-3β (GSK-3β), a kinase that induces tau hyperphosphorylation (**Figure 2h**). Because neurofibrillary tangle formation correlates with neuronal dysfunction in several neurodegenerative disorders including AD, Parkinson's disease and frontotemporal dementia, the impairment of aPKCs could aggravate neurodegeneration by the promotion of glycogen kinase synthase-3β-mediated neurofibrillary tangle formation.

Although toxic aggregates likely impair the functions of aPKCs, there is also evidence suggesting that kinases work against the neurotoxicity of the aggregates. For example, PKCι has been reported to protect neuronal cells from the toxic amyloid-β (Aβ) peptide-induced apoptosis (**Figure 2j**). Recently, aPKCs have been shown to modulate Aβ production. This modulation occurs through the phosphorylation of the engulfment adaptor PTB domain containing 1 (GULP1), which is an amyloid-β precursor protein (APP) that promotes Aβ generation. Such GULP1 phosphorylation by aPKCs attenuates the GULP1-APP interaction and thereby reduces Aβ production (Chau et al., 2019) (**Figure 2g**). Noteworthy, type 2 diabetes is a risk factor for Alzheimer's disease, and insulin plays an important role in the disorder through aPKCs. Activation of aPKCs by insulin has been shown to influence Aβ generation by altering the metabolism of APP and has been shown to reduce the production of Aβ in cells and Alzheimer's disease mouse models. The intranasal administration of insulin improves the cognitive performance of Alzheimer's disease patients (Lu and Xu, 2019). Moreover, recent studies have also highlighted the potential of metformin in the treatment of neurological disorders. Metformin enhances neurogenesis and facilitates memory formation in mouse models, an effect that is mediated through the activation of aPKC (Farr et al., 2019). In addition to Aβ toxicity, aPKCs have also been shown in cell models to antagonise the toxicity of expanded polyglutamine through the activation of extracellular signal-regulated kinases. aPKC levels increase significantly in motor neurons of an amyotrophic lateral sclerosis mouse model with the superoxide dismutase 1 mutation, which is proposed to exert a protective response against mutant superoxide dismutase 1 toxicity (Tury et al., 2014) (**Figure 2i**).

Conclusion: aPKCs participate in various neuronal signaling pathways that regulate neurite outgrowth, neuronal differentiation, neurogenesis and memory formation. Some of these processes have been proposed to be essential for neuroregeneration. Aberrant aPKC activities are reported in various neurodegenerative disorders. In fact, these kinases are proposed to exert protective effects against some neurotoxic peptides/proteins. As stated above, the relation of insulin and aPKCs provides an example of how these kinases might help mitigate the effects of neurodegenerative diseases. The therapeutic activation of aPKCs (or aPKC-regulated pathways) could help overcome the effects of neurodegenerative conditions that involve the impairment of kinase activities as aPKCs can trigger neuronal processes that associate with neuroregeneration and neuroprotection. Although further work on aPKCs is needed, the existing literature suggests a novel direction for the development of therapeutic approaches for neurodegenerative disorders through aPKCs.
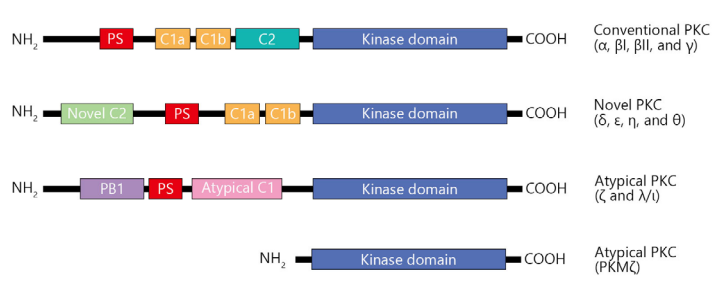
Figure 1 Schematic diagram of the structure of protein kinase C (PKC) isoforms.
The PKC family can be subdivided into three classes according to their domain composition. All PKC members contain a kinase domain at the C-terminus (blue). The conventional PKCs (α, βI, βII and γ) contain a pseudosubstrate domain (PS, red rectangle), consecutive C1 domains (orange) that binds diacylglycerol and phosphatidylserine, and a Ca^{2+}-binding C2 domain (green). The novel PKCs (δ, ε, η, and θ) have conserved C1 domains but a "novel" C2 domain (cyan) that does not bind Ca^{2+}. Atypical PKCs (ζ and ι/λ) feature a PB1 domain (purple) for protein-protein interaction and an "atypical" C1 domain (pink) that has no affinity to diacylglycerol or phosphatidylserine. PKMζ is a truncated form of PKCζ that lacks the N-terminal regulatory domains.
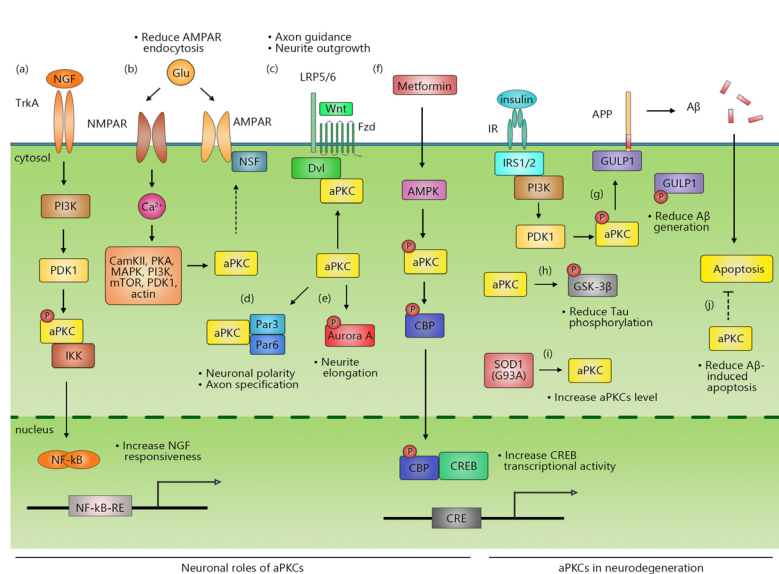
Figure 2 Neuronal roles of atypical protein kinases (aPKCs).
(a) aPKCs mediates nerve growth factor (NGF) response. NGF-TrkA-Src pathway stimulates aPKCs-IκB kinase (IKK) binding, leading to increase of nuclear factor kappa-light-chain-enhancer of activated B cells (nuclear factor-κB)-mediated gene transcription. (b) Following N-methyl-D-aspartate (NMDA) receptors (NMDAR) stimulation, a series of induction factors upregulate postsynaptic aPKCs level. aPKCs increase the surface expression of AMPAR via N-ethylmaleimide-sensitive factor (NSF)-dependent pathway that triggers LTP. (c) aPKCs are also involved in regulation of neuron morphology. Wnt enhances Dvl-aPKC interaction to stimulate neurite outgrowth. (d) aPKCs interact with Par3 and Par6 to form the polarity complex, which is essential for establishment of neuronal polarity and axon specification. (e) Additionally, aPKCs phosphorylate Aurora A at threonine 287 and activate its kinase activity. Activated Aurora A promotes microtubule extension during neurite outgrowth. (f) aPKCs control hippocampal neurogenesis through CBP-CREB pathway. AMP-activated protein kinase (AMPK) activates aPKCs through phosphorylation of PKC ζ/ι at threonine 410/413, which phosphorylate CBP serine 436 to promote CBP-CREB binding and CREB transcriptional activity. (g–j) aPKCs are implicated in neurodegenerative diseases. (g) Insulin activates downstream aPKCs to phosphorylate GULP1 threonine 35 and reduces GULP1-APP interaction. Such phosphorylation suppresses Aβ production from APP proteolytic cleavage. (h) aPKCs is implicated in tauopathy by the phosphorylation of the tau kinase GSK-3β Ser-9 which inactivates GSK-3β. (i) Elevated aPKCs level in ALS mouse model with mutated superoxide dismutase 1 (SOD1) (G93A) may be a protective response against mutant SOD1 toxicity. (j) aPKCs protect neuronal cells against Aβ-induced apoptosis.

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