Immunology
The Journal of cells, molecules, systems and technologies

British Society for immunology

IMMUNOLOGY REVIEW ARTICLE

Protein kinase C in the immune system: from signalling to chromatin regulation

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doi:10.1111/imm.12510
Received 24 March 2015; revised 29 June 2015; accepted 15 July 2015.
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Summary

Protein kinase C (PKC) form a key family of enzymes involved in signalling pathways that specifically phosphorylates substrates at serine/threonine residues. Phosphorylation by PKC is important in regulating a variety of cellular events such as cell proliferation and the regulation of gene expression. In the immune system, PKCs are involved in regulating signal transduction pathways important for both innate and adaptive immunity, ultimately resulting in the expression of key immune genes. PKCs act as mediators during immune cell signalling through the immunological synapse. PKCs are traditionally known to be cytoplasmic signal transducers and are well embedded in the signalling pathways of cells to mediate the cells' response to a stimulus from the plasma membrane to the nucleus. PKCs are also found to transduce signals within the nucleus, a process that is distinct from the cytoplasmic signalling pathway. There is now growing evidence suggesting that PKC can directly regulate gene expression programmes through a non-traditional role as nuclear kinases. In this review, we will focus on the role of PKCs as key cytoplasmic signal transducers in immune cell signalling, as well as its role in nuclear signal transduction. We will also highlight recent evidence for its newly discovered regulatory role in the nucleus as a chromatin-associated kinase.

Keywords: chromatin; epigenetics; immune system; protein kinase C; signal transduction.

Introduction

Protein kinase C (PKC) is a key family of enzymes involved in signalling pathways that specifically phosphorylates substrates at serine/threonine residues, influencing a variety of cellular events such as cell proliferation and the regulation of gene expression.[1,2] PKC is a subfamily of AGC (PKA, PKG and PKC) kinases, incorporating 10 kinase members that share a highly conserved catalytic kinase domain, and a less conserved regulatory domain

Abbreviations: BAF60c, Brg1/Brm-associated factor 60c; Bcl10, B-cell leukemia/lymphoma 10; BCR, B-cell receptor; Btk, Bruton's tyrosine kinase; CARMA1, caspase recruitment domain family (CARD)-containing membrane-associated guanylate kinase (MAGUK) protein 1; CIITA, class II transactivator; CREB, cAMP response element-binding protein; DAG, diacylglycerol; ERα, oestrogen receptor α; GLK, germinal centre kinase (GCK)-like kinase (MAP4K3); H1, histone H1; H2B, histone H2B; H3, histone H3; HEXIM1, hexamethylene-*bis*-acetamide-induced mRNA-encoded proteins 1; IFN, interferon; IKK, inhibitor of κB (IκB) kinase; IL, interleukin; IκB, inhibitor of κB; K, lysine; Ki-1/57, 57-000 MW human protein antigen recognized by the CD30 antibody Ki-1; MALT1, mucosa-associated lymphoid tissue 1; MyD88, myeloid differentiation primary-response protein 88; NF-κB, nuclear factor κB; NLS, nuclear localization signal; P, proline; PCAF, p300/CREB-binding protein-associated factor; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G; S, serine; S/T-P-S/T, SPT; SATB1, special AT-rich binding protein 1; STAT, signal transducer and activator of transcription; T, threonine; TAK1, transforming-growth-factor–activated kinase 1; TCR, T-cell receptor; Th, T helper; TIR, Toll–IL-1 receptor; TIRAP, Toll–IL-1 receptor domain-containing adaptor protein; TLR, Toll-like receptor; TRAF6, tumour necrosis factor receptor-associated factor 6; TRAM, TRIF-related adaptor molecule; TRIF, TIR domain-containing adaptor inducing interferon-β; Y, Tyrosine

responsible for binding to activators and to anchoring proteins.[2] Isoforms of PKC can be divided into three subclasses of serine/threonine kinases (classical, novel and atypical) according to structural motifs and activation requirements (Fig. 1).[1] Classical (also called conventional) PKC (cPKC) isoforms, including α, β (I and II) and γ, contain motifs for diacylglycerol (DAG) and calcium-dependent phospholipid binding, and so require both DAG and calcium for activation to occur.

Unlike cPKCs, in novel PKCs (nPKCs – δ, ε, η and θ), the C2 domain (also known as the V1 domain) is located at the N-terminus of the C1 domain and lacks the aspartic acid residues necessary for coordinating calcium ions.[3] Furthermore, the same lipids activate cPKCs and nPKCs but they can activate cPKCs only in the presence of calcium, this is in part due to the higher affinity of the C1 domain of nPKCs to DAG.[4] In contrast, atypical PKC isoforms (aPKCs – ζ and λ/ι) contain a single C1 domain and are therefore incapable of binding DAG and do not bind calcium. Atypical PKC contain a single zinc-finger motif within the C1 domain that can be bound by zinc-finger proteins.[5]

While PKCs act to phosphorylate substrates, the enzyme itself requires three ordered phosphorylations in order to be catalytically competent.[2] The first, rate-limiting phosphorylation occurs on the activation loop at T500 by phosphoinositide-dependent kinase, 3-phosphoinositide dependent protein kinase-1.[2] This then prompts the rapid phosphorylation of a turn motif at T641, which results in autophosphorylation at S660 on the hydrophobic motif. The fully phosphorylated PKCs are maintained in a catalytically inactive form mainly by intramolecular interactions such as that of the pseudosubstrate domain until the binding of cofactors such as phosphatidylserine, DAG and calcium, to the regulatory modules. All PKC enzymes are allosterically activated by phosphatidylserine, which binds to the C1 domain, but its affinity for membrane phospholipids is increased by the binding of DAG to the C1 domain and calcium-binding to the C2 domain, depending on the class of PKCs. As a result of the cofactor binding, the pseudosubstrate domain is released from the kinase core, allowing PKC to phosphorylate target substrates.[6]

The vast amount of published data presented on PKCs describes their function as cytoplasmic signal transducers, incorporated into the pathways of every mammalian cell to serve as an intermediatory between membrane binding and nuclear events.[1,7,8] PKCs are expressed in numerous tissue and cell types and although most PKC isoforms are ubiquitously expressed, some isoforms are expressed in a tissue-specific and cell-specific manner (Table 1). In the immune system, PKCs are important mediators of immune cell signalling through the immunological synapse. All PKC isoforms are expressed by immune cells, with the exception of PKCγ which is preferably expressed by the central nervous system.[9] Although ubiquitously expressed, the expression pattern and levels of each PKC isoform are cell-type specific, highlighting their specific function and non-redundant roles in the immune system. Recent data suggest that PKCs have additional, non-traditional roles as nuclear kinases, whereby these enzymes have the capacity to directly regulate gene expression programmes. In this review, we will focus on the role of specific PKCs as key cytoplasmic signal transducers in well-characterized immune cell signalling pathways in the innate and adaptive immune system. We will also discuss the role of PKC in the context of nuclear signal transduction and highlight recent evidence for its newly

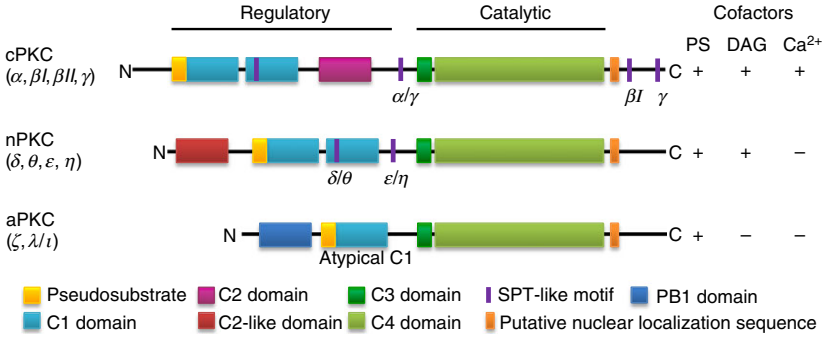

Figure 1. Schematic diagram of the primary structure of protein kinase C (PKC) family members. All PKC isoforms have the pseudosubstrate (yellow) and the C1 domain (blue) for diacylglycerol (DAG) (except for atypical PKCs) and phosphatidylserine (PS) binding in the regulatory region located at the N-terminal. They also have a catalytic domain, C3 (dark green) and C4 (light green), in the C-terminal region. Conventional PKCs (cPKC) have a C2 domain (pink) for the calcium (Ca^{2+}) dependent binding of anionic lipids such as phosphatidylinositol 4,5-biphosphate (PIP_2). Novel PKCs (nPKC) have a C2-like domain (red), which cannot bind Ca^{2+} or PIP_2. Atypical PKCs (aPKC) have an atypical C1 domain that cannot bind DAG and Phox/Bem domain 1 (PB1), which allows protein interactions. All PKC isoforms also have the putative nuclear localization sequence and all except for aPKCs show the presence of SPT-like motif. Different PKC isoforms have SPT-like motif present at different locations within their structure.

Table 1. Protein kinase C (PKC) isoform cell-specific expression and defects in knockout mice

PKC	Tissue expression[1]	Knockout mouse phenotype
PKCα	Ubiquitous, T cells, plasmacytoid dendritic cells (pDC)	T-cell activation and T-cell immunity defects[148]
PKCβ	Ubiquitous, B cells and mast cells	B-cell signalling and survival defects; mast cells defects[56,149]
PKCδ	Ubiquitous, B cells, mast cells, macrophages	B-cell homeostasis defects[150]
PKCε	Ubiquitous	Macrophage activation defect[16]
PKCη	Ubiquitous, T cells and macrophages	T-cell homeostasis and regulatory T cell function defects[151,152]
PKCθ	T cells, mast cells, platelets, skeletal muscle	T-cell activation defects[37,39]
PKCζ	Ubiquitous	B-cell receptor signalling defects; T helper type 2 response defects[153]
PKCλ/ι	Ubiquitous	Embryonic lethal[154]

[1]PKCγ is preferably expressed in the brain.

discovered regulatory role in the nucleus as a chromatin-associated kinase, a function that appears to be evolutionarily conserved.

PKC as cytoplasmic signal transducers

The PKCs are involved in regulating signal transduction pathways important for both innate and adaptive immunity, ultimately resulting in the expression of key immune genes. Different PKC isoforms are involved in distinct signalling pathways, with selective functions in a cell-specific manner. This review will focus on the Toll-like receptor (TLR) signalling in the innate system, T-cell receptor (TCR) signalling and B-cell receptor (BCR) signalling in the adaptive immune system as these pathways are very well characterized signalling pathways known in the literature. Furthermore, PKCs are known to be key signalling molecules in these pathways. Although different PKC isozymes are involved in these signalling pathways, we will highlight the key PKC isozymes implicated for each pathway in this review.

PKCε in TLR4 signalling

Toll-like receptors play a key role in the innate immune system by defending the host against microbial infection.[10] TLRs are a family of pattern recognition receptors that are present in many cell types, with most of the expression by macrophages, neutrophils and dendritic cells.[11] The best-studied TLR ligand is lipopolysaccharide, a component of the Gram-negative bacteria. It activates the innate immune system through binding with TLR4 to initiate two intracellular pathways: MyD88 (myeloid differentiation primary-response protein 88)-dependent and MyD88-independent pathways (Fig. 2a).[12] The MyD88-dependent pathway requires TIRAP [Toll–interleukin-1 (IL-1) receptor (TIR) domain-containing adaptor protein] to link MyD88 to TLR4 receptor, leading to the expression of inflammatory cytokines such as IL-1, IL-6 and tumour necrosis factor α. In contrast, the MyD88-independent pathway is mediated by TRIF [TIR domain-containing adaptor inducing interferon-β (IFN-β)] and TRAM (TRIF-related adaptor molecule) to induce expression of type I IFNs.[13] It is beyond the scope of this review to cover these pathways in detail hence we refer readers to the multiple reviews that explain the TLR4 signalling pathway in depth.[10,11,14,15]

Although PKC isoforms are involved at many levels of the TLR signalling cascade, we will focus on the involvement of PKCε in TLR4 signalling as PKCε is implicated as an important player in the TLR4 signalling pathway during macrophage activation.[7] The role of PKCε in host defence against bacterial infection was revealed through studies in PKCε knockout mice, where mice lacking PKCε have a diminished response to lipopolysaccharide stimulation, characterized by low levels of several cytokines, namely tumour necrosis factor-α and IL-1β.[16] Other studies show PKCε playing a role in both the MyD88-dependent and MyD88-independent pathways of TLR4 signalling (Fig. 2a).[17,18] In the MyD88-dependent pathway, lipopolysaccharide stimulation leads to PKCε recruitment to TLR4 and phosphorylation on S346 and S368 via MyD88.[17] These phosphorylations lead to binding with 14-3-3β, which is also MyD88 dependent. The phosphorylation event is important for downstream signalling as cells expressing mutant PKCε S346A/S368A were unable to activate nuclear factor-κB (NF-κB) upon TLR induction. This suggests that PKCε not only needs to be phosphorylated for its ability to bind to 14-3-3β, but it also exists as a complex with TLR, MyD88 and 14-3-3β to regulate gene expression. In the MyD88-independent pathway, PKCε is required for TLR4 activation via the TRAM substrate as phosphorylation of TRAM is disrupted in PKCε-deficient cells.[18] TRAM is localized to the plasma membrane in the unstimulated state but upon lipopolysaccharide stimulation, it is phosphorylated by PKCε on a serine residue near the N-terminal end.[14] This phosphorylation dissociates TRAM from the membrane, allowing it to then link TLR4 with TRIF.[7] The TLR4–TRAM–TRIF complex is necessary for activation of further downstream NF-κB and IFN regulatory factor-3/7 signalling pathways.[19]

The innate immunity provided by the TLR pathway is also required for the adaptive response by T-cell activation against antigens.[20–22] Cross-talk in signalling

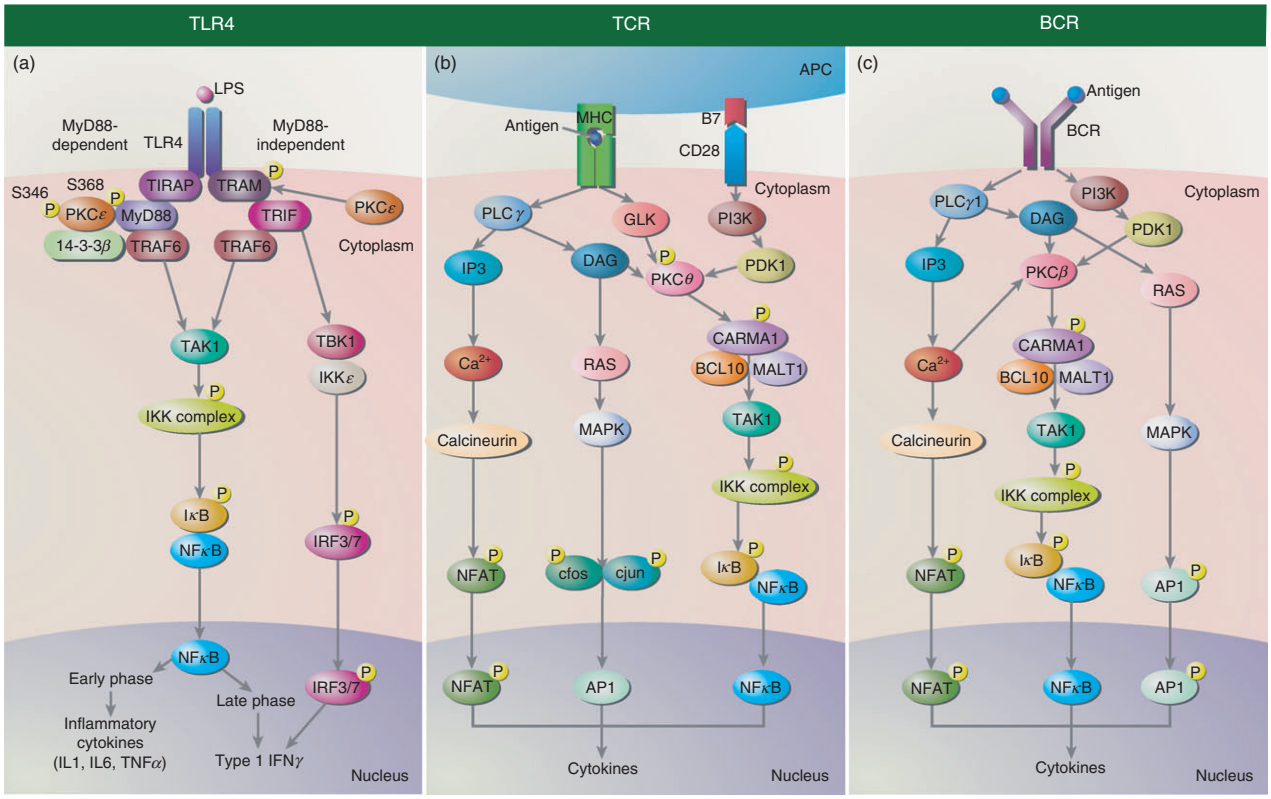

Figure 2. Protein kinase C (PKC) involvement in cytoplasmic signal transduction pathways in the immune system. (a) Protein kinase Cε (PKCε) is an important player in the Toll-like receptor 4 (TLR4) signalling pathway during macrophage activation. Binding of lipopolysaccharide (LPS) to the TLR4 initiates the activation of two intracellular pathways: myeloid differentiation primary-response protein 88 (MyD88)-dependent and MyD88-independent pathways. In the MyD88-dependent pathway, Toll–interleukin (IL)-1 receptor (TIR) domain-containing adaptor (TIRAP) links MyD88 to the TLR4 receptor. PKCε is then recruited to TLR4 via MyD88 and phosphorylated on serine 346/368. This phosphorylation leads to binding with 14-3-3β and the formation of a complex with TLR4, TIRAP, MyD88 and TNF receptor-associated factor 6 (TRAF6) as well. In the MyD88-independent pathway, TRIF-related adaptor molecule (TRAM) is phosphorylated by PKCε allowing TLR4 to link with TRIF. TRIF then recruits kinases such as transforming-growth-factor-activated kinase 1 (TAK1) (through TRAF6), TANK-binding kinase 1 (TBK1) and inhibitor of κB (IkB) kinase epsilon (IKKε) for activation of nuclear factor κB (NF-κB) and interferon regulatory factor (IRF)-3/7 signalling pathways to produce inflammatory cytokines and Type I interferons. (b) In T cell receptor (TCR) signalling, activation occurs when an antigen is presented by the antigen-presenting cell (APC) on the MHC is complexed with the TCR together with the binding of co-stimulatory molecules CD28 and B7. This leads to the activation of calcium signalling pathways and mitogen-activated protein kinase (MAPK) pathways through phospholipase Cγ (PLCγ), GCK-like kinase (GLK) activation and 3-phosphoinositide dependent protein kinase-1 (PDK1) activation through phosphoinositide 3-kinase (PI3K). Diacylglycerol (DAG) generated through PLCγ1 activation binds to protein kinase Cθ (PKCθ), which is also phosphorylated by PDK1 and GLK. Activation of PKCθ leads to the activation of NF-κB signalling pathways. (c) B cells are activated upon antigen binding to the B cell receptor (BCR). Similar pathways to TCR are activated but the key PKC isoform involved is PKCβ. In addition, calcium (Ca^{2+}) generated from PLCγ1 activation also acts to activate protein kinase Cβ (PKCβ). For both TCR and BCR signalling, the signalling pathways activated leads to the recruitment and activation of nuclear transcription factors [NF-κB, activator protein 1 (AP1) and nuclear factor of activated T cells (NFAT)] to then produce cytokines.

pathways is not uncommon but the specificity of the response depends on the stimulus provided and the cell type engaged to generate the appropriate immune response.

PKCθ in TCR signalling

The cells of the adaptive immune system form the immunological synapse with antigen-presenting cells to activate signal transduction pathways that induce gene expression programmes for lymphocyte function. In T cells, activation of T cells occurs when the TCR recognizes an antigen presented by the antigen-presenting cells. This leads to T-cell differentiation and is a process involving the activation of multiple pathways including PKC signalling (Fig. 2b). Following TCR–antigen-presenting cell complex formation, PKC localizes to the immunological synapse and subsequently stimulates the recruitment and activation of nuclear transcription factors (such as NF-κB, activator protein 1 and nuclear factor of activated T cells) required for induction of immune effec-

tor genes.[8] These effector genes are then expressed in a rapid and transient manner to produce cytokines, chemokines, cell surface molecules and growth factors, important for T-cell proliferation and differentiation. The details of the T-cell activation pathways have been reviewed extensively elsewhere.[23–28] Although other members of the PKC family can also be found in the immunological synapse of different T-cell subsets,[29] PKCθ is the most prominently studied PKC in TCR signalling since the discovery of its selective recruitment to the immunological synapse in effector T cells.[30] Furthermore, it is selectively expressed in T cells within the haematopoietic cell population.[31] Upon TCR activation, PKCθ localizes to the central supramolecular activation cluster of the immunological synapse at the plasma membrane.[32] The membrane translocation of PKCθ requires association with co-stimulatory molecule CD28 with the V3 domain of PKCθ.[33–35] The ability of PKCθ to segregate correctly to the central supramolecular activation cluster is dependent on the presence of CD28 as activated T cells from CD28-deficient mice were unable to form the mature immunological synapse with PKCθ, forming a diffuse pattern throughout the synapse instead.[33] The specific localization of PKCθ to the immunological synapse is critical for an effective T-cell activation and this translocated PKCθ is also enzymatically active.[30] Interestingly, the activity of PKCθ is also regulated by the intracellular redox state, in which the oxidized inactive form of PKCθ is recruited to the plasma membrane in naive T cells.[36]

The role of PKCθ in regulating T-cell function was initially characterized using PKCθ-knockout mice (Table 1).[37–39] Further studies on PKCθ-deficient T cells reveal that PKCθ performs different functions depending on the T-cell subpopulations. For example, PKCθ is required for a T helper type 2 (Th2) cell but not Th1 cell *in vivo* immune response against helminth infection and allergic airway inflammation.[40] However, contrasting studies in mouse experimental autoimmune encephalomyelitis show impaired Th1 responses in PKCθ-deficient mice suggesting that PKCθ is important for regulating both Th1 and Th2 responses but in an antigen-dependent and organ-specific manner.[41,42] These studies have also shown that PKCθ is required for the Th17-dependent development of experimental autoimmune encephalomyelitis, implicating PKCθ in controlling Th17 differentiation.[41,42] According to Kwon *et al.*,[43] PKCθ up-regulates signal transducer and activator of transcription 3 (STAT3) under Th17 priming conditions upon TCR stimulation with PMA. PKCθ promotes the activation of STAT3 by regulating the association of activator protein 1 and NF-κB transcription factors to STAT3 promoter.[43] More recently, PKCθ was found to be essential in suppressing Th1-typical genes such as *Stat4*, *Tbet* and *Ifng* during Th17 immune activation, as a way to stabilize the Th17 cell phenotype.[44] PKCθ is also involved in regulating immune memory. In a study on antiviral responses by $CD8^+$ T-cell responses, PKCθ is required for antigen recall responses upon *in vitro* infection by lymphocytic choriomeningitis virus and influenza virus.[45,46] Furthermore, the efficient and timely recruitment of PKCθ to the immunological synapse is critical for memory T-cell development.[47]

Interestingly, PKCθ also localizes to the immunological synapse in effector T cells to positively regulate cell function but activation of regulatory T cells sequesters PKCθ away from the immunological synapse, leading to negative regulation of induced regulatory T cells.[48,49] This negative regulation by PKCθ involves inhibiting differentiation of induced regulatory T cells through the AKT/Foxo1/3a pathway.[50] Hence, PKCθ plays a role in regulating the T-cell immune response through maintaining the equilibrium of T-cell subpopulations. However, the precise mechanism by which it deciphers the signals received within each cell subset to perform the cell-type-specific function is yet to be elucidated.

PKCβ in BCR signalling

Like T cells, B-cell activation leads to production of regulatory cytokines and chemokines to eliminate pathogens. However, B cells also present antigen to T cells and specialize in producing high-affinity antibodies and long-lived memory cells, generating rapid and long-lasting protection against secondary exposure to the same pathogen.[51] Activation of B cells occurs when an antigen binds to the BCR, initiating phosphorylation events by Src-family kinases as well as Syk and Bruton's tyrosine kinase (Btk)/Tec family kinases. This signals the organized assembly kinases and adaptor proteins forming the signalosome and activating multiple signalling cascades (Fig. 2c).[52] Secondary messengers such as DAG and inositol-1,4,5-triphosphate are generated as part of the BCR activation signalling cascade to initiate Ca^{2+} and PKC downstream signalling pathways, respectively, leading to activation of transcription factors (Myc, nuclear factor of activated T cells, NF-κB, activator protein 1) critical for B-cell function.[53] While B cells express multiple isoforms of PKC (α, β, δ, ε, η, ζ, and λ), PKCβ is the key PKC isoform that is important in BCR signalling.[54–58]

The role for PKCβ in regulating B-cell functions was first discovered through PKCβ gene knockout mice, which were shown to have impaired B-cell activation, inability to proliferate upon BCR stimulation and defects in T-cell-independent immune responses (Table 1).[58] The immunodeficiency traits exhibited by these PKCβ knockout mice are similar to those seen in Btk-deficient or X-linked immunodeficient mice, suggesting that Btk and PKCβ may be linked in BCR signalling.[59] Indeed, Btk has been shown to be important for NF-κB activation upon BCR engagement and is regulated by PKCβ in a negative

feedback mechanism.[60–62] Specifically, PKCβ directly phosphorylates Btk to down-regulate Btk kinase activity and alter its membrane localization in BCR signalling.[62] This result was also confirmed using a PKCβ-selective inhibitor, where inhibiting PKCβ kinase activity leads to an increase in Btk kinase activity to enhance calcium mobilization, so implicating PKCβ in the regulation of calcium release upon BCR activation.[63]

Another study used *N*-ethyl-*N*-nitrosurea-induced mutagenesis to generate PKCβ mutant mice (*Tilcara*) that have heterozygous mis-sense mutations.[64] The *Tilcara* mutant mice have single amino acid substitutions in conserved residues within the kinase domain of PKCβ. This results in an S552P mutation that is close to a docking site for the pseudosubstrate domain. These mice show impaired T-cell independent antibody responses, as seen in the PKCβ gene knockout mice.[58] Another effect of the *Tilcara* mutation is loss of active PKCβI but not PKCβII in B-cell protein lysates in homozygous mutants, implying that the region of mutation is important for PKCβI function. While the *Tilcara* mutant mice do not show as significant an effect phenotypically compared to PKCβ knockout mice, the S552P substitution occurs at an evolutionarily conserved residue, hence there could be changes occurring at the transcriptional level. It would be of great interest to examine how mutation at this conserved residue can affect BCR signalling on a genotypic level.

PKCβ is also involved in forming the BCR signalosome upon BCR engagement (Fig. 2c). PKCβ phosphorylates CARMA1 [caspase recruitment domain family (CARD)-containing membrane-associated guanylate kinase (MAGUK) protein 1] on S668 to lead to the formation and recruitment of the CARMA1, B-cell leukaemia/lymphoma 10 (Bcl10), and mucosa-associated lymphoid tissue 1 (MALT1) complex to lipid rafts to form part of the BCR signalosome.[65] PKCβ is also required in recruiting inhibitor of κB (IκB) kinase (IKK) to the CARMA1–Bcl10–MALT1 complex.[56] For IKK activation to occur, the adaptor protein CARMA1 is directly phosphorylated by PKCβ, which then brings IKK and another protein kinase transforming-growth factor-activated kinase 1 (TAK1) close together.[66,67] This allows TAK1 to phosphorylate IKK leading to its activation.[66] As a result, IκB is phosphorylated by IKK to lead to activation of NF-κB, hence demonstrating the critical role that PKCβ plays in BCR-dependent NF-κB signalling.

Hence, from all the examples shown in the different immune cells, PKC signalling pathways converge with other signalling pathways in the nucleus to regulate inducible gene transcription. Furthermore, only a specific isozyme has been discussed in this review but there are other isozymes that participate in each of the signalling pathways, in which crosstalk between the different isozymes could occur. Undoubtedly, the PKC signalling pathway is important in transducing signals in the cytoplasm upon immune cell activation but PKCs are also found in the nucleus, suggesting a dual role by PKC as nuclear signal transducers as well as cytoplasmic signal transducers.

PKC as nuclear signal transducers

While the mechanism of PKC signalling in the cytoplasm through the plasma membrane is very well characterized, relatively less is known about PKC signalling within the nucleus. Since the discovery of nuclear PKC in the nuclei of rat liver,[68] there is a growing body of evidence to support the presence of PKC in the nucleus, with specific expression of the isoforms depending on the cell type and differential distribution within subnuclear compartments.[69–71] The PKCs present in the cell nucleus are either translocated from the cytoplasm upon activation or exist constitutively within the nucleus.[72] In the immune system, translocation of PKC to the nucleus appears to be the main mechanism in which nuclear PKC regulates immune cell differentiation. This translocation occurs upon stimulus with differentiation agonists such as macrophage colony-stimulating factor, hexamethylene-bis-acetamide, vitamin D3, anti-*trans* retinoic acid, PMA and nerve growth factor (reviewed in ref. 71). Granulocyte–macrophage colony-forming cells treated with macrophage colony-stimulating factor show increased PKCα levels and stimulated its translocation to the nucleus, leading to macrophage differentiation.[73] Similarly, hexamethylene-bis-acetamide-induced differentiation of Friend erythroleukaemia cells requires the localization of PKCα to the nucleus as PKCα-antisense transfection prevented cell differentiation.[74] Also, the translocation of specific PKC isoforms is dependent on the stimulus used within the same cells.[75] This can be seen in human promyelocytic leukaemia HL-60 cell line, where vitamin D3 exposure leads to increased levels of PKCζ isoform while anti-*trans* retinoic acid treatment leads to an increase in PKCα and PKCζ isoform in the nucleus.[76,77] In contrast, HL-60 cells stimulated with PMA leads to the accumulation of PKCδ within the nucleus.[78]

As mentioned earlier, PKC is activated by second messengers generated from immune cell activation such as calcium and/or DAG depending on the PKC isoform. Interestingly, there are distinct second messenger signalling pathways in the cytoplasm and nucleus. Calcium released in the cytoplasm causes cytoplasmic PKC to translocate to the plasma membrane while calcium that is released within the nucleus translocates nuclear PKC to the nuclear envelope.[79] Also, there are separate pools of DAG produced by phospholipids localized within the cytoplasm or the nucleus.[80] Furthermore, stimulus-dependent production of DAG leads to selective translocation of specific PKC isoforms to the nucleus. For example, in

HL-60 cells, differentiation signals lead to DAG production in the nucleus by phospholipase D causing PKCα nuclear translocation. When proliferation stimulus is used, nuclear DAG is produced by phosphatidylinositol (4,5) biphosphate leading to PKCβII nuclear migration.[81] Hence, PKC in the cytoplasm and the nucleus are differentially regulated depending on its localization. Although PKC isoforms have been detected in the nucleus of immune cells in the resting state, it is unclear how it is being retained in the nucleus and whether it is structurally and functionally different from the translocated PKCs. It is postulated that PKC-binding proteins may play a role not only in the retention of PKC in the nucleus but also in the translocation of PKC into the nucleus.[75,82]

Nuclear translocation signals for nucleocytoplasmic shuttling

Proteins can be transported between the cytoplasm and the nucleus through nuclear pores located within the nuclear envelope. The ability of proteins to translocate into or out of the cell nucleus involves nuclear translocation signals such as nuclear localization signals (NLS) or nuclear export signals, respectively.[83] It can also occur through binding with proteins that have NLS as a way to enter the nucleus. The best characterized NLSs are classical NLS motifs, which can have a monopartite or bipartite motif.[84] PKC isoforms do not have the canonical NLS but contain a nuclear targeting motif that is similar to the classical bipartite NLS, forming a putative NLS that is conserved across the different PKC isoforms (Fig. 1).[85,86] In line with this, a putative NLS motif was proven to be functional for nuclear import of PKCδ to initiate apoptosis.[86] Upon induction with apoptotic agents in ParC5 cells, PKCδ is phosphorylated at Y64 and Y155, causing a conformational change to expose the NLS and allow the nuclear import factor, importin-α, to bind and facilitate nuclear import of PKCδ.[87] Atypical PKC was also shown to contain both a functional NLS and nuclear export signals within the zinc-finger domain, allowing for rapid shuttling between the nucleus and cytoplasm.[88] Similarly, aPKC is phosphorylated at Y256 upon nerve growth factor stimulation in PC12 cells, and bound by importin-α for nuclear translocation.[89] Hence, there seem to be similarities in the mechanism of translocation between PKC isoforms but this is quite likely to occur in a stimulus-specific and cell context-dependent manner.

Translocation of PKC into the nucleus occurs through mechanisms that are different from the proteins with canonical NLS.[90,91] For example, PKCα requires an intact cytoskeleton to translocate into the nucleus but a protein with canonical NLS is unaffected when the cytoskeleton is disrupted.[90] In addition, PKCα does not require nuclear import factors p97/importin/karyopherin β or GTP to transport into the nucleus, unlike proteins with canonical NLS.[91] Interestingly, aPKCs have isoform-specific regulation of nucleocytoplasmic shuttling. Even though both PKCζ and PKCι contain the same NLS, only PKCζ require the NLS for nuclear translocation while the ability of PKCι to translocate is independent of the NLS.[92] In contrast, using chimeric proteins, Seidl *et al.*[92] showed that PKCι requires the hinge region for nuclear localization but the hinge region of PKCζ excludes it from the nucleus. Undoubtedly, the requirement of the PKC NLS and the associated protein domains as well as nuclear import factors plays a role in nucleocytoplasmic shuttling of PKC. What is interesting is how different isoforms each have specific requirements for each of those features, which leads to isoform-specific localization of PKC, and hence isoform-specific regulation of nuclear function.

In addition to the NLS, another motif, the S/T-P-S/T (SPT-like) motif, was suggested to perform like a general NLS for signalling proteins that do not have the canonical NLS, implying that PKC could potentially be regulated by the SPT-like motif.[93] Using protein sequence alignment, Sutcliffe *et al.*[94] showed that novel and conventional PKC isoforms were predicted to have SPT-like motif present. The SPT-like motif appears to reside close to where the regulatory and kinase/catalytic domains are and deletions in the regulatory or kinase domains of PKC are shown to affect nuclear translocation.[95,96] Mutation studies substituting the SPT motif in PKCθ with an alternative motif that cannot be phosphorylated impaired the localization of PKCθ to the nucleus, suggesting that phosphorylation of SPT is required for nuclear translocation.[94] Interestingly, mutating the putative NLS motif has no effect on the distribution of PKC in the cell, suggesting that the SPT-like motif may be an alternative NLS motif used by signalling kinases that do not have the canonical NLS for nuclear transport.[94] Hence, it would be of interest to examine how PKC activation can affect the configuration of the protein folding/structure and as a consequence the exposure of the nuclear localization motifs for regulation.

The fact that there are numerous substrates described for nuclear PKC suggests that PKC could potentially regulate nuclear functions like gene transcription (reviewed in refs. 97,98). Indeed, some of the nuclear PKC substrates include chromatin factors such as histone (H1, H2B, H3), RNA polymerase II, transcription factors Fos and cAMP response element-binding protein (CREB), as well as architectural proteins such as high mobility group proteins, implicating PKC in directly regulating gene transcription in the nucleus.

PKC as nuclear chromatin regulators

Gene transcription is dynamically regulated through the chromatin structure by various epigenetic factors. The

fundamental unit of chromatin is the nucleosome: 147 base pairs of DNA wrapped around a histone octamer (two sets of H2A, H2B, H3 and H4) often with a linker histone (H1) present.[99,100] The accessibility of gene regulatory regions is dependent on whether the chromatin is in the open (permissive) or closed (repressive) state, so leading to either gene activation or repression, respectively. Control of chromatin accessibility hence gene expression involves transcriptional regulatory complexes such as transcription factors, histone variants, chromatin remodelling complexes and histone modifiers (reviewed in refs 101–103).

The earliest evidence of a signalling kinase involvement in directly regulating chromatin is in yeast where the key signalling kinase Hog1 was found to physically associated with promoter regions upon osmotic stress.[104] Since then, more evidence has revealed the role of signalling kinases as epigenetic regulators that can modify transcription factors, histones as well as histone modifiers in the nucleus (reviewed in ref. 105). This includes the PKC signalling family, which has been shown to have a role in directly regulating gene transcription through either phosphorylating specific histone residues, histone modifiers, transcription factors as well as other transcriptional regulatory factors or forming part of a transcriptional regulatory complex (Fig. 3, Table 2).

PKCδ as a histone kinase in immune-mediated apoptosis

Protein kinase Cδ regulates the immune system through the events of mitochondrial-dependent apoptosis by phosphorylating histones at apoptotic histone residues such as H2BS14, H3S10 and H3T45 (Fig. 3).[106–109] In B cells, the nuclear localization of a catalytically active PKCδ leads to phosphorylation of H2BS14 to result in apoptosis of resting B cells.[107] Similarly, in Sjögren's syndrome, interaction of B cells with human salivary gland cells causes translocation and activation of PKCδ into the nucleus to phosphorylate H2BS14 leading to a B-cell-mediated apoptosis of epithelial cells.[106] Using PKCδ small interfering RNAs in Jurkat T cells, Park *et al.*[109] showed that phosphorylation of H3S10 by PKCδ is required for DNA damage-induced apoptosis. *In vitro* studies in neutrophils showed that phosphorylation of H3T45 increases significantly in apoptotic cells and that this phosphorylation is mediated by PKCδ.[108] These systems focused on the pro-apoptotic function of PKCδ as a result of cellular stress but PKCδ can also be anti-apoptotic depending on the cell type. Specifically, PKCδ promotes the survival of cancer cells through established anti-apoptotic pathways including NF-κB.[110] However, whether the anti-apoptotic activity by PKCδ is regulated through histone phosphorylation is yet to be studied.

PKCθ association with histone modifiers in T-cell activation

In addition to phosphorylating histones, PKC can also interact with and phosphorylate histone modifiers (Table 2). Specifically in the immune system, PKCθ not only has a cytoplasmic signalling role during TCR activation, it is also present in the nucleus of T cells to regulate transcription of inducible immune genes.[94,111,112] In stimulated human Jurkat T cells, PKCθ associates with the N-terminal transcriptional activation domain of p300 and phosphorylates S384 on p300 leading to its transcriptional activation.[111] In a study by Sutcliffe *et al.*,[112] PKCθ does not appear to phosphorylate histones directly but instead forms a complex with RNA polymerase II, histone kinase mitogen and stress-activated protein kinase 1, adaptor protein 14-3-3ζ and histone demethylase lysine-specific demethylase 1 at the proximal promoter of inducible genes in human Jurkat T cells (Fig. 3). The assembly of this complex is dependent on NF-κB as inhibiting NF-κB impaired PKCθ, RNA polymerase II and lysine-specific demethylase 1 recruitment to immune gene promoters upon TCR activation.[94] This formation of an active transcriptional complex on key inducible genes can also be seen in breast cancer stem cells, where PKCθ acts as a molecular switch that regulates epithelial to mesenchymal transition.[112,113] Interestingly, inhibiting PKCθ in T cells leads to increased expression of microRNAs, small non-coding RNAs responsible for post-transcriptional repression of gene expression.[112] This negative regulation of microRNA genes by PKCθ involves NF-κB, both of which may be part of a repressive complex on microRNA genes to repress its transcription.[94] This nuclear activity further strengthens the notion that PKCθ is a key enzyme in the regulation of T-cell activation. So far only PKCθ is shown to associate and phosphorylate histone modifiers in the immune system. Given the key role that histone modifiers play in regulating inducible gene expression, it can be postulated that other PKC isoforms could potentially regulate histone modifiers through their kinase activity in the immune context.

Direct regulation of transcription factors by PKCs

Protein kinase C signalling in the cytoplasm activates downstream signalling cascades leading to transcription factor activation, and hence gene transcription (Fig. 2). In the nucleus, PKC plays a more direct role in regulating transcription factors through phosphorylating the factors directly or regulating interactions of transcriptional regulatory factors to lead to transcriptional activation of genes (Table 2). For example, monocyte induction by macrophage colony-stimulating factor leads to phosphorylation of NF-κB p65 at S276 by PKCα, which is important for transcriptional activation of NF-κB.[114] In dendritic cells,

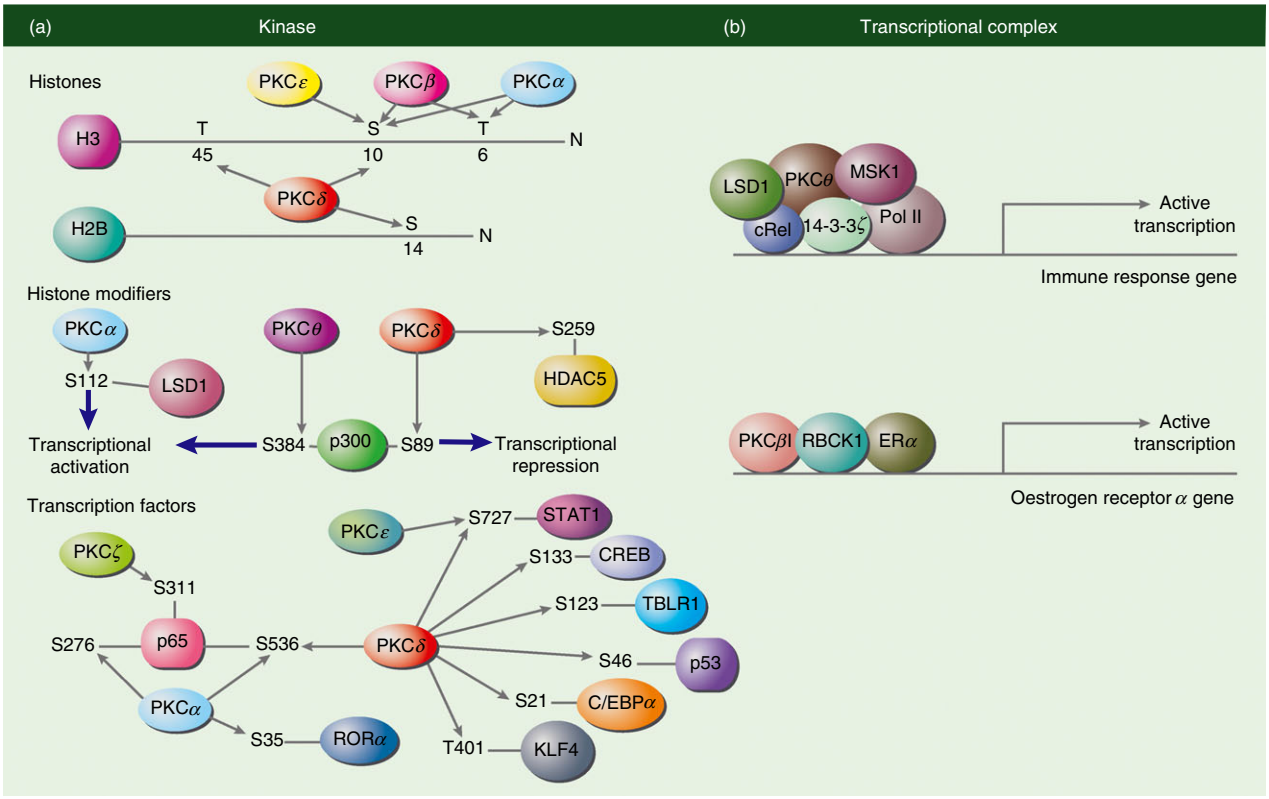

Figure 3. Protein kinase C (PKC) has a role as chromatin regulator in the nucleus. (a) PKC can act as a kinase to phosphorylate histones, histone modifiers or transcription factors at specific serine and/or threonine residues leading to transcriptional activation or repression. Both PKCα and PKCβ can phosphorylate histone H3 at threonine (T)6 and serine (S)10 while PKCε phosphorylates S10 only. PKCδ phosphorylates H3S10, H3T45 and H2BS14. PKCα phosphorylates S112 on lysine specific demethylase 1 (LSD1) leading to transcriptional activation. While PKCθ phosphorylates S384 on p300 causes gene activation, PKCδ phosphorylation on S389 of p300 leads to transcriptional repression. PKCδ can also phosphorylate S259 on histone deacetylase 5 (HDAC5). As for transcription factors, PKCα phosphorylates S276 and S536 on p65 and S35 on retinoic acid-related orphan nuclear receptor α (RORα). PKCζ phosphorylates S311 on p65. PKCδ phosphorylates T401 on Krüppel-like factor 4 (KLF4), S21 on CCAAT/enhancer-binding protein α (C/EBPα), S46 on p53, S123 on transducin β-like 1X-linked receptor 1 (TBLR4), S133 on cAMP response element-binding protein (CREB) and S727 on signal transducer and activator of transcription 1 (STAT1). PKCε can also phosphorylate STAT1 at S727. (b) PKC can also form a transcriptional complex on a gene promoter. For example, PKCθ forms a transcriptional complex with LSD1, mitogen and stress-activated protein kinase 1 (MSK1), RNA polymerase II (Pol II), 14-3-3ζ and cRel on immune response gene promoters to lead to transcriptional activation. PKCβI is recruited to the oestrogen receptor a promoter as part of a regulatory complex with RanBP-type and C3HC4-type zinc finger containing 1 (RBCK1) and oestrogen receptor α (ERα) to regulate ERα promoter gene expression.

PKCζ phosphorylation of p65 at S311 prevents binding of histone methyltransferase glucagon-like peptide to p65 at monomethylated K310, allowing expression of p65 target genes.[115] Similarly, IL-32β mediates PKCδ phosphorylation of CCAAT/enhancer-binding protein α at S21 to prevent binding of the CCAAT/enhancer-binding protein α to IL-10 promoter, hence activating the gene in human myeloid cells.[116]

Upon heat-shock induction, PKCε phosphorylates STAT1 at S727 in Jurkat T cells to activate the heat-shock protein 90β (hsp90β) gene.[117] While in macrophages, induction by IFN-γ leads to STAT1 phosphorylation by PKCδ at S727 to promote class II transactivator (CIITA) gene expression.[118] A similar study shows that CIITA gene expression in B cells is controlled by CREB phosphorylation by PKCδ.[119] The phosphorylation of CREB by PKCδ occurs at S133 upon B-cell activation.[120] Whereas in T cells, PKCθ is involved in the phosphorylation and binding of CREB to the IL-2 promoter.[121] In human Jurkat T cells, phosphorylation by PKC can regulate the interaction of special AT-rich binding protein 1 (SATB1) with either histone deacetylase 1 or p300/CREB-binding protein-associated factor (PCAF).[122] In the basal state, PKC-phosphorylated SATB1 at S185 associates with histone deacetylase 1, so acting as a repressor, but upon activation, dephosphorylated SATB1 associates with PCAF, leading to activation of the IL-2 gene. These studies show that nuclear PKCs directly regulate transcription factors through phosphorylation for transcriptional activation in the immune system. However, phosphorylation

Table 2. Chromatin-associated protein kinase C (PKC) substrates

Kinase	Histone	Histone modifier	Transcription factor	Other chromatin regulatory factors	Phosphorylated residue	Regulatory role
PKCα	H3				S10	ND
	H3				T6	Txn activation
		HDAC6				Txn activation
		LSD1			S112	Txn activation
			p65		S276/S536	Txn activation
			RORα		S35	Txn repression
			Sp1		S-P	Txn activation
PKCβ	H3				S10	Txn activation
		CBP				Txn activation
PKCβI	H3				T6	Txn activation
PKCβII		PCAF				Txn activation
				HMGB1		Cytokine secretion
PKCδ	H2B				S14	Pro-apoptotic
	H3				S10	Pro-apoptotic
	H3				T45	Pro-apoptotic
		HDAC5			S259	ND
		p300			S89	Txn repression
			C/EBPα		S21	Txn activation
			CREB		S133 (I)	Txn activation
			KLF4		T401	Txn repression
			p53		S46	Txn activation
			p65		S536 (I)	Txn activation
			STAT1		S727	Txn activation
			TBLR1		S123	Txn activation
				DNMT1		Proteasomal degradation
				hnRNPK	S302	Pro-apoptotic
				TIF1β	S473	Txn repression
PKCε	H1					Anchoring protein
	H3				S10	Txn activation
			STAT1		S727	Txn activation
				Hsp90β		Txn activation
PKCθ		MSK-1, LSD1 (C)		Pol II, 14-3-3ζ (C)		Txn activation
		p300			S384	Txn activation
			CREB			Txn activation
				Ki-1/57	T-P	Subcellular localization
PKCζ			p65		S311	Txn activation
				CDP/Cut		Txn repression
				Ki-1/57	T/S-P	Subcellular localization
				SHP-T55	T55	Txn repression
PKCζ/λ				BAF60c-S247	S247	Txn activation
PKC	H2A					ND
			SATB1			Txn activation
				HEXIM1	S158	PTEFb activation
				HMGB1		DNA bending
				Ki-1/57		Subcellular localization
				Pol II		Pol II activation

BAF60c, Brg1/Brm-associated factor 60c; C, transcriptional complex; C/EBPα, CCAAT/enhancer-binding protein α; CBP, CREB-binding protein; CDP/Cut, Cut-like homeodomain protein; CREB, cAMP response element-binding protein; H1, histone H1; H2B, histone H2B; H3, histone H3; HDAC, histone deacetylase; HEXIM1, hexamethylene bisacetamide-(HMBA)-induced mRNA-encoded proteins 1; HMGB1, high mobility group box 1; hnRNPK, heterogeneous nuclear ribonucleoprotein K; Hsp90β, heat-shock protein 90 β; I, interaction; Ki-1/57, 57-kDa human protein antigen recognized by the CD30 antibody Ki-1; KLF4, Krüppel-like factor 4; LSD1, lysine-specific demethylase 1; MSK1, mitogen and stress-activated protein kinase 1; ND, not determined; P, phosphorylation; PCAF, p300/CBP-associated factor; Pol II, RNA polymerase II; RORα, retinoic acid-related orphan nuclear receptor α; S, serine; SATB1, special AT-rich binding protein 1; SHP, small heterodimer partner; Sp1, specificity protein 1; STAT1, signal transducer and activator of transcription 1; T, threonine; TBLR1, transducin β-like 1X-linked receptor 1; TIFb, transcriptional intermediary factor 1β; Txn, transcriptional.

of transcription factors by PKCs can also lead to gene repression[123,124] but this has not been shown in the immune context.

Other PKC isoforms as chromatin regulators in non-immune systems

At present, there are no reported epigenetic activities by PKCγ and PKCη. Although not published to have epigenetic roles in immune cells, PKCα and PKCβ have been shown to act as histone kinases in other studies (Table 2).[125,126] A screen with 97 kinases using HeLa cells showed that PKCα, PKCβI and PKCβII can phosphorylate H3T6 but not H3T3, H3S10 or H3T11.[126] This is in line with a study using high-resolution nuclear magnetic resonance spectroscopy, where PKCα and both PKCβ isoforms could phosphorylate H3T6 and H3S10 but in a mutually exclusive manner (Fig. 3).[125] Using prostate cancer cells, Metzger *et al.*[126] showed that PKCβI phosphorylates H3T6, which blocks H3K4 demethylation by lysine-specific demethylase 1 during androgen receptor-dependent gene activation. In breast cancer cells, PKCβI is recruited to the oestrogen receptor α (ERα) promoter B as part of a regulatory complex with RanBP-type and C3HC4-type zinc finger containing 1 and ERα to regulate ERα gene expression (Fig. 3).[127] This recruitment of PKCβI is associated with the presence of PKCβI-dependent H3K4me2 and H3T6 phosphorylation though a direct causal effect has not been demonstrated in this model.[127] Given that PKCβ is specifically expressed in B cells, it can be postulated that PKCβ could potentially regulate immune gene expression as an epigenetic enzyme.

We have discussed examples of PKC isoforms functioning as epigenetic enzymes in neutrophils (PKCδ), macrophages (PKCα, PKCδ), myeloid cells (PKCδ), dendritic cells (PKCζ), B cells (PKCδ) and T cells (PKCδ, PKCε, PKCθ). Given the defects seen in immune cell homeostasis, activation and function in various PKC knockout studies (Table 1), it is highly possible that PKC isoforms that are not known to have an epigenetic function could potentially regulate the expression of key immune genes as epigenetic enzymes.

Therapeutic potential of PKCs in disease

As PKCs play a ubiquitous role in cell signalling, it is not surprising that dysregulation of PKC leads to disease such as diabetes, cancer, cardiovascular disease, dermatological disease, psychiatric diseases, neurological conditions and immune-mediated diseases.[128] The two main PKC isoforms implicated in human autoimmunity and inflammation diseases are PKCδ and PKCθ.[128] PKCδ deficiency is linked with autoimmune diseases involving B-cell development immunodeficiency.[129,130] While there are currently no drugs in development against PKCδ in autoimmune diseases, a peptide inhibitor of PKCδ, delcasertib (δV1-1), shows promising results in myocardial infarction with reduction of infarct size when administered in patients.[131] Furthermore, two published patents reported the potential use of antisense oligonucleotides against PKCδ to regulate its expression in infectious and autoimmune disease.[132] Hence, there are tools currently available that can be applied for therapeutic studies of PKCδ in the immune context.

While there are a number of PKC regulators in clinical trials for various diseases, the main research of autoimmune PKC activity and drug targeting is in T cells due to the pivotal role that T cells play in the immune response.[128] There is a growing body of evidence that PKCθ is indispensable for critical T-cell functions such as protective responses to pathogens. Hence PKCθ is less likely to result in off-target immunosuppression.[133] As a result, PKCθ, is highly studied as a potential therapeutic target.[9] The most advanced PKCθ inhibitor to be developed is sotrastaurin (AEB071), which is currently undergoing clinical trials for the treatment of psoriasis and organ transplantation.[134–138] Another promising pharmaceutical PKCθ inhibitor is compound 27, for which oral administration in mice led to a decreased IL-2 response.[139] Furthermore, similar compounds of PKCθ inhibitors developed by the same group were developed and tested in *in vivo* mouse models of colitis and multiple sclerosis and the mice treated with the PKCθ inhibitors showed improved general well-being and reduced disease severity.[140] Undoubtedly, these findings highlight the importance and great potential for development of small molecule inhibitors of PKCs in treating immune-mediated diseases.

Conclusion

The review highlights the contrasting roles of PKC in the cytoplasm compared with the nucleus. In the cytoplasm, active PKC bind membrane components to act as signalling molecules and phosphorylate different substrates to mediate activation of transcription factors required for immune gene expression via the signalling cascade. The phosphorylated substrates that may be anchored to membrane fractions by adaptor proteins and/or directly bind active PKC once phosphorylated, may be released to the cytoplasm and act in the cytoplasm or they may translocate to the nucleus upon phosphorylation. In the nucleus, PKCs directly phosphorylate histones/transcriptional regulatory factors or form complexes that associate with chromatin. Whereas the mechanism of PKC as a cytoplasmic signalling transducer is extensively studied, its role in the nucleus either as a signal transducer or chromatin regulator is not as well-studied.

Different activation mechanisms and subcellular localization of PKC can determine different functions but what remains to be determined is how translocation to subcellular locations is determined upon different stimuli.[71,75] Although local increases of DAG and calcium are shown to affect PKC translocation, it is unclear if this translocation is a diffusion mediated process due to local increases of specific lipids or calcium.[79–81] Alternatively, is translocation mediated by active transport aided by anchoring proteins such as receptors for activated C-kinases? The receptors for activated C-kinases anchor proteins bind directly with the C2 domain of activated PKC but this has only been shown for PKCβII and PKCε.[141,142] There are other proteins described as forming protein–protein interactions with PKC be it adapter proteins, anchor proteins or PKC substrates and these have great potential for therapeutic target development (reviewed in ref. 128).

It will also be interesting to uncover how the nuclear PKC and cytoplasmic PKC interpret external stimuli. Is there a different mechanism of activation that relays the message allowing them to perform their cell-specific function depending on their location within the cell? Also, current examples of PKC as epigenetic enzymes are found only in the adaptive immune system. There is no evidence as yet of PKC as an epigenetic enzyme in innate immune response but this is highly probable as there is evidence of PKC translocating to the nucleus in human monocytes.[143] Following from there, are the PKCs in the nucleus the same as the one in cytoplasm; i.e. do they perform the same function and are recycled or are there specific nuclear PKCs and cytoplasmic PKCs? Examples from another protein kinase family, mitogen-activated protein kinases, show that cytosolic and nuclear mitogen-activated protein kinases are regulated differently, and so a potentially similar mechanism could be present for PKCs.[144] Cleaved forms of PKC may be a further avenue of nuclear and cytoplasmic function differentiation as catalytic fragments of cleaved PKCδ and PKCβII isoforms have been suggested to accumulate in the nucleus.[145,146] Hence, nuclear translocation or retention could be mediated through cleavage of PKC isoforms, in which the catalytic domain could be retained for further phosphorylation or interaction with other proteins. In addition, phosphorylation of PKC is a key mechanism regulating PKC function. It remains to be determined which substrate for phosphorylation is critical for different outcomes depending on the context the isoform is in.

It would be essential to uncover the difference between nuclear and cytoplasmic PKCs as these differences could be used to design specific drug targets. Most existing PKC kinase inhibitors target the common catalytic ATP pocket and hence are not isozyme-selective. There are also isozyme-specific inhibitors and these tend to target PKC binding to receptors for activated C-kinases, but not all PKCs have been shown to interact with receptors for activated C-kinases and the key interaction sites are not known.[128] Hence, it is important to understand how and when PKCs translocate to the nucleus to enable the design of specific modulators of protein–protein interactions. This was shown in a proof of concept study where a peptide was developed that specifically inhibited the nuclear translocation of extracellular signal-regulated kinase 1/2 kinase to combat its function to induce cell proliferation in melanoma cells.[147] Structural studies of the nuclear PKC domains and the contribution of the nuclear import receptors will enhance our understanding of the mode of action of these kinases. This provides a strong basis for the design of high-affinity inhibitors capable of specifically blocking the nuclear translocation and function of PKCs in an isoform-specific manner. Specific inhibition of substrate interactions with specific PKC isoforms could pave the way for more targeted therapeutics. As discussed, dysregulation of PKC leads to the development of disease and particularly with PKC being involved in multiple signalling pathways, it is even more critical to uncover how PKC functions in its different roles to build on the knowledge and understanding for future therapeutic target development.

Acknowledgements

This work was supported by NHMRC Project Grant (APP1025718) awarded to SR and also by a UC PDF Fellowship awarded to PSL in SR's laboratory.

Disclosure

The authors declare no conflict of interest.

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