PKC and the control of localized signal dynamics

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Abstract | Networks of signal transducers determine the conversion of environmental cues into cellular actions. Among the main players in these networks are protein kinases, which can acutely and reversibly modify protein functions to influence cellular events. One group of kinases, the protein kinase C (PKC) family, have been increasingly implicated in the organization of signal propagation, particularly in the spatial distribution of signals. Examples of where and how various PKC isoforms direct this tier of signal organization are becoming more evident.

Allosteric mechanism
A mechanism by which a protein is regulated by a change in its shape and activity after binding an effector molecule at a site other than its active site.

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Understanding the complex network of information relays that is known as signal transduction has become more important, as evidence of aberrant signalling is increasingly documented in diseases such as cancer[1]. Signal transduction is defined by the sense and connectivity of signalling networks, as well as their spatial and dynamic properties that are known to markedly influence signalling outputs. Protein kinases are key transducers in such networks and are considered to be important candidates for the design of molecularly targeted therapeutics[2].

Kinases were first identified as cellular mediators of hormones that regulate metabolic processes, and this provided early evidence for the existence of kinase cascades, specifically the cAMP-dependent protein kinase (PKA)–phosphorylase kinase cascade[3], which in skeletal muscle is associated with the 'fight or flight' response. On the basis of these early studies it was rationalized that such regulatory organization was required as it provided tremendous signal amplification through sequential catalytic steps in the regulatory cascade. Findings over the past 25 years have shown that protein kinases have regulatory roles in all aspects of eukaryotic cell function, which has led to a more sophisticated view of the action of kinases and kinase cascades. Pertinent to this Review, the mutual organization of protein kinases and other transducers has marked effects on their actions, influencing efficiency, specificity and to some extent negating their catalytic promiscuity. This organization of signalling events is driven by the colocalization or sequestration of the signal transducers themselves through the support of protein scaffolds and membrane domains, or more broadly in organelles.

Protein scaffolds are involved in spatially organizing signal transducers, and several are known to be used by protein kinases. One of the best understood protein scaffolds is Ste5p, which is responsible for organizing the pheromone signal transduction pathway in *Saccharomyces cerevisiae* (reviewed in REF. 4). This scaffold binds all four kinases of the mitogen-activated protein kinase (MAPK) cascade in the pheromone response pathway, conferring specificity and efficiency to their action. Such higher-order organizational inputs are not necessarily static or passive. In the case of Ste5p, interaction with the MAPK kinase kinase (MAPKKK) (in this case Ste11p) positively regulates MAPKK activity through an allosteric mechanism[4]. Protein scaffolds are themselves regulated, as documented for the A-kinase anchor proteins (AKAPs), a class of scaffolds for PKA the phosphorylation of which can regulate the release of binding partners. For example, PKA-mediated phosphorylation of AKAP-Lbc (also known as AKAP13), which controls a protein kinase Cη (PKCη)-to-PKD cascade, allows the release of PKCη-activated PKD from the scaffolded complex[5]. Regulated scaffold behaviour is well understood for protein tyrosine kinase-linked receptors, in which phosphorylation of the receptor itself, or of an associated adaptor, creates docking sites for phosphotyrosine recognition domains (for example, SRC homology 2 and PTB domains; see REF. 6) that are modularly embedded in downstream transducers which are recruited to propagate signals. The assembly of such activated receptor complexes, and their trafficking from the plasma membrane through endomembrane compartments to lysosomes or recycling compartments, is a vivid illustration of the dynamic and spatial distribution of signalling scaffolds.

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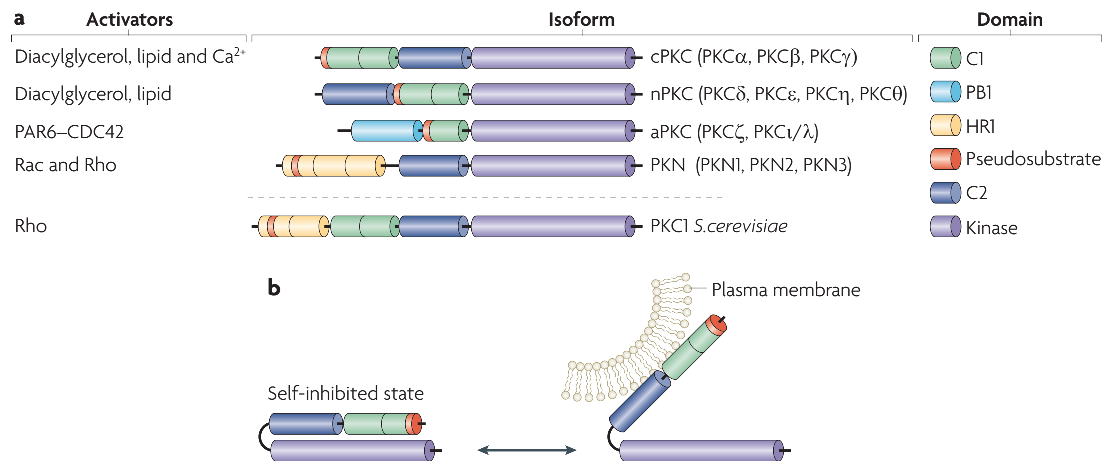

Figure 1 | **The protein kinase C superfamily. a** | A schematic of the domain structure of the mammalian protein kinase C (PKC) family members is shown above the *Saccharomyces cerevisiae* PKC1. In mammals the PKC family members can be divided into four structurally and functionally distinct subgroups according to their regulatory domains. These are the classical isoforms (cPKC), novel isoforms (nPKC), atypical isoforms (aPKC) and the PKC-related kinases (known as PKN). Note that aPKCι is referred to as aPKCλ in mice. PKCs are regulated by auto-inhibition through their pseudosubstrate sites; this inhibition is relieved by different activator proteins (as indicated), depending on their subgroup. **b** | A cPKC is shown in its self-inhibited state, with the pseudosubstrate site binding to the substrate-binding pocket in the kinase domain. When the regulatory domain is recruited to plasma membranes through Ca^{2+}, phospholipid and diacylglycerol, autoinhibition is relieved and the catalytic domain of cPKC is free to exert its action on target substrates. Conserved region 1 (C1) can confer binding of diacylglycerol and phospholipids, and C2 confers binding to phospholipids. Phox/Bem domain 1 (PB1) acts as a dimerization domain in various proteins. Homology region 1 (HR1) confers small-GTPase binding properties to PKCs.

A class of acutely regulated, typically membrane-localized protein kinases, the PKC family (FIG. 1; BOX 1), is emerging as essential for the control of aspects of higher-level signal organization. These kinases are themselves subject to regulation through recruitment to scaffolds, such as AKAP-Lbc (reviewed in REF. 7) and the *Drosophila melanogaster* scaffold InaD. In *D. melanogaster* InaD, which was originally identified in a screen of mutant flies for genes involved in photoreception, has been shown to interact with an eye-specific PKC isoform and other components of the photoreception signalling machinery to control vision[8].

It is not simply that PKC isoforms can be recruited to scaffolds with other transducers that is important, but also that they can control the behaviour of scaffolded complexes by influencing their assembly or disassembly and their subcellular localization, without necessarily being an integral part of the complex. As the spatial and dynamic aspects of signal transduction are becoming increasingly more important in our view of cellular physiology, it is timely to review examples of PKC isoform action in controlling these events. In this Review we provide a brief background on the PKC family, covering general properties and pathways. This is followed by a few selected examples of particular PKC isoforms being engaged in signal relays that influence the dynamic and localized behaviour of signals and signalling complexes. It is not our intention to cover the plethora of PKC-interacting proteins and scaffolds, which have been extensively discussed (see REFS 9–12). We emphasize that the processes illustrated here are intended to exemplify the dynamic nature of signalling platforms and the roles of PKC isoforms in imposing this dynamism. Specifically, the examples relate to homotypic and heterotypic cell–cell recognition and cell–extracellular matrix (ECM) interactions that regulate cell migration. Finally, we address the problem of how it is possible to convert descriptions of spatially resolved signals into an understanding of causative relationships for these distributed signals.

The PKC family

The Ser/Thr PKC family comprises ~2% of the human kinome. PKCs are broadly conserved in eukaryotes, ranging in complexity from a single isoform in budding yeast (*S. cerevisiae*) to 5 isoforms in *D. melanogaster* and 12 in mammals[13]. The large number of mammalian PKC family members, and their broadly overlapping substrate specificities, has presented a challenge when attempting to assign specific functions to members of this class of kinases. Although a degree of redundancy has been suggested through mouse genetic experiments, increasing evidence supports individual, non-redundant, albeit often subtle roles for many members of this family[14].

All PKC isoforms share a highly conserved carboxy-terminal kinase domain that is linked by a hinge region to a more divergent amino-terminal regulatory domain (FIG. 1a). When inactive, PKC is auto-inhibited by a pseudosubstrate sequence that is present in the regulatory domain, which occupies the substrate binding pocket in the otherwise functional kinase domain[15]. PKC is activated when second messengers and/or allosteric effectors bind to its regulatory domain, typically at the

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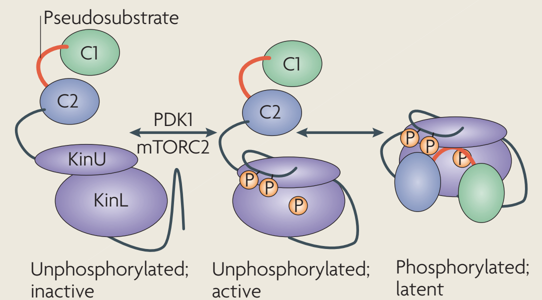
Box 1 | **Priming active protein kinase C**



Maintaining the latent state of protein kinase C (PKC) isoforms requires that the catalytic domain is in a form that can bind protein substrates and also undergo auto-inhibition by pseudosubstrate binding (see the figure; latent state). To achieve this competent state the kinase domains are phosphorylated on three (conventional PKCs and novel PKCs) or two (atypical PKCs and PKNs) Ser or Thr residues[112]. These modifications stabilize the active kinase domain conformers, which enables the conversion to the inhibited latent state and confers optimal catalytic activity following de-inhibition (see the figure; active state).

It is implicit in this conceptual view of PKC isoform function that unphosphorylated forms are in catalytically incompetent 'open' conformations (with fully exposed regulatory conserved region 1 (C1) and C2 domains) and that, although these conformers may be in part sequestered by chaperones[113], they can also markedly affect cell behaviour through their constitutively exposed binding domains (for examples see REF. 114) (see the figure; inactive state).

The priming of PKC isoforms seems to require two upstream kinases. One is phosphoinositide-dependent protein kinase 1 (PDK1)[115,116], which is also responsible for the phosphorylation of many other Ser/Thr protein kinases of the AGC kinase family[117]. PDK1 phosphorylates the activation loop in kinase domains, a variably structured region that typically lies between the upper (KinU) and lower (KinL) lobes of kinase domains and influences their mutual alignment to determine catalytic potential. The second kinase is the mammalian target of rapamycin 2 complex (mTORC2)[118,119]. mTORC2 controls the phosphorylation of the turn motif and hydrophobic sites (when present) in the carboxy-terminal tails of these kinases. On the basis of the structural solution to the PKCβ kinase domain[120], it can be concluded that these two C-terminal phosphorylation sites help to stabilize an active conformer of the kinase domain by docking the C-terminal tail of the protein with KinU, limiting the relative orientation of KinU to KinL; these form discrete subdomains in the kinase.

plasma membrane (FIG. 1b). This disrupts the docking of the regulatory kinase domain, which displaces the bound pseudosubstrate region from the active site, allowing the activation of PKC [16,17].

The PKC family can be divided into four structurally and functionally distinct subgroups specified by their divergent regulatory domains (FIG. 1a). The conventional PKCs (cPKCs) comprise PKCα, PKCβ and PKCγ. This family is activated by a combination of diacylglycerol and phospholipid binding to their conserved region 1 (C1) domains and Ca^{2+}-dependent phospholipid binding to their C2 domains. The novel PKCs (nPKCs), which include PKCδ, PKCε, PKCθ and PKCη, are similarly activated by diacylglycerol and phospholipids, but they do not respond directly to Ca^{2+} (reviewed in REF. 13). The atypical PKCs (aPKCs; PKCι (known as PKCλ in mice) and PKCζ) do not depend on Ca^{2+} or diacylglycerol for activation, but are instead allosterically activated by an interaction of their Phox/Bem 1(PB1) domain with the partitioning defective 6 (PAR6)–CDC42 complex, which is involved in specifying cell polarity[18] (see below). The PKN subfamily members (PKN1, PKN2 and PKN3) share a related G-protein dependent allosteric mode of regulation and contain homology region 1 (HR1) motifs instead of a regulatory PB1 domain. Bivalent engagement of the HR1a and HR1b motifs[19] by the Rho-family GTPases Rho or Rac (BOX 2) disengages the PKN pseudosubstrate and results in kinase activation[20]. Recent evidence indicates that the C-terminus of PKN1 may also be required for its activation by Rho[21]. In addition to these specific inputs, other regulatory processes influence the function of PKCs. These include the covalent modification (specifically phosphorylation) of PKCs and their interaction with specific binding partners, which can modulate the requirements for allosteric inputs or in some cases bypass them all together, as seen for some scaffold interactions (reviewed in REF. 11).

Phox/Bem 1 (PB1) domain
Protein module that can bind to target proteins through a PB1–PB1 domain interaction.

Antigen-presenting cell
A cell of the immune system (a macrophage, dendritic cell or B cell) that stimulates immune responses by displaying antigens on its surface to other cells of the immune system (T cells).

The modular nature of this family — PKCs have a conserved kinase domain coupled to a series of differentially activated regulatory domains — allows PKC activity to be deployed with spatial and temporal specificity. It also allows PKC activity to be directed by multiple inputs, including localized (membrane limited) second messenger production and interaction with membrane-anchored small G proteins, scaffolds and accessory proteins. As a result the PKC family is centrally involved in the spatial control of signal transduction in cells.

PKC action in cell–cell contacts

Cell–cell interactions can involve heterologous or homologous cell recognition. Heterologous recognition occurs when interactions are formed between different cell types, such as a T cell and an antigen-presenting cell (APC) (see below). Homologous cell recognition occurs between cells of the same origin, as exemplified by cells forming a polarized epithelial layer. PKC isoforms have key roles in controlling the assembly and disassembly of localized signalling complexes in both types of cell recognition; two specific examples are discussed below.

PKCθ regulates T cell recognition of APCs. The interaction of an effector T cell with an APC occurs through multiple cell–cell interactions. These interactions centre on the ability of the T cell receptor (TCR) to recognize a processed peptide antigen that is presented to it bound to the major histocompatibility complex (MHC) class I

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Box 2 | **G proteins**

The function of all members of the protein kinase C (PKC) family is influenced by G proteins. This is in part indirect through G-protein coupled receptors (GPCRs), which operate through heterotrimeric G proteins to control phospholipase C allosterically[121], and the subsequent generation of the PKC second messenger diacylglycerol. PKCs can also be activated directly by G proteins, specifically the small GTPases. For atypical PKCs (aPKCs) this regulation is through CDC42 and Rac, which are small GTPases that act through the aPKC-associated partitioning defective 6 (PAR6)[52]. In the case of PKNs, the small GTPases Rho and Rac can bind to them directly[19,20]. The small GTPases (similarly to their larger cousins) are a family of proteins that switch between active GTP-bound and inactive GDP-bound forms through their intrinsic GTPase activity and nucleotide exchange. The cycling of small GTPases between these two states is regulated by three sets of proteins: guanine nucleotide exchange factors, GTPase-activating proteins (GAPs) and guanine nucleotide dissociation inhibitors (GDIs) (reviewed in REF. 122). Small GTPases interact with and activate downstream effector proteins when bound to GTP (for example, CDC42–GTP, but not CDC42–GDP, interacts with PAR6 to regulate aPKCs[53]). All small GTPases are isoprenylated at their carboxyl termini (with either farnesyl or geranyl–geranyl) and associate with the membrane in their active states. Their action as signal relays is due to a combination of their ability to recruit targets to the membrane and to have allosteric effects on them. For aPKC, CDC42–GTP and Rac–GTP work by relieving the allosteric inhibition exerted on this kinase by its interaction with PAR6 (REFS 52,53). For PKNs the effect of Rho–GTP and Rac–GTP is directly allosteric and triggers pseudosubstrate release from the catalytic domain[123].

T_H1
(T helper 1). A subset of CD4+ T helper cells that produce the cytokines interferon-γ, interleukin-2 (IL-2) and IL-12 and promote cell-mediated immunity.

T_H2
(T helper 2). A subset of CD4+ T helper cells that produce cytokines such as interleukin-4 (IL-4), IL-5, IL-6, IL-10 and IL-13, leading to activation of humoral immune responses.

T_H17
(T helper 17). A subset of CD4+ T helper cells that produce cytokines such as interleukin-17 (IL-17), IL-21 and IL-22. They are thought to be important in inflammatory and autoimmune diseases.

Supramolecular activation complex
Areas of the immunological synapse in which T cell receptors, integrins and other cell surface proteins have segregated into distinct areas.

Total internal reflection fluorescence
A microscope exploiting evanescent wave excitation of the thin region (~100nm) at the contact area between a specimen and the glass coverslip (of distinct refractive index).

or class II on the APC[22]. This recognition event triggers T cell activation, which is characterized by asymmetric T cell division and differentiation processes that are determined by the nature of the antigen. The differentiated CD4+ T helper (T_H) cell subsets that are generated following initial antigen recognition (including T_H1, T_H2 and T_H17 cells) then execute effector functions that are relevant to the pathogen eliciting the response. The initial pathogen trigger relies on TCR-associated and TCR-proximal signalling events that are partly regulated by PKCθ and aPKCs[23,24].

PKCθ is highly, but not exclusively, expressed by T cells[25,26]. This pattern of expression led to investigation into the role of PKCθ in T cell proliferation and survival in *ex vivo* models, which revealed that PKCθ is involved in the activation of the transcription factors nuclear factor-κB (NF-κB), nuclear factor of activated T cells (NFAT) and activator protein 1 (AP1) (reviewed in REF. 27). None of these transcription factors is known to be involved in steering the differentiation of CD4+ T cells towards different subsets. By contrast, *in vivo* studies indicate that PKCθ is required for specific T cell differentiation pathways. PKCθ is not required for the differentiation and effector function of T_H1 cells in response to the intracellular bacterium *Leischmania major*[28] and viruses[29]. However, in the absence of PKCθ T_H2 cell differentiation is defective, which compromises immune responses to helminths. A similar defect in the absence of PKCθ is associated with T_H17 cell differentiation in response to allergens[30]. The evidence thus indicates that PKCθ has a key role in the differentiation of CD4+ T cells into certain T helper subsets.

The initiating T cell activation event that precedes the subsequent division and differentiation to T_H cell subsets is triggered by TCR-mediated recognition of a peptide-bound MHC molecule on the surface of an APC. This event, and the activation of various co-stimulatory receptors (for example, the T cell co-receptor CD28 following binding to its target cell ligands CD80 or CD86 (REF. 31)), leads to the assembly of a signalling synapse that comprises distinct subdomains at the membrane. These subdomains make up the supramolecular activation complex (SMAC[32]), which assembles as a series of concentric membrane domains comprising a central SMAC (cSMAC), a peripheral SMAC (pSMAC) and a distal SMAC (dSMAC)[33]. The contact area between the T cell and the APC is known as the immunological synapse, reminiscent of the terminology used for neuronal connections. Signalling proteins that mark these membrane subdomains in the T cell include the TCR and the SRC family tyrosine kinase LCK in the cSMAC, lymphocyte function-associated antigen 1 (LFA1; also known as α4β7 integrin) in the pSMAC and the transmembrane phosphotyrosine phosphatase CD45 in the dSMAC[34]. Robust T cell stimulation is associated with the accumulation of PKCθ in the cSMAC[35]. This recruitment requires PKCθ to interact with the coiled-coil domain of the scaffold protein CARD-containing MAGUK protein 3 (CARMA1; also known as CARD11)[36]. After being recruited to the cSMAC, PKCθ is phosphorylated by LCK on Tyr90 (REF. 37) and autophosphorylated on Thr219 (REF. 38). Both of these modifications are required to maintain PKCθ in the immunological synapse. Furthermore, studies in model systems indicate roles for PKCθ in controlling NF-κB, NFAT and AP1 through the phosphorylation, recruitment and/or activation of other receptor complex proteins such as the adaptor protein B cell lymphoma 10 (which is required for NF-κB activation), the tyrosine kinase–phospholipase C (PLC) pathway proteins IL-2-inducible T cell kinase (ITK), tyrosine kinase expressed in hepatocellular carcinoma (TEC) and PLCγ1 (which activate NFAT) and the MAPKKK SPAK (which activates AP1)[39].

In addition to its ability to regulate co-recruited signal transducers in the SMAC, PKCθ seems to control the stability of the immunological synapse itself. Live imaging of the components of the immunological synapse can be carried out in model systems using total internal reflection fluorescence (TIRF) microscopy to follow T cells that come into contact with surfaces that are modified to mimic APCs. This has been done for wild-type and PKCθ-knockout T cells interacting with immobilized T cell agonists. The evidence indicates that the immunological synapse is dynamic in wild-type cells, with components moving in and out of the synapse. By contrast, in PKCθ-knockout cells the synapses are considerably more stable[40]. The differences in the dynamics of these signalling events influence signal strength, duration and signal location. It is well established that the pattern of signal strength influences T cell responses, such that anergy ensues in response to weak signal input, whereas strong signals trigger T cell differentiation and effector function[30]. It is likely that the range of effects of PKCθ on T cell differentiation and function *in vivo* reflects its influence on SMAC dynamics relating to the distinct strengths of signal that are required to drive specific differentiation pathways.

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PKC, cell polarity and the Par complex. Polarity is essential for a range of normal cellular functions, including asymmetric cell division, the maintenance of epithelial integrity and cell migration. In epithelial cells, polarity is established by transmembrane proteins that act as cell surface organizers to bring cortical proteins together and provide the scaffold for intercellular sub-apical adhesion structures, which are known as tight junctions in mammals and septate junctions in *D. melanogaster*. These sub-apical junctions physically separate the apical and basolateral membrane domains of cells and require ongoing, active sorting of proteins from these domains for their maintenance and for continued apical–basal polarity[41]. Loss of apical–basal polarity in epithelial cells is a core feature of advanced malignancy. aPKCs have increasingly been implicated in mediating cell polarity. They are apically located in polarized cells, they are associated with cell junctions and are necessary for apical membrane development[42–45]. Furthermore, aPKCs can regulate the composition and localization of polarity complexes though a combination of scaffold and catalytic activities.

The aPKC control of polarity complexes is well illustrated by the interaction between the Par complex and lethal giant larvae (LGL; including LGL1 and LGL2) (FIG. 2a). The Par complex is well conserved in metazoans[42,46,47]. The proteins in the Par complex (the mammalian orthologues are aPKCs, PAR6 and PAR3) were first identified in studies of *Caenorhabditis elegans*, in which knockdown of the genes encoding PKC-3, PAR-6 and PAR-3 led to the abnormal process of symmetrical division of the fertilized zygote and improper segregation of polar granules (posterior markers of maternal origin); these partitioning defects are the hallmarks of the par phenotype[43,44,48–50]. PAR6 and aPKCs bind through their respective PB1 domains (reviewed in REF. 51) to form a stable heterodimer. RAC1 and CDC42 (which are Rho family GTPases) activate the PAR6–aPKC heterodimer by releasing PAR6-induced inhibition of aPKCs[52,53]. The active PAR6–aPKC can then bind PAR3, which interacts with sub-apical junction proteins. The binding of PAR6–aPKC to PAR3 is mediated by the catalytic domain of aPKCs and the PDZ domain of PAR6 (REFS 54,55). Thus, a tertiary Par complex is formed, which contributes to sub-apical junction formation and is itself localized (through PAR3 binding) to junctional proteins, including junctional adhesion molecule A, nectin 1 and nectin 3 (REFS 56–58).

LGL is one of a group of basolaterally located tumour suppressor proteins that competes with PAR3 for binding to PAR6–aPKC in both *D. melanogaster* and mammals[59,60]. LGL is phosphorylated by aPKC when LGL is in a complex with PAR6–aPKC and aPKC is active; this leads to the separation of LGL from the complex and translocation from the membrane[60,61] (FIG. 2a). LGL is therefore excluded from apical cell locations in an aPKC-dependent manner. Phosphorylation of LGL by aPKC can also lead to an autoinhibitory intramolecular interaction of LGL between its LGL and N-terminal domains[62,63]. The aPKC-induced phosphorylation of LGL is potentiated by an interaction between aPKC and the LGL-associated protein p32, a regulatory protein that can cause actin-enrichment of

Anergy
The impaired or absent ability of an immune cell to respond to specific antigens.

Tight junction
Closely associated area of two cells, the membranes of which join to form a barrier to fluids and molecules.

Basolateral membrane
The layer of plasma membrane of epithelial cells that forms its basal (base) and lateral (side) surfaces.

Apical–basal polarity
The unequal distribution of proteins and other materials between the apical side (facing the exterior) and the basal side (facing the interior) in epithelial cells.

PDZ domain
A protein-interaction domain (also known as DHR or GLGF domain) that is often found in multi-domain scaffolding proteins and holds together signalling complexes.

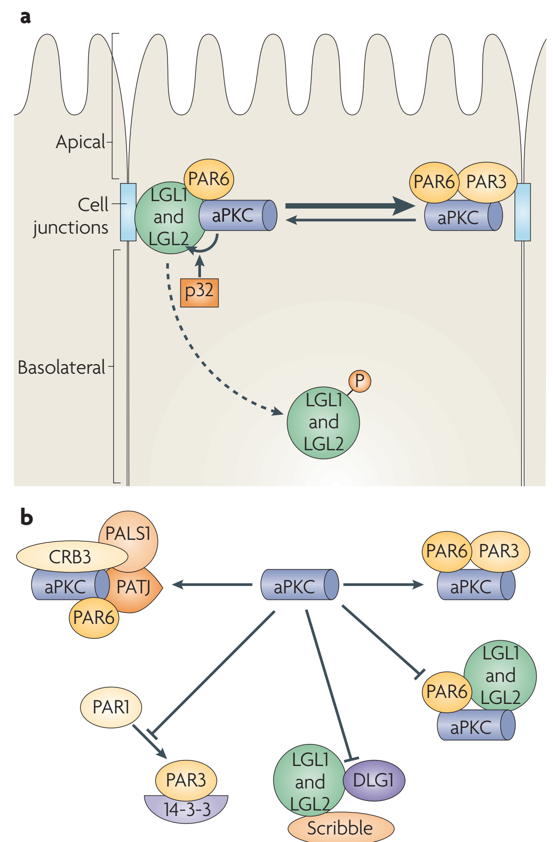

Figure 2 | **Protein kinase C and polarizing signals.** **a** | In polarized epithelial cells atypical protein kinase C (aPKC) isoforms contribute to the establishment of polarity and are localized apically in a complex with the polarity proteins partitioning defective 6 (PAR6) and PAR3. PAR3 competes for aPKC–PAR6 binding with lethal giant larvae 1 (LGL1) and LGL2. However, aPKC-induced phosphorylation of LGL1 and LGL2, which is promoted by p32, causes LGL1 and LGL2 to dissociate from cell junctions and accumulate in the basolateral region. **b** | Five different complexes involved in polarizing behaviour are regulated by aPKC isoforms. The influence that aPKC isoforms have on these complexes is indicated by the arrows and blocks. Some of the protein complexes compete for binding to common proteins such as PAR3, PAR6 and LGL1 and LGL2, leading to a process of mutual exclusion that is crucial for spatial restriction. In addition, aPKC-mediated phosphorylation of certain proteins in the polarity complexes affects their activity and/or retention in these complexes, as observed for LGL1 and LGL2. In mammals, the proteins they phosphorylate are: protein associated with LIN-7 1 (PALS1); crumbs protein homologue 3 (CRB3); PALS1-associated tight junction protein (PATJ); PAR1; PAR3, PAR6; LGL1; LGL2; Scribble; discs large 1 (DLG1); and the scaffold proteins 14-3-3.

the apical membrane and disruption of polarity when overexpressed in Madin–Darby canine kidney cells[64].

When phosphorylated by aPKC, and thus restricted to the basolateral regions of mammalian epithelial cells, LGL1 and LGL2 form a complex with the membrane bound proteins Scribble and discs large 1 (DLG1)[65] (FIG. 2b). In *D. melanogaster*, these three proteins have

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been shown to depend on each other to establish polarity[66]. Mammalian LGL may also contribute to epithelial polarity by interacting with the basolaterally located syntaxin 4, which has been implicated in promoting exocytosis and the polarized delivery of molecules to the basolateral membrane[67]. The Par complex and LGL are by no means the only polarity complexes the interaction of which is mediated by aPKCs. Other examples include the crumbs complex and PAR1 (FIG. 2b).

Evidently, cells have developed an intricate signalling network on the basis of spatial restriction and mutual exclusion to regulate polarity. As summarized above, aPKCs are crucial for this control. They act as scaffolds that interact predominantly with apically located complexes and influence their action through their kinase activity. A series of basolaterally located polarity complexes are likely to form only transient complexes with apically based proteins, as they are prevented from stable assembly by aPKC-induced phosphorylation. In line with the theme of this Review, aPKC isoforms in this context determine the dynamics of these signalling complexes.

Inappropriate input from a regulator of polarity, such as aPKC, is a potential route for carcinogenesis. Indeed, aPKC overexpression or altered localization correlates with a poor prognosis in numerous human epithelial cancers[68–71], and altered global expression levels of PAR6 and p32 have been implicated in human malignancy[64,72]. Further study of these proteins, and other putative disease-associated polarity proteins, is needed to establish how altered levels and subcellular distribution of polarity proteins contributes to and is prognostic of disease.

Localized PKC actions in migration

PKC isoforms have been implicated in numerous migratory models because they regulate properties such as cytoskeletal dynamics (reviewed in REF. 73) and the function of various cell surface proteins that are engaged in cell–ECM interactions and migration. These cell surface proteins include syndecan 4, which interacts with PKCα[74], β1 integrin, which is trafficked under the control of PKCα and PKCε[75,76], and CD44 (also known as gp85), which is also regulated by PKC isoforms[77] and is a direct substrate for purified brain PKC (typically a cPKC mixture)[78]. Two systems in particular illustrate the spatial influence of PKC isoforms on signal outputs in migration, one involving the cPKC and nPKC-mediated control of MET signalling and the second involving aPKC-mediated control of integrin behaviour in migration. Integrins are heterodimers made up of α- and β-chains and are integral for the interaction of cells with ECM proteins. The strength and dynamics of these interactions determine how effectively cells adhere to a substrate and migrate (reviewed in REF. 79).

cPKC, nPKC and control of the signal generator MET. MET, the receptor for hepatocyte growth factor (HGF), stimulates several cell functions such as cell growth, migration and survival. MET overexpression or gain of function mutations are thought to have a role in tumour progression to metastatic disease in various cancers[80–83]. Following HGF binding MET gets rapidly internalized through a typical receptor endocytic pathway involving clathrin and the GTPase dynamin[84,85]. It is then recruited to early endosome antigen 1 (EEA1)-positive peripheral early endosomes, where it remains active and capable of signalling[86–91]. Without the localization of MET to endosomes, HGF-mediated stimulation of downstream effectors, such as extracellular signal-regulated kinase 1 (ERK1) and ERK2, is suboptimal[91]. PKCε can negatively regulate MET signalling by phosphorylating Ser985 in the MET juxtamembrane region, which is associated with reduced Tyr MET phosphorylation and hence inefficient recruitment of downstream effectors[92]. However, PKCε also exerts a positive influence on MET signalling to ERK1 and ERK2 by promoting their recruitment to focal complexes, where they mediate HGF-induced cell migration. It is not yet clear how PKCε influences the delivery of ERK1 and ERK2 to focal complexes. However, drawing a parallel from the finding that platelet-derived growth factor promotes the recruitment of β3 integrin and ERK1 to focal complexes by supporting the β3 integrin–ERK1 association[93], PKCε might regulate the localization of ERK1 and ERK2 by controlling the delivery of a β1 integrin–ERK1 or ERK2 complex to the plasma membrane. This would be consistent with the finding that PKCε controls β1 integrin recycling[76]. Once delivered to the plasma membrane, active ERK1 and ERK2 are thought to regulate focal adhesion dynamics through the phosphorylation of paxillin and other focal adhesion targets (see below).

From the peripheral endosomes, PKCα promotes activated MET trafficking along microtubules (FIG. 3), causing the progressive accumulation of MET in a perinuclear endomembrane compartment. This behaviour is consistent with a role for PKCα in promoting trafficking to the perinuclear compartment, which results in the accumulation of markers of this trafficking pathway (recently reviewed in REF. 94). This post-endocytic trafficking is not required for MET degradation, but it is necessary for the efficient activation and nuclear accumulation of the oncogene and transcription factor signal transducer and activator of transcription 3 (STAT3)[95]. When nuclear uptake of STAT3, which is required for STAT3 to reach its target genes, is inhibited with the drug STA-21 (also known as deoxytetrangomycin), HGF-induced migration is also inhibited[95]. Owing to the action of phosphotyrosine phosphatases, STAT3 is only weakly activated by MET at the plasma membrane and peripheral endosomes. Once proximal to the nucleus, activated MET can trigger the nuclear translocation of STAT3 (REF. 95). Thus MET signalling to STAT3 requires PKCα-regulated MET trafficking to the perinuclear endomembrane compartment (FIG. 3).

aPKCs control localized signals in migratory cells. aPKCζ and aPKCι seem to be key modulators of cell migration through their ability to promote highly localized signalling events. *In vivo* aPKC, as part of the activated Par complex (see above), is required for the migration of neuroblasts and axons in *C. elegans*[96]. aPKCs are also important for *Xenopus laevis* gastrulation. XGAP, a GTPase-activating

Clathrin
A protein that forms a lattice-shaped coating on coated pits and coated vesicles during endocytosis.

Dynamin
A large GTPase involved in the scission of nascent vesicles from parent membranes.

Early endosome
Small irregularly shaped intracellular vesicle to which endocytosed molecules are initially delivered.

Focal adhesion
Large macromolecular assembly through which both mechanical force and regulatory signals are transmitted between the cell and the extracellular matrix.

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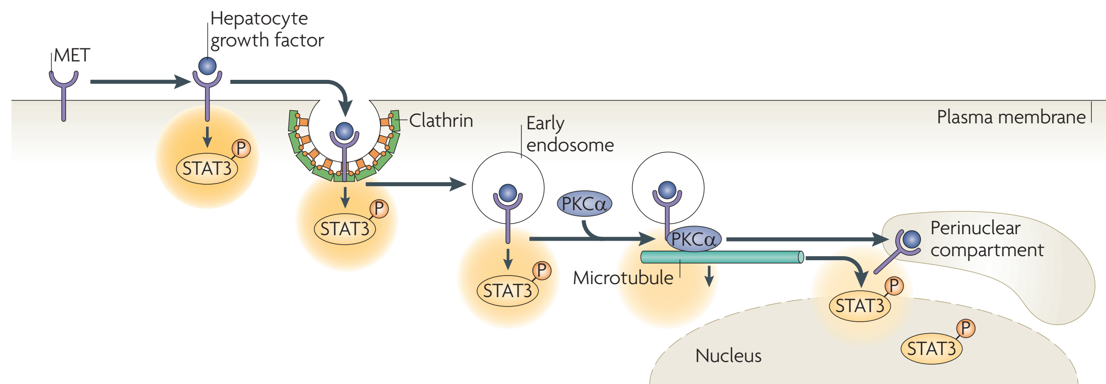

Figure 3 | **Protein kinase C isoforms influence MET signals.** The stimulation of HeLa cells with hepatocyte growth factor activates MET at the plasma membrane. The active receptor then undergoes clathrin-mediated endocytosis and accumulates transiently in early endosomes. From there, ligand-bound, activated MET traffics along a microtubule network and depends on PKCα to accumulate in the perinuclear compartment. The receptor has a limited range of influence (indicated by the orange circle) and has to traffic to the perinuclear compartment to promote entry of phosphorylated STAT3 into the nucleus. Activated MET can trigger the phosphorylation of signal transducer and activator of transcription 3 (STAT3) in these compartments, competing with STAT3 phosphotyrosine phosphatases. However, the ability of MET to induce STAT3 phosphorylation at the plasma membrane or the early endosome is not sufficient to overcome the action of phosphotyrosine phosphatases to induce the nuclear accumulation of phosphorylated STAT3. By contrast, when activated MET reaches the perinuclear compartment the local phosphorylation of STAT3 is sufficient to drive nuclear accumulation.

protein for ADP ribosylation factors, seems to control a prominent set of cell movements during *X. laevis* gastrulation that are known as convergent extension. XGAP achieves this by restricting the localization of Par proteins and aPKC at the protrusive regions[97]. Furthermore, during *D. melanogaster* border cell migration cells need to maintain their apical-basal polarity through the aPKC–PAR3–PAR6 complex to organize the border cell cluster and allow migration. The aPKC–PAR3–PAR6 complex maintains distinct protein distributions in different parts of the migrating border cells[98].

There are numerous models of cell migration in which aPKC isoforms have been implicated. One of these elegantly illustrates the role of the aPKCs in organizing signals. In the normal rat kidney cell model aPKCζ and aPKCι are required for cell migration[99], as is the exocyst protein complex[100] (FIG. 4a). The exocyst complex consists of 8 proteins, which are mainly associated with membranes. During normal rat kidney cell migration, the localization of aPKCs and the exocyst complex at the leading edge are mutually dependent, and aPKCζ and aPKCι interact with the exocyst complex through the scaffold kidney and brain protein (KIBRA; also known as WWC1). Functionally, the aPKC–KIBRA–exocyst complex is instrumental in triggering the activation of ERK1, ERK2 and Jun N-terminal kinase 1 (JNK1) at the leading edge of migrating normal rat kidney cells[99]. Evidence indicates that ERK1, ERK2 and JNK1 activation in this compartment controls focal adhesion dynamics; however, global inhibition of ERK1, ERK2 or JNK1 activation do not distinguish their roles at the leading edge from those in other compartments, for example in the nucleus. Hence, assigning phenotypic consequences to localized effectors is at best correlative (see below). Reflecting the spatial resolution of these events, the aPKC–KIBRA–exocyst complex controls the activity of JNK1 at leading edge of the migrating cell specifically, but does not regulate the activity of JNK1 in the nucleus. The aPKC–KIBRA–exocyst-dependent activation of JNK1, ERK1 and ERK2 at the leading edge is necessary for the MAPK-induced phosphorylation of the cytoskeletal protein paxillin in this compartment. In turn, the phosphorylation of paxillin at the plasma membrane is associated with changes in the dynamics of focal adhesion complexes, which is predicted to determine the rate of migration. These events explain in part the requirement for aPKC function in normal rat kidney cell migration.

In an astrocyte migration model, localized aPKCζ activity acts through the microtubule motor protein dynein to determine Golgi and centrosome polarity (FIG. 4b). In migrating astrocytes, the activation of integrins at the newly formed leading edge allows the recruitment and activation of the PAR6–aPKCζ complex through the polarized recruitment and activation of the small GTPase CDC42 (REFS 101,102), which directly targets PAR6. During astrocyte migration, integrins that are activated at the front of the cell activate CDC42 and the PAR6–aPKCζ complex, which leads to the subsequent reorientation of the centrosome — the main microtubule-organizing centre of the cell. The activation of aPKCζ can lead to the phosphorylation and inactivation of the Ser/Thr protein kinase glycogen synthase kinase 3β (GSK3β; the role of this specific phosphorylation event in cetrosome orientation is questioned by data in REF. 102), and causes adenomatous polyposis coli (a tumour suppressor protein that is a direct substrate of GSK3β) to associate with microtubule plus ends at the leading edge[103,104]. The PAR6–PKCζ complex also regulates the spatially localized association of mammalian DLG1 and adenomatous polyposis coli to control cell polarization in this astrocyte model. Activation of the

Convergent extension
A process during gastrulation in which layers of cells converge and extend by a rearrangement of the cells of the ventral part of the epithelium towards the ventral midline.

Leading edge
The area of a motile cell that is closest to the direction of movement.

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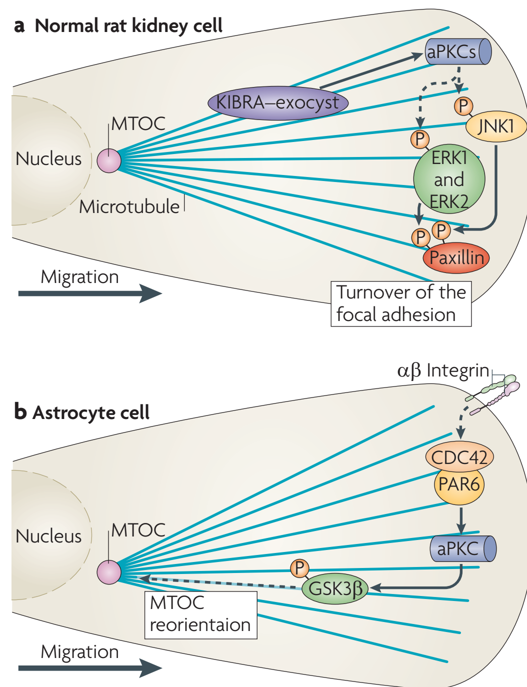

Figure 4 | **Localized signals controlled by PKCζ and PKCι during cell migration.** Studies in different cell types illustrate key targets for atypical protein kinase c (aPKC) isoforms in controlling migration. **a** | Polarized delivery of the kidney and brain protein (KIBRA)–exocyst complex to the leading edge of migrating normal rat kidney cells depends on PKCζ and PKCι. Reciprocally, the localization of these aPKCs at the leading edge depends on the exocyst. The aPKCζ–KIBRA–exocyst or aPKCι–KIBRA–exocyst complex is required for the localized phosphorylation and activation of Jun N-terminal kinase 1 (JNK1), extracellular signal-regulated kinase 1 (ERK1) and ERK2 at the leading edge of migrating cells, which acts to control the phosphorylation of the cytoskeletal protein paxillin. **b** | In migrating astrocytes α- and β-integrins activate CDC42 at the leading edge. In turn, active, GTP-bound CDC42 recruits and activates the partitioning defective 6 (PAR6)–PKCζ complex in this compartment. PKCζ then phosphorylates and inactivates glycogen synthase kinase 3β (GSK3β); this allows the reorientation of the microtubule-organizing centre (MTOC), which is required for directed cell migration.

PAR6–aPKCζ complex by CDC42 at the leading edge of migrating cells promotes both the localized association of adenomatous polyposis coli with microtubule plus ends and the assembly of DLG1-containing puncta in the plasma membrane. The physical interaction between adenomatous polyposis coli and DLG1 is required for the polarization of the microtubule cytoskeleton[105].

Perspectives

The examples illustrated here show the importance of PKCs in controlling spatial resolution in signalling processes. Even though signalling cascades are often controlled simply by the presence, absence or activation state of proteins, the effect of signal location can be as important as signal strength, or indeed help to determine net strength, as we have discussed in this Review using the MET and STAT3 example. The localization of signalling molecules to subcellular environments that contain the appropriate downstream targets is essential and to a great extent determines the output of a particular signalling cascade.

Despite the recognition of the importance of localized signalling, our ability to locally interfere with signals to provide evidence of necessity and sufficiency has been limited by a lack of appropriate experimental methodologies. Many high-resolution microscopy approaches have been developed, such as TIRF, fluorescence recovery after photobleaching (FRAP), fluorescence resonance energy transfer (FRET) and stimulated emission depletion (STED), for visualizing and quantifying distribution and interactions of individual molecules in a spatial and temporal context (reviewed in REF. 106). Such approaches have been employed to monitor the localization of fluorescently tagged PKC isoforms in cells and also to follow their activity, either by autophosphorylation[107] or through a reporter[108]. In the second case, the reporter is a genetically encoded device that changes its conformation and consequently FRET behaviour after it has been phosphorylated by PKC. Such analyses can lend substantial weight of evidence to defining roles of PKCs in controlling specific events in particular compartments. However, these imaging approaches are mostly descriptive of localized processes and do not provide information of the requirement for the protein in the particular location.

Strategies are necessary that allow the manipulation of spatial and dynamic behaviour in a physiological context. Pharmacological agents or small interfering RNAs that interfere with signalling cascades typically have global effects that are often chronic (knockdown or knockout) and ignore spatial resolution. However, a drug-dependent dimerization approach, originating in the Schreiber laboratory[109], is a promising tool to overcome these spatial restrictions. It exploits the mechanism of action of the immunosuppressive drug rapamycin, which binds FK506-binding protein 12 (FKBP12) and, as a complex with FKBP12, interacts with the FKBP rapamycin-binding (FRB) domain on mammalian target of rapamycin (mTOR), thereby disrupting its function. As modular binding domains, FKBP12 and the FRB from mTOR can be fused to other proteins, which allows the recruitment of any genetically encoded enzymatic activity to any protein marker-defined compartment, thereby leading to the modification of local behaviour in cellular systems. This approach has been used to manipulate specific membrane phosphoinositide compartments[110,111]. One of these studies used the recruitment of the inositol lipid phosphatase myotubularin 1 into RAB5-positive endosomes to locally dephosphorylate phosphatidylinositol 3-phosphate and phosphatidylinositol 3,5 bisphosphate, which shows that these lipids determine the normal maturation of this endosomal compartment and the flux of certain receptors through it[110]. This strategy is broadly applicable to localized intervention in many different signalling contexts and promises to provide information on the consequences of localized actions.

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Although the examples of PKC control exerted on cell–cell contacts and migratory behaviours are distinct, they do share a common theme in that they both involve regulated scaffold functions. The signalling synapses associated with both immune cell receptor signalling and tight junctions have echoes in the signalling interfaces associated with receptors engaged in cell–ECM and cell–growth factor interactions. In all these situations the evidence indicates that PKC isoforms control the localization, assembly and/or disassembly of these signalling platforms.

1. Velculescu, V. E. Defining the blueprint of the cancer genome. *Carcinogenesis* **29**, 1087–1091 (2008).
2. Capdeville, R., Buchdunger, E., Zimmermann, J. & Matter, A. Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug. *Nature Rev. Drug Discov.* **1**, 493–502 (2002).
3. DeLange, R. J., Kemp, R. G., Riley, W. D., Cooper, R. A. & Krebs, E. G.. Activation of skeletal muscle phosphorylase kinase by adenosine triphosphate and adenosine 3′, 5′-monophosphate. *J. Biol. Chem.* **243**, 2200–2208 (1968).
4. Elion, E. A. The Ste5p scaffold. *J. Cell Sci.* **114**, 3967–3978 (2001).
5. Carnegie, G. K., Smith, F. D., McConnachie, G., Langeberg, L. K. & Scott, J. D. AKAP-Lbc nucleates a protein kinase D activation scaffold. *Mol. Cell* **15**, 889–899 (2004).
6. Pawson, T. Regulation and targets of receptor tyrosine kinases. *Eur. J. Cancer* **38**, S3–S10 (2002).
7. Perkins, G. A. *et al.* PKA, PKC, and AKAP localization in and around the neuromuscular junction. *BMC Neurosci.* **2**, 17 (2001).
8. Shieh, B. H., Parker, L. & Popescu, D. Protein kinase C (PKC) isoforms in *Drosophila*. *J. Biochem.* **132**, 523–527 (2002).
9. Sim, A. T. & Scott, J. D. Targeting of, PKA, PKC and protein phosphatases to cellular microdomains. *Cell Calcium* **26**, 209–217 (1999).
10. Csukai, M. & Mochly-Rosen, D. Pharmacologic modulation of protein kinase C isozymes: the role of RACKs and subcellular localisation. *Pharmacol. Res.* **39**, 253–259 (1999).
11. Jaken, S. & Parker, P. J. Protein kinase C binding partners. *Bioessays* **22**, 245–254 (2000).
12. Poole, A. W., Pula, G., Hers, I., Crosby, D. & Jones, M. L. PKC-interacting proteins: from function to pharmacology. *Trends Pharmacol. Sci.* **25**, 528–535 (2004).
13. Mellor, H. & Parker, P. J. The extended protein kinase C superfamily. *Biochem. J.* **332** , 281–292 (1998).
14. Leitges, M. Functional PKC *in vivo* analysis using deficient mouse models. *Biochem. Soc. Trans.* **35**, 1018–1020 (2007).
15. Pears, C. J., Kour, G., House, C., Kemp, B. E. & Parker, P. J. Mutagenesis of the pseudosubstrate site of protein kinase C leads to activation. *Eur. J. Biochem.* **194**, 89–94 (1990).
16. Nalefski, E. A. & Newton, A. C. Membrane binding kinetics of protein kinase C βII mediated by the C2 domain. *Biochemistry* **40**, 13216–13229 (2001).
17. Oancea, E. & Meyer, T. Protein kinase C as a molecular machine for decoding calcium and diacylglycerol signals. *Cell* **95**, 307–318 (1998).
18. Suzuki, A. *et al.* Atypical protein kinase C is involved in the evolutionarily conserved par protein complex and plays a critical role in establishing epithelia-specific junctional structures. *J. Cell Biol.* **152**, 1183–1196 (2001).
19. Flynn, P., Mellor, H., Palmer, R., Panayotou, G. & Parker, P. J. Multiple interactions of PRK1 with RhoA. Functional assignment of the Hr1 repeat motif. *J. Biol. Chem.* **273**, 2698–2705 (1998).
20. Shibata, H. *et al.* Characterization of the interaction between RhoA and the amino-terminal region of PKN. *FEBS Lett.* **385**, 221–224 (1996).
21. Lim, W. G. *et al.* The very C-terminus of PRK1/PKN is essential for its activation by RhoA and downstream signaling. *Cell Signal.* **18**, 1473–1481 (2006).
22. Triantafilou, K., Triantafilou, M. & Fernandez, N. Molecular associations and microdomains in antigen-presenting cell-T-cell interactions. *Crit. Rev. Immunol.* **20**, 359–373 (2000).
23. Martin, P. *et al.* Control of T helper 2 cell function and allergic airway inflammation by PKCζ. *Proc. Natl Acad. Sci. USA* **102**, 9866–9871 (2005).
24. Yang, J. Q., Leitges, M., Duran, A., Diaz-Meco, M. T. & Moscat, J. Loss of PKC λ/ι impairs Th2 establishment and allergic airway inflammation *in vivo*. *Proc. Natl Acad. Sci. USA* **106**, 1099–1104 (2009).
25. Baier, G. *et al.* Molecular-cloning and characterization of pkc-θ, a novel member of protein kinase c (pkc) gene family expressed predominantly in hematopoietic cells. *J. Biol. Chem.* **268**, 4997–5004 (1993).
26. Osada, S. *et al.* A new member of the protein kinase C family, nPKC theta, predominantly expressed in skeletal muscle. *Mol. Cell. Biol.* **12**, 3930–3938 (1992).
27. Manicassamy, S., Gupta, S. & Sun, Z. Selective function of PKC-θ in T cells. *Cell. Mol. Immunol.* **3**, 263–270 (2006).
28. Marsland, B. J., Soos, T. J., Spath, G., Littman, D. R. & Kopf, M. Protein kinase C θ is critical for the development of *in vivo* T helper (Th)2 cell but not Th1 cell responses. *J. Exp. Med.* **200**, 181–189 (2004).
29. Marsland, B. J. *et al.* Innate signals compensate for the absence of PKC-θ during *in vivo* $CD8^+$ T cell effector and memory responses. *Proc. Natl Acad. Sci. USA* **102**, 14374–14379 (2005).
30. Marsland, B. J. & Kopf, M. T-cell fate and function: PKC-θ and beyond. *Trends Immunol.* **29**, 179–185 (2008).
31. Rao, K. L., Varalakshmi, C., Kumari, A. L. & Khar, A. Interaction between B.7 and CD28 costimulatory molecules is essential for the activation of effector function mediating spontaneous tumour regression. *Scand. J. Immunol.* **49**, 633–640 (1999).
32. Monks, C. R., Freiberg, B. A., Kupfer, H., Sciaky, N. & Kupfer, A. Three-dimensional segregation of supramolecular activation clusters in T cells. *Nature* **395**, 82–86 (1998).
33. Altman, A. & Villalba, M. Protein kinase C-θ (PKCθ): it's all about location, location, location. *Immunol. Rev.* **192**, 53–63 (2003).
34. Freiberg, B. A. *et al.* Staging and resetting T cell activation in SMACs. *Nature Immunol.* **3**, 911–917 (2002).
35. Schaefer, B. C., Kappler, J. W., Kupfer, A. & Marrack, P. Complex and dynamic redistribution of NF-κB signaling intermediates in response to T cell receptor stimulation. *Proc. Natl Acad. Sci. USA* **101**, 1004–1009 (2004).
36. Sommer, K. *et al.* Phosphorylation of the CARMA1 linker controls NF-κB activation. *Immunity* **23**, 561–574 (2005).
37. Liu, Y. *et al.* Regulation of protein kinase C θ function during T cell activation by Lck-mediated tyrosine phosphorylation. *J. Biol. Chem.* **275**, 3603–3609 (2000).
38. Thuille, N. *et al.* Critical role of novel Thr-219 autophosphorylation for the cellular function of PKCθ in T lymphocytes. *EMBO J.* **24**, 3869–3880 (2005).
39. Hayashi, K. & Altman, A. Protein kinase C θ (PKC θ): a key player in T cell life and death. *Pharmacol. Res.* **55**, 537–544 (2007).
40. Dustin, M. L. T-cell activation through immunological synapses and kinapses. *Immunol. Rev.* **221**, 77–89 (2008).
41. Tepass, U., Theres, C. & Knust, E. crumbs encodes an EGF-like protein expressed on apical membranes of Drosophila epithelial cells and required for organization of epithelia. *Cell* **61**, 787–799 (1990).
42. Hung, T. J. & Kemphues, K. J. PAR-6 is a conserved PDZ domain-containing protein that colocalizes with PAR-3 in *Caenorhabditis elegans* embryos. *Development* **126**, 127–135 (1999).
43. Tabuse, Y. *et al.* Atypical protein kinase C cooperates with PAR-3 to establish embryonic polarity in *Caenorhabditis elegans*. *Development* **125**, 3607–3614 (1998).
44. Watts, J. L. *et al. par-6*, a gene involved in the establishment of asymmetry in early *C. elegans* embryos, mediates the asymmetric localization of PAR-3. *Development* **122**, 3133–3140 (1996).
45. Horikoshi, Y. *et al.* Interaction between PAR-3 and the aPKC-PAR-6 complex is indispensable for apical domain development of epithelial cells. *J. Cell Sci.* **122**, 1595–1606 (2009).
46. Joberty, G., Petersen, C., Gao, L. & Macara, I. G. The cell-polarity protein Par6 links Par3 and atypical protein kinase C to Cdc42. *Nature Cell Biol.* **2**, 531–539 (2000).
47. Izumi, Y. *et al.* An atypical PKC directly associates and colocalizes at the epithelial tight junction with ASIP, a mammalian homologue of *Caenorhabditis elegans* polarity protein PAR-3. *J. Cell Biol.* **143**, 95–106 (1998).
48. Kemphues, K. J., Priess, J. R., Morton, D. G. & Cheng, N. S. Identification of genes required for cytoplasmic localization in early *C. elegans* embryos. *Cell* **52**, 311–320 (1988).
49. Kemphues, K. PARsing embryonic polarity. *Cell* **101**, 345–348 (2000).
50. Ono, Y. *et al.* Protein kinase C ζ subspecies from rat brain: its structure, expression, and properties. *Proc. Natl Acad. Sci. USA* **86**, 3099–3103 (1989).
51. Moscat, J., Diaz-Meco, M. T., Albert, A. & Campuzano, S. Cell signaling and function organized by PB1 domain interactions. *Mol. Cell* **23**, 631–640 (2006).
52. Lin, D. *et al.* A mammalian PAR-3-PAR-6 complex implicated in Cdc42/Rac1 and aPKC signalling and cell polarity. *Nature Cell Biol.* **2**, 540–547 (2000).
53. Yamanaka, T. *et al.* PAR-6 regulates aPKC activity in a novel way and mediates cell-cell contact-induced formation of the epithelial junctional complex. *Genes Cells* **6**, 721–731 (2001).
54. Bilder, D., Schober, M. & Perrimon, N. Integrated activity of PDZ protein complexes regulates epithelial polarity. *Nature Cell Biol.* **5**, 53–58 (2003).
55. Hirose, T. *et al.* Involvement of ASIP/PAR-3 in the promotion of epithelial tight junction formation. *J. Cell Sci.* **115**, 2485–2495 (2002).
56. Takekuni, K. *et al.* Direct binding of cell polarity protein PAR-3 to cell-cell adhesion molecule nectin at neuroepithelial cells of developing mouse. *J. Biol. Chem.* **278**, 5497–5500 (2003).
57. Ebnet, K. *et al.* The cell polarity protein ASIP/PAR-3 directly associates with junctional adhesion molecule (JAM). *EMBO J.* **20**, 3738–3748 (2001).
58. Mizuno, K. *et al.* Self-association of PAR-3-mediated by the conserved N-terminal domain contributes to the development of epithelial tight junctions. *J. Biol. Chem.* **278**, 31240–31250 (2003).
59. Yamanaka, T. *et al.* Mammalian Lgl forms a protein complex with PAR-6 and aPKC independently of PAR-3 to regulate epithelial cell polarity. *Curr. Biol.* **13**, 734–743 (2003).
60. Betschinger, J., Mechtler, K. & Knoblich, J. A. The Par complex directs asymmetric cell division by phosphorylating the cytoskeletal protein Lgl. *Nature* **422**, 326–330 (2003).
61. Yamanaka, T. *et al.* Lgl mediates apical domain disassembly by suppressing the PAR-3-aPKC-PAR-6 complex to orient apical membrane polarity. *J. Cell Sci.* **119**, 2107–2118 (2006).
62. Betschinger, J., Eisenhaber, F. & Knoblich, J. A. Phosphorylation-induced autoinhibition regulates the cytoskeletal protein Lethal (2) giant larvae. *Curr. Biol.* **15**, 276–282 (2005).
63. Hutterer, A., Betschinger, J., Petronczki, M. & Knoblich, J. A. Sequential roles of Cdc42, Par-6, aPKC, and Lgl in the establishment of epithelial polarity during *Drosophila* embryogenesis. *Dev. Cell* **6**, 845–854 (2004).
64. Bialucha, C. U., Ferber, E. C., Pichaud, F., Peak-Chew, S. Y. & Fujita, Y. p32 is a novel mammalian Lgl binding protein that enhances the activity of protein kinase Cζ and regulates cell polarity. *J. Cell Biol.* **178**, 575–581 (2007).
65. Kallay, L. M., McNickle, A., Brennwald, P. J., Hubbard, A. L. & Braiterman, L. T. Scribble associates with two polarity proteins, Lgl2 and Vangl2, via distinct molecular domains. *J. Cell. Biochem.* **99**, 647–664 (2006).
66. Bilder, D. & Perrimon, N. Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. *Nature* **403**, 676–680 (2000).

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67. Musch, A. *et al.* Mammalian homolog of *Drosophila* tumor suppressor lethal (2) giant larvae interacts with basolateral exocytic machinery in Madin-Darby canine kidney cells. *Mol. Biol. Cell* **13**, 158–168 (2002).
68. Eder, A. M. *et al.* Atypical PKCι contributes to poor prognosis through loss of apical-basal polarity and cyclin E overexpression in ovarian cancer. *Proc. Natl Acad. Sci. USA* **102**, 12519–12524 (2005).
69. Fields, A. P. & Regala, R. P. Protein kinase C ι: human oncogene, prognostic marker and therapeutic target. *Pharmacol. Res.* **55**, 487–497 (2007).
70. Kojima, Y. *et al.* The overexpression and altered localization of the atypical protein kinase C λ/ι in breast cancer correlates with the pathologic type of these tumors. *Hum. Pathol.* **39**, 824–831 (2008).
71. Regala, R. P. *et al.* Atypical protein kinase Cι plays a critical role in human lung cancer cell growth and tumorigenicity. *J. Biol. Chem.* **280**, 31109–31115 (2005).
72. Nolan, M. E. *et al.* The polarity protein Par6 induces cell proliferation and is overexpressed in breast cancer. *Cancer Res.* **68**, 8201–8209 (2008).
73. Larsson, C. Protein kinase C and the regulation of the actin cytoskeleton. *Cell Signal.* **18**, 276–284 (2006).
74. Keum, E. *et al.* Syndecan-4 regulates localization, activity and stability of protein kinase C-α. *Biochem. J.* **378**, 1007–1014 (2004).
75. Ng, T. *et al.* PKCα regulates β1 integrin-dependent cell motility through association and control of integrin traffic. *EMBO J.* **18**, 3309–3923 (1999).
76. Ivaska, J., Whelan, R. D., Watson, R. & Parker, P. J. PKCε controls the traffic of β1 integrins in motile cells. *EMBO J.* **21**, 3608–3619 (2002).
77. Legg, J. W., Lewis, C. A., Parsons, M., Ng, T. & Isacke, C. M. A novel PKC-regulated mechanism controls CD44 ezrin association and directional cell motility. *Nature Cell Biol.* **4**, 399–407 (2002).
78. Kalomiris, E. L. & Bourguignon, L. Y. Lymphoma protein kinase C is associated with the transmembrane glycoprotein, GP85, and may function in GP85-ankyrin binding. *J. Biol. Chem.* **264**, 8113–8119 (1989).
79. Hood, J. D. & Cheresh, D. A. Role of integrins in cell invasion and migration. *Nature Rev. Cancer* **2**, 91–100 (2002).
80. Birchmeier, C., Birchmeier, W., Gherardi, E. & Vande Woude, G. F. Met, metastasis, motility and more. *Nature Rev. Mol. Cell Biol.* **4**, 915–925 (2003).
81. Lengyel, E., Sawada, K. & Salgia, R. Tyrosine kinase mutations in human cancer. *Curr. Mol. Med.* **7**, 77–84 (2007).
82. Ma, P. C., Maulik, G., Christensen, J. & Salgia, R. c-Met: structure, functions and potential for therapeutic inhibition. *Cancer Metastasis Rev.* **22**, 309–325 (2003).
83. Trusolino, L. & Comoglio, P. M. Scatter-factor and semaphorin receptors: cell signalling for invasive growth. *Nature Rev. Cancer* **2**, 289–300 (2002).
84. Kermorgant, S., Zicha, D. & Parker, P. J. Protein kinase C controls microtubule-based traffic but not proteasomal degradation of c-Met. *J. Biol. Chem.* **278**, 28921–28929 (2003).
85. Hammond, D. E., Carter, S. & Clague, M. J. Met receptor dynamics and signalling. *Curr. Top. Microbiol. Immunol.* **286**, 21–44 (2004).
86. Vieira, A. V., Lamaze, C. & Schmid, S. L. Control of EGF receptor signaling by clathrin-mediated endocytosis. *Science* **274**, 2086–2089 (1996).
87. Chow, J. C., Condorelli, G. & Smith, R. J. Insulin-like growth factor-I receptor internalization regulates signaling via the Shc/mitogen-activated protein kinase pathway, but not the insulin receptor substrate-1 pathway. *J. Biol. Chem.* **273**, 4672–4680 (1998).
88. Zhang, Y., Moheban, D. B., Conway, B. R., Bhattacharyya, A. & Segal, R. A. Cell surface Trk receptors mediate NGF-induced survival while internalized receptors regulate NGF-induced differentiation. *J. Neurosci.* **20**, 5671–5678 (2000).
89. Howe, C. L., Valletta, J. S., Rusnak, A. S. & Mobley, W. C. NGF signaling from clathrin-coated vesicles: evidence that signaling endosomes serve as a platform for the Ras-MAPK pathway. *Neuron* **32**, 801–814 (2001).
90. Hayes, S., Chawla, A. & Corvera, S. TGF β receptor internalization into EEA1-enriched early endosomes: role in signaling to Smad2. *J. Cell Biol.* **158**, 1239–1249 (2002).
91. Kermorgant, S., Zicha, D. & Parker, P. J. PKC controls HGF-dependent c-Met traffic, signalling and cell migration. *EMBO J.* **23**, 3721–3734 (2004).
92. Hashigasako, A., Machide, M., Nakamura, T., Matsumoto, K. & Nakamura, T. Bi-directional regulation of Ser-985 phosphorylation of c-met via protein kinase C and protein phosphatase 2A involves c-Met activation and cellular responsiveness to hepatocyte growth factor. *J. Biol. Chem.* **279**, 26445–26452 (2004).
93. Roberts, M. S., Woods, A. J., Shaw, P. E. & Norman, J. C. ERK1 associates with $\alpha_v\beta_3$ integrin and regulates cell spreading on vitronectin. *J. Biol. Chem.* **278**, 1975–1985 (2003).
94. Alvi, F., Idkowiak-Baldys, J., Baldys, A., Raymond, J. R. & Hannun, Y. A. Regulation of membrane trafficking and endocytosis by protein kinase C: emerging role of the pericentrion, a novel protein kinase C-dependent subset of recycling endosomes. *Cell. Mol. Life Sci.* **64**, 263–270 (2007).
95. Kermorgant, S. & Parker, P. J. Receptor trafficking controls weak signal delivery: a strategy used by c-Met for STAT3 nuclear accumulation. *J. Cell Biol.* **182**, 855–863 (2008).
96. Welchman, D. P., Mathies, L. D. & Ahringer, J. Similar requirements for CDC-42 and the PAR-3/PAR-6/PKC-3 complex in diverse cell types. *Dev. Biol.* **305**, 347–357 (2007).
97. Hyodo-Miura, J. *et al.* XGAP, an ArfGAP, is required for polarized localization of PAR proteins and cell polarity in *Xenopus* gastrulation. *Dev. Cell* **11**, 69–79 (2006).
98. Pinheiro, E. M. & Montell, D. J. Requirement for Par-6 and Bazooka in *Drosophila* border cell migration. *Development* **131**, 5243–5251 (2004).
99. Rosse, C., Formstecher, E., Boeckeler, K., Zhao, Y., Kremerskothen, J., White, M., Camonis, J. & Parker, P. J. An aPKC-Exocyst complex controls Paxillin phosphorylation and migration through localised JNK1 activation. *PLoS Biol.* **7**, e1000235 (2009).
100. Rosse, C. *et al.* RalB mobilizes the exocyst to drive cell migration. *Mol. Cell. Biol.* **26**, 727–734 (2006).
101. Cau, J. & Hall, A. Cdc42 controls the polarity of the actin and microtubule cytoskeletons through two distinct signal transduction pathways. *J. Cell Sci.* **118**, 2579–2587 (2005).
102. Schlessinger, K., McManus, E. J. & Hall, A. Cdc42 and noncanonical Wnt signal transduction pathways cooperate to promote cell polarity. *J. Cell Biol.* **178**, 355–361 (2007).
103. Etienne-Manneville, S. & Hall, A. Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKCζ. *Cell* **106**, 489–498 (2001).
104. Etienne-Manneville, S. & Hall, A. Cdc42 regulates GSK-3β and adenomatous polyposis coli to control cell polarity. *Nature* **421**, 753–756 (2003).
105. Etienne-Manneville, S., Manneville, J. B., Nicholls, S., Ferenczi, M. A. & Hall, A. Cdc42 and Par6-PKCζ regulate the spatially localized association of Dlg1 and APC to control cell polarization. *J. Cell Biol.* **170**, 895–901 (2005).
106. Meyer, T. & Teruel, M. N. Fluorescence imaging of signaling networks. *Trends Cell Biol.* **13**, 101–106 (2003).
107. Ng, T. *et al.* Imaging protein kinase Cα activation in cells. *Science* **283**, 2085–2089 (1999).
108. Gallegos, L. L., Kunkel, M. T. & Newton, A. C. Targeting protein kinase C activity reporter to discrete intracellular regions reveals spatiotemporal differences in agonist-dependent signaling. *J. Biol. Chem.* **281**, 30947–30956 (2006).
109. Ho, S. N., Biggar, S. R., Spencer, D. M., Schreiber, S. L. & Crabtree, G. R. Dimeric ligands define a role for transcriptional activation domains in reinitiation. *Nature* **382**, 822–826 (1996).
110. Fili, N., Calleja, V., Woscholski, R., Parker, P. J. & Larijani, B. Compartmental signal modulation: endosomal phosphatidylinositol 3-phosphate controls endosome morphology and selective cargo sorting. *Proc. Natl Acad. Sci. USA* **103**, 15473–15478 (2006).
111. Varnai, P., Thyagarajan, B., Rohacs, T. & Balla, T. Rapidly inducible changes in phosphatidylinositol 4,5-bisphosphate levels influence multiple regulatory functions of the lipid in intact living cells. *J. Cell Biol.* **175**, 377–382 (2006).
112. Parekh, D. B., Ziegler, W. & Parker, P. J. Multiple pathways control protein kinase C phosphorylation. *EMBO J.* **19**, 496–503 (2000).
113. Gao, T. & Newton, A. C. The turn motif is a phosphorylation switch that regulates the binding of Hsp70 to protein kinase, C. *J. Biol. Chem.* **277**, 31585–31592 (2002).
114. Cameron, A. J., Procyk, K. J., Leitges, M. & Parker, P. J. PKC α protein but not kinase activity is critical for glioma cell proliferation and survival. *Int. J. Cancer* **123**, 769–779 (2008).
115. Chou, M. M. *et al.* Regulation of protein kinase C ζ by PI 3-kinase and PDK-1. *Curr. Biol.* **8**, 1069–1077 (1998).
116. Le Good, J. A. *et al.* Protein kinase C isotypes controlled by phosphoinositide 3-kinase through the protein kinase PDK1. *Science* **281**, 2042–2045 (1998).
117. Mora, A., Komander, D., van Aalten, D. M. & Alessi, D. R. PDK1, the master regulator of AGC kinase signal transduction. *Semin. Cell Dev. Biol.* **15**, 161–170 (2004).
118. Facchinetti, V. *et al.* The mammalian target of rapamycin complex 2 controls folding and stability of Akt and protein kinase, C. *EMBO J.* **27**, 1932–1943 (2008).
119. Ikenoue, T., Inoki, K., Yang, Q., Zhou, X. & Guan, K. L. Essential function of TORC2 in PKC and Akt turn motif phosphorylation, maturation and signalling. *EMBO J.* **27**, 1919–1931 (2008).
120. Grodsky, N. *et al.* Structure of the catalytic domain of human protein kinase C β II complexed with a bisindolylmaleimide inhibitor. *Biochemistry* **45**, 13970–13981 (2006).
121. Rhee, S. G. Regulation of phosphoinositide-specific phospholipase, C. *Annu. Rev. Biochem.* **70**, 281–312 (2001).
122. Heasman, S. J. & Ridley, A. J. Mammalian Rho GTPases: new insights into their functions from *in vivo* studies. *Nature Rev. Mol. Cell Biol.* **9**, 690–701 (2008).
123. Kitagawa, M., Shibata, H., Toshimori, M., Mukai, H. & Ono, Y. The role of the unique motifs in the amino-terminal region of PKN on its enzymatic activity. *Biochem. Biophys. Res. Commun.* **220**, 963–968 (1996).

Competing interests statement

The authors declare no competing financial interests.

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