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Molecular control of atypical Protein Kinase C: tipping the balance between self-renewal and differentiation

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Abstract

Complex organisms are faced with the challenge of generating and maintaining diverse cell types, ranging from simple epithelia, to neurons and motile immune cells[1–3]. To meet this challenge, a complex set of regulatory pathways controls nearly every aspect of cell growth and function, including genetic and epigenetic programming, cytoskeleton dynamics, and protein trafficking. The far reach of cell fate specification pathways makes it particularly catastrophic when they malfunction, both during development and for tissue homeostasis in adult organisms. Furthermore, the promise of stem cells as a therapeutic derives from their ability to deftly navigate the multitude of pathways that control cell fate[4]. How the molecular components that make up these pathways function to specify cell fate is beginning to become clear. Work from diverse systems suggests that the atypical Protein Kinase C (aPKC) is a key regulator of cell fate decisions in metazoans[5–7]. Here we examine some of the diverse physiological outcomes of aPKC's function in differentiation, along with the molecular pathways that control aPKC, and those that are responsive to changes in its catalytic activity.

Graphical Abstract

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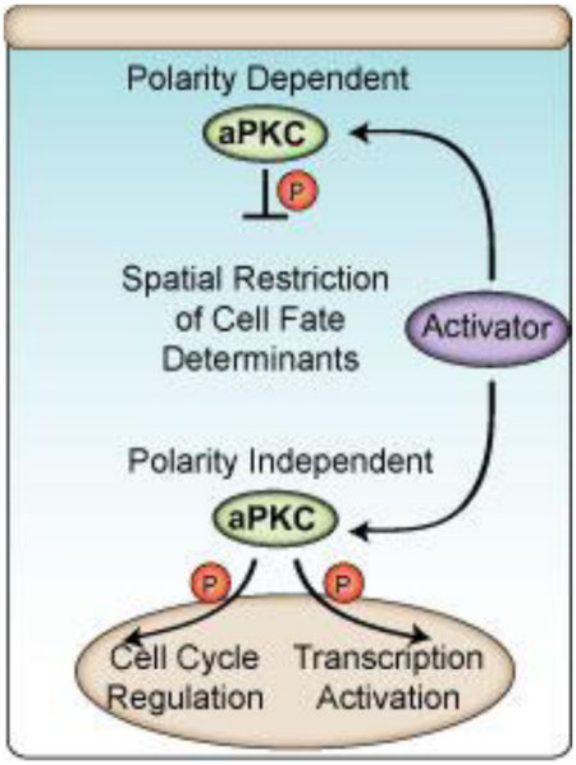

PKC family kinases are ubiquitous components of cellular signaling pathways[8,9]. In animals, PKCs are commonly divided into three subfamilies (yeast contain a single PKC), including the conventional, novel, and atypical (Figure 1)[8]. This last group contains the iota (lambda in mice) and zeta isoforms in mammals, and a single isoform in flies and worms. All family members contain a catalytic domain at the COOH-terminus connected to NH_2-terminal regulatory domains (Figure 1A). The downstream pathways that are regulated by each isoform is primarily determined by their kinase domain's specificity, which determines the repertoire of substrates that they can phosphorylate. Upstream regulation of PKCs is determined by phosphorylation of the kinase domain and allosteric mechanisms that depend on interactions with specific elements contained within the NH_2-terminal regulatory domain[8].

aPKC regulates differentiation in diverse physiological contexts

The physiological contexts in which aPKC participates in cellular differentiation are remarkably varied, suggesting that it is a central component of a fate specification machinery. In this section, we discuss several examples of systems where aPKC plays a known role in regulating cell fate with the goal of emphasizing the diverse physiology in which it can function.

In the mammalian pre-implantation embryo, aPKC activity is essential for development of extra-embryonic tissues such as the Primitive Endoderm (PrE)[10,11]. These tissues provide the connection with the mother and serve as signaling centers for subsequent embryonic patterning[12]. The PrE forms a highly organized epithelium at the exterior of the epiblast,

which ultimately forms the fetus. However, PrE cells are originally specified in an apparently stochastic manner, intermingled with epiblast cells. Intriguingly, aPKC is essential for not only specification of the PrE fate, but segregation of the mixed PrE and epiblast cells. Following segregation, aPKC causes PrE cells to become highly polarized and to promote pro-survival signals in correctly sorted cells[11].

In the fly central nervous system, aPKC regulates the balance between self-renewal of neuronal progenitors and their differentiation into neurons[5,6]. The *Drosophila* neuroblast (NB) is a cell that participates in the development of both the embryonic and larval nervous systems. NBs undergo repeated asymmetric cell divisions that produce as daughter cells a self-renewed NB and a Ganglion Mother Cell (GMC). The GMC subsequently divides once more which typically generates two cells that become neurons. NBs with incorrect levels of aPKC activity fail to asymmetrically divide and can exhibit characteristics of tumor cells, or alternatively can prematurely differentiate and concomitant loss of the progenitor pool. Excess aPKC activity leads to indefinite replication capacity[13], whereas NB quiescence or premature differentiation are associated with inadequate aPKC activity[14,15]. aPKC is also polarized in mammalian neurons and is required for axonal-dendritic differentiation during development[16,17]. Perturbing aPKC or regulator of aPKC localization and activity leads to improper number of axons and dendrites. In addition to its role in development, aPKC-mediated asymmetric cell division is also essential for homeostasis in the adult gut[18].

The central role of aPKC in cell fate determination is also supported by the severe consequences if it is improperly regulated. Overexpression of aPKC is observed in multiple cancers[19], including hepatocellular carcinoma, pancreatic adenocarcinoma, and breast cancer. It has recently been shown that excess aPKC activity can overcome contact inhibited growth in epithelial cells and is sufficient for transformation[20]. It is interesting to note that the aPKC iota/lambda and zeta isoforms may have distinct functions in regulating proliferation based on their requirement in different cell types. For example, the iota/lambda isoform promotes the growth and metastasis of triple-negative breast cancers, a subtype defined by the absence of estrogen receptor, progesterone receptor and epidermal growth factor receptor 2[21]. However, the zeta isoform is required for the mitogen induced growth of squamous cell carcinomas of the head and neck[22]. Whether these isoforms are indeed differentially regulated and/or act on distinct downstream pathways is an important outstanding question.

Cellular mechanisms of fate determination by aPKC

In this section, we examine the cellular mechanisms by which aPKC controls cell fate. As a regulator of cell polarity, aPKC is a member of the Par (partitioning defective) complex, which includes Par-3 (Bazooka in flies), and Par-6[23,24]. Polarity is essential for many aspects of cell function, including aPKC's role in cell fate specification in the *Drosophila* NB. Early in mitosis, asymmetrically dividing NBs begins to polarize such that by metaphase, aPKC and the rest of the Par complex localize to one half of the cell cortex, while neuronal fate specification factors localize to the other half[5,6]. Because the mitotic spindle is aligned with cortical polarity, the cytokinetic furrow bisects the two cortical domains: one daughter cell is formed from the cortex containing the Par complex, and the

other forms from the cortex with differentiation factors bound. aPKC is a key output of Par complex activity, as it phosphorylates downstream targets to displace them into the cytoplasm[25]. These substrates can localize to cortical regions that lack the Par complex but are removed from the cortex once they enter the Par domain (Figure 1B). For at least several aPKC substrates, this mechanism appears to involve phosphorylation of short motifs enriched for basic and hydrophobic residues that directly interact with phospholipids[26,27]. Phosphorylation of the motif alters its electrostatic character thereby reducing the affinity for the membrane and causing displacement of the substrate into the cytoplasm.

Activating aPKC at the NB apical cortex is critical for restricting neuronal fate determinants to the basal cortex. These proteins include the coiled-coiled protein Miranda with its cargo protein, the transcription factor, Prospero (Pros; Prox1 in mammals), and the translational regulator Brain Tumor (Brat; TRIM3 in mammals), as well as the Notch signaling regulator Numb[5,6]. Following mitosis, these determinants induce conversion into a ganglion mother cell (GMC) by preventing self-renewal and promoting differentiation. Pros is a homedomain transcription factor that translocates to the GMC nucleus and activates genes that specify differentiation while repressing genes that are necessary for self-renewal[5]. High Pros expression in NBs is sufficient to drive their differentiation[28] while intermediate levels induce quiescence[29]. Differentiation is aided by the translational repressor Brat, which regulates important proliferation signals including Cyclin E, β-Catenin, dMyc, and Mad[30–32], and the repressor of Notch signaling, Numb[14,33].

Besides excluding neuronal fate determinants from the self-renewed NB, aPKC also plays a direct role in maintaining NB fate. The transcription factor Zif represses NB formation and in NBs lacking Zif aPKC is unpolarized[34]. The *aPKC* gene contains Zif binding sites and Zif appears to repress *aPKC* expression. Furthermore, Zif is an aPKC substrate and phosphorylation prevents its entrance into the nucleus, forming a feedback loop that regulates aPKC expression and localization.

Regulation of aPKC during asymmetric cell division is controlled by a large network of regulatory factors. The Rho GTPase Cdc42 is a key regulator of the Par complex by binding [35–37]. The neoplastic tumor suppressor Lgl is a negative regulator of aPKC localization and helps ensure that aPKC is restricted to the proper cortical region[38,39]. Dynamin associated protein-160 (Dap160) regulates both aPKC localization and kinase activity[40]. It co-localizes with the Par complex at the apical cortex of dividing NBs and interacts with both aPKC and Par6. Dap160, through an unknown mechanism, also helps ensure that aPKC is properly polarized and does not enter the basal cortical domain. Other factors that control aPKC activity and localization include Clueless[41] and Canoe/afadin[42,43], although the mechanisms by which they do so are poorly understood.

Another important cellular role for aPKC is in orienting the division axis. Several recent reviews cover aPKC function in oriented cell divisions in detail[44,45].

Regulation of the cell cycle by aPKC

Cell fate specification can be tightly coupled to the cell division cycle. For example, in certain contexts a prolonged G1 cell cycle phase leads to differentiation, while a shortened G1 promotes proliferation (i.e. self-renewal)[46]. Recent evidence from the *Xenopus* neuroectoderm suggests that G1 is controlled in part by the inhibition of G1 specific cyclin/cdks[47]. Although many aPKC functions involve its activity at the cell cortex, aPKC is found in the nucleus of progenitor cells in this tissue[48] consistent with a role in transcriptional regulation. This seems to be the case for at least one cell-cycle regulatory protein in *Xenopus* progenitor cells, p27xic. p27xic is a CIP/KIP protein family of cyclin-dependent kinase inhibitors (CDKIs) that prevents the G1 to S transition by inhibiting cyclin-dependent kinase 2 (Cdk2) through binding and sequestering it from the nucleus (Figure 2A). In this manner, the level of p27xic expression in the progenitor cells can indirectly affect the decision to proliferate or differentiate by controlling G1 length. But what controls the level of p27xic? Recent work has demonstrated that p27xic is an aPKC substrate and phosphorylation regulates its ability to inhibit the G1 to S transition. Phosphorylation prevents p27xic's binding to Cdk2 providing a simple, but elegant method for coupling aPKC activity to cell cycle control, and ultimately the decision to proliferate or differentiate[49] (Figure 2B).

Transcriptional programming by aPKC

Hedgehog (HH) signaling is important for cell fate decisions that specify the animal body plan[50]. In the absence of HH ligand, Patched (Ptch) represses HH signaling through inhibition of the receptor Smoothened (Smo)[51] (Figure 3). When HH binds Ptch at the membrane, transcriptional activators such as GLI (Cubitus interruptus in *Drosophila*) become active[51]. This pathway can regulate stem cell proliferation versus differentiation decisions[52] and is often reactivated during the initiation and progression of cancers such as basal cell carcinomas (BCCs) and lung squamous cell carcinomas (LSCCs)[53,54]. Binding to Ptch requires numerous HH post-translational modifications including specific proteolysis followed by palmitoylation by HH acyl transferase (HHAT)[51]. Once HH ligand binds to Ptch, Ptch no longer inhibits Smo, resulting in translocation of GLI to the nucleus and subsequent activation of proliferative genes[50]. Recently, aPKC has been found to regulate multiple points within the HH pathway. Activity of aPKC leads to upregulation of the HH ligand, phosphorylation of the receptor Smoothened, and activation of the bifunctional transcriptional regulator of HH signaling, GLI[55–57] (Figure 3).

There are multiple mechanisms by which aPKC regulates HH signaling. First, expression of the HHAT enzyme is dependent on aPKC activity. This control occurs by aPKC's phosphorylation of SOX2, an important transcriptional regulator of stem cell maintenance. SOX2 modification by aPKC allows it to bind the HHAT promoter region[57] (Figure 3). This leads to an increase in functional HH ligands. Upregulation of HHAT by aPKC can be important for tumorigenic growth by maintaining stemness, as has been demonstrated for LSCC oncospheres[57].

HH signaling can also be regulated by aPKC downstream of the Ptch receptor. The GLI1 transcription factor is an aPKC substrate[55], and, as with SOX2, phosphorylation activates transcription of GLI1 target genes including aPKC itself (Figure 3). This positive feedback loop can lead to the development and progression of basal cell carcinomas (BCCs) independent of Smo activation of GLI1[55] (Figure 3). Currently, Smo inhibitors are used to treat BCCs but the tumors can develop resistance[50,58]. Inhibition of aPKC signaling inhibits BCC tumor-growth indicating that inhibitors could have therapeutic potential for treating BCCs[58]. In *Drosophila,* aPKC phosphorylates Smo and GLI (Cubitus interruptus in *Drosophila*) to polarize them basolaterally, thereby promoting HH signaling during early wing development[56]. However, the molecular mechanism by which aPKC activity is controlled during HH signaling remains unclear.

aPKC regulation of Wnt signaling

Many tissues, such as the epidermal and intestinal epithelia, undergo rapid turnover requiring constant differentiation from precursor cells for tissue maintenance. In mammalian epidermal models, aPKC regulates cell fate by ensuring proper division orientation[59]. Adult intestinal stem cells are continually replenishing the cells of the epithelium, which is turned over ever 3–5 days[60,61]. In these adult stem cell models, precise regulation of β-Catenin (Wnt signaling) and Yap (Hippo pathway) is required for maintenance of tissue homeostasis and prevention of tumor initiation and progression[1,62].

In the absence of Wnt ligands, β-Catenin is degraded by the “destruction” complex composed of the tumor suppressor adenomatous polyposis coli (APC), scaffolding protein Axin, glycogen synthase-3 (GSK-3β) and casein kinase 1 (CK1). While the complex is intact, β-Catenin is phosphorylated by GSK-3β and degraded by the proteasome[63]. In the absence of nuclear β-Catenin, downstream Wnt-dependent target genes are not transcribed, inhibiting proliferative and growth signals (Figure 4). When Wnt is bound to the receptor Frizzled (FZD) and a co-receptor, Axin is thought to be degraded and the destruction complex dissociates, concomitantly stabilizing β-Catenin levels, allowing for nuclear translocation and binding to co-activator TCF/LEF proteins. Ultimately, this leads to the transcription of Wnt-dependent target genes[63] (Figure 4). Wnt signaling has been implicated in polarity through interactions with the Par complex in migratory cells[64]. Recent work has shed light on how aPKC might be playing a direct roll in Wnt signaling.

aPKC has now been identified as a component of destruction complex that interacts with Yap and β-Catenin[65]. While best known for their role in Hippo pathway signaling[66], Yap and Yaz also interact with the destruction complex[62,67]. aPKC phosphorylates both β-Catenin and Yap, preventing their nuclear accumulation, thereby inhibiting Wnt and Hippo downstream targets required for proliferation and cell growth[65] (Figure 4). β-Catenin must be phosphorylated at its aPKC phosphorylation site (either by aPKC or another kinase) before GSK-3β can act on it[68,69]. Yap activity is increased by aPKC, in a manner that is at least partially independent from canonical Hippo signaling. In *Drosophila,* GSK-3β regulates polarity by phosphorylating aPKC, which targets it for proteasomal degradation[70] suggesting crosstalk between these pathways.

aPKC's regulation of JAK/Stat

Janus Kinase (JAK) and Signal transducer and activator of transcription (Stat) are important growth regulators that play a prominent role in development and tumor progression[71–73]. Numerous signaling pathways activate JAK/Stat by inducing JAK recruitment to Stat and subsequent Stat phosphorylation. During IL6 cytokine activation, phosphorylation of the Stat3 isoform by JAK leads to Stat3 nuclear translocation where it activates proliferation and survival genes and represses differentiation genes[74]. Stat3 has also been implicated in the maintenance of cancer stem cells (CSCs)[75,76].

In a recent study, aPKC activity was found to activate Stat3 in a mammalian model of breast cancer[77]. Activation occurs via aPKC's interaction with the NF-κb signaling pathway, which is up regulated in many human cancers[78]. In this system, aPKC becomes active in the cytoplasm after loss of polarity where it activates IKK ultimately causing increased IL6 production[79,80]. This leads to a positive feedback loop associated with proliferation and tumor progression (Figure 5). Up-regulation of IL6 by active aPKC in unpolarized cells also occurs in *Drosophila* models that combine polarity loss with oncogenic transformations[81]. In fact, constitutively active aPKC is sufficient to induce IL6 (Upd in *Drosophila*) expression, although the effect is dependent on the *Drosophila* ortholog of YAP (Yki)[81]. Whether or not aPKC induces IL6 through YAP via the canonical Hippo pathway signaling or as part of the destruction complex (i.e. Wnt signaling), remains to be resolved.

The above examples suggest that the output of aPKC activity is dependent on the cellular context. For example, while aPKC promotes self-renewal and cell growth in neuroblasts, it seems to inhibit self-renewal in the intestinal epithelium. This conundrum highlights the necessity of discovering and understanding the mechanisms that regulate aPKC activity in spatial and temporal manner in these diverse cell and organismal contexts.

Regulation of aPKC: localized activity

The cellular mechanisms by which aPKC regulates differentiation suggest a high degree of both catalytic and spatial control[9]. For example, the cortical exclusion of fate determinants in polarized neuroblasts during asymmetric cell division requires that aPKC activity is not only tightly coupled to the cell cycle, but that it is localized to a specific cortical domain. The central role of aPKC in many differentiation pathways means that incorrect activity levels could lead to improper fate specification or proliferation, as described in the previous sections. In general, PKC family enzymes are controlled primarily by kinase domain phosphorylation and allosteric mechanisms (Figure 1A). In aPKCs, the kinase domain is phosphorylated at the activation loop and turn motif[8]. A third site present in other PKC isoforms, known as the hydrophobic motif, is a non-phosphorylatable residue in aPKCs. The activation loop is modified by Phosphoinositide-Dependent Kinase 1 (PDK1)[8]. For some time, PKC turn motifs were thought to be modified as the result of autophosphorylation, but elegant work by Parker and co-workers demonstrated that the turn motif is phosphorylated by an exogenous kinase[82]. Interestingly, at least in some contexts, this kinase can be the mammalian target of rapamycin 2 complex (mTORC2)[83,84]. However, the physiological role of these phosphorylations in regulating aPKC remains unclear. They may be constitutive,

"priming" modifications[85], and recent structural evidence even suggests that they may not be required for activity[86].

In addition to modification of the kinase domain by phosphorylation, PKCs can also be regulated through allosteric mechanisms by binding of upstream pathway components to the NH_2-terminal regulatory domain[9]. Although aPKCs have a different complement of upstream regulators compared to their conventional and novel counterparts, they share several important regulatory elements (Figure 1A). Perhaps most important is the "pseudosubstrate", which has many of the sequence characteristics of a normal substrate so that it can bind in the kinase domain active site, but an alanine at the position that would be phosphorylated prevents progression through the catalytic cycle[8,87]. Determining how the pseudosubstrate is removed from the kinase domain's active site is a key part of understanding PKC activation mechanisms, but other domains, such as the C1, may also directly repress kinase activity[87,88]. The C1 cysteine rich domain is directly COOH-terminal to the pseudosubstrate in all PKCs, and in the single structure of a full-length PKC, the C1 binds a lobe of the kinase domain where it could potentially inhibit activity[89]. aPKC's regulatory domain is distinguished from the other family members by the presence of a PB1 domain that heterodimerizes with certain PB1s from other proteins, and a COOH terminal PDZ ligand sequence[8,90–92].

The Rho GTPase Cdc42 indirectly regulates aPKC by binding to the Par complex member Par-6. GTP-bound Cdc42 interacts with the semi-crib and PDZ domain of Par-6 causing a conformational change, which is essential for aPKC polarization[35–37]. Par-6 contains a PB1 domain that binds aPKC's PB1 and this interaction, via an unknown mechanism, displaces the pseudosubstrate from the kinase domain active site[87,9293] (Figure 6). Interestingly, Par-6 is overexpressed in breast cancer cells and induces their proliferation[94]. Par-6 is also required to recruit aPKC to the cortex, where lipid binding can play a direct role in the activation of aPKC downstream of phosphatidylionositol 3-kinase (PI3K) by binding phosphatidylinositol 3,4,5-phosphate (PIP_3)[95–97]. The lipid ceramide also activates aPKC by directly interacting with the kinase domain, an interaction that is important for junction formation in epithelia and signaling during cellular stress conditions[98,99]. Coupling of aPKC protein-protein and protein-lipid interactions to activation provides an elegant mechanism for ensuring that aPKC is active at the right place and time. Cdc42 may also play a direct role in controlling aPKC's kinase activity as the Par-6 semi-CRIB and PDZ are important for full activation of aPKC by Par-6, further coupling aPKC localization and activity to the NB apical cortex[35,37,100]. In the *Drosophila* neuroblast, loss of either Par-3 or Par-6 leads to improper aPKC localization, defective asymmetric cell division, and improper development[15,35,101].

While Cdc42 and Par-6 are critical for increasing the amount of cortically localized, active aPKC, the neoplastic tumor suppressor Lgl is an important repressor of localization and activity that helps ensure the basal cortical domain remains free of aPKC (and therefore bound to neuronal fate determinants)[38,39]. The mechanism by which Lgl inhibits aPKC has remained enigmatic. In NBs lacking Lgl activity, aPKC activity is no longer restricted to the apical cortex leading to an increase in proliferation and a loss of apico-basal polarity[39].

aPKC counteracts Lgl's repression by phosphorylating it and displacing it into the cytoplasm[38]. How Lgl inhibits aPKC's localization to the basal cortex remains unknown.

Concluding remarks

How cellular diversity is generated during development is one of the most fundamental questions in Biology. Once development is complete, homeostasis requires the constant activity of progenitor cells to replenish rapidly turned over differentiated products. Each of these processes is highly intertwined with proliferation pathways, such that defects are commonly associated with tumorigenesis. Our understanding of the molecular mechanisms that control cell fate decisions is still in its infancy, but it is now clear that the atypical members of the PKC kinase family are involved in many aspects of fate specification. Some of these functions relate to aPKC's activity in regulating cell polarity, but there are newly identified polarity-independent aPKC functions (both in normal and pathological Biology) that are essential for conferring proper cell identity. We expect that many more aPKC substrates and downstream pathways remain to be found, and that fitting them into the puzzle of cell fate determination will help provide a more complete picture of this fundamental process. Furthermore, the mechanisms that govern the localized activity of aPKC are just now being uncovered and will no doubt be important for understanding the diversity of physiological contexts in which aPKC functions.

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Highlights

- atypical Protein Kinase C (aPKC) regulates stem cell fate decision using multiple mechanisms.
- aPKC polarizes fate determinant proteins during asymmetric cell division.
- aPKC shortens the cell cycle to promote cell proliferation.
- aPKC phosphorylates conserved transcription factors to regulate cell fate decisions.
- aPKC controls cell fate decision by polarity dependent and independent mechanisms.

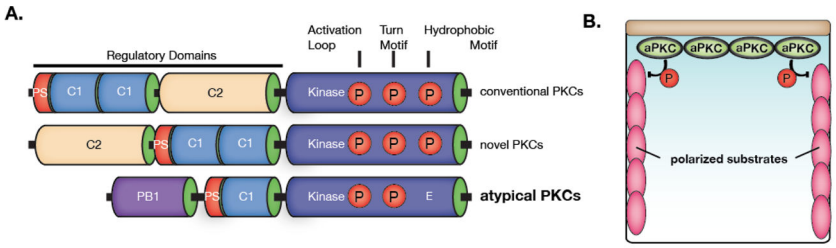

Figure 1.

PKC family kinases and regulation and function of atypical Protein Kinase C. A. Schematic of the protein kinase C family showing domain architectures, demonstrating both common and unique aspects of each PKC family member (PS = pseudosubstrate; C1 and C2 are cysteine rich domains; PB1 Phox/Bem1 domain). B. Schematic of Par-mediated polarity mechanism. aPKC generates cellular polarity through phosphorylation and exclusion of cortically localized substrates (pink).

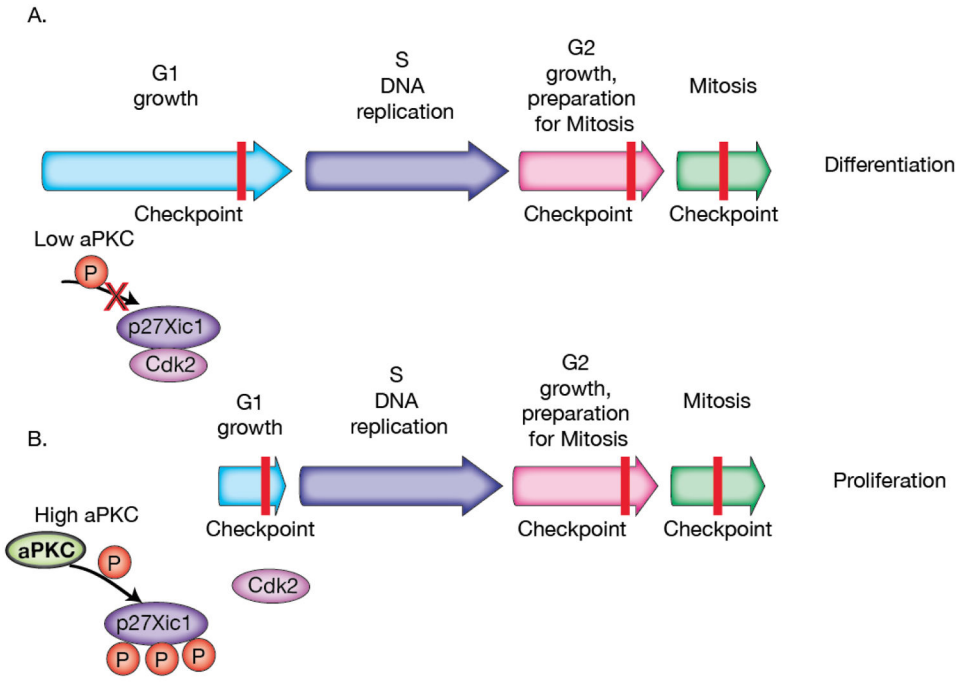

Figure 2.

aPKC regulation of the cell cycle. A. When aPKC levels are low, p27Xic1 is able to elongate the G1 to S transition by binding to Cdk2, which can lead to differentiation in *Xenopus* neuroectoderm progenitor cells. B. When aPKC levels are high, p27Xic1 phosphorylation by aPKC blocks p27Xic1 binding of Cdk2, shortening the G1 to S transition to promote proliferation.

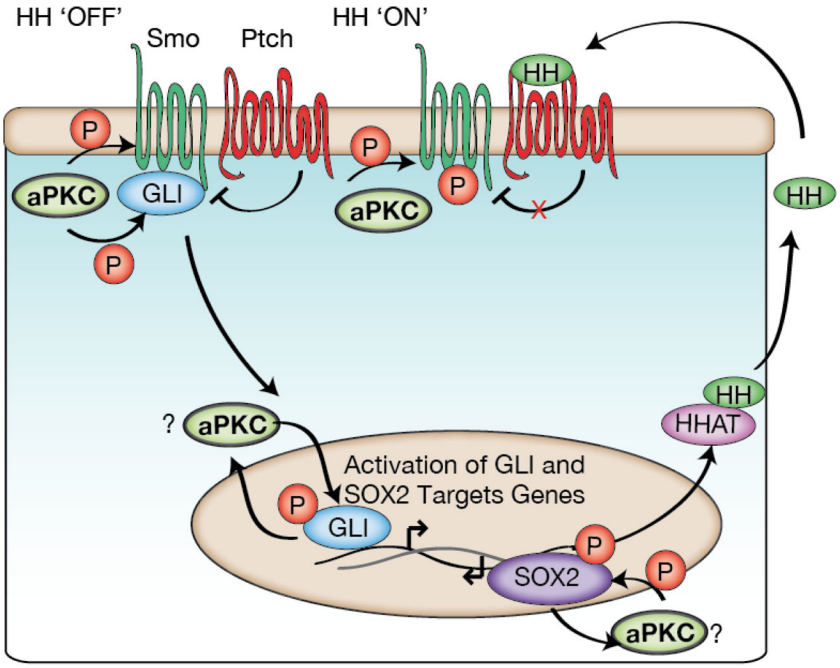

Figure 3.

aPKC regulation of Hedgehog signaling. In basal cell carcinomas (BCCs) and lung squamous cell carcinomas (LSCCs) aPKC is able to phosphorylate GLI (BCCs) and SOX2 (LSCCs) transcription factors. These phosphorylations can lead to positive feedback, upregulating HH signaling genes including HHAT and aPKC itself. This activation can occur independently of HH ligand receptor binding. In the *Drosophlia* developing wing, aPKC phosphorylates the Smoothened receptor to regulate its activity and its subsequent proper development.

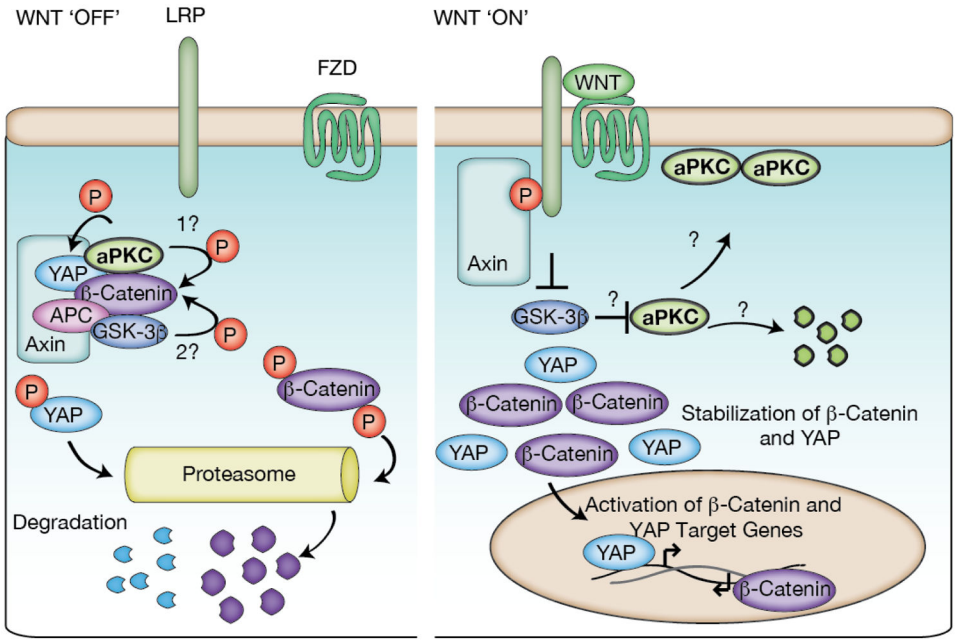

Figure 4.

aPKC regulation of Wnt signaling. aPKC is part of the destruction complex, where it can phosphorylate Catenin to prime it for (2) GSK-3 phosphorylation and subsequent proteasomal degradation. aPKC is also able to phosphorylate YAP, leading to proteasomal degradation. Loss of aPKC or Wnt binding leads to disassembly of the destruction complex and activation of Wnt signaling favoring a proliferative state. The fate of aPKC once the destruction complex is inactivated is unknown.

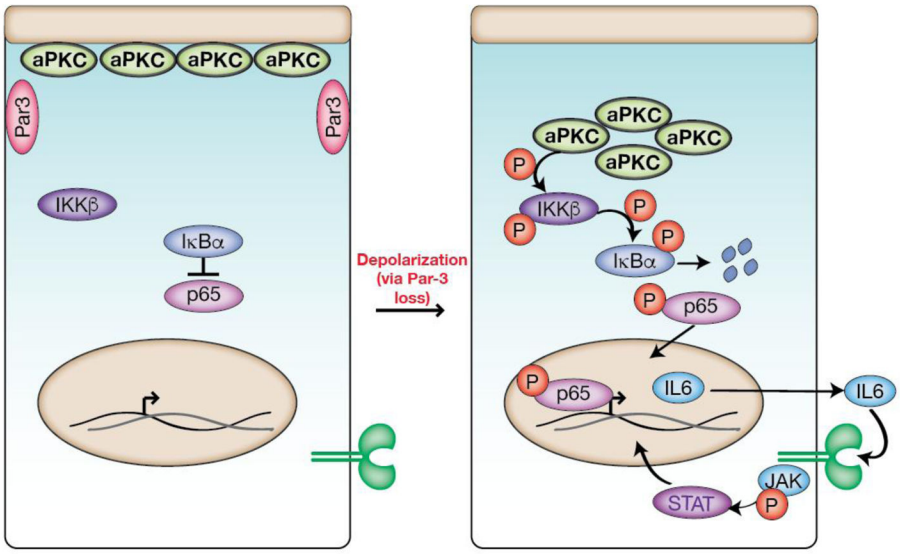

Figure 5.

aPKC regulation of JAK/Stat signaling. Loss of polarity leads to cytoplasmic aPKC which causes activation of IKKβ, degradation of IB, and translocation of p65 to the nucleus to upregulate IL6 production. The increase in IL6 leads to a positive feedback loop with JAK/Stat3 signaling, which, when unregulated, leads to proliferation and tumor progression in a breast cancer model.

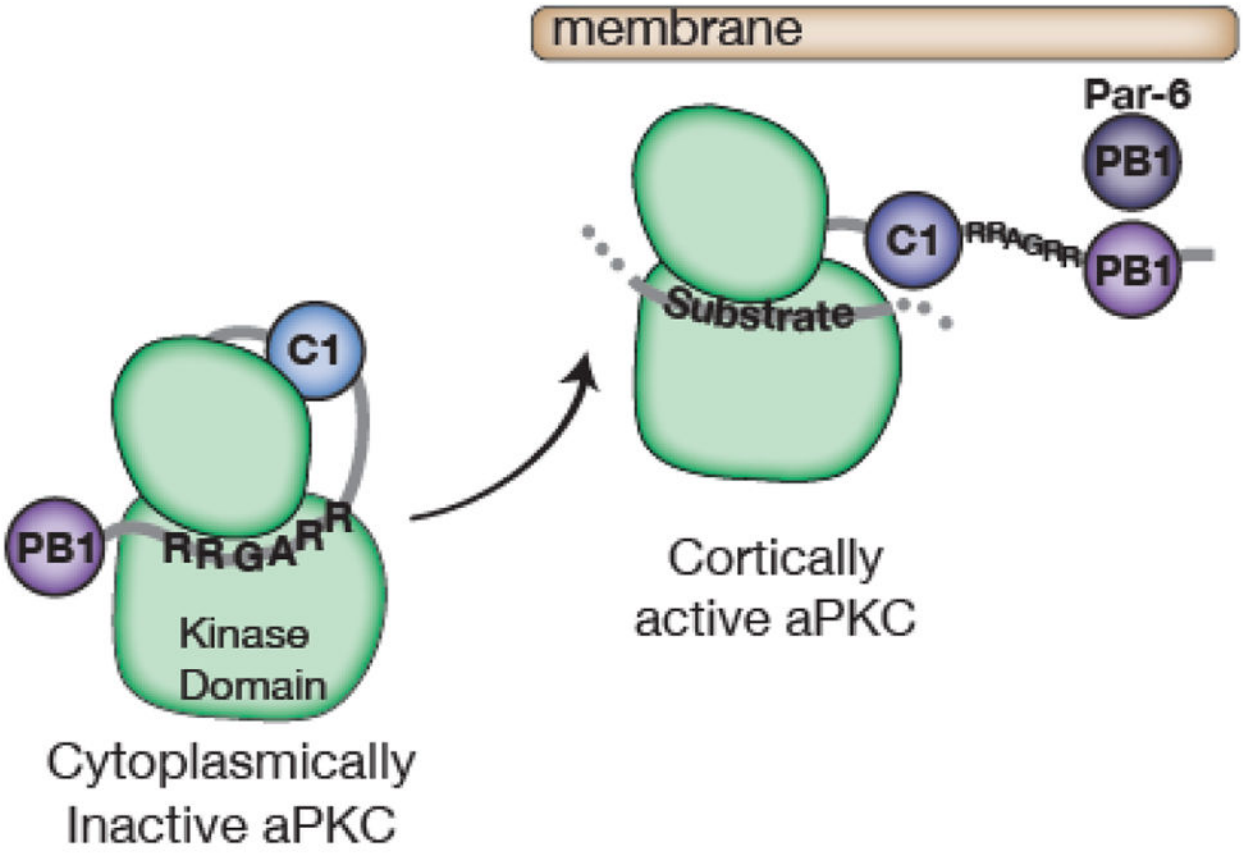

Figure 6.

Regulation of aPKC localization and activity. Par-6's interaction with aPKC's PB1 domain disrupts the pseudosubstrate's (sequence = RRGARR) inhibition of the kinase domain. The C1 domain may also play a role in regulating aPKC kinase activity.