

Review

PKC ζ at the crossroad of NF- κ B and Jak1/Stat6 signaling pathways

J Moscat^{*,1}, P Rennert² and MT Diaz-Meco¹¹ Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain² Biogen-Idec, 14 Cambridge Center, Cambridge, MA 02142, USA^{*} Corresponding author: J Moscat, Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas, Universidad Autónoma de Madrid, Canto Blanco, 28049 Madrid, Spain. Tel: +34 914978039; Fax: +34 917616184; E-mail: jmoscat@cbm.uam.esReceived 19.9.05; revised 24.10.05; accepted 25.10.05; published online 02.12.05
Edited by G Kroemer

Abstract

The atypical protein kinase C (PKC) isoforms (aPKC) have been implicated in the regulation of a number of essential signaling events. Early studies using dominant-negative mutants suggested that they are important intermediaries in the activation of the canonical nuclear factor (NF)- κ B pathway. More recent data using knockout mice genetically demonstrate that in fact the PKC ζ isoform is essential for the adequate activation of this cascade both upstream and downstream the I κ B kinase complex. In this review, we summarize the mechanistic details whereby the aPKC pathway regulates important cellular functions and how this is achieved by the ability of these kinases to interact with different protein regulators and adapters, as well as to impinge in NF- κ B-independent signaling cascades such as the Janus kinase-1/signal transducer and activator of transcription 6 system, which plays a critical role in T-cell-mediated hepatitis and asthma.

Cell Death and Differentiation (2006) 13, 702–711.

doi:10.1038/sj.cdd.4401823; published online 2 December 2005

Keywords: atypical PKCs; NF- κ B; apoptosis; inflammation; cancer; p62/Sequestosome; Par4; Th2 polarization; asthma**Abbreviations:** BCR, B-cell receptor; CBP, CREB-binding protein; COPD, chronic obstructive pulmonary disease; CREB, cyclic AMP-binding protein; Dif, dorsal-related immunity factor; EGF, epidermal growth factor; ERK, extracellular responsive kinase; IFN- γ , interferon- γ ; Ig, immunoglobulin; I κ B, inhibitor of κ B; IKK, I κ B kinase; IL, interleukin; Jak1, Janus kinase-1; JNK, Jun kinase; KID, kinase-inducible transactivation domain; LPS, lipopolysaccharide; LT, lymphotoxin; MEK, MAPK/ERK kinase; MSK-1, mitogen- and stress-activated protein kinase 1; NF-AT, nuclear factor of activated T cells; NF- κ B, nuclear factor- κ B; Par-4, prostate androgen responsive-4; Par-6, partitioning defective-6; PI-PLC, phosphoinositide-specific phospholipase C; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C;RANK-L, receptor activator of NF- κ B-ligand; RIP, receptor-interacting protein; Stat6, signal transducer and activator of transcription 6; TCR, T-cell receptor; Th, T helper; TNF α , tumor necrosis factor α ; TRAF, TNF receptor-associated factor; UBA, ubiquitin associated; XIAP, X-linked mammalian inhibitor of apoptosis protein

Introduction

Cell signaling is an exciting field of research because it not only will give us a full understanding of important biological processes in health and disease but also is serving to identify novel and more selective therapeutic targets to design the medicines of the future. The mechanisms regulating specificity and crosstalk, two important aspects of cell signal transduction, need to be investigated if we want to know how a cell works under the control of complex activating/repressing networks. Specificity is especially relevant when we are dealing with kinases, such as the atypical protein kinase C (aPKC), which are tremendously promiscuous in the selection of their substrates.¹ Therefore, for these enzymes to function efficiently and selectively, there must be ways to constrain their activity and, more importantly, their ability to specifically target substrates at a given time and cell location during activation. The second concept, crosstalk, is likewise important since cells are confronted with complex biological situations, such as embryonic development or inflammation, in which a single kinase may be required to perform a variety of functions by targeting different substrates. Unraveling the mechanisms underlying both processes at a molecular level is of paramount importance to understand how information flows through the different pathways, and how cells are able to cope with complex biological or pathophysiological conditions. A detailed understanding of these phenomena will certainly offer new venues to design better and more selective drugs for the treatment of complex diseases such as cancer and inflammation.

The Atypical and the Other PKCs

The protein kinase C family of isozymes is formed by a large number of members that are grouped in three subfamilies (Figure 1), depending on their structure and mechanism of regulation.² All the isoforms have relatively well-conserved kinase domains at their C-terminus and a clearly divergent regulatory region at the N-terminus. The topology of the regulatory domain serves to ascribe a given PKC isotype to a particular subfamily. Thus, the classical isoforms have a phospholipid and a Ca²⁺-binding region and a double zinc-finger domain that have been shown to account for the ability of these PKC subtypes to be regulated by Ca²⁺ and lipid second messengers, such as diacylglycerol, which are

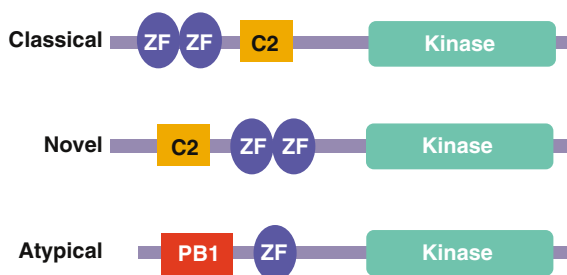


Figure 1 Schematic representation of the different PKC subfamilies. C2, C2 domain; PB1, PB1 dimerization domain; ZF, zinc-finger

generated as a consequence of the activation of the phosphoinositide-specific phospholipase Cs (PI-PLC).² Genetic evidence of a functional interaction between PI-PLC and the classical PKC isoforms has been obtained in studies of B-cell activation in the immune system. Thus, the genetic ablation of PLC- γ 2 produces defects in B-cell function similar to those induced as a consequence of the genetic inactivation of PKC- β .^{3,4} On the other hand, genetic and biochemical studies have functionally linked a novel PKC isoform, PKC θ , as a *bona fide* downstream target of PLC activation in mature T cells.⁵ The so-called novel PKC subfamily is composed of members that, in contrast to the classical isoforms, are insensitive to Ca²⁺ but, like the classical, can be activated by phorbol esters which are stable pharmacological analogs of diacylglycerol.⁶ The reason why phorbol esters activate the classical and novel PKCs may be due to the fact that their regulatory domains harbor a double zinc-finger motif.⁷ The atypical PKCs are insensitive to Ca²⁺ and diacylglycerol probably because they lack the Ca²⁺-binding region and have only a single zinc-finger domain, which suggests that their mechanism of activation must be necessarily different from that of the other PKC isoforms. Two different isoforms belong to the atypical subfamily: PKC ζ and PKC $\lambda/1$. They are highly related especially in their catalytic domain, but must clearly play different roles based on the different phenotypes of the respective knockout mice. Thus, PKC $\lambda/1$ -/- mice are embryonic lethal at very early stages probably due to defects in cell polarity (Diaz-Meco and Moscat, unpublished observations), whereas the PKC ζ -/- mice are born in Mendelian ratios, indicating that, whereas PKC $\lambda/1$ plays a role in the developing embryo, the PKC ζ isoform does not seem involved in such a function.⁸

aPKC Cellular Functions in Cell Growth and Apoptosis

The initial studies to address the role of the aPKCs in cell function focused on the impact that their inhibition may have in the control of cell proliferation, and relied on experiments in which cells were microinjected with inhibitor peptides based on the sequence of the aPKC kinase's pseudosubstrate.⁹ Those studies led to propose that the aPKCs were important players in the control of cell growth and survival. Subsequent transfection experiments using dominant-negative mutants of these kinases supported this notion and indicated that the target of their action was the nuclear factor (NF)- κ B path-

way.¹⁰ This was considered to be of potential great relevance as NF- κ B is critically involved in cell survival and differentiation, two important cellular parameters in immunity and inflammation.¹¹ However, both strategies, the pseudosubstrate peptides and the dominant-negative mutants, are not totally specific when one intends to establish the selective role played by PKC ζ versus PKC $\lambda/1$, because the pseudosubstrate sequences are identical in both isoforms and the dominant-negative mutant experiments may be impinging pathways other than those physiologically relevant for each atypical PKC isotype, a caveat of all such overexpression studies. The first independent indication that the aPKCs may in fact be relevant in apoptosis came from a completely unbiased study aimed at identifying potentially relevant regulators of these PKCs by searching for interacting protein partners using the two-hybrid system in yeast.¹² This strategy led to the identification of the proapoptotic protein prostate androgen responsive-4 (Par-4) as an atypical PKC-binding protein. This protein binds both aPKCs and inhibits their enzymatic activity.¹² This is very interesting because Par-4 had been previously identified by Sells and co-workers in screenings designed to isolate genes involved in apoptosis.¹³ Importantly, the overexpression of Par-4 in cell model systems inhibits NF- κ B activation and activates apoptosis.¹⁴ This is consistent with, and constitutes an independent confirmation of, the proposed role of the atypical PKCs in NF- κ B activation. The genetic inactivation of Par-4 in mice allowed the generation of Par-4-/- embryonic fibroblasts, and experiments, which confirmed the role of Par-4 as a naturally occurring negative regulator of NF- κ B and positive inducer of apoptosis.¹⁵ Thus, Par-4-/- embryonic fibroblasts show enhanced NF- κ B transcriptional activity and elevated levels of the NF- κ B target and antiapoptotic protein, X-linked mammalian inhibitor of apoptosis protein (XIAP). Interestingly, the upregulation of XIAP by NF- κ B was reported to downregulate Jun kinase (JNK) and the subsequent apoptotic response.¹⁶ Consistently, JNK activation was severely inhibited in Par-4-/- cells in keeping with the fact that they have enhanced NF- κ B activity and XIAP levels.^{15,17} Considering the link between decreased apoptosis and tumor progression, Par-4 should be important from the point of view of cancer and its potential therapeutics. In this regard, in good agreement with this notion, tumor cells and tissues display dramatically reduced levels of Par-4, suggesting that Par-4 expression is a roadblock for tumors to develop.¹⁸ The functional relevance of this finding is highlighted by observations that restoration of Par-4 levels in Ras-transformed cells to the values of the parental nontransformed cells makes the tumor cells more sensitive to the action of anticancer drugs both *in vitro* and *in vivo*.¹⁸ This suggests that Par-4 behaves as a tumor suppressor gene. Proof of this assertion is the fact that Par-4-/- mice are prone to spontaneously develop tumors with the endometrium and the prostate particularly sensitive to the development of proliferative lesions.¹⁹ Consistent with these observations, it has been shown recently that PKC λ contributes to poor prognosis in ovarian cancer.²⁰ Interestingly, like in the embryonic fibroblasts, the loss of Par-4 leads to enhanced expression of XIAP in both the uterus and prostate,¹⁹ validating *in vivo* the Par-4/PKC ζ /NF- κ B/XIAP axis as a critical controller of tumor development.

Genetic Inactivation of PKC ζ Reveals its Role in the Control of RelA Transcriptional Activity

The genetic inactivation of RelA and I κ B kinase (IKK β), two major mediators of NF- κ B activation, is lethal due to tumor necrosis factor (TNF) α -dependent liver apoptosis during gestation.¹¹ However, PKC ζ gene-deficient mice do not display liver toxicity, although NF- κ B and IKK activation is impaired in lung extracts from mice injected with TNF α and lipopolysaccharide (LPS).⁸ The lung results suggest that PKC ζ may be upstream of IKK in this pathway. However, the analysis of this cascade in embryonic fibroblasts reveals the surprising observation that, whereas the expression of at least some NF- κ B-dependent genes is inhibited in PKC ζ ^{-/-} cells, the activation of IKK or the nuclear translocation of NF- κ B is not affected.⁸ Also, apoptosis in response to TNF α is increased in PKC ζ ^{-/-} fibroblasts, which is a clear hallmark of NF- κ B deficiency.⁸ Therefore, PKC ζ is required for NF- κ B transcriptional activity, but not for the initial steps of the activation of this pathway. This is in line with recent evidences indicating that the regulation of NF- κ B takes place at different steps of the pathway and not only through the regulation of IKK. In this regard, the phosphorylation of the RelA subunit of the NF- κ B complex has received considerable attention. The pioneer work of Ghosh *et al.*²¹ identified Ser276 as a critical residue for the control of the transcriptional activity due to its ability to recruit, when phosphorylated, the transcriptional cofactor cyclic AMP-binding protein (CREB)-binding protein (CBP). The initial work of Ghosh suggested that Ser276 is a protein kinase A (PKA)-targeted residue, but TNF α or the other cytokines implicated in NF- κ B activation do not appear to be potent activators of this kinase. Studies have indicated that Ser276 may be constitutively phosphorylated at least in some cells,²² whereas recent reports suggest that mitogen- and stress-activated protein kinase 1 (MSK-1) may be the Ser276 kinase activated by TNF α in cell culture experiments.²³ However, more *in vivo* studies should be carried out to firmly confirm these initial cellular observations. Other studies suggest that Ser536 is phosphorylated by the IKK complex, but this site seems to negatively regulate the activity of RelA by modulating its proteasomal degradation, playing a role in the resolution of inflammation by the IKK α subunit of the IKK complex.²⁴

The loss of PKC ζ impairs RelA phosphorylation in response to TNF α , which suggest that this kinase is in fact a RelA kinase. Recent data demonstrate that PKC ζ directly interacts with NF- κ B once this is released from I κ B α following IKK action phosphorylates and Ser311.²⁵ This, like in the case of Ser276 phosphorylation, serves to recruit CBP allowing κ B-dependent transcription.²⁵ Interestingly, PKA-driven phosphorylation of Ser133 in the transcription factor CREB was early shown to mediate the recruitment of CBP. Ghosh *et al.*²⁶ noted that the amino-acid sequence surrounding Ser133 in CREB and that around Ser276 in RelA show a high degree of homology. This region in CREB, termed kinase-inducible transactivation domain (KID), contacts the KIX domain of CBP.²⁷ Phosphorylated Ser133 is located in the N-terminus of KID and makes the contacts required for KID-KIX stabiliza-

tion.²⁸ Of note, whereas Ser276 (in RelA) and Ser133 (in CREB) lie at different ends of the homologous region, Ser311 in RelA is located at the same end as Ser133 in CREB. The implications of this are unclear and must wait the availability of protein cocrystallization data. In any case, the fact that both Ser276 and Ser311 are required for CBP recruitment and taking into account that they seem to be independently targeted by PKA and PKC ζ , respectively, suggests that both sites may regulate independently CBP binding and NF- κ B transcription in response to separate pathways. Further *in vivo* studies should address this important question.

The Atypical PKC ζ in the *Drosophila* Immune Response

The NF- κ B pathway is remarkably conserved in *Drosophila*, controlling not only development but also the innate immune response.²⁹ Thus, the RelA homologs in *Drosophila*, dorsal-related immunity factor (Dif) and Dorsal, have been shown to be necessary for the synthesis of the antimicrobial peptide drosomycin in response to the activation of the Toll pathway by fungal pathogens. This pathway involves the adapter Tube, the kinase Pelle and the phosphorylation, and subsequent degradation of the inhibitor of κ B (I κ B) homolog, named Cactus. Pelle resembles IRAK and is required for Cactus degradation and the subsequent release of Dif for its nuclear translocation. However, the kinase directly involved in the activation of Cactus still remains to be discovered. Parallel to this pathway, there is another one in *Drosophila* that involves the kinase dTAK1, which acts upstream the *Drosophila* IKK complex and that serves to control the degradation of Relish. Relish is the fly homolog of NF- κ B1/NF- κ B2 and is required for the synthesis of dipterin in response to bacterial infection. Interestingly, knocking down PKC ζ with RNAi in *Drosophila* cells dramatically inhibits drosomycin expression but not that of dipterin, indicating that PKC ζ is located specifically in the Toll antifungal pathway.³⁰ Remarkably, PKC ζ downmodulation does not affect Cactus or Relish degradation, but it does inhibit the drosomycin promoter activity of a luciferase reporter plasmid. Therefore, in *Drosophila*, like in mammalian cells, PKC ζ is necessary for NF- κ B transcriptional activity, but not for the control of the nuclear translocation of RelA or Dorsal/Dif. In this regard, PKC ζ is capable of phosphorylating directly Dif, which suggests a high degree of conservation of PKC ζ in the NF- κ B pathways.³⁰ Notably, recent data confirmed these observations by direct injection of double-stranded RNA of *Drosophila* PKC ζ and Ref(2)P into *Drosophila* adult flies.³¹

PKC ζ and the Adaptive Immune System

The phenotypic analysis of PKC ζ ^{-/-} mice reveals significant alterations in the development of secondary lymphoid organs in young animals.⁸ Thus, although the overall structure of the spleen of these mice was preserved, there was a detectable defect in the marginal zone with smaller B-cell follicles in the white pulp. In addition to these alterations, there were also defects in peripheral and mesenteric lymph nodes and in Peyer's Patches showing impaired segregation between the

T- and B-cell zones and a decrease in the follicular dendritic cells.⁸ This phenotype is complex and reminiscent of those of mutant mice for the TNF α receptor-1 (TNFR-1) and the lymphotoxin β -receptor (LT β -R). In fact, the activation of κ B-dependent genes is impaired in PKC ζ ^{-/-} fibroblasts not only when activated by TNF α or interleukin (IL)-1 but also in response to LT- β .⁸ In this regard, other KO mice for NF- κ B family members display defects in the development of secondary lymphoid tissues. Thus, p52^{-/-} mice as well as IKK α mice genetically engineered to express a kinase-dead mutant of IKK α , or knockout mice for the IKK α upstream kinase NIK, all show altered splenic architecture as well as impaired B-cell responses.³² The p52^{-/-} mice show reduced formation of follicular dendritic cells, and alterations in the germinal centers and the marginal zone.³³ On the other hand, studies on p50^{-/-} mice also reveal the role of this protein in the development of marginal zone B-lymphocytes.^{32,34} The deletion of the gene encoding for receptor activator of NF- κ B ligand (RANK-L) demonstrates the role of this cytokine in the development of lymph nodes, and Peyer's Patches, whereas signaling through the LT- β receptor is likewise necessary for the development of these secondary lymphoid organs as well as for a normal splenic architecture.³⁵ The TNFR-1^{-/-} mice, although have normal lymph nodes, are unable to form Peyer's Patches properly.^{35,36} On the other hand, the double TNF α receptor/RelA knockout mice have a more profound phenotype characterized by lack of lymph nodes, Peyer's Patches, and organized spleen microarchitecture.³⁷ The congruence of phenotypes of the PKC ζ ^{-/-} mice and mice genetically deficient in TNFR family and NF- κ B components suggests that PKC ζ plays a role in signal transduction in one or more of these pathways, that is, TNFR-1, LT β -R, and RANK. However, it should be emphasized that the loss of PKC ζ does not lead to a total blockade in the development of the secondary lymphoid organs but just a delay, because as the mice age the defects in lymph nodes disappear and those of the Peyer's Patches become much less dramatic in adult mice.

The development of secondary lymphoid organs is controlled by dynamic interactions between stromal and hematopoietic cells. Interestingly, young PKC ζ ^{-/-} mice displaying defects in these organs also show a reduced percentage of mature B cells in peripheral and mesenteric lymph nodes, whereas in Peyer's Patches of these mutant mice the content of immature B cells was significantly increased.⁸ Potentially relevant for this observation is the fact that B cells from PKC ζ ^{-/-} mice display increased spontaneous apoptosis and reduced proliferative responses when challenged through the B-cell receptor (BCR), but not by other activators such as CD40 or LPS.³⁸ This is specific for B cells, since the activation of T cells through the T-cell receptor (TCR) is not affected by the loss of PKC ζ .³⁸ These observations are in keeping with the fact that these mice show a small, although reproducible, inhibition of serum immunoglobulin (Ig)M production when challenged with a T-independent antigen.³⁸ Surprisingly, the response to a T-dependent challenge was also impaired in the PKC ζ ^{-/-} mice.³⁸ Since T cells respond normally to an anti-CD3 stimulation, this observation suggests that PKC ζ may be required for some kind of T-cell function but not directly downstream of the TCR signaling cascade. An interesting

aspect of the role of the atypical PKCs in the control of adaptive immune response was discovered when the phenotype of the Par-4^{-/-} mouse was analyzed.¹⁵ The loss of Par-4 leads to hyperactivation of both atypical PKCs and, therefore, its analysis will shed light on the role of both PKCs *in vivo*. The first striking observation was that these mice have a dramatically enlarged spleen due to a general increase in the number of splenocytes, without detectable alterations in the percentages of B cells or in CD4⁺ or CD8⁺ T cells.¹⁷ In addition, the proportions of different subpopulations of B cells were not affected by the loss of Par-4, indicating that it may be a negative regulator of B- and T-cell proliferation, but not of the maturation programs of these cells. Interestingly, and consistent with the data from the PKC ζ ^{-/-} mice, the Par-4-deficient B cells hyperproliferate when challenged through the BCR, indicating that Par-4 is a *bona fide* negative regulator of PKC ζ in B cells, since the B-cell phenotype of both mice is just opposite.¹⁷ Because the loss of PKC ζ does not affect T-cell proliferation, the observation that Par-4-deficient cells hyperproliferate in response to a TCR-dependent challenge was a surprise. However, since Par-4 targets both PKC ζ and PKC $\lambda/1$, these observations may suggest that PKC $\lambda/1$, but not PKC ζ , is critically involved in TCR signaling. This would account for the T-cell phenotype of Par-4^{-/-} mice. Consistent with this notion, Par-4 is induced during long-term incubation of T lymphocytes with anti-CD3 plus anti-CD28 and its loss leads to the hyperactivation of NF- κ B, in keeping with the hypothesis that the aPKCs play a key role in the activation of this important transcription factor.¹⁷ How PKC $\lambda/1$ regulates NF- κ B in T cells in the context of other more established NF- κ B activators and adapters, including PKC θ , is unclear and must wait until conditional PKC $\lambda/1$ knockout mice can be investigated. In any case, the loss of Par-4 promotes the hyperproliferation of T cells, probably – although not yet proven – through the stimulation of PKC $\lambda/1$ and NF- κ B.

PKC ζ in Th2 Polarization and IL-4 Signaling

Another unanticipated discovery from the analysis of the Par-4-deficient mice was the potential implication of the aPKCs in the control of T-cell polarization programs. It is well established that naïve CD4⁺ T cells can differentiate in response to antigen stimulation into two distinct subsets of effector cells, Th1 and Th2, which display distinct cytokine profiles and immune-regulatory functions.³⁹ Th1 cells produce interferon- γ (IFN- γ) and IL-2, and are essential for cell-mediated immune responses against intracellular pathogens.³⁹ Th2 cells produce a different set of cytokines, including IL-4, IL-5, IL-10, and IL-13, and are important in the control of humoral immunity and allergy.⁴⁰ The investigation of the signaling cascades that control Th2 differentiation and function is of paramount importance because it may lead to the identification of novel therapeutic targets for asthma and other allergic diseases.⁴¹

The differentiation program for Th2 cells is controlled essentially by the GATA-3 transcription factor, whose induction is required and sufficient for Th2 polarization.⁴² In addition, other transcription factors such as c-Maf, nuclear

factor of activated T cells (NF-AT)c1, and NF- κ B also contribute to this differentiation process, although evidence exists that, at least in the case of NF- κ B, its actions are mediated by GATA-3 induction.⁴² In contrast, Th1 polarization is controlled by T-bet.⁴² A number of regulators and adapters have been genetically implicated in the Th2 differentiation process. Of special relevance for this review is PKC θ , since it is a member of the large family of PKCs, whose deletion in mice suggests its role in Th2 function *in vitro* and *in vivo*.^{43–45} However, in contrast to PKC ζ , PKC θ has been shown to play a broader role in T-cell activation, including that of naïve T cells.⁵ Thus, several reports demonstrate that PKC θ is critical for mature T-cell proliferation most likely acting upstream of the adapters Bcl-10 and Malt1.^{46,47} However, further studies are required to totally establish the mechanism of action of this important PKC.

Interestingly, the induction of GATA-3 during Th2 polarization depends on signals emanating from the TCR and the IL-4 signaling cascade.⁴² Genetic evidences demonstrate the critical role played by this latter pathway, which involves the transcription factor signal transducer and activator of transcription 6 (Stat6), *in vitro* and *in vivo*.⁴² However, it should be borne in mind that IL-4 is a Th2-secreted cytokine and, paradoxically, is required for the Th2 polarization process. Therefore, IL-4 is required for the generation of the cells that are at the same time its producer. This apparent contradiction can be resolved if it is taken into account that there are sources other than the Th2 cells for the production of IL-4 and that, in fact, Th2 differentiation can be induced through IL-4-independent pathways such as those regulated by Notch ligands. In any event, it is clear that Th2 responses are dramatically reduced in Stat6 $-/-$ mice, which reinforces the notion that the IL-4 signaling cascade is at least important for Th2 differentiation not only *in vitro* but also *in vivo*.⁴² The first indication that PKC ζ could be involved in Th2 polarization came from the studies on the Par-4 $-/-$ mice. Thus, when CD4 $+$ T cells were incubated during a prolonged period of time in the presence of anti-CD3 plus anti-CD28, it was apparent that secretion of IFN- γ , a hallmark of Th1 polarization, was not affected in the Par-4 $-/-$ cell cultures, but that of IL-4 was significantly increased, suggesting that an aPKC could be involved in IL-4 production by T cells and consequently in Th2 polarization.¹⁷ If this were the case, these observations could explain at least in part the inability of PKC ζ -deficient mice to mount an optimal immune response when challenged with a T-dependent antigen, which was inconsistent with the fact that PKC ζ - $-/-$ naïve T cells did not show proliferative defects.³⁸ More importantly, serum IgE levels were dramatically reduced in PKC ζ - $-/-$ mice, suggesting a potential defect in IL-4 function, since this is an important cytokine also for B-cell Ig isotype switching to IgE.³⁸ Therefore, it seems plausible that PKC ζ may play an important role during Th2 differentiation. In fact, when CD4 $+$ T cells were induced to differentiate *in vitro*, it became apparent that the loss of PKC ζ produced a dramatic inhibition in Th2 differentiation markers such as the secretion of IL-4 and other Th2 cytokines or the production of GATA-3.⁴⁸ Interestingly, PKC ζ is induced during Th2, but not Th1, polarization and the activation of Stat6 phosphorylation and nuclear translocation was also robustly inhibited in the PKC ζ - $-/-$ cells.⁴⁸ Bio-

chemical evidence locates PKC ζ downstream the IL-4 receptor (IL-4R), but not as a direct target of TCR-derived signaling pathways, suggesting that PKC ζ is a novel essential element in IL-4 signal transduction.⁴⁸ This pathway has been investigated extensively and consists of the tyrosine kinase Janus kinase-1 (Jak1), which phosphorylates the IL-4R α -chain, creating a docking site for the SH2 domain of Stat6 that is then recruited and phosphorylated by the receptor-bound Jak1. Once tyrosine-phosphorylated, Stat6 dimerizes and translocates to the nucleus. Importantly, the activation of Jak1 by IL-4 addition is severely impaired in PKC ζ - $-/-$ cells, indicating that this kinase is required for an optimal activation of the pathway.⁴⁸ The residue phosphorylated by PKC ζ in Jak1 as well as the precise mechanism whereby this phosphorylation event controls Jak1 activation remain to be determined, but it is clear that there are additional cytokines that activate Jak1 independently of PKC ζ and that PKC ζ is not required for the stimulation of other Jak1-like tyrosine kinases, such as Jak3 or Tyk2. The structural constraints that account for this specificity have not been revealed yet, but suffice to say that PKC ζ is recruited to the IL-4R α complex most likely through its ability to interact with Jak1, although whether other still unidentified components of this complex may confer specificity to the PKC ζ recruitment to the IL-4R must await further research. The precise mechanism whereby PKC ζ regulates Jak1 needs to be clarified. This will require mapping the PKC ζ phosphorylation sites in Jak1, as well as cell transfection studies to determine the impact that mutation of these putative phosphorylation sites has in the formation of the Jak1/IL-4R α complex.

All these observations would have been of limited significance had *in vivo* data not been reported on the role of PKC ζ in physiological processes in which IL-4 and Th2 cells have been firmly established. One such process is allergic airway inflammation, or asthma. Compelling evidence demonstrated that the pathology of asthma is associated with aberrant activation of CD4 $+$ T cells differentiated along the Th2 lineage.⁴⁹ Therefore, if PKC ζ is relevant for Th2 differentiation, the induction of asthma in a model of ovalbumin-induced allergic airway disease should be impaired in PKC ζ - $-/-$ mice as compared to WT controls. In fact, the loss of PKC ζ inhibits most of the signs of asthma in this model, including the recruitment of eosinophils in the bronchoalveolar fluid, as well as secretion of Th2 cytokines by activated T cells, strongly suggesting that PKC ζ can be considered a therapeutic target in asthma.⁴⁸ However, it has been shown that PKC ζ is required for NF- κ B activation in response to TNF α in pulmonary artery endothelial cells,⁵⁰ a critical parameter in the expression of leukocyte adhesion molecules. Therefore, it could be argued that this function of PKC ζ in lung-resident cells could also contribute to its role during asthma. When Th2 cells from WT mice were adoptively transferred to PKC ζ -deficient mice, it was apparent that the requirement for PKC ζ in asthma was overcome, indicating that it is the function of PKC ζ in Th2 polarization that is critical for allergic airway inflammation, although a potential role of PKC ζ in the lung stroma cannot be ruled out in other pulmonary inflammatory pathologies.⁴⁸ Actually, the evidence that PKC ζ is heavily expressed in lung extracts under resting conditions would be consistent with a putative role of this

kinase in other pulmonary diseases, including chronic obstructive pulmonary disease (COPD) and lung cancer. Future studies should address this important question.

PKC ζ at the Crossroad of Inflammatory Pathways in the Liver

The fact that PKC ζ is able to play roles in two different signaling cascades (Stat6 and NF- κ B) suggests that this kinase may work as a finely tuned switch whose regulation determines the final outcome of a given inflammatory response. This is particularly true in circumstances in which Stat6 and NF- κ B play antagonistic roles. This concept is well exemplified in recent studies in which the signaling cascades activated during T-cell-mediated hepatitis in PKC ζ and Par-4 KO mice were investigated.⁵¹ This disease model is very interesting from the point of view of cell signal transduction as a whole network of cells and signaling molecules are set in motion in response to an immunological insult and, in addition, this serves as an important model system of a worldwide human health problem. This model is induced in response to the injection of concanavalin A (ConA), which leads to T-cell-mediated hepatitis with features of autoimmune hepatitis. This is important because the immune response is central to the pathogenesis of hepatitis whatever the etiology of the disease. As NF- κ B is important in the control of immunity and inflammation, the investigation of the role and function of this pathway in T-cell-mediated hepatitis is the subject of recent important studies. In addition, the liver is a particularly relevant tissue from the point of view of NF- κ B activation, since this transcription factor has been shown genetically to be essential in the control of TNF α -induced apoptosis during embryonic development.¹¹ Actually, both RelA $-/-$ and IKK β $-/-$ mice die of liver apoptosis during gestation. Is NF- κ B also playing a critical role in hepatocyte survival in adult mice subjected to an inflammatory insult? Two recent studies have addressed this important matter using liver-specific IKK β conditional KO mice and two models of inflammatory-mediated liver damage. The first model relates to the action of LPS, which provokes the release of TNF α that, in the absence of NF- κ B, induces lethality as consequence of liver failure. If NF- κ B is an important survival factor in TNF α -induced liver damage as the data from the classical studies of RelA $-/-$ and IKK β $-/-$ mice demonstrate, the prediction is that injection of LPS should induce lethality in the liver-specific conditional IKK β KO mice as compared to the WT controls. Surprisingly, both studies using these mutant mice show that the loss of IKK β in the liver does not sensitize this organ to LPS- or TNF α -induced cell death.^{52,53} However, liver NF- κ B activation is severely inhibited, as is the synthesis of a series of NF- κ B-dependent genes. These results suggest that inhibition of NF- κ B activation is not sufficient to induce hepatocyte death in response to even high doses of circulating TNF α . However, Luedde *et al.*⁵³ show that liver-specific IKK γ (Nemo) conditional KO mice experience massive liver apoptosis when injected with TNF α . These apparently paradoxical results could be explained if it is considered that the genetic inactivation of IKK γ leads to a more complete inactivation of NF- κ B than that observed in

IKK β conditional KO mice. An alternative explanation to these findings is that NF- κ B is important for the prevention of cell death induced by membrane-bound TNF α , which targets both TNF α receptor 1 and 2.⁵² In contrast, circulating TNF α , that is produced in LPS-injected mice, only targets receptor 1 and activates JNK less potently than membrane-bound TNF α . This is produced in ConA-injected mice, and activates JNK more potently than the circulating TNF α and therefore is a more powerful inducer of apoptosis in the absence of NF- κ B. In agreement with this model, Maeda *et al.*⁵² elegantly show that although the liver-specific IKK β conditional mice are resistant to LPS-induced lethality, they readily undergo massive liver apoptosis and lethality when injected with ConA. Therefore, one may predict that NF- κ B will play a beneficial role as a liver protective factor in pathologies such as immune-mediated fulminant hepatic failure. Since PKC ζ is important for NF- κ B activation, it would be of interest to determine whether these mice will be likewise resistant to LPS or circulating TNF α but sensitive to ConA-induced liver damage. Unexpectedly, ConA injection did not induce lethality or liver apoptosis in PKC ζ -deficient mice, despite the fact that NF- κ B activation was ablated in the mutant liver.⁵¹ These results were surprising and could only be explained if PKC ζ , in addition to being critical for ConA-induced NF- κ B, could also be important in a pathway required for liver inflammation and damage provoked by ConA-activated T cells (Figure 2). Interestingly, it has been genetically demonstrated that the IL-4/Stat6 pathway plays an essential role in the synthesis of eotaxin and IL-5, two important mediators of eosinophil recruitment and induction of liver damage.⁵⁴ In this regard, it is clear that the loss of PKC ζ severely inhibited the synthesis of IL-5 and eotaxin-1 in ConA-injected mice, as well as in primary mouse embryo fibroblasts challenged *in vitro* with IL-4, in agreement with the role of PKC ζ in Th2 polarization.⁵¹ Both mediators are essential players in the regulation of T-cell mediated hepatitis at the level of hepatocytes and NKT cells (Figure 3). This requirement of PKC ζ for the induction of these inflammatory mediators very well correlated with inhibited tyrosine phosphorylation of Jak1 and Stat6. However, when these pathways were analyzed in mice and cells deficient in the PKC ζ negative regulator, Par-4, ConA-induced liver injury was enhanced and the activation of the Stat6 pathway was more prominent than in WT mice.⁵¹ Therefore, activation of

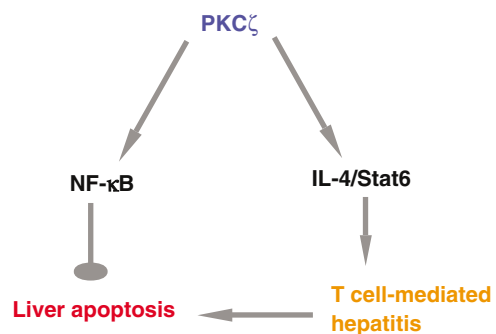


Figure 2 Potential involvement of PKC ζ in pathways other than NF- κ B in ConA-induced hepatitis. Liver apoptosis is positively promoted by the IL-4/Stat6 pathway and antagonized by NF- κ B

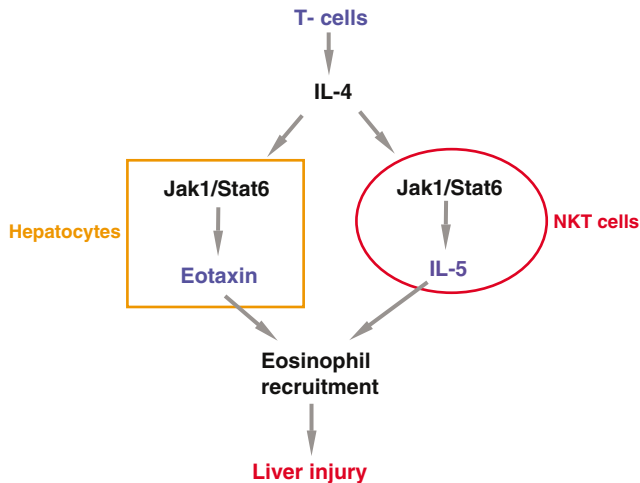


Figure 3 Mechanism of liver injury activated by IL-4. IL-4 activated Jak1/Stat6 in hepatocytes to produce eotaxin, whereas triggers IL-5 production by natural killer T cells (NKT). Both mediators promote liver eosinophil recruitment and damage

PKC ζ is required for ConA-induced hepatitis due to its role in the activation of Jak1, and the fact that NF- κ B is not activated is irrelevant under these conditions because liver damage does not occur. However, there are conditions at which PKC ζ becomes redundant for the activation of the IL4/Stat6 pathway in liver, such as, for example, when mice were challenged with high doses of ConA. Under these circumstances, Stat6 is activated in the absence of PKC ζ and the KO mice display more, not less, liver injury than the WT mice because NF- κ B activation is still severely reduced in the PKC ζ -deficient liver, but the Stat6 pathway is fully functional.⁵¹ Therefore, PKC ζ emerges as a critical control step that determines the final outcome of a biological complex process such as liver inflammation, thanks to its ability to control what in the livers are two antagonistic signaling pathways, and depending on a number of conditions such as the intensity of the signal. These observations reinforce the concept that considerable crosstalk must be established when cells 'have to address' intricate pathophysiological situations such as inflammation, in which a whole collection of signaling networks is set in motion.

Adapters and the Specificity During Cell Signaling

The PKCs are kinases that display little specificity *in vitro* and presumably *in vivo*, unless cells have mechanisms that impose specificity to the kinases' actions. As the aPKCs have been implicated in a relatively diverse series of functions, one hypothesis is that different adapter proteins that would serve to provide the required selectivity must exist.⁷ In other words, the aPKCs would be the 'catalytic subunit' of different signaling complexes designed to perform different functions. Two-hybrid screenings in yeast using the regulatory domain of PKC ζ as the bait identified p62 as a selective adaptor for the aPKCs.^{55,56} p62 interacts with PKC ζ and PKC $\lambda/1$, but not with

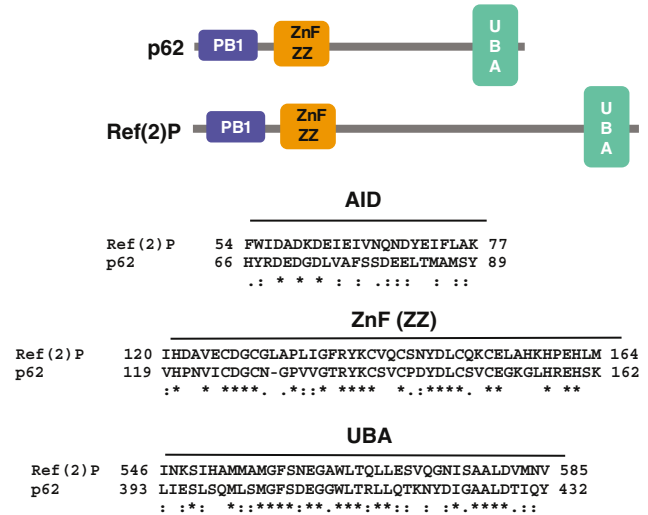


Figure 4 Sequence alignment of the different signaling modules of p62 and Ref(2)P. Overall structures of p62 and its *Drosophila* homolog Ref(2)P are shown. Also, the sequences of the AID region in the PB1 domains, the zinc-finger and the UBA domain alignments are shown. *, identical residues; ., conserved residue; :, highly conserved residues

any of the other closely related PKC family members. It is not a substrate and does not seem to significantly affect the intrinsic kinase activity of PKC ζ or of PKC $\lambda/1$. However, p62 has a number of domains that strongly suggested its role as a novel scaffold protein.⁵⁶ Interestingly, p62 was shown to be required for NF- κ B signaling in several systems^{57–59} including *Drosophila*, in which a functionally relevant homolog termed Ref(2)P was identified.³⁰ This protein has an overall structure very similar to that of p62 (Figure 4), suggesting that it may be a critical mediator of NF- κ B in *Drosophila* cells in partnership with PKC ζ . In fact, the knockdown of Ref(2)P with RNAi in *Drosophila* cells leads to the impairment of Drosomycin expression.^{30,31} Therefore, this indicates the remarkable state of conservation for the role of PKC ζ in the control of the transcriptional activity of NF- κ B downstream of I κ B degradation, which reinforces the importance of this kinase in the regulation of NF- κ B and the innate immune response.

Several studies demonstrated that p62 harbors at its N-terminal sequence a domain, named PB1,⁶⁰ whose crystal structure has been resolved and that accounts for the interaction with PKC ζ , which also has an N-terminal PB1 region.^{61–64} Therefore, the mechanism whereby PKC ζ binds p62 involves homotypic interactions between their respective PB1 regions. Solution structure and crystallographic and mutational analysis has revealed at the atomic level the interactions that govern the binding of the aPKCs to p62. More importantly, PB1 domains have also been identified in other scaffold proteins such as partitioning defective-6 (Par-6) and MAPK/ERK kinase (MEK)5.^{7,65} Par-6 has been shown genetically in *Caenorhabditis elegans* and *Drosophila* to be critically implicated in the control of cell polarity.^{66,67} Although genetic data have not yet been produced to prove the role of the aPKCs and Par-6 in different aspects of mammalian cell polarity, overexpression analysis indicate that the Par-6/aPKC complex can be implicated in, for example, the control

of epithelial–mesenchymal transition,⁶⁸ T-cell⁶⁹ and neuronal polarity,⁷⁰ or cell polarity in migrating astrocytes⁷¹ among others. On the other hand, the interaction of MEK5 with the aPKCs serves to regulate extracellular responsive kinase (ERK)5, which leads to c-Jun regulation and cell growth in response to certain mitogens such as epidermal growth factor (EGF).⁶⁵ Therefore, the formation of aPKC complexes with different adapters and scaffold proteins serves to confer specificity and plasticity to these kinase actions.

The mechanistic link between p62 and NF- κ B received further support when it was shown that TNF receptor-associated factor (TRAF)6 interacts with p62.⁵⁸ This is particularly relevant because TRAF6 is an important intermediary in the IL-1, NGF, and LPS signaling pathways controlling NF- κ B through still not totally clarified mechanisms likely involving K-63 ubiquitination of IKK γ .⁷² Interestingly, Ref(2)P interacts not only with *Drosophila* PKC ζ but also with the fly homolog of TRAF6, dTRAF2, reinforcing again the conservation of this pathway in flies.³⁰ In mammalian cells p62 also interacts with receptor-interacting protein (RIP), which mediates NF- κ B activation in response to TNF α .⁵⁷ Both interactions seem to be physiologically relevant, since down-regulation of p62 levels with antisense constructs leads to a significant reduction of NF- κ B activation in IL-1- or TNF α -activated cells. The generation of p62-deficient mice provided a unique opportunity to address the actual role of p62 *in vivo*. Interestingly, TRAF6 $-/-$ mice are osteopetrotic due to the critical role that TRAF6 plays in the control of osteoclastogenesis in response to RANK-L,^{73,74} which has also been shown genetically to be required for osteoclast differentiation *in vitro* and *in vivo*.⁷⁵ Interestingly, mutations in the gene coding for p62 are the cause of the Paget disease of bone (PDB), a genetic disorder characterized by aberrant osteoclastogenic activity.⁷⁶ Although the p62-deficient mice are not osteopetrotic, they show a clear impairment in osteoclastogenesis *in vivo* when injected with the calciotropic hormone PTHrP.⁷⁷ This suggests that although p62 may not be essential in basal bones for the control of osteoclastogenesis and bone remodeling, under induced conditions it may be important. In fact, p62-deficient osteoclast precursors respond poorly to RANK-L in cell cultures and are unable to produce a sustained NF- κ B response.⁷⁷ Therefore, p62 emerges as a critical cell-type-specific NF- κ B regulator in osteoclasts and a potential novel therapeutic target in osteoporosis and other bone diseases. As the PKC ζ mice does not seem to have any alterations in osteoclastogenesis,⁷⁷ it is likely that the other aPKC, PKC $\lambda/1$, is the essential aPKC component in this pathway although other possible mediators cannot be ruled out. In this regard, p62 harbors an ubiquitin associated (UBA) domain at its C-terminal region, which suggests its potential involvement in ubiquitin-mediated events, like, for example, the activation of the IKK complex. Ubiquitination of proteins of the NF- κ B pathway in a K63-type fashion serves to assemble signaling complexes not linked to proteasomal degradation, whereas ubiquitination with K48-linked chains leads to proteasome degradation and inactivation of signaling cascades.^{78,79} Recent data suggest that p62 preferably binds to K63-linked ubiquitinated proteins, which may open the possibility that p62 participates in the activation of the IKK complex through mechanisms other than, or in addition to,

those mediated by the aPKCs.⁸⁰ Future studies will address this important question. In any case, these observations highlight the importance of specificity during cell signaling through the existence of specific adapters that restrict the kinase's action, but at the same time allows enough crosstalk between pathways, necessary to regulate complex biological situations such as inflammation. If p62 were also implicated in the IL-4/Jak-1 pathway, an important question to be solved would be the precise mechanism whereby two completely different ligands, such as IL-4 and TNF α , can use the same PKC ζ cassette to perform their functions. This will require more biochemical and cell biology studies to investigate the cell kinetic and topology of the formation of the different signaling pathways when activated by the distinct stimuli.

Acknowledgements

We apologize for any contributions that could not be cited because of strict space limitations. JM is recipient of the Ayuda Investigación Juan March 2001. The Ramon Areces Foundation supports the Centro de Biología Molecular Severo Ochoa with an institutional grant.

References

1. Moscat J and Diaz-Meco MT (2002) The atypical PKC scaffold protein P62 is a novel target for anti-inflammatory and anti-cancer therapies. *Adv. Enzyme Regul.* 42: 173–179
2. Mellor H and Parker PJ (1998) The extended protein kinase C superfamily. *Biochem. J.* 332: 281–292
3. Moscat J, Diaz-Meco MT and Rennert P (2003) NF-kappaB activation by protein kinase C isoforms and B-cell function. *EMBO Rep.* 4: 31–36
4. Leitges M, Schmedt C, Guinamard R, Davoust J, Schaal S, Stabel S and Tarakhovskiy A (1996) Immunodeficiency in protein kinase cbeta-deficient mice. *Science* 273: 788–791
5. Sun Z, Arendt CW, Ellmeier W, Schaeffer EM, Sunshine MJ, Gandhi L, Annes J, Petrzilka D, Kupfer A, Schwartzberg PL and Littman DR (2000) PKC-theta is required for TCR-induced NF-kappaB activation in mature but not immature T lymphocytes. *Nature* 404: 402–407
6. Mellor H, Flynn P, Nobes CD, Hall A and Parker PJ (1998) PRK1 is targeted to endosomes by the small GTPase RhoB. *J. Biol. Chem.* 273: 4811–4814
7. Moscat J and Diaz-Meco MT (2000) The atypical protein kinase Cs. Functional specificity mediated by specific protein adapters. *EMBO Rep.* 1: 399–403
8. Leitges M, Sanz L, Martin P, Duran A, Braun U, Garcia JF, Camacho F, Diaz-Meco MT, Rennert PD and Moscat J (2001) Targeted disruption of the zetaPKC gene results in the impairment of the NF-kappaB pathway. *Mol. Cell* 8: 771–780
9. Berra E, Diaz-Meco MT, Dominguez I, Muncio MM, Sanz L, Lozano J, Chapkin RS and Moscat J (1993) Protein kinase C zeta isoform is critical for mitogenic signal transduction. *Cell* 74: 555–563
10. Diaz-Meco MT, Dominguez I, Sanz L, Dent P, Lozano J, Muncio MM, Berra E, Hay RT, Sturgill TW and Moscat J (1994) zeta PKC induces phosphorylation and inactivation of I kappa B-alpha *in vitro*. *EMBO J.* 13: 2842–2848
11. Ghosh S and Karin M (2002) Missing pieces in the NF-kappaB puzzle. *Cell* 109 (Suppl): S81–S96
12. Diaz-Meco MT, Muncio MM, Frutos S, Sanchez P, Lozano J, Sanz L and Moscat J (1996) The product of par-4, a gene induced during apoptosis, interacts selectively with the atypical isoforms of protein kinase C. *Cell* 86: 777–786
13. Sells SF, Wood Jr DP, Joshi-Barve SS, Muthukumar S, Jacob RJ, Crist SA, Humphreys S and Rangnekar VM (1994) Commonality of the gene programs induced by effectors of apoptosis in androgen-dependent and -independent prostate cells. *Cell Growth Differ.* 5: 457–466
14. Diaz-Meco MT, Lallena MJ, Monjas A, Frutos S and Moscat J (1999) Inactivation of the inhibitory kappaB protein kinase/nuclear factor kappaB

- pathway by Par-4 expression potentiates tumor necrosis factor alpha-induced apoptosis. *J. Biol. Chem.* 274: 19606–19612
15. Garcia-Cao I, Lafuente M, Criado L, Diaz-Meco M, Serrano M and Moscat J (2003) Genetic inactivation of Par4 results in hyperactivation of NF- κ B and impairment of JNK and p38. *EMBO Rep.* 4: 307–312
 16. Tang G, Minemoto Y, Dibling B, Purcell NH, Li Z, Karin M and Lin A (2001) Inhibition of JNK activation through NF- κ B target genes. *Nature* 414: 313–317
 17. Lafuente MJ, Martin P, Garcia-Cao I, Diaz-Meco MT, Serrano M and Moscat J (2003) Regulation of mature T lymphocyte proliferation and differentiation by Par-4. *EMBO J.* 22: 4689–4698
 18. Barradas M, Monjas A, Diaz-Meco MT, Serrano M and Moscat J (1999) The downregulation of the pro-apoptotic protein Par-4 is critical for Ras-induced survival and tumor progression. *EMBO J.* 18: 6362–6369
 19. Garcia-Cao I, Duran A, Collado M, Carrascosa MJ, Martin-Caballero J, Flores JM, Diaz-Meco MT, Moscat J and Serrano M (2005) Tumour-suppression activity of the proapoptotic regulator Par4. *EMBO Rep.* 6: 577–583
 20. Eder AM, Sui X, Rosen DG, Nolden LK, Cheng KW, Lahad JP, Kango-Singh M, Lu KH, Warneke CL, Atkinson EN, Bedrosian I, Keyomarsi K, Kuo WL, Gray JW, Yin JC, Liu J, Halder G and Mills GB (2005) Atypical PKC ζ 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
 21. Zhong H, May MJ, Jimi E and Ghosh S (2002) The phosphorylation status of nuclear NF- κ B determines its association with CBP/p300 or HDAC-1. *Mol. Cell* 9: 625–636
 22. Anrather J, Csizmadia V, Soares MP and Winkler H (1999) Regulation of NF- κ B RelA phosphorylation and transcriptional activity by p21(ras) and protein kinase C ζ in primary endothelial cells. *J. Biol. Chem.* 274: 13594–13603
 23. Vermeulen L, De Wilde G, Damme PV, Vanden Berghe W and Haegeman G (2003) Transcriptional activation of the NF- κ B p65 subunit by mitogen- and stress-activated protein kinase-1 (MSK1). *EMBO J.* 22: 1313–1324
 24. Lawrence T, Bebiec M, Liu GY, Nizet V and Karin M (2005) IKK α limits macrophage NF- κ B activation and contributes to the resolution of inflammation. *Nature* 434: 1138–1143
 25. Duran A, Diaz-Meco MT and Moscat J (2003) Essential role of RelA Ser311 phosphorylation by zetaPKC in NF- κ B transcriptional activation. *EMBO J.* 22: 3910–3918
 26. Zhong H, SuYang H, Erdjument-Bromage H, Tempst P and Ghosh S (1997) The transcriptional activity of NF- κ B is regulated by the IkappaB-associated PKAc subunit through a cyclic AMP-independent mechanism. *Cell* 89: 413–424
 27. Mayr B and Montminy M (2001) Transcriptional regulation by the phosphorylation-dependent factor CREB. *Nat. Rev. Mol. Cell Biol.* 2: 599–609
 28. Radhakrishnan I, Perez-Alvarado GC, Parker D, Dyson HJ, Montminy MR and Wright PE (1997) Solution structure of the KIX domain of CBP bound to the transactivation domain of CREB: a model for activator:coactivator interactions. *Cell* 91: 741–752
 29. Hoffmann JA (2003) The immune response of *Drosophila*. *Nature* 426: 33–38
 30. Avila A, Silverman N, Diaz-Meco MT and Moscat J (2002) The *Drosophila* atypical protein kinase C-ref(2)p complex constitutes a conserved module for signaling in the toll pathway. *Mol. Cell. Biol.* 22: 8787–8795
 31. Goto A, Blandin S, Royet J, Reichhart JM and Levashina EA (2003) Silencing of Toll pathway components by direct injection of double-stranded RNA into *Drosophila* adult flies. *Nucleic Acids Res.* 31: 6619–6623
 32. Bonizzi G and Karin M (2004) The two NF- κ B activation pathways and their role in innate and adaptive immunity. *Trends Immunol.* 25: 280–288
 33. Caamano JH, Rizzo CA, Durham SK, Barton DS, Raventos-Suarez C, Snapper CM and Bravo R (1998) Nuclear factor (NF)- κ B2 (p100/p52) is required for normal splenic microarchitecture and B cell-mediated immune responses. *J. Exp. Med.* 187: 185–196
 34. Cariappa A, Liou HC, Horwitz BH and Pillai S (2000) Nuclear factor kappa B is required for the development of marginal zone B lymphocytes. *J. Exp. Med.* 192: 1175–1182
 35. Locksley RM, Killeen N and Lenardo MJ (2001) The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell* 104: 487–501
 36. Pasparakis M, Alexopoulou L, Grell M, Pfizenmaier K, Bluethmann H and Kollias G (1997) Peyer's patch organogenesis is intact yet formation of B lymphocyte follicles is defective in peripheral lymphoid organs of mice deficient for tumor necrosis factor and its 55-kDa receptor. *Proc. Natl. Acad. Sci. USA* 94: 6319–6323
 37. Alcamo E, Hacohen N, Schulte LC, Rennert PD, Hynes RO and Baltimore D (2002) Requirement for the NF- κ B family member RelA in the development of secondary lymphoid organs. *J. Exp. Med.* 195: 233–244
 38. Martin P, Duran A, Minguet S, Gaspar ML, Diaz-Meco MT, Rennert P, Leitges M and Moscat J (2002) Role of zeta PKC in B-cell signaling and function. *EMBO J.* 21: 4049–4057
 39. Mosmann TR and Coffman RL (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol.* 7: 145–173
 40. Shuai K and Liu B (2003) Regulation of JAK-STAT signalling in the immune system. *Nat. Rev. Immunol.* 3: 900–911
 41. Bix M, Kim S and Rao A (2005) Immunology. Opposites attract in differentiating T cells. *Science* 308: 1563–1565
 42. Murphy KM and Reiner SL (2002) The lineage decisions of helper T cells. *Nat. Rev. Immunol.* 2: 933–944
 43. Salek-Ardakani S, So T, Halteman BS, Altman A and Croft M (2004) Differential regulation of Th2 and Th1 lung inflammatory responses by protein kinase C theta. *J. Immunol.* 173: 6440–6447
 44. Cannons JL, Yu LJ, Hill B, Mijares LA, Dombroski D, Nichols KE, Antonellis A, Koretzky GA, Gardner K and Schwartzberg PL (2004) SAP regulates T(H)2 differentiation and PKC-theta-mediated activation of NF- κ B1. *Immunity* 21: 693–706
 45. Marsland BJ, Soos TJ, Spath G, Littman DR and Kopf M (2004) Protein kinase C theta is critical for the development of *in vivo* T helper (Th)2 cell but not Th1 cell responses. *J. Exp. Med.* 200: 181–189
 46. Thome M, Thome M and Tschopp J (2004) CARMA1, BCL-10 and MALT1 in lymphocyte development and activation TCR-induced NF- κ B activation: a crucial role for Carma1, Bcl10 and MALT1. *Nat. Rev. Immunol.* 4: 348–359
 47. Huang Y and Wange RL (2004) T cell receptor signaling: beyond complex complexes. *J. Biol. Chem.* 279: 28827–28830
 48. Martin P, Villares R, Rodriguez-Mascarenhas S, Zaballos A, Leitges M, Kovac J, Sizing I, Rennert P, Marquez G, Martinez AC, Diaz-Meco MT and Moscat J (2005) Control of T helper 2 cell function and allergic airway inflammation by PKC ζ . *Proc. Natl. Acad. Sci. USA* 102: 9866–9871
 49. Elias JA, Lee CG, Zheng T, Ma B, Homer RJ and Zhu Z (2003) New insights into the pathogenesis of asthma. *J. Clin. Invest.* 111: 291–297
 50. Javaid K, Rahman A, Anwar KN, Frey RS, Minshall RD and Malik AB (2003) Tumor necrosis factor-alpha induces early-onset endothelial adhesivity by protein kinase C ζ -dependent activation of intercellular adhesion molecule-1. *Circ. Res.* 92: 1089–1097
 51. Duran A, Rodriguez A, Martin P, Serrano M, Flores JM, Leitges M, Diaz-Meco MT and Moscat J (2004) Crosstalk between PKCzeta and the IL4/Stat6 pathway during T-cell-mediated hepatitis. *EMBO J.* 23: 4595–4605
 52. Maeda S, Chang L, Li ZW, Luo JL, Leffert H and Karin M (2003) IKKbeta is required for prevention of apoptosis mediated by cell-bound but not by circulating TNFalpha. *Immunity.* 19: 725–737
 53. Luedde T, Assmus U, Wustefeld T, Meyer zu Vilsendorf A, Roskams T, Schmidt-Supprian M, Rajewsky K, Brenner DA, Manns MP, Pasparakis M and Trautwein C (2005) Deletion of IKK2 in hepatocytes does not sensitize these cells to TNF-induced apoptosis but protects from ischemia/reperfusion injury. *J. Clin. Invest.* 115: 849–859
 54. Jaruga B, Hong F, Sun R, Radaeva S and Gao B (2003) Crucial role of IL-4/STAT6 in T cell-mediated hepatitis: up-regulating eotaxins and IL-5 and recruiting leukocytes. *J. Immunol.* 171: 3233–3244
 55. Puls A, Schmidt S, Grawe F and Stabel S (1997) Interaction of protein kinase C zeta with ZIP, a novel protein kinase C-binding protein. *Proc. Natl. Acad. Sci. USA* 94: 6191–6196
 56. Sanchez P, De Carcer G, Sandoval IV, Moscat J and Diaz-Meco MT (1998) Localization of atypical protein kinase C isoforms into lysosome-targeted endosomes through interaction with p62. *Mol. Cell. Biol.* 18: 3069–3080
 57. Sanz L, Sanchez P, Lallena MJ, Diaz-Meco MT and Moscat J (1999) The interaction of p62 with RIP links the atypical PKCs to NF- κ B activation. *EMBO J.* 18: 3044–3053
 58. Sanz L, Diaz-Meco MT, Nakano H and Moscat J (2000) The atypical PKC-interacting protein p62 channels NF- κ B activation by the IL-1-TRAF6 pathway. *EMBO J.* 19: 1576–1586

59. Wooten MW, Seibenhener ML, Mamidipudi V, Diaz-Meco MT, Barker PA and Moscat J (2001) The atypical protein kinase C-interacting protein p62 is a scaffold for NF-kappaB activation by nerve growth factor. *J. Biol. Chem.* 276: 7709–7712
60. Ponting CP, Ito T, Moscat J, Diaz-Meco MT, Inagaki F and Sumimoto H (2002) OPR, PC and AID: all in the PB1 family. *Trends Biochem. Sci.* 27: 10
61. Hirano Y, Yoshinaga S, Ogura K, Yokochi M, Noda Y, Sumimoto H and Inagaki F (2004) Solution structure of atypical protein kinase C PB1 domain and its mode of interaction with ZIP/p62 and MEK5. *J. Biol. Chem.* 279: 31883–31890
62. Wilson MI, Gill DJ, Perisic O, Quinn MT and Williams RL (2003) PB1 domain-mediated heterodimerization in NADPH oxidase and signaling complexes of atypical protein kinase C with Par6 and p62. *Mol. Cell* 12: 39–50
63. Ito T, Matsui Y, Ago T, Ota K and Sumimoto H (2001) Novel modular domain PB1 recognizes PC motif to mediate functional protein–protein interactions. *EMBO J.* 20: 3938–3946
64. Terasawa H, Noda Y, Ito T, Hatanaka H, Ichikawa S, Ogura K, Sumimoto H and Inagaki F (2001) Structure and ligand recognition of the PB1 domain: a novel protein module binding to the PC motif. *EMBO J.* 20: 3947–3956
65. Diaz-Meco MT and Moscat J (2001) MEK5, a new target of the atypical protein kinase C isoforms in mitogenic signaling. *Mol. Cell. Biol.* 21: 1218–1227
66. Macara IG (2004) Par proteins: partners in polarization. *Curr. Biol.* 14: R160–R162
67. Ohno S (2001) Intercellular junctions and cellular polarity: the PAR–aPKC complex, a conserved core cassette playing fundamental roles in cell polarity. *Curr. Opin. Cell Biol.* 13: 641–648
68. Ozdamar B, Bose R, Barrios-Rodiles M, Wang HR, Zhang Y and Wrana JL (2005) Regulation of the polarity protein Par6 by TGFbeta receptors controls epithelial cell plasticity. *Science* 307: 1603–1609
69. Ludford-Menting MJ, Oliaro J, Sacirbegovic F, Cheah ET, Pedersen N, Thomas SJ, Pasam A, Lazzolino R, Dow LE, Waterhouse NJ, Murphy A, Ellis S, Smyth MJ, Kershaw MH, Darcy PK, Humbert PO and Russell SM (2005) A network of PDZ-containing proteins regulates T cell polarity and morphology during migration and immunological synapse formation. *Immunity* 22: 737–748
70. Shi SH, Jan LY and Jan YN (2003) Hippocampal neuronal polarity specified by spatially localized mPar3/mPar6 and PI 3-kinase activity. *Cell* 112: 63–75
71. Etienne-Manneville S and Hall A (2003) Cell polarity: Par6, aPKC and cytoskeletal crosstalk. *Curr. Opin. Cell Biol.* 15: 67–72
72. Sun L, Deng L, Ea CK, Xia ZP and Chen ZJ (2004) The TRAF6 ubiquitin ligase and TAK1 kinase mediate IKK activation by BCL10 and MALT1 in T lymphocytes. *Mol. Cell* 14: 289–301
73. Kobayashi N, Kadono Y, Naito A, Matsumoto K, Yamamoto T, Tanaka S and Inoue J (2001) Segregation of TRAF6-mediated signaling pathways clarifies its role in osteoclastogenesis. *EMBO J.* 20: 1271–1280
74. Lomaga MA, Yeh WC, Sarosi I, Duncan GS, Furlonger C, Ho A, Morony S, Capparelli C, Van G, Kaufman S, van der Heiden A, Itie A, Wakeham A, Khoo W, Sasaki T, Cao Z, Penninger JM, Paige CJ, Lacey DL, Dunstan CR, Boyle WJ, Goeddel DV and Mak TW (1999) TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. *Genes Dev.* 13: 1015–1024
75. Theill LE, Boyle WJ and Penninger JM (2002) RANK-L and RANK: T cells, bone loss, and mammalian evolution. *Annu. Rev. Immunol.* 20: 795–823
76. Laurin N, Brown JP, Morissette J and Raymond V (2002) Recurrent mutation of the gene encoding sequestosome 1 (SQSTM1/p62) in Paget disease of bone. *Am. J. Hum. Genet.* 70: 1582–1588
77. Duran A, Serrano M, Leitges M, Flores JM, Picard S, Brown JP, Moscat J and Diaz-Meco MT (2004) The atypical PKC-interacting protein p62 is an important mediator of RANK-activated osteoclastogenesis. *Dev. Cell* 6: 303–309
78. Xia ZP and Chen ZJ (2005) TRAF2: a double-edged sword? *Sci. STKE* 2005: pe7
79. Zhou H, Wertz I, O'Rourke K, Ultsch M, Seshagiri S, Eby M, Xiao W and Dixit VM (2004) Bcl10 activates the NF-kappaB pathway through ubiquitination of NEMO. *Nature* 427: 167–171
80. Seibenhener ML, Babu JR, Geetha T, Wong HC, Krishna NR and Wooten MW (2004) Sequestosome 1/p62 is a polyubiquitin chain binding protein involved in ubiquitin proteasome degradation. *Mol. Cell. Biol.* 24: 8055–8068