Lipid signalling in disease

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Abstract | Signalling lipids such as eicosanoids, phosphoinositides, sphingolipids and fatty acids control important cellular processes, including cell proliferation, apoptosis, metabolism and migration. Extracellular signals from cytokines, growth factors and nutrients control the activity of a key set of lipid-modifying enzymes: phospholipases, prostaglandin synthase, 5-lipoxygenase, phosphoinositide 3-kinase, sphingosine kinase and sphingomyelinase. These enzymes and their downstream targets constitute a complex lipid signalling network with multiple nodes of interaction and cross-regulation. Imbalances in this network contribute to the pathogenesis of human disease. Although the function of a particular signalling lipid is traditionally studied in isolation, this review attempts a more integrated overview of the key role of these signalling lipids in inflammation, cancer and metabolic disease, and discusses emerging strategies for therapeutic intervention.

Lipids have long since been recognized as signalling molecules that have the capacity to trigger profound physiological responses. Slow-reacting substance of anaphylaxis (SRS-A), now known to belong to the group of cysteinyl leukotrienes, was identified as early as 1930 (REF. 1); at about the same time, the vasodilating actions of prostaglandins were described[2]. Subsequently, arachidonic acid was identified as the source of leukotrienes and prostaglandins (BOX 1), a discovery that has led to our current understanding of eicosanoid signalling[3], and taught us that one and the same signalling lipid can provoke different cellular responses, depending on the precise cell type and underlying signalling network present in the target cell.
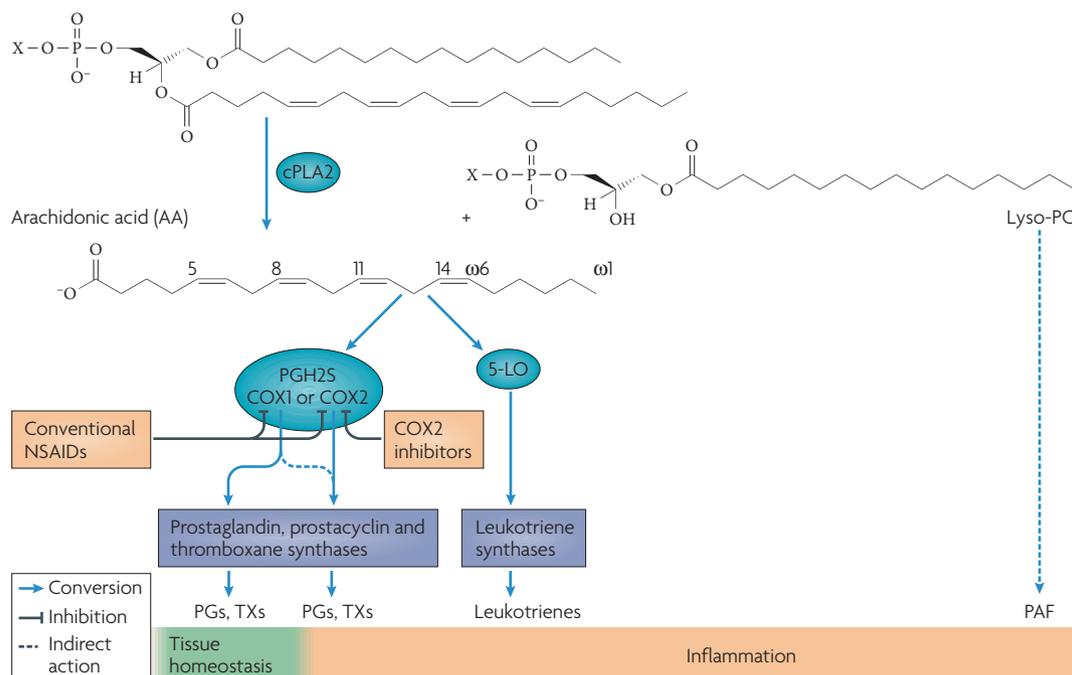
The study of the lipid components of cellular membranes, and in particular the turnover of phosphoinositides, culminated in the 1980s with the discovery that phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P$_2$) could be hydrolysed by phospholipase C (PLC) to diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (Ins(1,4,5)P$_3$). Both products were identified as second messengers that trigger the activation of protein kinase C (PKC; REF. 4) and the release of Ca^{2+} from internal stores, respectively. In parallel, inositol and phosphoinositide kinases have been identified; these enzymes generate a huge repertoire of soluble inositol polyphosphates and membrane polyphosphoinositide lipids. Phosphoinositide 3-kinase (PI3K), for example, which is regulated by cell-surface receptors, promotes the formation of phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P$_3$), a signalling lipid that modulates cell growth, proliferation and motility[5,6]. Interestingly,

nature has used several possible variations of the inositol head group to create highly specific docking sites for lipid-binding proteins — effector proteins that trigger signalling cascades or enzymes that further modify these lipids (BOX 2; see the review by Lemmon in this issue). Phosphoinositides have, therefore, gained importance as localization tags that are involved in the organization of membrane-bound signalling complexes[7].

Ceramides and sphingosines are lipids that have pro-apoptotic and antiproliferative actions[8]; when phosphorylated by lipid kinases, however, sphingosine is converted into sphingosine-1-phosphate (S1P), which promotes cell growth and proliferation by a still poorly understood intracellular action as well as by extracellular signalling through a set of different G protein-coupled receptors (GPCRs) [9–11] (BOX 3; see the review by Hannun and Obeid in this issue). Another lipid that is released from cells, lysophosphatidic acid (LPA), also functions in an autocrine and paracrine manner by binding to a family of GPCRs[12,13]. LPA, together with specific eicosanoids, can additionally modulate gene expression by binding to lipid-sensing transcription activators, such as the peroxisome proliferator-activated receptors (PPARs)[14,15] (BOX 4).

Imbalances of these major lipid signalling pathways contribute to disease progression in chronic inflammation, autoimmunity, allergy, cancer, atherosclerosis, hypertension, heart hypertrophy, metabolic and degenerative diseases, among others (FIG. 1). Owing to their complexity, these pathways are mostly studied and reviewed in isolation. However, many of these signalling lipids, their modifying enzymes and downstream targets

Box 1 | **From phospholipids to eicosanoid signalling**

The production of eicosanoids is initiated by the release of C20-polyunsaturated fatty acids, such as arachidonic acid (AA, C20:4), from phospholipids (X stands for a phospholipid headgroup; see figure) or diacylglycerol (not shown; see BOX 2). This hydrolysis is catalysed by cytosolic phospholipase A2 (cPLA2). Ca^{2+}-induced cPLA2 lipase action is the rate-limiting step in eicosanoid formation, and cells lacking cPLA2 are generally devoid of eicosanoids. Liberated fatty acids are then stereospecifically oxygenated either through the cyclic prostaglandin synthase pathway (prostaglandin H2 synthase (PGH2S), including cyclooxygenase (COX) activity) or through the linear lipoxygenase-dependent pathway (5-lipoxygenase; 5-LO), and are thus converted into one of four families of eicosanoids: the prostaglandins (PGs), prostacyclins, thromboxanes (TXs) and leukotrienes.

Eicosanoids have a short half-life, ranging from seconds to minutes. Their prime mode of action is mediated by binding to specific G protein-coupled receptors (GPCRs), many of which have been identified recently, enabling the rational development of specific receptor agonists and antagonists (see the accompanying Poster entitled 'Targeting lipid signalling in disease'). Eicosanoids can also bind to nuclear receptors. Eicosanoid signalling pathways are complex and are generally implicated in tissue homeostasis and/or inflammation. Prostanoids that are derived from ω−6 unsaturated fatty acids, such as AA, tend to give rise to the production of inflammatory mediators, whereas ω−3 unsaturated fatty acids are a source for anti-inflammatory and homeostatic prostanoids.

In addition, the lysolipids that are produced by the action of cPLA2 might themselves exert signalling functions and can be further processed: for example, when lysophosphatidylcholine (lyso-PC) is converted to platelet-activating factor (PAF, alkyl-acetyl phosphatidylcholine), which also supports inflammation. Anti-inflammatory drugs such as aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) function by blocking the cyclooxygenase-catalysed synthesis of prostanoids (prostacyclins, PGs and TXs)[137,138].

are common to multiple lipid signalling pathways, resulting in the formation of highly interconnected lipid signalling networks. As a consequence, many lipid signalling pathways are deregulated in different disease conditions. This review attempts to integrate the role of lipid signalling networks in inflammation, cancer and metabolic syndrome. Owing to the focus on integration, an in-depth discussion of each individual lipid signalling pathway is beyond the scope of this review. We will also provide a brief overview of strategies that are currently being used to pharmacologically manipulate these lipid signalling networks. The commonality of various different signalling components means that different diseases share common points of intervention — this has important consequences for therapy, as will be discussed

(see also the accompanying Poster entitled 'Targeting lipid signalling in disease'; URL in the box at the end of the article).
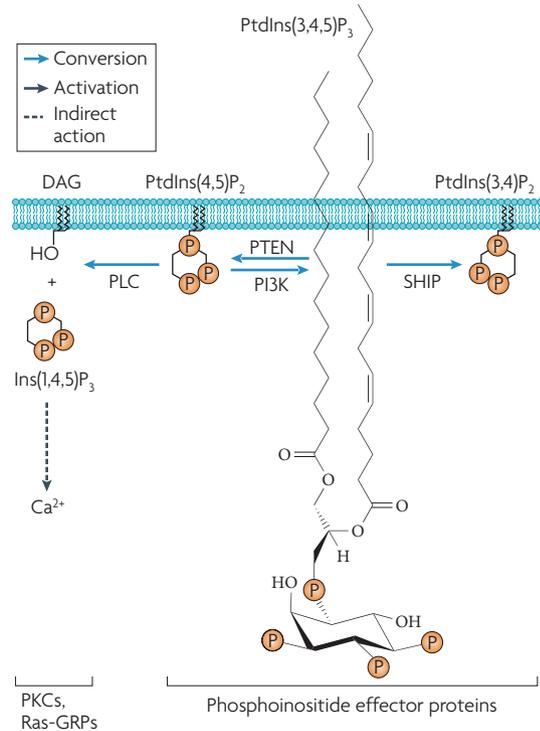
Lipid signalling in inflammation

Modulation of the levels and/or behaviour of lipids that are involved in promoting inflammation has been practised for centuries. Early records indicate that a brew of willow tree leaves was used by Hippocrates around 400 BC to relieve pain and fever. Many centuries later, this led from the clinical use of aspirin (synthesized by Felix Hoffmann at Bayer in 1897) to the development of modern non-steroidal anti-inflammatory drugs (NSAIDs) and highly selective cyclooxygenase-2 (COX2) inhibitors.

Box 2 | **Intracellular signalling by phosphoinositides**

The biological versatility of phosphatidylinositol (PtdIns) derives from its unique ability to be reversibly phosphorylated at three distinct positions of the inositol headgroup. Single or combinatorial phosphorylation of the 3, 4 and 5 positions on the inositol ring of PtdIns can generate at least seven unique phosphoinositides, which have diverse roles in receptor-mediated signal transduction, cytoskeletal remodelling, nuclear events and membrane trafficking. Lipid kinases, phosphatases and lipases that produce and degrade these signalling lipids spatially and temporally control the biological activity of phosphoinositides. Phosphoinositides signal through cytosolic effector proteins that have phosphoinositide-binding domains, which are frequently combined with additional modules that are involved in the formation of complex protein–protein and protein–lipid interaction networks[7] (see the review by Lemmon in this issue).

The plasma-membrane-localized phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2) represents a focal point in phosphoinositide-dependent signalling because it serves as the substrate for two powerful receptor-regulated signal-generating enzymes (see figure). Cleavage of PtdIns(4,5)P_2 by phosphoinositide-specific phospholipase C (PLC) produces two second messengers, membrane-associated diacylglycerol (DAG) and the soluble inositol-1,4,5-trisphosphate (Ins(1,4,5)P_3) (see the review by Michell in this issue). Protein kinase C (PKC) docks through its C1 domain onto DAG, whereas Ins(1,4,5)P_3 stimulates the release of Ca^{2+} ions from the endoplasmic reticulum (ER).

Alternatively, PtdIns(4,5)P_2 can be converted to PtdIns(3,4,5)P_3 by the class I phosphoinositide 3-kinases (PI3Ks). PtdIns(3,4,5)P_3 levels are negligible in resting cells, but can transiently and dramatically increase in response to growth-factor stimulation to mediate a wide variety of effects, including cell proliferation, migration, chemotaxis, phagocytosis and macropinocytosis, differentiation, survival and metabolic changes. PtdIns(3,4,5)P_3 functions by recruiting effectors that activate synergistic pathways. Prominent among these are guanine nucleotide-exchange factors and GTPase-activating proteins, which regulate the actin cytoskeleton, and phosphoinositide-dependent protein kinase and protein kinase B (PKB/Akt), which cooperate in the activation of important signalling cascades, such as the mammalian target of rapamycin (mTOR) pathway.

PtdIns(3,4,5)P_3 is dephosphorylated by two types of phosphatases: first, dephosphorylation at the 3 position by PTEN (phosphatase and tensin homologue deleted on chromosome ten) regenerates PtdIns(4,5)P_2 and is the 'off' signal. Second, dephosphorylation by 5-phosphatases such as SHIP1 and SHIP2 (Src-homology-2 (SH2) domain-containing inositol 5-phosphatase) generates PtdIns(3,4)P_2, which shares some actions with PtdIns(3,4,5)P_3 and can, for example, translocate PKB/Akt, but not Bruton's protein tyrosine kinase (Btk; see FIG. 2), to the plasma membrane. Polyphosphoinositides regulate a wide range of effector proteins that harbour PH, FYVE, PX and ENTH domains[139] (see also the review by Lemmon in this issue). Ras-GRP, Ras guanyl nucleotide-releasing protein.
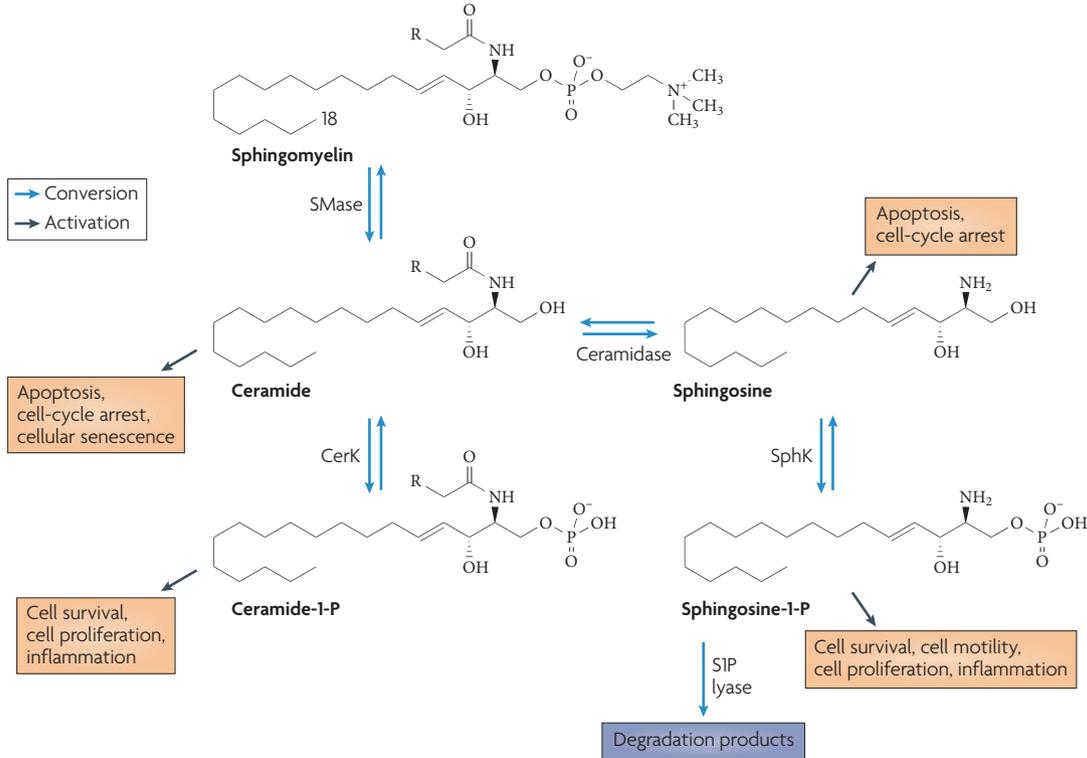
Players in inflammation. Chronic inflammation, auto-immunity and allergy arise from disturbances in vital host defence mechanisms. Lymphocytes detect antigens through T-cell and B-cell receptors (TCRs and BCRs), whereas immunoglobulin (Ig) receptors engage in neutrophil-dependent phagocytosis of foreign particles and in the activation of mast cells. Mast cells, macrophages and neutrophils can unleash irreversible tissue destruction. Independent of the cell type, immunoglobulin receptors (IgG receptors (Fcγ) in neutrophils and macrophages, IgE receptors (FcεRI) in mast cells) couple to similar signal cascades that differ in the exact identity, but not functionality, of the protein kinases and adaptor proteins that are involved, and culminate in an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$).

In mast cells and granulocytes, elevated $[Ca^{2+}]_i$ initiates the release of granule contents and the formation of prostaglandins, thromboxanes and leukotrienes, which is followed by the production of cytokines and chemokines. Although leukocytes (lymphocytes, mast cells, granulocytes, macrophages and neutrophils) have common features of activation, the activation of mast cells will be discussed as a representative example because lipid signalling has been investigated extensively in these cells.

From antigen receptors to PtdIns(4,5)P_2 turnover. Antigen−IgE complexes trigger the crosslinking of high-affinity IgE receptors and their association with lipid rafts on mast cells, initiating a cascade of protein tyrosine kinases. These phosphorylate immunoreceptor tyrosine-based activation motifs (ITAMs) on the cytosolic part of the IgE receptor and cause the translocation of the kinase Syk through its two Src-homology-2 (SH2)

Box 3 | Sphingolipid signalling from sphingomyelin to sphingosine-1-phosphate

Sphingolipids are characterized by the presence of a particular aliphatic aminoalcohol, sphingosine (also termed a long-chain base), instead of the glycerol that is found in glycerolipids. The turnover of sphingolipids can yield potent signalling molecules. Cleavage of sphingomyelin by sphingomyelinase (SMase) generates ceramide, which promotes apoptosis, cell-cycle arrest and cellular senescence (see REFS 140,141 and the review by Hannun and Obeid in this issue). Ceramide can be phosphorylated by ceramide kinase (CerK) to form ceramide-1-phosphate (C1P), which has mitogenic properties, antagonizes the pro-apoptotic action of ceramide and promotes inflammation[41]. Alternatively, ceramide can be cleaved by ceramidase to produce sphingosine, which again mediates apoptosis and cell-cycle arrest. Sphingosine can, in analogy to ceramide, be phosphorylated by one of two sphingosine kinases (SphK1 or -2) to form sphingosine-1-phosphate (S1P). Sphingosine kinases thus form a crucial part of the 'sphingolipid rheostat' to balance the pro-apoptotic actions of ceramide with the pro-survival action of S1P (see figure). S1P operates both intracellularly and extracellularly to function in cell survival, cell motility, cell proliferation and inflammation. The extracellular actions of S1P are mediated by members of the lysophospholipid receptor family of G protein-coupled receptors (S1P1–5; previously known as the Edg family of receptors). The nature of intracellular S1P receptors is still controversial, and intracellular S1P actions are probably also linked to ceramide levels that are regulated through *de novo* synthesis[142]. S1P is degraded by S1P lyase to form ethanolamine phosphate and hexadecanal.

domains (see FIG. 2 and REF. 16). Further phosphorylation of membrane-anchored proteins (such as linker for activation of T cells (LAT) and non-T-cell activation linker (NTAL)) and soluble adaptor proteins (such as GAB2) starts a first wave of lipid signalling by recruitment of so-called class IA PI3K heterodimers that comprise the regulatory subunit p85 and catalytic subunit of PI3K (PI3K$_c$) (FIG. 2).

The catalytic subunit of PI3Kδ (p110δ) has an important role downstream of the IgE receptor[17], but it also relays signals from the B-cell receptor and, to a lesser extent, the T-cell receptor[18]. Elevation of levels of PtdIns(3,4,5)P$_3$ by PI3K is crucial for the progression of antigen receptor signalling because the lipid recruits and activates proteins that contain pleckstrin homology (PH) domains — among them, Bruton's tyrosine kinase (Btk) and PLCγ. Activation of PLCγ through

the generation of Ins(1,4,5)P$_3$ causes Ca^{2+} release from internal stores. Usually, overstimulation of immune cells at low antigen concentrations is prevented by the SH2-domain-containing inositol 5-phosphatase-1 (SHIP1; for a review see REF. 19), which reduces the levels of PtdIns(3,4,5)P$_3$.

Through the second wave to the point of no return. If PtdIns(3,4,5)P$_3$ levels remain high, phospholipase D (PLD) and sphingosine kinase (SphK) are activated, and release phosphatidic acid (PA) and S1P. S1P has been reported to act intracellularly on Ca^{2+} stores[20], but a distinct intracellular receptor has not been identified to date[21]. Once Ca^{2+} stores are emptied, they signal to open store-operated Ca^{2+} channels (SOCCs) in the plasma membrane and trigger a massive influx of extracellular Ca^{2+}. This influx is required to initiate the activation of

Box 4 | Nuclear receptors

Some eicosanoids, as well as fatty acid derivatives and sterol derivatives, signal through nuclear hormone receptors, such as the farnesoid X receptor (FXR), liver X receptor (LXR) and peroxisome proliferator-activated receptors (PPARs). All of these lipid-sensing receptors heterodimerize with the retinoid X receptor (RXR) to activate a feed-forward metabolic cascade that maintains nutrient lipid homeostasis by governing the transcription of a common family of genes involved in lipid metabolism, storage, transport and elimination[78,143].

The PPARs (α, γ, δ) were originally identified in *Xenopus laevis* as receptors that induce peroxisome formation. They are activated by polyunsaturated fatty acids, eicosanoids and various synthetic ligands. Consistent with the distinct expression pattern of these isoforms, gene-knockout experiments have revealed that each PPAR subtype carries out a specific function in fatty acid homeostasis. PPARα-regulated genes function together to coordinate the complex metabolic changes that are necessary to conserve energy during fasting and feeding. This isoform is the main target of fibrate drugs, a class of amphipathic carboxylic acids that are used in cholesterol disorders as an adjunct to statins, and in disorders that feature high levels of triglycerides. PPARγ was identified initially as a key regulator in adipogenesis, but it is also important in cellular differentiation, insulin sensitization, atherosclerosis and cancer. Ligands for PPARγ include fatty acids and other arachidonic acid metabolites, antidiabetic drugs and triterpenoids (polyisoprenes containing six isoprene units). In contrast to PPARα, PPARγ promotes fat storage by increasing adipocyte differentiation and the transcription of several important lipogenic genes. PPARδ might affect lipid metabolism in peripheral tissue and its ligands include long-chain fatty acids and carboprostacyclin. Hereditary disorders of all PPAR isoforms have been described and generally lead to a loss of function and concomitant lipodystrophy, insulin resistance and/or acanthosis nigricans[144].

a plethora of Ca^{2+}-sensitive signalling enzymes such as PKCs, ceramide kinase (CerK), cytosolic phospholipase A2 (cPLA2), 5-lipoxygenase (5-LO) and prostaglandin H2 synthase (PGH2S), and to release histamine from mast cell granulae.

The activation of cPLA2 by Ca^{2+}-mediated translocation to the nuclear membrane is further supported by ceramide-1-phosphate (C1P; REF. 22), which is produced by CerK, and by the phosphorylation of cPLA2 by multiple protein kinases[23,24]. Activated cPLA2 then releases arachidonic acid — the source of prostaglandins, leukotrienes and thromboxanes. These eicosanoids mediate the classical features of inflammation and promote a wide range of diseases from chronic inflammation, allergy, cardiovascular disease and obesity to cancer and more[3,25]. Eicosanoids[26] and S1P[11,27] act extracellularly through GPCRs, and function in conjunction with chemokines, cytokines and histamine. These inflammatory mediators promote bronchial constriction, increased vascular permeability and diameter, and the invasion of leukocytes, which, when in excess, cause tissue destruction in late allergy and inflammation[19].

Dysregulated lipid signalling in cancer

In cancer that is derived from epithelial cells, early mutations increase cell growth and proliferation, which results in hyperplasia and adenoma. Malignant and fatal tumours develop when the cells can undergo contact-independent growth, become motile and cross the basal lamina to form metastases. Although tumour-cell-autonomous activation steps are important in cancer progression, signals derived from the tumour or stroma that influence, for example, endothelial cells (to mediate angiogenesis) and immune cells (for example, to avoid recognition and destruction) are equally crucial. Here, we provide a simplified overview of the intracellular and extracellular lipid-derived signals that function in tumour growth, and provide some insight into current attempts to modulate deviations in lipid synthesis in cancer (we will not cover other lipid-independent signal transduction pathways from the membrane to the nucleus[28,29]).

Phosphoinositides: from growth to metastasis. Many known oncogenes, such as mutated ERBB, insulin-like growth factor-1 (IGF1), c-Kit (stem-cell factor receptor), overexpressed chemokine receptors, mutated Ras, BCR–ABL and elevated levels of the proto-oncogene Src, to name but a few, provide constitutive input signals to PI3K[6,30]. The finding that the 3-lipid phosphatase PTEN (phosphatase and tensin homologue deleted on chromosome ten) is frequently mutated in numerous late-stage tumours finally demonstrated that elevated levels of PtdIns(3,4,5)P_3 contribute to oncogenesis[31,32]. PI3Kα has also been found to be mutated in colon, gastric and breast cancer[33], and structural studies have recently elucidated how key mutations can constitutively activate the PI3Kα complex by relieving the inhibitory action of the p85 regulatory subunit on the catalytic subunit p110α[34].

Of the numerous PH-domain-containing proteins (see the review by Lemmon in this issue), protein kinase B (PKB; also known as Akt) functions as an important node to relay signals from PI3K-generated PtdIns(3,4,5)P_3 during growth and proliferation (FIG. 3; for reviews see REFS 35,36). PKB/Akt is upregulated or mutated in some tumours, and a mutation in the phosphoinositide-binding domain of PKBα/Akt1 that causes it to associate with the plasma membrane and renders it constitutively active has been identified[37]. PKB/Akt increases transcription and translation through the tuberous sclerosis complex (TSC), the target of rapamycin (TOR) complex-1 (TORC1; REF. 38) and phosphorylation of glycogen synthase kinase-3β (GSK3β). PKB/Akt-controlled regulation of cyclin D1 and class O forkhead box (FOXO) transcription factors promotes cell-cycle entry, whereas PKB/Akt counteracts apoptosis through phosphorylation of the inhibitor of nuclear factor-κB (IκB) kinase (IKK), caspase-9 and the pro-apoptotic factor BAD. Elevation of PtdIns(3,4,5)P_3 levels also promotes the transition to malignancy, as several guanine nucleotide-exchange factors for Rho GTPases — for example, Vav and Tiam — are directed to the membrane by PH domains. The cytoskeletal rearrangements that are subsequently stimulated enhance cell migration and metastasis[39].

5

Sphingolipids modulate growth and survival. Ceramide concentrations increase in response to cellular stress, such as DNA damage, disruption of lysosomal compartments or exposure to apoptotic stimuli. Although ceramide can be synthesized *de novo*, it is rapidly produced from sphingomyelin by the stress-induced activation of neutral sphingomyelinase (nSMase) and acid sphingomyelinase (aSMase) (BOX 3), which occurs rapidly and can lead to apoptosis. Ceramide can promote the clustering of death receptors[40] and interferes with the relay of PI3K signals by activating protein phosphatases, such as ceramide-activated protein phosphatase (CAPP) (FIG. 3). Depending on the cell type and setting, however, ceramide can also mediate cell differentiation, cell-cycle arrest, apoptosis and senescence[41], or it can be hydrolysed by ceramidase to yield sphingosine.

Sphingolipid metabolites have been recognized as important modulators of cell survival, cell growth, migration and angiogenesis, and therefore have an important role in cancer progression. Sphingosine has pleiotropic actions, including the ability to attenuate signalling by integrins and mitogen-activated protein kinase (MAPK). A truncated form of PKCδ has been described as a sphingosine-dependent protein kinase (SDK1). Substrates of SDK1 include 14-3-3 proteins; phosphorylated 14-3-3 proteins are reported to form dimers, which masks their binding sites and renders them unable to bind to phosphorylated targets[42]. For example, BAD is phosphorylated by PKB/Akt, and is subsequently retained in the cytosol where it is inactivated by 14-3-3 proteins. When 14-3-3 proteins are phosphorylated, they dimerize and release BAD, which permeabilizes the outer mitochondrial membranes (reviewed in REF. 8). From this, it becomes clear that binding of 14-3-3 proteins to phosphorylated PKB/Akt substrates and SDK1 action on 14-3-3 proteins provide an intersection at which increased sphingosine intercepts with a branch of PI3K signalling.

Ceramide and sphingosine are mostly antiproliferative or pro-apoptotic, but they can be reversibly converted, respectively, to C1P by CerK and to S1P by SphK. C1P and S1P both promote growth and counteract apoptotic stimuli (FIG. 3). C1P positively affects signalling by PKB/Akt, PKA and PKCζ. C1P-mediated phosphorylation of PKB/Akt was reported to be sensitive to PI3K inhibitors, which places C1P upstream of PI3K[43]. The ceramide–C1P and sphingosine–S1P balance thus exerts control on PKB/Akt signalling upstream of PI3K and downstream of PKB/Akt (through 14-3-3 protein dimerization). At the same time, PI3K can operate upstream of SphK, as demonstrated in mast cells (FIG. 2). It is therefore likely that the sphingolipid and phosphoinositide conversions are linked and cooperate to decide over the life or death of a cell.

Lipid signalling in metabolic syndrome

Lipid signalling is key for the aetiology of inflammation and cancer, but it also occupies a central role in the progression of the metabolic syndrome. The metabolic syndrome is a combination of medical disorders, which are probably triggered by high-calorie diets and
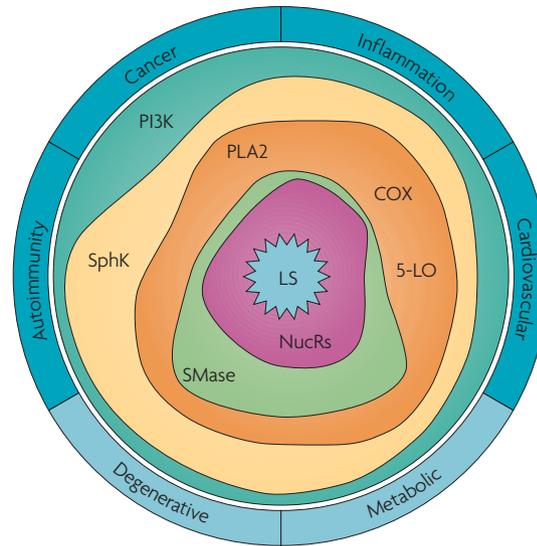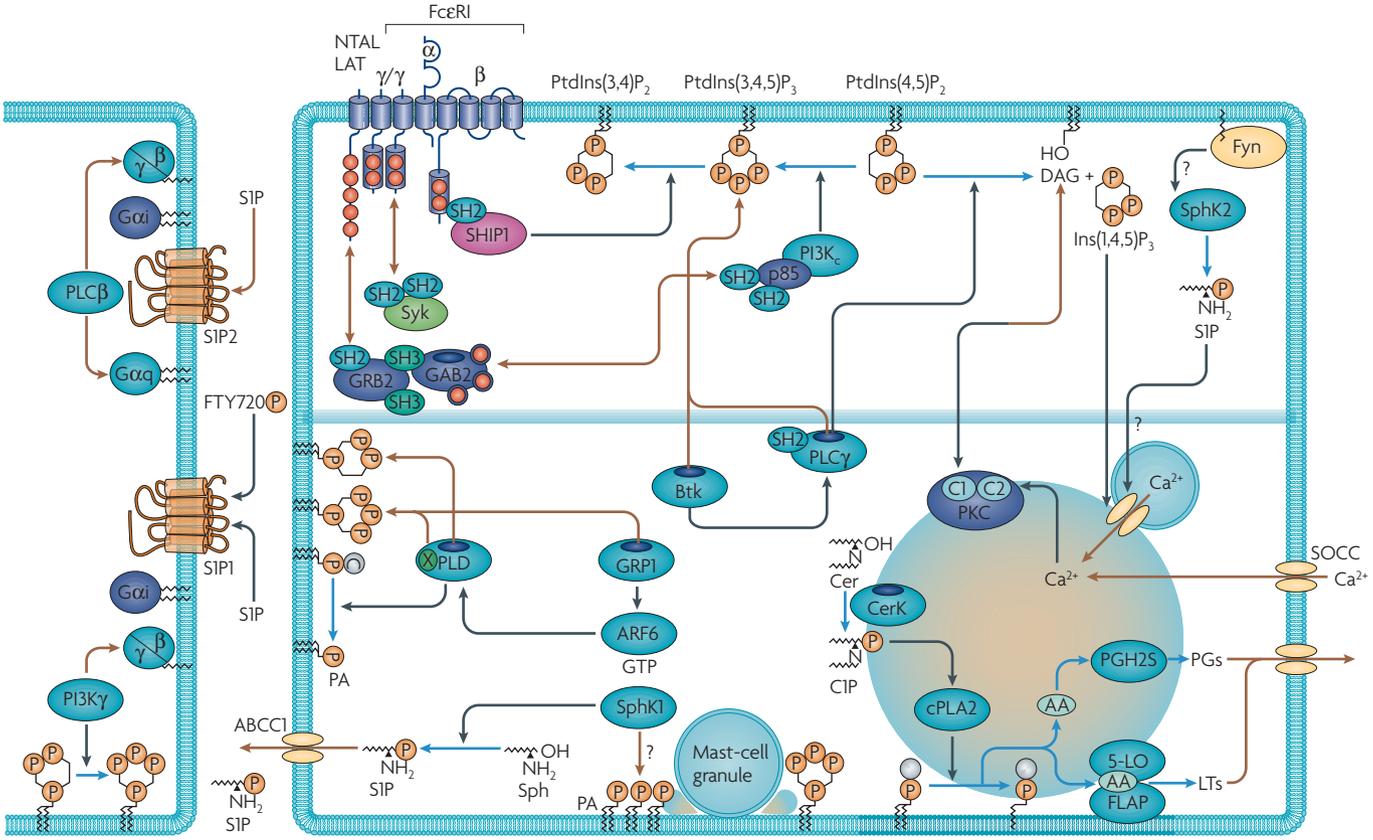

Figure 1 | **Mounting walls.** Lipid signalling (LS) is altered in a multitude of disease states and can dysregulate cell function in the immune system to provoke chronic inflammation, autoimmune reactions and allergy, contribute to cardiovascular disease, or promote tumour growth. Reduced rates of lipid signalling are often detected in degenerative and metabolic disease. The consequences of derailed lipid signalling can be therapeutically approached by inhibitors of lipid-modifying enzymes or lipid effector proteins (for example, nuclear receptors (NucRs)). Here, the width of such a 'therapeutic wall' — the space covered by a given coloured section between the centre (LS) and a respective disease — schematically indicates the power of protection that therapeutic inhibition of the marked lipid-modifying enzymes might provide. Indeed, drugs that target peroxisome proliferator-activated receptors (PPARs) or other NucRs, and enzymes involved in the generation of eicosanoids (phospholipase A2 (PLA2), cyclooxygenase (COX), 5-lipoxygenase (5-LO)), as well as sphingosine kinase (SphK), phosphatidylinositol 3-kinase (PI3K) signalling and sphingomyelinase (SMase) are currently developed or already on the market (see the accompanying Poster entitled 'Targeting lipid signalling in disease'). Numerous lipid-modifying strategies are currently under investigation, but little attention is attributed to the cross-interactions that occur in the lipid network. A better understanding of synergistic lipid actions might generate novel strategies for more effective treatment of the diseases outlined above.

physical inactivity, and result in an increased risk of developing cardiovascular disease and diabetes. The symptoms that accompany the metabolic syndrome include adipocyte proliferation, inflammation, cellular stress, increased production of reactive oxygen species (ROS) and insulin resistance (FIG. 4). Owing to the drastic increase of the incidence of obesity during recent decades, obesity and its associated disorders now constitute a serious health threat to western civilization[44]. Gaining a more detailed understanding of its aetiology is therefore important to identify potential points of intervention.

Figure 2 | Lipid signalling in inflammation and allergy. Crosslinking of high-affinity IgE receptors by IgE–antigen complexes initiates the phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) on the IgE receptor by the action of membrane-bound protein tyrosine kinases (for example, Fyn). Subsequently, Syk, which is recruited to phosphorylated ITAMs through its Src homology-2 (SH2) domains, mediates the phosphorylation of adaptor molecules such as LAT (linker for activation of T cells), NTAL (non-T-cell activation linker) and GAB2 (GRB2-associated binder), which together mediate the recruitment of class IA phosphoinositide 3-kinases (p85–PI3K$_c$ complex). At the membrane, PI3K generates phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P$_3$) from PtdIns(4,5)P$_2$. PtdIns(3,4,5)P$_3$ functions to recruit pleckstrin homology (PH)-containing proteins such as Bruton's tyrosine kinase (Btk), phospholipase Cγ (PLCγ) and GRP1 (general receptor for phosphoinositides-1). When activated by PtdIns(3,4,5)P$_3$, Btk phosphorylates PLCγ, which cleaves PtdIns(4,5)P$_2$ to form diacylglycerol (DAG) and Ins(1,4,5)P$_3$, completing the first wave of lipid signalling. Subsequently, Ins(1,4,5)P$_3$ releases Ca^{2+} from internal stores, while phospholipase D (PLD)[145,146] and sphingosine kinase (SphK) are activated downstream of PI3K. SphK reinforces Ca^{2+} release, eventually leading to the opening of store-operated Ca^{2+} channels (SOCCs) as soon as the intracellular Ca^{2+} pools are depleted. The resulting rise in the intracellular Ca^{2+} concentration (indicated by a yellow cloud) marks the full-scale activation of mast cells, and triggers degranulation of histamine-containing granules, as well as the production of prostaglandins (PGs) and leukotrienes (LTs) by the combined actions of cytosolic PLA2 (cPLA2), prostaglandin H2 synthase (PGH2S) and 5-lipoxygenase (5-LO). PGs, LTs and sphingosine-1-phosphate (S1P) exit the cell through transmembrane channels (for example, S1P through the ATP-binding cassette transporter ABCC1) and act on G protein-coupled receptors (GPCRs) that relay signals to effectors including PLCβ and PI3Kγ. AA, arachidonic acid; ARF6, ADP-ribosylation factor-6; FLAP, 5-LO activating protein; PA, phosphatidic acid; PC, phosphatidylcholine; SHIP1, SH2-domain-containing inositol 5-phosphatase-1.

Insulin signalling. The insulin signalling pathway is central to the metabolic syndrome because obesity is often accompanied by insulin resistance and type 2 diabetes. Insulin and IGF receptors belong to the receptor protein tyrosine kinase family and, unlike other receptors in this family (which act through ligand-dependent dimerization and autophosphorylation), mainly catalyse tyrosine phosphorylation on docking proteins to mediate their signalling. Among other substrates, insulin stimulates tyrosine phosphorylation of insulin receptor substrate (IRS)[45,46] proteins.

This mediates insulin action and is often attenuated in systemic insulin resistance, both in animal models and in human disease.

Under normal circumstances, IRS phosphorylation is linked to the activation of two main signalling pathways: first, the PI3K–PKB/Akt pathway, which is responsible for most of the metabolic actions of insulin, including uptake of glucose by the glucose transporter GLUT4 and glycogen synthesis; and second, the Ras–MAPK pathway, which cooperates with the PI3K pathway to control cell growth and differentiation. Control over insulin
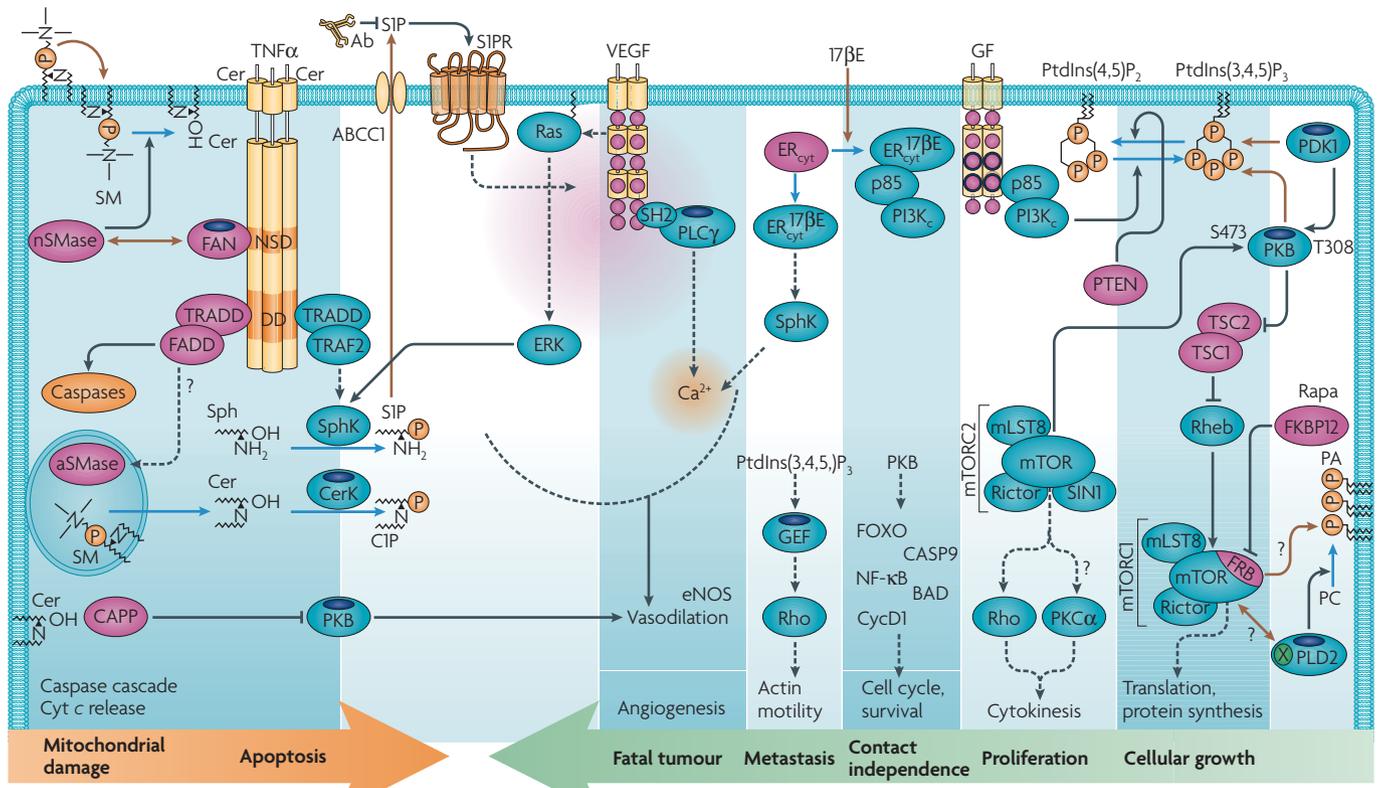
Figure 3 | **Pro- and anti-tumorigenic signals originate from membranes.** Growth factor (GF) receptors, cytokine and chemokine receptors and oncogenes trigger several signalling pathways that contribute to cancer progression. Only input signals that trigger lipid-modifying or lipid-binding proteins are shown. Class IA phosphatidylinositol 3-kinases (shown as the p85–PI3K$_c$ complex) can be activated by GF receptors directly, or by their phosphorylated substrates (not shown; for a review see REF. 5). Protein kinase B (PKB/Akt) is then translocated to PI3K-generated phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P$_3$) at the plasma membrane, where PKB/Akt is phosphorylated on Thr308 by phosphoinositide-dependent kinase-1 (PDK1) and on Ser473 by mammalian target of rapamycin complex-2 (mTORC2) to gain its full activity. Through the phosphorylation of TSC2 (tuberin) in the TSC2–TSC1 (hamartin) complex, PKB/Akt then releases the block on the GTPase Rheb to activate TORC1. TORC1 is a major relay to transcription and protein synthesis, and can be inhibited by the rapamycin (rapa)–FKBP12 complex. In cells that express the oestrogen receptor (ER$_{cyt}$), the addition of an ER ligand (17β-estradiol (17βE)) has been reported to induce the formation of a PI3K–ER complex. Similarly, 17βE was shown to induce sphingosine kinase (SphK) activity, which explains the cytosolic growth-promoting actions of ER. The vascular endothelial growth factor (VEGF)-induced activity of ERK can phosphorylate and activate SphK[147], which generates sphingosine-1-phosphate (S1P). This is especially relevant in endothelial cells, in which S1P supports signalling by VEGF receptors. This process enforces a feed-forward loop that supports angiogenesis. Counteracting GF signalling, both ceramide (Cer) and sphingosine (Sph) can trigger pro-apoptotic events. Ceramide promotes the lateral association of death receptors that contain death domains (DD) and enforces, for example, TNFα receptor signalling to the caspase cascade. Through the neutral sphingomyelinase (nSMase) activating domain (NSD), the TNFα receptor augments the conversion of sphingomyelin (SM) to ceramide. At the same time, ceramide and sphingosine activate protein phosphatases (for example, ceramide-activated protein phosphatase (CAPP)), which attenuate PKB/Akt activation by the PI3K pathway. Whereas these processes promote apoptosis, the TNFα receptor also has a growth-promoting output towards SphK activation. ABCC1, ATP-binding cassette subfamily C member-1 transporter; C1P, ceramide-1-phosphate; CASP9, caspase-9; CerK, ceramide kinase; CycD1, cyclin D1; Cyt c, cytochrome c; ERK, extracellular signal-regulated kinase; FAN, factor associated with nSMase; FADD, Fas-associated death domain; FOXO, class O forkhead box transcription factor; NF-κB, nuclear factor-κB; PC, phosphatidylcholine; PLC, phospholipase C; TRADD, tumour necrosis factor receptor-associated death domain protein.

signalling is achieved by an autoregulatory feedback loop whereby the S6 kinase-1 (S6K1), a downstream effector of mTOR, inhibits upstream elements (a process known as homologous desensitization[47,48]). Alternatively, signals from apparently unrelated pathways can inhibit insulin signalling through heterologous desensitization to coordinate catabolic processes with, for example, cellular stress or tissue inflammation.

Lipids and insulin resistance. One of the key causes of metabolic syndrome is the constant oversupply of energy-rich nutrients — particularly fatty acids, but also carbohydrates and proteins or amino acids. Elevated plasma levels of fatty acids alone, however, are sufficient to induce insulin resistance and to activate a pro-inflammatory response[49]. The availability of free fatty acids is sensed by PPARs, which are nuclear receptors
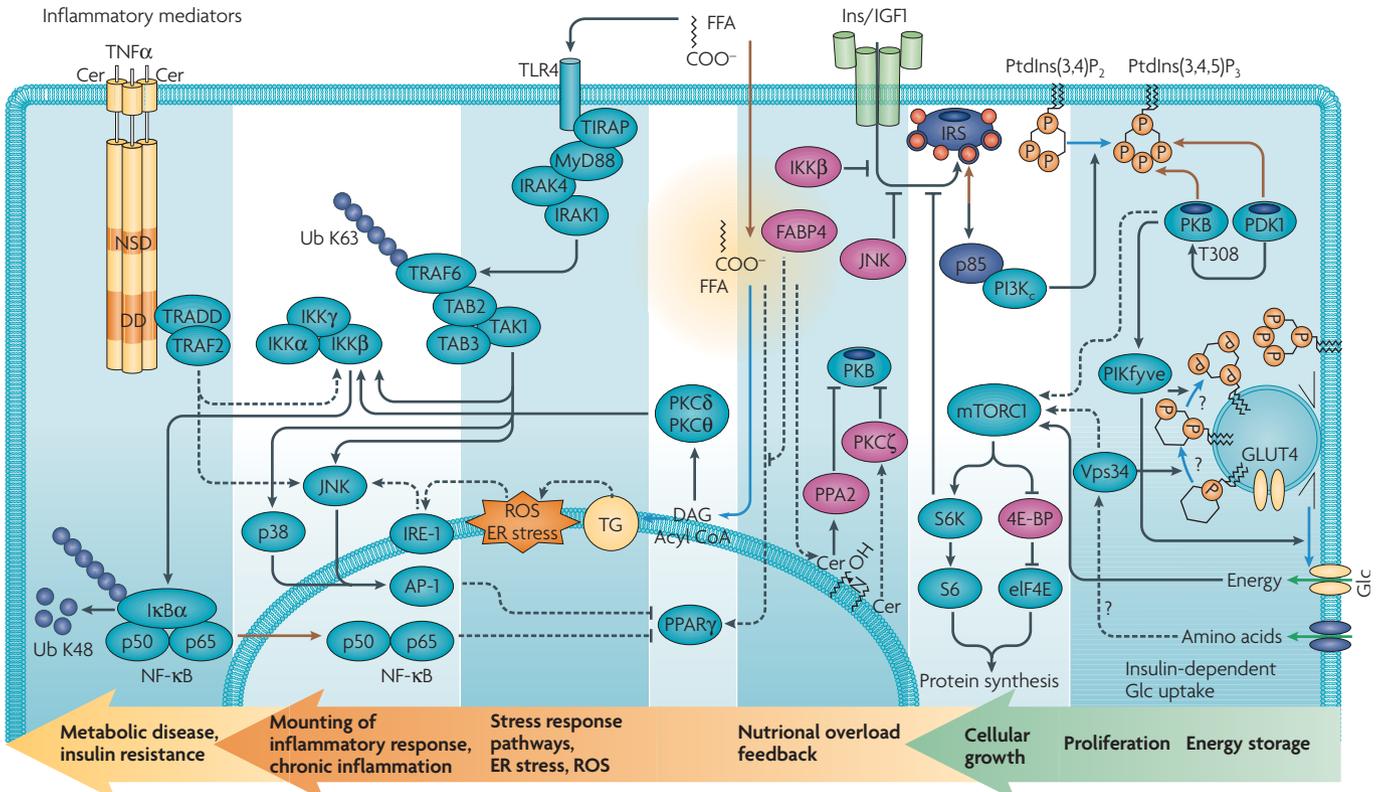
Figure 4 | **Excess fatty acids induce insulin resistance and metabolic syndrome.** Insulin controls glucose uptake through tyrosine phosphorylation of insulin receptor substrate (IRS) proteins. Activation of phosphatidylinositol 3-kinase (PI3K) and generation of phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3) activates protein kinase B (PKB/Akt), which, in turn, supports recycling of the glucose (Glc) transporter GLUT4 to the plasma membrane. The mammalian target of rapamycin complex-1 (mTORC1) coordinates protein synthesis with available nutrients and energy, and desensitizes the pathway through inhibitory phosphorylation of IRS. Excess levels of fatty acids increase the synthesis of ceramide and inhibit the activity of PKB/Akt through protein phosphatase-2A (PP2A) and protein kinase-ζ (PKCζ). Excess fatty acids also activate the nuclear receptor peroxisome proliferator-activated receptor (PPAR)-γ to induce adipocyte differentiation, which is dampened by the fatty acid-binding protein-4 (FABP4). Excess fatty acids induce increased lipid synthesis, further raising the levels of diacylglycerol (DAG), acyl CoA and triacylglycerol (TG). Elevated levels of DAG and acyl CoA result in activation of PKCδ and PKCθ and the subsequent induction of a pro-inflammatory programme, which culminates in the activation of nuclear factor-κB (NF-κB). The increased lipid load in the endoplasmic reticulum induces a stress response and leads to the production of reactive oxygen species (ROS). This leads to the activation of c-Jun N-terminal kinase (JNK) and induction of activator protein-1 (AP-1). Both molecules inhibit PPARγ, which further increases the detrimental action of elevated levels of fatty acids, and induces the synthesis of pro-inflammatory cytokines such as tumour necrosis factor-α (TNFα). This response is further amplified by activation of the Toll-like receptor-4 (TLR4) and its effectors by elevated plasma fatty acid levels, which then results in chronic inflammation. Both inhibitor of NF-κB kinase (IKK)-β and JNK further desensitize insulin responsiveness through inhibitory serine phosphorylation of IRS.

Hepatosteatosis
Fat accumulation in liver cells, which gives rise to fatty liver disease.

that control fatty acid storage, degradation and adipocyte differentiation. Another important group of molecules that coordinates lipid responses in adipocytes includes lipid chaperone proteins, such as fatty acid binding proteins (FABPs). Animals that lack FABP4 and FABP5 exhibit a fatty acid-resistant phenotype similar to that of mice and humans that are given PPARγ ligands, indicating that FABPs and PPARs might function in similar pathways to control the biological effects of lipids[50,51]. Remarkably, mice lacking FABP4 or FABP5, or animals treated with a small-molecule inhibitor of FABP4, are protected against almost every aspect of the metabolic syndrome, including visceral obesity, insulin resistance, hepatosteatosis and atherosclerosis[50,52–54].

Although our understanding of the precise mechanisms that underlie FABP action is incomplete, FABPs are known to modulate lipid composition and fluxes of fatty acids into newly synthesized lipids, which is probably a crucial feature of their biological function.

One lipid that is overproduced as a result of the increase in fatty acid concentrations is ceramide. Ceramide activates protein phosphatase-2A (PP2A), which, in turn, inactivates PKB/Akt and thereby attenuates the insulin response[55]. Moreover, recent results suggest that PKCζ, which is also activated by ceramide, inhibits PKB/Akt translocation to the cell membrane by phosphorylating Thr34 within the PH domain of PKB/Akt. In addition, ceramide blocks plasma membrane

translation of the PtdIns(3,4,5)P_3-binding protein general receptor for phosphoinositides-1 (GRP1), which catalyses guanine nucleotide exchange on ADP-ribosylation factors (ARFs) and thereby interferes with the PI3K-dependent regulation of vesicular transport and membrane remodelling[55]. Ceramide therefore attenuates insulin signalling, and thereby contributes to insulin resistance, through multiple pathways.

Lipid accumulation induces cellular stress. The accumulation of excess lipid, in particular saturated fatty acids and sterols, might induce cellular damage through stress on the membranes of lipid-metabolizing organelles, especially the endoplasmic reticulum (ER) and possibly mitochondria[56–58]. Under these conditions, the ER activates a complex response system known as the unfolded protein response (UPR) to restore the functional integrity of the organelle[59]. Remarkably, orally active chemical chaperones that stabilize protein conformation and improve ER folding capacity can alleviate ER stress and can function as potent antidiabetic agents, restoring glucose homeostasis in mouse models of type 2 diabetes[60]. The ER is also a major source of ROS and, consequently, oxidative stress[61,62]. Oxidative stress is emerging as a feature of obesity and an important factor in the development of insulin resistance, and is frequently associated with mitochondrial dysfunction[63–65].

Inflammation in metabolic syndrome. Lipid-derived signals, particularly eicosanoids and S1P, are important mediators of the inflammatory response. Inflammation itself, however, is a key feature of obesity and type 2 diabetes, as has become clear during the past decade[66]. Infiltration of adipose tissue by immune cells such as macrophages has recently been described in obese mice and humans[67,68]. Chronic inflammation is characterized by abnormal cytokine production[69], and tumour necrosis factor-α (TNFα) has been found to be overexpressed in the adipose tissue of obese mice[70]. TNFα is a pro-inflammatory cytokine that activates various signal transduction cascades, including many of the pathways that are discussed below as crucial inhibitors of insulin action. In obese mouse models with a targeted mutation in the gene encoding TNFα, insulin sensitivity and glucose homeostasis improved, which confirms that this inflammatory response has a vital role in the regulation of insulin action[71,72]. TNFα is also overexpressed in the adipose and muscle tissue of obese humans, and when exogenously administered, causes insulin resistance[73].

Elevated plasma levels of free fatty acids can also directly elicit a pro-inflammatory response by binding to Toll-like receptors (TLRs). TLRs belong to a family of pattern-recognition receptors that are essential in the innate immune system for activating the nuclear factor-κB (NF-κB)-dependent pro-inflammatory signalling pathway in response to microbial pathogens or nutrients, particularly fatty acids[74]. Although TLR4 recognizes lipopolysaccharide from Gram-negative bacteria as a ligand, it is also activated by free fatty acids[75].

Furthermore, mice that lack TLR4 are substantially protected from the ability of systemic lipid infusion to suppress insulin signalling in muscle and from insulin-mediated changes in systemic glucose metabolism[76].

Lipids coordinately regulate metabolic, innate immune and inflammatory processes[77]. Several transcription factors, particularly those of the PPAR and liver X receptor (LXR) families, seem to be crucial to integrate input from pathways involved in these processes. The ligands of all three PPAR family members suppress the production of pro-inflammatory cytokines, mostly by suppressing NF-κB[14,78,79].

How obesity impinges on insulin signalling. Mounting evidence indicates that activation of c-Jun N-terminal kinase (JNK), IKK and conventional PKC is central to the development of insulin resistance in response to obesity — mediated by the aforementioned fatty acid-induced ER stress, ROS production, TLR activation and inflammatory signalling by TNFα. JNK, IKK and PKC have all been reported to inhibit insulin action by phosphorylating IRS1 on serine[80–82]. Serine phosphorylation of IRS1 disrupts insulin-receptor signalling through several distinct mechanisms and blocks insulin action[83,84].

JNK is activated after exposure to cytokines, such as TNFα, as well as by free fatty acids through the TLR pathway and internal cues such as ER stress, all of which might underlie its obesity-induced activity[60,80,85]. There is a striking increase in JNK activity at crucial metabolic sites such as adipose and liver tissue in mouse models of obesity[86]. Most importantly, JNK deficiency protects mice from insulin resistance, fatty liver and diabetes[86], which implies that JNK inhibition might be a promising therapeutic avenue for diabetes[87–89].

Similarly, IKK is also activated through TLR4 and TNFα. Mice that are heterozygous for a loss-of-function mutation in IKKβ are partly protected from obesity-induced insulin resistance, and inhibition of IKKβ by high-dose salicylates improves insulin action in experimental models and humans[90].

PKC is the third important mediator between metabolic pathways and the inflammatory response[49,77,91]. Fatty acid metabolites such as fatty acid coenzyme A and DAG can activate PKCθ in muscles or PKCδ in the liver and inhibit insulin action[49,77]. Mice that are deficient in PKCθ are protected against fatty acid-induced insulin resistance, which confirms the contribution of this kinase to metabolic regulation *in vivo*[91]. At a mechanistic level, PKCθ is known to activate IKK and might contribute to insulin resistance through this pathway.

JNK, IKK and PKC also exert powerful effects on gene expression, including the promotion of inflammatory gene expression through the activation of activator protein-1 (AP-1) complexes and NF-κB. This activation results in downregulation of PPARγ-dependent genes, possibly through competition for common activators, thus inhibiting adipocyte differentiation and clearing of circulating fatty acids[92]. Notably, JNK–AP-1 and IKK–NF-κB are linked to components that sense and mediate ER stress and oxidative stress[93–95].

Taken together, free fatty acids seem to have a key role in inducing and mediating many of the symptoms that accompany the metabolic syndrome, including adipocyte proliferation, inflammation, cellular stress, increased ROS production and insulin resistance. A better characterization of the precise mechanisms by which these elevated levels of fatty acids result in the activation of key kinases such as JNK and PKC, and how they cause insulin resistance, will open new therapeutic opportunities to attenuate some of the pathologies and improve insulin responsiveness.

Therapeutic opportunities

Lipids in inflammation: points of interception. Strategies to attenuate chronic inflammation and allergy involve the interception of immune cell migration to target tissues and/or the blockage of signalling events, leading to the release of inflammatory mediators. PI3Kγ, the only class IB PI3K isoform, is activated by βγ subunits of trimeric G proteins that are dissociated by GPCRs to produce transient, but massive, elevations of $PtdIns(3,4,5)P_3$. This lipid kinase integrates multiple signals from chemokines, complement fragments, formylated bacterial peptides and other stimuli, and was shown to be essential for chemokine-induced leukocyte migration *in vivo*[96–98]. Therefore, PI3Kγ has become the subject of impressive pharmacological efforts as a target to treat inflammatory disease[5,99]. PI3Kγ inhibitors (for example, AS605240) have shown therapeutic effects in mouse models of rheumatoid arthritis[100] and systemic lupus erythematosus[101], in which the compounds attenuated the migration of neutrophils and lymphocytes, respectively, into inflamed tissue.

As outlined above, the PI3Kδ isoform is activated downstream of a protein tyrosine cascade that emerges from Ig receptors. In the absence of PI3Kδ activity — for example, in mice with a catalytically inactive p110δ catalytic subunit[17], or those treated with the PI3Kδ inhibitor IC87114 (REF. 102) — degranulation of mast cells is attenuated. As a result, these mice are protected against the deleterious effects of IgE and allergen exposure. Histamine acts on endothelia to increase vascular permeability and dilation, causing a drop in body temperature and blood pressure, which can result in a fatal anaphylactic shock.

Surprisingly, elimination of the PI3Kγ isoform protects mice against IgE–allergen-mediated anaphylaxis, as described for the loss of PI3Kδ activity above. PI3Kγ has not directly been linked to IgE–allergen-triggered clustering of FcεRI, but the IgE–allergen causes the release of adenosine from mast cells, which signals through GPCRs to activate PI3Kγ; the resulting peak in $PtdIns(3,4,5)P_3$ is essential for the *in vivo* responsiveness of mast cells[103]. In addition, genetic evidence suggests that modulation of PI3Kγ activity could be beneficial in cardiovascular disease[104], and dual PI3Kγ/δ inhibitors (for example, TG100-115) have entered clinical trials for myocardial infarction[105]. Owing to the above, PI3Kγ inhibitors have been enthusiastically dubbed the 'aspirin of the twenty-first century'[99], but single-specificity inhibitors for PI3Kγ and PI3Kδ still await validation for the treatment of human disease.

By contrast, a high degree of validation and feasibility has been reached for targeting the sphingosine kinase pathway. FTY720 (fingolimod), when phosphorylated by sphingosine kinase, functions as an S1P receptor agonist and targets four (out of the five) S1P receptors. FTY720 interferes with the exit of lymphocytes from lymph nodes, and therefore attenuates autoimmune disease (for example, multiple sclerosis)[106–108], organ rejection in transplantation, and is under consideration for other inflammatory and allergic conditions[27,109]. S1P1-specific agonists (SWE2871; REF. 110) and antagonists[111] have helped to define S1P1 as the main target of S1P on lymphocytes. S1P1 antagonists also modulate endothelial permeability[112], and thus also show some adverse effects.

Phospholipases A2, C and D have established roles in inflammation and allergy. Numerous efforts to target these enzymes have generated useful experimental inhibitors (see accompanying Poster ['Targeting lipid signalling in disease'](#)), but no marketable drugs so far. Overall, PLA2 levels are modulated by corticosteroids[113]. Downstream of cPLA2, the cyclooxygenase (COX) activity of prostaglandin H2 synthase is a classic target of NSAIDs. Treatment of rheumatoid disease with the selective COX2 inhibitor rofecoxib (Vioxx) was associated with serious cardiovascular complications[114], which raised fears that this result was coupled to COX2 as a drug target in general.

An alternative to inhibiting COX activity is targeting prostaglandin and thromboxane receptors. This inhibition has been achieved with ramatroban, marketed for allergic rhinitis, which blocks the TP receptor (this receptor binds thromboxane A2) and the chemoattractant receptor-homologous molecule that is expressed on Th2 lymphocytes (CRTH2; receptor for prostaglandin D2) in parallel[115]. Modulation of leukotriene action has also been shown to be effective against asthma and allergic rhinitis: zileton targets 5-LO with a limited potency, whereas montelukast, pranlukast and zafirlukast interfere with the action of cysteinyl leukotrienes (leukotrienes C4, D4 and E4) on cysteinyl leukotriene-1 and -2 receptors[116,117].

It is evident, therefore, that modulation of extracellular and intracellular lipid signalling is beneficial in the treatment of inflammation, autoimmunity and allergy. Novel strategies are in progress; it seems that it is often not necessary to completely block the output of a given lipid pathway, but that partial attenuation of the lipid network is sufficient to generate a beneficial effect for the patient.

Targeting lipids in cancer. Presently, the PI3K pathway can be therapeutically targeted at the level of TORC1 by rapamycin (sirolimus and its derivatives temsirolimus and everolimus; FIG. 3). Tumours with constitutively elevated $PtdIns(3,4,5)P_3$ (for example, owing to loss of PTEN or because of mutations in PI3Kα) are especially sensitive to rapamycin[118], but an important action of rapamycin-based drugs seems to be their effect on endothelial cells and angiogenesis[38]. Limited information is available concerning the *in vivo* isoform-specificity

of novel PI3K inhibitors, and molecules with proven anti-tumour activity in xenograft models (PI-103 (REFS 119,120) and ZSTK474 (REF. 121)) are broad-spectrum PI3K inhibitors, and might also owe some of their action to the inhibition of mammalian TOR (mTOR). NVP-BEZ235 has recently entered clinical trials as a treatment for solid tumours[122]. It is not clear whether or not targeting single PI3K isoforms will be successful in cancer therapy, and whether PI3K inhibitors will be effective as single agents. Collective data indicate that they will be of value in combination therapy: PI3K inhibitors have been shown to reverse resistance to trastuzumab (herceptin, a monoclonal antibody directed against ERBB2), which was caused by the loss of PTEN[123].

As explained above, S1P is of major importance in the migration of lymphocytes out of secondary lymphatic organs. In cancer, S1P promotes cell growth and survival, angiogenesis, vascular maturation and mediates cell migration (FIG. 3). Using a monoclonal antibody with high affinity for S1P, Visentin and colleagues[124] have demonstrated that providing a sink for S1P is sufficient to block angiogenesis and endothelial cell migration in response to vascular endothelial growth factor (VEGF) and basic fibroblast growth factor. The fact that the growth of an S1P-insensitive melanoma xenograft was inhibited indicates that removal of S1P hits a central signalling hub, which could prevent angiogenesis in various cancers (for an excellent comment see REF. 125). To further clarify the mechanism of action of S1P removal, it would be interesting to compare the S1P antibodies with SphK inhibitors, which also have anti-proliferative activity[126], and are also expected to cause a build-up of intracellular sphingosine. An alternative strategy for lowering S1P levels might be the activation of S1P lyase[127], an ER-localized enzyme, which cleaves S1P to yield ethanolamine phosphate and hexadecanal. Indeed, S1P lyase was reported to promote apoptosis and to be downregulated in colon cancer[128].

An action that is independent of FTY720 phosphorylation was reported in chronic myelogenous leukaemia (CML) cells, in which the fusion protein BCR–ABL drives proliferation: here, FTY720 triggered the activation of the protein phosphatase PP2A and, consequently, apoptosis[129]. Interestingly, PP2A regulates PKCs and PKB/Akt, providing another crossover point with PI3K signalling.

Sphingolipid and PI3K signalling not only control the same molecular targets, but the pathways also converge at the level of physiological responses. As an example, S1P (probably through S1P receptors[130]) and PtdIns(3,4,5)P_3 (through PKB/Akt) both lead to the activation of endothelial nitric oxide synthase (eNOS) and the release of nitric oxide (NO·) radicals. NO· acts through the NO–cyclic GMP pathway and, to some degree, through the formation of reactive nitrogen species to dilate blood vessels, decrease the blood–tumour barrier and to promote angiogenesis. This is clearly underlined by the lack of VEGF-induced angiogenesis in the absence of eNOS (reviewed in REF. 131). Based on the above, and on the fact that PI3K also acts downstream of the VEGF receptor, it becomes clear how PI3K inhibitors develop their anti-angiogenic activity[120].

Understanding the sphingolipid and polyphospho-inositide balance and their interconnection might therefore provide novel opportunities to achieve a stringent control of proliferation and growth of tumour cells and endothelia in angiogenesis, which is needed to interfere with the dissemination of adaptable and motile cancer cells.

Targeting metabolic syndrome. Given the complex aetiology of the metabolic syndrome, preventing the disease and its progression is a challenging task that must be addressed from various angles, preferably starting with the prevention of the intake of excess nutritional lipids to tame chronic inflammatory responses. Although a simple measure, it is often not applicable. Targeting the hormone-sensitive lipase (HSL), which liberates free fatty acids for cellular uptake, is an option that has been realized with the use of orlistat. Similarly, small-molecule inhibitors of FABP4 have produced promising results in preventing metabolic syndrome in high-fat diet animal models, and raises hopes that this strategy might advance further[50,52–54]. Cellular stress that is caused by an excess of fatty acids can be alleviated using orally active chemical chaperones that act to stabilize protein conformation and improve the protein folding capacity of the ER. Such small molecular chaperones have indeed been proven to restore glucose homeostasis in preclinical models of type 2 diabetes[60].

Nutrient overload also triggers alterations in gene expression, which are mediated by nuclear receptors as described above. Here, agonists for PPARs have a prototypic role, and rosiglitazone has been successfully used to manage insulin resistance. However, this treatment comes at a price because rosiglitazone is associated with an increased risk of myocardial infarction[132].

Both the inflammatory branch of metabolic disease and insulin resistance can eventually be targeted by JNK. JNK deficiency has been reported to protect mice from insulin resistance, fatty liver and diabetes[86], and inhibition of JNK is considered to be a promising therapeutic avenue[87–89]. Similarly, mouse models harbouring null mutations in IKKβ have validated that this kinase impacts insulin signalling, and inhibition of IKKβ by high-dose salicylates improves insulin action in experimental models and humans[90].

Long-term complications in type 2 diabetes, such as diabetes-induced retinopathy, are the subject of clinical trials with PKCβ inhibitors (LY-333531; also known as ruboxistaurin)[133,134]. It has also been claimed that PKCβ null mice — although they are hyperphagic — display an increased insulin sensitivity and fatty acid oxidation[135]. If applicable to humans, pharmacological manipulation of metabolic turnover could provide ways to lower visceral fat mass without reducing caloric intake. Whether this is desirable is, of course, another question, and maybe the best answer to this was given ~200 years ago by J. A. Brillet-Savarin: "Tell me what you eat, and I will tell you what you are".

Conclusions and perspectives

From the data presented above, it is clear that pathways that generate and respond to signalling lipids such as activation of PI3K, sphingolipid turnover, eicosanoid production and PPAR-dependent gene regulation have important roles in inflammation, cancer and metabolic syndrome. Lipid signalling events are shared among these different diseases; for example, metabolic syndrome involves inflammatory processes to develop into type 2 diabetes, atherosclerosis or cardiovascular disease; and cancer utilizes increased lipid signalling to progress from benign to malignant stages.

Lipid signalling cascades are complex, sometimes redundant and highly interconnected. Nevertheless, signalling lipid-generating enzymes have been and are still being targeted pharmacologically to alleviate the symptoms or even progression of the different diseases. In cases in which the modulation of one lipid signalling pathway does not suffice therapeutically, combinatorial treatments are being considered and may prove effective. Although such approaches can be explored experimentally *in vivo* or even clinically, the data to predict patient benefit are relatively scarce. This poor predictability of the *in vivo* response is, at least in part, caused by our incomplete understanding of the dominant and permissive properties of interacting lipid signalling pathways.

In addition, the tools that are available to follow the dynamics of agonist-modulated production of the lipid signal, to monitor the precise chemical modification of the lipid and its spatial organization are technically limited and hamper a more comprehensive description of these pathways and their interconnectivity. Unlike proteins, the function of which can largely be addressed individually, lipid actions are frequently masked by a large 'steady-state' mass represented by the structural lipids in the membrane, which makes it difficult to detect the transient lipid signal. Fluorescent probes — mostly based on lipid-binding protein domains such as PH domains — have been established for phosphoinositides[136], but many of the other lipid mediators are still difficult to trace. Further advances in these fields and their integration for therapeutic use are, therefore, likely to benefit significantly from improved time-resolved methods to monitor lipid signals.

If this is achieved, we can substantiate what becomes apparent in this review: the lipid signalling pathways, studied in-depth in isolation elsewhere, do not operate independently, but mostly act in concert to control cell death, stasis or proliferation and cellular activity. Lipid signalling is context dependent and understanding the semantics of this molecular language is a challenge for the future.

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DATABASES
UniProtKB: http://beta.uniprot.org/uniprot
5-LO | aSMase | CerK | COX2 | cPLA2 | nSMase | PGH2S | PKB | PLC | PLD | SphK

FURTHER INFORMATION
Matthias P. Wymann's homepage:
http://pages.unibas.ch/dbmw/biochemie
Roger Schneiter's homepage:
http://www.unifr.ch/biochem/default.php
Genetics Home Reference: http://ghr.nlm.nih.gov
Mouse Genome Informatics: http://www.informatics.jax.org
Lysosomal Storage Diseases:
http://www.lysosomallearning.com
Clinical Trials: http://clinicaltrials.gov
Lipid Metabolites and Pathways Strategy:
www.lipidmaps.org
The Aspirin Foundation:
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DrugBank: http://redpoll.pharmacy.ualberta.ca/drugbank//
Novartis Oncology: Targeted Kinase Inhibitors – BEZ235
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