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REVIEW ARTICLE

cAMP guided his way: a life for G protein-mediated signal transduction and molecular pharmacology—tribute to Karl H. Jakobs

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Abstract

Karl H. Jakobs, former editor-in-chief of Naunyn-Schmiedeberg's Archives of Pharmacology and renowned molecular pharmacologist, passed away in April 2018. In this article, his scientific achievements regarding G protein-mediated signal transduction and regulation of canonical pathways are summarized. Particularly, the discovery of inhibitory G proteins for adenylyl cyclase, methods for the analysis of receptor-G protein interactions, GTP supply by nucleoside diphosphate kinases, mechanisms in phospholipase C and phospholipase D activity regulation, as well as the development of the concept of sphingosine-1-phosphate as extra- and intracellular messenger will presented. His seminal scientific and methodological contributions are put in a general and timely perspective to display and honor his outstanding input to the current knowledge in molecular pharmacology.

Keywords Heterotrimeric G protein · Low molecular mass GTP binding protein · Adenylyl cyclase · Phospholipase C · Phospholipase D · Calcium-dependent signaling · Sphingosine-1-phoshate

Abbreviations

$A_{2A}AR$	adenosine receptor type 2A
ARF	ADP-ribosylation-factor
cAMP	cyclic AMP
$[Ca^{2+}]_I$	intracellular Ca^{2+} concentration
DHS	D,L-threo-dihydrosphingosine
DMS	*N*,*N*-dimethylsphingosine
Epac	exchange protein directly activated by cAMP
GAP	GTPase-activating protein
GEF	guanine nucleotide exchange factor
GPCR	G protein-coupled receptor
GppNHp	guanosine-5′-[(β,γ)-imido]triphosphate
GTPγS	guanosine-5′-[γ-thio]triphosphate
HL-60	human leukemia cell line
M_2	M_2 muscarinic acetylcholine receptor
M_3	M_3 muscarinic acetylcholine receptor
NDP	nucleoside diphosphate
NDPK	nucleoside diphosphate kinase
NEM	*N*-ethylmaleimide
NTP	nucleoside triphosphate
PA	phosphatidic acid
PGE1	prostaglandin E1
PIP_2	phosphatidylinositol-4,5-bisphosphate
PIP5 kinase	phosphatidylinositol-4-phosphate-5-kinase
PMA	phorbol 12-myristate 13-acetate
PKA	protein kinase A
PKC	protein kinase C
PLC	phospholipase C
PLD	phospholipase D
PTX	pertussis toxin

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RGS	regulator of G protein signaling
ROCK	Rho-dependent protein kinase
S1P	sphingosine-1-phosphate
SPC	sphingosylphosphorylcholine
SphK	sphingosine kinase

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On April 6, 2018, Professor Karl H. Jakobs, former editor-in-chief of Naunyn-Schmiedeberg's Archives of Pharmacology, passed away in his hometown Heidelberg, unexpectedly at the age of 76 years. Karl H. Jakobs was born in 1941 in the small village of Rissenthal, Saarland. He started to study medicine at Heidelberg University in 1965 and joined the Institute of Pharmacology of the University to work on his MD thesis. He graduated in 1971 and continued working in the Institute as a postdoctoral scholar. In 1982, he was promoted to associate professor of pharmacology and in 1984 to full professor. In 1991, he changed to the University of Duisburg-Essen, where he was appointed as Chair of Pharmacology at the Essen University Hospital. He was head of the Institute of Pharmacology in Essen until his retirement in 2007. From 1992 until 2007, Karl H. Jakobs was member of the Editorial Board of Naunyn-Schmiedeberg's Archives of Pharmacology and served as its editor-in-chief until 2002. During his time as editor, he guided the journal very well and initiated its change from a journal mainly recognized in the German Pharmacological Society to an internationally recognized platform to publish studies in experimental pharmacology. Naunyn-Schmiedeberg's Archives of Pharmacology is extremely grateful to Karl H. Jakobs for his long-lasting service and will dearly miss his input.

Karl H. Jakobs was a gifted academic scholar and scientist. He inspired numerous young MD and PhD students for experimental pharmacology focused on the molecular mechanisms of drug action. His curiosity and passion for science were catching, and quite a few of his scholars became well-known scientists and even pharmacologists themselves. He not only inspired them scientifically but also provided them with an example of a highly educated and caring human being with a look for the broader picture. Five of his former scholars and his scientific mentor teamed up to write this review on his scientific work and seminal contributions to G protein-mediated signal transduction and molecular pharmacology, which in many cases are still standing solid today and thus helped to shape our current knowledge.

1 G_i proteins as signal transducers

1.1 A G protein distinct from G_s mediates G protein-coupled receptor-induced inhibition of adenylyl cyclase

When Karl H. Jakobs started his career in pharmacology and joined the small signal transduction group of Günter Schultz in the Heidelberg University Institute of Pharmacology as a medical student, the institute was still located in Heidelberg's main street in the old anatomy building with a Bunsen statue on its front. The group had access to the roof of the building, overlooking the city, the castle, and the river Neckar. By the end of the 60s, signal transduction thinking was largely along Earl Sutherland's concept with hormones and neurotransmitters stimulating adenylyl cyclase (still wrongly named adenylate cyclase at the time (cf. footnote in (Toro et al. 1987)) to increase the intracellular concentration of cyclic AMP (cAMP) (Hardman et al. 1971). Karl H. Jakobs started working on adenylyl cyclase and setup an assay for determining the enzyme's activity in cell-free systems; such an assay was not available in Europe at the time. Using this setup, he showed cAMP-increasing effects for the antidiuretic hormone and parathyroid hormone in rat kidney homogenates (Jakobs and Schultz 1970; Jakobs et al. 1972).

In contrast to the many hormones and neurotransmitters stimulating cAMP formation, only few hormones had been reported to decrease cAMP in cells or tissues. The molecular mechanism was unknown in the 60s. In the early 70s, the only signal transduction components known were receptors (nowadays termed GPCRs), a GTP-binding and GTP-hydrolyzing G protein (which was also and variably referred to as N-protein (guanine-nucleotide-binding) (Iyengar and Birnbaumer 1982) and G/F (according to its stimulation by guanine nucleotides and fluoride ions) (Northup et al. 1980) at the time) and adenylyl cyclase (Fig. 1). As cAMP-lowering hormones stimulated the high affinity (i.e., low K_m) GTPase activity in membranes to a greater extent than the adenylyl-cyclase-stimulating hormones, one idea was that cAMP-lowering hormones favor the inactivation of the G protein by accelerating the hydrolysis of the bound GTP. Karl H. Jakobs however proposed another concept, i.e., the existence of a G protein different from the stimulatory G_s, an inhibitory G_i. In 1976, he was able to show, for the first time, that adrenaline caused adenylyl cyclase inhibition in a cell-free system, i.e., in a human platelet homogenate (Jakobs et al. 1976). Soon thereafter, hormonal adenylyl cyclase inhibition was demonstrated by muscarinic acetylcholine receptors in cardiac membranes (Jakobs et al. 1979), by angiotensin II and α_2-adrenoceptor agonists in rat liver membranes (Jard et al. 1981), and by prostaglandin E_1 (PGE1), adrenaline, and nicotinic acid in hamster fat cell membranes (Aktories et al. 1979; Aktories et al. 1980a; Aktories et al. 1980b; Jakobs et al. 1981). The involvement of an inhibitory G_i protein in these hormonal effects was supported by the requirement of the presence of GTP in the various systems (Jakobs et al. 1978; Aktories et al. 1979; Jakobs et al. 1979). Similarly important for the model of two distinct G proteins mediating hormonal stimulation and inhibition, respectively, of adenylyl cyclase, was the finding that adenylyl-cyclase-inhibiting hormones stimulated a high affinity GTPase (Aktories and Jakobs 1981), which was first observed in human platelet membranes with adrenaline in the presence of propranolol to exclude effects on β-adrenoceptors. The stimulation of the low K_m GTPase occurred with exactly the same agonist concentration dependency as the inhibition of adenylyl cyclase. In line with these results, inhibitors of fat cell adenylyl cyclase including PGE_1, adenosine derivatives, adrenaline in the presence of propranolol, and nicotinic acid stimulated a high affinity GTPase (Aktories et al. 1982b). Support for "distinct regulatory sites involved in opposing hormonal regulation" of adenylyl cyclase activity was obtained in studies with cysteine-modifying reagent *N*-ethylmaleimide (NEM), showing an uncoupling of the inhibitory regulation by this compound, whereas adenylyl cyclase stimulation was not

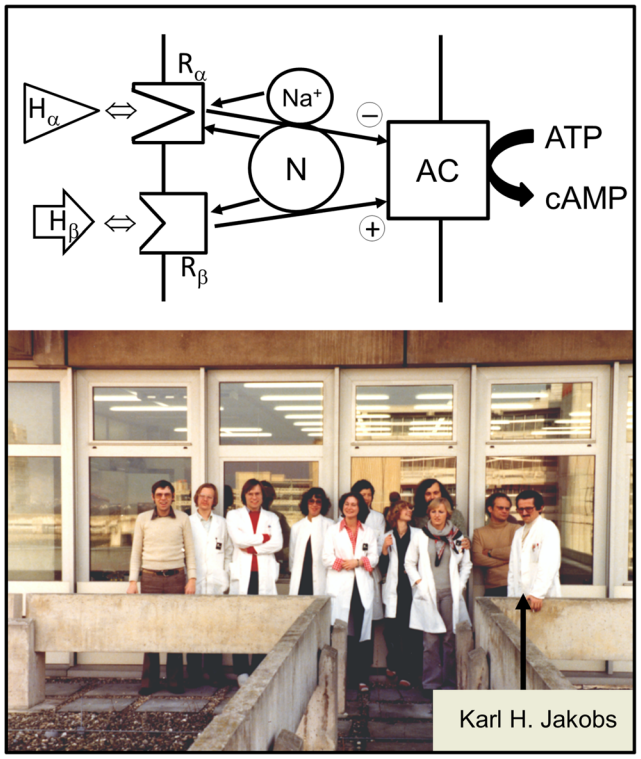

Fig. 1 Understanding of the regulation of adenylyl cyclase activity by stimulatory and inhibitory hormones at the beginning of the 1980s. Upper panel: Scheme adapted from (Jakobs and Schultz 1980). H, hormone; R, receptor; α and β. α- and β-adrenergic, representing examples of inhibitory and stimulatory receptors, respectively. N, regulatory guanine nucleotide binding protein(s); AC, catalytic moiety of the adenylyl cyclase. The existence of a second inhibitory G protein is not indicated yet. The lower panel shows members of the group on the balcony of the new institute in Heidelberg at that time

impaired (Jakobs et al. 1982). A pivotal finding by Karl H. Jakobs and co-workers providing strong support to the concept of two separate adenylyl cyclase regulating G proteins was the occurrence of a somatostatin-induced inhibition of adenylyl cyclase in membranes of lymphoma S49 cyc^- cells, which have a defect in the gene encoding and, hence, lack G_s (Jakobs et al. 1983; Jakobs and Schultz 1983). The idea of the existence of a G_i was further supported by Lutz Birnbaumer's data, showing the inhibition of the maximally forskolin-stimulated S49 cyc^- adenylyl cyclase by stable GTP analogues (Hildebrandt et al. 1982). In retrospect, cyc^- and other mutant S49 lymphoma cells (cf. (Salomon and Bourne 1981)) played an exciting and fundamental role in studies on G protein regulation of adenylyl cyclase in several laboratories at that time. Using these cells for a complementation assay, Alfred Gilman and colleagues purified components of stimulatory G proteins (Northup et al. 1983a; Northup et al. 1983b).

1.2 Bacterial toxins to dissect G_s- and G_i-mediated signaling

Other important tools to study adenylyl cyclase inhibition and the role of G proteins were two bacterial protein toxins, cholera toxin, and pertussis toxin. Cholera toxin had already been known at the time to persistently activate G_s by ADP-ribosylation (Cassel and Selinger 1977b). Klaus Aktories and Karl H. Jakobs showed that in platelet membranes, where PGE_1 stimulates and adrenaline inhibits adenylyl cyclase, cholera toxin inhibited PGE_1-stimulated GTP hydrolysis, but had no effect on the adrenaline-stimulated high affinity GTPase activity (Aktories et al. 1982a). Again, these data underlined the different nature of G protein-mediated inhibition and stimulation of adenylyl cyclase. Of major importance for further studies on the inhibitory regulation of adenylyl cyclase was the discovery of pertussis toxin (PTX, at that time called islet-activating factor). Michio Ui and Toshiaki Katada showed that this toxin ADP-ribosylated a 41-kDa protein that was involved in the regulation of adenylyl cyclase, but was different from the cholera toxin substrate G_s (Katada and Ui 1982). Thus, PTX became a most desirable tool. Therefore, Klaus Aktories in and Karl H. Jakobs decided to purify the toxin and used it successfully in numerous studies to clarify mechanisms involved in hormonal inhibition of adenylyl cyclase (Aktories et al. 1983a; Aktories et al. 1983b; Kather et al. 1983; Wuster et al. 1984; Hackenthal et al. 1985; Kather et al. 1985). After having left the group of Karl H. Jakobs and Günter Schultz, Klaus Aktories continued to work on bacterial toxins modifying signal transduction intermediates, which has meanwhile led to a much better understanding of pathological mechanisms as well as variety of extremely useful experimental tools (for review see (Aktories 2011)). As for adenylyl cyclase inhibition, it is now clear that the modes of regulation of the enzyme are by far more complicated than initially envisioned. This is mostly, albeit not solely, due to the multiplicity of adenylyl cyclase isoforms and the manifold ways of their regulation through G protein subunits, Ca^{2+} and Ca^{2+}-regulated proteins, protein kinases, and several other regulatory proteins not belonging to these categories (Sadana and Dessauer 2009; Halls and Cooper 2017).

Groundbreaking studies with thrombin in platelet membranes added another important extension to the concept of G protein-mediated hormonal inhibition of adenylyl cyclase. It was well known that, in contrast to adrenaline, thrombin caused a persistent aggregation of platelets. Aktories and Jakobs showed for the first time that thrombin behaves like a typical adenylyl cyclase inhibitory agonist: it inhibited adenylyl cyclase in a GTP-dependent manner, stimulated a high affinity GTPase, and its actions were sensitive to PTX (Aktories and Jakobs 1984). Further studies suggested that thrombin acts on yet another G protein(s) distinct from G_s and G_i, thereby predicting the coupling of thrombin receptors to multiple G proteins (Grandt et al. 1986). This, at that time, again changed and extended the view of the transmembrane signaling functions of G proteins and paved the way to the identification of the great variety of heterotrimeric G proteins as we describe them nowadays (Wettschureck and Offermanns 2005).

Taken together, the early work of Karl H. Jakobs set scientific milestones for the understanding of the hormonal control of adenylyl cyclase especially of its inhibitory regulation.

1.3 $G\alpha_i$ as substrate for protein kinase C

It should be noted that one of the most highly cited papers of Karl H. Jakobs describes work on the regulation of G_i by protein kinase C (PKC). These studies showed that the Gα-subunit of G_i is a physiological substrate for PKC and that its function in transducing inhibitory hormonal signals to adenylyl cyclase is altered by phosphorylation (Katada et al. 1985). Recent evidence suggest that this modification might be involved in heterologous desensitization of G_i-coupled GPCRs (Chu et al. 2010) and unusual coupling modes to adenylyl cyclase inhibition (Ventimiglia et al. 2008; Chakraborti et al. 2013).

1.4 $G\alpha_{i2}$ is the major pertussis toxin substrate of neutrophils

In the late 1980s, it became clear that the role of heterotrimeric G proteins is not only confined to regulation of cellular processes via altered levels of cAMP but also extends to regulating other effects, e.g., inositol-phospholipid-specific phospholipase C (PLC) (Williamson 1986). It quickly became clear that based on their differential sensitivity to PTX, at least two distinct G proteins are involved in the latter regulation. Neutrophils from various mammalian species were found to

harbor the PTX-sensitive pathway, since treatment with the toxin blocked all cellular functions known to be triggered by PLC-stimulating GPCRs, e.g., those for fMet-Leu-Phe, leukotriene B4, complement C5a, and platelet-activating factor. The inhibitory effects of PTX were not explained by toxin-dependent alterations of cAMP levels. All three PTX substrates known at that time, G_i, G_o, and transducin (G_t), had been implicated along the way in regulating PLC in neutrophils. Immunochemical studies revealed that the Gα subunit of the main major PTX substrate of human neutrophil plasma membranes differs from the hitherto known Gα subunits. In 1985, Gierschik et al. set out in the Jakobs laboratory to purify this protein(s) from bovine neutrophil membranes for its immunochemical and initial biochemical characterization as a novel Gα protein (Gierschik et al. 1986). Nowadays, it is clear that the purified protein was mainly made up of $G\alpha_{i2}$ and a minor portion of $G\alpha_{i3}$. To clarify which of the two $G\alpha_i$ subunits present in granulocyte membranes functionally interacted with activated formyl peptide receptors, Gierschik and Jakobs developed a novel approach, i.e., GPCR-mediated [^{32}P]ADP-ribosylation of $G\alpha_i$ proteins by cholera toxin. The protocol does not rely on reconstitution techniques and works well in native plasma membranes. The results of the initial and a subsequent paper showed that it was in fact both $G\alpha_{i2}$ and $G\alpha_{i3}$, which coupled to chemotactic-peptide-activated receptors (Fig. 2) (Gierschik and Jakobs 1987; Gierschik et al. 1989a). As the third $G\alpha_i$ protein, $G\alpha_{i1}$, is absent from both HL-60 cells and several other cell types in which inhibition of adenylyl cyclase occurs, the results showed that the primary structure of $G\alpha_i$ proteins alone does not suffice to determine which effector mechanism is regulated by a given G_i protein.

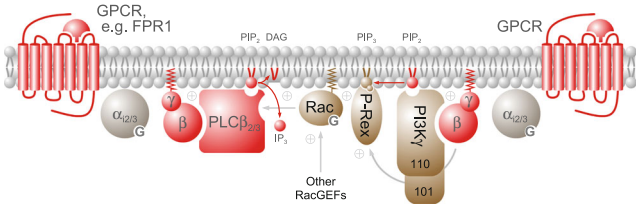

Fig. 2 Stimulation of PLCβ$_{2/3}$ by G-protein βγ subunits. G-Protein-coupled receptors (GPCR) coupled to pertussis-toxin-sensitive G proteins, e.g., the formyl peptide receptor FPR1 in neutrophils, mediate formation of free Gβγ subunits following their activation by agonist, e.g., formyl peptide, binding. Direct activation of PLCβ$_2$ and PLCβ$_3$ by Gβγ was shown by Peter Gierschik in the Jakobs laboratory in the late 80ties (see text). Activated PLCβ isozymes hydrolyze phosphatidylinositol 4,5-bisphosphate (PIP_2) to produce the two second messengers, D-*myo*-inositol-1,4,5-trisphosphate (IP_3) and *sn*-1,2-diacylglycerol (DAG). In subsequent years, Peter Gierschik showed in his own lab that PLCβ$_2$ is also activated by Rac1, Rac2, and, to a lesser extent, by Cdc42Hs (Illenberger et al. 1998). Others have extended these findings to PLCβ$_3$ and Rac3 (Snyder et al. 2003). Rac-mediated stimulation of PLCβ isozymes may be the basis for regulation of PLCβ isozymes in neutrophils and, possibly, other cells by Rac guanine nucleotide exchange factors, e.g., the PIP_3-dependent Rac exchanger P-Rex (Baker et al. 2016) and/or by other Rac GEFs, e.g., the Gβγ-regulated RhoG-ELMO-Dock complex (Xu and Jin 2017) or the protein-tyrosine-kinase-regulated Vav proteins (Bustelo 2014). In intact cells, PLCβ appears to be under multimodal control involving, Gβγ, Rac, and, possibly, other proteins, such as the WD40 protein WDR26 (Sun et al. 2013). For example, the ability of agonist-activated formyl peptide receptors to stimulate PLCβ$_2$ in HL60 granulocytes was markedly attenuated by expression of dominant-negative Cdc42N17 (Rabiet et al. 2002). Likewise, homozygous inactivation of the Rac2 gene in mice causes a reduction of the formyl-peptide-dependent rise in $[Ca^{2+}]_i$ in Gr1$^+$ Mac1$^+$ myeloid cells, which was particularly striking at low concentrations of formyl peptide (Walliser and Gierschik, unpublished results). For further details see text

2 Analysis of GPCR-G protein interactions

2.1 Constitutive basal activity of unoccupied G_i-coupled GPCRs

Given the fact that the GPCR for the chemotactic formyl peptides in cultured HL-60 cells differentiated along the granulocytic lineage is coupled to PTX-sensitive G_i proteins, which mediate PLC activation but not to adenylyl cyclase inhibition, this system was ideally suited as a model system to study the pharmacology of a bona fide PLC-stimulating GPCR. In a series of papers, the group of Karl H. Jakobs showed that the functions of this receptor are profoundly modified by both mono- and divalent cations (Gierschik et al. 1989b; Gierschik et al. 1989c; Herrmann et al. 1989; Gierschik et al. 1991; Kupprion et al. 1993). For example, the group demonstrated that two independent divalent metal binding sites are present on the formyl-peptide-receptor-G protein complex, one on the receptor and one on the G protein, to mediate an increase of agonist affinity of the receptor and enhanced agonist-mediated G protein activation, respectively (Gierschik et al. 1988). The results also suggested that the formyl peptide receptor, similar to many other G_i-protein-coupled receptors, contains a unique binding site for monovalent cations, which is most sensitive to Na^+ ions and present at an intracellular domain of either the receptor itself or some associated protein different from the G_i protein. From today's point of view, the studies on Na^+ regulation of the formyl-peptide-receptor-G protein interaction in native plasma membrane preparations are remarkable, because they indicated that a single monovalent cation binding site was involved in regulating the interaction of the receptor with both the chemotactic peptide agonist and the G_i protein. This suggestion was based on the observation that basal GTPase activity was markedly reduced by both Na^+ and PTX treatment. It was well established at that time that the ADP-ribosylation of G_i by PTX suppressed the GPCR-G protein interaction, but none of the "intrinsic" G_i protein functions (i.e., GDP release, GTP binding, GTP hydrolysis, and Gα-Gβγ subunit dissociation (reviewed in (Gierschik 1992)). Hence, these findings suggested that G proteins interact with and are activated by receptors even in the absence of agonists and (b) that Na^+ ions uncouple unoccupied, but intrinsically active GPCRs from the interaction and activation G protein. In

retrospect, the significance of these findings and their interpretation is related to the fact that credit for the discovery of the concept of constitutively active GPCRs has so far almost exclusively been given to Costa and Herz (e.g., Bond and Ijzerman 2006), based on their publication on antagonists with negative intrinsic activity at δ opioid receptors in 1989 (Costa and Herz 1989), i.e., at exactly the same time as the paper from the Jakobs group on constitutive activity of unoccupied GPCRs. The latter concept was in fact only subsequently suggested by Costa and colleagues (Costa et al. 1990).

2.1.1 Allosteric regulation of GPCRs by cations

The allosteric regulation of GPCRs by cations has very recently gained a marked increase in attention, given the fact that insights into the underlying mechanisms and their structural requirements may provide novel strategies for the design of innovative GPCR drugs targeting the relevant allosteric sites (Thal et al. 2018). Although many Na^+-regulated GPCRs have been known for decades (Gierschik et al. 1988), the structural properties of the GPCR Na^+ binding site remained unexplored until the first structural identification of such a site on the human adenosine A_{2A} receptor (A_{2A}AR) (Liu et al. 2012). Here, a singly Na^+ ion was found to be present in a water cluster in the middle of the seven transmembrane helix bundle, with Na^+ complexed by five oxygen atoms, two side chain oxygens, $O^{\delta 1}$ of Asp52$^{2.50}$ and O^{γ} of Ser91$^{3.39}$, and three water oxygens. The central water cluster itself is held in place by Asp52$^{2.50}$, Ser91$^{3.39}$, and five other highly conserved residues. All seven residues are present in the corresponding positions in the human formyl peptide receptor. Interestingly, high high-affinity A_{2A}AR agonist binding and the presence of Na^+ were found to be mutually exclusive (Liu et al. 2012), providing a structural rationale for the decrease in the number of high affinity formyl peptide receptors and the decrease in the EC_{50} of formyl peptide to stimulate high-affinity GTPase observed by the Jakobs group much earlier (Gierschik et al. 1989b). Mutational replacement of the two key Na^+-binding residues in A_{2A}AR, Asp52$^{2.50}$, and Ser91$^{3.39}$ by alanine residues not only either abrogated (Asp52Ala$^{2.50}$) or reduced (Ser91Ala$^{3.39}$) the negative allosteric effect of Na^+ on agonist binding in HEK293T cells but also either diminished (Ser91Ala$^{3.39}$) if not entirely obliterated (Asp52Ala$^{2.50}$) A_{2A}AR agonist-mediated adenylyl cyclase stimulation altogether. Furthermore, basal, agonist-independent cAMP formation was markedly enhanced in the presence of the Ser91Ala$^{3.39}$ mutant receptor, such that the amplitude of the A_{2A}AR agonist effect was markedly diminished (Massink et al. 2015). Note that these functional effects have already been attributed, more than 25 years earlier, to the actions of Na^+ on GPCRs like the formyl peptide receptor and many other GPCRs by the Jakobs group (Gierschik et al. 1988; Gierschik et al. 1989b).

Very recently, the allosteric Na^+ site of A_{2A}AR has been shown to specifically interact with one of its four activation microswitches, the NPxxY$^{7.53}$ motif present towards the end of the 7th A_{2A}AR transmembrane helix (White et al. 2018). "Microswitch activation" denotes rotamer changes in side chains that are highly conserved between GPCRs (Katritch et al. 2013) and is thought to play a key role among the various phases of signal transmission through the GPCR transmembrane helix bundle. Within this sequence of events, "microswitch activation" follows "agonist engagement" and "signal propagation" to be followed by "G protein coupling" (Wescott et al. 2016). According to the model put forward by White et al. (2018), coordination of Na^+ between helices II, III, VI, and VII (i) decreases the strength of agonist-mediated (and, presumably, at least to some extent, agonist-independent) helix-helix interactions and (ii) also modulates the conformation of the orthosteric ligand binding pocket to diminish its agonist (but not antagonist) affinity. On the basis of these considerations, small molecules interacting with the Na^+-binding site of specific GPCRs are currently reasoned to be attractive candidates for allosteric antagonists of several clinically important GPCRs like the A_{2A}AR (Massink et al. 2016), the leukotriene B4 receptor subtype BLT1 (Hori et al. 2018), and the dopamine D_2 receptor (Draper-Joyce et al. 2018).

2.2 GPCR-regulated GTPγS binding in membranes and permeabilized cells

As it was evident that the GTP analog guanosine-5′-[γ-thio]triphosphate (GTPγS) cannot (or only very poorly) be hydrolyzed by G proteins and [^{35}S]-labeled version became available, the binding of [^{35}S]GTPγS was used in the characterization of purified G proteins (Bokoch et al. 1984; Sternweis and Robishaw 1984). Subsequently, the agonist-stimulated binding of [^{35}S]GTPγS was used to probe mechanisms of GPCR activation in reconstituted systems containing partially purified G proteins and GPCRs (Asano et al. 1984; Kurose et al. 1986). Based on the findings on the regulation G_i protein-GPCR interaction described above, the group of Karl H. Jakobs was the first to adapt this method to be used in native membranes (Hilf et al. 1989). In this seminal study, porcine atrial membranes enriched in the M_2 muscarinic acetylcholine receptor (M_2) were used to study carbachol stimulated [^{35}S]GTPγS binding to pertussis toxin-sensitive G proteins of the G_i family. Several important attributes of the [^{35}S]GTPγS binding assay that are applicable to the assay in general were already described in this study. There is an absolute requirement for Mg^{2+} ions to see agonist-induced binding. The presence of a second guanine nucleotide, preferably GDP, and Na^+ ions is helpful to suppress basal binding and unoccupied receptor activity. Thus, a quantitatively significant stimulated-to-basal-ratio was obtained, which then allows for

the generation of agonist-concentration-dependent-response curves. Interestingly, the method could also be used to uncover the inverse agonist nature of "antagonists," such as atropine by the concentration dependent inhibition of [^{35}S]GTPγS binding to the unstimulated M_2-receptor-containing membranes (Hilf and Jakobs 1992b). In the following years, the method was adapted to a variety of membranous and solubilized systems (Gierschik et al. 1991; Liebmann et al. 1991; Gachet et al. 1992a; Gachet et al. 1992b; Hilf and Jakobs 1992a; Kupper et al. 1992; Liebmann et al. 1992; Wieland et al. 1992a; Wieland et al. 1992b; Offermanns et al. 1994; Wieland et al. 1994; Siffert et al. 1995) and to measure agonist-induced [^{35}S]GTPγS to native G_s, $G_{i/o}$, $G_{q/11}$, and G_t proteins by optimizing the experimental conditions (Wieland and Jakobs 1994). In the following, the assay was further developed to be used in cells permeabilized with different agents (Wieland et al. 1995). Using [^{35}S]GTPγS binding in permeabilized Sf9 insect cells and baculovirus-mediated expression of GPCRs and heterotrimeric G proteins, it was later shown that such system could be used as a modular system to study GPCR-G protein interaction (Kühn et al. 2002). The method has been modified by others, for example to be used in brain slices (Sim et al. 1995) and for ultra-high-throughput screening of unknown GPCR agonists (Johnson et al. 2008). It thus has become a valid method in pharmacological research, which is frequently used in different settings even 30 years after its first use in porcine atrial membranes (Cechova et al. 2018; Gado et al. 2018; Sim-Selley et al. 2018).

Interestingly, the [^{35}S]GTPγS binding assay also allowed to obtain information on the stoichiometry of the GPCR/G-protein interaction in native plasma membranes. In membranes of neutrophil-like differentiated HL-60 cells, Karl H. Jakobs and co-workers compared the number of chemotactic-peptide-sensitive [^{35}S]GTPγS binding sites to the number of chemotactic formyl peptide receptors present in these membranes. They provided evidence that a single formyl peptide receptor is capable of catalyzing the binding of [^{35}S]GTPγS to and thus the activation of multiple (up to 20) G_i proteins in native plasma membranes (Gierschik et al. 1991). Importantly, these results demonstrated for the first time the catalytic activation of non-retinal G proteins by a ligand-activated GPCR in native conditions. The findings not only supported earlier kinetic but also indirect data on the behavior of the β-adrenoceptor in turkey erythrocytes (Tolkovsky and Levitzki 1978) but also confirmed conclusions from in vitro reconstitution studies performed in synthetic phospholipid vesicles (Pedersen and Ross 1982; Hekman et al. 1984). Nevertheless, the number of G_i molecules activated per activated formyl peptide receptor is lower than the number of G_t molecules activated by a single light-activated rhodopsin in native retinal rod outer segment membranes (~ 500) (Kwok-Keung Fung and Stryer 1980); most likely due to fact that the abundance of G_t in the latter membrane is at least 10-fold higher than the abundance of G_i proteins in granulocyte membranes (Heitzmann 1972; Camps et al. 1992b; Pugh and Lamb 2000). Hence, non-retinal, ligand-binding GPCRs were shown to be similar to light-activated rhodopsin, containing all-trans-retinal as covalently bound receptor agonist, in terms of their ability to catalytically activate G_i proteins in native plasma membranes.

2.3 GPCR-induced high affinity GTPase

Before the optimization of the GPCR-induced [^{35}S]GTPγS binding assay by Karl H. Jakobs and co-workers, the classical method to determine agonist-induced heterotrimeric G protein activation was the measurement of high-affinity steady-state GTP hydrolysis (Cassel and Selinger 1976). Although this method has been used successfully in many laboratories, it often failed due to high rates of "unspecific background GTP hydrolysis." By transferring the insights obtained from studying the GPCR-G_i protein interaction and agonist-induced [^{35}S]GTPγS binding to the measurement of GPCR-stimulated high-affinity GTPase (Gierschik et al. 1989b; Gierschik et al. 1989c; Hilf and Jakobs 1989; Hilf and Jakobs 1992a), this method was optimized too. Together with the agonist-induced [^{35}S]GTPγS binding, the optimized GPCR-stimulated high affinity GTP hydrolysis assay thus became a hallmark method to study GPCR-induced heterotrimeric G protein activation (Gierschik et al. 1994; Wieland and Jakobs 1994).

2.4 GPCR-induced guanosine triphosphate release

In line with older data reporting an agonist-induced release of the stable GTP analog guanosine-5′-[(β,γ)-imido]triphosphate (GppNHp) from heterotrimeric G proteins (Cassel and Selinger 1977a; Citri and Schramm 1982; Motulsky and Insel 1983), the group of Karl H. Jakobs was also the first to show that also the binding of GTPγS to heterotrimeric G proteins is not as "irreversible" as anticipated before. Like the well-known induction of the dissociation of GDP from an interacting heterotrimeric G protein by the agonist-stimulated GPCR, such stimulated receptors can also induce a release of bound [^{35}S]GTPγS in the presence of a competing guanine nucleotide (Hilf and Jakobs 1992a; Hilf et al. 1992; Kupprion et al. 1993), raising questions about the dissociation of heterotrimeric G proteins into a GTP-bound Gα-subunit and a Gβγ-dimer. The possibility of a GPCR-induced release of GTP from activated G proteins to be functionally relevant for signal transduction in living cells, however, was disputed, and the occurrence of such a GTPγS or GppNHp release was attributed to using membranes or solubilized fractions and rather artificial conditions. Using FRET-based protein-protein interaction methods, a recent report (Hommers et al. 2010) showed that indeed G proteins might be put in reverse mode by an agonist-induced release of GTP, which

apparently attenuated the activation of the effector protein; in this case, the GIRK channel, by trapping nucleotide-free G proteins at the agonist-activated GPCR upon strong stimulation. Still, this study is not a proof that such a mechanism is relevant at regular expression levels of receptors, G proteins and effectors, but the accordance with the about 20 years older data from the group of Karl H. Jakobs is intriguing.

3 Interaction of nucleoside diphosphate kinases with heterotrimeric G proteins

3.1 Localized GTP supply for G protein activation

Since the work of Northup et al. (1980), it was clear that G proteins in the inactive state are heterotrimers and GDP is bound in the Gα subunit. It has been proven even by co-crystallization efforts (Rasmussen et al. 2011) that agonist-activated GPCRs induce the release of GDP from G protein upon agonist binding, and GTP, which is enzymatically kept at much higher intracellular concentrations than GDP, subsequently binds to the empty nucleotide-binding pocket in the Gα-subunit. It is generally accepted that the conformational change induced by GTP binding allows the GTP-bound Gα-subunit as well as the Gβγ-dimer to interact with their respective effector proteins. After the hydrolysis of GTP to GDP and inorganic phosphate by the intrinsic GTPase activity present in the Gα subunit, the G protein returns to its inactive heterotrimeric state. A single G protein can be repeatedly activated by an agonist-ligated GPCR, and many GPCRs also interact with more than one G protein subtype. Therefore, based on its high hydrolysis rate, the GTP concentration might locally drop drastically, whereas GDP increases accordingly. A replenishment of GTP nearby or at the site of G protein activation would thus secure an optimal functionality of the signaling cascade (Fig. 3, left panel). Kimura and Shimada were the first to report data for a membrane attached form of the enzyme nucleoside diphosphate kinase (NDPK) and association with glucagon receptors and G_s in rat liver membranes. NDPKs are ubiquitously expressed enzymes that catalyze the transfer of the γ-phosphate from nucleoside 5′-triphosphates (NTP) to nucleoside 5′-diphosphates (NDP) by a ping pong mechanism involving the formation of a high energy phosphate intermediate on a catalytic histidine residue (Morera et al. 2002; Tepper et al. 1994). To date, it is evident that NDPK activity resides in the four class I Nme proteins, Nme1-Nme4, which are also named NDPK A, B, C, and D. In mammalian tissues, the enzyme is mainly cytosolic and forms heterohexamers composed of different combinations of the three major isoforms, NDPK A, B, and C (Gilles et al. 1991; Janin et al. 2000). NDPK D has specific functions in mitochondria. In the late 1980s,

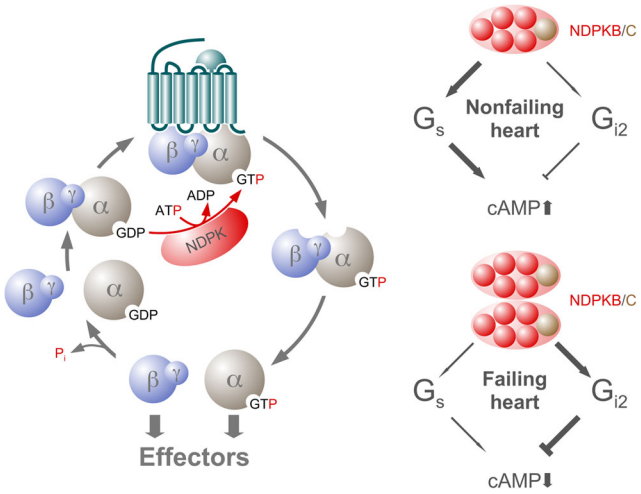

Fig. 3 Role of nucleoside diphosphate kinase in the regulation of heterotrimeric G protein activity. Heterotrimeric G protein cycle between a GDP-bound, inactive trimer, and an active state consistent of the GTP-bound Gα-subunit and the Gβγ dimer. Upon agonist stimulation, the activated GPCR induces the release of GDP from Gα to which then GTP can bind. The activation is terminated by GTP hydrolysis through the GTPase activity of Gα. The concept that NDPK activity is present at the plasma membrane and replenishes GTP from ATP and GDP was already proven by Karl H. Jakobs and others in the 1990s (left panel). Further research identified a complex formation of NDPK B and NDPK C with Gβγ and the possibility of GPCR-independent activation G proteins by NDPKs. Recent data indicate that especially NDPK C is required for the interaction with G_s and G_i proteins in cardiomyocytes and contributes to development of heart failure. Whereas in the non-failing heart, NDPK C is preferentially associated with G_s; NDPK–G_i complexes are predominantly formed and enriched at the plasma membrane in heart failure, resulting in a tonic suppression of cAMP formation (right panel). For further details see text

these details where unknown, but the concept that a membranous form of the enzyme forms a complex with heterotrimeric G proteins and supply these GTPases with GTP was an attractive hypothesis. Karl H. Jakobs was the first to extend this concept to G_i proteins and the inhibition of adenylyl cyclase in human platelet membranes (Jakobs and Wieland 1989). He and his co-workers were the first to take advantage that the poorly hydrolysable GTPγS is formed from ATPγS and GDP by NDPK and thus induces a permanent activation of heterotrimeric G proteins (Seifert et al. 1988; Wieland and Jakobs 1989). They could show that human platelet and myeloid differentiated human leukemia (HL-60) cell membranes contain membrane-bound NDPK activity to allow for basal as well as GPCR-induced activation of effector enzymes (Wieland and Jakobs 1989) and regulation of high-affinity agonist binding to GPCRs by the formed GTPγS (Wieland et al. 1991a), respectively. The group additionally showed that the GTPγs formed by NDPK was more potent than exogenously added GTPγS in regulating high affinity agonist binding indicating a close association of NDPK with G proteins (Wieland and Jakobs 1992). Using G_i/G_o preparations from bovine brain

and purified G_t from bovine retina, it was shown that NDPK B, but not NDPK A, is found in a complex with the Gβγ dimers of these G proteins (Cuello et al. 2003). In a collaboration with the group of Feraydoon Niroomand, which reported before a role of NDPKs in the activation of G_i proteins in human heart failure (Lutz et al. 2001), that the permanent overexpression of NDPK B in a cardiac myocyte derived cell line induced the complex formation with G_s and increased the G_s dependent adenylyl cyclase activity (Hippe et al. 2003). After having left the group of Karl H. Jakobs, Thomas Wieland continued to work on the interaction of NDPKs with heterotrimeric G proteins (Hippe et al. 2007; Hippe et al. 2009). Recent work provides evidence that the NDPK isoform interacting with G_s or G_i are NDPK B/NDPK C heterooligomers. Apparently, NDPK C is required for the binding to Gβγ dimer and mediates the transport of NDPK-G protein complexes to the plasma membrane (Abu-Taha et al. 2017). In the non-failing heart, NDPK C is preferentially found to be associated with G_s (Fig. 3, right panel). Similar as G_i, however, the expression of NDPK C is up-regulated in failing human hearts, and apparently, NDPK-G_i complexes are predominantly formed in heart failure. This switch in prevalence apparently contributes to the chronic suppression of cAMP formation and loss in contractility seen in heart failure and is also in accordance with the data reported by the Niroomand group (Lutz et al. 2001).

3.2 NDPKs acts as protein histidine kinase on Gβ subunits

Already in the beginning of the 1990s, the group of Karl H. Jakobs found that the Gβ subunit of the retinal G protein transducin (G_t) can be thiophosphorylated by GTPγS (Wieland et al. 1991b). As described for NDPKs, the bound thiophosphate is apparently highly energetic and can be re-transferred onto GDP, thus leading to GTPγS formation and subsequent G protein activation in the presence of an at that time unknown membranous co-factor (Wieland et al. 1991b; Wieland et al. 1992c). Using GTP as phosphate donor, the formation of an intermediate phosphoamidate on a histidine residue of the Gβ subunits of heterotrimeric G proteins and a preferential transfer of the phosphate onto GDP was established (Wieland et al. 1993). The first evidence that the missing co-factor acting as protein histidine kinase on the Gβ-subunit is NDPK was presented in 2003 (Cuello et al. 2003). His266 in the $Gβ_1$-subunit was identified as the phosphorylated amino acid. A homologous histidine residue is conserved in the mammalian $Gβ_1$-$Gβ_4$ isoforms, but not in $Gβ_5$. That NDPKs, especially NDPK B, are not only NTP/NDP transphosphorylases but also protein histidine kinases has been confirmed beyond doubt. Several specific protein substrates and counteracting phosphatases have been reported (for review see Attwood and Wieland 2015). Obviously, complex formation and subcellular localization govern the specificity of NDPKs acting as protein histidine kinases. With regard to the interaction of NDPK B/NDPK C with G_s and G_i, the intermediate phosphorylation of the Gβ-subunit on His266 by NDPKs is apparently required for a constitutive, basal activation of heterotrimeric G proteins (Hippe et al. 2011).

4 Regulation of phospholipase C and D activities

4.1 The stimulation of PLC by G_i proteins is mediated Gβγ

Given the fact that PLC was already known at the time to be, at least partially, cytosolic in myeloid-differentiated HL-60 cells, cytosol preparations of these cells were used to establish a system in which a GTPγS-stimulated inositol phosphate formation was observed (Camps et al. 1992b). It was anticipated that this stimulation was either mediated by a Gα-subunit of a heterotrimeric G protein or monomeric GTPases, which were already known to be present in the cytosol of granulocytes at the time. In order to discriminate between the two possibilities, the effect of purified, detergent-free retinal $G_tβγ$ was examined. Rather than causing an inhibition by interfering with a Gα-mediated PLC stimulation or having no effect on a stimulation caused by a monomeric GTPase (see Fig. 2), $G_tβγ$ markedly stimulated the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2). The effect of $G_tβγ$ was (i) unrelated to a putative modification of the artificial phospholipid substrate used in these assays, (ii) not only limited to HL-60 cells but also observed in peripheral blood granulocytes, and (iii) specific for only one of the PLC isoforms present in HL-60 cell cytosol. The stimulatory effect was not only limited to the farnesylated $G_tβγ$ but also observed for the more lipophilic, detergent-solubilized Gβγ from bovine brain carrying a geranylgeranyl moiety. Importantly, the Gβγ-induced stimulation was fully reversed by the addition of purified, GDP-bound $G_tα$, a method meanwhile often used to prove for the active involvement of Gβγ even in complex signaling pathways (Zhou et al. 2007; Zhou et al. 2008). In retrospect, it is important to note that these results were obtained at a time when regulation of G protein effectors, e.g., ion channels, by Gβγ rather than Gα was a highly controversial issue. Claims to the former were met with extremely high skepticism (e.g., Birnbaumer and Brown 1987). Several points of criticism were raised, e.g., the use of detergents, insufficient quality of the purified Gβγ, and the requirement of relatively high concentrations to obtain stimulation. In fact, even in today's literature, the question

whether or not Gβγ is active signaling molecule is listed among major resolved controversies in neuroscience, now well suited to engage students to read primary literature (Brasier 2017). As a corollary of this conflict, it was extremely difficult at the time to publish these findings in a renowned journal. The manuscript, upon serial submission to a variety of journals, received many highly conservative, dogmatic, and, in hindsight, even sub-intellectual comments. It took the contributing scientists in the group of Karl. H. Jakobs plenty of patience, strength, even stamina, to cope with these tribulations. In the end, the work was published in the European Journal of Biochemistry and, following its presentation at national and international meetings, several other groups published very similar findings (Blank et al. 1992; Boyer et al. 1992; Park et al. 1993; Smrcka and Sternweis 1993). This ultimately led to a highly recognized paper (Camps et al. 1992a) and provided the group with full scientific credit for their findings. The original paper was finally highlighted as a hot paper in The Scientist in 1994 (Camps 1994) and its main finding, the $G_i\beta\gamma$-mediated stimulation of PLCβ isoforms, and is now contained in major textbooks and included on signal transduction charts found on the walls of many laboratories. Peter Gierschik left the group of Karl H. Jakobs in 1992 and continued working on G protein and monomeric GTPase-mediated signaling in his own group, first in Heidelberg and later in Ulm. The regulation of PLC isoforms by monomeric GTPases is still pursued further in his laboratory.

4.2 Regulation of PLD activity

4.2.1 Differential regulation of PLD activity by PTX-insensitive G proteins

In the beginning of the 1990s, the group of Karl H. Jakobs got interested in the molecular mechanisms underlying the GPCR-mediated regulation of phospholipase D (PLD) (Offermanns et al. 1994; Schmidt et al. 1994; Rümenapp et al. 1996). Pioneering work dating from 1948 had indicated that PLD represents a distinct, phosphatidylcholine-specific phosphodiesterase activity leading to the formation of the bioactive lipid mediator phosphatidic acid (PA). It took until the mid-1980s to bring PLD signaling to the very forefront of biomedical research through the fundamental discovery that PLD is rapidly and dramatically activated in response to a variety of external stimuli, such as hormones, neurotransmitters, and growth factors (Cockcroft 2001; Exton 2002). To date, this bioactive lipid mediator is implicated in the regulation of a subset of cellular responses as diverse as calcium mobilization, rearrangements of the actin cytoskeleton, and mitogenesis. PA bears the capacity to directly interact with a huge variety of cellular signaling molecules, thereby inducing both changes in their subcellular localization and activity, and subsequently forming a rather dynamic network of signaling hubs (Tanguy et al. 2018). In the early 90s, the group of Karl H. Jakobs used HEK293 cells stably expressing different subtypes of muscarinic acetylcholine receptors as prototypical GPCRs. In these cells, the M_3 muscarinic acetylcholine (M_3) receptor was shown to couple, via pertussis toxin-insensitive G proteins to activation of both PLC and PLD (Fig. 4) (Offermanns et al. 1994; Schmidt et al. 1994). This mode of coupling was not restricted to the HEK293 cell model. A later collaboration with the group of Thomas Wieland demonstrated that activation of PLD GPCRs for endothelin-1, thrombin, and noradrenaline endogenously expressed in neonatal rat cardiomyocytes required the activation of PTX- insensitive G proteins (Fahimi-Vahid et al. 2002; Gosau et al. 2002) Treatment with PTX did, however, not allow to discriminate between the G_q and G_{12} family of G proteins, which were already implicated in PLD regulation (Dhanasekaran and Dermott 1996; Exton 2002; Xie et al. 2002). Hence, Thomas Wieland suggested to use regulators of G protein signaling (RGS) proteins, which act as GTPase-activating proteins (GAPs) promoting GTP hydrolysis and deactivation of Gα subunits (Wieland and Chen 1999), thus representing efficient experimental tools allowing the G protein-subtype-specific inhibition of agonist responses. To specifically discriminate between G_q-and G_{12}-family-member-dependent signaling, two members of the RGS family were of particular interest: RGS4, which has been shown to exhibit GAP activity for G_q-, but not for G_{12}-family proteins (Huang et al. 1997), and the N-terminal RGS homology domain of Lsc (Lsc-RGS), the murine homolog of a the guanine nucleotide exchange factor (GEF) p115RhoGEF. The latter has been shown to be a GAP specifically for G_{12}-, but not for G_q-family members (Kozasa et al. 1998; Wieland and Mittmann 2003). By overexpression of these RGS proteins, the group of Karl H. Jakobs showed that the coupling of M_3 receptors in HEK293 cells to PLC relies on G_q family members, whereas the stimulation of PLD was dependent on the activity of G_{12} class proteins (Rümenapp et al. 2001). Subsequent studies in neonatal rat cardiomyocytes using a similar approach confirmed that RGS proteins are valuable tools to dissect coupling mechanisms of distinct, endogenously expressed GPCRs to PLC and PLD (Fahimi-Vahid et al. 2002, Gosau et al. 2002). The method is meanwhile generally accepted and widely used to analyze complex PTX-insensitive signaling pathways even in primary cells such as endothelial cells and cardiomyocytes (Carbajo-Lozoya et al. 2012; Del Galdo et al. 2013; Toth et al. 2018).

Using the prototypical model of the M_3-receptor-expressing HEK293 cells, the group of Karl H. Jakobs analyzed the regulation of PLD activity in great detail and far beyond the activation heterotrimeric G proteins. The carbachol-induced activation of PLD was largely independent of the concomitant activation of PLC and its subsequent signals like the elevation of intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) and the

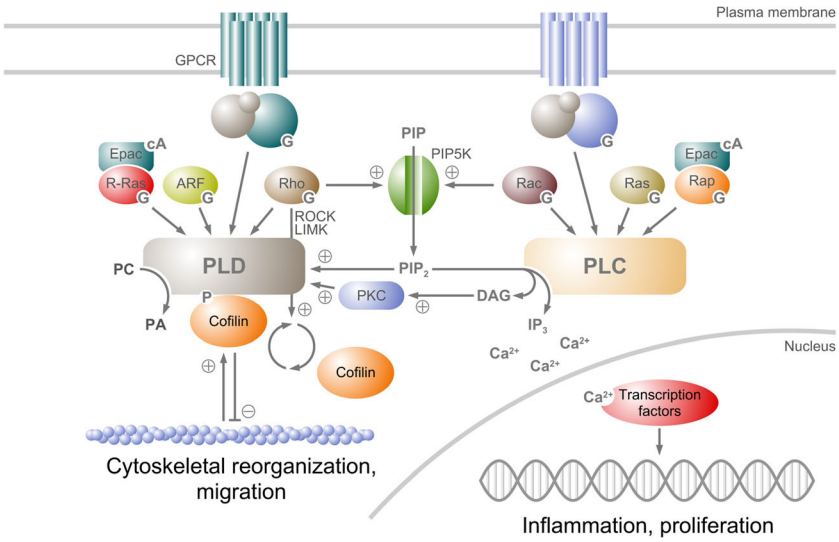

Fig. 4 Regulation of phospholipase D and phospholipase C activities by GPCRs. A variety of distinct GPCRs couple to PLD and PLC via heterotrimeric G proteins and members of the low molecular weight GTP binding protein superfamily (Rho, Rac, Rap, R-Ras, Ras, ARF). G_i- and G_q-family members directly stimulate PLCβ isoforms via direct interaction with Gβγ- or Gα-subunits, respectively. G proteins additionally activate Rac and Ras via Gβγ-dependent pathways. Epac senses the rise in cellular cAMP (cA) concentrations in response to the activation G_s-coupled GPCRs and subsequently initiates the stimulation of PLCε. PLC activation induces inositol trisphosphate (IP_3)-mediated rises in $[Ca^{2+}]_i$ and diacylglycerol (DAG)-mediated activation of PKC isoforms which subsequently activate PLD. G_{12} family members regulate PLD activity via activation of ARF and Rho proteins. Epac signals also to PLD via R-Ras. PLD cleaves the most abundant membrane lipid phosphatidyl choline (PC), thereby leading to the generation of the bioactive lipid phosphatidic acid (PA). PLD stimulation is sensitive to the cellular content of PIP_2 and the phosphorylation state of the actin regulator cofilin, both controlled by the Rho/ROCK-dependent pathways. For further details, see text

activation of PKC (Schmidt et al. 1994; Schmidt et al. 1995b). This finding was in accordance with later findings by others showing a PKC-independent activation of PLD in many other cell lines, including permanent cell lines like Mardin-Arby canine kidney cells and primary cells, such as rabbit aortic vascular smooth muscle cells (Cockcroft 2001, Exton 2002). Nevertheless, Karl H. Jakobs and co-workers showed in later research that PLC/PKC-dependent and -independent activation of PLD (Fig. 4) can occur within the same cell as a result of distinct signaling pathways initiated by specific GPCRs. For example, while the endothelin-1 type A receptor of neonatal cardiac myocytes activates PLD in a G_q/PLC/PKC-dependent manner, the protease-activated receptor 1 uses a G_{12}-family-member- and ADP-ribosylation-factor (ARF)-dependent pathway (Fahimi-Vahid et al. 2002).

4.2.2 Contribution of low molecular mass GTP-binding proteins to PLD regulation

Using the HEK293 cell model, the group described that various signal transduction components, including low molecular mass GTP-binding proteins, tyrosine kinases, and PIP_2, are involved as regulatory proteins or phospholipid cofactor in controlling the activity of PLD. Of the low molecular mass GTP-binding protein superfamily, a contribution of ARF and central role for Rho family members was revealed (Rümenapp et al. 1995; Schmidt et al. 1996b; Rümenapp et al. 1998). Studies with *Clostridium difficile* toxin B, which inactivates Rho, Rac, and Cdc42 and with the selective Rho-inactivating *Clostridium botulinum C3 exoenzyme*, showed that Rho proteins regulate the supply of PIP_2 (Schmidt et al. 1996b; Schmidt et al. 1996c) not only in HEK293 cells model but also in primary cells such as neonatal rat cardiac myocytes (Fahimi-Vahid et al. 2002, Gosau et al. 2002). These findings were in general agreement with reports from others indicating that PIP_2 profoundly increases the activity of PLD and supporting the role of the phospholipid as an essential PLD cofactor in vitro (Liscovitch et al. 1994; Exton 2002).

Activation of PKC with phorbol esters, such as phorbol 12-myristate 13-acetate (PMA), induced a marked PLD stimulation in HEK293 cells in a manner additive to the activation of the M_3 muscarinic receptor (Schmidt et al. 1994; Rümenapp et al. 1995; Rümenapp et al. 1997). In contrast to the carbachol induced PLD activation, the PMA stimulated PLD activity in HEK293 cells was by far less sensitive to the inactivation of Rho proteins by toxin B (Schmidt et al. 1996b). By using a distinct subset of clostridial toxins, namely *Clostridium sordellii* lethal toxin and the toxin B variant *C. difficile* toxin B-1470, which both inactivate Ras-like GTPases, the group of Karl H. Jakobs found that these toxins potently and specifically inhibited the PMA-stimulated PLD activity. In accordance with their substrate specificity, both toxins did not affect the supply of the PLD co-factor PIP_2 but blunted the activation of PLD by the Ras-like monomeric GTPases Ral (Schmidt

et al. 1998). A different class of membrane receptors, i.e., receptor tyrosine kinases, was identified as the source of the Ras/Ral-GEF/Ral signaling cascade and the subsequent stimulation PKC mediating the PMA-induced PLD activation in HEK293 cells (Voss et al. 1999).

4.2.3 The role of the PLD isozymes PLD1 and PLD2

The above summarized data, which indicate a rather complex regulation of PLD activity (Fig. 4) by multiple signaling pathways within the same, led to a general consent on the existence of different PLD isozymes in one cell type. The generation of catalytically inactive mutants of the two meanwhile identified isoforms of PLD, PLD1, and PLD2 represented a first crucial step to further characterize the coupling of membrane receptors to PLD1 and/or PLD2 (Exton 2002; Frohman 2015). Using these PLD mutants, the group of Karl H. Jakobs provided evidence that GPCRs can couple to both PLD1 and/or PLD2 depending on the activated signaling pathways. Receptor tyrosine kinases however couple to PLD2 (Voss et al. 1999; Lopez De Jesus et al. 2006; Han et al. 2007). These general principles of PLD regulation identified in the seminal studies performed in the group of Karl H. Jakobs were later confirmed and further extended by other research groups for a variety of cells and tissues (Frohman 2015). Based on the development on PLD-subtype-specific inhibitors from 2009 onwards, studies revealing receptor coupling to distinct PLD isozymes were performed in primary cells, tissues, and animal models, identifying these enzymes as interesting novel therapeutic targets (Frohman 2015; Brown et al. 2017).

4.2.4 Contribution of Rho-dependent protein kinases to PLD regulation

Besides identifying pathways leading to direct activation of PLD, the group of Karl H. Jakobs studied also indirect, co-factor-dependent regulation of PLD in great detail, again using their prototypical HEK293 cell model. The group for example showed that Rho proteins stimulate the activity of PIP_2-generating phosphatidylinositol-4-phosphate 5 (PIP5) kinases (Fig. 4) (Oude Weernink et al. 2000; Oude Weernink et al. 2004; Oude Weernink et al. 2007). Rho-dependent protein kinase (ROCK), a serine/threonine kinase, the activity of which depends on GTP-bound Rho proteins, regulates both PIP5 kinase and PLD (Oude Weernink et al. 2000), further supporting the concept of highly dynamic interconnected signaling networks. The activation of PIP5 kinase by the Rho/ROCK pathway and the subsequent generation of PIP_2 offered a link to not only both PLD (co-factor supply) and PLC (substrate supply) but also to the actin cytoskeleton. ROCK directly phosphorylates the serine/threonine kinase LIM kinase, which in turn phosphorylates and inactivates the actin depolymerization factor cofilin, ultimately leading to enhanced actin polymerization. Cofilin is the so far only known substrate of LIM kinase, and it was known that the cofilin-actin interaction is tightly controlled by phosphocycling. At the end of the 1990s, it was generally believed that phosphorylation of cofilin by LIM kinase at serine 3 in its actin binding domain leads to inactivation (Bamburg 1999; Chen et al. 2000). Studies In the group of Karl H. Jakobs changed the signaling dogma of cofilin profoundly. Indeed, phosphorylated cofilin exerts its biological activity by directly interacting with PLD1 leading to the rearrangement of the actin cytoskeleton (Fig. 4) (Han et al. 2007). To date, the concept of phosphocycling of cofilin is studied in the context of several chronic disorders, such as neurodegenerative diseases, and is linked to mitochondrial functions (Bernstein and Bamburg 2010; Bamburg 2011). The initial studies on cofilin phosphorylation and PLD were performed by Li Han and Martina Schmidt in the group of Karl H. Jakobs. Martina Schmidt left in 2004 and started her own group at the University in Groningen, The Netherlands. Li Han changed to the Military Academy for Disease Control in Beijing, China. Together with Martina Schmidt, he recently demonstrated that the phosphocycling of cofilin and its interaction with PLD are of crucial importance to the human host defense response to pathogens, such as *Listeria monocytgenes* and *Aspergillus fumigatus* (Han et al. 2011a; Han et al. 2011b; Li et al. 2012).

As shown for other areas in other chapters of this review, the work performed by Karl H. Jakobs on the regulation of PLD performed over more than 15 years was ground breaking and provided the scientific basis for translational studies now being performed from his former trainees and others.

4.3 Regulation of phospholipase C-epsilon (PLCε) activity

4.3.1 Requirement for a guanine nucleotide exchange protein activated by cAMP

In studies using *C. sordellii* lethal toxin and *C. difficile* toxin B-1470 mentioned above, the group of Karl H. Jakobs made the rather intriguing observation that treatment of M_3-receptor-expressing HEK293 cells with either toxin largely reduced the stimulation of PLC and the subsequent elevation in $[Ca^{2+}]_i$ (Schmidt et al. 1998; Voss et al. 1999; Schmidt et al. 2001). Both toxins inactivate the Ras-like proteins Ral, Rap, and the Rho family member Rac, whereas the additional inactivation of Ras is a feature of lethal toxin alone. In contrast to findings with Rho-inactivating toxins (Schmidt et al. 1996a; Schmidt et al.

1996b), the drop in the PLC response and the subsequent $[Ca^{2+}]_i$ elevation was not due to a reduction in the cellular supply of the PLC substrate PIP_2. Rather unexpectedly, it turned out that the low molecular mass GTP binding protein Rap was involved in the regulation of PLC activity (Fig. 5) (Schmidt et al. 2001; Evellin et al. 2002; Keiper et al. 2004; Stope et al. 2004). Interestingly, studies screening for novel cAMP-regulated factors found cAMP-binding domains in a putative RapGEF. This protein, now termed exchange protein directly activated by cAMP (Epac) indeed exhibited RapGEF activity stimulated by cAMP and therefore presented a novel cAMP effector (de Rooij et al. 1998; Kawasaki et al. 1998).

The crosstalk between Ca^{2+}- and cAMP-dependent signaling offers modes for the fine-tuning of crucial processes, such as neuronal learning and memory and cardiac excitation-contraction coupling on a cellular level (Rich et al. 2001; Beavo and Brunton 2002; Zaccolo et al. 2002; Schmidt et al. 2007; Grandoch et al. 2010). With regard to the regulation of PLC, earlier studies pointed to an inhibition of PLC activation by protein kinase A (PKA). Nevertheless, an increasing number of reports indicated that several G_s-coupled GPCRs are able to concomitantly stimulate PLC activity (Park et al. 1992; de la Pena et al. 1995; Lin et al. 1995; Liu and Simon 1996; Schmidt et al. 2004). In the late 1990s, mechanisms involving Rap proteins in PLC regulation were unknown. Nevertheless, the concept that GPCRs positively regulate PLC signaling in response to cAMP elevation was an attractive hypothesis. The group of Karl H. Jakobs reported that classically G_s-coupled GPCRs, e.g., recombinantly expressed β_2-adrenoceptors and endogenous GPCRs for PGE1 in HEK293 and N1E-115 neuroblastoma cells, respectively, induced PLC activation and subsequent Ca^{2+} mobilization, in a cAMP-dependent manner without contribution of the canonical cAMP effector PKA (Schmidt et al. 2001;

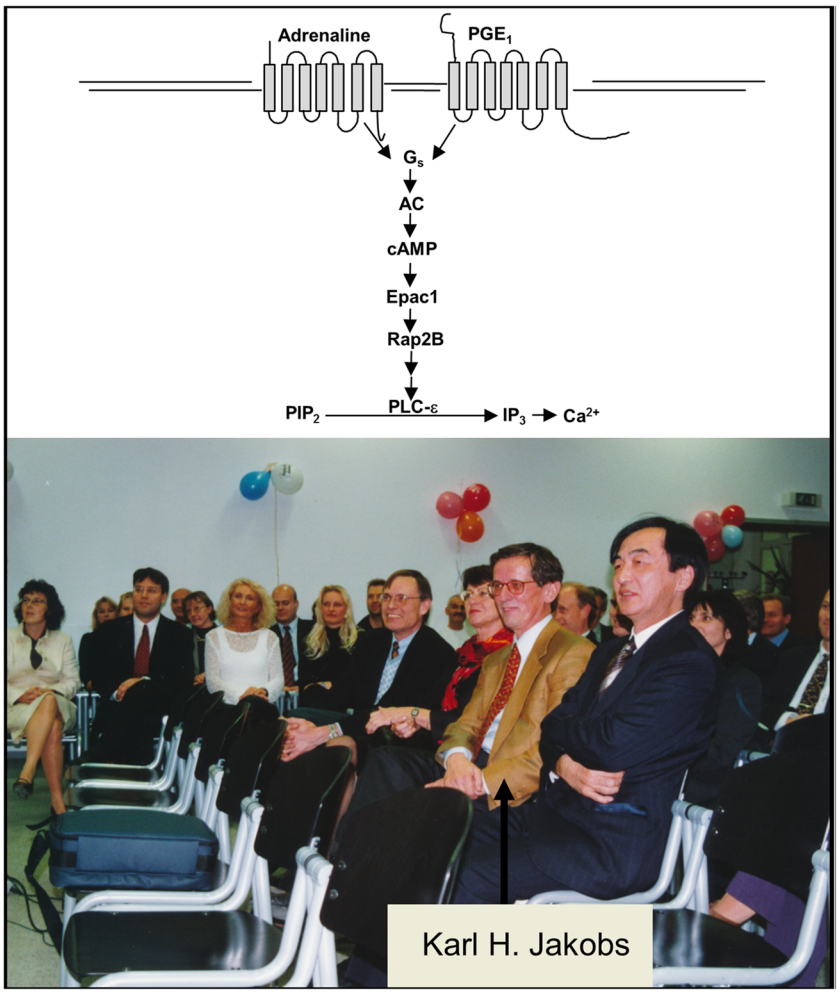

Fig. 5 Model for β_2-AR- and prostanoid-receptor-mediated phospholipase C and calcium signaling as proposed in 2001. Upper panel: Scheme adapted from (Schmidt et al. 2001). Receptors coupling to G_s stimulate adenylyl cyclase (AC), resulting in elevated cAMP levels and activation of Epac1. Epac1 then catalyzes GTP-loading on the low molecular weight GTP-binding protein Rap2B, which leads to PLC-ε activation. Lower panel: Picture taken at a scientific symposium in honor of Karl H. Jakobs' sixtieth birthday hold at the Institute in Essen in November 2001

Evellin et al. 2002; Keiper et al. 2004; Grandoch et al. 2010; Schmidt et al. 2013). The identification of PLCε (Lopez et al. 2001; Song et al. 2001), a novel PLC isoform containing both a RasGEF and Ras/Rap-binding domain within the context of the archetypical PLC structures, offered a plausible new candidate. Using mutants of different PLC isoforms, the group of Karl H. Jakobs was the first to show that PLCε was indeed the missing link in this novel signal cascade allowing for G_s-coupled GPCR-induced $[Ca^{2+}]_i$ elevation upon cAMP-induced Epac activation. In turn, Rap is activated by GTP-loading followed by PLCε stimulation (Fig. 5). As an additional proof of concept for this pathway, the G_s-dependent stimulation of PLCε was blunted upon co-stimulation of endogenously expressed G_i-coupled GPCRs in HEK293 and N1E-115 neuroblastoma cells (vom Dorp et al. 2004). In subsequent studies, it was shown that the M_3 receptor activates PLCβ_1 and PLCε simultaneously (Evellin et al. 2002). In addition, receptor tyrosine kinases were found to bear the capacity to activate PLCε (Stope et al. 2004). Thus, PLCε is to date regarded as a central signaling hub capable to integrate signals from a huge subset of membrane receptors (Bunney and Katan 2006; Smrcka et al. 2012). It was reported that PLCε-dependent signaling plays a role in various diseases, e.g., cancer and cardiac dysfunction (Wang et al. 2005; Schmidt et al. 2007; Dusaban and Brown 2015). These novel findings perfectly match earlier studies from members of the group of Karl H. Jakobs, reporting a link of PLCε to activation of extracellular signal-regulated kinases (Keiper et al. 2004).

4.3.2 Specific roles for the Epac isoforms Epac1 and Epac2

The specific Epac activator 8-pCPT-2'-O-Me-cAMP turned out as a valuable tool to assign functional responses diverse, such as calcium mobilization, proliferation, neurotransmitter and chemokine/cytokine release, migration, and apoptosis in many cell lines and primary cultures (Grandoch et al. 2009a; Grandoch et al. 2009b; Grandoch et al. 2010; Sand et al. 2010). To date, two isoforms of Epac1 and Epac2 have been identified. The latter one exists in several splice variants. The Epac proteins exhibit a very distinct expression profile, which is object to alterations during development and disease progression. In addition, Epac1 and Epac2 interact with very distinct subsets of signaling molecules, and it is generally accepted that specific Epac signalosomes drive specific cellular responses. Thus, using genetically modified cell and animal models, several functions could be specifically attributed to Epac1 and/or Epac2 (for recent review see Schmidt et al. 2013; Parnell et al. 2015; Robichaux 3rd and Cheng 2018). In the heart, Epac1 is involved in the development of hypertrophy and ischemia reperfusion injury, effects linked to the role Epac1 to maintain mitochondrial integrity. In contrast, Epac2 is involved in the progression of arrhythmias (Metrich et al. 2008; Fazal et al. 2017; Laudette et al. 2018). On the one hand, in the developing brain, Epac1, but not Epac2, defines the outgrowth of neurons (Munoz-Llancao et al. 2015; Munoz-Llancao et al. 2017). On the other hand, Epac2, but not Epac1, promoted the process of memory retrieval (Ostroveanu et al. 2010). Martina Schmidt now with the University of Groningen, The Netherlands focused on the role of Epac1 and Epac2 in the lung. Using Epac1 and Epac2 deficient mice, she demonstrated that Epac1 diminished remodeling processes in the lung, whereas Epac2 increased inflammatory responses (Oldenburger et al. 2012; Oldenburger et al. 2014). Based on these functional insights, from 2012 onwards, Epac1 and Epac2 inhibitors were developed (Schmidt et al. 2013, Parnell et al. 2015, Robichaux 3rd and Cheng 2018). In 2015, the first Epac1- and Epac2-specific activators were reported (Schwede et al. 2015). Whether these substances offer the possibility for specific therapeutic interventions on the level of Epac1 and Epac2 has to be proven. Nevertheless, the work on Epac initiated in the group of Karl H. Jakobs and others about 20 years ago opened the door for these options.

5 Guanine nucleotide exchange factors for RhoGTPases as mediators of heterotrimeric G protein-dependent signaling

Based on research on PLC and PLD regulation by low molecular mass GTP binding proteins of the Rho family (Rümenapp et al. 1996; Schmidt et al. 1996a; Rümenapp et al. 1997)), members of the group of Karl H. Jakobs got interested in Rho-protein activating GEFs and their regulation by heterotrimeric G protein-dependent-signaling mechanisms. The first RhoGEF, the group identified, was RhoGEF11, previously named PDZ-RhoGEF, KIAA0380, or GTRAP48 (Rümenapp et al. 1999). It is the third member of the p115RhoGEF family, specifically interacting with $G\alpha_{12}$ and $G\alpha_{13}$ proteins through their N-terminal regulator of G protein signaling (RGS) domain (Wieland and Mittmann 2003). The Jakobs group was the first to show that RhoGEF11 is indeed a GEF specific for RhoA-C, but not for Rac1 or Cdc42 (Rümenapp et al. 1999). RhoGEF11 has meanwhile been linked to several signaling pathways. For example, it is involved in the development of diet-induced obesity and type 2 diabetes (Chang et al. 2015), dendritic morphogenesis (Mizuki et al. 2016), and schizophrenia (Bauer et al. 2008; Mizuki et al. 2014). Jakobs and co-workers were also first to prove the RhoA-C GEF activity of RhoGEF17 (Rümenapp et al. 2002), RhoGEF18 (Blomquist et al. 2000), and RhoGEF25 (Lutz et al. 2004). RhoGEF17 is not interacting with heterotrimeric G proteins but was found to be activated by phosphorylation with cyclic GMP-dependent kinase I

(Lutz et al. 2013). It contains an actin-binding domain (Mitin et al. 2012), is apparently involved in the regulation of endothelial cell-cell adhesion (Mitin et al. 2013; Ngok et al. 2013), and is a risk gene for intracranial aneurysms (Yang et al. 2018). RhoGEF18 was originally named p114RhoGEF and was shown to act downstream of M_3 receptors (Blomquist et al. 2000). It was later shown to be activated by Gβγ dimers (Niu et al. 2003) and is important for epithelial cell morphogenesis (Terry et al. 2011) and tubulogenesis (Kim et al. 2015). It therefore plays a role in tumor cell migration (Terry et al. 2012) and metastasis (Song et al. 2013). RhoGEF25, originally named p63RhoGEF, is part of a family of RhoGEFs with two additional members, RhoGEF23, also named Trio, and RhoGEF24, also named Kalirin. In contrast, two RhoGEF25, the latter two proteins, exhibit a second N-terminal GEF domain activating Rac1. Their C-terminal RhoA-C specific GEF domains are homologous to that of RhoGEF25. They directly interact with and are stimulated by activated $G\alpha_q$ and $G\alpha_{11}$ (Lutz et al. 2005; Lutz et al. 2007). RhoGEF25 has meanwhile been shown to mediate $G_{q/11}$-mediated RhoA activation in vascular smooth muscle cells (Lutz et al. 2005, Lutz et al. 2007) and might play a role in the development of hypertension (Calo et al. 2014). It is also important for $G_{q/11}$-dependent RhoA activation in cardiac fibroblasts (Ongherth et al. 2015). Also in here, the work performed in the Jakobs' lab in the 1990 initiated important lines of research with outstretch to our current understanding.

6 Sphingolipid signaling

6.1 Sphingosine-1-phosphate as extracellular messenger and GPCR agonist

Sphingolipid research in Karl H. Jakobs' group started with the observation, made by Martina Schmidt, of a discrepancy between PLC activation and increases in intracellular free Ca^{2+} concentrations by the M_2 receptor in HEK293 cells: while PLC activation was fully insensitive to PTX, the increases in Ca^{2+} concentration were about 50% PTX sensitive, suggesting a Ca^{2+} mobilization pathway independent of PLC (Schmidt et al. 1995a). Chris van Koppen, at that time working in Karl H. Jakobs' department, suggested to use sphingosine-1-phosphate (S1P) as a stimulus, which then was considered as intracellular second messenger mediating cell proliferation and Ca^{2+} mobilization (Olivera and Spiegel 1993; Ghosh et al. 1994). Indeed, when added to HEK293 cells, S1P induced rapid and transient $[Ca^{2+}]_i$ increases, with a surprisingly low half-maximally effective concentration of about 2 nM. Looking at the concentration-response-curve, Karl H. Jakobs suggested testing the influence of PTX, with the idea that a GPCR was mediating the response. Indeed, S1P-induced Ca^{2+} increases in HEK293 and many other cells were PTX-sensitive. Together with Lutz Pott and Moritz Bünemann at the Ruhr-Universität Bochum, the group of Karl H. Jakobs provided the first evidence that extracellular S1P acted via GPCRs, as Bünemann could show that S1P-induced activation of $I_{K.ACh}$ in cardiac myocytes was mediated only from the outside of the plasma membrane in the presence of GTP (Bünemann et al. 1995; Van Koppen et al. 1996b). Later, S1P-GPCRs were identified by others as members of the endothelial differentiation gene family of GPCRs (Lee et al. 1998; Zondag et al. 1998). To date, five S1P receptor subtypes, termed $S1P_1$ - $S1P_5$, have been characterized (Fig. 6). They are nearly ubiquitously expressed and mediate a plethora of autocrine, paracrine, and systemic functions. The first-in-class $S1P_1$ receptor functional antagonist, fingolimod, has been approved for the treatment of multiple sclerosis (Brinkmann et al. 2010). At present, pharmacological and clinical development focuses on substances with better $S1P_1$ receptor selectivity and improved pharmacokinetic profiles for treatment of autoimmune and inflammatory disorders (Meyer zu Heringdorf et al. 2013; Proia and Hla 2015). Drugs targeting the other S1P-GPCRs are under consideration (see e.g., Blankenbach et al. 2016).

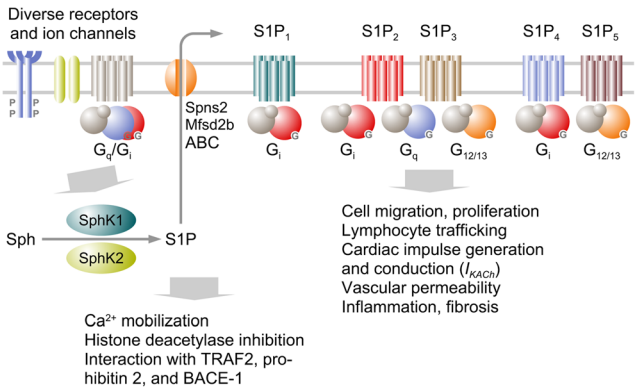

Fig. 6 Signaling by sphingosine-1-phosphate (S1P). S1P is generated from sphingosine by SphKs. There are two SphK isoforms which have several splice variants. SphKs are regulated transcriptionally and post-transcriptionally by a multitude of signals and receptors, e.g., growth factors, cytokines, and GPCRs (Chan and Pitson 2013). SphK1 can furthermore be activated by Ca^{2+} influx through voltage-dependent Ca^{2+} channels (Alemany et al. 2001). GPCR-induced SphK1 translocation to the plasma membrane is specifically mediated by $G\alpha_q$ (ter Braak et al. 2009). While it was initially thought that S1P acted as intracellular second messenger to induce cell proliferation and Ca^{2+} mobilization, Karl H. Jakobs' group provided first functional evidence that S1P acted as agonist at a specific high-affinity GPCR(s) (Van Koppen et al., 1996a). Meanwhile, five S1P-GPCRs have been identified, termed $S1P_{1-5}$ (Blaho and Hla 2014). Their differential expression and individual roles in diverse tissues and organs have been described in detail. A first-in-class $S1P_1$ functional antagonist has been approved for treatment of multiple sclerosis (Brinkmann et al. 2010). Also, proteins which transport S1P across the plasma membrane for secretion (Spns2, Mfsd2b, ABC transporters) have been identified. Nevertheless, there is still evidence that S1P also acts inside the cell, although there are only few studies that report direct intracellular targets of S1P (Strub et al. 2010). Further details, see text. TRAF2, tumor necrosis factor receptor-associated factor-2; BACE-1, β-site amyloid precursor protein cleaving enzyme-1

The activities of S1P and related lipids were further analyzed in different cell types in the group of Karl H. Jakobs. It was shown that S1P and sphingosylphosphorylcholine (SPC) induced Ca^{2+} signaling in bovine aortic endothelial cells, and that SPC activated a specifc G_i-coupled GPCR in human HL-60 leukemia cells (Meyer zu Heringdorf et al. 1996; Van Koppen et al. 1996). Together with the group of Ursula Ravens, in particular Herbert Himmel, it was demonstrated that the lysosphingolipids, psychosine, and glucopsychosine inhibited L-type Ca^{2+} currents in insulinoma cells in a guanine nucleotide-dependent manner (Himmel et al. 1998) and that S1P activated $I_{K.ACh}$ in human atrial myocytes (Himmel et al. 2000). In fact, the activation of $I_{K.ACh}$ with the ensuing bradycardia is an important side effect of fingolimod (Brinkmann et al. 2010). In human bladder carcinoma cells, S1P receptors inhibited LPA but enhanced thrombin-stimulated cell motility (Rümenapp et al. 2000). The $S1P_1$ receptor negatively regulated Ca^{2+} signaling by other agonists (Meyer zu Heringdorf et al. 2003b). Together with Burkhard Kleuser, Freie Universität Berlin, the influence of S1P and lysophosphatidic acid on human primary keratinocytes was shown (Lichte et al. 2008).

6.2 Sphingosine-1-phosphate as an intracellular lipid mediator

Following the original concept that S1P acted as a second messenger, Karl H. Jakobs and co-workers analyzed the influence of sphingosine kinase (SphK) inhibitors on M_2- and M_3-receptor-induced Ca^{2+} transients. In fact, the non-specific SphK inhibitors available at that time, *N*,*N*-dimethylsphingosine (DMS) and D,L-threo-dihydrosphingosine (DHS), inhibited the responses to M_2 and M_3 stimulation in a differential manner. Most importantly, intracellularly injected S1P mobilized Ca^{2+} from thapsigargin-sensitive stores (Meyer zu Heringdorf et al. 1998a). Likewise, the SPC-induced $[Ca^{2+}]_i$ rise in intact cells was found to be PTX-sensitive and stereoselective, while the SPC-induced release of Ca^{2+} in permeabilized cells was PTX-insensitive and induced with the same potency and efficacy by D-erythro- and L-threo-SPC (Meyer zu Heringdorf et al. 1998b; Meyer zu Heringdorf et al. 2002). Gabor Tigyi, University of Tennessee, Memphis, proposed to study the effects of intracellular S1P using caged S1P. Together with Gabor Tigyi and Karoly Liliom, Dagmar Meyer zu Heringdorf and Karl H. Jakobs showed that uncageing of caged S1P caused $[Ca^{2+}]_i$ elevations in cells that did not express S1P-GPCRs or in which the S1P-induced $[Ca^{2+}]_i$ response was fully blocked by PTX (Meyer zu Heringdorf et al. 2003a; Meyer zu Heringdorf 2004). These data proved that S1P can act as both an agonist at GPCRs and as an intracellular lipid mediator (Fig. 6).

The role of SphK in human HL-60 leukemia cells was studied by Regina Alemany in Karl H. Jakobs' group. She reported that formyl peptide and purinergic receptors activated SphK in these cells and that the SphK inhibitors, DMS and DHS, impaired GPCR-induced Ca^{2+} signaling as well as formyl peptide-induced enzyme release (Alemany et al. 1999; Alemany et al. 2000). Furthermore, depolarization-induced Ca^{2+} influx stimulated SphK activity, and overexpression of SphK1 augmented the depolarization-induced noradrenaline release in PC12 cells (Alemany et al. 2001). Interestingly, S1P-GPCRs were found to activate SphK (Meyer zu Heringdorf et al. 2001). While both G_i- and G_q-family coupled GPCRs stimulated SphK activity (Fig. 6), only stimulation of the latter or expression of constitutively active mutants of $G\alpha_q$ or $G\alpha_{11}$, but not of $G\alpha_i$, $G\alpha_{12}$, or $G\alpha_{13}$, induced a translocation of SphK1, but not of SphK2 to the plasma membrane (ter Braak et al. 2009). The study of vascular and renal effects of S1P and SPC was initiated in Essen by Martin Michel. Together with group of Karl H. Jakobs, he and Angela Bischoff studied the influence of these lipids on constriction of renal and mesenteric microvessels and on renal and mesenteric blood flow, diuresis, and natriuresis in rats (Bischoff et al. 2000a; Bischoff et al. 2000b; Bischoff et al. 2001). Martin Michel later continued his work on cardiovascular effects of S1P at the Academic Medical Center in Amsterdam. Research on S1P lyase was started by Dagmar Meyer zu Heringdorf (Claas et al. 2010), who continued to work on S1P-GPCRs, SphKs, and S1P lyase in her own group at the Goethe-University in Frankfurt am Main.

Taken together, Karl H. Jakobs and his research group made significant contributions during the exciting years of the discovery of the S1P signaling system, together with other groups, whose work cannot be cited here due to lack of space.

Fig. 7 Karl H. Jakobs during his last days as director of the Institute of Pharmacology at the University of Duisburg-Essen. He retired in 2007

Since that time, research on sphingolipid signaling has expanded immensely, and S1P-GPCR, SphKs and S1P lyase are considered as promising pharmacological targets.

7 Final remarks

Looking at the enormous amount of seminal and important scientific contributions during his scientific career, it is obvious that Karl H. Jakobs was an excellent and talented scientist. He was looking not only the details but had always a clear view on the broader picture, even beyond the boundaries of science. Most importantly, however, he was also what is called in German "ein guter Mensch." This is usually translated into English as being a good person. But, the meaning in German is much broader. From Yiddish "mensch" migrated as a loanword into American English, where it describes a particularly good person. Thus, "ein guter Mensch" is one of the finest compliments to be made for a human being. There is nothing to be added to this. Figure 7 shows this magnificent, but modest man as he would likely be remembered. We miss him dearly.

Author contribution KA, PG, DM, MS, GS, and TW reviewed the literature and wrote the manuscript. All authors read and approved the manuscript.

Compliance with ethical standards

The authors declare that there are no conflicts of interest.

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