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cAMP guided his way

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

Klaus Aktories¹ · Peter Gierschik² · Dagmar Meyer zu Heringdorf³ · Martina Schmidt⁴ · Günter Schultz⁵ · Thomas Wieland⁶

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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 be 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-phosphate

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	<i>N,N</i> -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	<i>N</i> -ethylmaleimide
NTP	nucleoside triphosphate
PA	phosphatidic acid
PGE ₁	prostaglandin E ₁
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
SIP	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 adenylylate 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.

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

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 PGE₁, 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

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 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 (Wettschurek 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_{α_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 G_{α_i} 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 G α subunit of the main major PTX substrate of human neutrophil plasma membranes differs from the hitherto known G α 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 G α protein (Gierschik et al. 1986). Nowadays, it is clear that the purified protein was mainly made up of G α_{i2} and a minor portion of G α_{i3} . To clarify which of the two G α_i subunits present in granulocyte membranes functionally interacted with activated formyl peptide receptors, Gierschik and Jakobs developed a novel approach, i.e., GPCR-mediated [³²P]ADP-ribosylation of G α_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 α_{i2} and G α_{i3} , which coupled to chemotactic-peptide-activated receptors (Fig. 2) (Gierschik and Jakobs 1987; Gierschik et al. 1989a). As the third G α_i protein, G α_{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 α_i proteins alone does not suffice to determine which effector mechanism is regulated by a given G α_i protein.

2 Analysis of GPCR-G protein interactions

2.1 Constitutive basal activity of unoccupied G α_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 α_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

Fig. 2 Stimulation of PLC $\beta_{2/3}$ by G-protein $\beta\gamma$ 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 G $\beta\gamma$ subunits following their activation by agonist, e.g., formyl peptide, binding. Direct activation of PLC β_2 and PLC β_3 by G $\beta\gamma$ was shown by Peter Gierschik in the Jakobs laboratory in the late 80ties (see text). Activated PLC β isozymes hydrolyze phosphatidylinositol 4,5-bisphosphate (PIP₂) to produce the two second messengers, D-*myo*-inositol-1,4,5-trisphosphate (IP₃) and sn-1,2-diacylglycerol (DAG). In subsequent years, Peter Gierschik showed in his own lab that PLC β_2 is also activated by Rac1, Rac2, and, to a lesser extent, by Cdc42Hs (Illenberger et al. 1998). Others have extended these findings to PLC β_3 and Rac3 (Snyder et al. 2003). Rac-mediated stimulation of PLC β isozymes may be the basis for regulation of PLC β isozymes in neutrophils and, possibly, other cells by Rac guanine nucleotide exchange factors, e.g., the PIP₃-dependent Rac exchanger P-Rex (Baker et al. 2016) and/or by other Rac GEFs, e.g., the G $\beta\gamma$ -regulated RhoG-ELMO-Dock complex (Xu and Jin 2017) or the protein-tyrosine-kinase-regulated Vav proteins (Bustelo 2014). In intact cells, PLC β appears to be under multimodal control involving G $\beta\gamma$, 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 PLC β_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 [Ca²⁺]_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

G protein activation, respectively (Gierschik et al. 1988). The results also suggested that the formyl peptide receptor, similar to many other G α_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 α_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 α_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 α_i by PTX suppressed the GPCR-G protein interaction, but none of the “intrinsic” G α_i protein functions (i.e., GDP release, GTP binding, GTP hydrolysis, and G α -G $\beta\gamma$ 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 $\text{A}_{2\text{A}}$ receptor ($\text{A}_{2\text{A}}\text{AR}$) (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, $\text{O}^{\delta 1}$ 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 $\text{A}_{2\text{A}}\text{AR}$ 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 $\text{A}_{2\text{A}}\text{AR}$, 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}) $\text{A}_{2\text{A}}\text{AR}$ 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 $\text{A}_{2\text{A}}\text{AR}$ 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 $\text{A}_{2\text{A}}\text{AR}$ 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 $\text{A}_{2\text{A}}\text{AR}$ 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 $\text{A}_{2\text{A}}\text{AR}$ (Massink et al. 2016), the leukotriene B₄ receptor subtype BLT1 (Hori et al. 2018), and the dopamine D₂ receptor (Draper-Joyce et al. 2018).

2.2 GPCR-regulated GTP γ S binding in membranes and permeabilized cells

As it was evident that the GTP analog guanosine-5'-[γ -thio]triphosphate (GTP γ S) cannot (or only very poorly) be hydrolyzed by G proteins and [³⁵S]-labeled version became available, the binding of [³⁵S]GTP γ S was used in the characterization of purified G proteins (Bokoch et al. 1984; Sternweis and Robishaw 1984). Subsequently, the agonist-stimulated binding of [³⁵S]GTP γ S 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 M₂ muscarinic acetylcholine receptor (M₂) were used to study carbachol stimulated [³⁵S]GTP γ S binding to pertussis toxin-sensitive G proteins of the G_i family. Several important attributes of the [³⁵S]GTP γ S 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]GTP γ S 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; Liebmman et al. 1991; Gachet et al. 1992a; Gachet et al. 1992b; Hilf and Jakobs 1992a; Kupper et al. 1992; Liebmman 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]GTP γ S 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]GTP γ S 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]GTP γ S 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]GTP γ S 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]GTP γ S 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]GTP γ S 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]GTP γ S 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]GTP γ S 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 GTP γ S 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]GTP γ S 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 $G\alpha$ -subunit and a $G\beta\gamma$ -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 GTP γ S 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 $G\alpha$ 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 $G\alpha$ -subunit. It is generally accepted that the conformational change induced by GTP binding allows the GTP-bound $G\alpha$ -subunit as well as the $G\beta\gamma$ -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 $G\alpha$ 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 $G\alpha$ -subunit and the $G\beta\gamma$ dimer. Upon agonist stimulation, the activated GPCR induces the release of GDP from $G\alpha$ to which then GTP can bind. The activation is terminated by GTP hydrolysis through the GTPase activity of $G\alpha$. 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 $G\beta\gamma$ 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 were 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 $GTP\gamma S$ is formed from $ATP\gamma S$ 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 $GTP\gamma S$ (Wieland et al. 1991a), respectively. The group additionally showed that the $GTP\gamma S$ formed by NDPK was more potent than exogenously added $GTP\gamma S$ 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 $G\beta\gamma$ 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 $G\beta\gamma$ 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 $G\beta$ subunits

Already in the beginning of the 1990s, the group of Karl H. Jakobs found that the $G\beta$ subunit of the retinal G protein transducin (G_t) can be thiophosphorylated by GTP γ S (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 GTP γ S 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 $G\beta$ 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 $G\beta$ -subunit is NDPK was presented in 2003 (Cuello et al. 2003). His266 in the $G\beta_1$ -subunit was identified as the phosphorylated amino acid. A homologous histidine residue is conserved in the mammalian $G\beta_1$ - $G\beta_4$ isoforms, but not in $G\beta_5$. 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 $G\beta$ -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 $G\beta\gamma$

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 GTP γ S-stimulated inositol phosphate formation was observed (Camps et al. 1992b). It was anticipated that this stimulation was either mediated by a $G\alpha$ -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 $G_t\beta\gamma$ was examined. Rather than causing an inhibition by interfering with a $G\alpha$ -mediated PLC stimulation or having no effect on a stimulation caused by a monomeric GTPase (see Fig. 2), $G_t\beta\gamma$ markedly stimulated the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP₂). The effect of $G_t\beta\gamma$ 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 $G_t\beta\gamma$ but also observed for the more lipophilic, detergent-solubilized $G\beta\gamma$ from bovine brain carrying a geranylgeranyl moiety. Importantly, the $G\beta\gamma$ -induced stimulation was fully reversed by the addition of purified, GDP-bound $G_t\alpha$, a method meanwhile often used to prove for the active involvement of $G\beta\gamma$ 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 $G\beta\gamma$ rather than $G\alpha$ 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 $G\beta\gamma$, and the requirement of relatively high concentrations to obtain stimulation. In fact, even in today's literature, the question

whether or not $G\beta\gamma$ 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 PLC β 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 $G\alpha$ 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 (IP₃)-mediated

risks in [Ca²⁺]_i and diacylglycerol (DAG)-mediated activation of PKC isoforms which subsequently activate PLD. G₁₂ 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 PIP₂ 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 G₁₂-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 PIP₂, 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 PIP₂ (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 PIP₂ 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 M₃ 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 PIP₂ 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 PIP₂-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 PIP₂ 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 monocytogenes* 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 M₃-receptor-expressing HEK293 cells with either toxin largely reduced the stimulation of PLC and the subsequent elevation in [Ca²⁺]_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 held 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 PLC ϵ (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 PLC ϵ 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 PLC ϵ stimulation (Fig. 5). As an additional proof of concept for this pathway, the G $_s$ -dependent stimulation of PLC ϵ 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 PLC β_1 and PLC ϵ simultaneously (Evellin et al. 2002). In addition, receptor tyrosine kinases were found to bear the capacity to activate PLC ϵ (Stope et al. 2004). Thus, PLC ϵ 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 PLC ϵ -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 PLC ϵ 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-Llanca et al. 2015; Munoz-Llanca 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 α_{12} and G α_{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 $G\beta\gamma$ 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 S1P₁ - S1P₅, have been characterized (Fig. 6). They are nearly ubiquitously expressed and mediate a plethora of autocrine, paracrine, and systemic functions. The first-in-class S1P₁ 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 S1P₁ 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 S1P₁ 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 specific 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 $\text{I}_{\text{K,ACh}}$ in human atrial myocytes (Himmel et al. 2000). In fact, the activation of $\text{I}_{\text{K,ACh}}$ 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 $[\text{Ca}^{2+}]_i$ 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 uncaging of caged S1P caused $[\text{Ca}^{2+}]_i$ elevations in cells that did not express S1P-GPCRs or in which the S1P-induced $[\text{Ca}^{2+}]_i$ 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 $\text{G}\alpha_q$ or $\text{G}\alpha_{11}$, but not of $\text{G}\alpha_i$, $\text{G}\alpha_{12}$, or $\text{G}\alpha_{13}$, 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 loan-word 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.

References

- Abu-Taha IH, Heijman J, Hippe HJ, Wolf NM, El-Armouche A, Nikolaev VO, Schafer M, Wurtz CM, Neef S, Voigt N, Baczko I, Varro A, Muller M, Meder B, Katus HA, Spiger K, Vettel C, Lehmann LH, Backs J, Skolnik EY, Lutz S, Dobrev D, Wieland T (2017) Nucleoside diphosphate kinase-C suppresses cAMP formation in human heart failure. *Circulation* 135:881–897
- Aktories K (2011) Bacterial protein toxins that modify host regulatory GTPases. *Nat Rev Microbiol* 9:487–498
- Aktories K, Jakobs KH (1981) Epinephrine inhibits adenylate cyclase and stimulates a GTPase in human platelet membranes via alpha-adrenoceptors. *FEBS Lett* 130:235–238
- Aktories K, Jakobs KH (1984) N_i -Mediated inhibition of human platelet adenylate cyclase by thrombin. *Eur J Biochem* 145:333–338
- Aktories K, Jakobs KH, Schultz G (1980a) Nicotinic acid inhibits adipocyte adenylate cyclase in a hormone-like manner. *FEBS Lett* 115:11–14
- Aktories K, Schultz G, Jakobs KH (1979) Inhibition of hamster fat cell adenylate cyclase by prostaglandin e1 and epinephrine: requirement for GTP and sodium ions. *FEBS Lett* 107:100–104
- Aktories K, Schultz G, Jakobs KH (1980b) Regulation of adenylate cyclase activity in hamster adipocytes. Inhibition by prostaglandins, α -adrenergic agonists and nicotinic acid. *Naunyn Schmiedeberg's Arch Pharmacol* 312:167–173
- Aktories K, Schultz G, Jakobs KH (1982a) Cholera toxin inhibits prostaglandin E_1 but not adrenaline-induced stimulation of GTP hydrolysis in human platelet membranes. *FEBS Lett* 146:65–68
- Aktories K, Schultz G, Jakobs KH (1982b) Stimulation of a low K_m GTPase by inhibitors of adipocyte adenylate cyclase. *Mol Pharmacol* 21:336–342
- Aktories K, Schultz G, Jakobs KH (1983a) Adenylate cyclase inhibition and GTPase stimulation by somatostatin in S49 lymphoma cyc^- variants are prevented by islet-activating protein. *FEBS Lett* 158:169–173
- Aktories K, Schultz G, Jakobs KH (1983b) Islet-activating protein prevents nicotinic acid-induced GTPase stimulation and GTP but not GTP γ S-induced adenylate cyclase inhibition in rat adipocytes. *FEBS Lett* 156:88–92
- Aleman R, Kleuser B, Ruwisch L, Danneberg K, Lass H, Hashemi R, Spiegel S, Jakobs KH, Meyer zu Heringdorf D (2001) Depolarisation induces rapid and transient formation of intracellular sphingosine-1-phosphate. *FEBS Lett* 509:239–244
- Aleman R, Meyer zu Heringdorf D, van Koppen CJ, Jakobs KH (1999) Formyl peptide receptor signaling in HL-60 cells through sphingosine kinase. *J Biol Chem* 274:3994–3999
- Aleman R, Sichelschmidt B, Zu Heringdorf DM, Lass H, van Koppen CJ, Jakobs KH (2000) Stimulation of sphingosine-1-phosphate formation by the P2Y $_2$ receptor in HL-60 cells: Ca^{2+} requirement and implication in receptor-mediated Ca^{2+} mobilization, but not MAP kinase activation. *Mol Pharmacol* 58:491–497
- Asano T, Pedersen SE, Scott CW, Ross EM (1984) Reconstitution of catecholamine-stimulated binding of guanosine 5'-O-(3-thiotriphosphate) to the stimulatory GTP-binding protein of adenylate cyclase. *Biochemistry* 23:5460–5467
- Attwood PV, Wieland T (2015) Nucleoside diphosphate kinase as protein histidine kinase. *Naunyn Schmiedeberg's Arch Pharmacol* 388:153–160
- Baker MJ, Pan D, Welch HC (2016) Small GTPases and their guanine-nucleotide exchange factors and GTPase-activating proteins in neutrophil recruitment. *Curr Opin Hematol* 23:44–54
- Bamburg JR (1999) Proteins of the ADF/cofilin family: essential regulators of actin dynamics. *Annu Rev Cell Dev Biol* 15:185–230
- Bamburg JR (2011) *Listeria monocytogenes* cell invasion: a new role for cofilin in co-ordinating actin dynamics and membrane lipids. *Mol Microbiol* 81:851–854
- Bauer D, Gupta D, Haroutunian V, Meador-Woodruff JH, McCullumsmith RE (2008) Abnormal expression of glutamate transporter and transporter interacting molecules in prefrontal cortex in elderly patients with schizophrenia. *Schizophr Res* 104:108–120
- Beavo JA, Brunton LL (2002) Cyclic nucleotide research—still expanding after half a century. *Nat Rev Mol Cell Biol* 3:710–718
- Bernstein BW, Bamburg JR (2010) ADF/cofilin: a functional node in cell biology. *Trends Cell Biol* 20:187–195
- Birnbaumer L, Brown AM (1987) G protein opening of K^+ channels. *Nature* 327:21–22
- Bischoff A, Czyborra P, Fetscher C, Meyer zu Heringdorf D, Jakobs KH, Michel MC (2000a) Sphingosine-1-phosphate and sphingosylphosphorylcholine constrict renal and mesenteric microvessels in vitro. *Br J Pharmacol* 130:1871–1877
- Bischoff A, Czyborra P, Meyer zu Heringdorf D, Jakobs KH, Michel MC (2000b) Sphingosine-1-phosphate reduces rat renal and mesenteric blood flow in vivo in a pertussis toxin-sensitive manner. *Br J Pharmacol* 130:1878–1883
- Bischoff A, Meyer zu Heringdorf D, Jakobs KH, Michel MC (2001) Lysosphingolipid receptor-mediated diuresis and natriuresis in anaesthetized rats. *Br J Pharmacol* 132:1925–1933
- Blaho VA, Hla T (2014) An update on the biology of sphingosine 1-phosphate receptors. *J Lipid Res* 55:1596–1608

- Blank JL, Brattain KA, Exton JH (1992) Activation of cytosolic phosphoinositide phospholipase C by G-protein $\beta\gamma$ subunits. *J Biol Chem* 267:23069–23075
- Blankenbach KV, Schwalm S, Pfeilschifter J, Meyer zu Heringdorf D (2016) Sphingosine-1-phosphate receptor-2 antagonists: therapeutic potential and potential risks. *Front Pharmacol* 7:167
- Blomquist A, Schworer G, Schabowski H, Psoma A, Lehnen M, Jakobs KH, Rümenapp U (2000) Identification and characterization of a novel Rho-specific guanine nucleotide exchange factor. *Biochem J* 352(Pt 2):319–325
- Bokoch GM, Katada T, Northup JK, Ui M, Gilman AG (1984) Purification and properties of the inhibitory guanine nucleotide-binding regulatory component of adenylate cyclase. *J Biol Chem* 259:3560–3567
- Bond RA, Ijzerman AP (2006) Recent developments in constitutive receptor activity and inverse agonism, and their potential for GPCR drug discovery. *Trends Pharmacol Sci* 27:92–96
- Boyer JL, Waldo GL, Harden TK (1992) $\beta\gamma$ -Subunit activation of G-protein-regulated phospholipase C. *J Biol Chem* 267:25451–25456
- Brasier DJ (2017) Three scientific controversies to engage students in reading primary literature. *J Undergrad Neurosci Educ: JUNE: a Publication of FUN, Faculty for Undergraduate Neuroscience* 16: R13–R19
- Brinkmann V, Billich A, Baumrucker T, Heining P, Schmouder R, Francis G, Aradhye S, Burtin P (2010) Fingolimod (FTY720): discovery and development of an oral drug to treat multiple sclerosis. *Nat Rev Drug Discov* 9:883–897
- Brown HA, Thomas PG, Lindsley CW (2017) Targeting phospholipase D in cancer, infection and neurodegenerative disorders. *Nat Rev Drug Discov* 16:351–367
- Bünemann M, Brandts B, zu Heringdorf DM, van Koppen CJ, Jakobs KH, Pott L (1995) Activation of muscarinic K^+ current in guinea-pig atrial myocytes by sphingosine-1-phosphate. *J Physiol* 489(Pt 3): 701–707
- Bunney TD, Katan M (2006) Phospholipase C ϵ : linking second messengers and small GTPases. *Trends Cell Biol* 16:640–648
- Bustelo XR (2014) Vav family exchange factors: an integrated regulatory and functional view. *Small GTPases* 5:9
- Calo LA, Davis PA, Pagnin E, Dal Maso L, Maiolino G, Seccia TM, Pessina AC, Rossi GP (2014) Increased level of p63RhoGEF and RhoA/Rho kinase activity in hypertensive patients. *J Hypertens* 32: 331–338
- Camps M (1994) Hot papers: biochemist Montserrat Camps discusses her paper on the regulation of phosphoinositide-specific phospholipase C by signal-transducing G-proteins. *Scientist* 8:16
- Camps M, Carozzi A, Schnabel P, Scheer A, Parker PJ, Gierschik P (1992a) Isozyme-selective stimulation of phospholipase C- β 2 by G protein $\beta\gamma$ -subunits. *Nature* 360:684–686
- Camps M, Hou C, Sidiropoulos D, Stock JB, Jakobs KH, Gierschik P (1992b) Stimulation of phospholipase C by guanine-nucleotide-binding protein $\beta\gamma$ subunits. *Eur J Biochem* 206:821–831
- Carbajo-Lozoya J, Lutz S, Feng Y, Kroll J, Hammes HP, Wieland T (2012) Angiotensin II modulates VEGF-driven angiogenesis by opposing effects of type 1 and type 2 receptor stimulation in the microvascular endothelium. *Cell Signal* 24:1261–1269
- Cassel D, Selinger Z (1976) Catecholamine-stimulated GTPase activity in turkey erythrocyte membranes. *Biochim Biophys Acta* 452:538–551
- Cassel D, Selinger Z (1977a) Catecholamine-induced release of [3 H]-Gpp(NH)p from turkey erythrocyte adenylate cyclase. *J Cyclic Nucleotide Res* 3:11–22
- Cassel D, Selinger Z (1977b) Mechanism of adenylate cyclase activation by cholera toxin: inhibition of GTP hydrolysis at the regulatory site. *Proc Natl Acad Sci U S A* 74:3307–3311
- Cechova K, Hlouskova M, Javorkova E, Roubalova L, Ujcikova H, Holan V, Svoboda P (2018) Up-regulation of μ -, δ - and κ -opioid receptors in concanavalin A-stimulated rat spleen lymphocytes. *J Neuroimmunol* 321:12–23
- Chakraborti S, Roy S, Mandal A, Chowdhury A, Chakraborti T (2013) Role of PKC- ξ in NADPH oxidase-PKC- α - $G_i\alpha$ axis dependent inhibition of β -adrenergic response by U46619 in pulmonary artery smooth muscle cells. *Arch Biochem Biophys* 540:133–144
- Chan H, Pitson SM (2013) Post-translational regulation of sphingosine kinases. *Biochim Biophys Acta* 1831:147–156
- Chang YJ, Pownall S, Jensen TE, Mouaaz S, Foltz W, Zhou L, Liadis N, Woo M, Hao Z, Dutt P, Bilan PJ, Klip A, Mak T, Stambolic V (2015) The Rho-guanine nucleotide exchange factor PDZ-RhoGEF governs susceptibility to diet-induced obesity and type 2 diabetes. *eLife* 4:e06011
- Chen H, Bernstein BW, Bamberg JR (2000) Regulating actin-filament dynamics in vivo. *Trends Biochem Sci* 25:19–23
- Chu J, Zheng H, Zhang YH, Loh HH, Law PY (2010) Agonist-dependent μ -opioid receptor signaling can lead to heterologous desensitization. *Cell Signal* 22:684–696
- Citri Y, Schramm M (1982) Probing of the coupling site of the β -adrenergic receptor. Competition between different forms of the guanyl nucleotide binding protein for interaction with the receptor. *J Biol Chem* 257:13257–13262
- Claas RF, ter Braak M, Hegen B, Hardel V, Angioni C, Schmidt H, Jakobs KH, Van Veldhoven PP, Meyer zu Heringdorf D (2010) Enhanced Ca^{2+} storage in sphingosine-1-phosphate lyase-deficient fibroblasts. *Cell Signal* 22:476–483
- Cockcroft S (2001) Signalling roles of mammalian phospholipase D1 and D2. *Cell Mol Life Sci: CMLS* 58:1674–1687
- Costa T, Herz A (1989) Antagonists with negative intrinsic activity at δ opioid receptors coupled to GTP-binding proteins. *Proc Natl Acad Sci U S A* 86:7321–7325
- Costa T, Lang J, Gless C, Herz A (1990) Spontaneous association between opioid receptors and GTP-binding regulatory proteins in native membranes: specific regulation by antagonists and sodium ions. *Mol Pharmacol* 37:383–394
- Cuello F, Schulze RA, Heemeyer F, Meyer HE, Lutz S, Jakobs KH, Niroomand F, Wieland T (2003) Activation of heterotrimeric G proteins by a high energy phosphate transfer via nucleoside diphosphate kinase (NDPK) B and G β subunits. Complex formation of NDPK B with G $\beta\gamma$ dimers and phosphorylation of His-266 in G β . *J Biol Chem* 278:7220–7226
- de la Pena P, del Camino D, Pardo LA, Dominguez P, Barros F (1995) Gs couples thyrotropin-releasing hormone receptors expressed in *Xenopus* oocytes to phospholipase C. *J Biol Chem* 270:3554–3559
- de Rooij J, Zwartkruis FJ, Verheijen MH, Cool RH, Nijman SM, Wittinghofer A, Bos JL (1998) Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. *Nature* 396:474–477
- Del Galdo S, Vettel C, Heringdorf DM, Wieland T (2013) The activation of RhoC in vascular endothelial cells is required for the S1P receptor type 2-induced inhibition of angiogenesis. *Cell Signal* 25:2478–2484
- Dhanasekaran N, Dermott JM (1996) Signaling by the G_{12} class of G proteins. *Cell Signal* 8:235–245
- Draper-Joyce CJ, Verma RK, Michino M, Shonberg J, Kopinathan A, Klein Herenbrink C, Scammells PJ, Capuano B, Abramyan AM, Thal DM, Javitch JA, Christopoulos A, Shi L, Lane JR (2018) The action of a negative allosteric modulator at the dopamine D_2 receptor is dependent upon sodium ions. *Sci Rep* 8:1208
- Dusaban SS, Brown JH (2015) PLC ϵ mediated sustained signaling pathways. *Adv Biol Regul* 57:17–23
- Evellin S, Nolte J, Tysack K, Vom Dorp F, Thiel M, Weernink PA, Jakobs KH, Webb EJ, Lomasney JW, Schmidt M (2002) Stimulation of phospholipase C- ϵ by the M_3 muscarinic acetylcholine receptor mediated by cyclic AMP and the GTPase Rap2B. *J Biol Chem* 277: 16805–16813

- Exton JH (2002) Phospholipase D-structure, regulation and function. *Rev Physiol Biochem Pharmacol* 144:1–94
- Fahimi-Vahid M, Gosau N, Michalek C, Han L, Jakobs KH, Schmidt M, Roberts N, Avkiran M, Wieland T (2002) Distinct signaling pathways mediate cardiomyocyte phospholipase D stimulation by endothelin-1 and thrombin. *J Mol Cell Cardiol* 34:441–453
- Fazal L, Laudette M, Paula-Gomes S, Pons S, Conte C, Tortosa F, Sicard P, Sainte-Marie Y, Bisserier M, Lairez O, Lucas A, Roy J, Ghaleh B, Fauconnier J, Miale-Perez J, Lezoualc'h F (2017) Multifunctional mitochondrial epac1 controls myocardial cell death. *Circ Res* 120:645–657
- Frohm MA (2015) The phospholipase D superfamily as therapeutic targets. *Trends Pharmacol Sci* 36:137–144
- Gachet C, Cazenave JP, Ohlmann P, Hilf G, Wieland T, Jakobs KH (1992a) ADP receptor-induced activation of guanine-nucleotide-binding proteins in human platelet membranes. *Eur J Biochem* 207:259–263
- Gachet C, Savi P, Ohlmann P, Maffrand JP, Jakobs KH, Cazenave JP (1992b) ADP receptor induced activation of guanine nucleotide binding proteins in rat platelet membranes—an effect selectively blocked by the thienopyridine clopidogrel. *Thromb Haemost* 68:79–83
- Gado F, Di Cesare Mannelli L, Lucarini E, Bertini S, Cappelli E, Digiaco M, Stevenson LA, Macchia M, Tuccinardi T, Ghelardini C, Pertwee RG, Manera C (2018) Identification of the first synthetic allosteric modulator of the CB₂ receptors and evidence of its efficacy for neuropathic pain relief. *J Med Chem*
- Ghosh TK, Bian J, Gill DL (1994) Sphingosine 1-phosphate generated in the endoplasmic reticulum membrane activates release of stored calcium. *J Biol Chem* 269:22628–22635
- Gierschik P (1992) ADP-ribosylation of signal-transducing guanine nucleotide-binding proteins by pertussis toxin. *Curr Top Microbiol Immunol* 175:69–96
- Gierschik P, Bouillon T, Jakobs KH (1994) Receptor-stimulated hydrolysis of guanosine 5'-triphosphate in membrane preparations. *Methods Enzymol* 237:13–26
- Gierschik P, Falloon J, Milligan G, Pines M, Gallin JJ, Spiegel A (1986) Immunochemical evidence for a novel pertussis toxin substrate in human neutrophils. *J Biol Chem* 261:8058–8062
- Gierschik P, Jakobs KH (1987) Receptor-mediated ADP-ribosylation of a phospholipase C-stimulating G protein. *FEBS Lett* 224:219–223
- Gierschik P, McLeish K, Jakobs KH (1988) Regulation of G-protein-mediated signal transfer by ions. *J Cardiovasc Pharmacol* 12(Suppl 5):S20–S24
- Gierschik P, Moghtader R, Straub C, Dieterich K, Jakobs KH (1991) Signal amplification in HL-60 granulocytes. Evidence that the chemotactic peptide receptor catalytically activates guanine-nucleotide-binding regulatory proteins in native plasma membranes. *Eur J Biochem* 197:725–732
- Gierschik P, Sidiropoulos D, Jakobs KH (1989a) Two distinct Gi-proteins mediate formyl peptide receptor signal transduction in human leukemia (HL-60) cells. *J Biol Chem* 264:21470–21473
- Gierschik P, Sidiropoulos D, Steisslinger M, Jakobs KH (1989b) Na⁺ regulation of formyl peptide receptor-mediated signal transduction in HL 60 cells. Evidence that the cation prevents activation of the G-protein by unoccupied receptors. *Eur J Pharmacol* 172:481–492
- Gierschik P, Steisslinger M, Sidiropoulos D, Herrmann E, Jakobs KH (1989c) Dual Mg²⁺ control of formyl-peptide-receptor–G-protein interaction in HL 60 cells. Evidence that the low-agonist-affinity receptor interacts with and activates the G-protein. *Eur J Biochem* 183:97–105
- Gilles AM, Presecan E, Vonica A, Lascu I (1991) Nucleoside diphosphate kinase from human erythrocytes. Structural characterization of the two polypeptide chains responsible for heterogeneity of the hexameric enzyme. *J Biol Chem* 266:8784–8789
- Gosau N, Fahimi-Vahid M, Michalek C, Schmidt M, Wieland T (2002) Signalling components involved in the coupling of α_1 -adrenoceptors to phospholipase D in neonatal rat cardiac myocytes. *Naunyn Schmiedeberg's Arch Pharmacol* 365:468–476
- Grandoch M, Bujok V, Fleckenstein D, Schmidt M, Fischer JW, Weber AA (2009a) Epac inhibits apoptosis of human leukocytes. *J Leukoc Biol* 86:847–849
- Grandoch M, Roscioni SS, Schmidt M (2010) The role of Epac proteins, novel cAMP mediators, in the regulation of immune, lung and neuronal function. *Br J Pharmacol* 159:265–284
- Grandoch M, Rose A, ter Braak M, Jendrosseck V, Rubben H, Fischer JW, Schmidt M, Weber AA (2009b) Epac inhibits migration and proliferation of human prostate carcinoma cells. *Br J Cancer* 101:2038–2042
- Grandt R, Aktories K, Jakobs KH (1986) Evidence for two GTPases activated by thrombin in membranes of human platelets. *Biochem J* 237:669–674
- Hackenthal E, Aktories K, Jakobs KH (1985) Mode of inhibition of renin release by angiotensin II. *J Hypertens Suppl: Official Journal of the International Society of Hypertension* 3:S263–S265
- Halls ML, Cooper DMF (2017) Adenylyl cyclase signalling complexes—pharmacological challenges and opportunities. *Pharmacol Ther* 172:171–180
- Han L, Stope MB, de Jesus ML, Oude Weernink PA, Urban M, Wieland T, Rosskopf D, Mizuno K, Jakobs KH, Schmidt M (2007) Direct stimulation of receptor-controlled phospholipase D1 by phosphocofillin. *EMBO J* 26:4189–4202
- Han X, Yu R, Ji L, Zhen D, Tao S, Li S, Sun Y, Huang L, Feng Z, Li X, Han G, Schmidt M, Han L (2011a) InlB-mediated *Listeria monocytogenes* internalization requires a balanced phospholipase D activity maintained through phospho-cofilin. *Mol Microbiol* 81:860–880
- Han X, Yu R, Zhen D, Tao S, Schmidt M, Han L (2011b) β -1,3-Glucan-induced host phospholipase D activation is involved in *Aspergillus fumigatus* internalization into type II human pneumocyte A549 cells. *PLoS One* 6:e21468
- Hardman JG, Robison GA, Sutherland EW (1971) Cyclic nucleotides. *Annu Rev Physiol* 33:311–336
- Heitzmann H (1972) Rhodopsin is the predominant protein of rod outer segment membranes. *Nat New Biol* 235:114
- Hekman M, Feder D, Keenan AK, Gal A, Klein HW, Pfeuffer T, Levitzki A, Helmreich EJ (1984) Reconstitution of β -adrenergic receptor with components of adenylate cyclase. *EMBO J* 3:3339–3345
- Herrmann E, Gierschik P, Jakobs KH (1989) Neomycin induces high-affinity agonist binding of G-protein-coupled receptors. *Eur J Biochem* 185:677–683
- Hildebrandt JD, Hanoune J, Bimbaumer L (1982) Guanine nucleotide inhibition of cyc^s S49 mouse lymphoma cell membrane adenylyl cyclase. *J Biol Chem* 257:14723–14725
- Hilf G, Gierschik P, Jakobs KH (1989) Muscarinic acetylcholine receptor-stimulated binding of guanosine 5'-O-(3-thiotriphosphate) to guanine-nucleotide-binding proteins in cardiac membranes. *Eur J Biochem* 186:725–731
- Hilf G, Jakobs KH (1989) Activation of cardiac G-proteins by muscarinic acetylcholine receptors: regulation by Mg²⁺ and Na⁺ ions. *Eur J Pharmacol* 172:155–163
- Hilf G, Jakobs KH (1992a) Activation of solubilized G-proteins by muscarinic acetylcholine receptors. *Cell Signal* 4:787–794
- Hilf G, Jakobs KH (1992b) Agonist-independent inhibition of G protein activation by muscarinic acetylcholine receptor antagonists in cardiac membranes. *Eur J Pharmacol* 225:245–252
- Hilf G, Kupprion C, Wieland T, Jakobs KH (1992) Dissociation of guanosine 5'-[γ -thio]triphosphate from guanine-nucleotide-binding regulatory proteins in native cardiac membranes. Regulation by nucleotides and muscarinic acetylcholine receptors. *Eur J Biochem* 204:725–731

- Himmel HM, Meyer zu Heringdorf D, Graf E, Dobrev D, Kortner A, Schuler S, Jakobs KH, Ravens U (2000) Evidence for Edg-3 receptor-mediated activation of $I_{K_{ACH}}$ by sphingosine-1-phosphate in human atrial cardiomyocytes. *Mol Pharmacol* 58:449–454
- Himmel HM, Meyer zu Heringdorf D, Windorfer B, van Koppen CJ, Ravens U, Jakobs KH (1998) Guanine nucleotide-sensitive inhibition of L-type Ca^{2+} current by lysosphingolipids in RINm5F insulinoma cells. *Mol Pharmacol* 53:862–869
- Hippe HJ, Abu-Taha I, Wolf NM, Katus HA, Wieland T (2011) Through scaffolding and catalytic actions nucleoside diphosphate kinase B differentially regulates basal and β -adrenoceptor-stimulated cAMP synthesis. *Cell Signal* 23:579–585
- Hippe HJ, Luedde M, Lutz S, Koehler H, Eschenhagen T, Frey N, Katus HA, Wieland T, Niroomand F (2007) Regulation of cardiac cAMP synthesis and contractility by nucleoside diphosphate kinase B/G protein $\beta\gamma$ dimer complexes. *Circ Res* 100:1191–1199
- Hippe HJ, Lutz S, Cuello F, Knorr K, Vogt A, Jakobs KH, Wieland T, Niroomand F (2003) Activation of heterotrimeric G proteins by a high energy phosphate transfer via nucleoside diphosphate kinase (NDPK) B and $G\beta$ subunits. Specific activation of $G_s\alpha$ by an NDPK B. $G\beta\gamma$ complex in H10 cells. *J Biol Chem* 278:7227–7233
- Hippe HJ, Wolf NM, Abu-Taha I, Mehringer R, Just S, Lutz S, Niroomand F, Postel EH, Katus HA, Rottbauer W, Wieland T (2009) The interaction of nucleoside diphosphate kinase B with $G\beta\gamma$ dimers controls heterotrimeric G protein function. *Proc Natl Acad Sci U S A* 106:16269–16274
- Hommers LG, Klenk C, Dees C, Bünemann M (2010) G proteins in reverse mode: receptor-mediated GTP release inhibits G protein and effector function. *J Biol Chem* 285:8227–8233
- Hori T, Okuno T, Hirata K, Yamashita K, Kawano Y, Yamamoto M, Hato M, Nakamura M, Shimizu T, Yokomizo T, Miyano M, Yokoyama S (2018) Na^+ -mimicking ligands stabilize the inactive state of leukotriene B4 receptor BLT1. *Nat Chem Biol* 14:262–269
- Huang C, Hepler JR, Gilman AG, Mumby SM (1997) Attenuation of Gi- and Gq-mediated signaling by expression of RGS4 or GAIP in mammalian cells. *Proc Natl Acad Sci U S A* 94:6159–6163
- Illenberger D, Schwald F, Pimmer D, Binder W, Maier G, Dietrich A, Gierschik P (1998) Stimulation of phospholipase C- β 2 by the Rho GTPases Cdc42Hs and Rac1. *EMBO J* 17:6241–6249
- Iyengar R, Birnbaumer L (1982) Hormone receptor modulates the regulatory component of adenylyl cyclase by reducing its requirement for Mg^{2+} and enhancing its extent of activation by guanine nucleotides. *Proc Natl Acad Sci U S A* 79:5179–5183
- Jakobs KH, Aktories K, Schultz G (1979) GTP-dependent inhibition of cardiac adenylyl cyclase by muscarinic cholinergic agonists. *Naunyn Schmiedeberg's Arch Pharmacol* 310:113–119
- Jakobs KH, Aktories K, Schultz G (1981) Inhibition of adenylyl cyclase by hormones and neurotransmitters. *Adv Cyclic Nucleotide Res* 14:173–187
- Jakobs KH, Aktories K, Schultz G (1983) A nucleotide regulatory site for somatostatin inhibition of adenylyl cyclase in S49 lymphoma cells. *Nature* 303:177–178
- Jakobs KH, Lasch P, Minuth M, Aktories K, Schultz G (1982) Uncoupling of alpha-adrenoceptor-mediated inhibition of human platelet adenylyl cyclase by N-ethylmaleimide. *J Biol Chem* 257:2829–2833
- Jakobs KH, Saur W, Schultz G (1976) Reduction of adenylyl cyclase activity in lysates of human platelets by the α -adrenergic component of epinephrine. *J Cyclic Nucleotide Res* 2:381–392
- Jakobs KH, Saur W, Schultz G (1978) Inhibition of platelet adenylyl cyclase by epinephrine requires GTP. *FEBS Lett* 85:167–170
- Jakobs KH, Schultz G (1970) Effects of various hormones and drugs on adenylyl cyclase of rat kidney. *Naunyn-Schmiedeberg's Archiv für Pharmakologie* 266:364–365
- Jakobs KH, Schultz G (1980) Actions of hormones and neurotransmitters at the plasma membrane: inhibition of adenylyl cyclase. *Trends Pharmacol Sci* 1:331–333
- Jakobs KH, Schultz G (1983) Occurrence of a hormone-sensitive inhibitory coupling component of the adenylyl cyclase in S49 lymphoma cyc⁻ variants. *Proc Natl Acad Sci U S A* 80:3899–3902
- Jakobs KH, Schultz K, Schultz G (1972) Inhibition of adenylyl cyclase preparations from rat kidney by calcium ions and various diuretics. *Naunyn Schmiedeberg's Arch Pharmacol* 273:248–266
- Jakobs KH, Wieland T (1989) Evidence for receptor-regulated phosphotransfer reactions involved in activation of the adenylyl cyclase inhibitory G protein in human platelet membranes. *Eur J Biochem* 183:115–121
- Janin J, Dumas C, Morera S, Xu Y, Meyer P, Chiadmi M, Cherfils J (2000) Three-dimensional structure of nucleoside diphosphate kinase. *J Bioenerg Biomembr* 32:215–225
- Jard S, Cantau B, Jakobs KH (1981) Angiotensin II and α -adrenergic agonists inhibit rat liver adenylyl cyclase. *J Biol Chem* 256:2603–2606
- Johnson EN, Shi X, Cassaday J, Ferrer M, Strulovici B, Kunapuli P (2008) A 1,536-well [³⁵S]GTP γ S scintillation proximity binding assay for ultra-high-throughput screening of an orphan galphai-coupled GPCR. *Assay Drug Dev Technol* 6:327–337
- Katada T, Gilman AG, Watanabe Y, Bauer S, Jakobs KH (1985) Protein kinase C phosphorylates the inhibitory guanine-nucleotide-binding regulatory component and apparently suppresses its function in hormonal inhibition of adenylyl cyclase. *Eur J Biochem* 151:431–437
- Katada T, Ui M (1982) ADP ribosylation of the specific membrane protein of C6 cells by islet-activating protein associated with modification of adenylyl cyclase activity. *J Biol Chem* 257:7210–7216
- Kather H, Aktories K, Schulz G, Jakobs KH (1983) Islet-activating protein discriminates the antilipolytic mechanism of insulin from that of other antilipolytic compounds. *FEBS Lett* 161:149–152
- Kather H, Bieger W, Michel G, Aktories K, Jakobs KH (1985) Human fat cell lipolysis is primarily regulated by inhibitory modulators acting through distinct mechanisms. *J Clin Invest* 76:1559–1565
- Katritch V, Cherezov V, Stevens RC (2013) Structure-function of the G protein-coupled receptor superfamily. *Annu Rev Pharmacol Toxicol* 53:531–556
- Kawasaki H, Springett GM, Mochizuki N, Toki S, Nakaya M, Matsuda M, Housman DE, Graybiel AM (1998) A family of cAMP-binding proteins that directly activate Rap1. *Science (New York, NY)* 282:2275–2279
- Keiper M, Stope MB, Szatkowski D, Bohm A, Tysack K, Vom Dorp F, Saur O, Oude Weermink PA, Evellin S, Jakobs KH, Schmidt M (2004) Epac- and Ca^{2+} -controlled activation of Ras and extracellular signal-regulated kinases by G_s -coupled receptors. *J Biol Chem* 279:46497–46508
- Kim M, MS A, Ewald AJ, Werb Z, Mostov KE (2015) p114RhoGEF governs cell motility and lumen formation during tubulogenesis through a ROCK-myosin-II pathway. *J Cell Sci* 128:4317–4327
- Kozasa T, Jiang X, Hart MJ, Sternweis PM, Singer WD, Gilman AG, Bollag G, Sternweis PC (1998) p115 RhoGEF, a GTPase activating protein for $G\alpha_{12}$ and $G\alpha_{13}$. *Science (New York, NY)* 280:2109–2111
- Kühn B, Christel C, Wieland T, Schultz G, Gudermann T (2002) G-protein $\beta\gamma$ -subunits contribute to the coupling specificity of the β 2-adrenergic receptor to G_s . *Naunyn Schmiedeberg's Arch Pharmacol* 365:231–241
- Kupper RW, Dewald B, Jakobs KH, Baggiolini M, Gierschik P (1992) G-protein activation by interleukin 8 and related cytokines in human neutrophil plasma membranes. *The Biochemical Journal* 282(Pt 2):429–434
- Kupprion C, Wieland T, Jakobs KH (1993) Receptor-stimulated dissociation of GTP[S] from G_i -proteins in membranes of HL-60 cells. *Cell Signal* 5:425–433

- Kurose H, Katada T, Haga T, Haga K, Ichiyama A, Ui M (1986) Functional interaction of purified muscarinic receptors with purified inhibitory guanine nucleotide regulatory proteins reconstituted in phospholipid vesicles. *J Biol Chem* 261:6423–6428
- Kwok-Keung Fung B, Stryer L (1980) Photolyzed rhodopsin catalyzes the exchange of GTP for bound GDP in retinal rod outer segments. *Proc Natl Acad Sci U S A* 77:2500–2504
- Laudette M, Zuo H, Lezoualc'h F, Schmidt M (2018) Epac function and cAMP scaffolds in the heart and lung. *J Cardiovasc Dev Dis* 5:E9
- Lee MJ, Van Brocklyn JR, Thangada S, Liu CH, Hand AR, Menzeleev R, Spiegel S, Hla T (1998) Sphingosine-1-phosphate as a ligand for the G protein-coupled receptor EDG-1. *Science (New York, NY)* 279:1552–1555
- Li X, Gao M, Han X, Tao S, Zheng D, Cheng Y, Yu R, Han G, Schmidt M, Han L (2012) Disruption of the phospholipase D gene attenuates the virulence of *Aspergillus fumigatus*. *Infect Immun* 80:429–440
- Lichte K, Rossi R, Danneberg K, ter Braak M, Kurschner U, Jakobs KH, Kleuser B, Meyer zu Heringdorf D (2008) Lysophospholipid receptor-mediated calcium signaling in human keratinocytes. *J Invest Dermatol* 128:1487–1498
- Liebmann C, Nawrath S, Schnittler M, Schubert H, Jakobs KH (1992) Binding characteristics and functional G protein coupling of muscarinic acetylcholine receptors in rat duodenum smooth muscle membranes. *Naunyn Schmiedeberg's Arch Pharmacol* 345:7–15
- Liebmann C, Schnittler M, Nawrath S, Jakobs KH (1991) High-affinity bradykinin receptor-catalyzed G protein activation in rat myometrium. *Eur J Pharmacol* 207:67–71
- Lin CW, Miller TR, Witte DG, Bianchi BR, Stashko M, Manelli AM, Frail DE (1995) Characterization of cloned human dopamine D1 receptor-mediated calcium release in 293 cells. *Mol Pharmacol* 47:131–139
- Liscovitch M, Chalifa V, Pertile P, Chen CS, Cantley LC (1994) Novel function of phosphatidylinositol 4,5-bisphosphate as a cofactor for brain membrane phospholipase D. *J Biol Chem* 269:21403–21406
- Liu M, Simon MI (1996) Regulation by cAMP-dependent protein kinase of a G-protein-mediated phospholipase C. *Nature* 382:83–87
- Liu W, Chun E, Thompson AA, Chubukov P, Xu F, Katritch V, Han GW, Roth CB, Heitman LH, IJ AP, Cherezov V, Stevens RC (2012) Structural basis for allosteric regulation of GPCRs by sodium ions. *Science (New York, NY)* 337:232–236
- Lopez De Jesus M, Stope MB, Oude Weernink PA, Mahlke Y, Borgermann C, Ananaba VN, Rimmbach C, Roskopf D, Michel MC, Jakobs KH, Schmidt M (2006) Cyclic AMP-dependent and Epac-mediated activation of R-Ras by G protein-coupled receptors leads to phospholipase D stimulation. *J Biol Chem* 281:21837–21847
- Lopez I, Mak EC, Ding J, Hamm HE, Lomasney JW (2001) A novel bifunctional phospholipase c that is regulated by G α_{12} and stimulates the Ras/mitogen-activated protein kinase pathway. *J Biol Chem* 276:2758–2765
- Lutz S, Freichel-Blomquist A, Rumenapp U, Schmidt M, Jakobs KH, Wieland T (2004) p63RhoGEF and GEFT are Rho-specific guanine nucleotide exchange factors encoded by the same gene. *Naunyn Schmiedeberg's Arch Pharmacol* 369:540–546
- Lutz S, Freichel-Blomquist A, Yang Y, Rumenapp U, Jakobs KH, Schmidt M, Wieland T (2005) The guanine nucleotide exchange factor p63RhoGEF, a specific link between G $_{q/11}$ -coupled receptor signaling and RhoA. *J Biol Chem* 280:11134–11139
- Lutz S, Mohl M, Rauch J, Weber P, Wieland T (2013) RhoGEF17, a rho-specific guanine nucleotide exchange factor activated by phosphorylation via cyclic GMP-dependent kinase I α . *Cell Signal* 25:630–638
- Lutz S, Mura R, Baltus D, Movsesian M, Kubler W, Niroomand F (2001) Increased activity of membrane-associated nucleoside diphosphate kinase and inhibition of cAMP synthesis in failing human myocardium. *Cardiovasc Res* 49:48–55
- Lutz S, Shankaranarayanan A, Coco C, Ridilla M, Nance MR, Vettel C, Baltus D, Evelyn CR, Neubig RR, Wieland T, Tesmer JJ (2007) Structure of G α_q -p63RhoGEF-RhoA complex reveals a pathway for the activation of RhoA by GPCRs. *Science (New York, NY)* 318:1923–1927
- Massink A, Gutierrez-de-Teran H, Lenselink EB, Ortiz Zacarias NV, Xia L, Heitman LH, Katritch V, Stevens RC, IJzerman AP (2015) Sodium ion binding pocket mutations and adenosine A $_{2A}$ receptor function. *Mol Pharmacol* 87:305–313
- Massink A, Louvel J, Adler I, van Veen C, Huisman BJ, Dijksteel GS, Guo D, Lenselink EB, Buckley BJ, Matthews H, Ranson M, Kelso M, IJzerman AP (2016) 5'-Substituted amiloride derivatives as allosteric modulators binding in the sodium ion pocket of the adenosine A $_{2A}$ receptor. *J Med Chem* 59:4769–4777
- Metrich M, Lucas A, Gastineau M, Samuel JL, Heymes C, Morel E, Lezoualc'h F (2008) Epac mediates β -adrenergic receptor-induced cardiomyocyte hypertrophy. *Circ Res* 102:959–965
- Meyer zu Heringdorf D (2004) Lysophospholipid receptor-dependent and -independent calcium signaling. *J Cell Biochem* 92:937–948
- Meyer zu Heringdorf D, Himmel HM, Jakobs KH (2002) Sphingosylphosphorylcholine-biological functions and mechanisms of action. *Biochim Biophys Acta* 1582:178–189
- Meyer zu Heringdorf D, Ihlefeld K, Pfeilschifter J (2013) Pharmacology of the sphingosine-1-phosphate signalling system. *Handb Exp Pharmacol* 239–253
- Meyer zu Heringdorf D, Lass H, Alemany R, Laser KT, Neumann E, Zhang C, Schmidt M, Rauen U, Jakobs KH, van Koppen CJ (1998a) Sphingosine kinase-mediated Ca $^{2+}$ signalling by G-protein-coupled receptors. *EMBO J* 17:2830–2837
- Meyer zu Heringdorf D, Lass H, Kuchar I, Lipinski M, Alemany R, Rumenapp U, Jakobs KH (2001) Stimulation of intracellular sphingosine-1-phosphate production by G-protein-coupled sphingosine-1-phosphate receptors. *Eur J Pharmacol* 414:145–154
- Meyer zu Heringdorf D, Liliom K, Schaefer M, Danneberg K, Jaggar JH, Tigyi G, Jakobs KH (2003a) Photolysis of intracellular caged sphingosine-1-phosphate causes Ca $^{2+}$ mobilization independently of G-protein-coupled receptors. *FEBS Lett* 554:443–449
- Meyer zu Heringdorf D, Niederdring N, Neumann E, Frode R, Lass H, Van Koppen CJ, Jakobs KH (1998b) Discrimination between plasma membrane and intracellular target sites of sphingosylphosphorylcholine. *Eur J Pharmacol* 354:113–122
- Meyer zu Heringdorf D, van Koppen CJ, Windorfer B, Himmel HM, Jakobs KH (1996) Calcium signalling by G protein-coupled sphingolipid receptors in bovine aortic endothelial cells. *Naunyn Schmiedeberg's Arch Pharmacol* 354:397–403
- Meyer zu Heringdorf D, Vincent ME, Lipinski M, Danneberg K, Stropp U, Wang DA, Tigyi G, Jakobs KH (2003b) Inhibition of Ca $^{2+}$ signalling by the sphingosine 1-phosphate receptor S1P $_1$. *Cell Signal* 15:677–687
- Mitin N, Rossman KL, Currin R, Anne S, Marshall TW, Bear JE, Bautch VL, Der CJ (2013) The RhoGEF TEM4 regulates endothelial cell migration by suppressing actomyosin contractility. *PLoS One* 8:e66260
- Mitin N, Rossman KL, Der CJ (2012) Identification of a novel actin-binding domain within the Rho guanine nucleotide exchange factor TEM4. *PLoS One* 7:e41876
- Mizuki Y, Takaki M, Okahisa Y, Sakamoto S, Kodama M, Ujike H, Uchitomi Y (2014) Human Rho guanine nucleotide exchange factor 11 gene is associated with schizophrenia in a Japanese population. *Human Psychopharmacol* 29:552–558
- Mizuki Y, Takaki M, Sakamoto S, Okamoto S, Kishimoto M, Okahisa Y, Itoh M, Yamada N (2016) Human Rho guanine nucleotide exchange factor 11 (ARHGEF11) regulates dendritic morphogenesis. *Int J Mol Sci* 18(1):67

- Morera S, Chiadmi M, LeBras G, Lascu I, Janin J (2002) Mechanism of phosphate transfer by nucleoside diphosphate kinase: X-ray structures of the phosphohistidine intermediate of the enzymes from *Drosophila* and *Dictyostelium*. *Biochemistry* 34(35):11062–11070
- Motulsky HJ, Insel PA (1983) ADP- and epinephrine-elicited release of [³H]guanylylimidodiphosphate from platelet membranes. Implications Recept-N_i Stoichiometry. *FEBS Lett* 164:13–16
- Munoz-Llancao P, de Gregorio C, Las Heras M, Meinohl C, Noorman K, Boddeke E, Cheng X, Lezoualc'h F, Schmidt M, Gonzalez-Billault C (2017) Microtubule-regulating proteins and cAMP-dependent signaling in neuroblastoma differentiation. *Cytoskeleton (Hoboken, NJ)* 74:143–158
- Munoz-Llancao P, Henriquez DR, Wilson C, Bodaleo F, Boddeke EW, Lezoualc'h F, Schmidt M, Gonzalez-Billault C (2015) Exchange protein directly activated by cAMP (EPAC) regulates neuronal polarization through Rap1B. *J Neurosci* 35:11315–11329
- Ngok SP, Geyer R, Kourtidis A, Mitin N, Feathers R, Der C, Anastasiadis PZ (2013) TEM4 is a junctional Rho GEF required for cell-cell adhesion, monolayer integrity and barrier function. *J Cell Sci* 126:3271–3277
- Niu J, Profirovic J, Pan H, Vaiskunaitis R, Voyno-Yasenetskaya T (2003) G protein $\beta\gamma$ subunits stimulate p114RhoGEF, a guanine nucleotide exchange factor for RhoA and Rac1: regulation of cell shape and reactive oxygen species production. *Circ Res* 93:848–856
- Northup JK, Smigel MD, Sternweis PC, Gilman AG (1983a) The subunits of the stimulatory regulatory component of adenylate cyclase. Resolution of the activated 45,000-Dalton α subunit. *J Biol Chem* 258:11369–11376
- Northup JK, Sternweis PC, Gilman AG (1983b) The subunits of the stimulatory regulatory component of adenylate cyclase. Resolution, activity, and properties of the 35,000-Dalton β subunit. *J Biol Chem* 258:11361–11368
- Northup JK, Sternweis PC, Smigel MD, Schleifer LS, Ross EM, Gilman AG (1980) Purification of the regulatory component of adenylate cyclase. *Proc Natl Acad Sci U S A* 77:6516–6520
- Offermanns S, Wieland T, Homann D, Sandmann J, Bombien E, Spicher K, Schultz G, Jakobs KH (1994) Transfected muscarinic acetylcholine receptors selectively couple to G_i-type G proteins and G_{q/11}. *Mol Pharmacol* 45:890–898
- Oldenburger A, Roscioni SS, Jansen E, Menzen MH, Halayko AJ, Timens W, Meurs H, Maarsingh H, Schmidt M (2012) Anti-inflammatory role of the cAMP effectors Epac and PKA: implications in chronic obstructive pulmonary disease. *PLoS One* 7:e31574
- Oldenburger A, Timens W, Bos S, Smit M, Smrcka AV, Laurent AC, Cao J, Hylkema M, Meurs H, Maarsingh H, Lezoualc'h F, Schmidt M (2014) Epac1 and Epac2 are differentially involved in inflammatory and remodeling processes induced by cigarette smoke. *FASEB J: Official Publication of the Federation of American Societies for Experimental Biology* 28:4617–4628
- Olivera A, Spiegel S (1993) Sphingosine-1-phosphate as second messenger in cell proliferation induced by PDGF and FCS mitogens. *Nature* 365:557–560
- Ongherth A, Pasch S, Wuertz CM, Nowak K, Kittana N, Weis CA, Jatho A, Vettel C, Tiburcy M, Toischer K, Hasenfuss G, Zimmermann WH, Wieland T, Lutz S (2015) p63RhoGEF regulates auto- and paracrine signaling in cardiac fibroblasts. *J Mol Cell Cardiol* 88:39–54
- Ostroveanu A, van der Zee EA, Eisel UL, Schmidt M, Nijholt IM (2010) Exchange protein activated by cyclic AMP 2 (Epac2) plays a specific and time-limited role in memory retrieval. *Hippocampus* 20:1018–1026
- Oude Weernink PA, Han L, Jakobs KH, Schmidt M (2007) Dynamic phospholipid signaling by G protein-coupled receptors. *Biochim Biophys Acta* 1768:888–900
- Oude Weernink PA, Schmidt M, Jakobs KH (2004) Regulation and cellular roles of phosphoinositide 5-kinases. *Eur J Pharmacol* 500:87–99
- Oude Weernink PA, Schulte P, Guo Y, Wetzel J, Amano M, Kaibuchi K, Haverland S, Voss M, Schmidt M, Mayr GW, Jakobs KH (2000) Stimulation of phosphatidylinositol-4-phosphate 5-kinase by Rho-kinase. *J Biol Chem* 275:10168–10174
- Park D, Jhon DY, Lee CW, Lee KH, Rhee SG (1993) Activation of phospholipase C isozymes by G protein $\beta\gamma$ subunits. *J Biol Chem* 268:4573–4576
- Park DJ, Min HK, Rhee SG (1992) Inhibition of CD3-linked phospholipase C by phorbol ester and by cAMP is associated with decreased phosphotyrosine and increased phosphoserine contents of PLC- γ 1. *J Biol Chem* 267:1496–1501
- Parnell E, Palmer TM, Yarwood SJ (2015) The future of EPAC-targeted therapies: agonism versus antagonism. *Trends Pharmacol Sci* 36:203–214
- Pedersen SE, Ross EM (1982) Functional reconstitution of β -adrenergic receptors and the stimulatory GTP-binding protein of adenylate cyclase. *Proc Natl Acad Sci U S A* 79:7228–7232
- Proia RL, Hla T (2015) Emerging biology of sphingosine-1-phosphate: its role in pathogenesis and therapy. *J Clin Invest* 125:1379–1387
- Pugh EN, Lamb TD (2000) Phototransduction in vertebrate rod and cones: molecular mechanisms of amplification, recovery, and light adaptation. In: G. SD, de Grip WJ, Pugh EN (eds.) *Handbook of biological physics*. pp. 183–255
- Rabiet MJ, Tardif M, Braun L, Boulay F (2002) Inhibitory effects of a dominant-interfering form of the Rho-GTPase Cdc42 in the chemoattractant-elicited signaling pathways leading to NADPH oxidase activation in differentiated HL-60 cells. *Blood* 100:1835–1844
- Rasmussen SG, DeVree BT, Zou Y, Kruse AC, Chung KY, Kobilka TS, Thian FS, Chae PS, Pardon E, Calinski D, Mathiesen JM, Shah ST, Lyons JA, Caffrey M, Gellman SH, Steyaert J, Skiniotis G, Weis WI, Sunahara RK, Kobilka BK (2011) Crystal structure of the β 2 adrenergic receptor-Gs protein complex. *Nature* 477:549–555
- Rich TC, Fagan KA, Tse TE, Schaack J, Cooper DM, Karpen JW (2001) A uniform extracellular stimulus triggers distinct cAMP signals in different compartments of a simple cell. *Proc Natl Acad Sci U S A* 98:13049–13054
- Robichaux WG 3rd, Cheng X (2018) Intracellular cAMP sensor EPAC: physiology, pathophysiology, and therapeutics development. *Physiol Rev* 98:919–1053
- Rümenapp U, Asmus M, Schablowski H, Woznicki M, Han L, Jakobs KH, Fahimi-Vahid M, Michalek C, Wieland T, Schmidt M (2001) The M3 muscarinic acetylcholine receptor expressed in HEK-293 cells signals to phospholipase D via G₁₂ but not G_q-type G proteins: regulators of G proteins as tools to dissect pertussis toxin-resistant G proteins in receptor-effector coupling. *J Biol Chem* 276:2474–2479
- Rümenapp U, Blomquist A, Schworer G, Schablowski H, Psoma A, Jakobs KH (1999) Rho-specific binding and guanine nucleotide exchange catalysis by KIAA0380, a dbl family member. *FEBS Lett* 459:313–318
- Rümenapp U, Freichel-Blomquist A, Wittinghofer B, Jakobs KH, Wieland T (2002) A mammalian Rho-specific guanine-nucleotide exchange factor (p164-RhoGEF) without a pleckstrin homology domain. *The Biochemical Journal* 366:721–728
- Rümenapp U, Geiszt M, Wahn F, Schmidt M, Jakobs KH (1995) Evidence for ADP-ribosylation-factor-mediated activation of phospholipase D by M3 muscarinic acetylcholine receptor. *Eur J Biochem* 234:240–244
- Rümenapp U, Lummen G, Virchow S, Hanske J, Meyer zu Heringdorf D, Jakobs KH (2000) Sphingolipid receptor signaling and function in human bladder carcinoma cells: inhibition of LPA- but enhancement

- of thrombin-stimulated cell motility. Naunyn Schmiedeberg's Arch Pharmacol 361:1–11
- Rümenapp U, Schmidt M, Geiszt M, Jakobs KH (1996) Participation of small GTP-binding proteins in M3 muscarinic acetylcholine receptor signalling to phospholipase D and C. Prog Brain Res 109:209–216
- Rümenapp U, Schmidt M, Olesch S, Ott S, Eichel-Streiber CV, Jakobs KH (1998) Tyrosine-phosphorylation-dependent and rho-protein-mediated control of cellular phosphatidylinositol 4,5-bisphosphate levels. The Biochemical Journal 334(Pt 3):625–631
- Rümenapp U, Schmidt M, Wahn F, Tapp E, Grannass A, Jakobs KH (1997) Characteristics of protein-kinase-C- and ADP-ribosylation-factor-stimulated phospholipase D activities in human embryonic kidney cells. Eur J Biochem 248:407–414
- Sadana R, Dessauer CW (2009) Physiological roles for G protein-regulated adenylyl cyclase isoforms: insights from knockout and overexpression studies. Neuro-Signals 17:5–22
- Salomon MR, Boume HR (1981) Novel S49 lymphoma variants with aberrant cyclic AMP metabolism. Mol Pharmacol 19:109–116
- Sand C, Grandoch M, Borgermann C, Oude Weemink PA, Mahlke Y, Schwindenhammer B, Weber AA, Fischer JW, Jakobs KH, Schmidt M (2010) 8-pCPT-conjugated cyclic AMP analogs exert thromboxane receptor antagonistic properties. Thromb Haemost 103:662–678
- Schmidt M, Huwe SM, Fasselt B, Homann D, Rümenapp U, Sandmann J, Jakobs KH (1994) Mechanisms of phospholipase D stimulation by M3 muscarinic acetylcholine receptors. Evidence for involvement of tyrosine phosphorylation. Eur J Biochem 225:667–675
- Schmidt M, Bienek C, van Koppen CJ, Michel MC, Jakobs KH (1995a) Differential calcium signalling by M2 and M3 muscarinic acetylcholine receptors in a single cell type. Naunyn Schmiedeberg's Arch Pharmacol 352:469–476
- Schmidt M, Fasselt B, Rümenapp U, Bienek C, Wieland T, van Koppen CJ, Jakobs KH (1995b) Rapid and persistent desensitization of M3 muscarinic acetylcholine receptor-stimulated phospholipase D. Concomitant sensitization of phospholipase C. J Biol Chem 270:19949–19956
- Schmidt M, Bienek C, Rümenapp U, Zhang C, Lummen G, Jakobs KH, Just I, Aktories K, Moos M, von Eichel-Streiber C (1996a) A role for rho in receptor- and G protein-stimulated phospholipase C. Reduction in phosphatidylinositol 4,5-bisphosphate by *Clostridium difficile* toxin B. Naunyn Schmiedeberg's Arch Pharmacol 354:87–94
- Schmidt M, Rümenapp U, Bienek C, Keller J, von Eichel-Streiber C, Jakobs KH (1996b) Inhibition of receptor signaling to phospholipase D by *Clostridium difficile* toxin B. Role of Rho proteins. J Biol Chem 271:2422–2426
- Schmidt M, Rümenapp U, Nehls C, Ott S, Keller J, Von Eichel-Streiber C, Jakobs KH (1996c) Restoration of *Clostridium difficile* toxin-B-inhibited phospholipase D by phosphatidylinositol 4,5-bisphosphate. Eur J Biochem 240:707–712
- Schmidt M, Voss M, Thiel M, Bauer B, Grannass A, Tapp E, Cool RH, de Gunzburg J, von Eichel-Streiber C, Jakobs KH (1998) Specific inhibition of phorbol ester-stimulated phospholipase D by *Clostridium sordellii* lethal toxin and *Clostridium difficile* toxin B-1470 in HEK-293 cells. Restoration by Ral GTPases. J Biol Chem 273:7413–7422
- Schmidt M, Evellin S, Weernink PA, von Dorp F, Rehmann H, Lomasney JW, Jakobs KH (2001) A new phospholipase-C-calcium signalling pathway mediated by cyclic AMP and a Rap GTPase. Nat Cell Biol 3:1020–1024
- Schmidt M, Oude Weemink PA, Vom Dorp F, Stope MB, Jakobs KH (2004) Mammalian phospholipase C. Adv Mol Cell Biol 33:433–453
- Schmidt M, Sand C, Jakobs KH, Michel MC, Weernink PA (2007) Epac and the cardiovascular system. Curr Opin Pharmacol 7:193–200
- Schmidt M, Dekker FJ, Maarsingh H (2013) Exchange protein directly activated by cAMP (epac): a multidomain cAMP mediator in the regulation of diverse biological functions. Pharmacol Rev 65:670–709
- Schwede F, Bertinetti D, Langerijs CN, Hadders MA, Wienk H, Ellenbroek JH, de Koning EJ, Bos JL, Herberg FW, Genieser HG, Janssen RA, Rehmann H (2015) Structure-guided design of selective Epac1 and Epac2 agonists. PLoS Biol 13:e1002038
- Seifert R, Rosenthal W, Schultz G, Wieland T, Gierschick P, Jakobs KH (1988) The role of nucleoside-diphosphate kinase reactions in G protein activation of NADPH oxidase by guanine and adenine nucleotides. Eur J Biochem 175:51–55
- Siffert W, Roskopf D, Moritz A, Wieland T, Kaldenberg-Stasch S, Kettler N, Hartung K, Beckmann S, Jakobs KH (1995) Enhanced G protein activation in immortalized lymphoblasts from patients with essential hypertension. J Clin Invest 96:759–766
- Sim-Selley LJ, Wilkerson JL, Burston JJ, Hauser KF, McLane V, Welch SP, Lichtman AH, Selley DE (2018) Differential tolerance to FTY720-induced antinociception in acute thermal and nerve injury mouse pain models: role of S1P receptor adaptation. J Pharmacol Exp Ther 366:509–518
- Sim LJ, Selley DE, Childers SR (1995) In vitro autoradiography of receptor-activated G proteins in rat brain by agonist-stimulated guanylyl 5'-[γ - 35 S]thio]-triphosphate binding. Proc Natl Acad Sci U S A 92:7242–7246
- Smrcka AV, Brown JH, Holz GG (2012) Role of phospholipase C ϵ in physiological phosphoinositide signaling networks. Cell Signal 24:1333–1343
- Smrcka AV, Sternweis PC (1993) Regulation of purified subtypes of phosphatidylinositol-specific phospholipase C β by G protein α and $\beta\gamma$ subunits. J Biol Chem 268:9667–9674
- Snyder JT, Singer AU, Wing MR, Harden TK, Sondek J (2003) The pleckstrin homology domain of phospholipase C- β 2 as an effector site for Rac. J Biol Chem 278:21099–21104
- Song C, Gao Y, Tian Y, Han X, Chen Y, Tian DL (2013) Expression of p114RhoGEF predicts lymph node metastasis and poor survival of squamous-cell lung carcinoma patients. Tumour Biol: the Journal of the International Society for Oncodevelopmental Biology and Medicine 34:1925–1933
- Song C, Hu CD, Masago M, Kariyai K, Yamawaki-Kataoka Y, Shibatohe M, Wu D, Satoh T, Kataoka T (2001) Regulation of a novel human phospholipase C, PLC ϵ , through membrane targeting by Ras. J Biol Chem 276:2752–2757
- Sternweis PC, Robishaw JD (1984) Isolation of two proteins with high affinity for guanine nucleotides from membranes of bovine brain. J Biol Chem 259:13806–13813
- Stope MB, Vom Dorp F, Szatkowski D, Bohm A, Keiper M, Nolte J, Oude Weemink PA, Roskopf D, Evellin S, Jakobs KH, Schmidt M (2004) Rap2B-dependent stimulation of phospholipase C- ϵ by epidermal growth factor receptor mediated by c-Src phosphorylation of RasGRP3. Mol Cell Biol 24:4664–4676
- Strub GM, Maceyka M, Hait NC, Milstien S, Spiegel S (2010) Extracellular and intracellular actions of sphingosine-1-phosphate. Adv Exp Med Biol 688:141–155
- Sun Z, Smrcka AV, Chen S (2013) WDR26 functions as a scaffolding protein to promote G $\beta\gamma$ -mediated phospholipase C β 2 (PLC β 2) activation in leukocytes. J Biol Chem 288:16715–16725
- Tanguy E, Kassas N, Vitale N (2018) Protein-phospholipid interaction motifs: a focus on phosphatidic acid. Biomolecules 8:20
- Tepper AD, Dammann H, Bominaar AA, Veron M (1994) Investigation of the active site and the conformational stability of nucleoside diphosphate kinase by site-directed mutagenesis. J Biol Chem 269:32175–32180

- ter Braak M, Danneberg K, Lichte K, Liphardt K, Ktistakis NT, Pitson SM, Hla T, Jakobs KH, Meyer zu Heringdorf D (2009) $G\alpha_q$ -mediated plasma membrane translocation of sphingosine kinase-1 and cross-activation of S1P receptors. *Biochim Biophys Acta* 1791:357–370
- Terry SJ, Elbediwy A, Zihni C, Harris AR, Bailly M, Charras GT, Balda MS, Matter K (2012) Stimulation of cortical myosin phosphorylation by p114RhoGEF drives cell migration and tumor cell invasion. *PLoS One* 7:e50188
- Terry SJ, Zihni C, Elbediwy A, Vitiello E, Leefa Chong San IV, Balda MS, Matter K (2011) Spatially restricted activation of RhoA signaling at epithelial junctions by p114RhoGEF drives junction formation and morphogenesis. *Nat Cell Biol* 13:159–166
- Thal DM, Glukhova A, Sexton PM, Christopoulos A (2018) Structural insights into G-protein-coupled receptor allostery. *Nature* 559:45–53
- Tolkovsky AM, Levitzki A (1978) Mode of coupling between the β -adrenergic receptor and adenylate cyclase in turkey erythrocytes. *Biochemistry* 17:3795
- Toro MJ, Montoya E, Birnbaumer L (1987) Inhibitory regulation of adenylyl cyclases. Evidence inconsistent with $\beta\gamma$ -complexes of G_i proteins mediating hormonal effects by interfering with activation of G_s . *Mol Endocrinol* (Baltimore, MD) 1:669–676
- Toth AD, Schell R, Levay M, Vettel C, Theis P, Haslinger C, Alban F, Werhahn S, Frischbier L, Krebs-Haupenthal J, Thomas D, Grone HJ, Avkiran M, Katus HA, Wieland T, Backs J (2018) Inflammation leads through PGE/EP3 signaling to HDAC5/MEF2-dependent transcription in cardiac myocytes. *EMBO Molecular Medicine* 10:e8536
- Van Koppen CJ, Meyer zu Heringdorf M, Laser KT, Zhang C, Jakobs KH, Bünemann M, Pott L (1996a) Activation of a high affinity G_i protein-coupled plasma membrane receptor by sphingosine-1-phosphate. *J Biol Chem* 271:2082–2087
- Van Koppen CJ, Meyer zu Heringdorf D, Zhang C, Laser KT, Jakobs KH (1996b) A distinct G_i protein-coupled receptor for sphingosylphosphorylcholine in human leukemia HL-60 cells and human neutrophils. *Mol Pharmacol* 49:956–961
- Ventimiglia MS, Rodriguez MR, Elverdin JC, Davio CA, Vatta MS, Bianciotti LG (2008) Atrial natriuretic factor intracellular signaling in the rat submandibular gland. *Regul Pept* 150:43–49
- vom Dorp F, Sari AY, Sanders H, Keiper M, Oude Weernink PA, Jakobs KH, Schmidt M (2004) Inhibition of phospholipase C- ϵ by G_i -coupled receptors. *Cell Signal* 16:921–928
- Voss M, Weernink PA, Haupenthal S, Moller U, Cool RH, Bauer B, Camonis JH, Jakobs KH, Schmidt M (1999) Phospholipase D stimulation by receptor tyrosine kinases mediated by protein kinase C and a Ras/Ral signaling cascade. *J Biol Chem* 274:34691–34698
- Wang H, Oestreich EA, Maekawa N, Bullard TA, Vikstrom KL, Dirksen RT, Kelley GG, Blaxall BC, Smrcka AV (2005) Phospholipase C ϵ modulates β -adrenergic receptor-dependent cardiac contraction and inhibits cardiac hypertrophy. *Circ Res* 97:1305–1313
- Wescott MP, Kufareva I, Paes C, Goodman JR, Thaker Y, Puffer BA, Berdougou E, Rucker JB, Handel TM, Doranz BJ (2016) Signal transmission through the CXCR4 chemokine receptor 4 (CXCR4) transmembrane helices. *Proc Natl Acad Sci U S A* 113:9928–9933
- Wettschureck N, Offermanns S (2005) Mammalian G proteins and their cell type specific functions. *Physiol Rev* 85:1159–1204
- White KL, Eddy MT, Gao ZG, Han GW, Lian T, Deary A, Patel N, Jacobson KA, Katritch V, Stevens RC (2018) Structural connection between activation microswitch and allosteric sodium site in GPCR signaling. *Structure* (London, England: 1993) 26:259–269 e255
- Wieland C, Jakobs KH, Wieland T (1994) Altered guanine nucleoside triphosphate binding to transducin by cholera toxin-catalysed ADP-ribosylation. *Cell Signal* 6:487–492
- Wieland T, Bremerich J, Gierschik P, Jakobs KH (1991a) Contribution of nucleoside diphosphokinase to guanine nucleotide regulation of agonist binding to formyl peptide receptors. *Eur J Pharmacol* 208:17–23
- Wieland T, Chen CK (1999) Regulators of G-protein signalling: a novel protein family involved in timely deactivation and desensitization of signalling via heterotrimeric G proteins. *Naunyn Schmiedeberg's Arch Pharmacol* 360:14–26
- Wieland T, Gierschik P, Jakobs KH (1992a) G protein-mediated receptor-receptor interaction: studies with chemotactic receptors in membranes of human leukemia (HL 60) cells. *Naunyn Schmiedeberg's Arch Pharmacol* 346:475–481
- Wieland T, Jakobs KH (1989) Receptor-regulated formation of GTP[γ S] with subsequent persistent G_s -protein activation in membranes of human platelets. *FEBS Lett* 245:189–193
- Wieland T, Jakobs KH (1992) Evidence for nucleoside diphosphokinase-dependent channeling of guanosine 5'-(γ -thio)triphosphate to guanine nucleotide-binding proteins. *Mol Pharmacol* 42:731–735
- Wieland T, Jakobs KH (1994) Measurement of receptor-stimulated guanosine 5'-O-(γ -thio)triphosphate binding by G proteins. *Methods Enzymol* 237:3–13
- Wieland T, Kreiss J, Gierschik P, Jakobs KH (1992b) Role of GDP in formyl-peptide-receptor-induced activation of guanine-nucleotide-binding proteins in membranes of HL 60 cells. *Eur J Biochem* 205:1201–1206
- Wieland T, Liedel K, Kaldenberg-Stasch S, Meyer zu Heringdorf D, Schmidt M, Jakobs KH (1995) Analysis of receptor-G protein interactions in permeabilized cells. *Naunyn Schmiedeberg's Arch Pharmacol* 351:329–336
- Wieland T, Mittmann C (2003) Regulators of G-protein signalling: multifunctional proteins with impact on signalling in the cardiovascular system. *Pharmacol Ther* 97:95–115
- Wieland T, Nürnberg B, Ulibarri I, Kaldenberg-Stasch S, Schultz G, Jakobs KH (1993) Guanine nucleotide-specific phosphate transfer by guanine nucleotide-binding regulatory protein β -subunits. Characterization of the phosphorylated amino acid. *J Biol Chem* 268:18111–18118
- Wieland T, Ronzani M, Jakobs KH (1992c) Stimulation and inhibition of human platelet adenylyl cyclase by thiophosphorylated transducin $\beta\gamma$ -subunits. *J Biol Chem* 267:20791–20797
- Wieland T, Ulibarri I, Gierschik P, Jakobs KH (1991b) Activation of signal-transducing guanine-nucleotide-binding regulatory proteins by guanosine 5'-[γ -thio]triphosphate. Information transfer by intermediately thiophosphorylated beta gamma subunits. *Eur J Biochem* 196:707–716
- Williamson JR (1986) Role of inositol lipid breakdown in the generation of intracellular signals. State of the art lecture. *Hypertension* (Dallas, Tex: 1979) 8:II140–II156
- Wuster M, Costa T, Aktories K, Jakobs KH (1984) Sodium regulation of opioid agonist binding is potentiated by pertussis toxin. *Biochem Biophys Res Commun* 123:1107–1115
- Xie Z, Ho WT, Spellman R, Cai S, Exton JH (2002) Mechanisms of regulation of phospholipase D1 and D2 by the heterotrimeric G proteins G_{13} and G_q . *J Biol Chem* 277:11979–11986
- Xu X, Jin T (2017) ELMO proteins transduce G protein-coupled receptor signal to control reorganization of actin cytoskeleton in chemotaxis of eukaryotic cells. *Small GTPases* 22:1–9
- Yang X, Li J, Fang Y, Zhang Z, Jin D, Chen X, Zhao Y, Li M, Huan L, Kent TA, Dong JF, Jiang R, Yang S, Jin L, Zhang J, Zhong TP, Yu F (2018) Rho guanine nucleotide exchange factor ARHGGEF17 is a risk gene for intracranial aneurysms. *Circ Genomic Precis Med* 11:e002099
- Zaccolo M, Magalhaes P, Pozzan T (2002) Compartmentalisation of cAMP and Ca^{2+} signals. *Curr Opin Cell Biol* 14:160–166

- Zhou XB, Lutz S, Steffens F, Korth M, Wieland T (2007) Oxytocin receptors differentially signal via G_q and G_i proteins in pregnant and nonpregnant rat uterine myocytes: implications for myometrial contractility. *Mol Endocrinol* (Baltimore, MD) 21:740–752
- Zhou XB, Wulfsen I, Lutz S, Utku E, Sausbier U, Ruth P, Wieland T, Korth M (2008) M2 muscarinic receptors induce airway smooth muscle activation via a dual, $G\beta\gamma$ -mediated inhibition of large conductance Ca^{2+} -activated K^+ channel activity. *J Biol Chem* 283: 21036–21044
- Zondag GC, Postma FR, Etten IV, Verlaan I, Moolenaar WH (1998) Sphingosine 1-phosphate signalling through the G-protein-coupled receptor Edg-1. *The Biochem J* 330(Pt 2):605–609

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