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Review

Pleckstrin homology (PH) like domains – versatile modules in protein–protein interaction platforms

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ABSTRACT

The initial reports on pleckstrin homology (PH) domains almost 20 years ago described them as sequence feature of proteins involved in signal transduction processes. Investigated at first along the phospholipid binding properties of a small subset of PH representatives, the PH fold turned out to appear as mediator of phosphotyrosine and polyproline peptide binding to other signaling proteins. While phospholipid binding now seems rather the exception among PH-like domains, protein–protein interactions established as more and more important feature of these modules. In this review we focus on the PH superfold as a versatile protein–protein interaction platform and its three-dimensional integration in an increasing number of available multidomain structures.

1. Introduction

The pleckstrin homology (PH) domain has been defined almost 20 years ago [1,2] as an approximately 100 residue sequence segment that is present twice in the protein kinase C (PKC) substrate pleckstrin [3] as well as in a variety of modular proteins involved in signaling, cytoskeletal organization, membrane trafficking and phospholipid processing [4–7]. The first structures [8,9] revealed the PH domain as a strongly bent seven stranded antiparallel β-sheet, closed on one end by a characteristic C-terminal α-helix [10] (Fig. 1). As a specialty, the structures of phospholipid binding PH domains are commonly polarized [11], a property not shared by many other PH-like domains. Many of the early investigated PH domains bind phospholipids or their head group derivatives. This biochemical function made them attractive membrane anchoring modules depending on the local phospholipid concentrations [4,12,13]. Along this line, initial functions of PH domains in Son of Sevenless (Sos), β-adrenergic receptor kinase (βARK) and insulin receptor substrate 1 (IRS1) pointed to roles in receptor binding [14–16]. It however turned out, that the apparent majority of PH domains in yeast do not show specific binding to phospholipid headgroups [17] and are not membrane targeted. Nor do phosphoinositides (PIs) alone define the location of those PH domains that actually are membrane targeted [17,18], pointing to PH-like domains being more versatile than initially expected. The elucidation of the structures of phosphotyrosine binding (PTB) [19,20] and the polyproline binding Enabled/Vasp (EVH1) [21] domains defined again the fold of the PH domain as a major *déjà vu*, thereby broadening the view for the functional PH-potential [11,22] along with its ligand binding repertoire (Fig. 1). While PH domains were known to occur in regulators of guanine nucleotide binding proteins (GNBPs), e.g. as an obligatory C-terminal association with the Dbl Homology (DH) RhoGEF catalyst [11], the discovery of the PH fold of Ran binding domain 1 (RBD1) made them direct interaction partners or effector modules with this class of proteins [23]. Furthermore, it turns out that also PI binding PH representatives are at the same time effector modules for small GNBPs [24–29] or of Gα-subunits [30]. Mutations within PH modules associated with human disease underscore the importance of these modules in human pathology [31–36]. It is believed that at least some of the disease associated missense mutations affect phospholipid binding and as a consequence potentially subcellular localization.

The structure of the PH domain can be operationally divided or 'split' in roughly two halves, containing a four-stranded N- and a three-stranded C-terminal β-sheet including terminal helix (Fig. 1).

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Abbreviations

Proteins

Akt1	(PKB) murine thymoma viral oncogene
Dbs	Dbl's big sister
Exo84	Exocyst complex 84 kDa subunit
FACT	Facilitates chromatin transcription
GEF	G-nucleotide exchange factor
GRK-2	G-protein coupled receptor kinase 2
Grb10	Growth factor receptor-bound protein 10
Grp1	General receptor of phosphoinositides 1
IRS1	Insulin receptor substrate 1
NDPP1	NPC-derived proline-rich protein 1
PDZ	PSD-95/Dlg/ZO-1
PLC	Phospholipase C
Rac	Ras related C3 botulinum toxin substrate 1
Ral	Ras-like
Ran	Ras-related nuclear protein
RanBP1/2	Ras-related nuclear protein binding protein 1/2,
Rtt106	Regulator of Ty1 transposition protein 106
SHC1	Src homology 2 domain-containing-transforming protein C1
SifA	Salmonella-induced filaments A
Skap-hom	Src kinase-associated phosphoprotein 2
SKIP	Salmonella-induced filaments A and kinesin-interacting protein
SOS	Son of sevenless
TFII	Transcription factor II

Domains

Ac	Acidic domain
AGC-K	cAMP-dependent, cGMP-dependent and protein kinase C family kinase
BEACH	BEige And Chediak-Higashi Syndrome
BSD	BTF2-like transcription factors, Synapse-associated proteins and DOS2-like proteins
CH	Calponin homology
CSRD	Cystein and serine rich domain
C2	Ca2+ -binding
DH	Dbl homology
DM	Dimerization
EF	EF-hand
G	GTPase domain
GAP	GTPase activating protein
GED	GTPase effector domain
Grb2-BD	Growth factor receptor-bound 2 binding domain
GRD	GAP related domain
MidD	Middle domain
RGS(L)	Regulator of G protein signaling (like)
PH	Pleckstrin homology
PK	Protein kinase
PRD	Proline-rich domain
PTB	Phosphotyrosine binding domain
SH2	Src homology 2
SH3	Src homology 3
Syn	Syndecane binding region
TIM	Triosephosphate isomerase barrel like
Tub	Tubulin binding region
WD40	Trp – Asp repeats of about 40 amino acids
WH1	Wiskott–Aldrich syndrome/Homer 1
ZF	Zinc finger

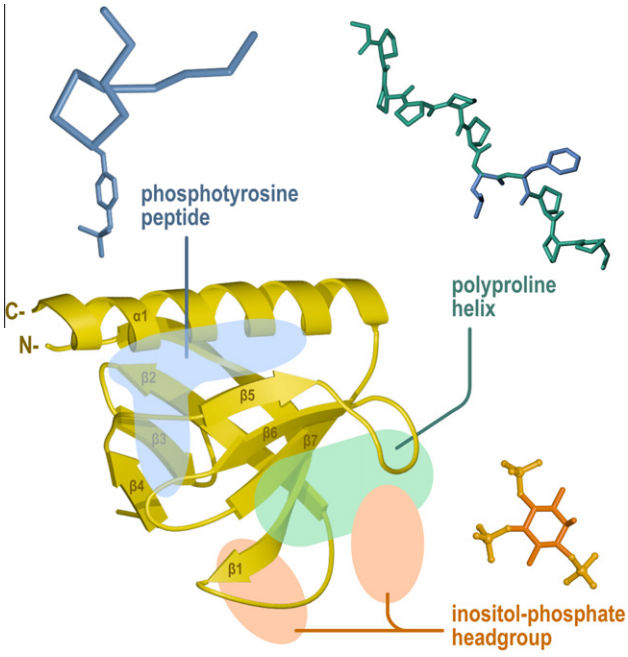

Fig. 1. The PH module and its canonical ligand binding sites. Besides protein interaction partners, the PH fold can accommodate binding sites for phosphorylated inositol head groups (orange), polyproline helices (green) and phosphotyrosine peptides (blue). The α-helix and β-strands of the PH module are labeled and numbered in dark yellow.

Examples of such split PH modules are found in Phospolipase Cγ (PLCγ) [37], α-syntrophin [38], Rho Kinase [39], Myosin X [40] and the Vps36 component of ESCRT-II [41]. A split PH domain has also been engineered on the basis of the PLCδ1 module [42] and reassembled to a functional phosphoinositide binding unit [43]. van Rossum et al. (2005) reported 'in trans' intermolecular interaction between PH domain halves derived from PLC-γ1 and a TRPC3 channel that elicits phospholipid binding and cell-surface expression of TRPC3 [44], although an NMR study could not confirm direct interaction of similar constructs purified from the recombinantly expressed PH portions [45].

In this review we will focus on PH-like examples that mediate protein–protein interaction or modulate cognate functions of their host proteins. We give preference to example systems where structural information is available. The availability of such information about the PH modules within whole molecular context sometimes reveals regulatory features not apparent from the isolated PH modules. We will also briefly describe examples where PH modules have been presented in association with other domains but where mechanistic knowledge is currently limited. Domain schemes of selected PH-like domain containing proteins are summarized in Supplementary Fig. 1.

2. PH-like modules in different molecular contexts and functions

As described above, PH modules occur in a wide variety of cellular environments. The following PH representatives are meant to highlight the PH fold as a versatile protein–protein interaction platform on an inter- as well as intramolecular level.

2.1. PH domains associated with guanine nucleotide exchange factors

The canonical Rho family specific guanine nucleotide exchange factors (GEFs) typically contain a Dbl homology (DH) domain harboring the GEF activity, which is commonly followed by a PH module [46]. The increased nucleotide exchange rates of some DH–PH constructs [47,48] suggest an at least supporting role of the PH portion for the DH GEF activity. Generally, the PH domain is located at the C-terminal DH helix, but subtle differences in the relative DH/PH orientation may lead to differential effects on nucleotide exchange. Along this line, PH residues conserved in a number of DH-PH modules have been observed to mediate interactions with the Rho component in the Cdc42:Dbs structure, suggesting that similar interactions may be present in other DH-PH-Rho complexes as well [49]. However, current structural data do not support a general role or mechanism of the PH domain in assisting DH mediated nucleotide exchange [46]. While in several Rho–RhoGEF structures (Dbs, Intersectin and LARG) the PH domain directly contacts the bound Rho protein [50,51] (Fig. 2a), no direct interaction has been observed between Rac1 and the PH domain of Tiam1 in the respective crystal structure [52]. In addition, the structure of the Sos derived DH–PH tandem rather suggests an autoinhibitory function for the PH portion, which appears to block the Rho binding face of the protein [53].

The role of PH domains in DH–PH membrane targeting is currently unclear. PH domains of common RhoGEFs bind lipids with low affinity and low specificity [54], disfavoring lipid binding at least as the only targeting mechanism. In addition, a number of DH–PH constructs apparently do not require phospholipid binding for membrane targeting [55–57]. It has also been proposed that the PH domain may play roles in allosteric regulation of the catalytic module [58].

Among the DH–PH modules, RhoGEF structures extending beyond DH–PH portions have been solved. A prominent example is Vav1, of which a structure containing its five N-terminal domains (Fig. 2b) [59] and one of the DH–PH-C1/ZF fragment in complex with Rac1 [60] are available. In both structures, the PH-C1 portion forms a single structural unit that packs against the C-terminal helix of the DH-portion, thereby stabilizing a catalytically competent conformation to promote nucleotide exchange.

The DH–PH module of Sos has been presented in the context of almost the entire Sos protein lacking only the C-terminal Grb2 binding domain [33] (Fig. 2c). In this structure the histone domain occludes the allosteric site [61,62] and stabilizes the DH–PH module in an autoinhibitory conformation [33]. The histone domain regulates the responsiveness of the PH domain to PIP_2 thereby constraining the activation of Sos at membranes. Furthermore, patient derived missense mutations associated with Noonan syndrome have been observed in the PH domain, underscoring the importance of the module. Noonan syndrome is a genetic disease which is characterized by deregulation of Ras signaling. Biochemical analysis indicates that the respective Sos mutants are upregulated in GEF activity, potentially due to uncoupling of inhibitory conformations of the histone and the DH–PH unit [33].

In addition to the DH–PH tandem regulating Rho family members, PH-like domains are also associated with many Arf-family specific nucleotide exchange factors of the Sec7 family [63,64]. In these proteins they are located at the C-terminal end of the Sec7 portion. Intriguingly recruitment of Arf6 to the plasma membrane occurs via direct interaction with the PH domain of Arno or Grp1 in a process requiring the presence of PIs. In addition, the Arf6–PH interaction was only observed in the presence of GTP, suggesting that Arno may act as an Arf6 effector module in this context, potentially to recruit ArfGEF activity to membrane compartments for the activation of Arf-isoforms [25]. The crystal structure of the Sec7-PH tandem reveals the Sec7 domain to be autoinhibited by a pseudo substrate mechanism. In the structure (Fig. 2d), the linker segment and portions of the C-terminal helix of the PH domain interact with the Arf-docking region normally accommodating the switch regions of Arf. The autoinhibition has been reported to be partially revertible presumably by binding of activated Arf6 to the PH portion, or by PKC mediated phosphorylation in the case of Cytohesin-1 [65].

The PH domain may also act like a potentially regulatory module binding to activated forms of other small GNBPs. PDZ-RhoGEF (PRG) is an unconventional RGS-domain containing Rho family GEF that can bind RhoA in two different ways. While the DH–PH tandem interacts with GDP-bound or nucleotide free RhoA to exert GEF activity, the PH domain can bind specifically to RhoA-GTP via a second binding surface. Structurally, the interaction between the PH domain and active RhoA-GTP essentially resembles a classic effector interface, involving the RhoA switch regions and complementary regions of the PH domain [26] (Figs. 2e, 3, Supplementary Fig. 2). The physiological relevance of the observed interaction has been discussed in view of existing models of a positive feeback loop, where allosteric binding of Ras-GTP enhances GEF activity [61]. However, GTP-bound RhoA does not stimulate PRG mediated nucleotide exchange on RhoA in solution. An alternative mechanism would involve enhanced localization of PRG in the presence of activated RhoA presumably involving other proteins at the plasma membrane [26].

2.2. PH domains as target modules of guanine nucleotide binding proteins

A number of signal transducing or signal regulatory components are directly controlled via activated GNBPs. Here we describe examples where a PH module serves as protein–protein interaction platform to accommodate the GNBP-activator.

2.2.1. Phospholipase C-β2/γ2 – activated by PH binding of activated Rho family members

Phospholipases (PLs) are paradigm effectors of heterotrimeric G proteins, catalyzing the hydrolysis of phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) to yield diacylglycerol and inositol 1,4,5-trisphosphate ($PI(1,4,5)P_3$). These second messenger molecules coordinate PKC activation and mobilization of intracellular calcium stores, leading to the regulation of a variety of signaling cascades [66]. Phospholipase C (PLC) isoforms are composed of a central catalytic triosephosphate isomerase (TIM) barrel domain flanked by a C-terminal C2, a coiled coil and an N-terminal PH domain, followed by an EF-hand module [66]. It has been shown that Rho family members directly activate these enzymes in a GTP-dependent manner [50,67,68,69]. Consistent with biochemical studies, Rac1 binds exclusively to the PH portion of PLC in the crystal structure of the Rac1–PLCβ2 complex [50,68], involving residues from the top of the PH β-sandwich (Fig. 2f). The structure of the split PH domain of PLCγ2 in complex with Rac1 involves primarily strand β5 along with areas derived from the C-terminal PH helix for stabilization [70]. While in PLCβ2 the Rho component binds its core β-strands approximately perpendicular to those of the PH portion, it is oriented roughly parallel to the PH derived β-sheet in PLCγ2 [24,70] (Fig. 3 (RhoA vs. Rac1), Supplementary Fig. 2). As expected for GNBP–effector interactions, in both cases the Rho component binds primarily via the switch regions involving hydrophobic as well as polar interactions. The PH domain of PLCβ2 tightly interacts with the core enzyme [24] in contrast to the situation in PLCδ where a flexible linker appears to tether the PH portion to the rest of the protein. Since the Rho component interacts exclusively with the PH portion of PLC and no conformational changes could be observed that would provide an activation mechanism, the authors proposed enhanced localization and enzyme orientation as the major drivers of enzyme activation [71].

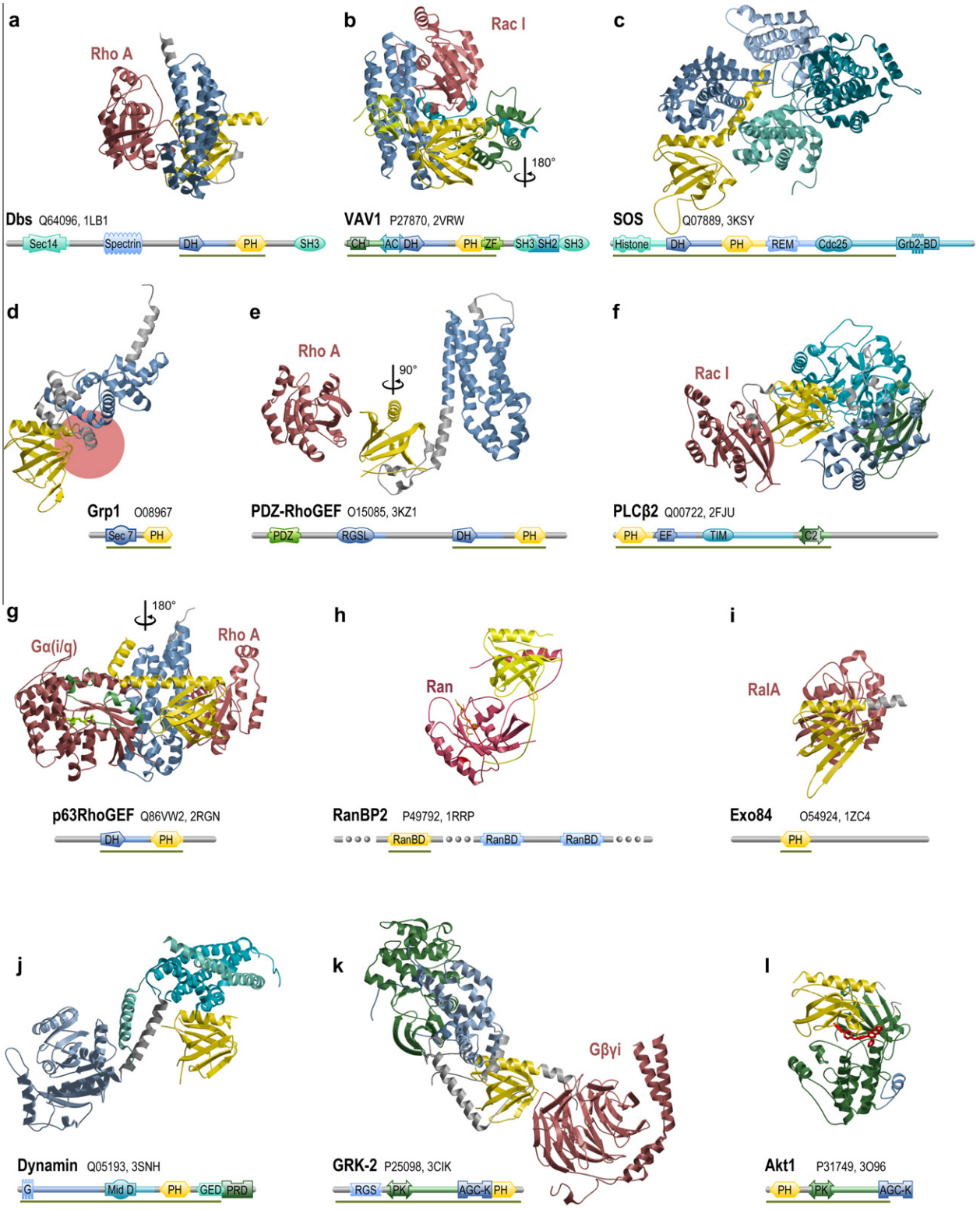

Fig. 2. Comparison of the intramolecular context of PH domains in multidomain proteins. The PH domains are displayed in yellow and have all the same orientation towards each other, unless indicated otherwise. Other domains are colored in blue/blue-green and protein binding partners in red /red-brown, corresponding to the respective domain scheme. Gray coloration indicates protein regions without a domain assignment. Nearly the whole surface of the core PH fold can serve as intramolecular interface to other domains. In the domain schemes, domains are drawn to relative scale and represented as combination of a labeled symbol and a color-matched bar. The protein region covered in the structural model is indicated with a green bar. Next to the protein name, the respective UniProtKB and PDB accession codes are given. (d) The red circle indicates the position of Arf1 in the ARNO:Arf1 complex (PDB: 1R8S) when superposed to Grp1 via the Sec7 domains, indicating that in an analog complex conformational changes would be necessary to avoid steric clashes.

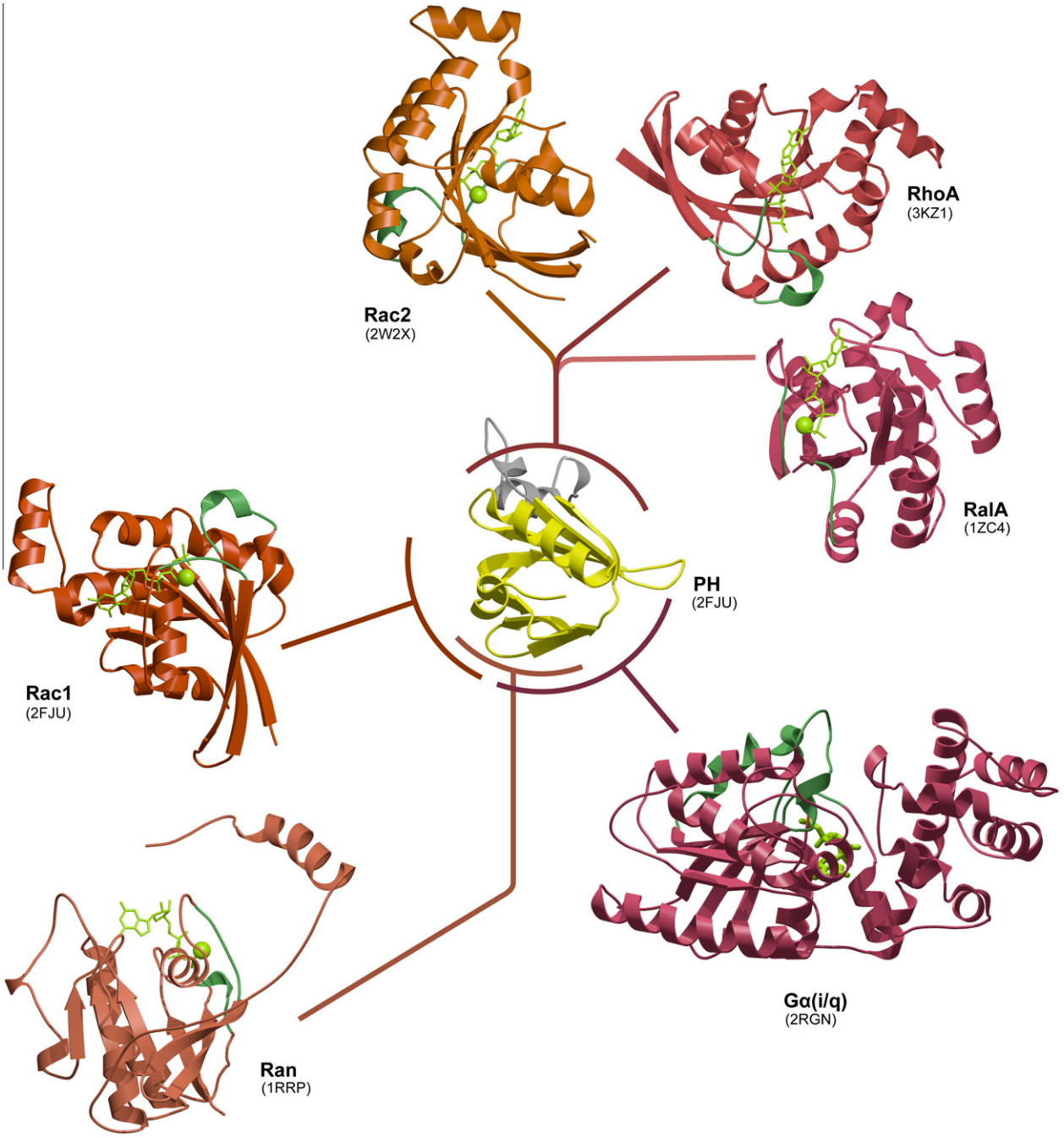

Fig. 3. Interaction between G-proteins and PH domains. Exploded assembly drawing of different PH–G-Protein complexes superposed with respect to the PH domains. Exemplarily, the PH domain of PLCβ2 is shown in yellow. Color matched lines indicate to which face of the PH domain the G-Proteins bind. The switch I and II regions of small G-proteins are colored in dark green, the nucleotide and metal ion in light green. PDB accession codes are given next to the protein names. Although the small G-proteins have overall the same fold, they have been observed binding PH domains in various orientations.

2.2.2. Gαq-p63RhoGEF–RhoA complex – PH mediated signaling cross talk from Gα to Rho

The Rho activator p63Rho-GEF is an effector of the Gαq associated heterotrimeric G-proteins [72], thereby linking Gαq receptor signaling to small GNBP activation. This is reflected in the crystal structure of the Gαq-p63RhoGEF-RhoA complex, where Gαq can be observed to contact the DH and PH domain of p63RhoGEF, but not RhoA. The regulatory PH domain, which presumably autoinhibits the GEF activity of the DH domain, is furthermore contacted by the effector regions of Gαq at a kinked, amphiphatic helical extension in the PH fold [30] (Fig. 2g, 3, Supplementary Fig. 2). In general, the interaction with Gαq seems to relieve the autoinhibition from the GEF activity of the DH-PH module, leading to an activation of RhoA. In addition to the engagement of Gαq and DH, the PH domain seems furthermore to support membrane association in this complex, by binding phospholipid headgroups via conserved basic residues in its N-terminal ß-strands.

2.2.3. The Ran binding domain – a PH-like module engaged for effector interaction with activated Ran

The small GNBP Ran is a critical component of the nucleocytoplasmic transport machinery [73] with important roles in the reas-

sembly of the nuclear envelope after mitosis [74]. RanBP2 is a component of the nuclear pore complex and acts like an effector of Ran, binding GTP-bound Ran via its Ran binding domains (RanBDs). The crystal structure of the Ran–RanBD1 complex shows the complex components in a 'molecular embrace', involving sequence motifs of the RanBD, the switch regions and the C-terminal DEEDL stretch that is critical for the protein–protein interactions of this complex [23] (Fig. 2h, 3, Supplementary Fig. 2). RanBP1 shares similarity with RanBDs and has been reported to act as a coactivator for the productive interaction with the cytoplasmic RanGAP [75]. In the corresponding structure of the Ran–RanGAP–RanBP1 complex, RanBP1 interacts primarily with the Ran components. This is comparable to the situation in the Ran–RanBD1 complex, displaying no interactions that might account for an enhancement of GAP activity [76]. This has been interpreted to mean that the C-terminal α-helical peptide of Ran is autoinhibitory to GTP hydrolysis in unbound activated Ran, but is kept in an extended i.e. non-inhibitory conformation in the complex [23]. Biochemical analyzes have shown that the RanBP1 primarily affects the dynamics of the protein–protein interactions rather than GAP catalysis, consistent with its binding site distant from the catalytic machinery [77].

2.2.4. Exo84 – subunit of the exocyst complex and PH-like Ral-effector module

The small GNBP Ral is a member of the Ras superfamily, functioning in cell proliferation, motility, protein sorting and cytoskeletal architecture. As part of extracellular signaling pathways, Ral proteins are involved in oncogenic transformation, endo/exocytotic processes and actin-cytoskeleton regulatory networks [78,79]. The exocyst or Sec6/8 complex is an octameric protein assembly that has important roles in tethering secretory vesicles to the plasma membrane. Two subunits of the complex, Sec5 and Exo84, have been shown to act as effectors of Ral-proteins [80,81]. The Ral-Sec6/8 interaction has essential targeting functions in the cell. The structure of a RalA-Exo84 complex reveals Exo84 as a largely unpolarized PH-like domain that uses primarily the C-terminal β-strands to interact with activated RalA in a rather extended interface using predominantly polar interactions. As expected for an effector–GNBP interaction, Ral uses the switch regions to contact the effector module (Fig. 2i, 3, Supplementary Fig. 2) [27].

2.3. Dynamin – PH insights from the near full-length structure

Dynamin is a modular GNBP essential for receptor mediated endocytosis [82]. It contains a central PH domain flanked by a G- and a GNBP effector domain (Fig. 2j). The PH domain of dynamin binds membrane associated $PI(4,5)P_2$ and mutations abolishing PIP_2 binding are defective in endocytosis [83,84]. Oligomerization of dynamin monomers is essential for its cellular function and positive cooperativity between dynamin and PIPs in stimulating the GTPase activity has been observed [85]. Contributions from intramolecular components have been proposed earlier, since GTPase activation can be achieved with soluble PIs i.e. in the absence of membranes [86]. Non-truncating mutations occurring in centronuclear myopahty (CNM) are clustered in the PH domain of dynamin 2, with some of them modulating GTPase activity [35]. In the structure of dynamin 1 lacking the carboxy-terminal proline rich domain, the PH domain interacts with the so-called stalk region, a helical assembly mediating oligomer formation (Fig. 2j) [87,88]. The presence of the other domains is apparently not associated with major structural changes in the PH module with respect to the isolated domain [89]. Several disease mutations [35] map to or in the vicinity of the stalk-PH interface. Some of them increased dynamin oligomerization rates in solution, suggesting that the stalk-PH interface modulates this activity [87]. Although the precise role of the PH domain in this process is currently unclear, the PH domain forms the inner ring structure in a model of a dynamin oligomeric turn [87].

2.4. G protein receptor kinase (GRK)-2 – PH module in desensitization

The G-protein receptor kinases (GRKs) are important components in the desensitization of G protein signaling and activation of alternative signaling pathways. GRK2 is composed of an N-terminal Regulator of G protein Signaling (RGS) homology (RH) domain followed by an AGC superfamily-like kinase and a C-terminal PH domain. Interaction of Gβγ with the PH domain increases the phosphorylation of activated G protein coupled receptors (GPCRs), presumably by a membrane recruitment mechanism increasing its local concentration [90,91]. The structure of GRK2–Gβγ complex shows the PH domain associated with the RH- domain as well as with the Gβγ subunit (Fig 2k), leaving the presumed phospholipid binding site exposed to interact with the plasma membrane [92]. The interaction with Gβγ is mediated by a continuous surface including strands β1, β4 and an extension of the C-terminal helix. While the sequence details of the interaction appear to be distinct in different PH domain containing GRKs, the overall structural features are characterized by a common concave surface complementary to the effector-binding region of Gβγ. In addition, the top of the Gβ propeller exposes a primarily acidic surface for PH interaction, consistent with PH binding areas containing predominantly basic residues [92]. Addition of the Gα subunit invokes interactions with the PH portion which is positioned at a distance and separated from the PH-Gβγ interface [93].

2.5. AKT/PKB – a PH domain containing S/T kinase module

AKT/PKB is a member of the S/T AGC kinase family with roles in cellular metabolism, cell growth, proliferation and survival. Deregulation of the AKT pathway has been implicated in a variety of human malignancies and AKT-inhibitors are under investigation as potential anti-cancer drugs in tumors of hyperactive AKT [94]. AKT isozymes consist of a central kinase domain that is preceded by an N-terminal PH domain [95,96]. While the PH domain binds PIP_2/PIP_3, the resulting interaction does not lead to kinase activation. Mutations affecting lipid binding block AKT-phoshporylation by PDK1, whereas removal of the PH domain abolishes the requirement for PIPs. PIP binding to the PH domain potentially induces conformational changes that allow AKT phosphorylation via PDK1. PH domains of AKT mediate membrane translocation that can be visualized after stimulation in a PI3K-dependent manner [97]. Consistently, a transforming mutation has been identified in the phospholipid binding pocket, suggestive to change the lipid binding profile of AKT and thereby inducing pathological membrane localization of the protein [31,32]. The structure of a PH-KINASE module has been presented in complex with a selective allosteric inhibitor [98]. In the structure, the PH domain employs portions of the C-terminal β-sheet to interact with the N-lobe next to the nucleotide binding pocket/cleft and with the C-lobe of the kinase module (Fig. 2l). PH-in and PH-out conformers of the PH domain have been defined with the former, corresponding to a closed inactive and the latter to an open and active conformation. The structure suggests that in the PH-in conformation AKT is unable to bind ATP or ATP-competitive inhibitors. In the co-crystal structure with the allosteric inhibitor bound, AKT is locked in a closed conformation with its phospholipid binding site blocked by the kinase domain. The authors suggest that the constitutive membrane localization observed with the E17K mutant is a result of a change in the interaction pattern along with a shift of the conformational equilibrium towards the PH-out form [98].

2.6. Neurofibromin – a PH module associated with a phospholipid-binding Sec14-like domain

A PH-like domain has been discovered in the RasGAP neurofibromin, the protein that is not functional in patients affected with the familial cancer syndrome neurofibromatosis type I (NF1) [99]. Adjacent to the central RasGAP domain, Neurofibromin contains a glycerophospholipid binding Sec14-PH module, that has been discovered by crystallographic analysis of the respective protein fragment [100,101] (Fig. 4a). Sec14-like domains have originally been described as globular lipid binding proteins in yeast where they serve as lipid shuttles between membrane compartments [102]. They are also present in a variety of modular signal regulators such as RhoGEFs, RhoGAPs and PTPases. [103]. The interaction between the two domains brings a PH derived hairpin-like protrusion in contact with a conserved helical lid segment of the Sec14 portion [100,101], that was proposed to control glycerophospholipid binding to the lipid binding cage in Sec14p [104]. Ligand binding to the respective region of the PH-like portion may induce conformational changes, thereby triggering release or closure of the helical lid (Fig. 4b). The precise nature of such a ligand is currently unclear. The integrity of the inter domain linker does not seem to be essential for the stability of the interface between the two domains, since a mutation essentially duplicating the linker does not affect the overall structure of the Sec14-PH module or the relative position of the two domains [105]. While glycreophospholipid binding of the Sec14-component is well established [101] and the Sec14-PH module binds phosphatidylinositol phosphates with moderate specificity in overlay assays [100], the function of the module and particularly of its PH portion is currently unclear. It is interesting to note that the N-terminal Sec14-like domain from Dbs located at its N-terminus also forms intramolecular interactions with the PH portion from its central DH–PH module and that these interactions seem to keep the Dbs protein in the non-transforming conformation. Removal of the Sec14-portion or a mutation preventing lipid binding is sufficient to translocate Dbs to the cell periphery, but not to activate transformation [106]. Whether the Sec14-PH module of neurofibromin has an influence on the GAP activity of neurofibromin is currently unclear.

2.7. PH modules in transcription, DNA repair and chromatin remodeling

Structural domains and folds are rarely confined to cellular compartments. Along this line PH-like domains have been found in proteins that function almost exclusively inside the cell nucleus. Likewise a histone-like domain has also been found in the signal regulatory protein Sos (see above).

2.7.1. The p62 subunit of TFIIH: a nuclear PH module with p53 and TFIIEα binding regions

The general transcription factor TFIIH is involved in transcription as well as DNA repair [107,108]. It is recruited to the pre-initiation complex through interaction between its p62 subunit and the carboxy terminal domain of TFIIEα. A strongly polarized PH-like domain was identified by structural analysis of a domain in the core subunit p62 that is essential for DNA repair activity by the nucleotide excision pathway, but not for complex assembly or basal transcription [109]. Most of the residues contributing to positively charge clustering are conserved among eukaryotic p62 sequences, suggesting common types of interactions among these regions. The XPG protein is involved in nucleotide excision repair and was retained in pull-down experiments, although detailed interaction studies were not reported [109]. The transactivation domain (TAD) of p53 specifically interacts with the PH portion of the p62 and Tfb1 subunits of human and yeast TFIIH. In the respective complex the TAD portion forms an amphipathic α-helix upon binding Tfb1 (Fig. 5a) [110]. A C-terminal acidic region of TFIIEα is required for native hTFIIH binding, with the p62 subunit binding strongly. TFIIEα shares its binding surface on the PH portion with

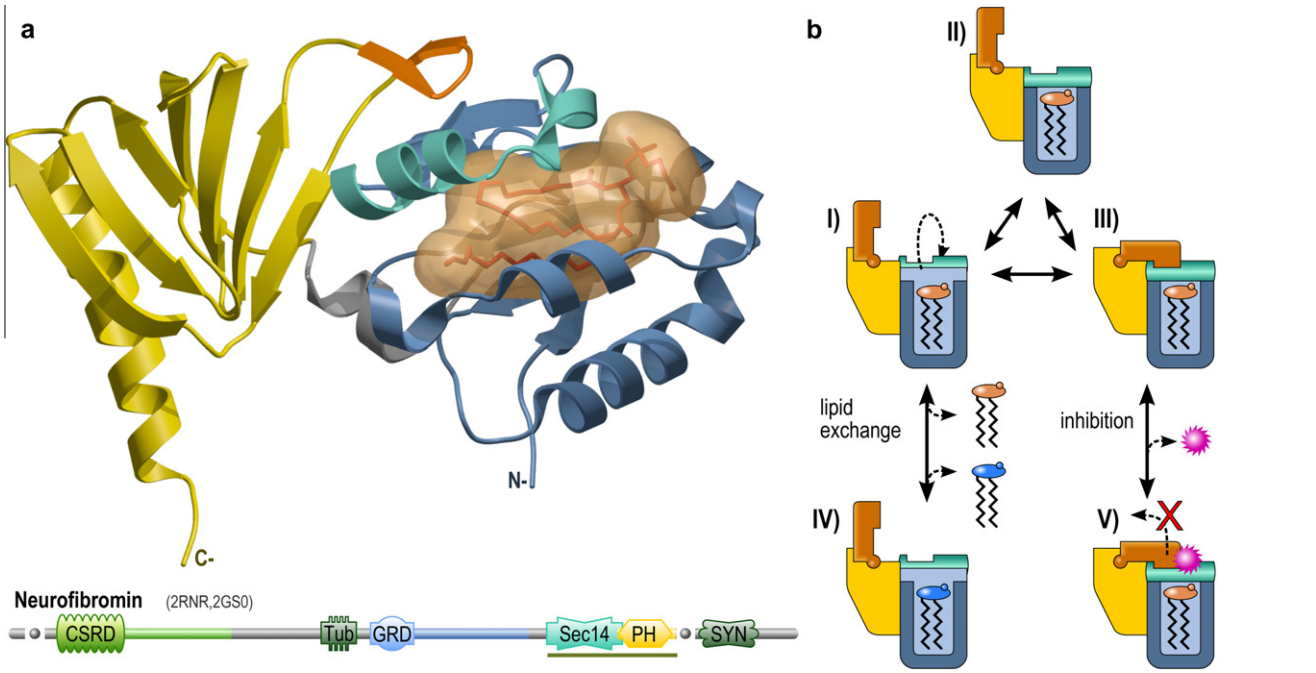

Fig. 4. The Sec14-PH module of Neurofibromin: intramolecular aspects. (a) Domain scheme of neurofibromin and ribbon representation of the Sec14-PH module. In the Sec14 domain, a glycerophospholipid is bound, depicted with both stick and molecular surface representation colored in brown–red. The Sec14 "lid" helix is shown in turquoise, the PH "lock" protrusion in brown. (b) Mechanistic scheme of lipid exchange: Sec14-PH can exchange the bound lipid against a different one in the presence of liposomes (I, IV). This suggests that the "lid" helix and the closely interacting "lock" protrusion are flexible (I,II,III). In vitro, lipid exchange can be blocked by small molecules binding the "lid"–"lock" interface (V).

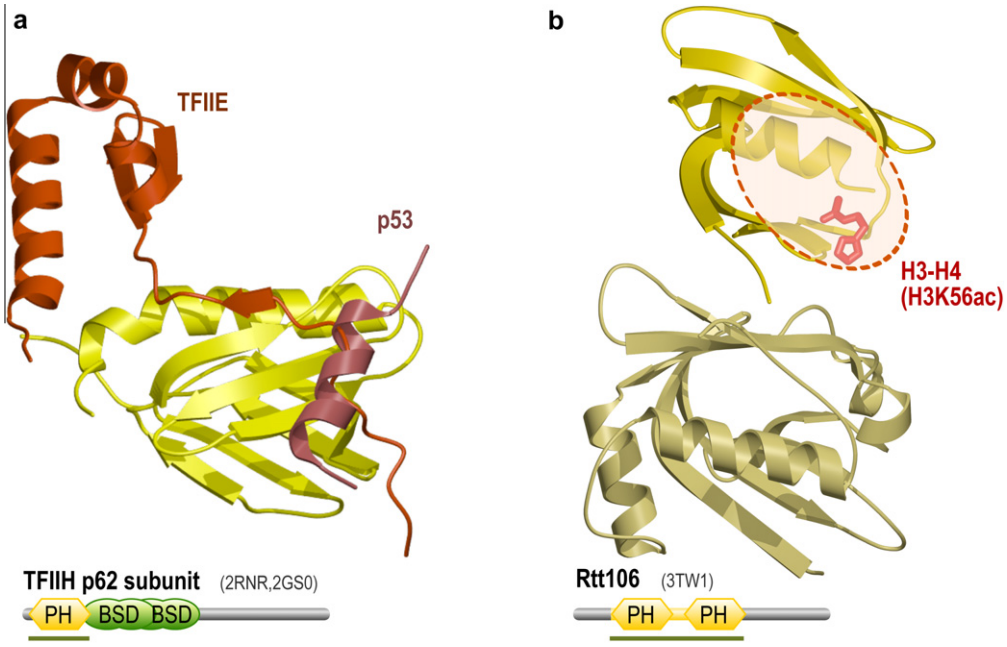

Fig. 5. Examples of nuclear PH modules. (a) Binding sites of TFIIE and p53 on the PH domain of the p62 TFIIH subunit. Binding of p53 and TFIIE is mutually exclusive due to an overlap of the binding sites. (b) Ribbon representation of Rtt106 bound to acetyl–histamine, indicating the binding site of K53 acetylated Histone H3 (red dotted line).

that of the TAD from p53 and of herpes simplex virus protein VP16 [110–112] with yet different modes of binding. The N-terminal region of the respective TFIIEα fragment becomes ordered upon interaction with the PH-portion. It thereby forms an additional strand on top of the core β-sheet and presents a number of consecutive acidic residues to interact with conserved basic contributions from the PH domain (Fig. 5a). The folded core of the acidic fragment does not show significant interactions with the PH-portion, consistent with the observations that constructs covering this core but lacking the preceding acidic tail do not interact with the PH module. Interestingly binding of monophosphorylated PIs to the PH-like domain of p62/Tfb1 has been reported with the binding region overlapping that of VP16 [111] although the physiological relevance of that finding is unclear.

2.7.2. yFACT, Rtt106p – PH-like-tandem arrangements in chromatin remodeling

The essential chromatin remodeling complex FACT (FAcilitates Chromatin Transcription) has important roles in transcription as well as DNA replication and consists of the three highly conserved subunits Spt16/Cdc68, Pob3 and Nhp6 [113]. The crystal structure of a proteolytic fragment of Pob3 (Pob3M) has revealed the presence of a closely associated tandem of PH-like domains, packing against each other with their β-sheets to generate an assembly in which the two PH-portions appear related by a translation [114] similar like in Rtt106 (see below, Fig. 5b). Mutational analysis of a surface patch lined by conserved residues pointed to a role in normal DNA replication and normal transcription. The authors find Pob3 interacting with replication protein A (RPA), a small β-barrel protein involved in DNA metabolism [114]. The mode of this interaction and the involved surfaces are currently unclear as is the role of the tandem association. The PH tandem found in Pob3 is sequence related to the histone chaperone Rtt106 that is involved in depositing H3K56-acetylated H3–H4 complex on replicating DNA [115]. A tandem of PH-like module also structurally resembling that seen in Pob3 binds the K56 containing region of H3, resulting in a complex tightening upon K56-acetylation. The binding region of the acetylated K56 was derived from a co-crystal-structure with acetyl-histamine along with chemical shift mapping [115] (Fig. 5b).

2.8. Other PH-modular assemblies

A number of other PH-like domains have been described recently in different cellular contexts and with different ligand binding properties, some revealed by their structure determination. In this section we briefly describe a few examples where the PH module appears in conjunction with another domain, although the significance of the involved interface is not established in all cases.

Skap-hom is an integrin-signaling adapter that mediates cytoskeletal interactions. It forms a dimer of two PH domains mediated by N-terminal coiled-coil dimerization segments. A PI-gated switch has been proposed in which the PH dimer has decreased PI affinity, presumably as a result of the stabilization of a conformation non-competent for IP-binding in the dimeric form [116] (Fig. 6a).

The BEACH domain (for beige and Chediak-Higashi Syndrome) is a ~300 residue protein module highly conserved in a large family of eukaryotic proteins, including Neurobeachin and CHS-protein. These are commonly large (>2000 amino acids) eukaryotic proteins with putative functions in vesicular transport or membrane dynamics [117]. The structure of the Neurobeachin BEACH domain reveals the presence of an N-terminal PH scaffold that tightly interacts with the BEACH domain core (Fig. 6b). The back-sheet (β-strands 5,6,7,1) of the PH portion is primarily involved in the highly conserved interface with the BEACH component and mutational analysis of the interface has confirmed the biochemical and structural relevance of the interaction. Little is known about binding partners of this PH-like domain [118].

Insulin receptor substrates (IRSs) are tyrosine phosphorylated by activated insulin receptors to associate with SH2 domain containing enzymes. Substrate targeting regions contain a PH followed by a PTB domain. The crystal structure of a PH–PTB tandem from IRS1 shows a tight association between the two modules in which the front sheet of the PH packs against the back of the PTB domain in a manner apparently different from those seen in other PH-tandems [119] (Fig. 6c).

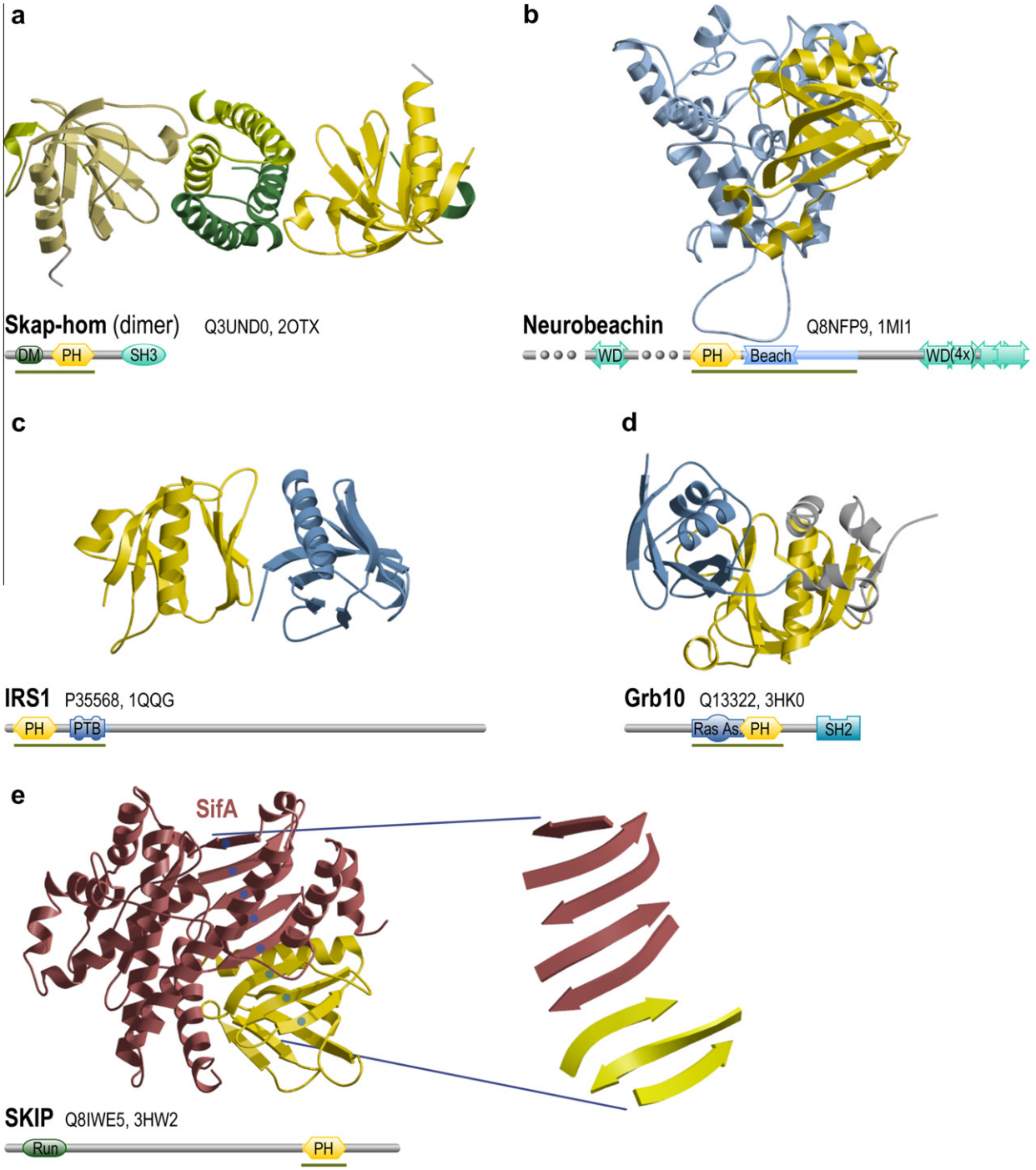

Fig. 6. Comparison of the intramolecular context of PH domains in multidomain proteins, focusing on selected arrangements containing two domains. Annotation and colors schemes are like in Fig. 2. The PH domains are not structurally aligned to each other to better visualize the neighboring domains. (e) SifA extends one β-sheet of the SKIP PH domain, as indicated with blue circles. A close up view of the augmented ß-sheet without the remaining parts of the two proteins is shown to the right, as indicated by blue lines.

RA-PH modules of grb-adapter proteins for receptor tyrosine kinases contain Ras association (RA) and PH domains in a tandem arrangement that are associated via β-augmentation. In this type of interaction two antiparallel β-sheets at the molecule edge adjoin to form a continuous β-sheet. The precise impact of the RA-PH interface is currently unclear [120] (Fig. 6d).

The bacterial virulence factor SifA (for Salmonella induced Filaments) is essential for *Salmonella* mediated pathogenesis [121] and has been shown to interact with the host protein SKIP via a PH-like domain at the C-terminal end of SKIP [122]. In the complex structure the N-terminal domain of SifA forms a continuous antiparallel β-sheet with the C-terminal β-sheet from the PH portion, as mediated by strand β5 [121,123] (Fig. 6e). Interestingly binding of SifA to the PH domain can be outcompeted specifically by the small GNBP Rab9 in a GTP-dependent manner, suggesting Rab9 as another G protein binder of the PH module. The Rab9/SKIP interface has not been defined so far [124].

3. Concluding remarks

Almost 20 years after the first reports about PH domains, it is becoming increasingly clear that with the PH superfold nature has provided a rather versatile module to interact with a variety of different ligands, using entirely different faces of the module. While PI binding can be observed in only few PH-like domains, it turns out that the PH superfold frequently serves as a protein–protein interaction platform, binding of which may elicit a variety of cellular responses. In particular, GNBPs are among the major PH interacting partners that bind primarily in a GTP-dependent manner. This defines the PH module as a common effector platform for GNBPs. The selection of examples described above suggests that the PH module is not confined to a particular cellular compartment or function, but is rather used in a variety of cellular contexts.

While the PH fold has originally been detected exclusively in eukaryotic proteins, the first bacterial examples have only recently appeared with the structures of three members of the previously uncharacterized protein family PF08000. Some of these PH-like domains formed pentamers and dodecamers in the crystal. The discovery of the PH fold in a bacterial background raises the possibility that PH-like domains may be rather ancient protein modules that adopted increasingly diverse molecular/biochemical functions [125].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2012.06.006.

References

[1] Mayer, B.J., Ren, R., Clark, K.L. and Baltimore, D. (1993) A putative modular domain present in diverse signaling proteins. Cell 73, 629–630.
[2] Haslam, R.J., Koide, H.B. and Hemmings, B.A. (1993) Pleckstrin domain homology. Nature 363, 309–310.
[3] Tyers, M., Haslam, R.J., Rachubinski, R.A. and Harley, C.B. (1989) Molecular analysis of pleckstrin: the major protein kinase C substrate of platelets. J. Cell. Biochem. 40, 133–145.
[4] Lemmon, M.A. and Ferguson, K.M. (2000) Signal-dependent membrane targeting by pleckstrin homology (PH) domains. Biochem. J. 350 (Pt 1), 1–18.
[5] Ingley, E. and Hemmings, B.A. (1994) Pleckstrin homology (PH) domains in signal transduction. J. Cell. Biochem. 56, 436–443.
[6] Musacchio, A., Gibson, T., Rice, P., Thompson, J. and Saraste, M. (1993) The PH domain: a common piece in the structural patchwork of signalling proteins. Trends Biochem. Sci. 18, 343–348.
[7] Gibson, T.J., Hyvonen, M., Musacchio, A., Saraste, M. and Birney, E. (1994) PH domain: the first anniversary. Trends Biochem. Sci. 19, 349–353.
[8] Macias, M.J., Musacchio, A., Ponstingl, H., Nilges, M., Saraste, M. and Oschkinat, H. (1994) Structure of the pleckstrin homology domain from β-spectrin. Nature 369, 675–677.
[9] Yoon, H.S., Hajduk, P.J., Petros, A.M., Olejniczak, E.T., Meadows, R.P. and Fesik, S.W. (1994) Solution structure of a pleckstrin-homology domain. Nature 369, 672–675.
[10] Saraste, M. and Hyvonen, M. (1995) Pleckstrin homology domains: a fact file. Curr. Opin. Struct. Biol. 5, 403–408.
[11] Blomberg, N., Baraldi, E., Nilges, M. and Saraste, M. (1999) The PH superfold: a structural scaffold for multiple functions. Trends Biochem. Sci. 24, 441–445.
[12] Lemmon, M.A., Ferguson, K.M. and Abrams, C.S. (2002) Pleckstrin homology domains and the cytoskeleton. FEBS Lett. 513, 71–76.
[13] Maffucci, T. and Falasca, M. (2001) Specificity in pleckstrin homology (PH) domain membrane targeting: a role for a phosphoinositide-protein co-operative mechanism. FEBS Lett. 506, 173–179.
[14] Karlovich, C.A., Bonfini, L., McCollam, L., Rogge, R.D., Daga, A., Czech, M.P. and Banerjee, U. (1995) In vivo functional analysis of the Ras exchange factor son of sevenless. Science 268, 576–579.
[15] Pitcher, J.A., Touhara, K., Payne, E.S. and Lefkowitz, R.J. (1995) Pleckstrin homology domain-mediated membrane association and activation of the β-adrenergic receptor kinase requires coordinate interaction with $G_{\beta\gamma}$ subunits and lipid. J. Biol. Chem. 270, 11707–11710.
[16] Myers Jr., M.G. et al. (1995) The pleckstrin homology domain in insulin receptor substrate-1 sensitizes insulin signaling. J. Biol. Chem. 270, 11715–11718.
[17] Yu, J.W. et al. (2004) Genome-wide analysis of membrane targeting by S. cerevisiae pleckstrin homology domains. Mol. Cell 13, 677–688.
[18] Lemmon, M.A. (2004) Pleckstrin homology domains: not just for phosphoinositides. Biochem. Soc. Trans. 32, 707–711.
[19] Zhou, M.M. et al. (1995) Structure and ligand recognition of the phosphotyrosine binding domain of Shc. Nature 378, 584–592.
[20] Eck, M.J., Dhe-Paganon, S., Trub, T., Nolte, R.T. and Shoelson, S.E. (1996) Structure of the IRS-1 PTB domain bound to the juxtamembrane region of the insulin receptor. Cell 85, 695–705.
[21] Prehoda, K.E., Lee, D.J. and Lim, W.A. (1999) Structure of the enabled/VASP homology 1 domain-peptide complex: a key component in the spatial control of actin assembly. Cell 97, 471–480.
[22] Lemmon, M.A., Ferguson, K.M. and Schlessinger, J. (1996) PH domains: diverse sequences with a common fold recruit signaling molecules to the cell surface. Cell 85, 621–624.
[23] Vetter, I.R., Nowak, C., Nishimoto, T., Kuhlmann, J. and Wittinghofer, A. (1999) Structure of a Ran-binding domain complexed with Ran bound to a GTP analogue: implications for nuclear transport. Nature 398, 39–46.
[24] Jezyk, M.R., Snyder, J.T., Gershberg, S., Worthylake, D.K., Harden, T.K. and Sondek, J. (2006) Crystal structure of Rac1 bound to its effector phospholipase C-β2. Nat. Struct. Mol. Biol. 13, 1135–1140.
[25] Cohen, L.A., Honda, A., Varnai, P., Brown, F.D., Balla, T. and Donaldson, J.G. (2007) Active Arf6 recruits ARNO/cytohesin GEFs to the PM by binding their PH domains. Mol. Biol. Cell 18, 2244–2253.
[26] Chen, Z., Medina, F., Liu, M.Y., Thomas, C., Sprang, S.R. and Sternweis, P.C. (2010) Activated RhoA binds to the pleckstrin homology (PH) domain of PDZ-RhoGEF, a potential site for autoregulation. J. Biol. Chem. 285, 21070–21081.
[27] Jin, R., Junutula, J.R., Matern, H.T., Ervin, K.E., Scheller, R.H. and Brunger, A.T. (2005) Exo84 and Sec5 are competitive regulatory Sec6/8 effectors to the RalA GTPase. EMBO J. 24, 2064–2074.
[28] Jaffe, A.B., Aspenstrom, P. and Hall, A. (2004) Human CNK1 acts as a scaffold protein, linking Rho and Ras signal transduction pathways. Mol. Cell. Biol. 24, 1736–1746.
[29] Kim, O., Yang, J. and Qiu, Y. (2002) Selective activation of small GTPase RhoA by tyrosine kinase Etk through its pleckstrin homology domain. J. Biol. Chem. 277, 30066–30071.
[30] Lutz, S. et al. (2007) Structure of $G_{\alpha q}$-p63RhoGEF-RhoA complex reveals a pathway for the activation of RhoA by GPCRs. Science 318, 1923–1927.
[31] Carpten, J.D. et al. (2007) A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. Nature 448, 439–444.
[32] Shoji, K. et al. (2009) The oncogenic mutation in the pleckstrin homology domain of AKT1 in endometrial carcinomas. Br. J. Cancer 101, 145–148.
[33] Gureasko, J., Kuchment, O., Makino, D.L., Sondermann, H., Bar-Sagi, D. and Kuriyan, J. (2010) Role of the histone domain in the autoinhibition and activation of the Ras activator Son of Sevenless. Proc. Natl. Acad. Sci. U S A 107, 3430–3435.
[34] Varnai, P., Rother, K.I. and Balla, T. (1999) Phosphatidylinositol 3-kinase-dependent membrane association of the Bruton's tyrosine kinase pleckstrin homology domain visualized in single living cells. J. Biol. Chem. 274, 10983–10989.
[35] Kenniston, J.A. and Lemmon, M.A. (2010) Dynamin GTPase regulation is altered by PH domain mutations found in centronuclear myopathy patients. EMBO J. 29, 3054–3067.
[36] Hyvonen, M. and Saraste, M. (1997) Structure of the PH domain and Btk motif from Bruton's tyrosine kinase: molecular explanations for X-linked agammaglobulinaemia. EMBO J. 16, 3396–3404.
[37] Walliser, C. et al. (2008) Rac regulates its effector phospholipase Cγ2 through interaction with a split pleckstrin homology domain. J. Biol. Chem. 283, 30351–30362.
[38] Yan, J., Wen, W., Xu, W., Long, J.F., Adams, M.E., Froehner, S.C. and Zhang, M. (2005) Structure of the split PH domain and distinct lipid-binding properties of the PH-PDZ supramodule of α-syntrophin. EMBO J. 24, 3985–3995.
[39] Wen, W., Liu, W., Yan, J. and Zhang, M. (2008) Structure basis and unconventional lipid membrane binding properties of the PH-C1 tandem of rho kinases. J. Biol. Chem. 283, 26263–26273.
[40] Lu, Q., Yu, J., Yan, J., Wei, Z. and Zhang, M. (2011) Structural basis of the myosin X PH1(N)-PH2-PH1(C) tandem as a specific and acute cellular PI(3,4,5)P(3) sensor. Mol. Biol. Cell 22, 4268–4278.
[41] Teo, H., Gill, D.J., Sun, J., Perisic, O., Veprintsev, D.B., Vallis, Y., Emr, S.D. and Williams, R.L. (2006) ESCRT-I core and ESCRT-II GLUE domain structures reveal role for GLUE in linking to ESCRT-I and membranes. Cell 125, 99–111.
[42] Ferguson, K.M., Lemmon, M.A., Schlessinger, J. and Sigler, P.B. (1995) Structure of the high affinity complex of inositol trisphosphate with a phospholipase C pleckstrin homology domain. Cell 83, 1037–1046.
[43] Sugimoto, K., Mori, Y., Makino, K., Ohkubo, K. and Morii, T. (2003) Functional reassembly of a split PH domain. J. Am. Chem. Soc. 125, 5000–5004.
[44] van Rossum, D.B., Patterson, R.L., Sharma, S., Barrow, R.K., Kornberg, M., Gill, D.L. and Snyder, S.H. (2005) Phospholipase Cγ1 controls surface expression of TRPC3 through an intermolecular PH domain. Nature 434, 99–104.
[45] Wen, W., Yan, J. and Zhang, M. (2006) Structural characterization of the split pleckstrin homology domain in phospholipase C-γ1 and its interaction with TRPC3. J. Biol. Chem. 281, 12060–12068.

[46] Rossman, K.L., Der, C.J. and Sondek, J. (2005) GEF means go: turning on RHO GTPases with guanine nucleotide-exchange factors. Nat. Rev. Mol. Cell Biol. 6, 167–180.
[47] Liu, X. et al. (1998) NMR structure and mutagenesis of the N-terminal Dbl homology domain of the nucleotide exchange factor Trio. Cell 95, 269–277.
[48] Kubiseski, T.J., Culotti, J. and Pawson, T. (2003) Functional analysis of the Caenorhabditis elegans UNC-73B PH domain demonstrates a role in activation of the Rac GTPase in vitro and axon guidance in vivo. Mol. Cell. Biol. 23, 6823–6835.
[49] Rossman, K.L., Worthylake, D.K., Snyder, J.T., Siderovski, D.P., Campbell, S.L. and Sondek, J. (2002) A crystallographic view of interactions between Dbs and Cdc42: PH domain-assisted guanine nucleotide exchange. EMBO J. 21, 1315–1326.
[50] Snyder, J.T., Singer, A.U., Wing, M.R., Harden, T.K. and Sondek, J. (2003) The pleckstrin homology domain of phospholipase C-β2 as an effector site for Rac. J. Biol. Chem. 278, 21099–21104.
[51] Kristelly, R., Gao, G. and Tesmer, J.J. (2004) Structural determinants of RhoA binding and nucleotide exchange in leukemia-associated Rho guanine-nucleotide exchange factor. J. Biol. Chem. 279, 47352–47362.
[52] Worthylake, D.K., Rossman, K.L. and Sondek, J. (2000) Crystal structure of Rac1 in complex with the guanine nucleotide exchange region of Tiam1. Nature 408, 682–688.
[53] Soisson, S.M., Nimnual, A.S., Uy, M., Bar-Sagi, D. and Kuriyan, J. (1998) Crystal structure of the Dbl and pleckstrin homology domains from the human Son of sevenless protein. Cell 95, 259–268.
[54] Snyder, J.T., Rossman, K.L., Baumeister, M.A., Pruitt, W.M., Siderovski, D.P., Der, C.J., Lemmon, M.A. and Sondek, J. (2001) Quantitative analysis of the effect of phosphoinositide interactions on the function of Dbl family proteins. J. Biol. Chem. 276, 45868–45875.
[55] Rossman, K.L., Cheng, L., Mahon, G.M., Rojas, R.J., Snyder, J.T., Whitehead, I.P. and Sondek, J. (2003) Multifunctional roles for the PH domain of Dbs in regulating Rho GTPase activation. J. Biol. Chem. 278, 18393–18400.
[56] Chen, R.H., Corbalan-Garcia, S. and Bar-Sagi, D. (1997) The role of the PH domain in the signal-dependent membrane targeting of Sos. EMBO J. 16, 1351–1359.
[57] Baumeister, M.A., Martinu, L., Rossman, K.L., Sondek, J., Lemmon, M.A. and Chou, M.M. (2003) Loss of phosphatidylinositol 3-phosphate binding by the C-terminal Tiam-1 pleckstrin homology domain prevents in vivo Rac1 activation without affecting membrane targeting. J. Biol. Chem. 278, 11457–11464.
[58] Baumeister, M.A., Rossman, K.L., Sondek, J. and Lemmon, M.A. (2006) The Dbs PH domain contributes independently to membrane targeting and regulation of guanine nucleotide-exchange activity. Biochem. J. 400, 563–572.
[59] Yu, B., Martins, I.R., Li, P., Amarasinghe, G.K., Umetani, J., Fernandez-Zapico, M.E., Billadeau, D.D., Machius, M., Tomchick, D.R. and Rosen, M.K. (2010) Structural and energetic mechanisms of cooperative autoinhibition and activation of Vav1. Cell. 140, 246–256.
[60] Rapley, J., Tybulewicz, V.L. and Rittinger, K. (2008) Crucial structural role for the PH and C1 domains of the Vav1 exchange factor. EMBO Rep. 9, 655–661.
[61] Margarit, S.M., Sondermann, H., Hall, B.E., Nagar, B., Hoelz, A., Pirruccello, M., Bar-Sagi, D. and Kuriyan, J. (2003) Structural evidence for feedback activation by Ras.GTP of the Ras-specific nucleotide exchange factor SOS. Cell 112, 685–695.
[62] Sondermann, H., Soisson, S.M., Boykevisch, S., Yang, S.S., Bar-Sagi, D. and Kuriyan, J. (2004) Structural analysis of autoinhibition in the Ras activator Son of sevenless. Cell 119, 393–405.
[63] Casanova, J.E. (2007) Regulation of Arf activation: the Sec7 family of guanine nucleotide exchange factors. Traffic 8, 1476–1485.
[64] Donaldson, J.G. and Jackson, C.L. (2011) ARF family G proteins and their regulators: roles in membrane transport, development and disease. Nat. Rev. Mol. Cell Biol. 12, 362–375.
[65] DiNitto, J.P., Delprato, A., Gabe Lee, M.T., Cronin, T.C., Huang, S., Guilherme, A., Czech, M.P. and Lambright, D.G. (2007) Structural basis and mechanism of autoregulation in 3-phosphoinositide-dependent Grp1 family Arf GTPase exchange factors. Mol. Cell 28, 569–583.
[66] Rhee, S.G. (2001) Regulation of phosphoinositide-specific phospholipase C. Annu. Rev. Biochem. 70, 281–312.
[67] Illenberger, D., Schwald, F., Pimmer, D., Binder, W., Maier, G., Dietrich, A. and Gierschik, P. (1998) Stimulation of phospholipase C-β2 by the Rho GTPases Cdc42Hs and Rac1. EMBO J. 17, 6241–6249.
[68] Illenberger, D., Walliser, C., Nurnberg, B., Diaz Lorente, M. and Gierschik, P. (2003) Specificity and structural requirements of phospholipase C-β stimulation by Rho GTPases versus G protein βγ dimers. J. Biol. Chem. 278, 3006–3014.
[69] Piechulek, T., Rehlen, T., Walliser, C., Vatter, P., Moepps, B. and Gierschik, P. (2005) Isozyme-specific stimulation of phospholipase C-γ2 by Rac GTPases. J. Biol. Chem. 280, 38923–38931.
[70] Bunney, T.D. et al. (2009) Structural insights into formation of an active signaling complex between Rac and phospholipase C-γ 2. Mol. Cell 34, 223–233.
[71] Hicks, S.N., Jezyk, M.R., Gershburg, S., Seifert, J.P., Harden, T.K. and Sondek, J. (2008) General and versatile autoinhibition of PLC isozymes. Mol. Cell 31, 383–394.
[72] Rojas, R.J., Yohe, M.E., Gershburg, S., Kawano, T., Kozasa, T. and Sondek, J. (2007) $G_{\alpha q}$ directly activates p63RhoGEF and Trio via a conserved extension of the Dbl homology-associated pleckstrin homology domain. J. Biol. Chem. 282, 29201–29210.
[73] Stewart, M. (2007) Molecular mechanism of the nuclear protein import cycle. Nat. Rev. Mol. Cell Biol. 8, 195–208.
[74] Hetzer, M., Gruss, O.J. and Mattaj, I.W. (2002) The Ran GTPase as a marker of chromosome position in spindle formation and nuclear envelope assembly. Nat. Cell Biol. 4, E177–E184.
[75] Bischoff, F.R., Krebber, H., Smirnova, E., Dong, W. and Ponstingl, H. (1995) Co-activation of RanGTPase and inhibition of GTP dissociation by Ran-GTP binding protein RanBP1. EMBO J. 14, 705–715.
[76] Seewald, M.J., Korner, C., Wittinghofer, A. and Vetter, I.R. (2002) RanGAP mediates GTP hydrolysis without an arginine finger. Nature 415, 662–666.
[77] Seewald, M.J., Kraemer, A., Farkasovsky, M., Korner, C., Wittinghofer, A. and Vetter, I.R. (2003) Biochemical characterization of the Ran-RanBP1-RanGAP system: are RanBP proteins and the acidic tail of RanGAP required for the Ran-RanGAP GTPase reaction? Mol. Cell. Biol. 23, 8124–8136.
[78] Feig, L.A. (2003) Ral-GTPases: approaching their 15 minutes of fame. Trends Cell Biol. 13, 419–425.
[79] Bodemann, B.O. and White, M.A. (2008) Ral GTPases and cancer: linchpin support of the tumorigenic platform. Nat. Rev. Cancer 8, 133–140.
[80] Moskalenko, S., Henry, D.O., Rosse, C., Mirey, G., Camonis, J.H. and White, M.A. (2002) The exocyst is a Ral effector complex. Nat. Cell Biol. 4, 66–72.
[81] Moskalenko, S., Tong, C., Rosse, C., Mirey, G., Formstecher, E., Daviet, L., Camonis, J. and White, M.A. (2003) Ral GTPases regulate exocyst assembly through dual subunit interactions. J. Biol. Chem. 278, 51743–51748.
[82] Ferguson, S.M. and De Camilli, P. (2012) Dynamin, a membrane-remodelling GTPase. Nat. Rev. Mol. Cell Biol. 13, 75–88.
[83] Vallis, Y., Wigge, P., Marks, B., Evans, P.R. and McMahon, H.T. (1999) Importance of the pleckstrin homology domain of dynamin in clathrin-mediated endocytosis. Curr. Biol. 9, 257–260.
[84] Lee, A., Frank, D.W., Marks, M.S. and Lemmon, M.A. (1999) Dominant-negative inhibition of receptor-mediated endocytosis by a dynamin-1 mutant with a defective pleckstrin homology domain. Curr. Biol. 9, 261–264.
[85] Tuma, P.L., Stachniak, M.C. and Collins, C.A. (1993) Activation of dynamin GTPase by acidic phospholipids and endogenous rat brain vesicles. J. Biol. Chem. 268, 17240–17246.
[86] Bottomley, M.J., Lo Surdo, P. and Driscoll, P.C. (1999) Endocytosis: How dynamin sets vesicles PHree! Curr. Biol. 9, R301–R304.
[87] Faelber, K. et al. (2011) Crystal structure of nucleotide-free dynamin. Nature 477, 556–560.
[88] Ford, M.G., Jenni, S. and Nunnari, J. (2011) The crystal structure of dynamin. Nature 477, 561–566.
[89] Ferguson, K.M., Lemmon, M.A., Schlessinger, J. and Sigler, P.B. (1994) Crystal structure at 2.2 A resolution of the pleckstrin homology domain from human dynamin. Cell 79, 199–209.
[90] Krupnick, J.G. and Benovic, J.L. (1998) The role of receptor kinases and arrestins in G protein-coupled receptor regulation. Annu. Rev. Pharmacol. Toxicol. 38, 289–319.
[91] Premont, R.T. and Gainetdinov, R.R. (2007) Physiological roles of G protein-coupled receptor kinases and arrestins. Annu. Rev. Physiol. 69, 511–534.
[92] Lodowski, D.T., Pitcher, J.A., Capel, W.D., Lefkowitz, R.J. and Tesmer, J.J. (2003) Keeping G proteins at bay: a complex between G protein-coupled receptor kinase 2 and $G_{\beta\gamma}$. Science 300, 1256–1262.
[93] Tesmer, V.M., Kawano, T., Shankaranarayanan, A., Kozasa, T. and Tesmer, J.J. (2005) Snapshot of activated G proteins at the membrane: the $G_{\alpha q}$-GRK2-$G_{\beta\gamma}$ complex. Science 310, 1686–1690.
[94] Liu, P., Cheng, H., Roberts, T.M. and Zhao, J.J. (2009) Targeting the phosphoinositide 3-kinase pathway in cancer. Nat. Rev. Drug Discov. 8, 627–644.
[95] Yang, J., Cron, P., Thompson, V., Good, V.M., Hess, D., Hemmings, B.A. and Barford, D. (2002) Molecular mechanism for the regulation of protein kinase B/Akt by hydrophobic motif phosphorylation. Mol. Cell 9, 1227–1240.
[96] Kannan, N., Haste, N., Taylor, S.S. and Neuwald, A.F. (2007) The hallmark of AGC kinase functional divergence is its C-terminal tail, a cis-acting regulatory module. Proc. Natl. Acad. Sci. U S A 104, 1272–1277.
[97] Leevers, S.J., Vanhaesebroeck, B. and Waterfield, M.D. (1999) Signalling through phosphoinositide 3-kinases: the lipids take centre stage. Curr. Opin. Cell Biol. 11, 219–225.
[98] Wu, W.I., Voegtli, W.C., Sturgis, H.L., Dizon, F.P., Vigers, G.P. and Brandhuber, B.J. (2010) Crystal structure of human AKT1 with an allosteric inhibitor reveals a new mode of kinase inhibition. PLoS ONE 5, e12913.
[99] Upadhyaya, M. and Cooper, D.N. (1998) Neurofibromatosis Type 1 from genotype to phenotype, Bios Scientific Publishers, Oxford, Washington DC.
[100] D'Angelo, I., Welti, S., Bonneau, F. and Scheffzek, K. (2006) A novel bipartite phospholipid-binding module in the neurofibromatosis type 1 protein. EMBO Rep. 7, 174–179.
[101] Welti, S., Fraterman, S., D'Angelo, I., Wilm, M. and Scheffzek, K. (2007) The sec14 homology module of neurofibromin binds cellular glycerophospholipids: mass spectrometry and structure of a lipid complex. J. Mol. Biol. 366, 551–562.
[102] Bankaitis, V.A., Mousley, C.J. and Schaaf, G. (2010) The Sec14 superfamily and mechanisms for crosstalk between lipid metabolism and lipid signaling. Trends Biochem. Sci. 35, 150–160.
[103] Phillips, S.E., Vincent, P., Rizzieri, K.E., Schaaf, G., Bankaitis, V.A. and Gaucher, E.A. (2006) The diverse biological functions of phosphatidylinositol transfer proteins in eukaryotes. Crit Rev Biochem Mol Biol 41, 21–49.

[104] Sha, B., Phillips, S.E., Bankaitis, V.A. and Luo, M. (1998) Crystal structure of the Saccharomyces cerevisiae phosphatidylinositol-transfer protein. Nature 391, 506–510.
[105] Welti, S., Kuhn, S., D'Angelo, I., Brugger, B., Kaufmann, D. and Scheffzek, K. (2011) Structural and biochemical consequences of NF1 associated nontruncating mutations in the Sec14-PH module of neurofibromin. Hum. Mutat. 32, 191–197.
[106] Kostenko, E.V., Mahon, G.M., Cheng, L. and Whitehead, I.P. (2005) The Sec14 homology domain regulates the cellular distribution and transforming activity of the Rho-specific guanine nucleotide exchange factor, Dbs. J Biol Chem. 280, 2807–2817.
[107] Thomas, M.C. and Chiang, C.M. (2006) The general transcription machinery and general cofactors. Crit. Rev. Biochem. Mol. Biol. 41, 105–178.
[108] Egly, J.M. and Coin, F. (2011) A history of TFIIH: two decades of molecular biology on a pivotal transcription/repair factor. DNA Repair (Amst) 10, 714–721.
[109] Gervais, V. et al. (2004) TFIIH contains a PH domain involved in DNA nucleotide excision repair. Nat. Struct. Mol. Biol. 11, 616–622.
[110] Di Lello, P. et al. (2006) Structure of the Tfb1/p53 complex: Insights into the interaction between the p62/Tfb1 subunit of TFIIH and the activation domain of p53. Mol. Cell 22, 731–740.
[111] Di Lello, P., Nguyen, B.D., Jones, T.N., Potempa, K., Kobor, M.S., Legault, P. and Omichinski, J.G. (2005) NMR structure of the amino-terminal domain from the Tfb1 subunit of TFIIH and characterization of its phosphoinositide and VP16 binding sites. Biochemistry 44, 7678–7686.
[112] Okuda, M., Tanaka, A., Satoh, M., Mizuta, S., Takazawa, M., Ohkuma, Y. and Nishimura, Y. (2008) Structural insight into the TFIIE-TFIIH interaction: TFIIE and p53 share the binding region on TFIIH. EMBO J. 27, 1161–1171.
[113] Singer, R.A. and Johnston, G.C. (2004) The FACT chromatin modulator: genetic and structure/function relationships. Biochem. Cell Biol. 82, 419–427.
[114] VanDemark, A.P., Blanksma, M., Ferris, E., Heroux, A., Hill, C.P. and Formosa, T. (2006) The structure of the yFACT Pob3-M domain, its interaction with the DNA replication factor RPA, and a potential role in nucleosome deposition. Mol. Cell 22, 363–374.
[115] Su, D. et al. (2012) Structural basis for recognition of H3K56-acetylated histone H3-H4 by the chaperone Rtt106. Nature 483, 104–107.
[116] Swanson, K.D., Tang, Y., Ceccarelli, D.F., Poy, F., Sliwa, J.P., Neel, B.G. and Eck, M.J. (2008) The Skap-hom dimerization and PH domains comprise a 3'-phosphoinositide-gated molecular switch. Mol. Cell 32, 564–575.
[117] De Lozanne, A. (2003) The role of BEACH proteins in Dictyostelium. Traffic 4, 6–12.
[118] Jogl, G., Shen, Y., Gebauer, D., Li, J., Wiegmann, K., Kashkar, H., Kronke, M. and Tong, L. (2002) Crystal structure of the BEACH domain reveals an unusual fold and extensive association with a novel PH domain. EMBO J. 21, 4785–4795.
[119] Dhe-Paganon, S., Ottinger, E.A., Nolte, R.T., Eck, M.J. and Shoelson, S.E. (1999) Crystal structure of the pleckstrin homology-phosphotyrosine binding (PH-PTB) targeting region of insulin receptor substrate 1. Proc. Natl. Acad. Sci. U S A 96, 8378–8383.
[120] Depetris, R.S., Wu, J. and Hubbard, S.R. (2009) Structural and functional studies of the Ras-associating and pleckstrin-homology domains of Grb10 and Grb14. Nat. Struct. Mol. Biol. 16, 833–839.
[121] Diacovich, L. et al. (2009) Interaction between the SifA virulence factor and its host target SKIP is essential for Salmonella pathogenesis. J. Biol. Chem. 284, 33151–33160.
[122] Boucrot, E., Henry, T., Borg, J.P., Gorvel, J.P. and Meresse, S. (2005) The intracellular fate of Salmonella depends on the recruitment of kinesin. Science 308, 1174–1178.
[123] Ohlson, M.B., Huang, Z., Alto, N.M., Blanc, M.P., Dixon, J.E., Chai, J. and Miller, S.I. (2008) Structure and function of Salmonella SifA indicate that its interactions with SKIP, SseJ, and RhoA family GTPases induce endosomal tubulation. Cell Host Microbe 4, 434–446.
[124] Jackson, L.K., Nawabi, P., Hentea, C., Roark, E.A. and Haldar, K. (2008) The Salmonella virulence protein SifA is a G protein antagonist. Proc. Natl. Acad. Sci. U S A 105, 14141–14146.
[125] Xu, Q. et al. (2010) Bacterial pleckstrin homology domains: a prokaryotic origin for the PH domain. J. Mol. Biol. 396, 31–46.