Pascal Maurice and Ralf Jockers

The melatonin receptors and their associated protein complexes

Introduction

Melatonin (N-acetyl-5-methoxytryptamine), the hormone of darkness, is synthesized during the night by the vertebrate pineal gland. The circadian rhythm of pineal melatonin synthesis and release is regulated by the biological clock located, in mammals, in the suprachiasmatic nucleus (SCN) of the hypothalamus that project to the pineal gland *via* a multi-synaptic pathway (Reppert and Weaver, 2002). The clock rhythm is entrained to a 24 h period by environmental light (the photoperiod) that is directly sensed by the retina and conveyed to the SCN *via* the retino-hypothalamic tract. In humans and all other diurnal species, this correlates with plasma levels of melatonin that exhibit a circadian rhythm with high levels at night and low levels during the day. Melatonin is also produced by other tissues, such as the retina, the gastrointestinal tract, skin, lymphocytes and bone marrow. In mammals, melatonin regulates a wide-range of physiological processes including circadian and seasonal rhythms (seasonal breeding), hibernation, ovarian and retinal physiology, as well as immunity, sleep disorders, oncogenesis and depression (Pandi-Perumal et al., 2008).

The melatonin receptor family

Two mammalian high-affinity receptors for melatonin, MT_1 and MT_2, have been cloned and characterized (Reppert et al., 1994, 1995). They belong to the superfamily of G protein-coupled receptors (GPCRs) and show high homology at the amino-acid level (about 55% overall and 70% within transmembrane domains). A third receptor, Mel1c, the first melatonin receptor to be cloned from *Xenopus laevis* immortalized melanophores, was shown to be restricted to non-mammalian species including birds, chicken and fish (Ebisawa et al., 1994). Recently, the orphan receptor GPR50, also known as melatonin-related receptor, which shares 45% identity with MT_1 and MT_2 (Reppert et al., 1996) was proposed to be the mammalian ortholog of Mel1c (Dufourny et al., 2008). During evolution, this mammalian receptor lost its affinity for melatonin and acquired a long C-terminal tail (C-tail). Another melatonin binding site with lower affinity, initially called MT_3, was later characterized as the enzyme quinone reductase (QR) 2 (Zhao et al., 1997; Nosjean et al., 2001). Inhibition of the catalytic activity of QR2 by melatonin is suggested to participate in the anti-oxidant activities and protective effects of melatonin (Jockers et al., 2008). However, further studies are needed to better understand the relationship between MT_3/QR2 and melatonin.

The G protein-mediated signal transduction pathways triggered by MT_1 and MT_2 receptors have been well characterized using different mammalian cell lines expressing recombinant receptors or various primary cell cultures and tissues, and will not be discussed here. The reader is referred to two recent reviews in the field (Jockers et al., 2008; Pandi-Perumal et al., 2008). An important discovery of the last ten years is that, in addition to G proteins, GPCRs, including melatonin receptors, can interact with a wide range of either soluble or transmembrane proteins, forming GPCR-associated protein complexes (GAPCs) (Daulat et al., 2009). Indeed, the nature of these GAPCs can determine its targeting to a specific cellular compartment, its association with other signaling or structural proteins and the fine-tuning of its signal transduction such as desensitization and resensitization. In addition, depending on the extra- and intra-cellular environment and the physiological state of the cell, the composition and dynamics of these GAPCs vary, thus participating in the dynamic regulation of GPCR function. Therefore, the identification of GAPCs constitutes an important step towards the development of new drugs that could be used to disrupt or strengthen specific interactions between GPCRs and their associated proteins. In this review, we will report recent results obtained in the field of GAPCs of the melatonin receptor family. The reader is referred to table 1 and 2 for the identified components of MT_1 and MT_2 melatonin receptor GAPCs, respectively.

Proteomic approaches for the identification of GAPCs of melatonin receptors

Most identified GAPCs were discovered using the carboxy-terminal domain of GPCRs in yeast two-hybrid and protein micro-array assays, mainly to circumvent problems associated with the hydrophobic nature of full-length GPCRs. However, these efficient technologies only allow the discovery of proteins that directly interact with the bait. In addition, these approaches do not detect interactions that depend on more than one subdomain or the native receptor structure. To obtain an overview of the GAPCs able to directly and indirectly interact with the

Table 1
Identified components of MT_1 melatonin receptor GAPCs

Protein name	Molecular mass (kDa)	Identified with MT_2	Approach used	References
Membrane proteins				
GPR50 (melatonin-related receptor)	67	+	immunoprecipitation/ BRET	Levoye et al., 2006
Leucine-rich repeat-containing protein 59	35	-	entire receptor	Daulat et al., 2007
Membrane-associated progesterone receptor component 1	22	-	entire receptor	Daulat et al., 2007
Solute carrier family 4, sodium bicarbonate cotransporter, member 5	124	-	C-tail	Maurice et al., 2008
Similar to transient receptor potential cation channel, subfamily M, member 6	248	-	C-tail	Maurice et al., 2008
Transmembrane protein 33	28	-	entire receptor	Daulat et al., 2007
Vomeronasal receptor 1 A12	35	-	C-tail	Maurice et al., 2008
Signal transduction				
14-3-3 protein beta	28	-	C-tail	Maurice et al., 2008
14-3-3 protein zeta	28	-	C-tail	Maurice et al., 2008
2',3'-cyclic-nucleotide 3'-phosphodiesterase	48	-	entire receptor	Daulat et al., 2007
Adenylate kinase isoenzyme 1	22	-	C-tail	Maurice et al., 2008
Casein kinase II subunit alpha	45	+	C-tail	Maurice et al., 2008
CIPP	199	-	PDZ protein microarray	Stiffler et al., 2007
COP9 signalosome subunit 4	47	-	C-tail	Maurice et al., 2008
Dual specificity protein phosphatase 3	20	+	C-tail	Maurice et al., 2008
Elongation factor 1-gamma	50	-	entire receptor	Daulat et al., 2007
GRK2/3	80	+	C-tail	Maurice et al., 2008
Guanine nucleotide-binding protein $G_{\beta 1}$	37	+	entire receptor	Daulat et al., 2007
Guanine nucleotide-binding protein $G_{\beta 4}$	37	+	entire receptor	Daulat et al., 2007
Guanine nucleotide-binding protein $G_{\alpha i1}$	40	+	entire receptor	Daulat et al., 2007
Guanine nucleotide-binding protein $G_{\alpha i2}$	40	+	entire receptor	Daulat et al., 2007
Guanine nucleotide-binding protein $G_{\alpha i3}$	40	+	entire receptor/C-tail	Daulat et al., 2007
Insulin receptor substrate 4	134	+	entire receptor C-tail	Daulat et al., 2007 Maurice et al., 2008
MUPP1	220	-	PDZ protein microarray C-tail	Stiffler et al., 2007 Maurice et al., 2008
nNOS (NOS1)	155	-	C-tail	Maurice et al., 2008
p21-Rac1	21	-	entire receptor	Daulat et al., 2007
PKC zeta 2	47	+	C-tail	Maurice et al., 2008
Protein phosphatase 2A regulatory subunits A alpha/beta	58	-	C-tail	Maurice et al., 2008
Protein phosphatase 2A catalytic subunit beta isoform (Ppp2cb)	32	-	C-tail	Maurice et al., 2008
PSD-95	80	-	C-tail	Maurice et al., 2008
Rabphilin 3A-like (Noc2)	34	-	C-tail	Maurice et al., 2008
Ras-related protein RAP-1A	21	-	entire receptor	Daulat et al., 2007
Regulator of G-protein signaling 20 (RGSZ1)	27	-	C-tail	Maurice et al., 2008
Ubiquitin-specific peptidase 5 (isopeptidase T)	97	-	C-tail	Maurice et al., 2008
Ubiquitin thiolesterase (Otub1 protein)	31	-	C-tail	Maurice et al., 2008
Cytoskeleton				
Filamin A	278	+	entire receptor	Daulat et al., 2007
ARP1 actin-related protein 1 homolog B	42		C-tail	Maurice et al., 2008
CLIP-associating protein 1	44	-	C-tail	Maurice et al., 2008
Cofilin-1	19	-	C-tail	Maurice et al., 2008
Cofilin-2	19	-	C-tail	Maurice et al., 2008

Protein name	Molecular mass (kDa)	Identified with MT_2	Approach used	References
Cytoplasmic linker protein 2	112	-	C-tail	Maurice et al., 2008
Destrin	19	-	C-tail	Maurice et al., 2008
Dynamin	85	-	C-tail	Maurice et al., 2008
Dynamin 1	96	-	C-tail	Maurice et al., 2008
Glial fibrillary acidic protein	47	-	C-tail	Maurice et al., 2008
Glial maturation factor beta	17	+	C-tail	Maurice et al., 2008
Kinesin	101	-	C-tail	Maurice et al., 2008
Myosin heavy polypeptide	222	-	C-tail	Maurice et al., 2008
Tubulin alpha	50	+	C-tail	Maurice et al., 2008
Tubulin beta	50	+	C-tail	Maurice et al., 2008
Tubulin gamma	50	+	C-tail	Maurice et al., 2008
Biosynthesis				
Calnexin	68	+	entire receptor	Daulat et al., 2007
78-kDa glucose-regulated protein	72	+	entire receptor	Daulat et al., 2007
Calreticulin	48	-	entire receptor	Daulat et al., 2007
Protein-disulfide isomerase A6	48	+	entire receptor	Daulat et al., 2007
Traffic, chaperone, stress response				
Chaperonin subunit 5 epsilon	60	-	C-tail	Maurice et al., 2008
Chaperonin subunit 2 beta	58	-	C-tail	Maurice et al., 2008
DnaJ homolog, subfamily B, member 11	41	-	C-tail	Maurice et al., 2008
Ras-related protein Rab-10	23	-	entire receptor	Daulat et al., 2007
Vesicle-associated membrane protein associated protein B/C	27	-	entire receptor	Daulat et al., 2007
Others				
Brain glycogen phosphorylase	96	+	C-tail	Maurice et al., 2008
Collapsin response mediator protein (CRMP) 1	62	-	C-tail	Maurice et al., 2008
Dihydropyrimidinase-like 2	63	+	C-tail	Maurice et al., 2008
Heterogeneous nuclear ribonucleoprotein A0	31	-	entire receptor	Daulat et al., 2007
HTRA1	51	-	PDZ protein microarray	Stiffler et al., 2007

MT_1 and MT_2 receptors, we have developed two complementary proteomic approaches based on 1) the tandem affinity purification (TAP) strategy and entire receptors as bait (Daulat et al., 2007), and 2) peptide affinity chromatography and GPCR subdomain (Maurice et al., 2008).

The TAP method, initially designed for large-scale purification of soluble protein complexes (Rigaut et al., 1999; Gavin et al., 2002) relies on the expression of the TAP-tagged target protein in the host cell. The TAP tag is composed of two affinity modules separated by a cleavage site for the Tobacco Etch Virus (TEV) protease leading to the recovery of the protein of interest and its associated protein complexes by a two-step purification protocol. In our study, MT_1 and MT_2 receptors were tagged at their C-terminus, with a TAP tag consisting of two immunoglobulin binding units of protein A from *Staphylococcus aureus*, a TEV cleavage site, and a calmodulin binding peptide, and expressed in human embryonic kidney (HEK) 293 cells (Daulat et al., 2007). The complexes were first immobilized on IgG-coated beads, and then specifically eluted by the addition of the TEV protease. The recovered material was further bound to calmodulin-coated beads in the presence of calcium and finally eluted with ethylene glycol tetraacetic acid. Eluted proteins were separated by 1-dimensional electrophoresis and identified by mass spectrometry. With this approach, we were able to identify 21 and 17 proteins associated with MT_1 and MT_2, respectively.

GAPCs can interact with intracellular loops, transmembrane regions and the C-tail of the receptor. This latter represents one of the most attractive targets for identification of GAPCs and in the past, much effort was focused on the identification of GAPCs binding to the receptor C-tail. Indeed, the sequence, length and binding motifs within this domain are specific to each GPCR. In addition, many splice variants of GPCRs show sequence variations within their C-tail, and important post-translational modifications, such as palmitoylation and phosphorylation, also take place within this domain. The second proteomic approach applied to melatonin receptors com-

bined the use of chemically synthesized 6xHis-tagged peptides encompassing the entire MT_1 and MT_2 receptor C-tail, with immobilized metal affinity chromatography (IMAC) followed by 1- and 2-dimensional electrophoresis, mass spectrometry identification and immunoblotting to systematically identify GAPCs from mouse brain that interact with the C-tail of both receptors (Maurice et al., 2008). With this approach, 40 and 22 proteins that specifically interact with the C-tails of MT_1 and MT_2 respectively were identified.

A careful analysis of the advantages and drawbacks of both approaches shows that they are more complementary than mutually exclusive. The TAP-tag approach using entire receptors allows the purification of protein complexes formed in intact cells, under native conditions, and at physiological expression levels. Potential drawbacks of this approach are the hindrance of the TAP-tag if placed at the C-terminus (thus masking possibly the recruitment of GAPCs interacting with the extremity of the C-tail such as PDZ-domain proteins) and the restriction of the interactome due to the cell line used. Advantages of the subdomain approach include the identification of GAPCs formed in a specific tissue, thus giving rise to the possibility of comparing GAPCs from different tissues, and under patho-physiological conditions or after *in vivo* pharmacological treatments. The main limitations of this approach are the potential loss of the native secondary structure of the bait and the loss of GAPCs whose binding depends on more than one GPCR subdomain (*e.g.* heterotrimeric G proteins). Accordingly, identified interacting partners need to be confirmed in intact cells using the entire GPCR to definitively eliminate the possibility of false positives.

We will report below the GAPCs of melatonin receptors identified by these two proteomic strategies and their main functions in the regulation of GPCR function.

GAPCs of the melatonin MT_1 and MT_2 receptors

Signaling proteins

Using the TAP strategy, we were able to identify 21 and 17 proteins associated with MT_1 and MT_2, respectively. MT_1 was shown to interact with various signaling proteins such as the small GTPases of the Rho family Rac-1 and Rap-1A. Activation of Rap and Rac-1 upon stimulation of various GPCRs has been already reported (Maillet et al., 2003; Pelletier et al., 2003; Weissman et al., 2004). Recently, Rac-1, together with beta-arrestin-1 and NADPH oxidase, was shown to be involved in the early phase activation of p38 MAPK mediated by β_2 adrenergic receptors (Gong et al., 2008). A role of Rac-1 in the regulation of the surface expression of PAR1 has also been reported (Yufu et al., 2005). Further GAPCs of MT_1 are the 2',3'-cyclic-nucleotide 3'- phosphodiesterase and the elongation factor 1-γ. This phosphodiesterase belongs to the PDE3A family and is involved in the degradation of cAMP and cGMP, two second messengers produced following activation of melatonin receptors (Petit et al., 1999; Lugnier, 2006). Elongation factors have been reported to modulate GPCR function by direct interaction with the receptor (McClatchy et al., 2002; Cho et al., 2003).

A common binding partner of the MT_1 and MT_2 receptors was also discovered: the insulin receptor substrate (IRS) 4. IRS proteins are key mediators in insulin signaling and play a central role in maintaining basic cellular functions such as growth, survival, and metabolism (Sesti et al., 2001). The role of IRS4 is less well documented. Recent findings have demonstrated that phosphorylation of IRS4 can be mediated by PKCζ (Lee et al., 2008), a PKC isoform (*e.g.* PKCζ2) which interacts with the C-tail of both receptors (Maurice et al., 2008), and that phosphorylation of IRS4 induced by insulin can be modulated by angiotensine II (Villarreal et al., 2006).

Specific GAPCs of the MT_2 receptor were also identified by the TAP approach, including catenin δ1 (also known as p120 catenin) and the protein phosphatase 2C (PP2C) isoform γ. Interestingly, catenin δ1 was also identified as specifically interacting with the C-tail of MT_2 (Maurice et al., 2008). Catenins are regulator of cadherin stability and important modulators of Rho GTPase activity (Reynolds, 2007). This protein was shown to interact with mGluR1 receptors and dissociate upon activation of the receptor by L-glutamate (Jones et al., 2002). However, its specific role in GPCR signaling is currently unknown. The role of PP2C in GPCR signaling is also not well documented. PP2C has been shown to bind selectively to mGluR3 and to dynamically regulate the receptor by dephosphorylation of the mGluR3 C-tail (Flajolet et al., 2003).

Using the subdomain approach, we were able to identify additional GAPCs that specifically interact with the C-tail of MT_1 and MT_2. Common GAPCs include casein kinase II (CK2) subunit α, the dual specificity phosphatase 3 and PKC ζ2. The serine/threonine protein kinase CK2 has been involved in phosphorylation and regulation of the M3 muscarinic receptor by specifically coupling the GPCR to the Jun-kinase pathway (Torrecilla et al., 2007). Moreover, CK2 consensus sites within GPCR C-tails have been proposed to target GPCRs to the β-arrestin-dependent pathway (Hanyaloglu et al., 2001). More recently, CK2 has been involved in RhoA phosphorylation and inhibition of arterial contractions induced by AT_2 receptor activation (Guilluy et al., 2008). Dual specificity phosphatase 3 (DSP3) is known to dephosphorylate protein substrates containing both phosphotyrosine and phosphoserine or phosphothreonine residues, with

specificity for the MAPKs ERK2 and c-Jun NH_2-terminal kinase (JNK) (Ducruet et al., 2005). PKC ζ2 is a member of the atypical PKC subfamily specifically expressed in the mouse brain (Hirai and Chida, 2003; Hirai et al., 2003). However, its role in the regulation of GPCR function remains unknown.

Among the GAPCs that were specifically recruited by the C-tail of MT_1, we identified two members of the ubiquitin-specific processing proteases involved in the deubiquitination pathway: otubain 1 and ubiquitin-specific peptidase 5 (also known as isopeptidase T). Protein ubiquitination is a biological process targeting proteins for degradation *via* the proteasome. Ubiquitination has another major role in the endocytosis and subsequent trafficking of plasma membrane proteins (Wojcikiewicz, 2004). In addition, the subunit 4 of COP9, a multiprotein complex of the ubiquitin-proteasome pathway, was also identified. Its role in GPCR signaling has not been reported so far. The β isoform of the catalytic subunit of protein phosphatase 2A (PPP2cb) was also identified. PP2A is a highly conserved serine/threonine phosphatase that plays pivotal roles in diverse cellular functions (Janssens and Goris, 2001). PP2A has been shown to co-immunoprecipitate with mGluR5 and to regulate mGluR5-dependent MEK/ERK phosphorylation in neurons (Mao et al., 2005), and there is evidence for a direct interaction between PP2A and the C-tail of GPCR (Evans et al., 2008). In addition, PP2A has been shown to regulate H2 receptor resensitization (Fernandez et al., 2008) and myocyte contraction responses under β_1 adrenergic receptor stimulation by limiting PKA phosphorylation (De Arcangelis et al., 2008). Adaptor proteins have been also identified: the 14-3-3 proteins β et ζ and the regulator of G protein signaling 20 (also known as RGSZ1). The interaction between RGS20 and MT_1 was recently validated by co-immunoprecipitation experiments from transfected HEK293 cells and from ovine pituitary *pars tuberalis* expressing both proteins endogenously and shown to regulate the speed of MT_1 signaling (Maurice et al., 2008). We further characterized the molecular determinants of the interaction and demonstrated that MT_1, $G\alpha_{i1}$ and RGS20 form a preassociated ternary complex that rearranges upon agonist stimulation and where $G\alpha_{i1}$ and RGSZ1 interact directly and independently with MT_1 (Maurice et al., unpublished data). Finally, two additional candidates: the adenylate kinase isoenzyme 1 and the Rab GTPase effector Rabphilin 3A-like (also known as Noc2). However, their role in the regulation of GPCR function has not been reported.

In contrast to the C-tail of MT_1, that of MT_2 appears to recruit less GAPCs. This is shown by the number of GAPCs identified in the TAP approach and suggests that MT_1 is the predominant receptor that recruited a greater number of GAPCs. Two GAPCs specific for the MT_2 C-tail were identified: the clathrin heavy chain and the tyrosine-protein phosphatase, non-receptor type 13 (PTPN13). PTPN13 is a member of the FERM and PSD-95/Disc-large/Zona occludens-1 (PDZ) domain-containing PTP family. PTPN13 is a large protein that, in addition to a PTP domain, contains one FERM domain, five PDZ domains and a non-catalytic C-lobe domain. Interestingly, mice that express a PTP domain-deleted form of PTPN13 exhibit impaired motor nerve repair and axon branching, and retinal ganglion cell neurite initiation and survival (Wansink et al., 2004; Lorber et al., 2005). To date, the participation of PTPN13 in the regulation of GPCR function has never been reported. Clathrin is a vesicle coat protein involved in the assembly of membrane and cargo into transport vesicles at the plasma membrane and on certain intracellular organelles. Many GPCRs internalize through the clathrin-coated vesicle endocytic pathway. The clathrin heavy chain has been reported to interact with the C-tail of another GPCR such as for the histamine H2 receptor (Xu et al., 2008). Recently, clathrin has been shown to be required for phosphorylation and internalization of β_2 adrenergic receptors mediated by GRK2 (Mangmool et al., 2006). Interestingly, GRK2/3 was also identified by immunoblot screening as interacting proteins of both receptor C-tails (Maurice et al., 2008). The main role of GRKs is to recognize and phosphorylate agonist-activated GPCRs. Receptor phosphorylation triggers the binding of arrestins, which block the activation of G proteins, leading to rapid desensitization of the receptor. In addition to these phosphorylation-dependent processes, GRKs may also contribute in modulating cellular responses due to its ability to interact with a variety of proteins involved in signaling and trafficking such as Gαq and Gβγ subunits, PI3K or clathrin (Ribas et al., 2007).

PDZ-domain proteins

One interesting feature of the MT_1 receptor is the presence of a class III recognition motif for PDZ domains at its C-terminal extremity (D-S-V). PDZ domains are protein interaction modules that are specialized for binding to C-terminal peptide motifs of proteins and are typically involved in the assembly of multiprotein complexes that participate in receptor signaling and determine binding of these complexes to membrane subdomains (*e.g.* tight junctions) (Sheng and Sala, 2001; Nourry et al., 2003). Moreover, these domains have been shown to represent promising targets for drug discovery (Dev, 2004).

Using protein microarrays and quantitative fluorescence polarization, Stiffler et al. (Stiffler et al., 2007) characterized the binding selectivity of 157 mouse PDZ domains with respect to 217 genome-encoded peptides encompassing the 10 C-terminal residues of mouse proteins, including the MT_1 receptor. By this approach, the

C-terminal extremity of the MT_1 receptor was shown to interact with the channel-interacting PDZ domain protein CIPP, the serine protease HTRA1 and MUPP1. CIPP is composed of four PDZ domains and was identified as a potential partner of inward rectifier K^+ family channels, *N*-methyl-D-aspartate (NMDA) receptors, neurexins and neuroligins (Kurschner et al., 1998). In addition, CIPP has been shown to interact with different types of serotonin receptors and transporters (Becamel et al., 2004; Joubert et al., 2004; Chanrion et al., 2007) and with proteins involved in actin and tubulin remodeling (Alpi et al., 2009). MUPP1 is a multi-PDZ domain protein composed of 13 PDZ domains. This protein was shown to interact with other GPCRs, including the 5-HT_{2C} receptor (Becamel et al., 2001), metabotropic γ-aminobutyric acid B ($GABA_B$) receptor 2 (Balasubramanian et al., 2007) and recently the somatostatin receptor 3 (SSTR3) (Liew et al., 2009). We also reported interaction between MUPP1 and MT_1 using a yeast two hybrid screen approach with the C-tail of MT_1 as bait (Guillaume et al., 2008). Interaction between MUPP1 and MT_1 was confirmed in the ovine pituitary *pars tuberalis* and from mouse brain lysates, and shown to stabilize the $MT_1/G_{\alpha i}$ complex promoting efficient $G_{\alpha i}$-dependent signaling of MT_1 (Guillaume et al., 2008; Maurice et al., 2008). Another PDZ domain-containing protein described as a potential interacting candidate of the MT_1 receptor was the neuronal NO synthase (nNOS) (Stricker et al., 1997). Using a C-terminal peptide display strategy, Stricker and colleagues screened 13 billion distinct C-terminal peptides to select sequences specific to the PDZ domain of nNOS and found that the positive peptides had a D-X-V C-terminal consensus sequence. Searching the non-redundant protein database NCBInr revealed 484 matches, including the glutamate receptor 6 and the MT_1 receptor. Interaction between nNOS and the C-tail of MT_1 was confirmed in mouse brain lysates (Maurice et al., 2008). nNOS has been sporadically shown to participate in GPCR signaling. However, since Stricker's publication and to our knowledge, no additional information has been reported so far concerning the possibility that nNOS can directly or indirectly interact with the C-tail of GPCRs. Indirect recruitment of nNOS to NMDA receptors has been demonstrated through a PDZ/PDZ interaction with PSD-95 (Christopherson et al., 1999) and shown to significantly enhance NO production upon stimulation by NMDA in chinese hamster ovary (CHO) cells (Ishii et al., 2006). PSD-95 is a prototypical scaffolding protein highly enriched in the postsynaptic density that belongs to the membrane-associated guanylate kinase family (Kim et Sheng, 2004). We also reported interaction of the MT_1 C-tail with PSD-95 (Maurice et al., 2008). Interaction between PSD-95 and GPCRs has been already described for somatostatin receptors SSTR4 and SSTR1 (Christenn et al., 2007), the serotonin 5-HT_{2A} and 5-HT_{2C} receptors (Becamel et al., 2002, 2004), and dopamine D1 receptors (Zhang et al., 2007). The coupling of PSD-95 with the C-tail of GPCR has been shown to regulate the surface expression and intracellular trafficking of receptors and to modulate receptor-mediated signaling (Gavarini et al., 2006; Zhang et al., 2007).

The MT_2 receptor also contains a putative PDZ domain-binding motif at its C-terminal extremity (-A-D-A-L), but no PDZ domain-containing protein was identified for MT_2, questioning the functionality of this motif.

Cytoskeletal proteins

In addition to signaling proteins, many cytoskeletal proteins were identified as GAPCs for melatonin receptors. Among these proteins, filamin A was identified as a common member of MT_1- and MT_2-associated complexes. Filamin A is an actin-binding protein that stabilizes the three-dimensional network of actin filaments, linking them to cellular membranes and maintains the integrity of the cell cytoskeleton. Filamin A has been reported to interact with several other GPCRs *via* either their C-tail or the third intracellular loop, including dopamine D2/D3 (Li et al., 2000, 2001), calcium-sensing (Awata et al., 2001; Hjalm et al., 2001), mu-opioid (Onoprishvili et al., 2003), calcitonin (Seck et al., 2003), and more recently P2Y2 (Yu et al., 2008) receptors. Interaction between GPCR and filamin A has been shown to regulate receptor trafficking (Onoprishvili et al., 2003), endocytic sorting and recycling (Seck et al., 2003), and spreading and migration of vascular smooth muscle cells (Yu et al., 2008). Other cytoskeletal proteins that interact with MT_1 and MT_2 receptors are tubulins and the glial maturation factor (GMF) β. α- and β-tubulins constitute the basic building block of microtubules, a major component of the cytoskeleton involved in many essential processes (McKean et al., 2001). α- and β-tubulins have been shown to interact with the C-tails of mGluR7a and mGluR1a, respectively (Ciruela et al., 1999; Saugstad et al., 2002). By our proteomic approaches, we identified α-, β-, and γ-tubulins as GAPCs of MT_1 and MT_2 receptors. We also demonstrated that microtubule dynamics modulate melatonin receptor function through their actions on $G_{\alpha i}$ proteins and impact on downstream signaling cascades (Jarzynka et al., 2006), strengthening the notion of a close interaction between melatonin receptors and microtubules. GMF β was identified as a growth and differentiation factor acting on neurons as well as glial cells (Lim et al., 1987) and first considered as a neurotrophic factor. However, GMF was also reported as an intracellular regulator of cell signal transduction and notably activates p38 MAP kinases and the NF-κB transcription factor in astrocytes (Lim et al., 2000). Interestingly, GMF has been reported to be phosphorylated by

PKC and CK2 (Zaheer and Lim, 1996, 1997), two proteins identified as GAPCs of both melatonin receptors. To date, interaction between GMF and GPCR has never been reported in the literature.

Similar to signaling proteins, the combination of both proteomic approaches also showed that MT_1 interacts with a greater number of cytoskeletal proteins than MT_2. The microtubule-associated protein 2 (MAP2) was the only cytoskeletal protein identified as specifically recruited by the MT_2 C-tail. MAP2 is a major agent responsible for promoting assembly and preservation of dendritic microtubules, which are the principal cytoskeletal constituents involved in growth and maintenance of dendrites (Dehmelt and Halpain, 2004, 2005). MAP2 can bind both microtubules and F-actin and changes in phosphorylation of MAP2 are known to affect its function. MAP2 has been reported as GAPCs of mGluR5 (Farr et al., 2004) and its phosphorylation state modulated by dopamine D1 receptors through a PKA-associated intracellular signaling pathway (Song et al., 2002).

In contrast, the MT_1 C-tail was shown to associate with a plethora of cytoskeletal proteins, including actin-binding proteins from the ADF (Actin-Depolymerizing Factor)/cofilin family: destrin and cofilins 1 and 2. ADF/cofilin proteins are important regulators of actin dynamics. The binding of these proteins to F-actin, which is regulated negatively by phosphorylation, influences actin filament turnover. To our knowledge, the identification of ADF/cofilin proteins as GAPCs has never been reported. However, it was recently shown that cofilin is involved in the $GABA_A$ receptor trafficking by dopamine D4 receptors (Graziane et al., 2009). Indeed, it was suggested that D4 receptor activation increases cofilin activity, presumably *via* its dephosphorylation, resulting in actin depolymerization and causing a decrease in the myosin-based transport of $GABA_A$ receptor clusters to the cell surface (Graziane et al., 2009). In addition, cofilin has been shown to participate in agonist-stimulated β-adrenergic receptor internalization (Volovyk et al., 2006). Involvement of cofilin in MT_1 internalization following melatonin stimulation remains to be determined. Another actin-binding protein recruited by the C-tail of MT_1 is the actin-related protein (ARP) 1 homolog B. ARP1 is part of a multiprotein complex known as dynactin, which is required for most, if not all, types of cytoplasmic dynein activity in eukaryotes. Dynactin binds dynein directly and allows the motor to traverse the microtubule lattice over long distances (Schroer, 2004). Other important regulators of cytoskeleton dynamics are microtubule-associated proteins. Whereas the C-tail of MT_2 specifically associates with MAP2, that of MT_1 interacts with another class of microtubule-associated proteins: the cytoplasmic linker protein 2 (CLIP2) and CLIP-associating protein 1 (or CLASP1). These proteins, called +TIPs for "plus-end tracking proteins", are known to associate with the tips of microtubules and regulate the dynamics of microtubule (Schuyler and Pellman, 2001; Galjart, 2005). To date, direct interaction between +TIPs and GPCRs has never been reported. Another microtubule-associated protein identified as binding protein of the MT_1 C-tail is kinesin. The kinesin superfamily proteins are motor proteins that transport membranous organelles, protein complexes and mRNAs to specific destinations along microtubules while hydrolyzing ATP for energy. Kinesins are also involved in various intracellular trafficking events such as axonal transport and chromosome segregation (Hirokawa and Noda, 2008). Involvement of kinesin in the regulation of GPCR function has never been reported. However, kinesin has been shown to be involved in the regulation of NMDA currents by $5\text{-}HT_{1A}$ receptors in rat prefrontal cortex neurons (Yuen et al., 2005). The C-tail of MT_1 also recruits the myosin heavy chain. Myosins are a large family of motor proteins responsible for actin-based motility. Interaction between a myosin heavy chain (IIA) and GPCR C-tail was reported for the chemokine CXCR4 and CCR5 receptors (Rey et al., 2002). This protein regulates endocytosis of CXCR4 and forms a complex with the C-tail of CXCR4 and β-arrestin (Rey et al., 2007). Another cytoskeletal protein known to be involved in GPCR endocytosis (Sever, 2002) and identified as a binding protein of MT_1 C-tail is dynamin. This GTPase protein regulates the endocytosis of numerous GPCRs following agonist activation. Finally, the MT_1 C-tail also interacts with the glial fibrillary acidic protein (GFAP), an intermediate filament protein thought to be specific for astrocytes in the central nervous system. Interestingly, MT_1 and MT_2 receptors have been reported to be present in mouse astrocytes and to modulate calcium signaling (Peters et al., 2005), and melatonin to reduce the GFAP mRNA and protein levels in neural stem cells (Kong et al., 2008). Involvement of GFAP in the regulation of GPCR function is not known.

Membrane proteins and heterodimerization

Not only soluble but also transmembrane proteins have been identified as GAPCs in numerous studies. Major advances have been made in the search for GPCR coupling to and regulation of ionic channels. Interestingly, these interactions often involve the C-tail of the GPCR. For instance, an interaction between the C-tail of D5 receptor and the ionotropic $GABA_A$ receptor has been reported (Liu et al., 2000). Similarly, the D1 receptor C-tail has been shown to physically interact with *N*-methyl-D-aspartate (NMDA) receptor (Lee et al., 2002). Interestingly, the physical interaction between these different receptors enables the modulation of each receptor function. In the same manner, a direct coupling between nociceptin (ORL1) re-

ceptors and N-type Ca^{2+} channel Cav2.2 *via* both C-tails has been demonstrated and shown to mediate inhibition of N-type channel currents and internalization of both ORL1 and Cav2.2 upon prolonged exposure of nociceptin (Beedle et al., 2004; Altier et al., 2006). Finally, two recent studies have provided evidence of a direct interaction between the mGluR5a and NMDA receptors (Perroy et al., 2008) and between D1 receptor and Cav2.2 (Kisilevski et al., 2008).

Melatonin receptors also couple to membrane proteins. Our two proteomic approaches identified several membrane proteins as GAPCs of MT_1, including the membrane-associated progesterone receptor component 1. This protein belongs to the membrane-associated progesterone receptor family. The signal transduction pathways induced by binding of progesterone to membrane-associated progesterone receptor component 1 have not been described to date, although motifs for tyrosine kinase, kinase binding, SH2 and SH3 have been predicted from the amino acid sequence (Thomas, 2008). Another membrane protein that interacts with the MT_1 receptor is a sodium bicarbonate cotransporter (*e.g.* solute carrier family 4, sodium bicarbonate cotransporter member 5). Sodium bicarbonate cotransporters are indispensable in acid-base homeostasis and could have a role in cell pH regulation (Bernardo et al., 2006). A vomeronasal receptor (*e.g.* vomeronasal receptor 1 A12) was identified. Vomeronasal receptors are GPCRs that bind pheromones and are responsible for various behavioral and neuroendocrine responses between individuals (Rouquier et Giorgi, 2006). Heterodimerization between this GPCR and MT_1 receptor remains to be confirmed. The MT_2 receptor was shown to interact with a Na/K-ATPase (*e.g.* Na/K ATPase α3 subunit). The Na/K-ATPase is a complex of integral membrane proteins that actively transports sodium and potassium across the cell plasma membrane, and maintains chemical gradients of these ions. In addition to pumping ions, the Na/K-ATPase has been shown to regulate the function of protein kinases and to act as a scaffold. Interestingly, this newly discovered signaling function of the Na/K-ATPase appears to play an important role in the pathogenesis of many cardiovascular diseases (Xie and Xie, 2005).

Beside the potential interaction with these membrane proteins, melatonin receptors have been shown to form homo- and heterodimers. Indeed, the MT_1 and MT_2 receptors were among the first GPCRs, whose homo- and heterodimerization have been demonstrated by bioluminescence resonance energy transfer (BRET) in transfected cells (Ayoub et al., 2002). The propensity of MT_2 homodimer formation is 3- to 4-fold lower than that of MT_1/MT_2 heterodimer and MT_1 homodimer formation (Ayoub et al., 2004), suggesting that MT_2 may be preferentially engaged into heterodimers in cells co-expressing equimolar quantities of both receptors. Other GPCRs have also been tested for their ability to heterodimerize with melatonin receptors. Whereas the CCR5 chemokine, β2-adrenergic (Ayoub et al., 2002, 2004) and serotonin 5-HT_4 (Berthouze et al., 2005) receptors do not heterodimerize with melatonin receptors, we demonstrated that the orphan GPR50 does (Levoye et al., 2006). Whereas this constitutive heterodimerization between GPR50 and MT_2 does not modify MT_2 function, GPR50 completely antagonizes the function of MT_1 within the heterodimer. Co-expression of GPR50 with MT_1 dose-dependently decreases by more than 50% the binding of melatonin to MT_1 in transfected cells. In addition, our data also demonstrated that MT_1 is devoid of G protein coupling in the presence of GPR50, a phenomenon that might depend on the presence of the long C-tail of GPR50 and the constitutive interaction of the GPR50/MT_1 heterodimer with β-arrestins (Levoye et al., 2006).

Others proteins

Other proteins belonging to chaperone or stress proteins or proteins involved in biosynthesis/trafficking have been identified by our proteomic approaches (table 1 and 2). It

Table 2
Identified components of MT_2 melatonin receptor GAPCs

Protein name	Molecular mass (kDa)	Identified with MT_1	Approach used	References
Membrane proteins				
GPR50 (melatonin-related receptor)	67	+	immunoprecipitation/ BRET	Levoye et al., 2006
Integral membrane protein 2C	30	-	entire receptor	Daulat et al., 2007
Na^+/K^+ ATPase alpha 3 subunit	113	-	C-tail	Maurice et al., 2008
Na^+/K^+ ATPase 3 (fragment)	33	-	C-tail	Maurice et al., 2008
Similar to WD repeat membrane protein	150	-	C-tail	Maurice et al., 2008

Protein name	Molecular mass (kDa)	Identified with MT_1	Approach used	References
Signal transduction				
14-3-3 protein tau	28	-	entire receptor	Daulat et al., 2007
Casein kinase II alpha subunit	45	+	C-tail	Maurice et al., 2008
Catenin delta-1 (p120-catenin)	102	-	entire receptor/C-tail	Daulat et al., 2007 Maurice et al., 2008
Clathrin heavy polypeptide	194	-	C-tail	Maurice et al., 2008
Dual specificity phosphatase 3	21	+	C-tail	Maurice et al., 2008
Guanine nucleotide-binding protein $G_{\beta 1}$	37	+	entire receptor	Daulat et al., 2007
Guanine nucleotide-binding protein $G_{\beta 4}$	37	+	entire receptor	Daulat et al., 2007
Guanine nucleotide-binding protein $G_{\alpha i1}$	40	+	entire receptor	Daulat et al., 2007
Guanine nucleotide-binding protein $G_{\alpha i2}$	40	+	entire receptor	Daulat et al., 2007
Guanine nucleotide-binding protein $G_{\alpha i3}$	40	+	entire receptor/C-tail	Daulat et al., 2007 Maurice et al., 2008
Insulin receptor substrate 4	134	+	entire receptor	Daulat et al., 2007
MKIAA0034 protein (clathrin heavy chain)	192	-	C-tail	Maurice et al., 2008
Phosphatidylinositol 4-kinase alpha	231	-	entire receptor	Daulat et al., 2007
PKC zeta 2	47	+	C-tail	Maurice et al., 2008
Protein phosphatase 2C gamma isoform	59	-	entire receptor	Daulat et al., 2007
Tyrosine-protein phosphatase, nonreceptor type 13	272	-	C-tail	Maurice et al., 2008
Cytoskeleton				
Filamin A	278	+	entire receptor	Daulat et al., 2007
Glial maturation factor beta	17	+	C-tail	Maurice et al., 2008
Microtubule-associated protein 2 (MAP2)	203	-	C-tail	Maurice et al., 2008
Tubulin alpha	50	+	C-tail	Maurice et al., 2008
Tubulin beta	50	+	C-tail	Maurice et al., 2008
Tubulin gamma	50	+	C-tail	Maurice et al., 2008
Biosynthesis				
Calnexin	68	+	entire receptor	Daulat et al., 2007
78-kDa glucose-regulated protein	72	+	entire receptor	Daulat et al., 2007
Protein-disulfide isomerase A6	48	+	entire receptor	Daulat et al., 2007
Traffic, chaperone, stress response				
Coatomer protein complex, alpha subunit	138	+	entire receptor	Daulat et al., 2007
Vacuolar sorting protein 35	92	+	entire receptor	Daulat et al., 2007
Heat shock protein 9	74	-	C-tail	Maurice et al., 2008
Heat shock protein 105 kDa (HSP-E7I)	96	-	C-tail	Maurice et al., 2008

Protein name	Molecular mass (kDa)	Identified with MT_1	Approach used	References
Others				
Brain glycogen phosphorylase	96	+	C-tail	Maurice et al., 2008
Crystallin mu	34	-	C-tail	Maurice et al., 2008
Dihydropyrimidinase-like 2	63	+	C-tail	Maurice et al., 2008
Calpain 3 (mUp76)	77	-	C-tail	Maurice et al., 2008

is interesting to note that most of the identified proteins that are involved in receptor biosynthesis were present in both receptor-associated complexes. This is expected because all GPCRs are suspected to follow the same biosynthetic pathway. However, identified proteins involved in trafficking clearly differ between MT_1 and MT_2, indicating different trafficking behavior.

Perspectives and concluding remarks

This review demonstrates the diversity of GAPCs that are able to interact with the melatonin receptor family, whose functions can be independent from or synergistic to G protein signaling pathways. Although the majority of these GAPCs remain to be validated notably in tissues expressing both proteins endogenously, this clearly increases the complexity of our initial view of the G protein-dependent melatonin receptor signaling. Moreover, it is important to note that these GAPCs are also dynamically regulated with respect to their molecular composition and tissue localization, thus contributing to a dynamic regulation of melatonin receptor function. MT_1 and MT_2 receptors associate clearly with a different repertoire of GAPCs providing additional evidence for the existence of different functional roles for these two receptor subtypes. According to our results, differences are expected not only for receptor signaling but also for receptor trafficking, a phenomenon that is still poorly understood for melatonin receptors. Further investigation will be required to complete our view of melatonin receptor-associated protein networks and the functional role of its components.

Acknowledgements

This work was supported by grants from SERVIER, the Fondation Recherche Médicale (“Equipe FRM”), Fondation pour la Recherche sur le Cerveau (FRC) Neurodon, Institut National de la Santé et de la Recherche Médicale (INSERM), Centre National de la Recherche Scientifique (CNRS). We are grateful to Patty Chen for comments on the manuscript.

References

Alpi E, L-andi E, Barilari M, Serresi M, Salvadori P, Bachi A, Dente L (2009). Channel-interacting PDZ protein “CIPP” interacts with proteins involved in cytoskeletal dynamics. Biochem J in press.

Altier C, Khosravani H, Evans RM, Hameed S, Peloquin JB, Vartian BA, Chen L, Beedle AM, Ferguson SS, Mezghrani A, Dubel SJ, Bourinet E, McRory JE, Zamponi GW (2006). ORL1 receptor-mediated internalization of N-type calcium channels. Nat Neurosci 9:31–40.

Awata H, Huang C, Handlogten ME, Miller RT (2001). Interaction of the calcium-sensing receptor and filamin, a potential scaffolding protein. J Biol Chem 276:34871–34879.

Ayoub MA, Couturier C, Lucas-Meunier E, Angers S, Fossier P, Bouvier M, Jockers R (2002). Monitoring of ligand-independent dimerization and ligand-induced conformational changes of melatonin receptors in living cells by bioluminescence resonance energy transfer. J Biol Chem 277:21522–21528.

Ayoub MA, Levoye A, Delagrange P, Jockers R (2004). Preferential formation of MT1/MT2 melatonin receptor heterodimers with distinct ligand interaction properties compared with MT2 homodimers. Mol Pharmacol 66:312–321.

Balasubramanian S, Fam SR, Hall RA (2007). GABAB receptor association with the PDZ scaffold Mupp1 alters receptor stability and function. J Biol Chem 282:4162–4171.

Bécamel C, Figge A, Poliak S, Dumuis A, Peles E, Bockaert J, Lubbert H, Ullmer C (2001). Interaction of serotonin 5-hydroxytryptamine type 2C receptors with PDZ10 of the multi-PDZ domain protein MUPP1. J Biol Chem 276:12974–12982.

Bécamel C, Alonso G, Galéotti N, Demey E, Jouin P, Ullmer C, Dumuis A, Bockaert J, Marin P (2002). Synaptic multiprotein complexes associated with 5-HT(2C) receptors: a proteomic approach. EMBO J 21:2332–2342.

Bécamel C, Gavarini S, Chanrion B, Alonso G, Galéotti N, Dumuis A, Bockaert J, Marin P (2004). The serotonin 5-HT2A and 5-HT2C receptors interact with specific sets of PDZ proteins. J Biol Chem 279:20257–20266.

Beedle AM, McRory JE, Poirot O, Doering CJ, Altier C, Barrere C, Hamid J, Nargeot J, Bourinet E, Zamponi GW (2004). Agonist-independent modulation of N-type calcium channels by ORL1 receptors. Nat Neurosci 7:118–125.

Bernardo AA, Bernardo CM, Espiritu DJ, Arruda JA (2006). The sodium bicarbonate cotransporter: structure, function, and regulation. Semin Nephrol 26:352–360.

Berthouze M, Ayoub M, Russo O, Rivail L, Sicsic S, Fischmeister R, Berque-Bestel I, Jockers R, Lezoualc'h F (2005). Constitutive dimerization of human serotonin 5-HT4 receptors in living cells. FEBS Lett 579:2973–2980.

Chanrion B, Mannoury la Cour C, Bertaso F, Lerner-Natoli M, Freissmuth M, Millan MJ, Bockaert J, Marin P (2007). Physical interaction between the serotonin transporter and neuronal nitric oxide syn-

thase underlies reciprocal modulation of their activity. Proc Natl Acad Sci USA 104:8119–8124.

Cho DI, Oak MH, Yang HJ, Choi HK, Janssen GM, Kim KM (2003). Direct and biochemical interaction between dopamine D3 receptor and elongation factor-1Bbetagamma. Life Sci 73:2991–3004.

Christenn M, Kindler S, Schulz S, Buck F, Richter D, Kreienkamp HJ (2007). Interaction of brain somatostatin receptors with the PDZ domains of PSD-95. FEBS Lett 581:5173–5177.

Christopherson KS, Hillier BJ, Lim WA, Bredt DS (1999). PSD-95 assembles a ternary complex with the N-methyl-D-aspartic acid receptor and a bivalent neuronal NO synthase PDZ domain. J Biol Chem 274:27467–27473.

Ciruela F, Robbins MJ, Willis AC, McIlhinney RA (1999). Interactions of the C terminus of metabotropic glutamate receptor type 1alpha with rat brain proteins: evidence for a direct interaction with tubulin. J Neurochem 72:346–354.

Daulat AM, Maurice P, Froment C, Guillaume JL, Broussard C, Monsarrat B, Delagrange P, Jockers R (2007). Purification and identification of G protein-coupled receptor protein complexes under native conditions. Mol Cell Proteomics 6:835–844.

Daulat AM, Maurice P, Jockers R (2009). Recent methodological advances in the discovery of GPCR-associated protein complexes. Trends Pharmacol Sci 30:72–78.

De Arcangelis V, Soto D, Xiang Y (2008). Phosphodiesterase 4 and phosphatase 2A differentially regulate cAMP/protein kinase a signaling for cardiac myocyte contraction under stimulation of beta1 adrenergic receptor. Mol Pharmacol 74:1453–1462.

Dehmelt L, Halpain S (2004). Actin and microtubules in neurite initiation: are MAPs the missing link? J Neurobiol 58:18–33.

Dehmelt L, Halpain S (2005). The MAP2/Tau family of microtubule-associated proteins. Genome Biol 6:204.

Dev KK (2004). Making protein interactions druggable: targeting PDZ domains. Nat Rev Drug Discov 3:1047–1056.

Ducruet AP, Vogt A, Wipf P, Lazo JS (2005). Dual specificity protein phosphatases: therapeutic targets for cancer and Alzheimer's disease. Annu Rev Pharmacol Toxicol 45:725–750.

Dufourny L, Levasseur A, Migaud M, Callebaut I, Pontarotti P, Malpaux B, Monget P (2008). GPR50 is the mammalian ortholog of Mel1c: evidence of rapid evolution in mammals. BMC Evol Biol 8:105.

Ebisawa T, Karne S, Lerner MR, Reppert SM (1994). Expression cloning of a high-affinity melatonin receptor from Xenopus dermal melanophores. Proc Natl Acad Sci USA 91:6133–6137.

Evans BJ, Wang Z, Mobley L, Khosravi D, Fujii N, Navenot JM, Peiper SC (2008). Physical association of GPR54 C-terminal with protein phosphatase 2A. Biochem Biophys Res Commun 377:1067–1071.

Farr CD, Gafken PR, Norbeck AD, Doneanu CE, Stapels MD, Barofsky DF, Minami M, Saugstad JA (2004). Proteomic analysis of native metabotropic glutamate receptor 5 protein complexes reveals novel molecular constituents. J Neurochem 91:438–450.

Fernandez N, Monczor F, Baldi A, Davio C, Shayo C (2008). Histamine H2 receptor trafficking: role of arrestin, dynamin, and clathrin in histamine H2 receptor internalization. Mol Pharmacol 74:1109–1118.

Flajolet M, Rakhilin S, Wang H, Starkova N, Nuangchamnong N, Nairn AC, Greengard P (2003). Protein phosphatase 2C binds selectively to and dephosphorylates metabotropic glutamate receptor 3. Proc Natl Acad Sci USA 100:16006–16011.

Galjart N (2005). CLIPs and CLASPs and cellular dynamics. Nat Rev Mol Cell Biol 6:487–498.

Gavarini S, Bécamel C, Altier C, Lory P, Poncet J, Wijnholds J, Bockaert P, Marin P (2006). Opposite effects of PSD-95 and MPP3 PDZ proteins on serotonin 5-hydroxytryptamine2C receptor desensitization and membrane stability. Mol Biol Cell 17:4619–4631.

Gavin AC, Bösche M, Krause R, Grandi P, Marzioch M, Bauer A, Schultz J, Rick JM, Michon AM, Cruciat CM, Remor M, Höfert C, Schelder M, Brajenovic M, Ruffner H, Merino A, Klein K, Hudak M, Dickson D, Rudi T, Gnau V, Bauch A, Bastuck S, Huhse B, Leutwein C, Heurtier MA, Copley RR, Edelmann A, Querfurth E, Rybin V, Drewes G, Raida M, Bouwmeester T, Bork P, Seraphin B, Kuster B, Neubauer G, Superti-Furga G (2002). Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature 415:141–147.

Gong K, Li Z, Xu M, Du J, Lv Z, Zhang Y (2008). A novel protein kinase A-independent, beta-arrestin-1-dependent signaling pathway for p38 mitogen-activated protein kinase activation by beta2-adrenergic receptors. J Biol Chem 283:29028–29036.

Graziane NM, Yuen EY, Yan Z (2009). Dopamine D4 receptors regulate GABAA receptor trafficking via an actin/cofilin/myosin-dependent mechanism. J Biol Chem in press.

Guillaume JL, Daulat AM, Maurice P, Levoye A, Migaud M, Brydon L, Malpaux B, Borg-Capra C, Jockers R (2008). The PDZ protein mupp1 promotes Gi coupling and signaling of the Mt1 melatonin receptor. J Biol Chem 283:16762–16771.

Guilluy C, Rolli-Derkinderen M, Loufrani L, Bourgé A, Henrion D, Sabourin L, Loirand G, Pacaud P (2008). Ste20-related kinase SLK phosphorylates Ser188 of RhoA to induce vasodilation in response to angiotensin II Type 2 receptor activation. Circ Res 102:1265–1274.

Hanyaloglu AC, Vrecl M, Kroeger KM, Miles LE, Qian H, Thomas WG, Eidne KA (2001). Casein kinase II sites in the intracellular C-terminal domain of the thyrotropin-releasing hormone receptor and chimeric gonadotropin-releasing hormone receptors contribute to beta-arrestin-dependent internalization. J Biol Chem 276:18066–18074.

Hirai T, Niino YS, Chida K (2003). PKC zeta II, a small molecule of protein kinase C zeta, specifically expressed in the mouse brain. Neurosci Lett 348:151–154.

Hirai T, Chida K (2003). Protein kinase Czeta (PKCzeta): activation mechanisms and cellular functions. J Biochem 133:1–7.

Hirokawa N, Noda Y (2008). Intracellular transport and kinesin superfamily proteins, KIFs: structure, function, and dynamics. Physiol Rev 88:1089–1118.

Hjälm G, MacLeod RJ, Kifor O, Chattopadhyay N, Brown EM (2001). Filamin-A binds to the carboxyl-terminal tail of the calcium-sensing receptor, an interaction that participates in CaR-mediated activation of mitogen-activated protein kinase. J Biol Chem 276:34880–34887.

Ishii H, Shibuya K, Ohta Y, Mukai H, Uchino S, Takata N, Rose JA, Kawato S (2006). Enhancement of nitric oxide production by association of nitric oxide synthase with N-methyl-D-aspartate receptors via postsynaptic density 95 in genetically engineered Chinese hamster ovary cells: real-time fluorescence imaging using nitric oxide sensitive dye. J Neurochem 96:1531–1539.

Janssens V, Goris J (2001). Protein phosphatase 2A: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling. Biochem J 353:417–439.

Jarzynka MJ, Passey DK, Ignatius PF, Melan MA, Radio NM, Jockers R, Rasenick MM, Brydon L, Witt-Enderby PA (2006). Modulation of melatonin receptors and G-protein function by microtubules. J Pineal Res 41:324–336.

Jockers R, Maurice P, Boutin JA, Delagrange P (2008). Melatonin receptors, heterodimerization, signal transduction and binding sites: what's new? Br J Pharmacol 154:1182–1195.

Jones SB, Lanford GW, Chen YH, Morabito M, Kim K, Lu Q (2002). Glutamate-induced delta-catenin redistribution and dissociation from postsynaptic receptor complexes. Neuroscience 115:1009–1021.

Joubert L, Hanson B, Barthet G, Sebben M, Claeysen S, Hong W, Marin P, Dumuis A, Bockaert J (2004). New sorting nexin (SNX27) and NHERF specifically interact with the 5-HT4a receptor splice variant: roles in receptor targeting. J Cell Sc 117:5367–5379.

Kim E, Sheng M (2004). PDZ domain proteins of synapses. Nat Rev Neurosci 5:771–781.

Kisilevsky AE, Mulligan SJ, Altier C, Iftinca MC, Varela D, Tai C, Chen L, Hameed S, Hamid J, Macvicar BA, Zamponi GW (2008). D1 receptors physically interact with N-type calcium channels to regulate channel distribution and dendritic calcium entry. Neuron 58:557–570.

Kong X, Li X, Cai Z, Yang N, Liu Y, Shu J, Pan L, Zuo P (2008). Melatonin regulates the viability and differentiation of rat midbrain neural stem cells. Cell Mol Neurobiol 28:569–579.

Kurschner C, Mermelstein PG, Holden WT, Surmeier DJ (1998). CIPP, a novel multivalent PDZ domain protein, selectively interacts with Kir4.0 family members, NMDA receptor subunits, neurexins, and neuroligins. Mol Cell Neurosci 11:161–172.

Lee FJ, Xue S, Pei L, Vukusic B, Chéry N, Wang Y, Wang YT, Niznik HB, Yu XM, Liu F (2002). Dual regulation of NMDA receptor functions by direct protein-protein interactions with the dopamine D1 receptor. Cell 111:219–230.

Lee S, Lynn EG, Kim JA, Quon MJ (2008). Protein kinase C-zeta phosphorylates insulin receptor substrate-1, -3, and -4 but not -2: isoform specific determinants of specificity in insulin signaling. Endocrinology 149:2451–2458.

Levoye A, Dam J, Ayoub MA, Guillaume JL, Couturier C, Delagrange P, Jockers R (2006). The orphan GPR50 receptor specifically inhibits MT1 melatonin receptor function through heterodimerization. EMBO J 25:3012–3023.

Li M, Bermak JC, Wang ZW, Zhou QY (2000). Modulation of dopamine D(2) receptor signaling by actin-binding protein (ABP-280). Mol Pharmacol 57:446–452.

Liew CW, Vockel M, Glassmeier G, Brandner JM, Fernandez-Ballester GJ, Schwarz JR, Schulz S, Buck F, Serrano L, Richter D, Kreienkamp HJ (2009). Interaction of the human somatostatin receptor 3 with the multiple PDZ domain protein MUPP1 enables somatostatin to control permeability of epithelial tight junctions. FEBS Lett 583:49–54.

Lim R, Hicklin DJ, Ryken TC, Miller JF (1987). Endogenous immunoreactive glia maturation factor-like molecule in astrocytes and glioma cells. Brain Res 430:49–57.

Lim R, Zaheer A, Yorek MA, Darby CJ, Oberley LW (2000). Activation of nuclear factor-kappaB in C6 rat glioma cells after transfection with glia maturation factor. J Neurochem 74:596–602.

Lin R, Karpa K, Kabbani N, Goldman-Rakic P, Levenson R (2001). Dopamine D2 and D3 receptors are linked to the actin cytoskeleton via interaction with filamin A. Proc Natl Acad Sci USA 98:5258–5263.

Liu F, Wan Q, Pristupa ZB, Yu XM, Wang YT, Niznik HB (2000). Direct protein-protein coupling enables cross-talk between dopamine D5 and gamma-aminobutyric acid A receptors. Nature 403:274–280.

Lorber B, Hendriks WJ, Van der Zee CE, Berry M, Logan A (2005). Effects of LAR and PTP-BL phosphatase deficiency on adult mouse retinal cells activated by lens injury. Eur J Neurosci 21:2375–2383.

Lugnier C (2005). Cyclic nucleotide phosphodiesterase (PDE) superfamily: a new target for the development of specific therapeutic agents. Pharmacol Ther 109:366–398.

Maillet M, Robert SJ, Cacquevel M, Gastineau M, Vivien D, Bertoglio J, Zugaza JL, Fischmeister R, Lezoualc'h F (2003). Crosstalk between Rap1 and Rac regulates secretion of sAPPalpha. Nat Cell Biol 5:633–639.

Mangmool S, Haga T, Kobayashi H, Kim KM, Nakata H, Nishida M, Kurose H (2006). Clathrin required for phosphorylation and internalization of beta2-adrenergic receptor by G protein-coupled receptor kinase 2 (GRK2). J Biol Chem 281:31940–31949.

Mao L, Yang L, Arora A, Choe ES, Zhang G, Liu Z, Fibuch EE, Wang JQ (2005). Role of protein phosphatase 2A in mGluR5-regulated MEK/ERK phosphorylation in neurons. J Biol Chem 280:12602–12610.

Maurice P, Daulat AM, Broussard C, Mozo J, Clary G, Hotellier F, Chafey P, Guillaume JL, Ferry G, Boutin JA, Delagrange P, Camoin L, Jockers R (2008). A generic approach for the purification of signaling complexes that specifically interact with the carboxyl-terminal domain of G protein-coupled receptors. Mol Cell Proteomics 7:1556–1569.

McClatchy DB, Knudsen CR, Clark BF, Kahn RA, Hall RA, Levey AI (2002). Novel interaction between the M4 muscarinic acetylcholine receptor and elongation factor 1A2. J Biol Chem 277:29268–29274.

McKean PG, Vaughan S, Gull K (2001). The extended tubulin superfamily. J Cell Sci 114:2723–2733.

Nosjean O, Nicolas JP, Klupsch F, Delagrange P, Canet E, Boutin JA (2001). Comparative pharmacological studies of melatonin receptors: MT1, MT2 and MT3/QR2. Tissue distribution of MT3/QR2 . Biochem Pharmacol 61:1369–1379.

Nourry C, Grant SG, Borg JP (2003). PDZ domain proteins: plug and play! Sci STKE 2003:RE7.

Onoprishvili I, Andria ML, Kramer HK, Ancevska-Taneva N, Hiller JM, Simon EJ (2003). Interaction between the mu opioid receptor and filamin A is involved in receptor regulation and trafficking. Mol Pharmacol 64:1092–1100.

Pandi-Perumal SR, Trakht I, Srinivasan V, Spence DW, Maestroni GJ, Zisapel N, Cardinali DP (2008). Physiological effects of melatonin: role of melatonin receptors and signal transduction pathways. Prog Neurobiol 85:335–353.

Pelletier S, Duhamel F, Coulombe P, Popoff MR, Meloche S (2003). Rho family GTPases are required for activation of Jak/STAT signaling by G protein-coupled receptors. Mol Cell Biol 23:1316–1333.

Perroy J, Raynaud F, Homburger V, Rousset MC, Telley L, Bockaert J, Fagni L (2008). Direct interaction enables cross-talk between ionotropic and group I metabotropic glutamate receptors. J Biol Chem 283:6799–6805.

Peters CM, Ghilardi JR, Keyser CP, Kubota K, Lindsay TH, Luger NM, Mach DB, Schwei MJ, Sevcik MA, Mantyh PW (2005). Tumor-induced injury of primary afferent sensory nerve fibers in bone cancer pain. Exp Neurol 193:85–100.

Petit L, Lacroix I, de Coppet P, Strosberg AD, Jockers R (1999). Differential signaling of human Mel1a and Mel1b melatonin receptors through the cyclic guanosine 3'-5'-monophosphate pathway. Biochem Pharmacol 58:633–639.

Reppert SM, Weaver DR, Ebisawa T (1994). Cloning and characterization of a mammalian melatonin receptor that mediates reproductive and circadian responses. Neuron 13:1177–1185.

Reppert SM, Godson C, Mahle CD, Weaver DR, Slaugenhaupt SA, Gusella JF (1995). Molecular characterization of a second melatonin receptor expressed in human retina and brain: the Mel1b melatonin receptor. Proc Natl Acad Sci USA 92:8734–8738.

Reppert SM, Weaver DR, Ebisawa T, Mahle CD, Kolakowski LF Jr (1996). Cloning of a melatonin-related receptor from human pituitary. FEBS Lett 386:219–224.

Reppert SM, Weaver DR (2002). Coordination of circadian timing in mammals. Nature 418:935–941.

Rey M, Vicente-Manzanares M, Viedma F, Yáñez-Mó M, Urzainqui A, Barreiro O, Vázquez J, Sánchez-Madrid F (2002). Cutting edge: association of the motor protein nonmuscle myosin heavy chain-IIA with the C terminus of the chemokine receptor CXCR4 in T lymphocytes. J Immunol 169:5410–5414.

Rey M, Valenzuela-Fernández A, Urzainqui A, Yáñez-Mó M, Pérez-Martínez M, Penela P, Mayor F Jr, Sánchez-Madrid F (2007). Myosin IIA is involved in the endocytosis of CXCR4 induced by SDF-1alpha. J Cell Sci 120:1126–1133.

Reynolds AB (2007). p120-catenin: Past and present. Biochim Biophys Acta 1773:2–7.

Ribas C, Penela P, Murga C, Salcedo A, García-Hoz C, Jurado-Pueyo M, Aymerich I, Mayor F Jr (2007). The G protein-coupled receptor kinase (GRK) interactome: role of GRKs in GPCR regulation and signaling. Biochim Biophys Acta 1768:913–922.

Rigaut G, Shevchenko A, Rutz B, Wilm M, Mann M, Séraphin B (1999). A generic protein purification method for protein complex characterization and proteome exploration. Nat Biotechnol 17:1030–1032.

Rouquier S, Giorgi D (2007). Olfactory receptor gene repertoires in mammals. Mutat Res 616:95–102.

Saugstad JA, Yang S, Pohl J, Hall RA, Conn PJ (2002). Interaction between metabotropic glutamate receptor 7 and alpha tubulin. J Neurochem 80:980–988.

Schroer TA (2004). Dynactin. Annu Rev Cell Dev Biol 20:759–779.

Schuyler SC, Pellman D (2001). Microtubule "plus-end-tracking proteins": The end is just the beginning. Cell 105:421–424.

Seck T, Baron R, Horne WC (2003). Binding of filamin to the C-terminal tail of the calcitonin receptor controls recycling. J Biol Chem 278:10408–10416.

Sesti G, Federici M, Hribal ML, Lauro D, Sbraccia P, Lauro R (2001). Defects of the insulin receptor substrate (IRS) system in human metabolic disorders. FASEB J 15:2099–2111.

Sever S (2002). Dynamin and endocytosis. Curr Opin Cell Biol 14:463–467.

Sheng M, Sala C (2001). PDZ domains and the organization of supramolecular complexes. Annu Rev Neurosci 24:1–29.

Song ZM, Undie AS, Koh PO, Fang YY, Zhang L, Dracheva S, Sealfon SC, Lidow MS (2002). D1 dopamine receptor regulation of microtubule-associated protein-2 phosphorylation in developing cerebral cortical neurons. J Neurosci 22:6092–6105.

Stiffler MA, Chen JR, Grantcharova VP, Lei Y, Fuchs D, Allen JE, Zaslavskaia LA, MacBeath G (2007). PDZ domain binding selectivity is optimized across the mouse proteome. Science 317:364–369.

Stricker NL, Christopherson KS, Yi BA, Schatz PJ, Raab RW, Dawes G, Bassett DE Jr, Bredt DS, Li M (1997). PDZ domain of neuronal nitric oxide synthase recognizes novel C-terminal peptide sequences. Nat Biotechnol 15:336–342.

Thomas P (2008). Characteristics of membrane progestin receptor alpha (mPRalpha) and progesterone membrane receptor component 1 (PGMRC1) and their roles in mediating rapid progestin actions. Front Neuroendocrinol 29:292–312.

Torrecilla I, Spragg EJ, Poulin B, McWilliams PJ, Mistry SC, Blaukat A, Tobin AB (2007). Phosphorylation and regulation of a G protein-coupled receptor by protein kinase CK2. J Cell Biol 177:127–137.

Villarreal RS, Alvarez SE, Ayub MJ, Ciuffo GM (2006). Angiotensin II modulates tyr-phosphorylation of IRS-4, an insulin receptor substrate, in rat liver membranes. Mol Cell Biochem 293:35–46.

Volovyk ZM, Wolf MJ, Prasad SV, Rockman HA (2006). Agonist-stimulated beta-adrenergic receptor internalization requires dynamic cytoskeletal actin turnover. J Biol Chem 281:9773–9780.

Wansink DG, Peters W, Schaafsma I, Sutmuller RP, Oerlemans F, Adema GJ, Wieringa B, van der Zee CE, Hendriks W (2004). Mild impairment of motor nerve repair in mice lacking PTP-BL tyrosine phosphatase activity. Physiol Genomics 19:50–60.

Weissman JT, Ma JN, Essex A, Gao Y, Burstein ES (2004). G-protein-coupled receptor-mediated activation of rap GTPases: characterization of a novel Galphai regulated pathway. Oncogene 23:241–249.

Wojcikiewicz RJ (2004). Regulated ubiquitination of proteins in GPCR-initiated signaling pathways. Trends Pharmacol Sci 25:35–41.

Xie Z, Xie J (2005). The Na/K-ATPase-mediated signal transduction as a target for new drug development. Front Biosci 10:3100–3109.

Xu AJ, Kuramasu A, Maeda K, Kinoshita K, Takayanagi S, Fukushima Y, Watanabe T, Yanagisawa T, Sukegawa J, Yanai K (2008). Agonist-induced internalization of histamine H2 receptor and activation of extracellular signal-regulated kinases are dynamin-dependent. J Neurochem 107:208–217.

Yu N, Erb L, Shivaji R, Weisman GA, Seye CI (2008). Binding of the P2Y2 nucleotide receptor to filamin A regulates migration of vascular smooth muscle cells. Circ Res 102:581–588.

Yuen EY, Jiang Q, Chen P, Gu Z, Feng J, Yan Z (2005). Serotonin 5-HT1A receptors regulate NMDA receptor channels through a microtubule-dependent mechanism. J Neurosci 25:5488–5501.

Yufu T, Hirano K, Bi D, Hirano M, Nishimura J, Iwamoto Y, Kanaide H (2005). Rac1 regulation of surface expression of protease-activated receptor-1 and responsiveness to thrombin in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 25:1506–1511.

Zaheer A, Lim R (1996). In vitro inhibition of MAP kinase (ERK1/ERK2) activity by phosphorylated glia maturation factor (GMF). Biochemistry 35:6283–6288.

Zaheer A, Lim R (1997). Protein kinase A (PKA)- and protein kinase C-phosphorylated glia maturation factor promotes the catalytic activity of PKA. J Biol Chem 272:5183–5186.

Zhang J, Vinuela A, Neely MH, Hallett PJ, Grant SG, Miller GM, Isacson O, Caron MG, Yao WD (2007). Inhibition of the dopamine D1 receptor signaling by PSD-95. J Biol Chem 282:15778–15789.

Zhao Q, Yang XL, Holtzclaw WD, Talalay P (1997). Unexpected genetic and structural relationships of a long-forgotten flavoenzyme to NAD(P)H:quinone reductase (DT-diaphorase). Proc Natl Acad Sci USA 94:1669–1674.