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Dawn of a New RAMPage

D. Stephen Serafin[*], **Natalie R. Harris**[*], **Natalie R. Nielsen**[*], **Duncan I. Mackie**, **Kathleen M. Caron, PhD**[1]

Department of Cell Biology & Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

Abstract

Receptor activity-modifying proteins (RAMPs) interact with G-protein-coupled receptors (GPCRs) to modify their functions, imparting significant implications upon their physiological and therapeutic potentials. A resurging interest in identifying RAMP-GPCR interactions has recently been fueled by coevolution studies and orthogonal technological screening platforms. These new studies reveal previously unrecognized RAMP-interacting GPCRs, many of which expand beyond Class B GPCRs. The consequences of these interactions on GPCR function and physiology lays the foundation for new molecular therapeutic targets, as evidenced by the recent success of Erenumab. Here, we highlight recent papers that uncovered novel RAMP-GPCR interactions, human RAMP-GPCR disease-causing mutations, and RAMP-related human pathologies, paving the way for a new era of RAMP-targeted drug development.

Keywords

Receptor activity-modifying proteins (RAMPs); G-protein-coupled receptor; Erenumab; CGRP; Adrenomedullin; Coevolution

Historical Paradigm of RAMP-GPCR Signaling

G-protein-coupled receptors (GPCRs) are the most tractable and druggable class of proteins, comprising approximately 30% of all approved therapeutics [1-3]. These seven-transmembrane pass receptors mediate intercellular communication through the binding of endogenous or exogenous ligands eliciting subsequent receptor conformational changes to release bound G-proteins. Over the past decade, the field of GPCR biology has experienced a dramatic revitalization with the discovery of biased signaling, spawning a new generation of allosteric drugs. Similarly, in the last three years, exciting evidence has emerged for novel GPCR interactions with a class of single-transmembrane proteins called receptor activity-modifying proteins (RAMPs), ushering in a new RAMPage that will revolutionize the GPCR field (BOX 1) [4].

[1]**Correspondence:** kathleen caron@med.unc.edu (K.M. Caron).

[*]Indicates equal contributing authors.

Dynamic regulation of GPCR function by RAMPs involves receptor-dependent alterations in GPCR trafficking, recycling, signal transduction, post-translational modifications, and G-protein coupling. Consequently, RAMPs impart pharmacological and physiological diversity to GPCR function [4-9]. Recent reports, reviewed herein, provide compelling evidence for RAMP-GPCR pairings within most GPCR subfamilies, but the pharmacologic, physiological, and pathological consequences of these interactions remain largely unknown. With the discovery of two disease-associated human mutations in the AM-CLR-RAMP2 signaling axis and the accumulated wealth of physiologic knowledge gleaned from genetic mouse models, the recent groundbreaking RAMP pharmacologic advancements are primed to drive new translational explorations of RAMP-GPCR functions in human biology and disease.

Previous reviews have comprehensively described the pharmacology, physiology, and protein structure of RAMP-GPCR interactions [10-12]. However, considering the rapid progression of new discoveries related to RAMPs, the purpose of this review is to i) highlight recent evidence supporting a global RAMP-GPCR interactome, ii) illuminate the substantial physiological functions imparted by RAMP-GPCR interactions in animal models and humans, iii) speculate on the therapeutic implications of such interactions, and iv) provide future perspectives as to the trajectory of the multi-faceted study of RAMP-GPCR interactions.

RAMP-GPCR Coevolution: Emerging Evidence of a Global RAMP-GPCR Interactome

As of 2016, there were only 11 reported RAMP-GPCR interactions [12]. Further, 9 of these 11 known RAMP-GPCR pairings involved Class B GPCRs, with the remaining 2 RAMP-GPCR pairs involving Class A and Class C receptors [13-15]. Considering Class B GPCRs only account for approximately 5% of the non-odorant GPCRs, one can speculate that the breadth of RAMP-GPCR pairings is more extensive than previously thought.

In support of this hypothesis, Barbash et al. 2017 provided compelling evidence for widespread RAMP-GPCR pairings [16, 17]. They utilized genomic and transcriptomic data to perform coevolution (see Glossary) and co-expression analyses to interrogate global RAMP-GPCR interactions. A protein phylogenetic analysis, based upon the supposition that protein-protein interactions which convey a fitness advantage will coevolve, was employed by this study [18]. This approach is advantageous in predicting RAMP-GPCR interactions because it is unburdened by technical limitations such as heterologous overexpression systems or antibody specificity, problems that have plagued previous biochemical studies of RAMP-GPCR pairs [12].

From their analysis, Barbash et al. 2017 concluded that RAMPs and GPCRs showed substantial evidence of coevolution. Specifically, they found that RAMPs and GPCRs had orthologs present within the same species and had correlated phylogenetic trees [16]. Further, Barbash et al. hypothesized that RAMP1 and RAMP3 have redundant GPCR interactions due to similar N-terminal sequence homology and hydrophobicity [4, 16]. To this end, the authors report a positive correlation, in the same species, between the

expression of specific GPCRs and RAMP1 and the same GPCRs and RAMP3. Additionally, they report an inverse relationship for RAMP2 compared to RAMP1 or RAMP3, suggesting that RAMP2 coevolved with a distinct group of GPCRs. To provide further support of global RAMP-GPCR interactions, the authors analyzed gene expression data from human tissues to test the hypothesis that interacting proteins will exhibit similar expression patterns. The authors found a positive correlation between RAMP and GPCR expression patterns which was significantly higher than expected by chance. The authors conclude that RAMPs and GPCRs showed substantial evidence of coevolution, and that RAMP1 and RAMP3 may have redundant functions, in contrast to independent roles of RAMP2. However, this protein coevolution analysis is limited by the inability to distinguish direct versus indirect protein interactions; coevolved proteins may be members of the same complex or broadly associated within the same signaling pathway [18].

To expand upon their bioinformatic findings, Barbash et al. 2019 developed an experimental approach to validate several predicted RAMP-GPCR pairings. Here, the authors overexpressed RAMP2 in HEK293T cells and employed a modified multiplexed error-correcting fluorescence in situ hybridization (MERFISH) assay and whole exome expression profiling to measure GPCR transcript levels [17, 19]. This work was based on the hypothesis that overexpression of RAMP2 would result in expression changes of putative-interacting-GPCRs [20-22]. The authors focused on RAMP2 as it is unplagued by the possible functional redundancy between RAMP1 and RAMP3. Use of these RAMPs may be complicated by cell compensatory mechanisms which would confound data analysis. Using the modified MERFISH assay, the authors found that 5 of the 14 GPCRs analyzed resulted in significant expression changes upon RAMP2 over-expression. Interestingly, all five responsive GPCRs were Class A GPCRs. A correlation was found between RAMP2 overexpression induced changes in GPCR gene expression and the extent of coevolution [16, 17]. Next, the authors used whole exome expression profiling to probe global GPCR expression changes in response to RAMP2 overexpression and concluded that there was a global downregulation of GPCR expression upon RAMP2 expression. These results support the 2017 phylogenetic analysis and overarching hypothesis of global RAMP-GPCR interactions.

Expanding the RAMP Repertoire

The early groundwork for RAMP-GPCR interactions focused on Class B GPCR receptors, which revealed that RAMPs interact with calcitonin receptor (CT) [23], calcitonin receptor-like receptor (CLR) [4], corticotropin-releasing factor receptors (CRF) [8], glucagon receptor [24, 25], parathyroid hormone receptors [25], secretin receptor [26], and pituitary adenylate cyclase activating peptide (PACAP) receptors [8, 25]. The characterization of these interactions uncovered diverse functions of RAMPs including alteration of ligand binding, specificity, and potency, best exemplified by CT [23] and CLR [4]. RAMPs were also shown to alter receptor trafficking and modulate G-protein coupling and secondary messengers, exemplified by VPAC1 [25]. Additional studies evaluated the interactions of RAMPs with the Class A receptor **GPER/GPR30** (described in the ‘Physiological and Pathophysiological Roles of RAMPs’ section below) [27], and the Class C receptor, **calcium-sensing receptor (CaSR)** [13]. These seminal papers identifying RAMP-GPCR

interactions relied heavily on over-expression of both a RAMP and a GPCR followed by detection of an increase in either RAMP or GPCR cell surface expression or their colocalization at the plasma membrane. Detection was primarily achieved using fluorescent-activated cell sorting (FACs), immunofluorescence microscopy, and ELISA assays [4, 7, 8, 13, 25, 26]. Later studies began to implement proximity-based approaches to detect direct RAMP-GPCR interactions, such as **bioluminescence resonances energy transfer (BRET)** assays [26, 27]. Commonly, these results were validated using a combination of radioligand binding, coimmunoprecipitation and western blot analysis, and interrogation of downstream G-protein signaling [4, 7, 8, 13, 23, 25-27].

While early studies were successful in interrogating specific RAMP-GPCR interactions, the coevolution studies described above prompt the need to systematically screen all GPCRs for putative interactions with RAMPS using biochemical, pharmacological, and physiological assays. Recently, two independent studies developed screening platforms to identify previously unrecognized RAMP-GPCR interactions. In the first study, Lorenzen et al. 2019 adapted a **multiplexed suspension bead array (SBA)** immunoassay to screen for putative interactions between RAMPs and Class B GPCRs, as well as a small cohort of non-Class B GPCRs [28]. Interactions were identified between at least one RAMP and members of the adhesion family of receptors, several orphan GPCRs (i.e. GPR182 and GPR4), members of the chemokine receptor family (part of Class A GPCRs), and Class B GPCRs. However, the functional and physiological consequences of these interactions were not explored.

A second study by Mackie et al. 2019 screened 24 chemokine receptors within the broader Class A family for possible interactions with RAMPs [29]. Leveraging a BRET screening methodology and FACs-based cell surface expression screening, the authors identified multiple putative chemokine receptor-RAMP interactions, including interactions with the sub-family of atypical chemokine receptors (ACKRs) (TABLE 1). Further, the authors chose to investigate the cellular consequences of one of the newly discovered RAMP-GPCR interactions between the atypical chemokine receptor **ACKR3** and RAMP3. ACKR3 functions as a decoy receptor for the ligands CXCL12/SDF-1, CXCL11, and **adrenomedullin** [30-33]. The authors found that RAMP3 alters the decoy activity of ACKR3 through a Rab4-dependent recycling mechanism to promote plasma membrane re-sensitization of ACKR3. The exciting physiological consequences of this interaction is discussed in the following ‘Physiological and Pathophysiological Roles of RAMPs’ section.

These studies highlight the breadth of putative RAMP-GPCR interactions and accentuate the current lack of studies aimed at validating or characterizing the functional significance of these interactions *in vitro* and *in vivo*. Screening platforms, like those described above, provide an invaluable tool to further interrogate RAMP-GPCR interactions on a large scale. Of note, both groups (Lorenzen and Mackie) utilized proximity ligation assay (PLA) to validate RAMP-GPCR interactions [28, 29]. This technique has traditionally been used over the last decade in the GPCR field to validate *ex vivo* GPCR heterodimers [34-36]. Future identification of novel RAMP-GPCR interactions can leverage existing PLA protocols and other non-traditional *in vitro* approaches for new exploratory and validation studies. As new RAMP-GPCR interactions are identified and biochemically validated, the implications of

RAMP regulation of GPCR pharmacology and cellular functions will continue to be unraveled.

Physiological and Pathophysiological Roles of RAMPs

The aforementioned bioinformatic and biochemical screening approaches revealed exciting new RAMP-GPCR interactions. However, despite the diverse and widespread tissue expression of RAMPs, the field has been largely unsuccessful in linking specific RAMP-GPCR pairings to physiological and pathological phenotypes [37]. This is due in large part to a lack of rigorous in situ detection approaches. To date, much of our current understanding of the physiological role of RAMPs has been gleaned through global and conditional RAMP knockout mice. These studies consistently show that RAMPs play essential roles in the cardiovascular, lymphatic, immune, endocrine, and central and peripheral nervous systems [38-44]. Global genetic knockout of *RAMP2* results in embryonic lethality marked by excessive fluid accumulation in the embryo [45-49], while global knockout of *RAMP1* or *RAMP3* leads to viable offspring with mild phenotypes[46]. Further, studies using haploinsufficient RAMP2 mice linked RAMP2 to the endocrine and skeletal systems [46]. In the following sections, we have chosen to highlight new discoveries made since 2018 that spotlight the influence of RAMP-GPCR interactions on physiology and pathology.

RAMP1

RAMP1 has been most intensely studied due to its role in the CGRP signaling axis. CGRP is a neuropeptide that signals through the CGRP receptor (RAMP1-CLR) [4]. The CGRP receptor is expressed on a variety of cell types [50, 51] (Figure 1A-B) and activation via CGRP results in potent vasodilation that has been clinically linked to migraine pathology [52] (Figure 1C). The successful therapeutic targeting of the CGRP receptor for the treatment of migraines is discussed in a later section titled ‘Therapeutic Targeting of RAMP-GPCRs’. The recent generation of sophisticated genetically engineered mouse models, such as the inducible neuronal overexpression of human RAMP1 (hRAMP1/Nestin-Cre) model, have proven invaluable to explore the physiological roles of RAMPs. Sabharwal et al. 2019 utilized the hRAMP1/Nestin mice to examine whether the known antihypertensive effects of CGRP were mediated primarily through neuronal or vascular CGRP receptors [53]. Cardiovascular phenotyping revealed that hRAMP1/Nestin mice display no baseline phenotypes, but in two hypertension models showed a reduction in the development of hypertension [53]. This paper highlighted an underappreciated neuronal role for the RAMP1-CLR signaling axis in mediating protection against hypertension.

Recently, the role of the RAMP1-CLR signaling axis in peripheral neurons has been expanded. An established link between CGRP and the digestive system indicates that CGRP-responsive neurons innervate multiple intestinal cells, including the epithelia, vasculature, smooth muscle, and enteric nerves [54]. Expanding upon these findings, Davis et al. 2019 linked RAMP1-CLR signaling to lymphatic innervation and lipid uptake in the intestinal lacteals [55]. This was accomplished using two different mouse models, 1) an inducible lymphatic-specific deletion of *Calcrl* (*Calcrl*$^{fl/fl}$/*Prox1-CreER*^{T2}), and 2) global

deletion of RAMP1 (*RAMP1*$^{-/-}$). The authors found that mice with lymphatic deletion of CLR, subjected to a high fat diet, displayed defective lacteal lipid transport, suppressed fat accumulation, and reduction and mis-patterning of the enteric nerve cage surrounding the lacteals. Similar results were found in the *RAMP1* $^{-/-}$ mice, which presented with dilated lymphatics and similar enteric nerve mis-patterning and density reduction (Figure 1D).

The dysregulation of the CGRP-RAMP1-CLR signaling axis in peripheral neurons and the gastrointestinal system has recently been linked to human disease. Pauza et al. 2019 associated dysregulation of CGRP-RAMP1-CLR signaling with diverticular disease (DD) pathology. DD, a common large bowel disease, is characterized by impaired colonic motility accompanied with expansive remodeling of the enteric nervous system. An imbalance in neuromuscular transmission has been recognized as a major contributing factor in the development of DD [56-58]. Considering the link between CGRP innervation and colonic motility, the authors investigated changes in CGRP and CLR-RAMP1 expression on enteric ganglia from colon biopsies from healthy individuals, asymptomatic and symptomatic DD patients. The authors hypothesized that changes in CGRP signaling within discrete colon structures would result in motility impairments in DD patients. Interestingly, DD samples showed a decrease in CGRP peptide expression with an associated increase in CLR expression [58]. This study demonstrated that pathologic alterations to CGRP signaling leads to neuro-muscular signaling imbalances in DD colons, and that targeting this signaling axis may prove to be an effective treatment strategy for DD (Figure 1E).

Neuropeptides, such as Neuromedin U (NMU) and CGRP, have garnered much attention in the immunology field through the discovery that neuro-immune crosstalk can influence allergic inflammation and shape innate lymphocyte responses during infection [59, 60]. Both NMU and CGRP are specifically upregulated in distinct populations of Type 2 innate lymphoid cells (ILC2) during an innate immune response and can modulate the inflammatory response of these cells during allergy or helminth infection [60]. Similar to NMU, Nagashima et al. 2019 found that CGRP and the CGRP receptor (RAMP1-CLR) were upregulated in discrete ILC2 populations in response to helminth infection (Figure 1F) [59]. Specifically, CGRP was found to modulate the cytokine production of these cells and constrain the magnitude of the innate immune response. The authors showed that inhibition of CGRP signaling, through the use of RAMP1 deficient mice and cells, resulted in improved ILC2 responses to helminth infection [59]. This suggests that the CGRP-RAMP1-CLR signaling axis is a viable pharmacological target during helminth infection.

RAMP2

A groundbreaking paper by Mackie et al. 2018 identified the first instance of a disease-causing mutation that disrupts a RAMP-GPCR interaction. Specifically, they identified a recessive mutation in human *Calcr*, which impairs the interaction between CLR and RAMP2 and is required for adrenomedullin signaling. [61]. The mutation was identified in a consanguineous family who presented with a high incidence of fetal demise due to excessive fluid accumulation associated with lymphatic insufficiency, a condition known as **nonimmune hydrops fetalis** (NIHF). This mutation is an in-frame deletion of the highly conserved valine 205 (V205del) within the first extracellular loop of CLR (Figure 2A).

Homozygous V205del resulted in NIHF, while the heterozygous carrier exhibits female subfertility (Figure 2B). *In vitro*, CLR V205del showed defective RAMP2-CLR receptor oligomerization, plasma membrane localization of RAMP2-CLR, and cAMP signaling. A prior role for the AM-CLR-RAMP2 signaling axis in the development of NIHF was established utilizing murine global knockout models of AM, RAMP2 and CLR [45-49]. To further define the role of this signaling axis in lymphatics, Mackie et al. generated two independent murine models of lymphatic *Calcrl* loss: a constitutive *Calcrl*$^{fl/fl}$;*Lyve1-Cre* and inducible *Calcrl*$^{fl/fl}$;*Prox1-CreER*^{T2}. These models recapitulated clinical phenotypes: lymphatic growth arrest, hypoplastic jugular lymph sacs, and dilated dermal lymphatics. Further, loss of RAMP2, modeled by rescue of the global RAMP2 knockout mouse with an endothelial expressed RAMP2 transgene (*RAMP2*$^{-/-}$ *Tg*), was shown to recapitulate the lymphatic and NIHF phenotypes of the *Calcrl* knockout mouse models. This study successfully translated clinical identification of a *Calcrl* mutation to a structurally and biochemically validated AM-CLR-RAMP2 signaling axis in lymphatic development.

In 2019, Gong et al. identified novel mutations within RAMP2 linked to primary open-angle glaucoma (POAG) [62]. POAG is one of the most common types of glaucoma and irreversible blindness. Taking advantage of the rapidly advancing repertoire of genome profiling tools, Gong et al. sought to identify novel POAG-causing genes and variants through exome sequencing analysis of 398 patients with POAG and 2010 control individuals. From their analysis, they identified 6 heterozygous pathologic variants in *Ramp2* (Figure 2A). The authors probed the functional effects of these variants *in vitro* and found that mutant RAMP2 formed intracellular aggregates, indicative of protein trafficking defects. Further, they found decreased cAMP expression, a known output of functional adrenomedullin-RAMP2-CLR-cAMP signaling. Gong et al. utilized a heterozygous RAMP2 knockout mouse to analyze the effects of RAMP2 haploinsufficiency in the eye. These mice displayed retinal ganglion death and reduced sensitivity of the retina to produce cAMP in response to adrenomedullin stimulation [62]. This study identified and linked *Ramp2* mutations to impaired AM-RAMP2-CLR cAMP signaling in retinal ganglion cells, thereby laying the groundwork for development of RAMP2 targeting strategies for the treatment of POAG (Figure 2C).

RAMP3

Recent years have witnessed significant advances in defining the role of RAMP3 in physiology and pathology. RAMP3 is unique amongst the RAMPs and differs from RAMP1 and RAMP2 in several ways. First, RAMP3 is capable of trafficking to the plasma membrane even in the absence of over-expression of a cognate GPCR [25, 29, 63]. Secondly, unlike RAMP1 and RAMP2, the N-terminus of RAMP3 contains four N-glycosylation sites and its intracellular C-terminus harbors a PDZ binding motif which has been shown to regulate receptor recycling [63-65]. Finally, as described below, the repertoire of RAMP3-assocaited GPCRs appears to be quite broad and impart evident physiological functions.

For example, one of the first studies to describe a RAMP interaction beyond the Class B receptors was performed by Barrick et al. 2012. Here, the authors describe an interaction

between RAMP3 and the Class A GPCR GPER/GPR30, whose ligand is estradiol (Figure 3A) [27]. The effect of RAMP3 on GPER/GPR30 signaling was investigated *in vivo* in the context of heart disease by crossing RAMP3 knockout mice onto a cardiac disease-prone genetic background (RenTgMK;*RAMP3*$^{-/-}$). These mice were treated with a GPER/GPR30 agonist, resulting in a significant reduction in cardiac hypertrophy and perivascular fibrosis that was both RAMP3- and sex-dependent (Figure 3B).

Recently, Mackie et al. 2019 further demonstrated the consequences of deleting RAMP3 during development [29]. Using a BRET screening assay, RAMP3 was found to interact with the atypical chemokine receptor, ACKR3, which is a decoy receptor for the chemokine ligands CXCL12/SDF1 and CXCL11 as well as adrenomedullin (Figure 3C). The authors showed *in vitro* that RAMP3 was required for ACKR3 rapid endosome recycling and its subsequent re-sensitization at the plasma membrane. In addition, the developmental consequences of this interaction were shown using knockout mouse models of the AM-ACKR3-RAMP3 decoy signaling axis (*ACKR3*$^{-/-}$ and *RAMP3*$^{-/-}$). In the absence of RAMP3, the chemotactic gradients established by ACKR3 decoy functions fail to be established, thereby disrupting normal guided cell migration during angiogenesis in murine retinal development (Figure 3D).

The initial generation of RAMP3 knockout mice was reported by Dackor et al. 2007, where *Ramp3*$^{-/-}$ mice were surprisingly viable and overtly phenotypically normal [46]. However, aged mice displayed decreased weight, suggesting an age-dependent induction of RAMP3 in normal metabolism. A dozen years later, two studies evaluated the levels of adrenomedullin 2/intermedin (AM2), a ligand for RAMP3-CLR, in the context of obesity (Figure 3E). The authors found an inverse relationship between AM2/intermedin plasma levels and the degree of adiposity in both obese mice and humans [66, 67]. Expanding on these findings, an additional paper probed the AM2-RAMP3-CLR signaling axis by examining the expression of AM2/intermedin, RAMP3, and CLR in obese patients with or without type 2 diabetes [68]. In this work, the authors profiled the mRNA expression of *Adm2*, *Calcrl*, *Ramp1*, *Ramp2*, and *Ramp3* in adipose tissue. They found a significant increase in *Adm2* mRNA expression in adipose tissue from non-diabetic and diabetic obese patients compared to controls, in contrast to the decreased plasma concentration of AM2/intermedin previously found in obese individuals. Interestingly, significantly higher levels of *Ramp1* and *Ramp3* mRNA were noted in the non-diabetic obese samples in comparison to both the controls and diabetic obese samples. *In vitro* experiments using human preadipocytes exposed to a fat microenvironment showed an increase in *AM2* mRNA expression but a decrease in AM2/intermedin secretion, recapitulating clinical findings. RAMP3 was not profiled in *these in vitro* experiment. Taken together, these studies provide a link between AM2-RAMP3-CLR perturbations and obesity and specifically identify significant downregulation of *Ramp3* mRNA in non-diabetic obesity (Figure 3F).

Another recent clinical study examined RAMP3 gene polymorphisms in women. The authors looked at bone density and body mass composition and found that amongst women with RAMP3 polymorphisms, fat mass tended to be higher only in the elderly women in this group[69]. This suggests that variations in RAMP3 expression may contribute to age related changes in body composition.

Therapeutic Targeting of RAMP-GPCRs

Considering the rapidly-expanding cohort of GPCRs that functionally interact with RAMPs and the breadth of physiological systems affected by RAMP signaling axes, it stands to reason that the protein-protein interface of a RAMP-GPCR interaction could be exploited for therapeutic benefit. Indeed, the therapeutic tractability of targeting RAMP-GPCR pairs has been recently established through the generation of monoclonal antibodies targeting CGRP or its receptor (RAMP1-CLR) for the treatment of migraines. CGRP has been implicated in migraine pathology due to its location in peripheral and central neurons, and its role as a potent vasodilator and nociception transmitter [70, 71]. Although the pathophysiology of migraines is multifactorial, it is generally thought that the activation and sensitization of the trigeminal system, which regulates blood flow and pain transmission in the head, contributes heavily to migraine symptomology [72, 73]. CGRP is the most abundant neuropeptide released in the trigeminal nerve and due to its short plasma half-life, likely exerts its effects near its release site at the vessel wall [73]. The CGRP receptor (RAMP1-CLR) is expressed throughout the trigeminal system, including in neurons and endothelial cells [73-75]. Migraines are thought to be the result of neurogenic inflammation which triggers the release of CGRP, leading to vasodilation of the peripheral and central nervous system and subsequent pathologic activation of the trigeminal system [75]. In support of this model of migraine pathophysiology, blood levels of CGRP are elevated during active migraine episodes and are further elevated in patients who experience chronic migraine in comparison to patients with episodic migraine [52, 76-78]. Consequently, recent therapies aimed at blocking CGRP activity, either using the small molecule inhibitors classified as “-gepants” [79] or using monoclonal antibodies against CGRP, have experienced great success and received FDA approval by effectively reducing the number of migraine days experienced by chronic migraine sufferers [80].

The essential role of RAMP1 in migraine pathology has been demonstrated *in vivo* using pre-clinical RAMP1 overexpression transgenic animals. While there are many methods used to simulate migraines in rodents, including adding an inflammatory stimulus directly onto the dura mater of the meninges, there are few genetic models which mimic the clinical symptoms associated with migraines [81]. One such genetic mouse model was designed to overexpresses human RAMP1 in glia and dura under the inducible Nestin-Cre promoter (hRAMP1/Nestin mice), thereby increasing the availability of CGRP receptor to bind CGRP and induce migraine [82]. Intracerebroventricular injection of CGRP into hRAMP1/Nestin mice resulted in various migraine phenotypes including photophobia and decreased motor activity in the dark [83]. Collectively, these pre-clinical animal studies solidified the importance of RAMP1 in migraine pain.

The groundbreaking therapy **Erenumab**, used as a treatment for migraines, is the first FDA-approved monoclonal antibody targeting a GPCR (Figure 1C). Specifically, the epitope was strategically designed to target the interface of the CGRP receptor RAMP1-CLR rather than RAMP2- or RAMP3-CLR, which form adrenomedullin receptors [84]. Thus, Erenumab is highly selective for the CGRP receptor, with no measurable activity for other CGRP family receptors [85]. Targeting of the RAMP1-CLR interface is supported by the crystal structure of RAMP1-CLR ectodomains bound with a CGRP antagonist (olcegepant or telcagepant) at

a peptide-binding cleft within the RAMP1-CLR interface, which disrupts the RAMP1-CLR interaction to impair CGRP signaling [86]. In fact, the first structure of a full-length RAMP-GPCR in complex with its Gs-protein and ligand was CGRP in complex with RAMP1-CLR (BOX 2). These structures support that RAMP-mediated ligand specificity can be attributed to stabilization of receptor complexes [87]. Future studies analyzing the structure of RAMP1-CLR bound to Erenumab would both clarify and underscore the importance of RAMPs in the stabilization of activated GPCR conformations and provide a structural framework for the development of new biologics exploiting tractable RAMP-GPCR interfaces.

The clinical success of Erenumab to target novel RAMP-GPCR interfaces to treat disease illustrates a massive therapeutic and commercial opportunity to target other RAMP-GPCRs dysregulated in disease. GPCRs are the most tractable and druggable class of proteins and represent the largest family of targets for approved drugs, which have classically been targeted using small molecules [1-3]. Yet in 2018, 5 of the top 10 best-selling prescription drugs were monoclonal antibodies, not small molecules [88]. This demonstrates the current disconnect between historical GPCRs targeting strategies, and the growing shift in the pharmaceutical market towards biologic therapies. Recent reports exploring the global diversity of RAMP-GPCR interactions coupled with the established role that RAMPs dynamically regulate GPCRs necessitates that drug discovery efforts must take into consideration RAMP-GPCR interactions. TABLE 1 includes FDA approved drugs that target RAMP-interacting GPCRs. It is striking to note that 66% of RAMP-interacting GPCRs listed in TABLE 1 have no approved FDA drug. It will be interesting to see how new RAMP-GPCR pairings will guide future drug development efforts.

Concluding Remarks and Future Perspectives

The last several years has marked an exciting time for the RAMP-GPCR field, where RAMP-GPCR coevolution studies hypothesized a global RAMP-GPCR interactome. Importantly, in 2019, we witnessed the experimental validation of these bioinformatic studies with the identification of previously unrecognized RAMP-GPCR interactions using two screening platforms (BRET and SBA). These studies effectively expanded the list of RAMP-interacting GPCRs from 11 to 44 receptors that span Class A, B, C and Adhesion family of GPCRs (TABLE 1). The newly expanded repertoire of RAMP-GPCR interactions alludes to a much more pronounced regulation of the entire GPCR family by RAMPs. In the coming years, it will be exciting to see how others expand upon these findings to further validate the global RAMP-GPCR interactome hypothesis. Such findings are likely to have broad physiological and pathological consequences considering the already established role of RAMPs in human health. This is most apparent with the recent success of Erenumab, a new anti-CGRP therapy, that was designed to specifically target the RAMP1-CLR interface, for the treatment of migraine. Further explorations into RAMP-GPCR signaling in human pathological conditions may help reveal new disease mechanisms and pharmacologically tractable targets for a variety of diseases (see Outstanding Questions). Moving forward, it has become both apparent and essential to consider RAMP-GPCR pairings for the development of more efficacious, selective, and context dependent therapies.

GLOSSARY

ACKR3:
The atypical chemokine receptor 3, also known as C-X-C chemokine receptor type 7 (CXCR7). It is a decoy receptor that binds the chemokines CXCL11 and CXCL12 to induce β-arrestin recruitment and ligand internalization. ACKR3 is also a decoy receptor for adrenomedullin (AM) to cause β-arrestin recruitment.

Adrenomedullin (AM):
It is a 52-amino acid peptide and member of the calcitonin/calcitonin gene-related peptide (CGRP) family. It is a potent vasodilator that plays a regulatory role in the cardiovascular and lymphatic systems through activation of CLR paired with RAMP2 or RAMP3.

AM2:
A member of the calcitonin/calcitonin gene-related peptide (CGRP) family, also known as intermedin, that is expressed in both the peripheral and central nervous systems.

BRET:
Bioluminescence resonances energy transfer is a method to measure protein-protein interactions. It is based upon the resonance energy transfer from a bioluminescent-taggeddonor protein to a fluorescently-tagged-acceptor protein.

CaSR:
The calcium-sensing receptor is a class C GPCR that regulates calcium homeostasis through the binding of extracellular calcium. It is expressed primarily in the parathyroid and kidney.

CGRP:
It is a 37 amino acid neuropeptide and member of the calcitonin/calcitonin gene-related peptide (CGRP) family. It is a potent vasodilator that functions though binding to its cognate CGRP receptor (RAMP1-CLR). It is expressed in both the peripheral and central nervous system.

CLR/*Calcrl*-
Calcitonin receptor-like receptor is a member of the class B GPCRs.

Coevolution analysis:
A method to identify putative protein-protein interactions that is based upon the supposition that two proteins that interact will coevolve due to evolutionary pressure that preserves the interaction. The analysis looks for co-acquisition of mutations in interacting proteins and correlations in protein phylogenetic trees.

Erenumab:
A CGRP-receptor antagonist approved by the FDA in 2018 for the treatment of migraines. It is a monoclonal antibody selective for the CGRP receptor.

GPER/GPR30:

Originally known as the orphan G-protein-coupled receptor 30 (GPR30) and later re-named the G-protein-coupled estrogen receptor 1 (GPER) when it was found to bind Estradiol to cause cyclic AMP, inositol trisphosphate (IP3) and calcium signaling.

HEK293T:
Transformed Human embryonic kidney cell line that have very low endogenous RAMP expression. They are commonly used to investigate RAMP-GPCR interactions due to their high transfection efficiency.

NIHF:
Nonimmune hydrops fetalis is a condition characterized by fluid accumulation in the extravascular and body cavity of a fetus.

PDZ motif:
Regarding RAMP3, it is a four amino acid sequence (DTLL) on the RAMP3 C-terminus that promotes interactions with RAMP3 and other PDZ proteins, such as NHERF1. These interactions can affect receptor internalization and trafficking of RAMP3-GPCR pairs.

SBA:
A suspension bead array (SBA) immunoassay, which consists of magnetic, bar-coded beads conjugated to specific antibodies, to capture and detect target protein epitopes from complex cell lysates.

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Outstanding Questions:

- Will the global RAMP-GPCR interactome hypothesis be validated by biochemical assays?
- Do RAMPs interact with discrete GPCRs or is there broad redundancy in their respective interactomes?
- In cells that express multiple RAMPs and RAMP-interacting GPCRs, how are cell surface presentation and signaling selectively determined, coordinated, and regulated?
- Are there are other proteins associated with RAMP-GPCR pairs?
- Are RAMP1 and RAMP3 redundant or will future research reveal context dependent signaling biases?
- Are validated RAMP-GPCR interactions cellular, tissue, and context-specific?
- Disease causing mutations in RAMP2 have set a precedence. Are there yet unidentified mutations or polymorphisms in RAMPs with physiological consequences?
- How will insights gained from RAMP-GPCR high resolution atomic structures influence future drug discovery efforts?
- Will RAMP-GPCR interactions be more tractable via biologies or small molecules? Is there a role for allosterism?
- Is the failure to account for RAMP-GPCR interactions during target identification and drug development influencing the rampant attrition rate of GPCR-targeted pharmaceutical therapies?
- Will context-dependent identification of RAMP-GPCR interactions allow for development more of targeted therapies?

Highlights:

- RAMPs interact with GPCRs to regulate receptor function.
- RAMP-GPCR coevolution studies suggest that RAMPs globally interact with GPCRs.
- In the past year, two studies have expanded the RAMP-GPCR interactome using both SBA and BRET methodologies.
- Human mutations in RAMP-GPCR pairings have been identified and linked to human disease, including a mutation in CLR, associated with hydrops fetalis, and mutations in RAMP2, associated with glaucoma.
- For the first time, the FDA has approved a GPCR-directed antibody against RAMP1-CLR, which reduces the number of migraine days patients experience.
- The expanded RAMP-GPCR interactome and pivotal success of the first targeted therapy of RAMP-GPCR pairs has ushered in a new RAMPage.

BOX 1:

Structural determinants of RAMP function

RAMPs are single-pass transmembrane proteins that consist of an ~100 amino acid N-terminal extracellular domain (ECD) and a short ~9 amino acid C-terminal intracellular domain. Amino acid multiple alignment indicates that RAMP1, RAMP2, and RAMP3 are approximately 31% homologous, yet 56% similar, which supports both redundant and independent functions between RAMP family members [4, 16]. Functional differences between RAMPs can be attributed to differences within their N-terminal and C-terminal domains to alter ligand binding and G-protein coupling, respectively [91, 92]. For example, the RAMP2 N-terminal domain contains an additional 28 amino acids not found in either RAMP1 or RAMP3 [16]. Conversely, the RAMP3 C-terminal domain contains a type-1 **PDZ motif** (DTLL) that mediates interactions with the NA+/H+ exchange regulatory factor (NHERF) and N-ethylmaleimide-sensitive factor (NSF) to alter GPCR pharmacology [63, 64]. Important novel insights into GPCR ligand affinity and selectivity, as well as the role of RAMPs in modulation of GPCR pharmacology, are being reported through structural analysis of crystal and cryo-EM structures.

BOX 2:

New Insights into RAMP-GPCR structure

Over the past decade, insights into how RAMPs impart pharmacological diversity to GPCR function and ability to bind cognate ligands has been elucidated through structural analyses. However, until recently, these conclusions have been limited due to partial structures consisting of GPCRs and RAMP ECD domains. While valuable in that they allowed insight into how RAMPs and GPCR interact, these structural advancements were not equipped to depict how RAMPs impart peptide selectively. For a thorough review of class B GPCR structure along with commentary on structures of RAMP1-CLR and RAMP2 ECD domains, we recommend Hay and Pioszak 2016 [12].

Recently, Liang et al. 2018 reported a full cryo-EM structure depicting active, G-protein-coupled complex; a RAMP-GPCR pair bound to endogenous ligand and in complex with heterotrimeric G-proteins [87]. Specifically, using Volta phase plate cryo-electron microscopy, they obtained a 3.3 A structure of human CGRP receptor (RAMP1-CLR) bound to its ligand CGRP and the Gs-protein heterotrimer. Regarding the RAMP1-CLR interface, the authors found that 23% of the surface of RAMP1 is buried within the interface of CLR. Structural, mutagenesis, and molecular dynamic stimulations highlight the key function of RAMP1 is in the stabilization of the CLR extracellular loop 2. Regarding the RAMP1-CGRP interface, the authors found that while the CGRP peptide interacts extensively with the RAMP1-CLR complex with 61.5% of CGRP's surface buried, there are limited direct interactions between RAMP1 and CGRP peptide. These interactions are between the C-terminus of the CGRP peptide and RAMP1 residues $F83^{R}$-$P85^{R}$ [93]. Further, mutagenesis and modeling experiments accentuated the importance of the ECL2 conformation for CGRP activation of its receptor [9]. Taken together, the authors conclude that RAMP1 likely acts as an allosteric regulator of CLR and functions to stabilize the ECD and ECL2 of CLR to make CLR conformationally amendable for GCRP binding and presentation to the CLR core.

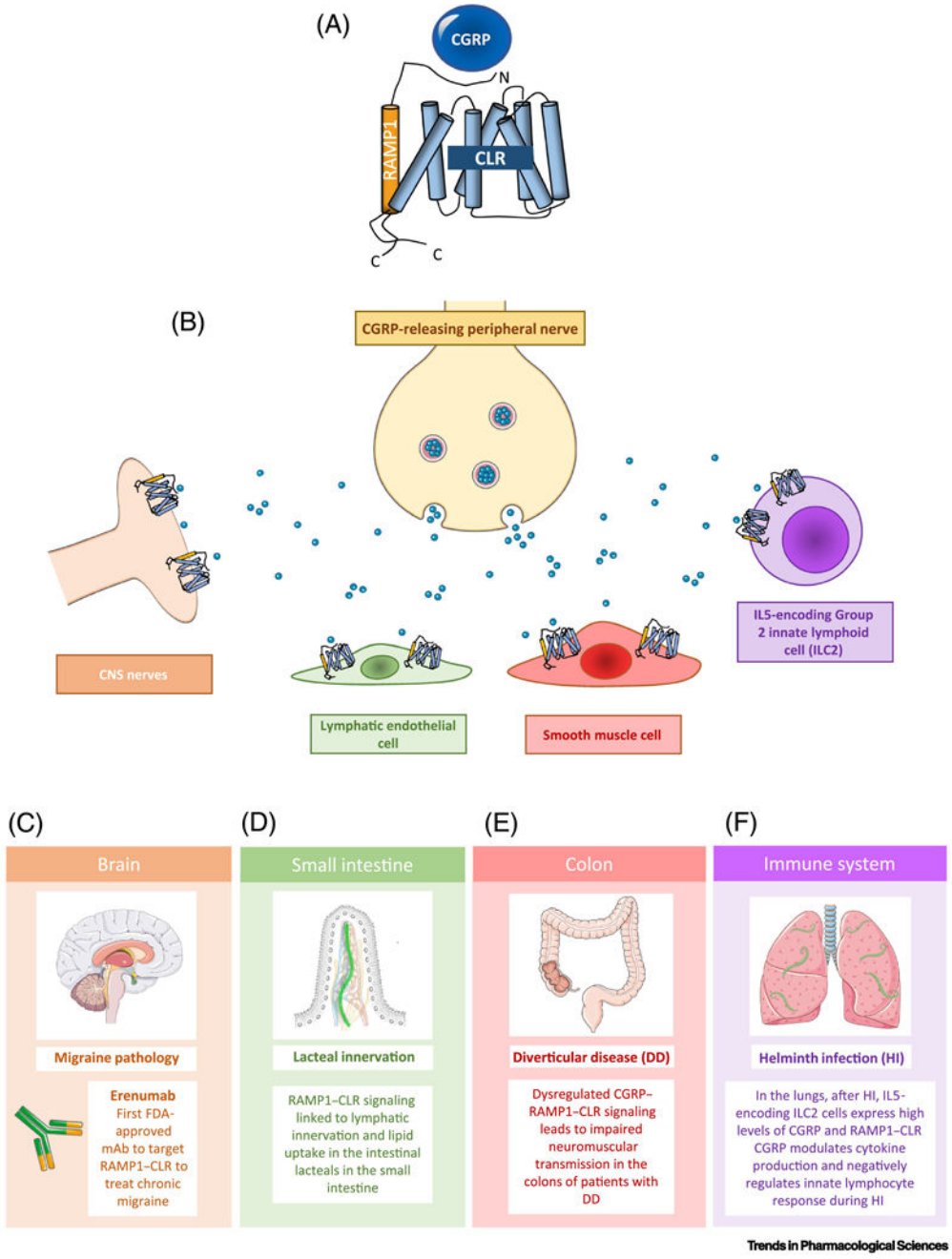

Figure 1: Role of RAMP1-CLR in disease

(A) Graphical representation of the CGRP receptor components CLR and RAMP1. **(B)** Schematic depicting release of CGRP from peripheral nerves and its potential targets including lymphatic endothelial cells, CNS nerves, and smooth muscle cells. **(C)** CGRP can also stimulate CNS nerves, and other cell types in the CNS, to incite migraines. Erenumab, the first FDA-approved monoclonal antibody against a GPCR, targets the RAMP1-CLR receptor to help treat migraine. **(D)** CGRP can bind to its receptor on lymphatic endothelial cells, which is linked to lacteal innervation and lipid uptake in the small intestine. **(E)** CGRP can also bind to receptors on smooth muscle cells, which can have physiological effects in

the colon. Human studies have shown dysregulation of CGRP signaling in patients with diverticulitis disease (DD), highlighting the importance of this signaling axis in human disease. **(F)** CGRP is specifically upregulated in a distinct population of Type 2 Innate Lymphoid Cells found in the lungs in response to specific cytokine cues released by lung-infiltrated *N. brasiliensis* worms during helminth infection and constrains the magnitude of the innate immune response.

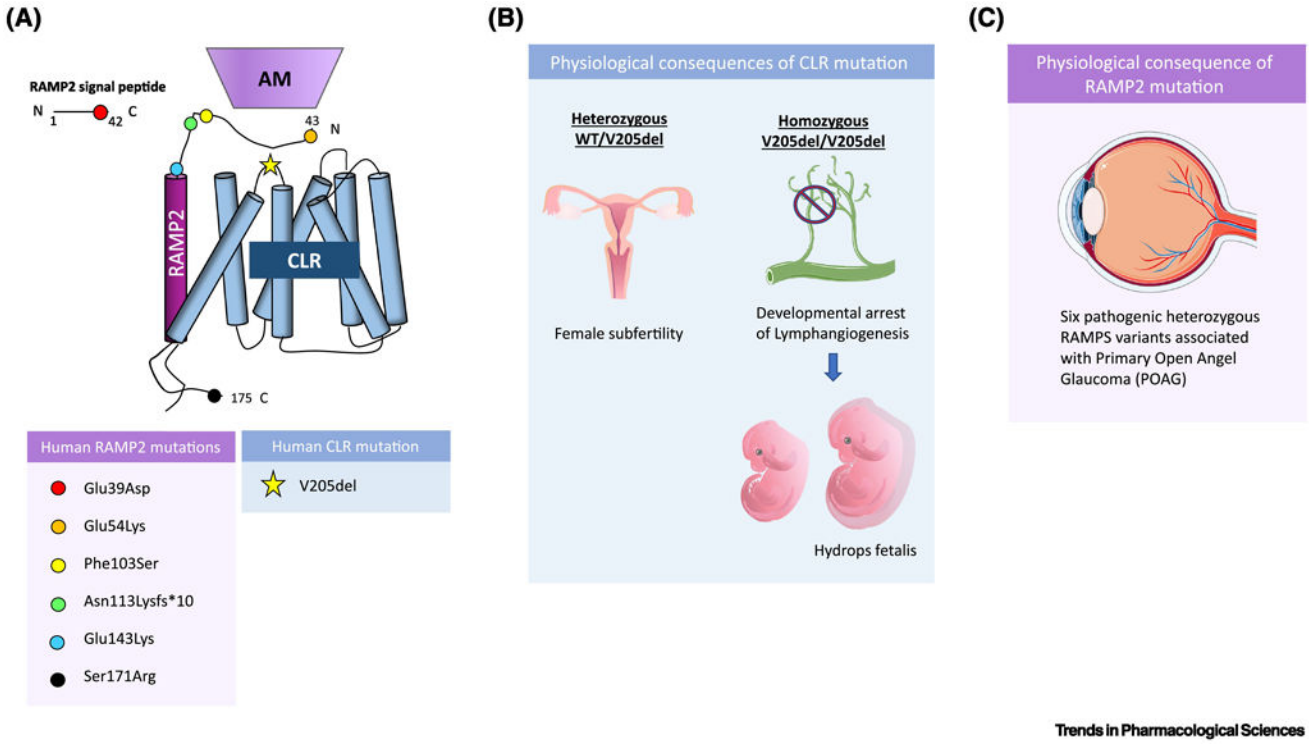

Figure 2. Recently identified human mutations in the AM-RAMP2-CLR signaling axis.

(A) As of 2019, in humans, a single pathologic mutation was identified in CLR, specifically on extracellular loop 1 of the receptor, and 6 heterozygous pathologic RAMP2 variants were identified, with mutations on the cleaved signaling peptide of RAMP2, and both the extracellular and cytosolic portion of the membrane-localized protein, with no mutations identified in the transmembrane domain. These mutations are spatially displayed on a schematic of CLR bound to RAMP2 and adrenomedullin, with each colored circle corresponding to one of the six identified RAMP2 variants and the star corresponding to the identified CLR mutation. **(B)** In humans, homozygous deletion of Valine 205 in CLR results in developmental arrest of lymphangiogenesis associated with lethal fluid accumulation, known as non-immune hydrops fetalis, while heterozygous carriers display female subfertility. Lymphatic deletion of CLR in mice results in similar phenotypes as seen in humans. **(C)** 6 pathologic RAMP2 variants were identified in an exome sequencing study of patients with primary open angle glaucoma (POAG) and each of the 6 variants were linked to functional impairments in AM-RAMP2-CLR signaling and deleterious effects on retinal ganglion nerve health.

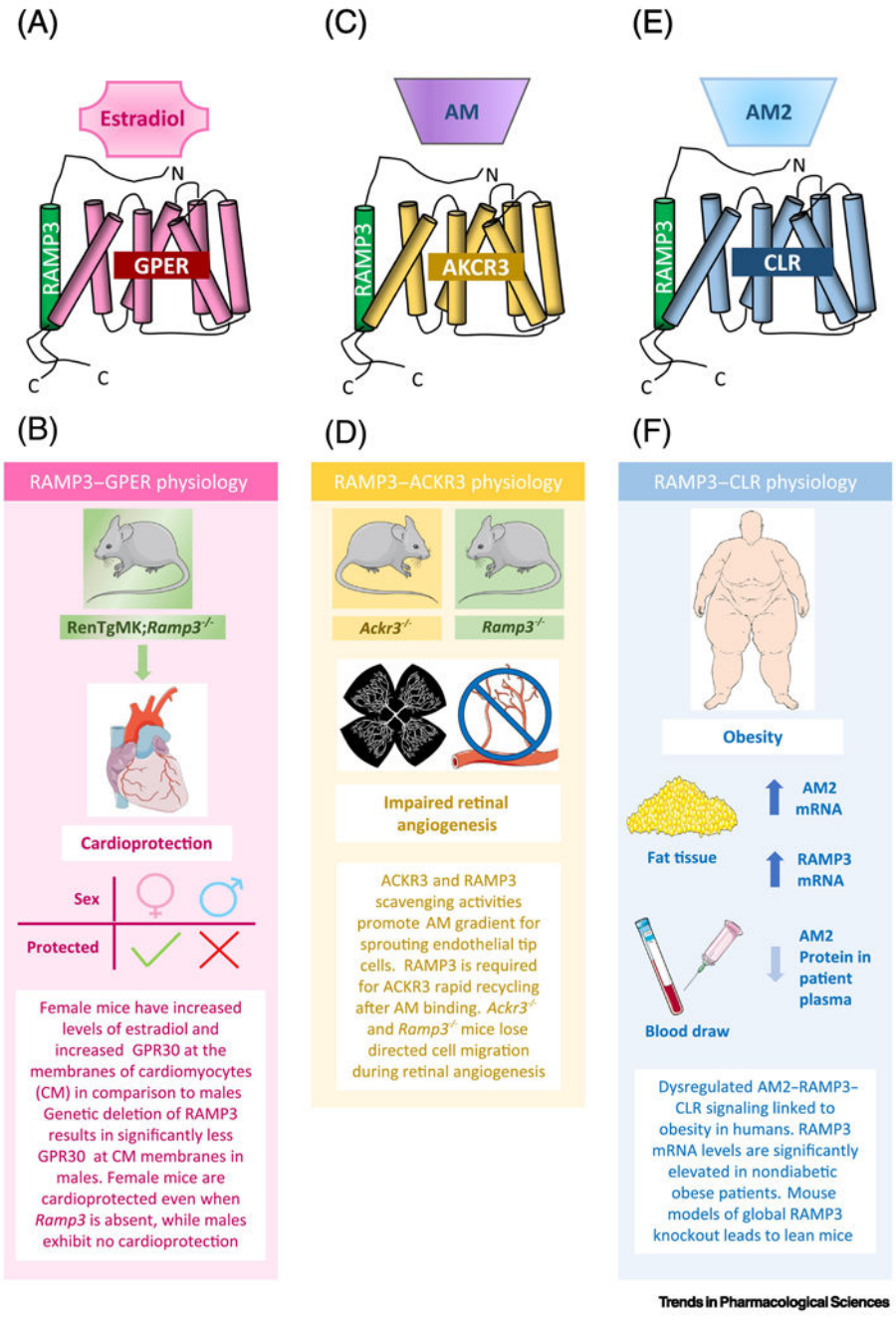

Figure 3: Pathophysiological roles of RAMP3.

(A) Depiction of the receptor complex RAMP3-GPER/GPR30 and its ligand estradiol. **(B)** Murine studies looked at the link between RAMP3-GPER/GPR30 and heart disease by crossing RAMP3 knockout mice onto a heart disease-prone genetic background. This *in vivo* activation of GPER/GPR30 resulted in significant reduction in heart disease parameters that was both RAMP3 and sex dependent. **(C)** The RAMP3-ACKR3 receptor is a decoy-receptor for the ligand adrenomedullin (AM), in that it binds AM, but does not result in G-protein signaling. **(D)** Recently, RAMP3 was shown to alter the decoy activity of ACKR3 through a recycling mechanism, which promoted plasma membrane re-sensitization of ACKR3. This

decoy activity was shown to be important during guided cell migration in murine retinal angiogenesis. **(D)** RAMP3 can also interact with CLR to form a receptor for adrenomedullin 2 (AM2). **(E)** This signaling axis was investigated in obese and non-obese human patients, where it was found that RAMP3 mRNA levels were increased in obese patients, which correlates with RAMP3 knockout mice phenotypes. These studies highlight the importance of continuing to study RAMP3 in human disease, particularly metabolic disorders.

Table 1:

Currently identified RAMP-interacting GPCRs, methods of interaction, identification, and FDA approved drugs[a]

Official IUPHAR Receptor Name	Class	RAMP	Cell Line	Identification Method	FDA Approved Drugs[b]	Clinical Indication	Ref.
Chemerin receptor 1	A	RAMP2, RAMP3	HEK293T, COS-7	BRET assay, FACS	N/A[c]	---	[29]
CCR1	A	RAMP1, RAMP2, RAMP3	HEK293T, COS-7	BRET assay	N/A	---	[29]
CCR2	A	RAMP2, RAMP3	HEK293T, COS-7	BRET assay	N/A	---	[29]
CCR3	A	RAMP1, RAMP2, RAMP3	HEK293T, COS-7	BRET assay	N/A	---	[29]
CCR4	A	RAMP1, RAMP2, RAMP3	HEK293T, COS-7	BRET assay	mogamuli zumab	Cutaneous T-cell Lymphoma (Mycosis Fungoides, Sézary Syndrome)	[29]
					plerixafor	non-Hodgkin lymphoma, multiple myeloma	
CCR5	A	RAMP1, RAMP2, RAMP3	HEK293T, COS-7	BRET assay, FACS	maraviroc	HIV/AIDS	[29]
					ibalizuma b	HIV/AIDS	
CCR6	A	RAMP1, RAMP2, RAMP3	HEK293T, COS-7	BRET assay	N/A	---	[29]
CCR7	A	RAMP2, RAMP3	HEK293T, COS-7	BRET assay, FACS	N/A	---	[29]
CCR8	A	RAMP2, RAMP3	HEK293T, COS-7	BRET assay	N/A	---	[29]
CCR9	A	RAMP1, RAMP2, RAMP3	HEK293T, COS-7	BRET assay	N/A	---	[29]
CCR10	A	RAMP1, RAMP2, RAMP3	HEK293T, COS-7	BRET assay, FACS	N/A	---	[29]
CXCR1	A	RAMP1, RAMP2, RAMP3	HEK293T, COS-7	BRET assay	N/A	---	[29]
CXCR2	A	RAMP2, RAMP3	HEK293T, COS-7	BRET assay, FACS	N/A	---	[29]
CXCR3	A	RAMP2, RAMP3	HEK293T, COS-7	BRET assay, FACS	N/A	---	[29]
		RAMP2, RAMP3	HEK293 FreeStyle	SBA immunoassay			[28]
CXCR4	A	RAMP1, RAMP3	HEK293T, COS-7	BRET assay	plerixafor	non-Hodgkin lymphoma, multiple myeloma	[29]
CXCR5	A	RAMP3	HEK293T, COS-7	BRET assay, FACS	N/A	---	[29]

Official IUPHAR Receptor Name	Class	RAMP	Cell Line	Identification Method	FDA Approved Drugs[b]	Clinical Indication	Ref.
CXCR6	A	RAMP3	HEK293T, COS-7	BRET assay	N/A	---	[29]
CX_3CR1	A	RAMP1, RAMP2, RAMP3	HEK293T, COS-7	BRET assay, FACS	N/A	---	[29]
XCR1	A	RAMP1, RAMP2, RAMP3	HEK293T, COS-7	BRET assay, FACS	N/A	---	[29]
ACKR1	A	RAMP1, RAMP2, RAMP3	HEK293T, COS-7	BRET assay, FACS	N/A	---	[29]
ACKR2	A	RAMP1, RAMP3	HEK293T, COS-7	BRET assay, FACS	N/A	---	[29]
ACKR3	A	RAMP1, RAMP2, RAMP3	HEK293T, COS-7	BRET assay, FACS, PLA, Confocal microscopy	plerixafor	non-Hodgkin lymphoma, multiple myeloma	[29]
		RAMP2, RAMP3	HEK293 FreeStyle	SBA immunoassay			[28]
ACKR4	A	RAMP2, RAMP3	HEK293T, COS-7	BRET assay	N/A	---	[29]
ACKR5	A	RAMP3	COS-7	FACS	N/A	---	[29]
GPR4	A	RAMP1, RAMP2, RAMP3	HEK293T, HEK293 FreeStyle	SBA immunoassay, PLA	N/A	---	[28]
GPR182	A	RAMP1, RAMP2, RAMP3	HEK293T, HEK293 FreeStyle	SBA immunoassay, PLA	N/A	---	[28]
GPER/ GPR30	A	RAMP3	HEK293T	BRET assay, Co-IP, Confocal microscopy	estradiol (estrogen receptor agonist; naturally occurring)	Oral contraceptives, treatment of menopausal and perimenopausal symptoms, and hypoestrogenism	[27]
CT receptor	B	RAMP1, RAMP2, RAMP3	COS-7, CHO-P	Radioligand binding, Crosslinking analysis, Confocal microscopy	pramlintide	Type I & Type II Diabetes	[23]
		RAMP1, RAMP2, RAMP3	HEK293T, HEK293 FreeStyle	SBA immunoassay			[28]
Calcitonin receptor like receptor	B	RAMP1, RAMP2, RAMP3	*Xenopus* oocytes, HEK293T	Radioligand binding, FACS, Crosslinking analysis	eptinezumab, fremanez umab, glacanezumab, erenumab	Chronic Migraine	[4]
		RAMP1, RAMP2, RAMP3	HEK293T, COS-7	BRET assay, FACS, PLA, Confocal microscopy			[25, 29]
		RAMP1, RAMP2, RAMP3	HEK293T, HEK293 FreeStyle	SBA immunoassay, PLA			[28]
CRF_1 receptor	B	RAMP2	HEK293S, CHO-K1	ELISA	N/A	---	[8]
		RAMP2, RAMP3	HEK293 FreeStyle	SBA immunoassay	N/A	---	[28]

Official IUPHAR Receptor Name	Class	RAMP	Cell Line	Identification Method	FDA Approved Drugs[b]	Clinical Indication	Ref.
CRF_2 receptor	B	RAMP3	HEK293 FreeStyle	SBA immunoassay	N/A	---	[28]
GHRH receptor	B	RAMP2, RAMP3	HEK293 FreeStyle	SBA immunoassay	sermorelin	Growth hormone deficiency or growth failure, prevention of HIV-induced weight loss	[28]
GIP receptor	B	RAMP1, RAMP2, RAMP3	HEK293 FreeStyle	SBA immunoassay	N/A	---	[28]
GLP-1 receptor	B	RAMP1, RAMP2, RAMP3	HEK293 FreeStyle	SBA immunoassay	exenatide, sorafenib, lixisenatide, mecaserm in rinfabate, dulaglutide, albiglutide, conivaptan, lenalidomide	Type II Diabetes	[28, 89]
GLP-2 receptor	B	RAMP1, RAMP2, RAMP3	HEK293 FreeStyle	SBA immunoassay	teduglutide	Short Bowel Syndrome	[28]
Glucagon receptor	B	RAMP2	HEK293, COS-7	Confocal microscopy, radioligand binding	glucagon recombinant, glucagon hydrochloride, oxyphenb utazone, chlordiaze poxide	Type II Diabetes	[7, 8, 25]
		RAMP1, RAMP2, RAMP3	HEK293 FreeStyle	SBA immunoassay	N/A	---	[28]
Secretin receptor	B	RAMP3	COS-7, CHO-K1	Bimolecular fluorescence complementation, BRET assay	secretin synthetic porcine, ezetimibe, pegfilgrastim	Treat High Blood Cholesterol, Lipid Abnormalities	[26]
		RAMP1, RAMP2, RAMP3	HEK293 FreeStyle	SBA immunoassay			[28]
PTH1 receptor	B	RAMP2	HEK293, COS-7	Confocal microscopy	teriparatide, abaloparatide	Osteoporosis	[25, 89]
		RAMP1, RAMP2, RAMP3	HEK293T, HEK293 FreeStyle	SBA Immunoassay, PLA			[28, 89]
PTH2 receptor	B	RAMP3	HEK293, COS-7	Confocal microscopy	teriparatide, recombin ant parathyroid hormone	Osteoporosis	[25, 89]
		RAMP1, RAMP2, RAMP3	HEK293 FreeStyle	SBA Immunoassay			[28, 89]
PAC_1 receptor	B	RAMP1, RAMP2, RAMP3	HEK293 FreeStyle	SBA Immunoassay	N/A	---	[28]
$VPAC_1$ receptor	B	RAMP1, RAMP2, RAMP3	HEK293, COS-7	Confocal microscopy	N/A	---	[25]
		RAMP2, RAMP3	HEK293 FreeStyle	SBA Immunoassay			[28]

Official IUPHAR Receptor Name	Class	RAMP	Cell Line	Identification Method	FDA Approved Drugs[b]	Clinical Indication	Ref.
$VPAC_2$ receptor	B	RAMP1, RAMP2, RAMP3	HEK293S, CHO-K1	ELISA	N/A	---	[8]
		RAMP2, RAMP3	HEK293 FreeStyle	SBA Immunoassay			[28]
CaS receptor	C	RAMP1, RAMP3	COS-7, HEK293	Confocal microscopy, Co-IP	etelcalcetide	Secondary Hyperparathyroidism	[13]
ADGRF 5	Adhesion Family	RAMP1, RAMP2, RAMP3	HEK293 FreeStyle	SBA immunoassay	N/A	---	[28]

[a] IUPHAR: The International Union of Basic and Clinical Pharmacology, RAMP: Receptor activity-modifying protein, BRET: Bioluminescence Resonance Energy Transfer, FACS: Fluorescenceactivated cell sorting, SBA: Suspension bead array, PLA: Proximity ligation assay, Co-IP: Coimmunoprecipitation.

[b] To determine whether each RAMP-interacting GPCR listed in the table had an associated FDA approved drug, each GPCR was cross referenced against the public resource DrugBank [90], a database which combines drug data with drug target information, and a recent review profiling trends in GPCR drug discovery through 2017.

[c] N/A acronym in the Drug column stands for None Approved and is meant to denote that for the given GPCR there are no currently approved therapies directed at this GPCR in DrugBank.