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Review

The G protein-coupled receptor N-terminus and receptor signalling: N-tering a new era

James L.J. Coleman *, Tony Ngo, Nicola J. Smith *

Molecular Pharmacology Group, Victor Chang Cardiac Research Institute, Darlinghurst, NSW 2010, Australia
St Vincent's Clinical School, University of New South Wales, Darlinghurst, NSW 2010, Australia

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ABSTRACT

G protein-coupled receptors (GPCRs) are a vast family of membrane-traversing proteins, essential to the ability of eukaryotic life to detect, and mount an intracellular response to, a diverse range of extracellular stimuli. GPCRs have evolved with archetypal features including an extracellular N-terminus and intracellular C-terminus that flank a transmembrane structure of seven sequential helices joined by intracellular and extracellular loops. These structural domains contribute to the ability of a GPCR to be correctly synthesised and inserted into the cell membrane, to interact with its cognate ligand(s) and to couple with signal-transducing heterotrimeric G proteins, allowing the activated receptor to selectively modulate a number of signalling cascades. Whilst well known for its importance in receptor translation and trafficking, the GPCR N-terminus is underexplored as a participant in receptor signalling. This review aims to discuss and integrate recent advances in knowledge of the vital roles of the GPCR N-terminus in receptor signalling.

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1. Introduction

G protein-coupled receptors (GPCRs) are paramount to the ability of eukaryotic organisms to detect and respond to stimuli in their extracellular environment; a property that perhaps made GPCRs integral to the evolution and diversification of multicellular eukaryotic life itself [1]. Each GPCR has evolved remarkable specificity towards biological detection of discrete stimuli including photons, ions, odorants, nucleotides, amino acids, peptides and proteins [2], association with which biases the membrane-spanning GPCR towards an active conformation, facilitating interaction with heterotrimeric G proteins which transduce the once *extracellular* signal along a number of potential *intracellular* signalling pathways [3]. Crucial to the ability of GPCRs to serve as a nexus between extracellular and intracellular signals is their membrane-traversing structure; a GPCR consists of an extracellular N-terminus, seven serial transmembrane helices joined by intracellular and extracellular loops, and an intracellular C-terminus [3]. Whilst we refer the reader to a number of comprehensive reviews of the relationship between the GPCR structural domains and receptor function [3,4], the GPCR N-terminus is an often overlooked and ever-evolving area of research, with increasing evidence that it is truly a dynamic participant

* Corresponding authors at: Molecular Pharmacology Group, Victor Chang Cardiac Research Institute, Darlinghurst, NSW 2010, Australia.
E-mail addresses: j.coleman@victorchang.edu.au (J.L.J. Coleman), n.smith@victorchang.edu.au (N.J. Smith).

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in the exquisitely regulated mechanisms of GPCR signalling. This review will briefly introduce the *traditional* paradigm of N-terminus function before exploring more recent knowledge of N-terminus-mediated GPCR signalling, including both protease-modulated and –independent processes, primarily in the context of the rhodopsin-like (class A) receptors.

2. Traditional roles of the G protein-coupled receptor N-terminus

The importance of the GPCR N-terminus is typically understood in the context of receptor trafficking, from the endoplasmic reticulum (ER) and Golgi apparatus [5], or as being involved in ligand binding for secretin-like (class B) [6] and glutamate-like (class C) GPCRs [7]. Regarding trafficking, the GPCR N-terminus can be subject to post-translational modification in the form of N-linked glycosylation occurring at the motif NxS/T, proving important for correct integration of a number of GPCRs into the cell membrane [5]. Notable examples include the β_2-adrenoceptors [8], vasoactive intestinal peptide 1 receptor [9], angiotensin II type 1 (AT_1) receptor [10], the melanin-concentrating hormone receptor 1 [11] and the melanocortin 2 receptor [12] - all displaying reduced surface expression in response to site-directed mutagenesis of N-terminal, N-linked glycosylation motifs.

Another means by which the GPCR N-terminus may influence surface expression is by possessing a hydrophobic signal peptide sequence; present on 5–10% of all GPCRs and typically essential for correct protein translation and export from the ER for these receptors [13]. GPCRs without an N-terminal signal peptide are thought to rely on uncleaved signal anchor sequences, commonly located within the first transmembrane domain, that are recognized by the signal recognition particle (SRP) following synthesis by cytosolic ribosomes, leading to a temporary arrest of translation. This ultimately mediates transfer of the ribosome and nascent protein to the ER translocon complex, where translation resumes, and the GPCR is co-translationally inserted into the ER membrane. For these receptors, the N-terminus is synthesised in the cytoplasm before the signal anchor sequence is synthesised and recognized, so must be post-translationally translocated through the ER membrane [13]. This represents a challenge for GPCRs with longer N-termini or those with a high abundance of positively charged amino acids (Lys and Arg) in their N-termini and, concordantly, GPCRs with these properties more commonly possess an N-terminal hydrophobic signal peptide, such that the N-terminus can be translocated into the ER as it is synthesised [14]. Similarly, the presence of stably folded domains in the N-terminus may prohibit post-translational translocation, hence the postulated role of the N-terminal signal peptide for the endothelin B (ET_B) receptor [15]. These signal peptides are typically cleaved off by the signal peptidases of the ER following translation, but not in all cases. The corticotropin-releasing factor receptor 2 is such an example, possessing an uncleaved signal peptide that reduces receptor expression at the plasma membrane and inhibits receptor dimerisation (as the signal peptide carries bulky N-linked glycans) [16]. In contrast, the predicted signal peptide of the α_{2C}-adrenoceptor has been reported to remain uncleaved, resulting in an increase in binding-competent receptor in the cell membrane [17].

In addition to these roles in receptor surface expression and trafficking, the N-terminus also regulates ligand-binding for many GPCRs, though primarily outside of the rhodopsin-like class. For peptide ligands of the secretin-like GPCRs, the ligand first binds to the N-terminus before interacting with the receptor's transmembrane domain, exemplified by parathyroid hormone and its cognate receptor [18]. The glutamate-like receptors show yet another distinct mode of ligand binding, as seen in the metabotropic glutamate receptors, for which ligand binding takes place solely within the N-terminus, with these receptors having so-called 'venus fly trap' domains on their extensive N-termini [19].

Despite these well characterised roles for N-termini, ongoing research is elucidating new ways in which the N-terminus can *directly* contribute to the signalling capacity of GPCRs. For the purposes of this review, GPCRs with signalling activity that can be attributed to the presence of the receptor's N-terminus (or defined regions within) have been categorised as being either protease-*modulated* or protease-*independent* and these classifications will now be explored further.

3. The N-terminus and protease-modulated GPCR signalling

Perhaps no better example of protease-modulated GPCR signalling exists than the protease activated receptors (PARs) (Table 1). PARs contain their own ligand within their N-terminus but this 'tethered ligand' is only accessible and able to activate the receptor upon N-terminal proteolysis immediately upstream of the ligand sequence [20] (Fig. 1A). The first discovered PAR was PAR1, reported in 1991 to be the receptor for the serine protease thrombin [21,22], which until that point was considered primarily as a catalyst for the formation of fibrin in blood clotting. Vu et al. [21] found that microinjection of mRNA from thrombin-responsive Dami cells conferred thrombin-induced Ca^{2+} release to *Xenopus* oocytes. Using size fractionation of mRNA and size-selected cDNA library synthesis, the thrombin receptor was subsequently cloned, with the receptor cRNA then shown to induced a 100-fold greater thrombin-induced Ca^{2+} response in *Xenopus* oocytes than the unenriched Dami cell cRNA [21]. Further characterization of this thrombin-receptor cDNA identified the encoded protein to be 425 amino acids long, with an N-terminus of approximately 75 amino acids that intriguingly contained the motif LDPRS – noted as strongly resembling the thrombin-cleavage site within zymogen protein C (LDPRI) and suggesting thrombin can cleave this newly identified receptor between Arg41 and Ser42. Indeed, mutation of Arg41 to alanine abolished receptor-induced Ca^{2+} release in *Xenopus* oocytes, yet elegantly, signalling was restored to wild-type levels following addition of an exogenous peptide mimicking the region of the N-terminus distal to the hypothesised cleavage site (SFLLRNPNKDYEPF) [21]. It was shown soon after that only the first seven of these residues are necessary for the agonist activity of the peptide [23].

Following on from the discovery of a novel N-terminal-dependent mechanism of GPCR activation, several more protease-activated receptors were discovered. Trypsin was shown to cause activation of a receptor identified in a mouse genomic library as having 28% amino acid similarity to mouse PAR1, subsequently named PAR2, with a trypsin-cleavage site identified on the N-terminus between Arg34 and Ser35 [24]. A similar Ca^{2+} efflux assay was used in *Xenopus* oocytes to show that cRNA encoding a PAR2 receptor with a mutation to interrupt the putative trypsin cleavage site was functionally silent, yet addition of a peptide corresponding to the six amino acids distal to the site (SLIGRL) restored signalling [24]. Discovery of PAR3 [25] and PAR4 [26] followed suit, both being substrates for N-terminal proteolysis by thrombin, similar to PAR1. Whilst discussed in detail elsewhere [20,27], thrombin and trypsin are not the only enzymes that may activate the PARs, with a plethora of other proteases shown to cleave PARs at their canonical sites for activation. Of these other proteases, metalloproteases (including the matrix metalloproteases [MMPs] and a disintegrin and metalloproteases [ADAMs]) warrant specific mention, and their role in the regulation of a number of other GPCRs will be discussed later in this review. Importantly, the discovery of the PARs challenged traditional paradigms of GPCR signalling and demonstrated new roles for proteases in physiology.

Whilst PARs become activated following protease-mediated unveiling of a tethered agonist on the GPCR N-terminus, the thyroid-stimulating hormone (TSH) receptor exists as an example of a GPCR that may be activated by protease-mediated removal of an inhibitory N-terminal domain, *without* implicating a tethered agonist. The TSH receptor consists of two subunits [28], shown to result from a metalloprotease-mediated cleavage between the membrane-spanning domain and the large N-terminal extracellular domain of the receptor, which occurs at the cell surface [29]. The propensity of an HA-tagged