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From cradle to twilight: The carboxyl terminus directs the fate of the A_{2A}-adenosine receptor☆

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ABSTRACT

The extended carboxyl terminus of the A_{2A}-adenosine receptor is known to engage several proteins other than those canonically involved in signalling by GPCRs (i.e., G proteins, G protein-coupled receptor kinases/GRKs, arrestins). The list includes the deubiquinating enzyme USP4, α-actinin, the guanine nucleotide exchange factor for ARF6 ARNO, translin-X-associated protein, calmodulin, the neuronal calcium binding protein NECAB2 and the synapse associated protein SAP102. However, if the fate of the A_{2A}-receptor is taken into account — from its birthplace in the endoplasmic reticulum to its presumed site of disposal in the lysosome, it is evident that many more proteins must interact with the A_{2A}-adenosine receptor. There are several arguments that support the conjecture that these interactions will preferentially occur with the carboxyl terminus of the A_{2A}-adeonsine receptor: (i) the extended carboxyl terminus (of 122 residues=) offers the required space to accommodate companions; (ii) analogies can be drawn with other receptors, which engage several of these binding partners with their C-termini. This approach allows for defining the nature of the unknown territory. As an example, we posit a chaperone/coat protein complex-II (COPII) exchange model that must occur on the carboxyl terminus of the receptor. This model accounts for the observation that a minimum size of the C-terminus is required for correct folding of the receptor. It also precludes premature recruitment of the COPII-coat to a partially folded receptor. This article is part of a Special Issue entitled: "Adenosine Receptors".

1. Introduction

1.1. Accessory proteins/GPCR interacting proteins

G protein-coupled receptors (GPCRs) were originally defined by their interaction with their eponymous signalling partner, the heterotrimeric G proteins; they were later appreciated to share a common structural backbone, the heptahelical arrangement of their transmembrane segments. Desensitization of GPCRs was also appreciated to occur via a conserved mechanism, namely the recruitment of GRKs (G protein-coupled receptor kinases) that phosphorylated the agonist-bound receptor and arrestins that trapped the phosphorylated receptors and thus precluded access of the cognate G protein(s). In addition, the non-visual arrestins (arrestin-2 and -3=β-arrestin1- and -2) serve as adapters for recruiting the clathrin coat components and to recruit additional signaling molecules; this allows the internalized receptor to trigger a second wave of signals that are G protein-independent [1]. While these common properties have been clarified over the past three decades, it has also been appreciated that individual receptors differ widely in the range of physiological responses that they elicit although they engage similar or identical signaling components (*i.e.*, heterotrimeric G protein(s), GRKs and arrestins).

From a structural perspective, this observation is not surprising: there is a large variation in the primary sequence of GPCRs; conserved elements can be found in the hydrophobic core and in the intracellular loops. However, it is worth noting that even the "business ends" are remarkably divergent: the G protein interaction site is formed by the intracellular loops and the segment adjacent to the last transmembrane helix (termed helix 8, because it runs as short helix perpendicular to the helices of the hydropohobic core). Sequence gazing fails to unequivocally predict which heterotrimeric G protein(s) a given receptor couples to. In addition, GPCRs differ widely in the presence or absence of additional structural elements. Based on these they can be arranged into 5 groups termed GRAFS for glutamate, rhodopsin, adhesion, frizzled, and secretin (based on prototypical representatives of individual families; see [2]. The phylogenetic assignment of a common GPCR ancestor also proves useful when tested on an evolutionary scale in species ranging from plants and fungi to mammals [3]. Large differences exist in the N- and C-termini of GPCR classes. The N-termini are extracellular and may act as additional ligand docking site. The C-termini are of particular interest, because–in many instances–they afford the association of GPCRs with a variety of additional proteins that are neither heterotrimeric G proteins, GRKs or arrestins. For lack of a better term, these proteins are referred to as accessory proteins or

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GPCR-interacting proteins (GIPs); GIPs are very diverse; they range from small soluble signalling proteins (e.g., calmodulin) to peripheral membrane proteins like the PDZ-containing scaffolds (e.g. PICK1 — protein interacting with C-kinase) and transmembrane proteins (e.g., RAMP1-3, receptor activity modifying proteins 1-3, other GPCRs) [4].

1.2. Docking to the C-terminus of the A_{2A}-receptor

This review focuses on the A_{2A}-adenosine receptor, which has many unusual properties: it is a member of the rhodopsin class of GPCR's like its closest relatives, the other three adenosine receptor — the G_i/G_o-coupled A_1- and A_3-adenosine receptors and the G_s/G_q-coupled A_{2B}-adenosine receptors [5]. In contrast to the other three adenosine receptors and in contrast to many other GPCRs of the rhodopsin family, the A_{2A}-receptor has an unusually long intracellular carboxyl terminus: 122 amino acids in the human A_{2A}-receptor versus 40, 38 and 34 amino acids of the A_{2B}-receptor and of the A_{1}- and A_{3}-receptors, respectively (Fig. 1). The C-terminus of the A_{2A}-receptor also lacks the canonical cysteine at the end of helix 8, i.e., the segment adjacent to transmembrane helix 7. One or two cysteines are present at this position in virtually all other rhodopsin-like GPCRs. The cysteine residue(s) represent(s) a (putative) palmitoylation site (Fig. 1). Palmitoylation is known to mediate protein–protein interactions and to anchor peripheral membrane proteins or protein segments at the membrane [6]. The C-terminus of the A_{2A} receptor may be more flexible because it is not constrained by a lipid anchor. These two features, relative length and lateral flexibility, may combine to afford the interaction of the A_{2A} receptor with many additional accessory proteins, other than G proteins, G protein receptor kinases (GRKs) or β-arrestins. The X-ray crystal structure of the A_{2A}-receptor [7] visualizes helix 8 as a folded structure up to leucine^{308}. It is not possible to infer any additional information about the C-terminus because residues beyond alanine^{317} were eliminated by truncation. In the crystal, helix 8 is stabilized by interactions with transmembrane helix 1. The juxtamembrane segment (helix 8 plus a few residues up to position 311) is required for proper folding of the receptor. The rest of the C-terminus (~100 amino acids) is dispensable for ligand binding [7,8] and for G protein coupling [9].

It is not clear, how the conformational change induced by binding of agonists and antagonists is relayed to the carboxyl terminus. Structural information is only available for the antagonist-bound form of the A_{2A}-adenosine receptor [7]. The best studied receptor is rhodopsin: there is little evidence that helix 8 unwinds or changes its conformation in response to activation. In fact, the crystal structure of an active (=retinal-free) opsin does not indicate any rearrangement within helix-8 [10]. In fact, a C-terminal peptide of $G\alpha_t$ (the α-subunit of transducin) is accommodated by a cavity between the transmembrane helices while helix-8 is displaced by rigid body motion [11]. Similarly, distance measurements by electron paramagnetic resonance also indicate that helix 8 is maintained in light activated rhodopsin in solution [12,13]. Beyond helix 8, the carboxyl terminus of rhodopsin does not appear to contain any ordered structure. Regardless of whether it is examined in the inactive dark state or in the light-activated metarhodopsin-II form, the carboxyl terminus is highly flexible and mobile and reminiscent of an unfolded peptide [14]. Unfolded protein segments are intuitively unappealing. However, they are much more common than generally appreciated [15,16]. Assuming that a large portion of the carboxyl terminus of the A_{2A}-receptor is also devoid of a rigid structure, the following mechanism can be invoked to account for transmitting information on the activity state of the receptor to the carboxyl terminus: the high flexibility of the carboxyl terminus allows the A_{2A}-receptor to fold back and interact with the loops connecting the hydrophobic transmembrane helices. By these transient interactions segments of the carboxyl terminus may therefore sample the receptor conformation. There is little experimental evidence to support this conjecture for the A_{2A}-receptor other that serial truncation reduces cell surface expression of the receptor [7–9]. However, this issue has been investigated more systematically with the A_1-adenosine receptor: truncation of the carboxyl terminus causes a folding problem, i.e., the receptor is retained in the endoplasmic reticulum in an inactive, binding incompetent state [17]. This deficiency can be remedied by receptor antagonist [18] (see also below). At the very least, this provides circumstantial evidence for a connection between the hydrophobic core, where the ligand binding site resides and the carboxyl terminus.

There are at least six accessory proteins that have been documented to bind to the C-terminus [19–21] and the list is growing. Examples are (i) the ubiquitin-specific protease 4 (USP4) [22], (ii) a guanine nucleotide exchange factor of the small monomeric G proteins of the ADP-ribosylation factor (ARF) family (ARNO/cytohesin-2) [23,24], (iii) translin-X-associated protein [25], (iv) α-actinin [26]. We have identified several additional candidate proteins (e.g., Neuroendocrine-Disks large homolog 3/Synapse-associated protein 102 (NE-DLG/ SAP102); 14.3.3 protein-θ/τ and astrin (unpublished observations). Although the C-terminus may exist in an extended conformation and thus provide a lot of

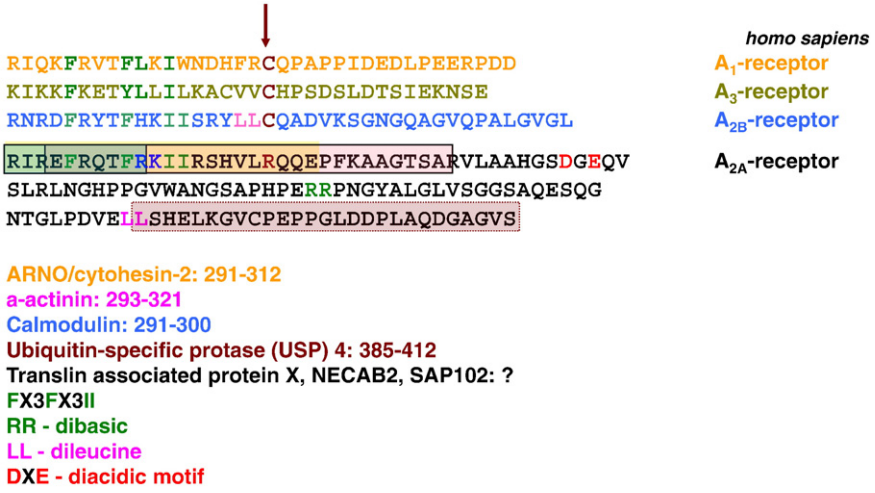

Fig. 1. The C-terminus of the human A_{2A}-receptor compared to the C-termini of the three other G protein coupled adenosine receptors. The carboxyl terminus of the A_{2A}-adenosine receptor (bottom, 122 amino acids) is much longer than that of the human A_1-receptor (36 amino acids), A_3-receptor (34 amino acids) and A_{2B}-receptors (40 amino acids). The first amino acid is the R/K at the end of helix 7, *i.e.*: R^{291} in the human A_1-receptor, K^{285} in the human A_3-receptor, R^{293} in the A_{2B}-receptor and R^{291} in the A^{2A}-receptor. The phenylalanine/leucine/isoleucine (FXXXFXXXLL)-related related motifs are highlighted in all receptors. The arrow marks the position of the conserved cysteine, which is the site of palmitoylation and which is absent in the A_{2A}-receptor. The significance of the dileucine motif is not clear, it is absent in several species orthologs (e.g., rat and cattle). Similarly, the diacidic motif is present in mammals but is, for instance absent in the two A_{2A}-receptors of the zebra fish. The known binding sites for A_{2A}-receptor interacting proteins are marked by colour code in the C-terminus of the A_{2A}-receptor.