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REVIEW

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The A_{2A}-adenosine receptor: a GPCR with unique features?

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The A_{2A}-adenosine receptor is a prototypical G_s-coupled receptor. However, the A_{2A}-receptor has several structural and functional characteristics that make it unique. In contrast to the classical model of collision coupling described for the β-adrenergic receptors, the A_{2A}-receptor couples to adenylyl cyclase by restricted collision coupling and forms a tight complex with G_s. The mechanistic basis for this is not clear; restricted collision coupling may arise from the interaction of the receptor with additional proteins or due to the fact that G protein-coupling is confined to specialized membrane microdomains. The A_{2A}-receptor has a long C-terminus (of >120 residues), which is for the most part dispensable for coupling to G_s. It was originally viewed as the docking site for kinases and the β-arrestin family to initiate receptor desensitization and endocytosis. The A_{2A}-receptor is, however, fairly resistant to agonist-induced internalization. Recently, the C-terminus has also been appreciated as a binding site for several additional 'accessory' proteins. Established interaction partners include α-actinin, ARNO, USP4 and translin-associated protein-X. In addition, the A_{2A}-receptor has also been reported to form a heteromeric complex with the D_2-dopamine receptor and the metabotropic glutamate receptor-5. It is clear that (i) this list cannot be exhaustive and (ii) that all these proteins cannot bind simultaneously to the receptor. There must be rules of engagement, which allow the receptor to elicit different biological responses, which depend on the cellular context and the nature of the concomitant signal(s). Thus, the receptor may function as a coincidence detector and a signal integrator.
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Abbreviations: ARF, ADP-ribosylation factor; ARNO, ARF-nucleotide binding site opener; FRET, fluorescence/Foerster resonance energy transfer; GRK, G-protein-coupled receptor kinase; GPCR, G-protein-coupled receptor; MAP kinase, mitogen-activated protein kinase; mGluR5, metabotropic glutamate receptor 5; USP4, ubiquitin-specific protease 4

Adenosine receptor classification

G-protein-coupled receptors (GPCRs) for adenosine, which either inhibited (A_1) or stimulated (A_2) cAMP accumulation, were first identified in the 1970s (van Calker *et al*., 1979) and differentiated—by structure–activity relationship and other pharmacological criteria—from the direct action of adenosine at the catalytic centre of AC, the so-called P-site (Londos *et al*., 1980). The further subclassification of A_2-adenosine receptors was introduced later to account for the receptor that required low concentrations of adenosine and adenosine analogues for the stimulation of AC; this was termed the A_{2A} receptor (Bruns *et al*., 1986). The A_{2A} receptor has one additional close relative the A_{2B} receptor, which also signals via G_s, and two more distant relatives termed A_1- and A_3-adenosine receptors, which interact with pertussis-toxin-sensitive G proteins of the G_i and G_o family. These receptors are encoded by distinct genes and are classified based on their affinities for adenosine analogues and methylxanthine antagonists (Klinger *et al*., 2002a).

Interest in the A_{2A} receptor has been rekindled by findings that suggest that both agonism at this receptor and its inhibition by specific antagonists may be of therapeutic relevance. Examples are highlighted in the accompanying reviews (Brown, 2008; Sitkovsky, 2008). The A_{2A}-adenosine receptor, however, is also of interest, because it has many features that do not readily fit into the accepted paradigms of GPCR signalling and desensitization; in addition, the receptor binds many proteins other than G_s. This review will focus on these three points.

Precoupling, tight coupling and restricted collision coupling

In contrast to β-adrenergic receptors (or rhodopsin), which engage their signalling cascade by collision coupling, the A_{2A} receptor has long been known to couple to AC by restricted

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collision coupling. Restricted collision coupling refers to the inability of the receptor to access all G_s molecules and thus activate the full complement of all AC moieties in the cell membrane. Originally, these observations were made with the avian A_2 receptor in turkey erythrocytes (Tolkovsky and Levitzki, 1978; Braun and Levitzki, 1979). Restricted collision coupling, however, was also observed in human platelets, which express A_{2A} receptors (Gross and Lohse, 1991; Lohse *et al.*, 1991). The A_{2A} receptor forms a tight complex with G_s; this complex is resistant to dissociation by guanine nucleotides. In addition, the receptor can be solubilized in a complex with G_s in the absence of agonist (Nanoff *et al.*, 1991; Nanoff and Stiles, 1993). Thus, the A_{2A} receptor appears to be precoupled to G_s. Precoupling, that is, formation of a stable complex between receptor and G protein, by definition, gives rise to restricted collision coupling.

It is also worth noting that, when measured directly by fluorescence/Foerster resonance energy transfer (FRET), the kinetics of G protein activation do not differ substantially between β_1-adrenergic receptor and A_{2A}-adenosine receptor (Hein *et al.*, 2006). These experiments, however, cannot decide on the existence or absence of precoupling. First, in the ground state (that is, in the absence of agonist), A_{2A} receptor and G_s may be in a complex that does not support efficient FRET. Agonist binding reorients both receptor and G_s into a tightly packed complex, in which energy transfer is supported. Second, when compared to the time constants required for G protein activation (and formation of the high-affinity complex of agonist, receptor and G protein), diffusion of the receptor does not appear to be rate limiting; ternary complexes formed by fusion proteins of receptor and α-subunits of G protein are kinetically indistinguishable from those formed by the individual components (Waldhoer *et al.*, 1999). Although precoupling of the A_{2A} receptor to G_s may provide a mechanistic basis for restricted collision coupling, it remains unclear as to why the A_{2A} receptor would form a tight complex with G_s. Earlier reconstitution experiments did not suggest that the A_{2A} receptor (Nanoff *et al.*, 1994) had a substantially higher affinity for G_s than the β_1- or β_2-adrenergic receptors (Freissmuth *et al.*, 1991). The formation of a tight receptor/G_s complex and the restricted mobility of the A_{2A} receptor may, however, arise from the interaction with additional proteins (see below); the receptor may, for instance, be concentrated in membrane subdomains, because it is tethered to the actin cytoskeleton via its binding partner α-actinin (Burgueño *et al.*, 2003). Alternatively, the interaction between A_{2A}-adenosine receptor and G_s may be limited to membrane microdomains ('lipid rafts'). We have examined these conjectures and have observed that the actin cytoskeleton did not account for the restricted mobility of the A_{2A} receptor but that coupling of the A_{2A} receptor to G_s was contingent on the presence of cholesterol. In contrast, activation of an alternative signalling pathway (namely ARNO (ARF-nucleotide binding site opener), the guanine nucleotide exchange factor for ADP-ribosylation factor (ARF)6; Gsandtner *et al.*, 2005) was not impaired by removal of cholesterol (Charalambous *et al.*, manuscript submitted).

The other issue is why should have receptors evolved to differ in their coupling modes, that is, collision coupling versus restricted collision coupling. This question could simply be considered an academic preoccupation of little relevance. However, it is evident that collision coupling and restricted collision coupling confer different outcomes to adaptive changes; if a receptor operates by collision coupling, increasing the number of receptors primarily augments the sensitivity of the system. In other words, the concentration–response curve for agonists is shifted to the left, because all receptors have unrestricted access to all G proteins. In restricted collision coupling, where the receptor is allocated its particular lot of G proteins, there are no spare receptors. Changes in receptor number, therefore, translate in changes of the maximum response E_{max} rather than in EC_{50} values. Actually, this is precisely the finding that was obtained when the number of A_{2A} receptors was decreased by inactivation (Lohse *et al.*, 1991) or raised by facilitating its export from the endoplasmic reticulum (Milojevic *et al.*, 2006).

Desensitization and internalization of the A_{2A} receptor

For GPCRs, desensitization and receptor trafficking is important for regulation of the temporal and spatial aspects of their signalling properties. Both desensitization and internalization function to control signal termination and rely on the intracellular binding of GPCR kinases (GRKs) and arrestins. The basic mechanisms underlying desensitization of GPCRs is understood in considerable detail. The major insights have originally been obtained with two model GPCRs, rhodopsin and the β_2-adrenergic receptor; the agonist stabilizes the active conformation of the receptor, which is recognized by GRKs (GRK2–6, with GRK1 being the specialized rhodopsin kinase). Phosphorylation by GRKs of clustered serine/threonine residues creates docking sites that allow the recruitment of arrestins. The general arrestins, arrestin 2 and arrestin 3, are also referred to as β-arrestin 1 and β-arrestin 2, whereas cone and rod arrestins are confined to the visual system. Binding of arrestin 2 and arrestin 3 via its N-terminal phosphate sensor triggers a structural rearrangement that exposes their C terminus, which recruits components of the clathrin coat. Accordingly, binding of arrestins favours endocytosis of receptors via the clathrin-dependent pathway (reviewed by Gurevich and Gurevich, 2006). It is a matter of debate whether the A_{2A} receptor undergoes a desensitization/resensitization cycle under physiological conditions; short-term desensitization can be observed in various cell culture models (reviewed by Olah and Stiles, 2000). The structural requirements of the receptor that may be necessary for the rapid desensitization have been examined in receptor mutagenesis studies in chinese hamster ovary cells (Palmer and Stiles, 1997). The removal of 95 amino acids, including 10 potential phosphorylation sites, from the cytoplasmic tail of the A_{2A} receptor had no effect on the ability of the receptor to undergo rapid desensitization. Although a simultaneous mutation of Thr298 and Ser305 to Ala residues attenuated the desensitization observed in response to short-term agonist treatment, it did not block the ability to desensitize after long-term agonist exposure. Analysis of the individual mutation of

these residues revealed that mutation of Thr298 alone was sufficient to diminish both short-term desensitization and agonist-stimulated receptor phosphorylation. This is remarkable because efficient recruitment of arrestins typically requires a cluster of phosphates, which interact with the N-terminal phosphate sensor and thereby trigger the structural rearrangement that leads to the tight interaction between arrestin and receptor (Gurevich and Gurevich, 2006). This issue may be explained by the following two alternative scenarios: (i) negatively charged amino acids in the vicinity of Thr298 may substitute for phosphoserines/phosphothreonines and thus obviate the requirement for multiple phosphorylated residues for arrestin recruitment or (ii) phosphorylation of Thr298 may induce a conformational change, which, *per se*, suffices to disrupt G protein coupling; in this scenario, recruitment of arrestin would be dispensable. A precedent for this latter model is provided by the observation that PK-A-mediated desensitization of the β_2-adrenergic receptor is apparently not dependent on arrestin recruitment (Vaughan *et al.*, 2006).

Manipulations in the level of GRK2 affect the extent of desensitization of the A_{2A} receptor (Mundell *et al.*, 1997, 1998). However, the relevance of these observations has been questioned for the following reasons: (i) adenosine appears to signal tonically via the A_{2A} receptor; *in vivo*, many actions of adenosine are accounted for by the retaliatory metabolite concept that hypoxia and tissue damage lead to the accumulation and release of adenosine; adenosine acts via its receptors to orchestrate a concerted protective response, where the A_{2A} receptor plays a prominent role (Linden, 2005). From a teleological perspective, rapid desensitization is of questionable value in a situation where sustained signalling is vital to support long-term tissue repair. (ii) In most instances, it has been difficult to visualize internalization *in vivo* (Fredholm *et al.*, 2001). A representative experiment is shown in Figure 1, where the yellow fluorescent protein-labelled A_{2A} receptor was visualized together with cyan fluorescent protein-tagged Rab5 (a marker for early endocytotic vesicles). Even continuous administration of agonist did not result in any appreciable increase in basal A_{2A} receptor endocytosis. There are, however, reports that suggest that rapid internalization upon agonist binding can occur via an interaction with the actin-binding protein α-actinin (Burgueño *et al.*, 2003). α-Actinin is a component of the actin cytoskeleton that plays a central role by directly crosslinking actin molecules; so the presence of a complex involving A_{2A} receptor and α-actinin suggests that α-actinin may mediate receptor association with the actin cytoskeleton. The precise regulatory role of this interaction remains to be established.

Sustained G-protein-independent signalling via ARNO

The action of GRKs on agonist-liganded receptors and the subsequent binding of arrestins to the receptors are necessary to initiate desensitization and a second wave of long-term signalling events, the persistent stimulation of mitogen-activated protein kinase (MAP kinase) being a particularly well-studied example (Lefkowitz and Shenoy, 2005). The A_{2A}-adenosine receptor was first shown to cause a G_s-independent sustained stimulation of MAP kinase in human macrovascular endothelial cells (Sexl *et al.*, 1997), which is presumably related to its capacity to support endothelial cell proliferation (Sexl *et al.*, 1995). It was later appreciated that depending on the cell type studied, there was a G_s- and cAMP-dependent mechanism that supported MAP kinase activation, for example, in PC12 cells (Seidel *et al.*, 1999) and that required src-like kinases (Klinger *et al.*, 2002b), as well as a G_s-independent pathway that required $p21^{ras}$ (Seidel *et al.*, 1999). Heterotrimeric G proteins were, in fact, dispensable for the latter signal, as was dynamin-dependent internalization (Klinger *et al.*, 2002c). Clearly, based on these findings, it is safe to conclude that the A_{2A} receptor does not conform to the paradigm established for the β_2-adrenergic receptor. More recently, the G_s-independent signalling pathway was found to comprise ARNO and ARF6 (schematically represented in Figure 2); ARNO/cytohesin-2 was identified as an interactor that bound to the C terminus of the A_{2A} receptor in an yeast two-hybrid screen (Gsandtner *et al.*, 2005).

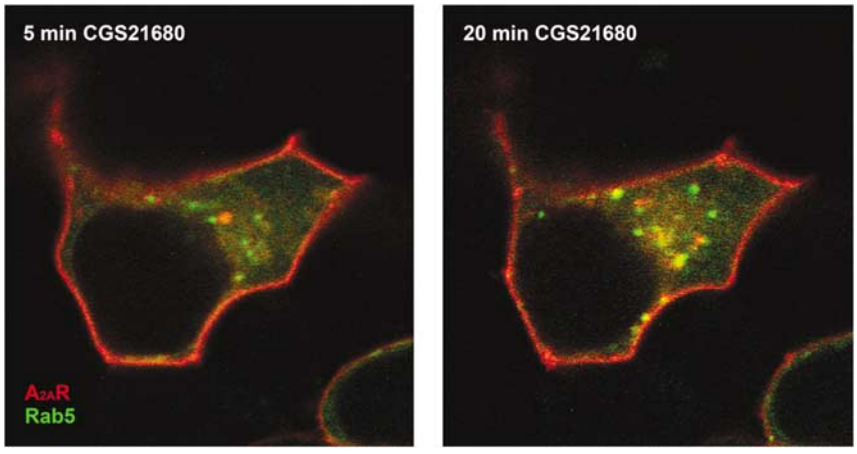

Figure 1 Human embryonic kidney cells were transiently transfected with plasmids coding for the fluorescence-tagged versions of the A_{2A} receptor and Rab5. Cells were then stimulated with the A_{2A}-specific agonist CGS2168. Images were taken at the indicated times using a confocal laser microscope. For better visual distinction in the overlay, fluorescent A_{2A} receptors were converted to red and Rab5 to green.

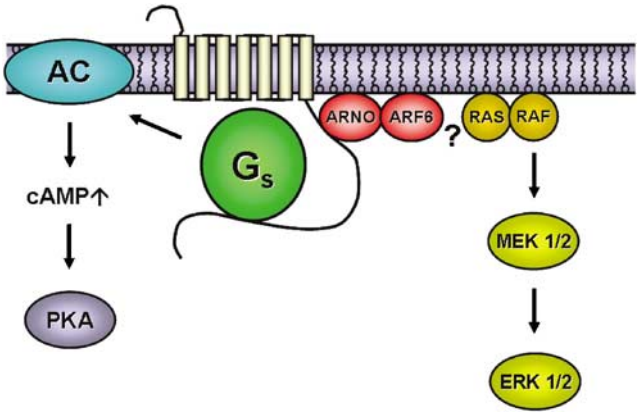

Figure 2 Schematic illustration of the signalling pathways of the A_{2A}-adenosine receptor. The link between ARF6 and Ras remains to be elucidated.

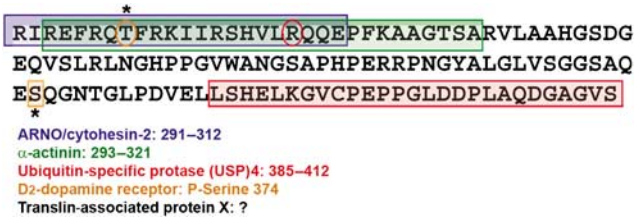

Figure 3 Scheme of interaction sites on the C terminus of the A_{2A}-adenosine receptor. Binding sites for α-actinin, ARNO, the D_2 receptor and USP4 have been tentatively assigned in Burgueño *et al.* (2003), Gsandtner *et al.* (2005), Woods and Ferré (2005) and Milojevic *et al.* (2006), respectively. The orange circle identifies a threonine important for short-term desensitization (Palmer and Stiles, 1997). The red circle depicts the arginine unique to the A_{2A} receptor. All other adenosine receptors have a cysteine in this position, which in most GPCRs is palmitoylated and thus stabilizes helix 8.

ARNO contains a sec7 domain, which acts as the guanine nucleotide exchange factor for ARF-family members. These small G proteins have been recently recognized to play a prominent role in endocytosis of GPCRs; many but not all receptors require ARF6 (originally identified as the cofactor necessary for cholera-toxin-mediated ADP-ribosylation of $G\alpha_s$) for internalization (Lahuna *et al.*, 2005; Houndolo *et al.*, 2005). ARF6 differs from the other members of the ARF family because it is primarily found at the cell membrane, where it regulates endocytosis through both the clathrin/dynamin-dependent and a less well-understood clathrin/caveolae-independent pathway. Two mechanistically conflicting models have been proposed, both involving the exchange factor ARNO. During internalization of the β_2-adrenergic receptor, arrestin binding precedes that of ARNO; in fact, ARNO is recruited by arrestin (Claing *et al.*, 2001; Lefkowitz and Shenoy, 2005). In contrast, for the luteinizing hormone/human chorionic gonadotropin receptor, arrestin binding is contingent on prior stimulation of ARNO, the ensuing activation of ARF6 required for the release of membrane-associated arrestin (Hunzicker-Dunn *et al.*, 2002). The A_{2A} receptor differs from these two paradigmatic examples because it binds ARNO/cytohesin-2 directly (Gsandtner *et al.*, 2005). Truncation experiments showed that the proximal 22 amino acids of the C terminus are sufficient for the interaction (highlighted in Figure 3). The classical pathway, activation of AC, was not altered by ARNO, and this was also true for agonist-induced desensitization. In contrast, expression of dominant-negative ARNO and dominant-negative ARF6 abrogated the sustained phase of MAP-kinase stimulation induced by the A_{2A} receptor. Thus, the binding of ARNO to the proximal portion of the C terminus provides one of the missing links to support the alternative—heterotrimeric G-protein-independent—signalling pathway of A_{2A} receptor.

Accessory proteins

In the last 15 years, it has been appreciated that GPCRs bind many additional proteins; these are being referred to as 'accessory proteins' for lack of a better description. The list includes proteins involved in selective retention in specialized membrane compartments (for example, dendritic spines) or components of signalling cascades (for example, PKs of the Src and Jak family, adapter proteins like Grb2 and Shc, and enzymes generating second messengers such as PLCβ1 and eNOS; for a detailed list, see Bockaert *et al.*, 2004). The A_{2A}-adenosine receptor can also claim a respectable list of accessory proteins (Gsandtner and Freissmuth, 2006) that bind to various portions of its C terminus (Figure 3). In addition, A_{2A} receptors, like other GPCRs, can form a heteromeric complex with at least one GPCR, namely, the D_2-dopamine receptor (see below), and it can transactivate the neurotrophin receptors TrkA and TrkB (Lee and Chao, 2001). The term transactivation of TK has been coined by analogy to its original use in the description of gene regulation: GPCRs can act in *trans*, that is, recruit signalling components by activation of TK receptors in the absence of cognate ligands of the latter (in this instance, in the absence of nerve growth factor and related neurotrophins). The molecular basis of transactivation is poorly understood, and it is specifically not clear whether the A_{2A} receptor forms a heteromeric complex with TrkA and/or TrkB.

Interaction of the A_{2A}-adenosine receptor with other GPCRs (D_2-dopamine receptor, mGluR5)

The first indirect evidence for a membrane-delimited interaction between the A_{2A} receptor and the D_2-dopamine receptor came from binding experiments that suggested that activation of the A_{2A} receptor interfered with coupling of the D_2-dopamine receptor to its cognate G proteins, presumably a mixture of G_i and G_o isoforms (Ferré *et al.*, 1991). Confocal laser microscopy showed that A_{2A} receptors and D_2-dopamine receptors colocalized to a large extent in the cell membranes of stably transfected neuroblastoma cells and in cultured striatal neurons; in addition, the existence of heteromeric complexes between these two receptors was confirmed in co-immunoprecipitation experiments (Hillion *et al.*, 2002). Evidence for a direct and specific interaction between A_{2A} and D_2 receptors was also obtained with a

quantitative bioluminescence resonance energy transfer analysis and sensitized emission FRET as well as acceptor photobleaching FRET analysis (Canals *et al.*, 2003; Kamiya *et al.*, 2003). This phenomenon may therefore constitute the molecular basis of the A_{2A}/D_2 receptor interactions, which give rise to mutual antagonism *in vitro* and *in vivo* (Fuxe *et al.*, 2005, 2007). These findings led to the idea that blockade of the A_{2A} receptor could be of value in the treatment of Parkinson's disease. In fact, istradefylline, a selective A_{2A} receptor antagonist has shown (modest) efficacy in phase II trials (Hauser *et al.*, 2003), which were considered promising enough to justify phase III clinical trials (Schapira *et al.*, 2006); related selective antagonists are also being tested (Schapira *et al.*, 2006). The metabotropic glutamate receptor 5 (mGluR5) has also been suggested to exist in a complex with the A_{2A} receptor in the striatopallidal neurons (Ferré *et al.*, 2002). This is thought to explain the complex interaction of mGluR5 and A_{2A} receptor ligands; the beneficial action of an mGluR5 antagonist is enhanced by blockage of A_{2A} receptor, but it is nevertheless contingent on the presence of A_{2A} receptors (and D_2 receptors) (Kachroo *et al.*, 2005). It has to be stressed that the interaction of D_2 and A_{2A} receptors does not uniformly result in mutual antagonism. Under appropriate conditions, the two receptors can also signal synergistically (Kudlacek *et al.*, 2003), and this has been proposed to be important for enhanced rewarding action of adenosine in the nucleus accumbens (Yao *et al.*, 2002). Finally, heterodimerization may affect the kinetics of desensitization; this has, for instance, been documented for the trafficking pattern of V_{1a}- and V_2-vasopressin receptors (Terrillon *et al.*, 2004). In fact, the A_{2A} receptor has been shown to co-internalize with the D_2-dopamine receptor (Hillion *et al.*, 2002), suggesting that the D_2-dopamine receptor confers a classical GRK/arrestin-dependent internalization mechanism to the A_{2A}-adenosine receptor.

Interactors that bind to the C terminus of the A_{2A} receptor

In contrast to many other GPCRs and, in particular, to the other members of the adenosine receptor family, the A_{2A} receptor has an unusually long intracellular C-terminal tail (122 amino acids in man compared with only 34 residues in the C terminus of the A_1-adenosine receptor). The juxtamembrane segment immediately adjacent to the seventh transmembrane helix is required for proper folding of the receptor. The rest of the C terminus (100 amino acids) is dispensable for ligand binding (Piersen *et al.*, 1994) and for G protein coupling (Klinger *et al.*, 2002c). The vast majority of group I (rhodopsin-like) GPCRs carry one (or two) palmitoylated cysteine(s) within the proximal portion of their C terminus (typically, approximately 20 residues away from the end of the seventh transmembrane helix). The palmitate thioester is thought to act as an additional anchor. This stabilizes the proximal segment in an α-helical conformation (referred to as helix 8 in the rhodopsin structure, which is oriented in a manner parallel to the membrane and perpendicular to helix 7). The A_{2A} receptor does not have any cysteine residue in the proximal segment; there is only a single cysteine in position 394 in the human receptor and this is absent in other species orthologues (for example, of rat and mouse). Instead of the canonical cysteine, the A_{2A} receptor carries an arginine residue in position 309 (circled in Figure 3). Thus, one is tempted to speculate that the C terminus of the A_{2A} receptor is more flexible because it is not constrained by a lipid anchor. These two features, relative length and flexibility, may combine to afford the interaction of the A_{2A} receptor with many additional proteins, other than G protein arrestins and kinases, that is, the so-called 'accessory' proteins.

In recent years, several interaction partners were identified in yeast two-hybrid interaction screens using the C terminus of the A_{2A}-adenosine receptor as a bait and the list is growing rapidly. Figure 3 gives an overview of accessory proteins that have been found to bind to the A_{2A} receptor. Although the C terminus may exist in an extended conformation and thus provide a lot of room for interaction, the size of the individual binding partners makes it unlikely that the C terminus can accommodate several partners simultaneously. This is highlighted in Figure 3 by the overlapping binding sites for α-actinin (Burgueño *et al.*, 2003) and ARNO (Gsandtner *et al.*, 2005). Thus, individual interactions are likely to be only transient. The nature of the regulatory inputs that promote and terminate these interactions is unknown, but Thr298 (highlighted by an orange circle in Figure 3) is strategically placed to regulate binding of ARNO and α-actinin. It is therefore conceivable that the interaction of the receptor with ARNO and/or α-actinin is regulated by phosphorylation of Thr298. Similarly, the hetero-oligomer between D_2 and A_{2A} receptors has been proposed to be stabilized by binding of the phosphorylated Ser374 (boxed in orange Figure 3) to an arginine-rich segment in the third intracellular loop of the D_2 receptor (Woods and Ferré, 2005). However, the authors used only synthetic peptides to show the stability of the arginine–phosphate electrostatic interaction. It remains to be demonstrated that the phosphorylation of Ser374 of the A_{2A} receptor occurs *in vivo* and that it is a prerequisite for complex formation between A_{2A} and D_2 receptors.

Finally, it is also conceivable that ubiquitin-specific protease (USP)4 may also participate in regulating interactions. The binding site of deubiquitinating enzyme USP4 on the A_{2A} receptor has been located within the last 50 amino acids of the C terminus and USP4 is required for export of the receptor from the endoplasmic reticulum (Milojevic *et al.*, 2006). It is, however, conceivable that USP4 may also regulate later events in the trafficking of the receptor. The endocytotic routing of GPCRs has been known to depend on the extent of their ubiquitination (Wojcikiewicz, 2004; Lefkowitz and Shenoy, 2005). Extensive ubiquitination favours trafficking of endosomes to late endosomes and eventual lysosomal degradation over recycling (Marchese and Benovic, 2001; Shenoy *et al.*, 2001). It is evident that for recycling receptors both phosphorylation and ubiquitination must be reversed. Dephosphorylation is thought to be accomplished in the endosome by protein phosphatase 2A. Very little is known about the enzymes responsible for deubiquitinating (Millard and Wood, 2006). If one examines

the sequence of the C terminus of the A_{2A} receptor in Figure 3, it is evident that the number of lysine residues to which ubiquitin may be attached is limited. Two of the three lysine residues fall into the region where ARNO and α-actinin are thought to bind. It is evident that the addition of a 76-amino-acid moiety may not be conducive for the interaction with either ARNO or α-actinin given that neither has an ubiquitin-interaction domain.

The presence of multiple binding sites for signalling molecules, scaffolding proteins and adapter proteins may allow the C terminus of the A_{2A} receptor to serve as a coincidence detector (the binding of agonist and a second signal must occur simultaneously for interactor recruitment or release), as a signal integrator (several inputs must accumulate sequentially before interactor recruitment or release) or as an alternative switch (depending on where the receptor resides or which accessory proteins are available, the receptor may employ different signalling pathways to elicit distinct biological responses). The presence of translin-associated protein X, for instance, allows the A_{2A} receptor to recruit p53-mediated cell cycle arrest and thus mediate differentiation of PC12 cells in a manner independent of G_s and the cAMP cascade (Sun *et al.*, 2006). At this point, recruitment of ARNO by the A_{2A} receptor is presumably counterproductive, as ARNO inhibits the hallmark of neuronal differentiation, the sprouting of dendrites and axons (Hernandez-Deviez *et al.*, 2002, 2004).

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Conflict of interest

The authors state no conflict of interest.

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