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Melanocortin receptors and their accessory proteins

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Abstract

The melanocortin receptor family consists of 5 members which belong to the GPCR superfamily. Their specific ligands, the melanocortins are peptide hormones which are formed by the proteolytic cleavage of the proopiomelanocortin (POMC) protein. It is now recognised that certain GPCRs require accessory proteins for their function. Like these GPCRs the melanocortin receptor family is also known to be associated with accessory proteins that regulate their function.

In this review we will summarise the accessory proteins involved in the function of the 5 melanocortin receptors and in particular focus on the melanocortin 2 receptor accessory protein (MRAP) which is crucial for the function of the MC2R.
1. Melanocortin receptors

The melanocortin peptides exert their numerous biological effects by activating receptors (melanocortin receptors-MCRs) that belong to the rhodopsin/β2-adrenergic -like Family A of the GPCR superfamily. The MSH receptor on melanocytes was the first to be cloned in 1992 by two independent groups (Mountjoy et al., 1992, Chhajlani and Wikberg, 1992). To date five MCRs have been identified and termed MC1R-MC5R in the order they were cloned. The melanocortin system regulates key physiological functions including pigmentation, steroidogenesis, energy balance, food intake and sexual behaviour (Gantz, and Fong, 2003). MCRs exhibit the general structural characteristics of the GPCR family, consisting of seven α-helical transmembrane domains that are interconnected by three alternating extra- and intracellular peptide loops. The MCRs are one of the smallest GPCRs known with short N and C-terminal ends. They are all positively coupled to adenylyl cyclase and mediate their effects primarily by activating the cAMP dependent signalling pathway. MCRs display many features that are similar to other GPCRs, including potential N-glycosylation sites in their N-terminal domains, recognition sites for protein kinase A and C phosphorylation and conserved cysteines in their C terminal ends (Wikberg et al, 2000; Abdel-Malek, 2001; Rana, 2003).

1.1 Melanocortin 1 receptor

The 317 amino acid human MC1R on chromosome 16q24.3 was the first melanocortin receptor to be identified (Chhajlani and Wikberg, 1992; Mountjoy et al., 1992). Expression of the MC1R is predominantly in melanocytes and melanoma tissue (Chhajlani and Wikberg, 1992; Mountjoy et al., 1992). It has also been found to be expressed in macrophages (Star et al., 1995), and in the brain in neurons of the periaqueductal grey matter (Xia et al., 1995). Because of the wide distribution of this receptor, it has been shown to be involved in a number of biological functions including pigmentation and anti-pyretic and anti-inflammatory actions. The relative affinity of the human MC1R for the melanocortin peptides is shown in table 1.

1.2 Melanocortin 2 receptor/ACTH receptor

The MC2R (human: 297 amino acids) on chromosome 18p11.2 is unique among the melanocortin receptors because it is only activated by adrenocorticotropic (ACTH). Activation of the MC2R results in an increase in cAMP, and PKA activity which is essential for promoting the expression of steroidogenic enzymes. In addition to the expression in the adrenal glands (Mountjoy et al., 1992), the MC2R has also been found to be expressed in skin
(Slominski et al., 1996). The mouse MC2R has been found to be expressed in adipocytes (Boston and Cone, 1996) and was found to be involved in lipolysis in response to ACTH (Boston, 1999).

Mutations in the MC2R are associated with about 25% of cases of Familial Glucocorticoid Deficiency (FGD). FGD, also known as hereditary unresponsiveness to ACTH or isolated glucocorticoid deficiency, is a rare autosomal recessive disorder that is characterised by severe cortisol deficiency, high plasma ACTH levels and a well preserved renin-angiotensin-aldosterone axis and hence normal mineralocorticoid levels (Clark and Weber, 1998). If untreated, patients can succumb to hypoglycaemia or overwhelming infection in infancy or childhood.

1.3 Melanocortin 3 receptor

The human MC3R (chromosome 20q13.2-q13.3), which is a 361 amino acid protein, shows no preference for the melanocortins binding them with equal affinity. The MC3R is the only MCR to be activated by γ-MSH with similar potency to other melanocortin ligands (table 1). The MC3R is predominantly expressed in the brain (Gantz et al, 1993a; Roselli-Rehfuss et al, 1993) with expression also in the placenta, stomach and pancreas (Gantz et al., 1993a). The MC3R has been found to play a potential role in energy metabolism, since MC3R-null mice show an increased body mass and a higher ratio of weight gain to food intake (Chen et al., 2000). MC3R has also been implicated in the inflammatory response (Getting and Perretti, 2000).

1.4 Melanocortin 4 receptor

The human MC4R (chromosome 18q22) which is a 322 amino acid protein shows certain similarities to the pharmacological profile of human MC1R, (table 1). The MC4R is expressed in brain, autonomic nervous system and spinal cord (Gantz et al., 1993b; Mountjoy and Wild, 1998). The MC4R has been found to be involved in erectile dysfunction (Martin and MacIntyre, 2004) and pain (Starowicz et al., 2003). Most importantly extensive studies have shown that MC4R is involved in controlling food intake and energy expenditure. Huszar and colleagues showed that MC4R-knockout mice have increased food intake and are severely obese (Huszar et al, 1997). Mutations in the MC4R were found to be associated with human obesity for the first time in 1998 (Vaisse et al, 1998; Yeo et al, 1998) when it was shown that heterozygous frameshift mutations in MC4R were linked to dominantly inherited obesity. Since then many missense and nonsense mutations have been found in individuals...
with severe obesity (Farooqi et al, 2000; Gu et al, 1999; Hinney et al, 1999; Vaisse et al, 1998; Wangensteen et al, 2009). Mutations in the MC4R are now considered to be the most common monogenic cause of human obesity. Recent Genome wide association studies identified common variants near the melanocortin 4 receptor associated with BMI, waist circumference and insulin resistance (Chambers et al, 2008; Loos et al, 2008).

1.5 Melanocortin 5 receptor

The 325-amino acid MC5R on chromosome 18p11.2 was the last melanocortin receptor to be cloned. See table 1 for the agonist binding properties of the MC5R. The MC5R is ubiquitously expressed in several tissues, including skin, adrenal gland, adipose tissue, kidney, lymph nodes, liver, skeletal muscle and exocrine glands (Barrett et al., 1994; Chen et al., 1997; Chhajlani et al., 1993; Gantz et al., 1994; Griffon et al., 1994; Labbe et al., 1994). It is implicated in the production of sebaceous lipids (Chen et al., 1997), protein and tear secretion by the lacrimal gland (Entwistle et al., 1990) and also has a role in immune regulation (Taylor and Namba, 2001).

See table 1 for the biological distribution and functions of the melanocortin receptor family.

2. GPCR function and accessory proteins

In order for GPCRs to function normally it is essential that they are successfully translocated across the endoplasmic reticulum (ER) and expressed at the cell surface thereby enabling them to bind to their ligands. Most GPCRs are able to be expressed at the cell surface although growing evidence shows that certain GPCRs are retained at the ER unable to traffic to the cell surface.

GPCRs have been found to interact with several proteins, including molecular chaperones that are established as proteins that assist in the folding, processing, or cellular transport of GPCRs through the secretory pathway. The homo/heterodimerisation between GPCRs has also been implicated in their cell surface expression. A few GPCRs have also recently been found to interact with membrane associated accessory proteins that are required for their function.

Some of these accessory proteins are implicated in the correct folding and trafficking of the GPCRs to the cell surface whilst others are also implicated in ligand binding and found to be associated throughout the life cycle of the receptor. The Drosophila cyclophilin gene Nina A
(neither inactivation nor after potential A) was the first accessory protein to be identified as being a requisite for the biogenesis of Rh1 which is the predominant rhodopsin in Drosophila (Shieh, 1989; Stamnes, 1991). RanBP2 (Ran binding protein 2) which is the mammalian homologue of Nina A is implied as a chaperone for the processing of opsins (Colley et al, 1991; Ferreira et al, 1996). ODR-4 (odorant defective receptor 4) is another accessory protein that has been found to be associated with targeting of the odorant receptors to the olfactory cilia in C. elegans chemosensory neurons (Dwyer et al, 1998; Gimelbrant et al, 1999). The receptor transporting proteins 1 and 2 (RTP 1 and RTP 2) are implicated in the function of the mammalian OR proteins, MOR203-1, OREG, OR-S46, olfr62 and rat 17 (Saito et al, 2004). The Dopamine receptor interacting protein 78 (Drip 78) is another accessory protein that is implicated in the trafficking of the D1 dopamine receptor (Bermak et al, 2001), AT1 angiotensin receptors and M2 muscarinic acetylcholine receptors (Leclere et al, 2002).

The receptor activity modifying proteins (RAMP 1, 2 and 3) are associated with the trafficking of the calcitonin like receptor (CLR) and thought to be linked throughout the life cycle of the CLR (McLatchie et al, 1998; Fraser et al, 1999; Kuwasako et al, 2000). RAMPs 1 and 3 have also been shown to be associated with calcitonin receptor (Christopoulos et al, 1999) and the calcium sensing receptor (Bouschet et al, 2005), and several other class B glucagon/secretin receptor family members may also interact with the RAMPs (Christopoulos et al, 2003).

See table 2 for a summary of GPCR accessory proteins and their relevant functions.

3. Accessory proteins for melanocortin receptor expression

Of the five melanocortin receptors only the MC2R fails to be expressed efficiently at the cell surface when heterologously expressed in cells. The functional characterisation of the human MC2R in heterologous cells has been extremely challenging due to the problems with expressing the receptor. Several groups have attempted to express and characterise the MC2R in a number of cell lines without success. Weber and colleagues used a highly optimised transfection protocol to transfect COS 7 cells with the MC2R although this result was confounded by the endogenous melanocortin receptor present in this cell line that was able to generate cAMP in response to ACTH (Weber et al., 1993). Others also attempted to transfect the Cloudman M3 melanoma cell line and again were unable to reliably characterise the MC2R because of the interference from the MC1R that is endogenously expressed in these cells (Naville et al., 1996).
However when MC2R was transfected into the Y6 and OS3 cell lines which are derived as sister lines to the mouse Y1 adrenocortical cell line, there was satisfactory expression (Yang et al., 1997; Elias et al., 1999). Studies using these cell lines have enabled the functional characterisation of the MC2R using radioligand binding assays as well as cAMP assays. This led to the suggestion that the MC2R may require an adrenal specific accessory factor for its function.

4. Identification of the melanocortin receptor accessory protein

Mutations in MC2R account for only about 25% of FGD cases (FGD type 1). In the other 75% of cases the receptor is normal (FGD type 2). In order to search for a gene associated with FGD type 2, a whole genome scan was carried out (Metherell et al., 2005). Genomic DNA from the parents and affected siblings of a family affected with FGD that had no mutations in the MC2R were used in this study.

Data analysis revealed a single candidate region in one family at 21q22.1. Microsatellite marker analysis confirmed the homozygosity (Metherell et al., 2005). None of the 30 known or predicted genes localised in this region provided an obvious candidate gene on the basis of their known or predicted function. Because of the highly adrenal specific nature of the disease, it was hypothesised that the expression pattern of the causative gene for FGD type 2 may possibly be limited to a few tissues including the adrenal cortex. RT-PCRs were carried out for all these genes using cDNA from human adrenal, brain and liver. A single gene was expressed only in the adrenal and not in the brain or liver (Metherell et al., 2005) and encoded a novel single transmembrane domain protein of unknown function. This protein was originally named fat tissue specific low molecular weight protein (FALP) by Xu and colleagues who reported its expression in adipocytes but not in preadipocytes as determined by two dimensional gel electrophoresis (Xu et al., 2002). Although the authors concluded that the function of this protein was not clear, they suggested that it might be involved in intracellular trafficking pathways. This gene was later renamed melanocortin receptor 2 accessory protein or MRAP because of its association with the MC2R function (Metherell et al., 2005). Mutations in MRAP were identified in about 20% of FGD type 2 cases.

4.1 MRAP structure

The MRAP protein is a small single transmembrane domain protein. The genomic sequence of human MRAP consists of 6 exons and spans a 23kb region on chromosome 21q22.1. In the human alternative splice variants give rise to two isoforms – MRAP-α and –β. MRAP α
encodes a protein of 172 amino acids and a predicted molecular weight of 19kDa, whilst
MRAP β encodes a protein of 102 amino acids and a predicted molecular weight of 14.1kDa.
These isoforms share identical N termini and transmembrane domains but differ at the C
termini. Both isoforms are expressed in the adrenal. MRAP shows little conservation between
species (Webb and Clark, 2010)

Transmembrane protein and membrane prediction tools such as TMPRED (Viklund and
Elllofsun, 2004) predict that MRAP is a type II integral membrane protein with its N-terminus
most likely to lie on the intracellular cytoplasmic surface of the membrane. However this
prediction varies for different MRAP splice variants and for MRAP from different species.
Using an MRAP construct with an epitope tag at the C terminus we showed that the C
terminus of MRAP was at the extracellular face of transfected cells (Metherell et al., 2005). In
subsequent studies using immunofluorescence microscopy on CHO cells transfected with
MRAP epitope tagged at the N and C termini, Sebag & Hinkle demonstrated that both ends of
MRAP were localized at the extracellular face of the plasma membrane (Sebag and Hinkle,
2007). They also identified that endogenous MRAP existed in this dual orientation by staining
for MRAP using antibodies raised against peptides from the N and C terminal ends of the
protein. Sebag and colleagues also analysed the glycosylation patterns of MRAP using an
artificial glycosylation site at the C terminus in order to further identify the dual topology of
MRAP (Sebag and Hinkle, 2007).

In addition to this using the co-immunoprecipitation technique we were also able to show that
MRAP exists as homodimeric structures which are resistant to dissociation by sodium
dodecyl sulphate and reducing agents (Cooray et al, 2008). This higher molecular weight
species detected at above 30kDa was confirmed as MRAP using mass spectrometry analysis
(Cooray et al, 2008).

Using bimolecular fluorescence complementation which incorporates a ‘split’ YFP approach
Sebag and colleagues also confirmed that MRAP exists as antiparallel dimers from the time
that it is synthesised at the ER and remains in this configuration throughout its translocation
to the cell surface (Sebag and Hinkle, 2009).

4.2 MRAP is essential for melanocortin 2 receptor function

Using a functional cAMP assay we showed that MRAP was needed in order for the MC2R to
be able to signal at the cell surface (Metherell et al., 2005). The necessity of MRAP for
MC2R signaling was further confirmed by RNA interference studies by knocking down the
expression of MRAP in the Y1 mouse adrenocortical cell line which expresses an endogenous MRAP and a functional MC2R (Cooray et al, 2008). The knockdown of MRAP in this cell line resulted in a reduction in the MC2R mediated signaling. This phenotype was rescued using a human MRAP construct that was resistant to silencing by the mouse MRAP siRNAs (Cooray et al, 2008). The interaction between MRAP and MC2R was shown using co-IPs (Metherell et al., 2005; Webb et al, 2009).

In an attempt to elucidate the domains of MRAP involved in MC2R function several deletions and mutations of different regions of the MRAP protein were created. A 27 amino acid region including residues 36-62 which almost equates to the transmembrane domain was identified as being required for MC2R binding whilst the tyrosine rich residues 9-24 of the N terminus of MRAP was essential for MC2R to be efficiently expressed at the cell surface (Webb et al, 2009). Sebag & Hinkle also showed that the four amino acid residues LDYI at positions 18-21 were needed for MC2R signaling and suggested that the MC2R may require MRAP in order to form a high affinity binding pocket for ACTH or that it may assist MC2R to bind to the Gs subunit of the heterotrimeric G protein complex (Sebag and Hinkle, 2009). Mutant MRAP constructs lacking the C terminus were found to function normally (Webb et al, 2009; Sebag and Hinkle, 2009). The C terminus of MRAP was not found to be essential for the trafficking or interaction with the MC2R. This is consistent with the observation that MRAP β has also been found to assist MC2R trafficking and function (Roy et al, 2007) and that MRAP α and MRAP β are identical in their N termini and transmembrane domains but only differ in their C termini.

4.3 The MRAP orthologue MRAP 2

A protein encoded by C6orf117 on human chromosome 6q14.3 is clearly related to MRAP and has been named MRAP2. Human MRAP2 consists of 4 exons and encodes a 23.5kDa protein which contains a single transmembrane domain and is over 36% identical to MRAP in the N-terminal and transmembrane regions, though they differ greatly at the C-terminus.

See Figure 1 for a sequences alignment of human MRAP α, β and MRAP 2.

MRAP 2 is expressed in the adrenal and brain (Chan, 2009 et al). Similarly to MRAP, MRAP 2 has also been found to exist in a dimeric form that is resistant to denaturing and reducing conditions as indicated by its appearance as a 48kDa protein on Western blots (Chan et al, 2009).
Like MRAP, MRAP2 was also found to assist MC2R to the cell surface as well enabling the receptor to respond to its ligand ACTH at $10^{-6}$M concentration. Sebag & Hinkle used the mouse MRAP2 along with MC2R to investigate its role in MC2R cell surface expression and function and found that although MRAP2 assists MC2R trafficking it is not needed for the MC2R signaling. The ACTH concentration used in these experiments was $10^{-7}$M (Sebag and Hinkle, 2009). They suggested that MRAP2 fails to assist MC2R signaling as it lacks the LDYI residues that are present in MRAP. The apparent contradiction in these findings between Chan et al and Sebag & Hinkle may be due to the difference in the dose of ACTH which may explain why MRAP2 cannot rescue ACTH resistance in FGD type 2 patients (with MRAP mutations).

4.4 MRAPs negatively regulate MC1R, MC3R, MC4R and MC5R function

MRAP interacts with all five melanocortin receptors as determined by co-immunoprecipitations (Chan et al, 2009). It is not required for the cell surface expression of the MC1R, MC3R, MC4R or MC5R but was found to partly reduce the expression of the MC4R and MC5R at the cell surface (Chan et al, 2009). Contrary to its role in enhancing MC2R function, MRAP was found to have a significant inhibitory role in the function of the MC1R, MC3R, MC4R and MC5R in cAMP assays in response to NDP-MSH (Chan et al, 2009). Sebag & Hinkle also suggested that MRAP impaired MC5R dimer formation using the bimolecular fluorescence complementation technique and co-IPs and proposed that the negative effect of MRAP in MC5R may be attributed to its action on the disruption of MC5R dimers (Sebag and Hinkle, 2009).

Similar to MRAP, MRAP2 was also shown to interact with all five melanocortin receptors using the co-immunoprecipitation technique (Chan et al, 2009). Chan et al also investigated the effect of MRAP2 on the function of MC1R, MC3R, MC4R and MC5R (Chan et al, 2009). Like MRAP, MRAP2 was also found to act as a negative regulator of these melanocortin receptors. The expression of MRAPs in human tissues has been found to be more widespread than the expression of the MC2R (Metherell et al., 2005, Chan et al., 2009). In particular both MRAP and MRAP2 have been found to be expressed in the hypothalamic region of the brain that expresses MC3R and MC4R (Lein et al, 2007, Gardiner et al.,2002) suggesting that it may regulate the function of these MCRs in the central nervous system.

5. Other accessory proteins involved in melanocortin receptor function

5.1 Attractin and Attractin-like –protein
Attractin (Attr), which is the gene mutated in the mouse mutant mahogany, encodes a type 1 transmembrane domain protein of 1428 amino acids. Attractin was believed to be involved in modulating the MC1R and MC4R function as mahogany mice are associated with dark coat colour and obesity. The association between Attractin, MC1R and the Agouti protein, which is an endogenous antagonist of the MC1R became clear when it was found that Attractin binds to the N terminal domain of Agouti protein (He et al, 2001). Attractin was believed to act as a co-receptor for Agouti presumably by assisting in the stability of the interaction between the C terminus of agouti and MC1R. In the brain attractin deficiency has been found to cause spongiform degeneration (He et al, 2001). However Attractin does not bind to the agouti related peptide (AGRP) which is an endogenous MC4R antagonist that is implicated in food intake (He et al, 2001). Attractin does not interact with the MC4R and does not show high levels of co-expression in the brain suggesting that it was unlikely to be involved in AGRP mediated MC4R signalling (Haqq et al, 2003).

Using a yeast two hybrid study to identify interacting partners for the MC4R (C terminus) in a mouse brain cDNA library, Haqq and colleagues identified a protein named attractin-like-protein (ALP) (Haqq et al, 2003). ALP is a transmembrane domain protein consisting of 1371 amino acids. The cytosolic tail of ALP is 63% identical to the mouse Attractin cytosolic tail. Specific regions of the C terminus of ALP, which differ from Attractin were implicated in the interaction with the C terminus of MC4R by GST-pulldown assays (Haqq et al, 2003). This interaction was believed to be specific as ALP did not bind the C terminus of the β2 adrenergic receptor. However it still remains to be seen whether ALP would interact with any of the other MCRs.

Although the authors presented no functional data for ALP-MC4R interaction, they showed co-localisation of the two proteins in numerous regions of the brain which are implicated in energy homeostasis. ALP was suggested to act as a co-receptor for AGRP thereby being involved in the MC4R signalling.

5.2 Mahogunin Ring Finger 1- MGRN1

Mgrn1 was identified by positional cloning of Mahoganoid (md), which is a mouse mutation that causes a similar phenotype to the mahoganin mouse. MGRN1 which is mutated in mahoganoid and its human orthologue KIAA0544 encodes a RING finger domain containing E-3 ubiquitin ligase. Unlike Attractin MGRN1 is an intracellular protein. It was suggested that MGRN1 might play a role in inhibiting MC1R signalling since the coat colour phenotype
of MC1R gain of function mutants is comparable to the \textit{md} phenotype. Perez-Oliva and colleagues recently showed that all four isoforms of human MGRN1 inhibited MC1R signalling (Perez-Oliva et al., 2009). They demonstrated that this inhibition was not due to the internalisation or ubiquitination of the receptor. MGRN1 was also found to be involved in inhibiting MC4R signalling. The authors suggested that the inhibition of MC1R and MC4R function by MGRN1 was most likely due to its competition with G\(\alpha\)s to bind to the receptor ligand complex (Perez-Oliva et al., 2009).

Two isoforms of MGRN1 were also found to be expressed in the nucleus in the presence of the MC1R and MC4R. It remains to be seen whether MGRN1 would also be involved in the function of the other 3 melanocortin receptors (Perez-Oliva et al., 2009).

See figure 2 for a summary of melanocortin receptor accessory proteins.

\textbf{6.0 Conclusion}

It is not known why only certain GPCRs and not others require accessory proteins for their functional expression. However it is possible that most of these GPCRs are expressed normally as they are assisted by as yet unidentified accessory proteins which are ubiquitously expressed in cells. Of the five melanocortin receptors only the MC2R fails to be expressed at the cell surface in the absence of an accessory protein. MRAP was found to be crucial for the cell surface expression and function of the MC2R whilst playing a negative role in the signalling of the other four melanocortin receptors. Like MRAP, MRAP 2 also assists MC2R function and has a negative impact on the function of the other four melanocortin receptors. Although the expression pattern of the MRAPs coincide with the expression of in particular MC4R and MC5R, there is no evidence to show that patients with MRAP mutations have increased MC4R/MC5R function. The exact mechanism by which MRAPs assist MC2R function still remains to be elucidated. However it is now well established that without MRAP, MC2R would not function. Several membrane permeant ligands known to act as pharmacoperones have been shown to assist in the signalling of GPCRs which would otherwise be retained at the ER. The action of the MRAP molecules on melanocortin receptor function may provide important targets in developing pharmacological agents that could potentially be used for treating disorders related to these receptors in the future.
Table 1: Biological distribution and functions of the melanocortin receptor family (adapted from Cone, R.D., 2006)

Table 2: GPCR accessory proteins and their functions

Figure 1: Alignment of human MRAP α, β and MRAP2: The majority of sequence conservation is seen within the N terminal and transmembrane domains.

Figure 2: Accessory proteins for the five melanocortin receptors:

A: Accessory proteins for the melanocortin 1 receptor: The MC1R is able to traffic to the cell surface in the absence of MRAP/MRAP2. Expression of MRAPs does not affect MC1R trafficking. However in the presence of MRAP or MRAP2, MC1R signalling is reduced in response to MSH. Binding of α-MSH to MC1R activates cAMP synthesis promoting the production of the pigment eumelanin in skin (black/brown).

Attractin acts as a co-receptor for the MC1R antagonist Agouti. Agouti antagonises binding of α-MSH to MC1R and inhibits eumelanogenic synthesis leading to the production of the red/yellow pigment, pheomelanin (yellow arrows). MGRN1 can reduce cAMP production from MC1R presumably by competition with Gas.

B: Accessory proteins for the melanocortin 2 receptor: The MC2R is retained at the ER in the absence of MRAP. MRAPs assist MC2R trafficking to the cell surface. Once at the cell surface MRAP enhances MC2R signalling resulting in steroidogenesis in the adrenal gland.

C: Accessory protein for the melanocortin 3 receptor: The MC3R can be expressed at the cell surface in the absence of MRAPs. MRAP has no significant effect on MC3R trafficking although it has been found to significantly reduce MC3R signalling.

D: Accessory proteins for the melanocortin 4 receptor: The MC4R does not require an accessory protein for cell surface expression. MRAPs can have a negative effect on MC4R trafficking as well as signalling. ALP is believed to act as a co-receptor for AGRP which antagonises binding of MSH to MC1R which can result in obesity. MGRN1 has also been
shown to reduce MC1R signalling as determined by reduced cAMP production.

**E:** Accessory proteins for the melanocortin 5 receptor: The MC5R is expressed at the cell surface without MRAPs. Expression of MC5R with MRAP results in reduced cell surface trafficking and signalling of the receptor.

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<tr>
<td>Calcitonin receptor (CTR)</td>
<td>RAMPs 1 &amp; 3</td>
<td>Activity modifier (allows binding to amylin in addition to calcitonin)</td>
<td></td>
</tr>
<tr>
<td>Calcium sensing receptor (CasR)</td>
<td>RAMPs 1 &amp; 3</td>
<td>Trafficking of receptor from ER to the Golgi and mediate terminal glycosylation</td>
<td></td>
</tr>
<tr>
<td>Melanocortin 2 receptor (MC2R)</td>
<td>MRAP α and &amp; MRAP 2</td>
<td>Trafficking and function of MC2R</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1

Transmembrane

hMRAPalpha: --------MANGTN-ASAPYYYSEYVLDDLPLVDDEKKLKAHKHSIVIAFWVSLAAPVVLFLILLYMSKASASPM
hMRAPbeta: --------MANGTN-ASAPYYYSEYVLDDLPLVDDEKKLKAHKHSIVIAFWVSLAAPVVLFLILLYMSKASASPM
hMRAP2: MSAQRLLSRTSQQGASNSDXTNBYEYELGEPVSFEGLKAKKHYSIVICFWVCLAVFVIRVMFFVLTDLTKTMGAPHQ

hMRAPalpha: RSPKHHQTCPW-----------SQCNLHICQKCLCRPLATSQAOASVEPKGRTGPDPLRC
hMRAPbeta: SFCNTSLLHSEVLEQTQA-ISCDFLQAP-----------R5
hMRAP2: DNAESSEKFRMNSFVSDFGRLPLEPKVFSRQGNEE3RSLFHACYNEVRLDRACKHCTAMPALDSVQLQBAIRS

hMRAPalpha: ESSSTLPAGGFQTHPTLLBL---------INGGFLVRKSEPPGDRTSCLQS-----172
hMRAPbeta: EGAA-----------------------103
hMRAP2: SQPPEEDNRMKFDIPNPSYNPDQNYFGEDDHSSEPIVLETKPLSTSHKDLD 205