Published in final edited form as:
Mol Cell Endocrinol. 2009 March 5; 300(1-2): 25–31. doi:10.1016/j.mce.2008.10.041.

Structure and Function of the Melanocortin2 Receptor Accessory Protein MRAP

Patricia M. Hinkle* and **Julien A. Sebag**
Department of Pharmacology and Physiology, University of Rochester Medical Center, Rochester, NY 14642

Abstract

The melanocortin2 (MC21), or ACTH receptor, requires MC2 receptor accessory protein (MRAP) for function, and individuals lacking MRAP are ACTH-resistant and glucocorticoid-deficient. MRAP facilitates trafficking of the MC2 receptor to the plasma membrane and is absolutely required for ACTH binding and stimulation of cAMP. MRAP, which contains a single transmembrane domain, has a unique structure, an antiparallel homodimer. It can be isolated from the plasma membrane in a complex with the MC2 receptor. A short sequence just aminoterminal to the transmembrane domain of MRAP is essential for dual topology, while the transmembrane region is not; both are necessary for function. Deletion or alanine-substitution of other aminoterminal regions yields MRAP mutants that promote surface expression of the MC2 receptor but not receptor signaling. These results identify two distinct actions of MRAP: to permit trafficking of the MC2 receptor, and to allow surface receptor binding and signaling.

Keywords

ACTH; cAMP; dual topology; GPCR; melanocortin receptor; MRAP

1. Introduction

ACTH actions in the adrenal gland are mediated by a G protein-coupled receptor (GPCR) known as the ACTH, or melanocortin2 (MC2), receptor (Mountjoy et al., 1992). The MC2 receptor is a member of the melanocortin receptor family that contains five structurally related receptors: MC1 (MSH), MC2 (ACTH), and MC3-5. It is unusual in responding to picomolar concentrations of ACTH but not to α-, β- or γ-MSH; both α-MSH and ACTH are potent agonists for the other four melanocortin receptors (Gantz and Fong, 2003). The molecular basis for differences in ligand specificity has not been established. Both MSH and ACTH are proteolytically cleaved from the precursor proopiomelanocortin (POMC).

Like all members of the melanocortin receptor family, the MC2 receptor signals via the G protein Gs to activate adenylyl cyclase, and ACTH exerts many, though not all, of its effects

[1]Abbreviations used: ER, endoplasmic reticulum; FGD, familial glucocorticoid activity; GPCR, G protein-coupled receptor; MC, melanocortin; MRAP, melanocortin2 receptor accessory protein; RAMP, receptor activity modifying protein; REEP, receptor expression enhancing protein; RTP, receptor transporting protein; YFP, yellow fluorescent protein.

*Corresponding author at: Department of Pharmacology and Physiology, University of Rochester Medical Center, Rochester, NY 14642, USA. Tel.: 585 275 4933; fax: 585 273 2652. *E-mail address*: E-mail: patricia_hinkle@urmc.rochester.edu.

by increasing cAMP (Schimmer et al., 2007). The MC2 receptor is a class A, rhodopsin-like GPCR, with the characteristic extracellular amino terminus, seven transmembrane segments, and cytoplasmic carboxylterminus. The extracellular and cytoplasmic tails of all of the melanocortin receptors are unusually short. In fact, the MC2 receptor is the smallest of the hundreds of identified GPCRs. The MC2 receptor has the canonical E/DRY sequence at the cytoplasmic end of the third transmembrane domain but lacks some other common features of rhodopsin-like receptors including a disulfide linkage between extracellular loops 1 and 2 (Yang et al., 2007).

Unlike the other melanocortin receptors, the MC2 receptor is not functional when expressed in heterologous cell lines typically used to study GPCR signaling (HEK293 and CHO cells, for example), although the receptor does traffic to the plasma membrane and respond to ACTH when it is expressed in adrenal cell lines or cell types such as melanocytes that normally express melanocortin receptors (Mountjoy et al., 1992; Noon et al., 2002; Forti et al., 2006). These features led to speculation that expression of a functional MC2 receptor requires an accessory protein. In 2005 Adrian Clark and his collaborators identified an accessory protein that is critical for ACTH signaling and named it melanocortin2 receptor accessory protein (MRAP) (Metherell et al., 2005).

2. Discovery of MRAP

Familial glucocorticoid deficiency (FGD) is an autosomal recessive, genetically heterogeneous disease (Clark et al., 2005; Clark and Metherell, 2006; Chan et al., 2008). Patients with FGD are resistant to ACTH and suffer from the consequences of a lack of adrenal glucocorticoids. Individuals with FGD have elevated ACTH and low or absent circulating cortisol, with normal mineralocorticoid levels and a normal renin-angiotensin system. FGD is often discovered in infancy when patients experience hypoglycemic episodes or other problems resulting from glucocorticoid deficiency. If glucocorticoids are not replaced, infants with FGD may succumb early in life. Interestingly, adequate replacement of glucocorticoids fails to suppress ACTH completely, and hyperpigmentation is usually present by several months of age and persists throughout life (Rumie et al., 2007). Hyperpigmentation is believed to result from activation of the MC1 (MSH) receptor by high concentrations of ACTH. The cause of the persistent elevation of ACTH is uncertain and may involve the loss of a short loop feedback exerted by ACTH via the MC2 receptor. Individuals with FGD do not experience adrenarche.

Mutations in the MC2 receptor are responsible for approximately a quarter of FGD cases, and FGD due to receptor mutations has been termed Type 1 (Clark et al., 2005; Clark and Metherell, 2006; Chan et al., 2008). Dozens of different MC2 receptor mutations can give rise to ACTH resistance (Chan et al., 2008). These mutations are found in multiple transmembrane segments and elsewhere in the receptor molecule.

Metherell et al. studied a group of individuals with FGD who had no mutations in the MC2 receptor (Metherell et al., 2005). They mapped a region of the genome associated with FGD and analyzed expression of 30 candidate genes in adrenal cortex versus liver and brain. This approach led to identification of a gene that was mutated in affected individuals. The gene encodes a small single transmembrane protein that was shown to be essential for the expression of functional MC2 receptor: the MC2 receptor accessory protein MRAP. The structures of the MC2 receptor and MRAP are shown schematically below in Fig. 5. MRAP had previously been identified as a transcript that increased upon differentiation of 3T3 fibroblasts to adipocytes, originally called fat cell-specific low molecular weight protein, or Falp (Xu et al., 2002). MRAP is the first GPCR accessory protein shown to be responsible for a human disease. Mutations in MRAP appear to be responsible for about 20% of FGD cases, and FGD due to MRAP deficiency is now termed Type 2 (Clark et al., 2005; Chan et al., 2008; Cooray et al.,

2008). At least nine different MRAP mutations have been identified in individuals with FGD Type 2. All of these are expected to encode either no protein or severely truncated MRAPs (Modan-Moses et al., 2006; Chan et al., 2008), giving no information about important regions of the molecule.

Phenotypically, FGD Types 1 and 2 are similar. Some individuals with FGD Type 1 are characterized by tall stature of uncertain cause (Imamine et al., 2005; Chan et al., 2008). A recently developed mouse model of FGD Type 1, the MC2 receptor knockout (Chida et al., 2007), has high circulating ACTH and absent cortisol. Most mice lacking the MC2 receptor die shortly after birth, probably as a result of hypoglycemia. The MC2 receptor-null mice differ from humans with FGD Type 1 in having low aldosterone and normal weight and length.

An unresolved question is whether MRAP acts as an essential accessory protein for any other GPCRs. If it does, one might expect that individuals lacking MRAP (FGD Type 2) would have more far-reaching deficits than individuals lacking only the MC2 receptor (FGD Type 1). This has not been observed in the relatively small group of patients with MRAP mutations available for study thus far. Instead, the known features of MRAP deficiency can be attributed to either high circulating ACTH (hyperpigmentation) or low cortisol secretion (hypoglycemia, etc.).

3. Structure of MRAP

MRAP is a small protein containing a single hydrophobic transmembrane domain. MRAP mRNA is found in adrenal cortex, lymph nodes, brain, testis, breast, thyroid and adipose tissue (Xu et al., 2002; Metherell et al., 2005). The protein is encoded by a single gene that contains 6 exons in the human (Xu et al., 2002; Metherell et al., 2005). Alternative splicing of human MRAP gives rise to two proteins, one of 172 amino acids, hMRAPα, and one of 102 residues, hMRAPβ. MRAPs α and β are identical in the 37 amino acid N-terminal domain, 23 amino acid transmembrane domain, and the first 9 residues of the C-terminal segment. The more distal regions of MRAPs α and β differ completely. Both α and β forms are present in adrenal tissue. Throughout evolution, the aminoterminal and transmembrane domains of MRAP have been highly conserved, whereas the carboxylterminal regions have diverged greatly. A careful analysis by Roy et al. (Roy et al., 2007) established that both MRAPα and MRAPβ can support cAMP generation by the MC2 receptor when the proteins are expressed in heterologous cells, with small quantitative differences in ACTH efficacy and potency.

Topology analysis programs such as TMHMM (Viklund and Elofsson, 2004) predict that MRAP is a type II integral membrane protein oriented with an exoplasmic carboxylterminus, i.e. with the C-terminus facing the inside of the endoplasmic reticulum (ER) and Golgi apparatus and facing the cell exterior when MRAP is localized on the plasma membrane. The strength of this prediction varies for different MRAP splice variants and for MRAP from different species. In their initial report identifying MRAP as an accessory protein for the MC2 receptor, Metherell et al. (Metherell et al., 2005) confirmed that the C-terminus of MRAP is on the extracellular face of transfected CHO cells using an MRAP construct bearing a C-terminal epitope tag. In subsequent studies, our laboratory made the unexpected discovery that *both* the N- and C-terminal ends of mouse MRAP face outward in CHO cells (Sebag and Hinkle, 2007). Roy et al. (Roy et al., 2007) noted that both ends of hMRAPβ are detectable on the exterior side of the plasma membrane of transiently transfected HEK293 cells.

Prior to the discovery of MRAP's dual topology, naturally occurring single transmembrane proteins were thought to be in either an N_{exo}-C_{cyt} (C-terminal cytoplasmic) or N_{cyt}-C_{exo} (N-terminal cytoplasmic) orientation, but not both. Dual orientation has been documented in eukaryotic cells for some engineered proteins (Beltzer et al., 1991), and a small fraction of a few native proteins is in an opposite orientation. We tagged MRAP at either the N- or the C-terminus with the V5 epitope. When the N-terminally tagged V5-MRAP was expressed in

CHO cells, the epitope was detected on the extracellular side of the membrane. When C-terminally tagged MRAP-V5 was tested, it was also localized on the extracellular face of the plasma membrane. Dual topology was detected over a wide range of MRAP expression levels, and the two orientations were present at roughly a 1:1 concentration (Sebag and Hinkle, 2007).

In a critical experiment, we established that *endogenous* MRAP in adrenocortical cells has dual orientation. We prepared antibodies against peptides from the N- and C-terminal segments of MRAP and stained live cells such that antibody could only bind to external epitopes. Both ends of MRAP were identified on the exoplasmic surface of ACTH-responsive Y1 adrenocortical cells (Sebag and Hinkle, 2007). As shown in Fig. 1, OS3 cells, an adrenal cell line that does not express MC2 receptor (Schimmer, 1972), also express both the N- and C-terminal regions of endogenous MRAP on the cell surface. These results confirm the dual topology of naturally expressed MRAP and prove that its structure is not dependent on the presence of MC2 receptor.

We analyzed MRAP topology further by examining glycosylation patterns in transfected CHO cells (Sebag and Hinkle, 2007). MRAP contains a single potential N-linked glycosylation site (Asn-X-Ser/Thr) at Asn3 in the aminoterminus. If the molecule is in the N_{exo} orientation, it can potentially be glycosylated. If it is in the C_{exo} orientation, glycosylation is impossible, because N-glycosylation can take place only on the inside of the ER and Golgi apparatus. If it has dual topology, then MRAP should be isolated as a mixture of glycosylated (N_{exo} orientation) and non-glycosylated (N_{cyt} orientation) species. This was confirmed by electrophoretic analysis of MRAP before and after enzymatic deglycosylation. When the glycosylation site was removed from the aminoterminus, no glycosylated MRAP was detected; when an artificial glycosylation site was added to the carboxylterminus, a mixture of glycosylated and nonglycosylated species was present. If potential glycosylation sites were present on both sides of the membrane, almost all MRAP was glycosylated. Furthermore, none of the MRAP was doubly glycosylated, ruling out a monotopic orientation in which both ends face outward. Glycosylation was not required for MRAP function.

MRAP oligomerization has been convincingly demonstrated. Cooray et al. found that a large fraction of MRAP expressed in CHO cells migrated at the size of a dimer during electrophoresis under denaturing conditions and used mass spectrometry to verify that the high molecular weight band contained MRAP (Cooray et al., 2008). When two MRAP constructs containing different epitope tags were co-expressed, immunoprecipitation with antibody against either tag precipitated both MRAP species, indicating that multiple MRAP molecules forms dimers or perhaps higher oligomers (Sebag and Hinkle, 2007; Cooray et al., 2008). In fact, this approach suggested that essentially all of the MRAP in a cell is in dimers.

Evidence that MRAP homodimers are in an antiparallel orientation came from our studies in which C-terminally tagged MRAP at the plasma membrane was selectively immunoprecipitated by adding antibodies to intact CHO cells. Precipitated cell surface MRAP was then analyzed by electrophoresis (Sebag and Hinkle, 2007). As discussed above, MRAP in the C_{exo} orientation cannot be glycosylated, yet cell surface C_{exo} MRAP immunoprecipitated with glycosylated MRAP. This could only occur if the molecules were in an antiparallel configuration. MRAP is the first single transmembrane protein shown to form dimers with an antiparallel orientation.

The remarkable topology of MRAP immediately raises the question of how dual orientation is achieved. One could imagine that MRAP is able to switch orientations, or flip, ending up in the most stable conformation, an antiparallel homodimer. This seems unlikely, though, because if MRAP flips readily, all of it should eventually spend time in an N_{exo}-C_{cyt} orientation, and

all of it should eventually be glycosylated. In most experiments, approximately half of cell surface MRAP is glycosylated (Sebag and Hinkle, 2007).

To ask if MRAP is in a dimer with dual orientation from the time it is first synthesized in the ER, we used a technique called bimolecular fluorescence complementation (Kerppola, 2006). We fused MRAP to split yellow fluorescent protein ("split YFP") molecules. MRAP was fused N-terminally to an incomplete YFP1 and C-terminally to a complementary YFP2 fragment. The results are shown schematically in Fig. 2. Neither fusion protein was fluorescent alone, but YFP1-MRAP and MRAP-YFP2 were fluorescent in the ER and on the plasma membrane when expressed together. Molecular complementation was not observed when the two YFP fragments were fused to the same end of MRAP, when the fragments were fused to MRAPΔ31-37, which does not form antiparallel homodimers (see below), or when YFP fragments were fused to receptor activity modifying protein 3 (RAMP3), a control single transmembrane protein with a single orientation (not depicted). The data indicate that parallel dimers do not form, unless these happen to be oriented in a manner that precludes interaction of the YFP fragments. More importantly, because the two YFP fragments cannot possibly form a fluorescent protein across a membrane, the findings provide strong confirmation for the unique antiparallel dimeric structure of MRAP and show that MRAP forms an antiparallel dimer when it is first synthesized in the ER.

The ability of MRAP to facilitate MC2 receptor trafficking and signaling begs the question of whether the two proteins interact directly or whether the actions of MRAP are indirect. MRAP and the MC2 receptor co-precipitate following lysis in nonionic detergents (Metherell et al., 2005; Sebag and Hinkle, 2007; Cooray et al., 2008), suggesting that MRAP and the receptor form a complex. MC2 receptor precipitates with MRAP in both the N_{exo}-C_{cyt} and N_{cyt}-C_{exo} orientations (Sebag and Hinkle, 2007). Such experiments cannot distinguish between direct MRAP-MC2 receptor binding and linkage through another protein, however. The functional complex may be larger than a single MC2 receptor with an MRAP dimer, because several other members of the melanocortin receptor family are known to form homodimers and heterodimers (Biebermann et al., 2003; Mandrika et al., 2005).

4. Function of MRAP

MRAP appears to be essential for MC2 receptor localization on the plasma membrane of CHO cells (Metherell et al., 2005; Sebag and Hinkle, 2007). In Fig. 3, we show an example of the effect of MRAP on MC2 receptor localization. The MC2 receptor was fused at its C-terminus to a red fluorescent protein (dubbed "tomato" for its spectral properties (Shaner et al., 2004)) and expressed with or without mMRAP. The MC2 receptor was trapped in the ER and Golgi apparatus in the absence of MRAP but largely present on the plasma membrane when expressed with the accessory protein. Total receptor concentrations measured by Western blotting are much higher in cells expressing MRAP (Sebag and Hinkle, 2007), probably because improperly processed MC2 receptor is subject to degradation by quality control mechanisms operative in the ER (Petaja-Repo et al., 2000). Roy et al. (Roy et al., 2007) found that MC2 receptor tagged with the myc epitope at the N-terminus was localized on the cell surface in the absence of MRAP in HEK293 cells, while an MC2 receptor-GFP fusion protein required MRAP for surface expression. These differences may be a consequence of differences in the MC2 receptor constructs tested in various studies, differences in the cell lines tested, or differences in expression levels. Based on reported B_{max} levels for ^{125}I-ACTH binding, the stable cell lines used by Roy et al. (Roy et al., 2007) expressed the MC2 receptor at 10-20 times the density found in adrenocortical cells (Buckley and Ramachandran, 1981) and at higher levels than those measured in transiently transfected cells where surface MC2 receptor expression is MRAP-dependent.

While there is some dispute about the extent to which MRAP is required for surface expression of MC2 receptor, there is no question that MRAP is required in every model for ACTH binding and signal transduction. The amount of cAMP accumulated in response to ACTH is negligible in cells expressing the MC2 receptor without MRAP (Metherell et al., 2005; Roy et al., 2007; Sebag and Hinkle, 2007). Importantly, partial knockdown of MRAP mRNA with RNAi causes a strong reduction in the ability of ACTH to signal in Y1 adrenal cells that express the MC2 receptor naturally (Cooray et al., 2008).

By deleting or mutating different regions of the small MRAP protein, it has been possible to elucidate the functions of various parts of the molecule. The results are summarized in Fig. 4. Not surprisingly, the nonconserved carboxylterminus of the molecule is not essential for function. The cAMP response to maximally effective concentrations of ACTH was decreased by less than half following deletion of 64 of the 67 amino acids in the C-terminus of mouse MRAP (Sebag and Hinkle, 2007). We made fusion proteins between MRAP and RAMP3, a control single transmembrane protein, and found that the ability of MRAP to promote MC2 receptor trafficking to the plasma membrane requires the transmembrane domain of MRAP. The ability of MRAP to facilitate MC2 receptor expression on the plasma membrane was reduced but not abolished following deletion of the first 30 of the 37 residues in the aminoterminus, while MRAP retained no activity after deltion of amino acids 31-37.

One of the major determinants of membrane protein orientation is positive charge in the juxtamembrane regions (von Heijne, 1986; Hartmann et al., 1989). A sequence with more positive charge near the transmembrane domain is more likely to be oriented toward the cytoplasm. We found that dual topology of MRAP requires a 7 amino acid basic region on the aminoterminal side of the protein (Fig. 4). Surprisingly, MRAP residues 29-37 can even confer dual topology to RAMP3 (without its natural signal peptide), which is otherwise in an exclusively N_{exo} orientation. As discussed below, RAMP3 is an accessory protein that regulates the expression and ligand specificity of calcitonin receptor-like receptor. The transmembrane segment of MRAP can be replaced with the transmembrane segment of RAMP3 without the loss of dual topology, although the RAMP3-MRAP chimera is not functional.

Detailed mutagenesis of the aminoterminal region of MRAP uncovered a series of MRAP mutants that facilitate expression of the MC2 receptor on the plasma membrane but do not permit ^{125}I-ACTH binding or ACTH stimulation of cAMP in CHO cells (Fig. 4, (Sebag and Hinkle, 2008)). MRAP amino acids 18-21, LDYI, are essential for MC2 receptor signaling. Because the interaction of a GPCR with its cognate G protein increases agonist affinity, it is uncertain whether the MC2 receptor requires MRAP residues 18-21 to form a high affinity binding pocket or to interact with Gs. These results establish that MRAP has two distinct functions in adrenocortical cells: to promote trafficking of the MC2 receptor, perhaps by acting as a chaperone and enhancing correct protein folding; and to allow surface receptor to bind ligand and signal via Gs to adenylyl cyclase (Fig. 5).

These dual functions of MRAP are reminiscent of the functions of receptor activity modifying proteins, or RAMPs (Hay et al., 2006; Parameswaran and Spielman, 2006; Sexton et al., 2006). There are three RAMPs that exert dramatic effects on the ligand specificity and trafficking of the calcitonin receptor-like receptor and the calcitonin receptor. Like MRAP, the RAMPs are single transmembrane proteins, but RAMPs have a classical signal sequence and are in a single N_{exo} orientation. RAMP1 does not reach the plasma membrane unless it is expressed with a receptor, yet certain mutations in the RAMP1 protein allow it to localize to the cell surface by itself, suggesting that RAMP1 contains an ER retention signal (Udawela et al., 2006). In contrast, MRAP assumes its unusual topology and reaches the plasma membrane with or without the MC2 receptor, although the distribution of MRAP through the ER, Golgi

apparatus, and plasma membrane appears to depend on the species and splice variant of MRAP. As is the case with MRAP, certain mutant RAMPs allow receptor trafficking but not signaling (Kuwasako et al., 2003; Parameswaran and Spielman, 2006). The responsible regions of MRAP and RAMPs do not share any obvious sequence homology.

5. Future Directions

Accessory proteins for GPCRs have been found in species ranging from *C. elegans* (Dwyer et al., 1998) to mammals. Some accessory proteins seem quite specific, such as DRIP78 needed for D1 dopamine receptor processing (Bermak et al., 2001). Others, such as receptor transporting proteins (RTP1, RTP2) and receptor expression enhancing protein (REEP), are required for expression and signaling of many of the receptors in the very large odorant and taste receptor families (Saito et al., 2004; Behrens et al., 2006). The topology and mechanism of action of these recently discovered proteins have not been thoroughly investigated, and some of these other accessory proteins may share MRAP's unusual structure. RAMPs have dramatic effects on the specificity of several receptors for agonists and antagonists (Hay et al., 2006; Parameswaran and Spielman, 2006; Sexton et al., 2006), and the possible impact of MRAP on MC2 receptor ligand specificity has not yet been explored. Future studies will also address the possibility that inactive forms of MRAP act in a dominant negative manner by dimerizing with wildtype MRAP. If dominant negative effects are potent, some MRAP mutations could give rise to dominant ACTH-resistance syndromes. It is noteworthy that a second gene encodes a protein highly homologous to MRAP (Metherell et al., 2005). Intriguingly, the second MRAP-like sequence has a deletion of precisely those residues found to be necessary for MRAP signaling in our studies (Fig. 4).

It will be of interest to determine whether MC2 receptor mutations that cause FGD Type 1 are defective in their ability to interact with MRAP. Many disease-causing mutant GPCRs are trapped in the ER and nonfunctional. In several cases, membrane permeant ligands have been shown to restore receptor trafficking and signaling (Bernier et al., 2004; Conn et al., 2007). If membrane permeant MC2 receptor ligands are developed, these could act as pharmacological chaperones and obviate the need for MRAP. Such a drug might be useful in patients with FGD Type 2, permitting physiological regulation of glucocorticoid synthesis and relief from the consequences of persistently elevated ACTH. In the opposite vein, a drug targeted against MRAP could block MC2 receptor signaling and be helpful in the management of Cushing's disease.

MRAP is not needed for expression of other melanocortin receptors on the cell surface (Sebag and Hinkle, 2007), but it coprecipitates with them[2]. These results hint that MRAP interacts with multiple receptors. The history of work on RAMPs may be illustrative in this regard. RAMPs were originally found to affect the trafficking and ligand specificity of the calcitonin receptor-like receptor, and subsequently found to affect the calcitonin receptor, then many class B GPCRs, and more recently even class C receptors (Christopoulos et al., 2003; Hay et al., 2006; Parameswaran and Spielman, 2006; Sexton et al., 2006). This experience suggests that MRAP will eventually be found to have a broader spectrum of activities than we currently appreciate.

Acknowledgements

This work was supported by National Institutes of Health grant DK19974 to P.M.H.

[2]J. A. Sebag and P. M. Hinkle, unpublished data.

References

Behrens M, Bartelt J, Reichling C, Winnig M, Kuhn C, Meyerhof W. Members of RTP and REEP gene families influence functional bitter taste receptor expression. J Biol Chem 2006;281:20650–20659. [PubMed: 16720576]

Beltzer JP, Fiedler K, Fuhrer C, Geffen I, Handschin C, Wessels HP, Spiess M. Charged residues are major determinants of the transmembrane orientation of a signal-anchor sequence. J Biol Chem 1991;266:973–978. [PubMed: 1985975]

Bermak JC, Li M, Bullock C, Zhou QY. Regulation of transport of the dopamine D1 receptor by a new membrane-associated ER protein. Nat Cell Biol 2001;3:492–498. [PubMed: 11331877]

Bernier V, Bichet DG, Bouvier M. Pharmacological chaperone action on G-protein-coupled receptors. Curr Opin Pharmacol 2004;4:528–533. [PubMed: 15351360]

Biebermann H, Krude H, Elsner A, Chubanov V, Gudermann T, Gruters A. Autosomal-dominant mode of inheritance of a melanocortin-4 receptor mutation in a patient with severe early-onset obesity is due to a dominant-negative effect caused by receptor dimerization. Diabetes 2003;52:2984–2988. [PubMed: 14633860]

Buckley DI, Ramachandran J. Characterization of corticotropin receptors on adrenocortical cells. Proc Natl Acad Sci U S A 1981;78:7431–7435. [PubMed: 6278474]

Chan LF, Clark AJ, Metherell LA. Familial glucocorticoid deficiency: advances in the molecular understanding of ACTH action. Horm Res 2008;69:75–82. [PubMed: 18059087]

Chida D, Nakagawa S, Nagai S, Sagara H, Katsumata H, Imaki T, Suzuki H, Mitani F, Ogishima T, Shimizu C, Kotaki H, Kakuta S, Sudo K, Koike T, Kubo M, Iwakura Y. Melanocortin 2 receptor is required for adrenal gland development, steroidogenesis, and neonatal gluconeogenesis. Proc Natl Acad Sci U S A 2007;104:18205–18210. [PubMed: 17989225]

Christopoulos A, Christopoulos G, Morfis M, Udawela M, Laburthe M, Couvineau A, Kuwasako K, Tilakaratne N, Sexton PM. Novel receptor partners and function of receptor activity-modifying proteins. J Biol Chem 2003;278:3293–3297. [PubMed: 12446722]

Clark AJ, Metherell LA. Mechanisms of disease: the adrenocorticotropin receptor and disease. Nat Clin Pract Endocrinol Metab 2006;2:282–290. [PubMed: 16932299]

Clark AJ, Metherell LA, Cheetham ME, Huebner A. Inherited ACTH insensitivity illuminates the mechanisms of ACTH action. Trends Endocrinol Metab 2005;16:451–457. [PubMed: 16271481]

Conn PM, Ulloa-Aguirre A, Ito J, Janovick JA. G protein-coupled receptor trafficking in health and disease: lessons learned to prepare for therapeutic mutant rescue in vivo. Pharmacol Rev 2007;59:225–250. [PubMed: 17878512]

Cooray SN, Almiro Do Vale I, Leung KY, Webb TR, Chapple JP, Egertova M, Cheetham ME, Elphick MR, Clark AJ. The melanocortin 2 receptor accessory protein exists as a homodimer and is essential for the function of the melanocortin 2 receptor in the mouse y1 cell line. Endocrinology 2008;149:1935–1941. [PubMed: 18162519]

Cooray SN, Chan L, Metherell L, Storr H, Clark AJ. Adrenocorticotropin resistance syndromes. Endocr Dev 2008;13:99–116. [PubMed: 18493136]

Dwyer ND, Troemel ER, Sengupta P, Bargmann CI. Odorant receptor localization to olfactory cilia is mediated by ODR-4, a novel membrane-associated protein. Cell 1998;93:455–466. [PubMed: 9590179]

Forti FL, Dias MH, Armelin HA. ACTH receptor: ectopic expression, activity and signaling. Mol Cell Biochem 2006;293:147–160. [PubMed: 16845490]

Gantz I, Fong TM. The melanocortin system. Am J Physiol Endocrinol Metab 2003;284:E468–474. [PubMed: 12556347]

Hartmann E, Rapoport TA, Lodish HF. Predicting the orientation of eukaryotic membrane-spanning proteins. Proc Natl Acad Sci U S A 1989;86:5786–5790. [PubMed: 2762295]

Hay DL, Poyner DR, Sexton PM. GPCR modulation by RAMPs. Pharmacol Ther 2006;109:173–197. [PubMed: 16111761]

Imamine H, Mizuno H, Sugiyama Y, Ohro Y, Sugiura T, Togari H. Possible relationship between elevated plasma ACTH and tall stature in familial glucocorticoid deficiency. Tohoku J Exp Med 2005;205:123–131. [PubMed: 15673970]

Kerppola TK. Visualization of molecular interactions by fluorescence complementation. Nat Rev Mol Cell Biol 2006;7:449–456. [PubMed: 16625152]

Kuwasako K, Kitamura K, Nagoshi Y, Cao YN, Eto T. Identification of the human receptor activity-modifying protein 1 domains responsible for agonist binding specificity. J Biol Chem 2003;278:22623–22630. [PubMed: 12684503]

Mandrika I, Petrovska R, Wikberg J. Melanocortin receptors form constitutive homo- and heterodimers. Biochem Biophys Res Commun 2005;326:349–354. [PubMed: 15582585]

Metherell LA, Chapple JP, Cooray S, David A, Becker C, Ruschendorf F, Naville D, Begeot M, Khoo B, Nurnberg P, Huebner A, Cheetham ME, Clark AJ. Mutations in MRAP, encoding a new interacting partner of the ACTH receptor, cause familial glucocorticoid deficiency type 2. Nat Genet 2005;37:166–170. [PubMed: 15654338]

Modan-Moses D, Ben-Zeev B, Hoffmann C, Falik-Zaccai TC, Bental YA, Pinhas-Hamiel O, Anikster Y. Unusual presentation of familial glucocorticoid deficiency with a novel MRAP mutation. J Clin Endocrinol Metab 2006;91:3713–3717. [PubMed: 16868047]

Mountjoy KG, Robbins LS, Mortrud MT, Cone RD. The cloning of a family of genes that encode the melanocortin receptors. Science 1992;257:1248–1251. [PubMed: 1325670]

Noon LA, Franklin JM, King PJ, Goulding NJ, Hunyady L, Clark AJ. Failed export of the adrenocorticotrophin receptor from the endoplasmic reticulum in non-adrenal cells: evidence in support of a requirement for a specific adrenal accessory factor. J Endocrinol 2002;174:17–25. [PubMed: 12098659]

Parameswaran N, Spielman WS. RAMPs: The past, present and future. Trends Biochem Sci 2006;31:631–638. [PubMed: 17010614]

Petaja-Repo UE, Hogue M, Laperriere A, Walker P, Bouvier M. Export from the endoplasmic reticulum represents the limiting step in the maturation and cell surface expression of the human delta opioid receptor. J Biol Chem 2000;275:13727–13736. [PubMed: 10788493]

Roy S, Rached M, Gallo-Payet N. Differential regulation of the human adrenocorticotropin receptor [melanocortin-2 receptor (MC2R)] by human MC2R accessory protein isoforms alpha and beta in isogenic human embryonic kidney 293 cells. Mol Endocrinol 2007;21:1656–1669. [PubMed: 17456795]

Rumie H, Metherell LA, Clark AJ, Beauloye V, Maes M. Clinical and biological phenotype of a patient with familial glucocorticoid deficiency type 2 caused by a mutation of melanocortin 2 receptor accessory protein. Eur J Endocrinol 2007;157:539–542. [PubMed: 17893271]

Saito H, Kubota M, Roberts RW, Chi Q, Matsunami H. RTP family members induce functional expression of mammalian odorant receptors. Cell 2004;119:679–691. [PubMed: 15550249]

Schimmer BP. Adenylate cyclase activity in adrenocorticotropic hormone-sensitive and mutant adrenocortical tumor cell lines. J Biol Chem 1972;247:3134–3138. [PubMed: 4337507]

Schimmer BP, Cordova M, Cheng H, Tsao A, Morris Q. A genome-wide assessment of adrenocorticotropin action in the Y1 mouse adrenal tumor cell line. Mol Cell Endocrinol 2007:265–266. 102–107.

Sebag JA, Hinkle PM. Melanocortin-2 receptor accessory protein MRAP forms antiparallel homodimers. Proc Natl Acad Sci U S A 2007;104:20244–20249. [PubMed: 18077336]

Sebag JA, Hinkle PM. Regions of melanocortin2 receptor accessory protein necessary for dual topology and MC2 receptor trafficking and signaling. J Biol Chem. in press

Sexton PM, Morfis M, Tilakaratne N, Hay DL, Udawela M, Christopoulos G, Christopoulos A. Complexing receptor pharmacology: modulation of family B G protein-coupled receptor function by RAMPs. Ann N Y Acad Sci 2006;1070:90–104. [PubMed: 16888151]

Shaner NC, Campbell RE, Steinbach PA, Giepmans BN, Palmer AE, Tsien RY. Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. Nat Biotechnol 2004;22:1567–1572. [PubMed: 15558047]

Udawela M, Christopoulos G, Morfis M, Christopoulos A, Ye S, Tilakaratne N, Sexton PM. A critical role for the short intracellular C terminus in receptor activity-modifying protein function. Mol Pharmacol 2006;70:1750–1760. [PubMed: 16912219]

Viklund H, Elofsson A. Best alpha-helical transmembrane protein topology predictions are achieved using hidden Markov models and evolutionary information. Protein Sci 2004;13:1908–1917. [PubMed: 15215532]

von Heijne G. The distribution of positively charged residues in bacterial inner membrane proteins correlates with the trans-membrane topology. Embo J 1986;5:3021–3027. [PubMed: 16453726]

Xu A, Choi KL, Wang Y, Permana PA, Xu LY, Bogardus C, Cooper GJ. Identification of novel putative membrane proteins selectively expressed during adipose conversion of 3T3-L1 cells. Biochem Biophys Res Commun 2002;293:1161–1167. [PubMed: 12054497]

Yang Y, Chen M, Kesterson RA Jr, Harmon CM. Structural insights into the role of the ACTH receptor cysteine residues on receptor function. Am J Physiol Regul Integr Comp Physiol 2007;293:R1120–1126. [PubMed: 17596328]

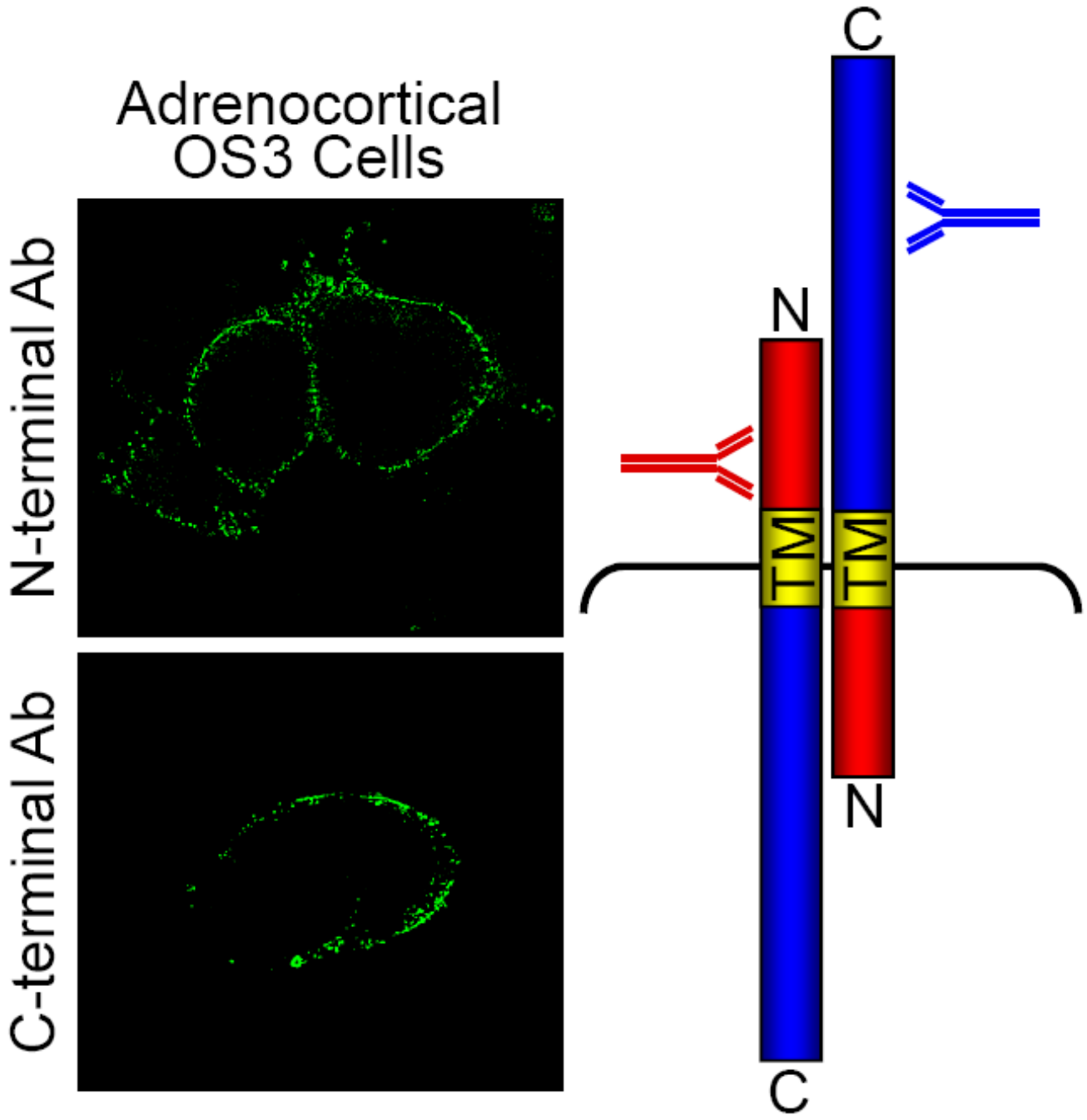

Fig. 1.

Topology of endogenous MRAP. Live OS3 cells, mouse adrenocortical cells that do not express the MC2 receptor (Schimmer, 1972), were stained with affinity-purified rabbit antibodies against the N-terminal (amino acids 18-32) or C-terminal (amino acids 89-108) domains of mouse MRAP, then washed and incubated with fluorescent secondary antibody. Antibodies were added to live cells and could bind only to external epitopes. Images were deconvoluted using Metamorph software. Control experiments showed that intracellular epitopes were not stained in this protocol (Sebag and Hinkle, 2007).

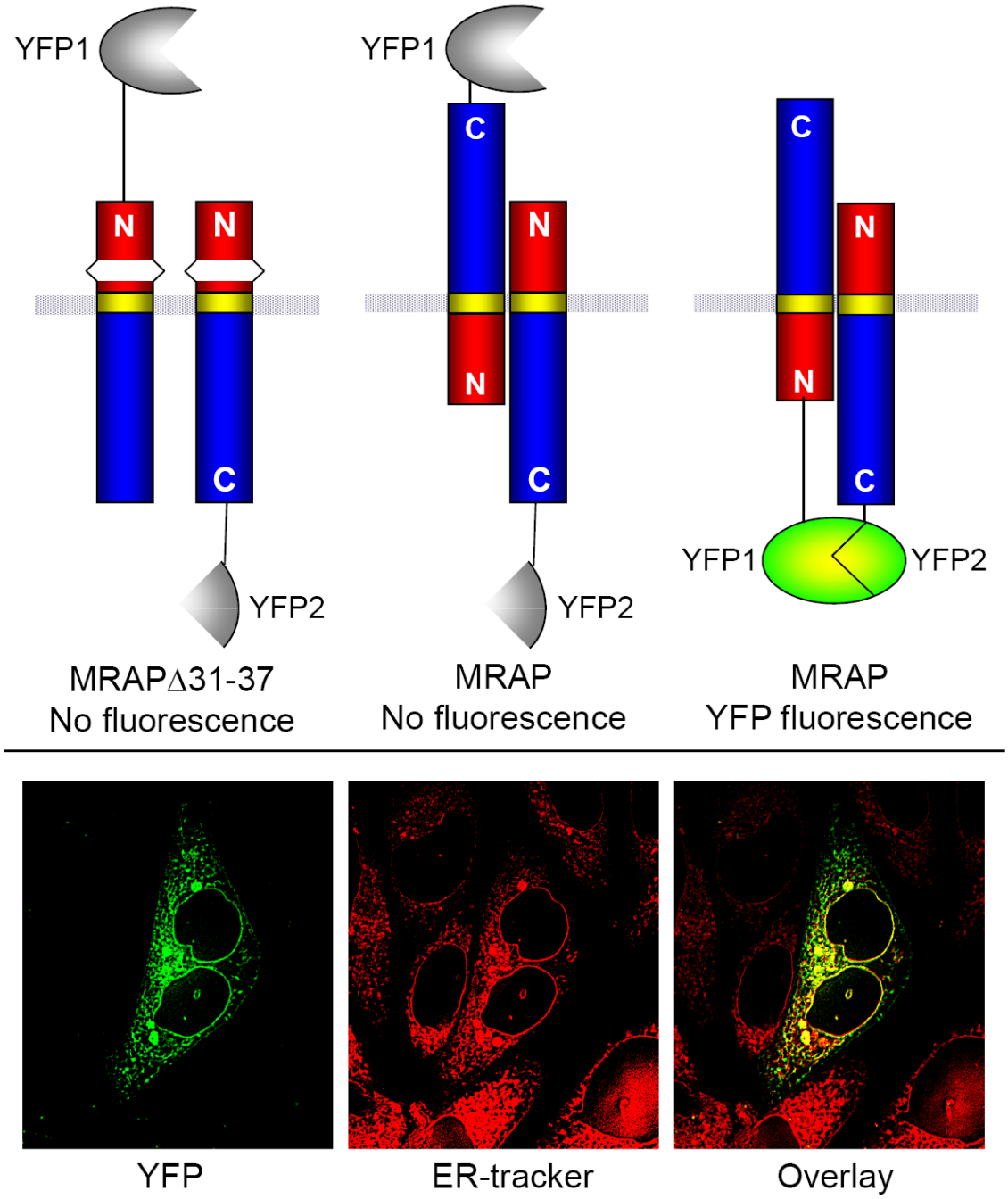

Fig. 2.

Top: Use of bimolecular fluorescence complementation to probe MRAP topology MRAP was fused to fragments of YFP as shown and the proteins expressed in CHO cells. *Bottom*: Cells were transiently transfected with MC2 receptor fused at the aminoterminus to the YFP1 fragment of YFP and MC2 receptor fused at the carboxylterminus to the YFP2 fragment. Cells were incubated with ER-tracker, which labels all cells, and imaged. As shown in Sebag and Hinkle (Sebag and Hinkle, 2008), strong fluorescence was observed in the ER and plasma membrane only when YFP fragments were present on opposite ends of the MRAP molecule.

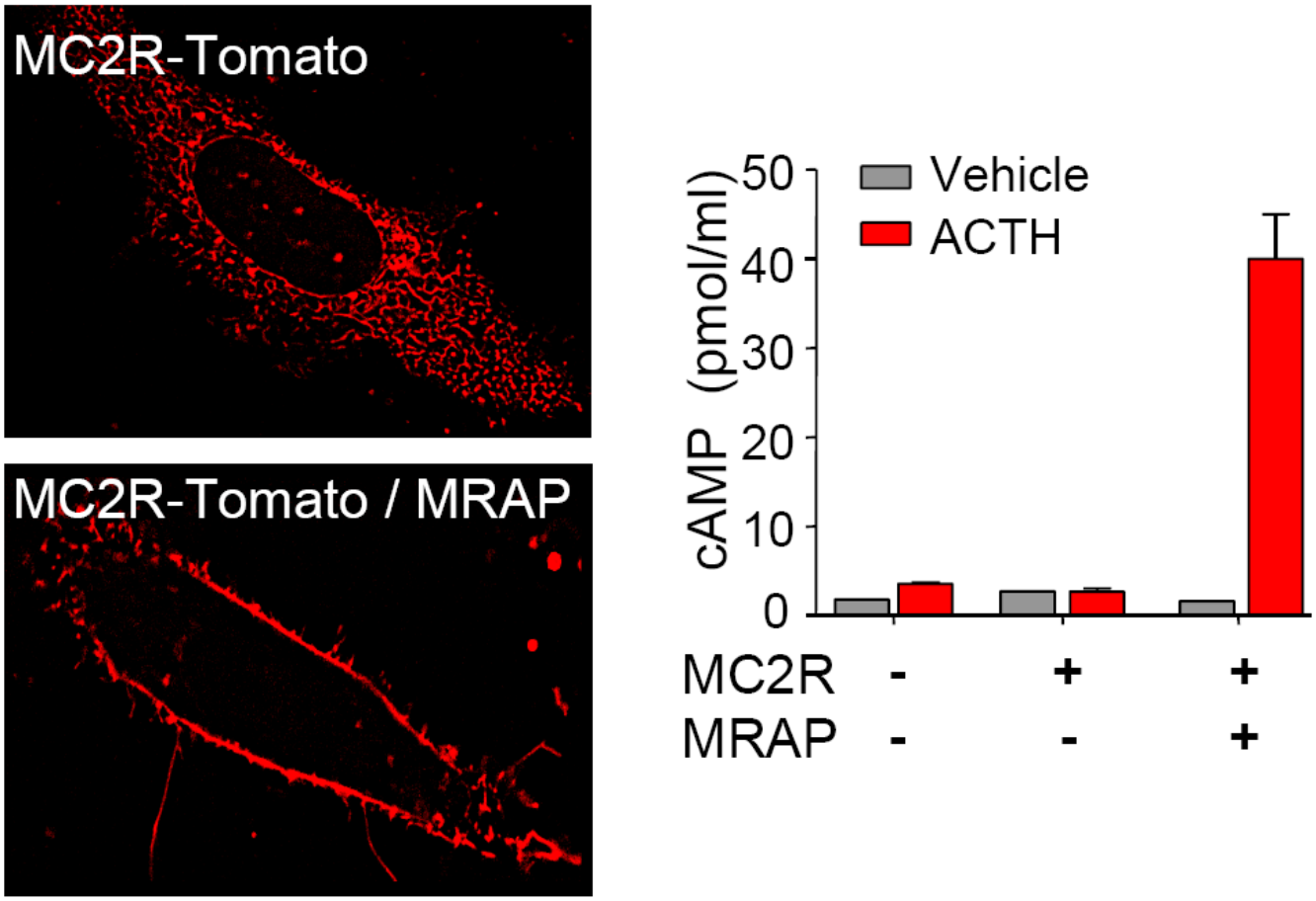

Fig. 3.

Effect of MRAP on MC2 receptor localization. *Left*: MC2 receptor fused at the C-terminus to tandem dimer tomato, a non-dimerizing red protein, was expressed in CHO cells with or without MRAP (Shaner et al., 2004). *Right*: Cells expressing MC2 receptor with or without MRAP were incubated for 20 min with vehicle or 100 nM ACTH, plus 0.1 mM isobutylmethylxanthine, and cAMP measured.

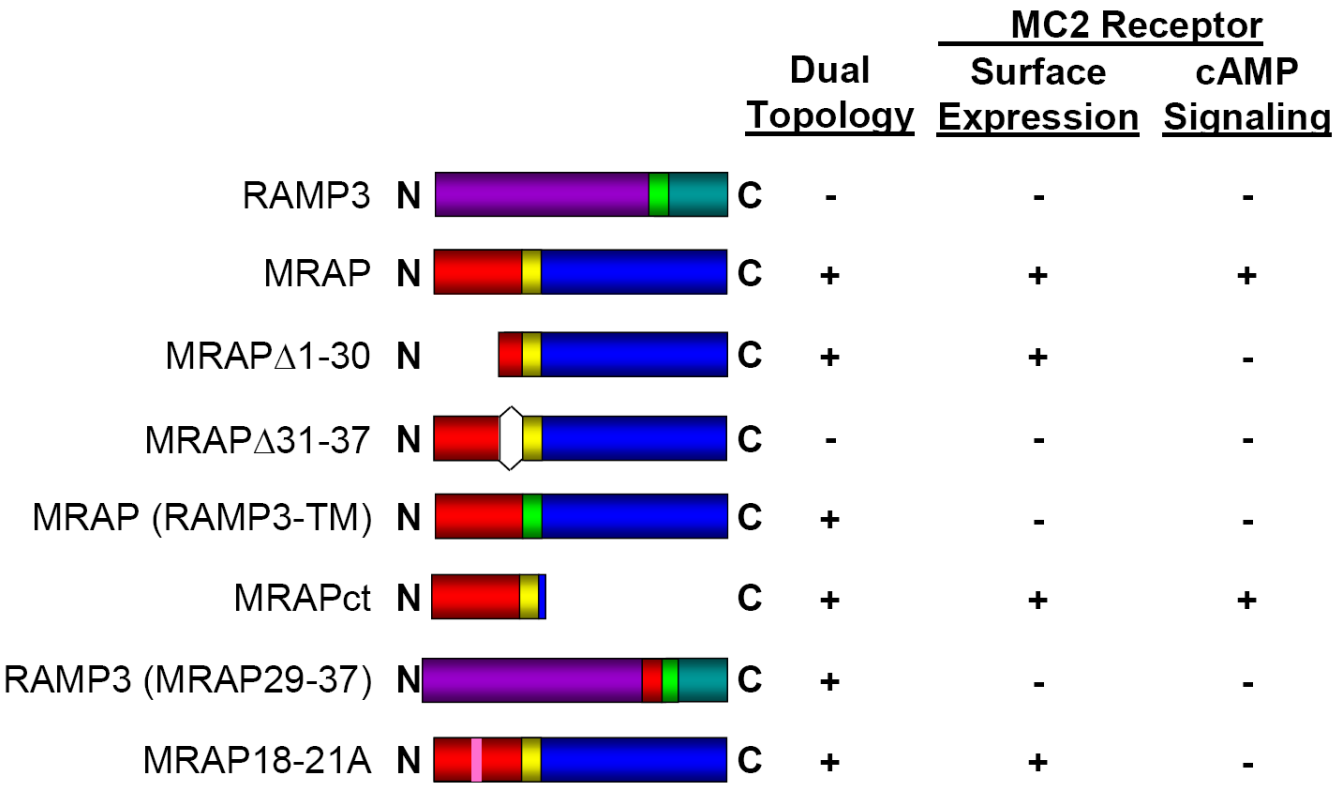

Fig. 4.

Importance of MRAP regions. CHO cells expressing wildtype MC2 receptor and various MRAP and RAMP3 constructs, as shown, were tested for dual orientation at the plasma membrane, surface expression of MC2 receptor and ACTH-stimulated cAMP. Data are summarized from Sebag and Hinkle (Sebag and Hinkle, 2007; Sebag and Hinkle, 2008).

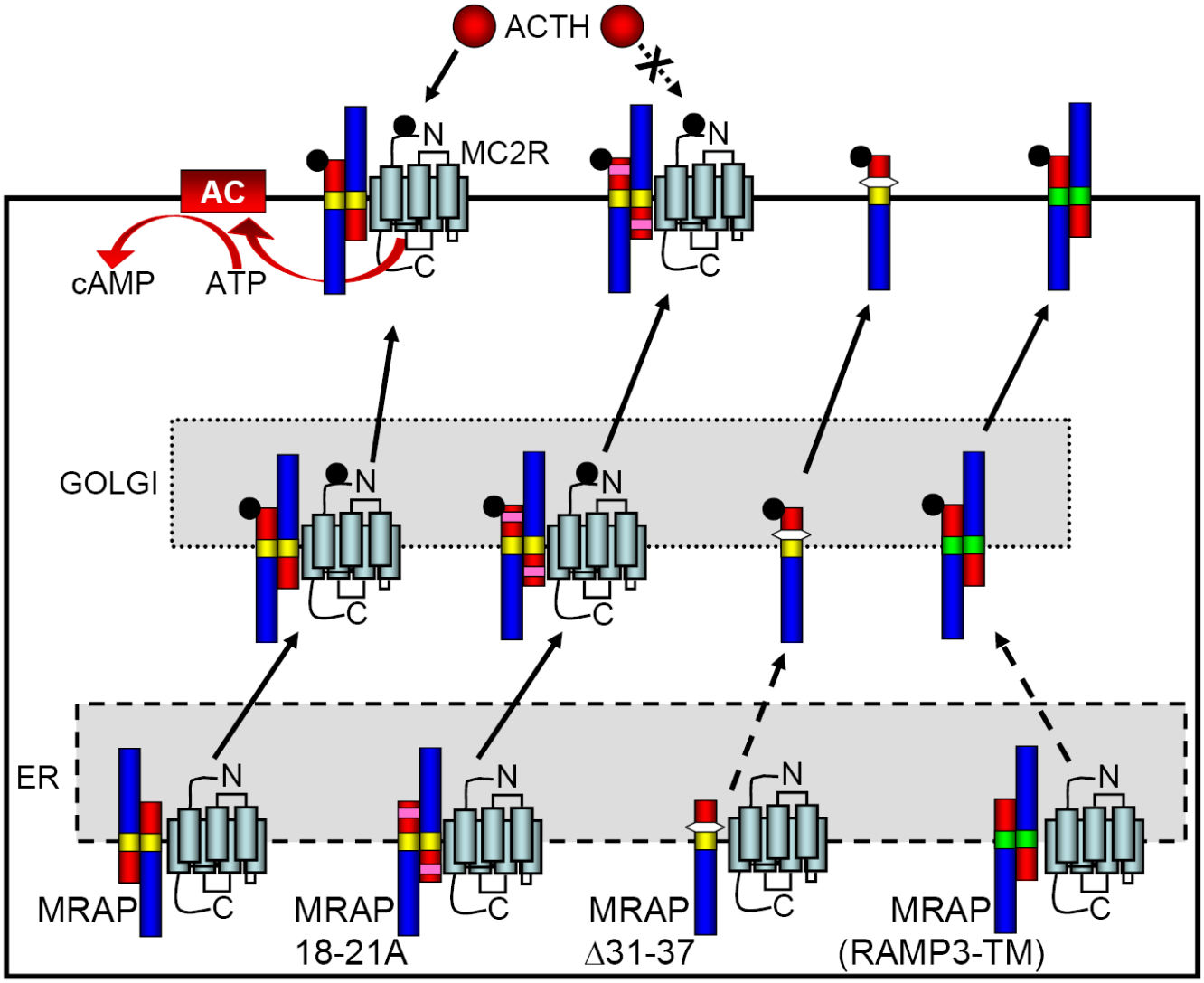

Fig. 5.

Model of MRAP action. Shown is a schematic representation of the two distinct roles of MRAP: facilitating MC2 receptor translocation to the plasma membrane, and permitting MC2 receptor to bind ACTH and signal via Gs to adenylyl cyclase (AC). Black circles depict glycosylated regions. Replacement of amino acids 18-21 (LYDI) with Ala residues yields a protein that promotes receptor trafficking but not signaling. Removal of amino acids 31-37 produces a nonfunctional monomeric protein with an N_{exo} orientation. Replacing the transmembrane domain of MRAP with the corresponding region from RAMP3 gives a protein with dual topology that is unable to facilitate MC2 receptor trafficking or signaling.