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Promiscuity among the MRAPs

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Abstract

The melanocortin 2 receptor accessory protein (MRAP) was originally discovered to be an essential co-receptor for the ACTH receptor/melanocortin 2 receptor, and it physically interacts with this receptor and is required for receptor trafficking and ligand binding. A related molecule, MRAP2, is mainly expressed in the CNS and appears to have a role with the melanocortin 4 receptor. Consistent with this is the observation that a massively obese phenotype develops when the *Mrap2* gene is deleted in mice. However, the characteristics of this phenotype differ from those of *Mc4r* deleted mice, and suggest that an additional role, possibly resulting from an interaction with other receptors is possible. In support of this, a functional interaction with the prokineticin receptors was recently reported. Evidence for other receptor interactions and aspects of the tissue distribution of MRAP and MRAP2 gene expression may indicate that these accessory proteins have a wider role than with the melanocortin receptors alone.

Introduction

Enormous financial and technical efforts are being expended in the drive to develop new and better drugs – many of which will be targeted at G protein-coupled receptors (GPCR). Massive small molecule library screening requires the availability of a target that most closely resembles the physiological target, and yet our understanding of the subtle regulatory factors that may influence these GPCRs is often not adequately represented in these screening procedures. It becomes particularly important therefore that efforts to properly understand any such receptor-associated factors are pursued. This view is well illustrated by considering the case of the melanocortin receptor accessory proteins (MRAPs).

The discovery of MRAP

An intact pituitary-adrenal axis is essential for normal healthy existence in mammals, and yet is surprisingly dependent on a number of unique components encoded by single genes such as the proopiomelanocortin gene and the receptor for adrenocorticotropin (ACTH).

The ACTH receptor – properly known as the melanocortin 2 receptor (MC2R) was cloned in 1992, and it was immediately apparent that it was very difficult to express a functional receptor in transfected cells. In their original paper Mountjoy et al (1992) only reported MC2R expression in a cell line that expressed an endogenous MC1R. Using a green fluorescent protein-tagged MC2R, we demonstrated that the hybrid protein seemed to be retained in the endoplasmic reticulum and failed to reach the cell surface (Noon et al, 2002). Significantly, cell lines derived from the murine adrenocortical tumour Y1 line which had developed unresponsiveness to ACTH action were found to be capable of expressing the transfected MC2R (Yang et al, 1997). This evidence suggested the existence of one or more adrenal-specific accessory factors that were required for receptor expression.

This hypothesis was shown to be correct in 2005 with the identification of a novel genetic cause of human ACTH insensitivity. Metherell et al, 2005 described a number of families in which a gene encoding a small single transmembrane domain protein was mutated. Co-transfection of this gene with the MC2R enabled a fully functional MC2R to be transported to the plasma membrane and to respond to ACTH stimulation by generating a cAMP signal. We named this the melanocortin 2 receptor accessory protein (MRAP) although, as will become apparent, this is occasionally and more helpfully referred to as MRAP1 and this terminology is used henceforth.

MRAP1 was most strongly expressed in adrenal tissues and cells, as well as in the gonad and adipose tissue. Human MRAP1 existed as one of two splice variants – MRAP1 α and β that had distinct 3' ends encoding different C-termini. We found MRAP1 existed as a very stable dimer that was relatively resistant to dissociation by detergents and reducing agents (Cooray et al, 2008). Remarkably, Sebag & Hinkle (2007) demonstrated using a number of techniques that this was an antiparallel homodimer in which one N-terminus was extracellular and the other intracellular. This structure is represented in the Figure. This seems to be a unique phenomenon in eukaryotic biology – although this topology has probably only very rarely been sought in other dimeric proteins.

It now appears that MRAP1 plays several key roles in expression of the MC2R. The MRAP1 dimer complexes with the receptor at the endoplasmic reticulum and this event is required for the receptor to be trafficked to the cell surface. During processing MRAP1 may also influence MC2R glycosylation (Kay et al, 2015). At the cell surface MRAP1 is required for ACTH to generate a G protein mediated signal (predominantly via $G\alpha_s$), and this is probably because the N-terminus of MRAP1 contributes to the recognition and binding of ACTH (Malik et al, 2015).

MRAP2

In our original paper describing MRAP1, we also pointed to the existence of a gene predicted to encode a related protein, which we called MRAP2 on the basis of its relatedness to MRAP1 (Metherell et al, 2005). As with MRAP, MRAP2 also naturally exists as an antiparallel homodimer but with a distinct tissue expression pattern principally in many areas of the CNS (Chan et al, 2009; Asai et al, 2013; Chaly et al, 2016). We demonstrated that MRAP2 interacted with all five of the melanocortin receptors in transfected cells (as does MRAP1) (Chan et al, 2009). MRAP2 supports trafficking of MC2R, although ACTH responsiveness is markedly weaker, such that substantially greater, supraphysiological concentrations of ACTH are required to activate this receptor (Gorrigan et al, 2011). Both MRAPs partially inhibited the signaling of the MC1R, MC3R, MC4R and MC5R (Chan et al, 2009). In the case of the inhibition of the MC5R there is evidence to suggest that MRAP2 inhibits MC5R homodimerisation (Sebag & Hinkle, 2009). Agulleiro et al (2013) demonstrated that the zebrafish MRAP2a, but not the related MRAP2b, was able to increase the responsiveness of Zf MC4R to ACTH without altering the MSH response and it is conceivable that modulation of agonist selectivity by MRAP2 could occur with other receptors or in other species.

Since both MRAP2 and the MC4R are expressed in the paraventricular nucleus of the hypothalamus, where it is well established that the MC4R has a key role in regulating satiety, the possibility that MRAP2 has a part to play in MC4R function and appetite regulation arises. Asai et al (2013) demonstrated that under certain transfection conditions, in which a 6:1 ratio of MRAP2 to MC4R expression vector was used, MRAP2 reduced the constitutive activity of the MC4R and enhanced the maximal effect of α-MSH stimulation. As this results in a greater change in signal for a given change in agonist dosage, this can be interpreted as “sensitizing” the MC4R to agonist. It is notable that the effect on constitutive activity was similar to a non-significant trend previously observed by Chan et al (2009), although this directly contrasted with the opposite effect – an increase in constitutive activity in the presence of MRAP2 - described by Kay et al, 2015. These contrasts should prompt caution in interpreting heterologous cell transient expression studies, particularly when discrepant concentrations of expression vector are required to demonstrate a result.

Arguably, a more physiological examination of this hypothesis is provided by the development of the MRAP2 gene deleted mouse (*Mrap2-/-)* which exhibited a severe obesity phenotype (Asai et al, 2013). A hypomorph *Mrap2* mouse generated using a different strategy and only expressing a fraction of normal *Mrap2* mRNA resulted in a very similar phenotype (Novoselova et al, 2016), as did deletion of *Mrap2* exclusively in the *Sim1* neurones of the paraventricular nucleus – implying that the mechanism was dependent solely on these neurons in the hypothalamus (Asai et al, 2013). These observations are highly suggestive of a vital role of MRAP2 with the MC4R.

However closer examination of the data shows that the phenotypes of the *Mc4r -/-* mice and the *Mrap2 -/-* mice are distinct. In particular, the synthetic MC4R agonist, MTII, is fully effective in *Mrap2 -/-* animals in contrast to its complete inactivity in *Mc4r -/-* mice (Asai et al, 2013), implying that the α-MSH induced satiety pathways are fully functional in *Mrap2* knockout animals. *Mc4r-/-* mice are hyperphagic and show reduced energy expenditure and insulin resistance, whereas no disturbance of food intake, energy expenditure or insulin and glucose regulation was seen in the *Mrap2-/-* mice. In fact male hypomorphic *Mrap2* mice show increased beam breaking activity compared to wild type littermates. Bone mineral density is unchanged in hypomorphic *Mrap2* mice in contrast to increased density seen in *Mc4r-/-* animals (Novoselova et al, 2016). These distinctions are summarized in Table 1.

Thus the evidence seems to suggest that although MRAP2 has a significant role in body weight maintenance, and that this role is delivered through the paraventricular nucleus of the hypothalamus, any influence on MC4R function is probably complicated by a role with other appetite regulating pathways.

Are the MRAPs promiscuous?

Phylogenetic studies demonstrate fairly conclusively that MRAP2 is the ancestral gene, being identifiable in the sea lamprey (*Petromyzon marinus*) Evidence for MRAP first arises at the time of the development of the teleosts (*Takifugu Rubripes*) or possibly in elasmobranchs including the elephant shark (*Callorhinchus milii*), and from this time both genes exist with a key role for MRAP in supporting MC2R function emerging by the time of the evolution of the zebrafish (*Danio rerio*) (Vastermark & Schioth, 2011). It is conceivable that although MRAP seems to have a vital role with the MC2R in mammals, there is no strong evidence that MRAP2 functions are restricted to the melanocortin receptors.

In support of the idea of a broader role for the MRAPs it is notable that Chan et al showed that MRAP2 interacted with the β_2-adrenergic receptor in transfected cells without apparently influencing its signaling capacity, but not with the AT1 angiotensin receptor (Chan et al, 2009). More recently, Chaly et al (2016) reported an interaction between the prokineticin 1 and 2 receptors (PKR1, PKR2) and MRAP2, and an inhibitory effect of MRAP2 when expressed *in vitro* with these receptors (although a 10:1 ratio of MRAP2 to PKR1 or PKR2 expression vector was used). These findings are particularly interesting in the light of the *Mrap2* knockout mouse studies. PKR2 is expressed in the arcuate nucleus and mediates a satiety effect that Chaly et al showed is independent of the MC4R anorectic effect. If MRAP2 has a physiological role in suppressing the PKR1 signal, one might expect the *Mrap2* knockout animal to show a lean phenotype, but it is argued that when this action is compounded with the potent reduction in satiety resulting from impaired MC4R action, the distinct phenotype observed by Asai et al and Novoselova et al results. Attractive as this hypothesis is, some questions remain, such as why the *Mrap2* knockout phenotype seems to arise from the paraventricular nucleus (as shown by the *Sim1* conditional knockout), whereas the PKR1 action occurs in the arcuate nucleus.

Thus evidence is beginning to emerge that MRAP2 may be more promiscuous than originally thought in having a range of receptor partners. Humans with a defective *MRAP1* gene exhibit a very clear adrenal failure phenotype without other consistent clinical problems. However, MRAP1 is expressed in tissues with little or no MC2R expression and the possibility of non-MC2R consequences may have to await the characterization of the *Mrap* -/- mouse.

This evidence of an expanding and more promiscuous role for the MRAPs resembles the way in which the RAMP proteins were initially believed to be calcitonin-like receptor specific accessory proteins, but which are now well-recognized to have a broader range of functions with several members of the Class 2 GPCRs (Hay et al, 2006). Further dissection of the *Mrap2* knockout model, the development and characterization of an *Mrap1* knockout and careful *in vitro* identification of further interacting partners of the MRAPs may reveal novel aspects of physiology, which may be important in supporting efforts to develop drugs that target these pathways.

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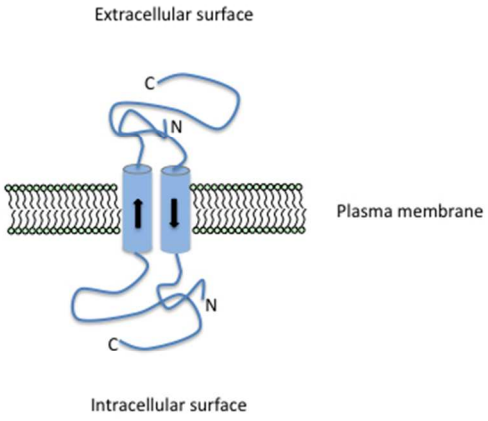
Figure Legend

Schematic representation of the antiparallel topology of the MRAP proteins that result in one N-terminus and one C-terminus being presented on each surface of the plasma membrane for each dimer.

Table 1
Comparison of the metabolic phenotype of the *Mc4r-/-* and *Mrap2-/-* mice.

Parameter	***Mc4r-/-* mouse**	***Mrap2-/-* mouse**
Weight	++++	+++
Hyperphagia	>20% ↑	No change
Energy expenditure	↓	No change
Movement	No change	↑
Insulin	+++ ↑	No change
Glucose tolerance	↑ at 10 - 14 weeks	No difference 12 – 28 weeks
Response to MTII	No response	Reduced feeding - same as wild type
Bone density	↑	No change



Schematic representation of the antiparallel topology of the MRAP proteins that result in one N-terminus and one C-terminus being presented on each surface of the plasma membrane for each dimer.
This structure is represented
254x190mm (72 x 72 DPI)