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RESEARCH HIGHLIGHT

Buckle up! How the nano-seatbelt MRAP1 fastens ACTH in its orthosteric seat

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Specific binding of the adrenocorticotropic hormone (ACTH) to the melanocortin-2 receptor (MC2R) requires the presence of the melanocortin-2 receptor accessory protein 1 (MRAP1) to induce stress responses via adrenal synthesis and secretion of glucocorticoids. In a recent study published in *Cell Research*, Luo et al. determined the cryo-EM structure of the ACTH-bound MC2R in complex with the heterotrimeric G protein G_s and MRAP1, which provides important new insights into the molecular mechanism of accessory protein-dependent regulation of ligand binding and signaling of G protein-coupled receptors.

The adrenocorticotropic hormone (ACTH) plays a central role in the regulation of glucocorticoid synthesis and secretion in the adrenal gland critical for the stress response in humans.[1] Together with the melanocyte-stimulating hormones (MSHs), α-, β- and γ-MSH, ACTH belongs to the melanocortins, a family of neuropeptide hormones that bind and signal through melanocortin receptors (MCRs). Five MCR subtypes have been identified, MC1R–MC5R, that are part of the superfamily of seven transmembrane G protein-coupled receptors (GPCRs).[2] Within the MCR family, MC2R is pharmacologically unique in that it is exclusively activated by ACTH. ACTH binding to MC2R, also referred to as the ACTH receptor, leads to coupling and activation of the heterotrimeric G protein G_s, which triggers cAMP production and subsequent stimulation of steroidogenesis. In contrast, the activity of the other MCR subtypes is not only modulated by ACTH, but also by binding of MSHs, resulting in diverse physiological responses, including pigmentation, energy homeostasis, cardiovascular regulation, inflammation, sexual function, exocrine secretion, and analgesia. Another unique feature of ACTH-mediated signaling via MC2R is its dependency on the melanocortin-2 receptor accessory protein 1 (MRAP1).[3] MRAP1 is a single transmembrane domain (TMD) protein that forms an antiparallel homodimer, which interacts with MC2R to regulate its expression, trafficking, ACTH binding, internalization, and recycling.[4] The physiological importance of this interaction is highlighted by inactivating mutations of the human *MRAP1* and *MC2R* genes that cause familial glucocorticioid deficiency, an autosomal recessive disease characterized by a defect in the ACTH-dependent stimulation of cortisol production in the adrenal cortex.[5] Despite the critical role of ACTH signaling for adrenal function and development, the precise mechanism of MRAP1-dependent ACTH binding to MC2R at the molecular level has remained unresolved.

To address this knowledge gap, Luo et al.[6] determined the structure of the ACTH-bound MC2R–G_s–MRAP1 complex with a global resolution of 3.3 Å using cryo-electron microscopy (cryo-EM) (Fig. 1a). In the structure, the peptide agonist ACTH binds within a large extracellular cavity of the receptor by adopting a U-shaped conformation that forms critical salt bridges, hydrogen bonds, and hydrophobic interactions with residues in the N-terminus, extracellular loop 3 (ECL3) and TM1–4 of MC2R. The 'message' motif (HFRW) of ACTH, which is conserved in all melanocortin hormones and crucial for MCR activation, is located at the bottom of this U-shaped binding pose pointing towards the receptor core (Fig. 1b). Peptide binding is further strengthened by calcium-mediated interactions between TM2 and TM3 of MC2R and ACTH, a structural feature that has been proposed to be of general importance for peptide–MCR interactions.[7]

The overall ligand binding pose of ACTH in the MC2R structure is found to be quite similar to the previously published structures of α-MSH in complex with MC1R and MC4R;[7,8] however, some significant differences are observed that contribute to the ligand binding specificity of MC2R (Fig. 1b). Sequence variations within the ligand-binding pocket and conformational differences in ECL1 between MC2R and the other MCR subtypes seem to lead to a less favorable polar and electrostatic environment as well as steric hindrance for peptide binding. As a result, receptor interactions with the N-terminal region of the bound peptide, which shares an identical amino acid sequence between ACTH (residues 1–13) and α-MSH, are weaker for the MC2R compared to MC1R and MC4R and, therefore, may preclude binding of α-MSH to this MCR subtype. However, in contrast to α-MSH, ACTH can specifically bind and activate MC2R because of its extended C-terminal region, including the 'address' motif (KKRR) that has been previously shown to be essential for agonist binding to MC2R.[9] Interestingly, in the ACTH–MC2R–G_s–MRAP1 complex structure, the C-terminal 'address' motif of ACTH does not interact with the receptor, as previously suggested, but points out of the ligand-binding pocket to form a short anti-parallel β-sheet and salt bridge-mediated interactions with the N-terminus of MRAP1, specifically with the LDYL motif, providing a structural explanation for the critical role of MRAP1 for ACTH binding to MC2R (Fig. 1c). Even though MRAP1 has been described to form an antiparallel homodimer, only the MRAP1 protomer with an extracellular N-terminus forms stable interactions with MC2R to facilitate ACTH binding, in agreement with previous studies.[10] MRAP1 engages ACTH and MC2R exclusively via the highly conserved TMD and the extracellular N-terminal loop. While the TMD of MRAP1 is in contact with TM5 and TM6 of MC2R, the N-terminal loop folds

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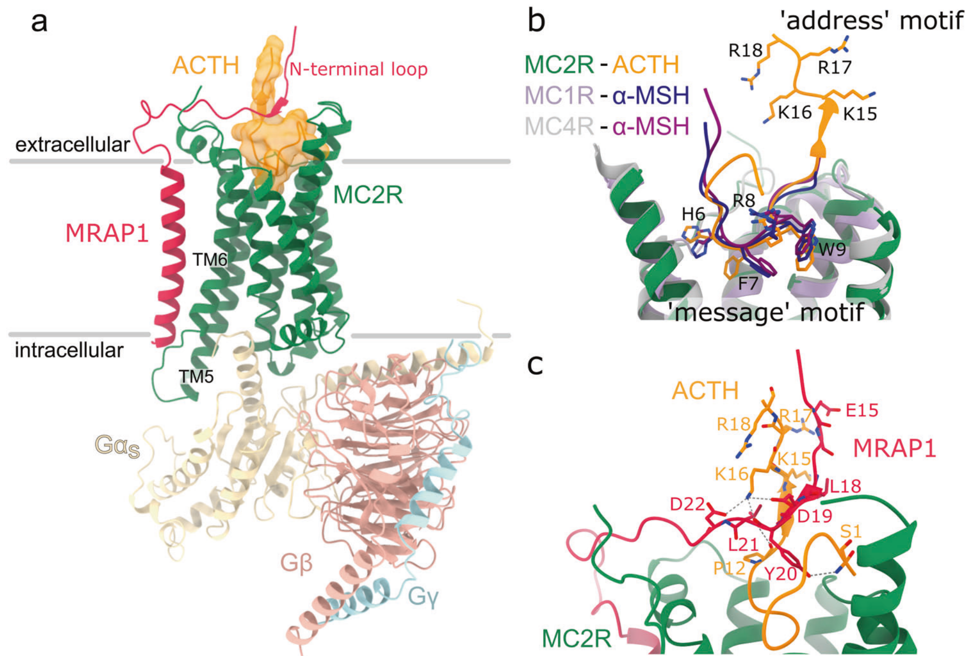

Fig. 1 Structure of the ACTH–MC2R–G_s–MRAP1 complex and the molecular basis of specific ACTH receptor binding. a Ribbon model of the ACTH–MC2R–G_s–MRAP1 complex. MRAP1 (pink) interacts with the extracellular face of the receptor (green) and the agonist ACTH (orange) to secure specific binding of the ligand. **b** Superimposition of the structures of ACTH bound to MC2R and α-MSH in complex with MC1R (PDB: 7F4D) and MC4R (PDB: 7F53), showing a less favorable binding pose of the N-terminal part of ACTH to MC2R that contributes to its ligand binding specificity. **c** Tight interactions between the unique 'address' motif of ACTH (orange) and the LDYL motif of MRAP1 (pink) secures binding of this ligand, but not α-MSH.

over the extracellular face of MC2R and the bound ACTH to reach the 'address' motif of the ligand. Conversely, the less conserved C-terminal part of MRAP1 does not undergo stable interaction with the receptor or the coupled G protein, which is consistent with previous studies showing that truncations of the C-terminus of MRAP1 only cause moderate effects on MC2R trafficking and ACTH binding.[11] Truncations of the entire N-terminal loop, however, has been previously found to diminish ACTH-dependent signaling, suggesting that it functions as a molecular seatbelt that specifically secures binding of ACTH to the MC2R via its unique 'address' motif.[11]

In conclusion, the structure of the ACTH–MC2R–G_s–MRAP1 complex offers new insight into the molecular determinants of the ligand binding specificity of the MC2R and the mechanism of MRAP1-assisted receptor activation. These results set the foundation for the discovery of new therapeutic strategies for diseases caused by an abnormality in the ACTH-MC2R-MRAP1 signaling pathway.

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ADDITIONAL INFORMATION

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