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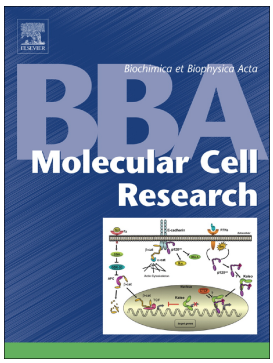

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Marta Abrisqueta, Concepción Olivares, Cecilia Herraiz, María Castejón-Griñán, Julia Sirés-Campos, José C. García-Borrón, Celia Jiménez-Cervantes

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Human Melanocortin 1 Receptor-Mediated Ubiquitination of Nonvisual Arrestins. Role of Mahogunin Ring Finger 1 E3 ligase.

Marta Abrisqueta, Concepción Olivares, Cecilia Herraiz, María Castejón-Griñán, Julia Sirés-Campos, José C García-Borrón and Celia Jiménez-Cervantes‡

Department of Biochemistry and Molecular Biology, School of Medicine, University of Murcia, and Instituto Murciano de Investigación Biosanitaria (IMIB). El Palmar-30120, Murcia, Spain.

‡Corresponding author, celiajim@um.es

Running title: MC1R-dependent β-arrestin ubiquitination

SUMMARY

Signaling from the melanocortin 1 receptor (MC1R), a Gs protein-coupled receptor (GPCR) crucial for melanocyte proliferation and differentiation, is regulated by cytosolic β-arrestins (ARRBs). MC1R signaling is also negatively modulated by the E3-ubiquitin ligase Mahogunin Ring Finger-1 (MGRN1), whose mutation causes hyperpigmentation, congenital heart defects and neurodegeneration in mice. We showed previously that although MC1R interacts stably with human ARRB1 or ARRB2, only ARRB2 mediates receptor desensitization and internalization. We analysed MC1R-dependent ARRB ubiquitination, and the possible role of MGRN1. ARRB1 expressed in heterologous cells or human melanoma cells migrated in SDS-PAGE as a 55 kDa protein whereas ARRB2 migrated as two major bands of apparent molecular weight near 45 and 55 kDa, with an intermediate mobility band occasionally detected. These forms were related by post-translational modification rather than by proteolysis. Presence of MC1R favoured expression of the 45 kDa protein, the form that interacted preferentially with MC1R. MC1R also mediated poly- or multimonoubiquitination of ARRB2. Ubiquitination was agonist-independent, but required a native MC1R conformation and/or normal receptor trafficking to the plasma membrane, as it was not observed for loss-of-function MC1R variants. In a heterologous expression system, MC1R-dependent ARRB ubiquitination was enhanced by overexpression of MGRN1 and was impaired by siRNA-mediated MGRN1 knockdown thus pointing to MGRN1 as the responsible E3-ligase. Co-immunoprecipitation experiments demonstrated interaction of MGRN1 and ARRBs in the presence of MC1R, suggesting a scaffolding role for the GPCR that may determine the selectivity of E3-ubiquitin ligase engagement and the functional outcome of ARRB ubiquitination.