β-arrestin1 Directs Substrate Lysine Selection and Linkage Type Specificity by E3 Ubiquitin Ligases

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β-arrestins are multifaceted adaptor proteins that regulate the desensitization, internalization, and signaling of G protein-coupled receptors (GPCRs). Arrestins interact with many signaling molecules, including E3 ubiquitin ligases, to facilitate ubiquitination of both GPCRs and non-GPCR binding partners. However, β-arrestins are not required for the ubiquitination of the chemokine receptor CXCR4, although they are believed to play a role in the ubiquitination of the non-GPCR interacting partner STAM1. STAM1 is an endosomal sorting protein, and ubiquitination of STAM1 regulates CXCR4 lysosomal trafficking and thus receptor abundance and signaling, yet very little is known concerning STAM1 ubiquitination. Here, we report that β-arrestin1 acts as an adaptor for the ubiquitination of STAM1 and dictates lysine selection and linkage-type specificity. We developed a cell-free, reconstituted system to study in vitro ubiquitination of STAM1 by β-arrestin1 via the E3 ligase AIP4. We provide evidence that β-arrestin1 dose-dependently increases ubiquitination of STAM1 by AIP4. This pattern is not observed when using a β-arrestin1 variant that is unable to bind to STAM1, consistent with β-arrestin1 serving as an adaptor for STAM1 ubiquitination by AIP4. Further establishing this adaptor role downstream of CXCR4 signaling, a pre-activated version of β-arrestin1 mimicking the receptor-bound conformation enhanced STAM1 ubiquitination. To learn more about STAM1 ubiquitination, we set out to define the ubiquitination sites and the type of polyubiquitin chains by mass spectrometry. Mass spectrometry analysis revealed that several lysine residues on STAM1 are modified by ubiquitin with potentially multiple common ubiquitin linkage types. In addition, we identified the potential formation of M1-linked chains, a type of ubiquitin linkage previously only described for one other E3 ubiquitin ligase. Further mass spectrometry and mutagenesis studies provide evidence that β-arrestin1 specifies M1-linked ubiquitin chain formation by AIP4 at Lys136 on STAM1. Using a combination of wild-type and lysine-free ubiquitin, we provide evidence that β-arrestin1 directs STAM1 modification with an anchor ubiquitin moiety at Lys136, which is then subsequently modified with M1-linked polyubiquitin chains. These data provide evidence for the first time that β-arrestin1 coordinates lysine selection and specifies M1-linked polyubiquitination of a substrate by an E3 ubiquitin ligase. This is the first report describing AIP4 conjugating M1-linked ubiquitin chains, making it the second E3 ligase known to conjugate such chains. Our study expands the roles of ubiquitin into GPCR signaling and trafficking.

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