A novel pre-early endosome sorting compartment is essential for spatio-temporal signalling of distinct receptors

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Membrane trafficking of G-protein coupled receptors (GPCRs) represents a key mechanism in defining cellular responses by controlling both the temporal and spatial parameters of cellular signalling. Many receptors internalize via clathrin-coated pits, traffic through signalling endosomes positive for APPL/RAB5 and are targeted to Rab5/EEA1-positive early endosomes (EE), the classic post-endocytic compartment from which internalized cargo are first sorted to distinct cellular fates. Utilising two GPCRs known to undergo clathrin-mediated endocytosis: the luteinizing hormone/chorionic gonadotrophin receptor (LHR) and the beta2-adrenergic receptor (B2AR), we observed that receptors are differentially organized to distinct levels in the early endocytic pathway. The B2ADR readily traffics to EE’s, however, the LHR trafficked to a pre-EE compartment, negative for both RAB5 and EEA1, but in part co-localizing with APPL1. Sequences in the LHR C-tail, containing a PDZ-interacting domain for GIPC, are both necessary and sufficient for its endosomal targeting. TIRF-imaging revealed that GIPC was recruited to the receptor at clathrin-coated pits and then enters endosomes positive for APPL. Loss of GIPC, via siRNA, enabled the LHR to traffic to EE’s but consequently inhibits its recycling. Moreover, by controlling LHR endosomal compartmentalization, GIPC determines the spatial and temporal signalling profile. All together, these results identify that internalized receptors can be sorted at a far earlier level of the endocytic pathway than previously anticipated, and that such organization is a key mechanism in spatio-temporal regulation of receptor signalling.