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A Signal Sequence Is Sufficient for Green Fluorescent Protein to Be Routed to Regulated Secretory Granules

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Abstract:

To investigate trafficking in neuroendocrine cells, green fluorescent protein (GFP) tags were fused to various portions of the preproneuropeptide Y (NPY) precursor. Two neuroendocrine cell lines, AtT-20 corticotrope tumor cells and PC-12 pheochromocytoma cells, along with primary anterior pituitary cells, were examined. Expression of chimeric constructs did not disrupt trafficking or regulated secretion of endogenous ACTH and prohormone convertase 1 in AtT-20 cells. Western blot and immunocytochemical analyses demonstrated that the chimeric constructs remained intact, as long as the Lys-Arg cleavage site within preproNPY was deleted. GFP was stored in, and released from, regulated granules in cells expressing half of the NPY precursor fused to GFP, and also in cells in which only the signal sequence of preproNPY was fused to GFP. Thus, in neuroendocrine cells, entering the lumen of the secretory pathway is sufficient to target GFP to regulated secretory granules.

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