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Review

Determinants for selective transport of exogenously expressed cargo proteins into regulated and constitutive secretory pathways

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ABSTRACT

Background: Newly synthesized secretory proteins are transported in vesicles and are sorted towards their appropriate destinations at trans-Golgi networks. It is thought that secretory proteins have sorting signals in their amino acid sequences that determine their destination. Therefore, many researchers have made efforts to identify these signals, using expression of exogenous cargo secretory proteins in various cell types. In this review, we will discuss these results in light of what will be necessary for future analysis of the sorting mechanisms.
Highlight: Endocrine and exocrine cells have two secretory pathways: regulated and constitutive. Sorting exogenously expressed proteins into these two pathways is dependent not only on the proteins themselves but also on the cell type. The results from in vivo experiments were more complex, where the same protein was delivered to different pathways in different cells.
Conclusion: In order to study the sorting mechanism for secretory proteins, some evaluation of the cells' ability to synthesize, transport, and store the proteins is required. The HaloTag method has proved promising for the quantification of transportation in the two pathways.

Contents

1. Introduction

After synthesis at the endoplasmic reticulum (ER), proteins are delivered to specific destinations where they perform their given functions. How are the destination and transport route determined? Sorting signals have been reported to be present in the amino acid sequence of proteins, such as a nuclear localization signal, an ER retention signal, and a lysosomal targeting signal. These signals ensure that the proteins are delivered to, remain in, and return to their target organelles. In cells that have cellular polarity, glycosylphosphahtydylinositol (GPI)-anchored membrane proteins are transported specifically to apical membranes. Secretory proteins have a signal peptide sequence in their N-terminus for translocation to the ER. The presence of

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Table 1
Destinations of exogenously expressed cargo proteins.

cell type	cell name	origin	cargo protein	stimulation	pathway	reference
constitutively secreting cell	L-cell	fibroblast	pro-insulin		constitutive	[1]
	CHO, COS, 3T3		pre-pro-vWF		constitutive	[3]
	BHK, psi-2	fibroblast	pancreatic polypeptide		constitutive	[2]
	Hepa	hepatocyte	pancreatic polypeptide		constitutive	[2]
	COS-1	fibroblast	pro-vasopressin		constitutive/granule	[4]
	(HepG2,		secretogranin II	A23187	granule-like	[4]
	HEK293, MDCK,		chromatogranin A		granule-like	[5]
	NIH3T3, CHO)		signal peptide + GFP		ER, Golgi	[5]
endocrine	AtT20	pituitary cell	pro-insulin	8-Br-cAMP	granule	[1]
			pre-pro-vWF		granule	[3]
			GP2		multivesicular bodies	[8]
			GP2-GPI-		constitutive	[7]
			amylase		constitutive	[7]
			PRP	8-Br-cAMP	granule	[9]
			amylase, PRP	8-Br-cAMP	granule/constitutive	[10]
			Cab_{308}-Myc	$BaCl_2$	constitutive	[13]
	RIN5F	insulinoma	pre-pro-vWF		granule	[3]
			GP2		multivesicular bodies	[8]
	INS-1	β-cell	signal peptide + GFP		granule > constitutive	[12]
			SEAP		constitutive > granule	[12]
			SEAP	high glucose	granule	[13]
			Cab_{308}-Myc		constitutive	[13]
	PC12	chromaffin	SEAP	$BaCl_2$	constitutive	[11]
exocrine	AR42J	pancreas exocrine cell	pancreatic polypeptide		granule	[2]
			GP2	cholecystokinin	granule	[8]
			GP2-GPI-	cholecystokinin	granule	[7]
			VSV-G		ER	[7]
			SEAP	cholecystokinin	granule	[15]
	parotid acinar primary culture	rat parotid gland	signal peptide + HaloTag	isoproterenol	granule	[16]
			signal peptide + DsRed		granule	[16]
in vivo	submandibular gland	mouse	erythropoietin	pilocalpine	serum	[21]
		mouse, rat	growth hormone	pilocalpine	saliva	[21]
		mouse	parathyroid hormone		serum	[22]
		rat	parathyroid hormone		saliva > serum	[22]
		mouse, rat	IgG Fc	pilocalpine	serum	[24]
	parotid gland	rat	parathyroid hormone		serum	[23]

*pre-pro-vWF: pre-pro form of von Willebrand factor.
*SEAP: secreted embryonic alkaline phosphatase.
*PRP: proline-rich protein.
*GP2-GPI-: GP2 deleted GPI-anchor.
*Cab_{308}-Myc: Cab45 replaced the C-terminal intracellular retention signal with Myc-tag.

a signal peptide sequence is the minimum requirement for secretion into the extracellular space. After entering the ER, secretory proteins are transported by vesicles to the Golgi apparatus and are sorted towards a specific destination at the trans-Golgi networks (TGN). Endocrine, neuroendocrine, and exocrine cells have two secretory pathways to the extracellular space: regulated and constitutive. Some proteins are stored in secretory granules (SGs) where they wait for secretagogues (regulated pathway), while others are secreted immediately after synthesis (constitutive pathway). Many studies have been performed to date (Table 1), but the mechanism that separates the two pathways is not yet clear. In the present paper, we focus on previous studies that analyzed the destinations of exogenously expressed cargo proteins in various types of cells: constitutively secreting, endocrine, and exocrine cells (Fig. 1). From these results, we will discuss the analysis that will be necessary in future studies.

2. Constitutively secreting cells

Cells other than endocrine, neuroendocrine, or exocrine cells have only a constitutive secretory pathway (Fig. 1A). When peptide hormones such as pro-insulin and pancreatic polypeptide are expressed in these constitutively secreting cells, they do not accumulate in the cell and are rapidly secreted [1, 2]. Studies that compared constitutively secreting cells with cells that have a regulated secretory pathway concluded that constitutively secreting cells do not have the ability to process and store peptide hormones. Expression of von Willebrand factor did not promote the generation of SGs or intracellular accumulation of the expressed protein [3]. In contrast, it has been reported that secretogranin II (SgII) and chromogranin A (CgA) induce the formation of granule-like structures in COS-1 cells, which do not naturally have SGs [4, 5]. Members of the granin family are localized to the SGs of endocrine and neuroendocrine cells and are essential for the transport of peptide hormones and neuropeptides. Elevation of intracellular calcium induced by the calcium ionophore A23187 increased the amount of SgII released from COS-1 cells, which supports the conclusion that they are being sorted into the regulated secretory pathway. Green fluorescent protein (GFP) fused to only the signal peptide sequence of SgII did not induce the formation of granule-like structures, and was detected in the ER and Golgi apparatus in COS-1 cells [5]. From these results, it can be concluded that the destination of cargo proteins is dependent on the proteins themselves. Aggregation of cargo proteins at the TGN

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