CHAPTER 7

Protein Sorting in Endosomes

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Abstract

Molecules delivered to endosomes by endocytosis or biosynthetic trafficking can be either recycled to the cell surface, transported to lysosomes, or shunted retrogradely to the biosynthetic pathway. The distinct fates of different endosomal cargo molecules point to the existence of sorting machineries able to distinguish between cargoes. In this review we will highlight recent studies that are beginning to elucidate the endosomal sorting machineries that recognize different cargoes, as well as individual sorting signals that specify their destinations.

Introduction

Endocytosed or biosynthesized molecules that transit through endosomes have several possible destinations (see Fig. 1). They can be either sorted for recycling to the plasma membrane, anterograde trafficking to the degradative lysosomes or retrograde transport to the trans-Golgi network of the biosynthetic pathway. Since different endosomal cargo molecules have distinct itineraries, efficient sorting mechanisms must exist that recognize specific cargoes. Accumulating evidence suggests that all the above-mentioned trafficking routes out of endosomes rely on the recognition of specific cargo sorting determinants by distinct endosomal sorting machineries. Here, we will discuss emerging data that are beginning to shed light on the sorting determinants of endosomal cargo proteins and the machineries that recognize them.

Endosomes As Sorting Stations in Intracellular Membrane Trafficking

The organisation of the endocytic pathway is reviewed elsewhere in this book (Chapter 1). For the purpose of this review, we will distinguish between early endosomes (EEs), recycling endosomes (REs) and late endosomes (LEs). As outlined in Figure 1, recycling to the plasma membrane can either occur directly from the EEs or indirectly via the RE, in processes controlled by the small GTPases Rab4 and Rab11, respectively. Typical examples of recycled membrane proteins include the receptors for transferrin and low-density lipoprotein. In EEs, sorting towards the degradative pathway also takes place, as exemplified by ligand-activated growth factor receptors such as the epidermal growth factor (EGF) receptor. Most membrane proteins destined for LEs and lysosomes are targeted into intraluminal vesicles that invaginate from the limiting membrane of the EE. Sorting to the trans-Golgi network (TGN) can occur from both EEs and LEs, most probably through different sorting machineries (see below).

The architecture of various types of endosomes reflects their specific purposes. For instance, the tubular morphology of REs and the "recycling" part of EEs ensures a high...
Figure 1. Protein sorting in the endocytic pathway of a nonpolarized cell. Upon endocytosis, cargo is transferred to the early endosome (EE). From here, cargo can be sorted for direct recycling to the plasma membrane (A), recycling via the recycling endosome (RE) (B), transport to the trans-Golgi network (TGN) (C) or transport to the late endosome (LE) (D). Sorting to the TGN can alternatively occur from the LE (E). Various trafficking steps are indicated by arrows whose line thickness reflects relative importance.

membrane-to-volume ratio. This favours an enrichment, and thus sorting, of endosomal membrane proteins with respect to soluble content, into transport carriers that leave the endosomal tubules. In this way, small tubules and vesicles that bud from the REs and the tubular regions of the EEs are efficient vehicles for membrane proteins, mostly targeted for recycling to the plasma membrane. Another peculiar geometric feature of endosomes is the invagination of the cisternal part of the endosome membrane to form intraluminal vesicles (see Fig. 1). Since the membrane of such vesicles is more accessible to digestion by lysosomal enzymes than the limiting membrane of LEs (probably due to differences in lipid compositions and lower abundance of highly glycosylated membrane proteins), such vesicles are ideally suited as vehicles for membrane proteins destined for degradation. The molecular machineries responsible for the formation of intraluminal vesicles, and for the sorting of membrane proteins into them, are beginning to emerge, and this will be discussed in the following sections (Table 1).

**Sorting to the Recycling Route**

The transferrin receptor (TfR) has served as a prototypic example of a recycling membrane protein. This receptor is constitutively endocytosed from clathrin-coated pits regardless of ligand binding. Upon reaching the EE, the TfR is very efficiently recycled to the plasma membrane. This recycling occurs both directly and via the RE. The finding that a truncated TfR lacking the whole cytoplasmic tail recycles at the same rate as the wild-type receptor initially led to the conclusion that endocytic recycling is signal independent. However, more recent studies have revealed that multiple receptors, including the TfR, contain bona fide recycling determinants. The TfR has been found to contain two phenylalanine-based signals which, when mutated to alanine, slow down TfR trafficking from and to the RE. These recycling sorting signals interact with ACAPl, a GTPase-activating protein for Arf6, which promotes cargo sorting to enhance TfR recycling. In addition, ACAPl interacts with cellubrevin, another recycling cargo