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# Protein trafficking in immune cells

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## Abstract

The majority of cells of the immune system are specialized secretory cells, whose function depends on regulated exocytosis. The latter is mediated by vesicular transport involving the sorting of specialized cargo into the secretory granules (SGs), thereby generating the transport vesicles; their transport along the microtubules and eventually their signal-dependent fusion with the plasma membrane. Each of these steps is tightly controlled by mechanisms, which involve the participation of specific sorting signals on the cargo proteins and their recognition by cognate adaptor proteins, posttranslational modifications of the cargo proteins and multiple GTPases and SNARE proteins. In some of the cells (i.e. mast cells, T killer cells) an intimate connection exists between the secretory system and the endocytic one, whereby the SGs are lysosome related organelles (LROs) also referred to as secretory lysosomes. Herein, we discuss these mechanisms in health and disease states.


***Keywords:*** Exocytosis; Endocytosis; GTPase; Immune cells; Mast cells; Sorting; Synaptotagmin

## Protein trafficking and immune cell function

Intracellular protein trafficking is a fundamental process in all eukaryotic cells, and even more so in immune cells. This is due to the fact that the essential function of these cells requires protein traffic, specifically regulated secretion, in order to carry out their role in killing target cells or in mediating inflammation. Immune cells secrete different molecules, such as cytokines, chemokines, and lysosomal enzymes, which regulate, among other functions, migration and lytic activity. In addition, fusion of secretory granules (SGs) with the plasma membrane (exocytosis) contributes to the membrane incorporation of proteins, such as the Fas ligand (FasL) that causes death of Fas receptor expressing target cells (Bossi and Griffiths, 1999). In addition, regulated exocytosis plays a crucial role in conversion of inactive, circulating neutrophils to fully activated cells capable of chemotaxis, phagocytosis, and bacterial killing (Faurschou and Borregaard, 2003). Another aspect that stresses the importance of protein traffic in immune cells is the fact that certain mutations or deficiencies, which involve defects in trafficking processes, affect mainly the immune system and the melanocytes, as is indicated by their association with immune syndromes and albinism.

Intracellular protein trafficking is basically divided into trafficking along the secretory and endocytic pathways, both of which are mediated by vesicular traffic. In fact, as will be depicted below, in the specialized secretory cells of the immune system, such as the mast cells and natural killer cells, an intimate connection exists between the endocytic and the regulated secretory pathways.

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In this review, we elaborate on the common mechanisms underlying vesicular traffic along both the secretory and endocytic systems with special emphasis on the mechanisms of protein sorting and regulated exocytosis, which are unique to cells of the immune system.

## The principles of vesicular transport

### Coat proteins

Protein transport in eukaryotic cells is mediated by vesicular traffic. During this process, cytosolic coat proteins are recruited to a donor membrane in order to induce budding of transport vesicles and concentration of cargo molecules within these vesicles. This concentration is mediated by the interaction of the coat proteins with specific sorting signals present within the cytoplasmic tails of the cargo proteins. Protein coats can be classified into three major families (Lewin and Mellman, 1998); (i) the "coatomer"/COPI complex, a heterotetradecameric protein complex responsible for the formation of vesicles in the retrograde transport, from the cis end of the Golgi to the ER, (ii) the COPII complex that mediates the antrograde transport from the ER to the Golgi (Kirchhausen, 1999; Lewin and Mellman, 1998), and finally (iii) the first and best characterized clathrin coat, which involves the assembly of clathrin, a three-legged shaped molecule that consists of three identical 190 kDa heavy chains and three 23–27 kDa light chains (Kim and Kim, 2000). The heavy chain is capable of interacting with a number of proteins, including the adaptor proteins (see below). The light chain has been proposed to regulate the assembly of clathrin triskelions (Mousavi et al., 2004). Recruitment and self-association of clathrin produces a polygonal clathrin lattice, which allows the formation of the coated pits (Traub, 2003). Clathrin coats play major roles in endocytic pathways including receptor-mediated endocytosis from the plasma membrane (Ehrlich et al., 2004), invagination into multivesicular bodies (MVBs) (Raiborg and Stenmark, 2002), mannose 6-phosphate receptor-mediated transport of lysosomal enzymes from the *trans*-Golgi network (TGN) to lysosomes (Poupon et al., 2008) and regulated exocytosis (Tooze and Tooze, 1986).

### Adaptor proteins

Recruitment of the COPI and COPII coat proteins to the donor membrane is mediated by the small GTPases ARF1 and SarI, respectively (Aridor et al., 2001; Donaldson et al., 1992). The assembly of clathrin coats is mediated by a family of clathrin-associated sorting proteins (CLASPs) (Ohno, 2006; Traub, 2005). These families of proteins are characterized by their ability to interact with both clathrin and with the integral membrane proteins, which are to serve as cargo of the clathrin-coated transport vesicles. Various adaptor protein (AP) complexes, differing in their cargo recognition specificities (see below), as well as in their subcellular locations, are used for each type of transport vesicle, thereby providing specificity in cargo packaging and trafficking (Kirchhausen, 1999, 2002; Lewin and Mellman, 1998; Traub, 2005).

The adaptor protein AP-2 was the first and still holds center stage in models of clathrin-dependent endocytosis (Traub, 2003). It consists of two adaptin heavy chains ($\alpha/\beta_2$) of ~100 kDa each, that can bind directly to clathrin, an ~50 kDa medium chain ($\mu_2$), which binds directly to the cargo, and a 20 kDa small chain ($\sigma_2$) (Kirchhausen, 1999, 2002; Lewin and Mellman, 1998; Traub, 2003, 2005). This complex has a 'Mickey Mouse' structure with a core consisting of the $\mu$ and $\sigma$ subunits, flanked by two 'ears' consisting of the carboxy-terminal domains of the two large subunits ($\alpha/\beta_2$), linked by flexible hinges (Robinson and Bonifacino, 2001). AP-2 also binds a number of accessory proteins including synaptotagmins (Syts) (Zhang et al., 1994) and arrestins (Laporte et al., 2000). Three of the subunits take part in clathrin coat assembly, while the role of $\sigma_2$ appears to be mainly structural (Traub, 2003). The $NH_2$ terminus of the AP-2 $\alpha$ subunit binds to PtdIns(4,5)$P_2$ thereby positioning AP-2 on the membrane.

While the AP-2 adaptor complex is associated with the plasma membrane, where it functions in the formation of endocytic clathrin-coated vesicles, its highly related AP-1 adaptor complex mediates protein transport from the TGN to early or late endosomes. AP-1 shares not only a great deal of structural similarity with AP-2, but also sequence homology suggesting conserved roles and interactions within cells and between species. Similar to the AP-2 complex, this adaptor comprises our subunits (~100 kDa $\alpha/\beta_1$, ~50 kDa $\mu_1$ and ~20 kDa $\sigma_1$), with the same characteristic as those of AP-2. A proteomic analysis of AP-1A-coated liposomes identified approximately 40 proteins, which might function in concert with the AP-1 complex to mediate transport. Among these proteins are included the small GTPase Rac1, its effectors, the Wave/Scar complex as well as the Rab family members Rab11 and Rab14 (Baust et al., 2006).

Two additional structurally related adaptors that have been subsequently identified, include the AP-4 complex, which also associates with TGN membranes (Barois and Bakke, 2005; Boehm et al., 2001), and the AP-3 complex.

Same as the AP-1 and AP-2 adaptor complexes, also the AP-3 adaptor complex contains four subunits. The latter include the 100 kDa $\delta$ and $\beta_3$, subunits, a ~50 kDa $\mu_3$ subunit and the 20 kDa $\sigma_3$. This adaptor complex