Molecular basis for the control of motor-based transport of MHC class II compartments

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Chapter 6

Summary and Discussion

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Late endosomal compartments have been in the past dismissively regarded as mere sinks where degradative processes occur. Emerging evidence points to crucial specialized roles played by these organelles in a wide range of cellular processes that go well beyond their conventional role in degradation, including cell signaling transduction and termination, and control of innate and adaptive immunity. Late endosomal compartments include late endosomes, lysosomes and the functionally specialized lysosomal-related organelles (LROs) cytolitic granules, phagosomes, and major histocompatibility complex class II (MHC II)-containing/-enriched compartments (MIICs) among others (reviewed in [1]) .

MHC II antigen presentation (reviewed in Chapter 1) is a prime example of how compartmentalization and functional specialization of the late endosomal route are exploited in complex cellular processes. MHC II molecules are assembled from α and β subunits in the endoplasmic reticulum (ER) to constitute a peptide-binding cleft. They are then chaperoned through the *trans* Golgi network and enter the endocytic pathway, thus gaining access to exogenous antigenic peptides there generated. Facilitated binding of ligand peptides to the peptide-binding groove of MHC II molecules occurs in subdomains of the highly specialized lysosomal organelle best-known as MIIC. Resultant MHC II-peptide complexes are ultimately transported to the plasma membrane for display at the cell surface. The MIICs use the microtubule cytoskeleton and motor proteins of opposite polarities for their intracellular transport. MHC II-peptide complexes that reach the cell surface can be recognized by $CD4^+$ T cells for immune responses to pathogens and tumors.

Most of the research conducted in the field of MHC II antigen presentation has focused on the events leading to the formation of MHC II-peptide complexes inside the specialized MIIC. Consistently, considerable effort has been geared toward the development of strategies to manipulate the generation of MHC II-peptide complexes. Although this has undoubtedly yielded significant results, extending our focus to include other aspects of the cell biology of antigen presentation, such as intracellular transport and trafficking of MHC II molecules, will almost certainly increase the spectrum of targets for therapeutic intervention in autoimmunity or manipulation of presentation of vaccine antigens, as well as the development of innovative modes to manipulate immunity against invasive pathogens and tumors.

The studies described in this thesis aimed at gaining a more advanced level of understanding of the molecular mechanisms that govern the intracellular transport of MIICs and LROs. Furthermore, the finding that cholesterol is, surprisingly, a messenger involved in this, provides a mechanistic explanation for the characteristic phenotypes observed in some lipid storage diseases (reviewed in [2]) like Niemann-Pick type C disease.

Here, a novel molecular mechanism, based on the studies presented in Chapters 2 and 3 of this thesis, is postulated and aims at explaining the complex pattern of intracellular motility exhibited by MIICs. We visualized already in 1996, by time-lapse imaging microscopy, the movement of MIICs carrying GFP-tagged MHC II molecules. These compartments exhibit microtubule-based movement from the Golgi area around the microtubule-organizing center (MTOC) toward the plasma membrane and move characteristically in a so-called bidirectional manner and in a stop-and-go fashion [3]. This is mediated by the alternate activities of the oppositely directed microtubule-based motor proteins dynein (powers microtubule-based

minus end-directed centripetal transport to the MTOC) and kinesins (power microtubule-based plus end-directed centrifugal transport to the plasma membrane) [4]. How the activities of the molecular motors are spatially and temporally coordinated is a long-standing question since.

We propose here that a signaling cascade initiated by the activation of the small GTPase Rab7 is responsible for the control of MIIC intracellular transport. Rab7 activation renders this small GTPase membrane-associated to specify the site for the recruitment of two of its soluble effectors—RILP and ORP1L. We show that the assembled tripartite Rab7-RILP-ORP1L complex is then responsible for governing the activity of the dynein-dynactin motor for centripetal microtubule transport. RILP not only establishes a positive feedback loop by lengthening the presence of Rab7 (and of its own, consequently) on late endosomal membranes through inhibition of the GTPase cycle of Rab7 but also interacts directly with the $p150^{Glued}$ subunit of dynein's adaptor dynactin to recruit this molecular motor to late endosomal membranes. For full activation of transport toward the minus end of microtubules, a second receptor on the surface of late endosomal membranes—βIII spectrin—is required. In an ORP1L-dependent mechanism, the Rab7-RILP-$p150^{Glued}$-dynein complex is targeted to βIII spectrin and centripetal transport ensues. Swift switching of transport direction by polarized motors (dynein and kinesins) is controlled by late endosomal cholesterol content which determines the conformational state of ORP1L. Notably, the conformation of ORP1L dictates the accumulation of ER integral membrane proteins—VAP-A and VAP-B—in late endosomal membranes to control the access of dynein-dynactin to the Rab7-RILP receptor and thus transport and intracellular positioning of MIICs and other LROs.

This model is consistent with the concept of Rab GTPases functioning as key regulators of transport of subcellular compartments by organizing the recruitment of soluble effector molecules, scaffolding proteins, and adaptor proteins to create tightly localized domains on specific membranes, a concept which is emerging as a common theme in the field (reviewed in [5], [6], [7]).

Another emerging paradigm in the field pertains to the role played by GTPases in orchestrating the coupling of each reaction with the next along the endocytic route (reviewed in [6], [8], [9]) in order to maintain directionality and organelle identity in the pathway. In Chapter 5, we propose that the Rab7-RILP-ORP1L complex operating in late endosomal transport also plays a regulatory role in tethering/docking and fusion of late endosomal bilayers downstream of motor-driven transport. We show that Vam6p, a specific tethering/docking factor that promotes fusion of late endosomal organelles [10], is recruited to Rab7-bearing membranes in a RILP-dependent manner. ORP1L controls this, thus influencing fusion of late endosomal compartments. Whether cholesterol and VAPs are also implicated in this mechanism remains to be determined.

Taken together these data suggest that a single RabGTPase-effectors complex—the Rab7-RILP-ORP1L complex—regulates both transport and fusion, thereby conveying spatiotemporal specificity to two consecutive processes in late endosomal trafficking.

While the findings described in this thesis provide novel insights into the regulation of intracellular trafficking of MIICs and LROs, they also raise a series of intriguing questions. This is particularly true for those pertaining to the fine-tuning of these seemingly robust regulatory mechanisms. The findings described in Chapter 4 could serve to illustrate this. We identified and characterized a naturally occurring splice variant of RILP (RILPsv) that competes with RILP for Rab7 but fails to recruit efficiently the dynein-dynactin motor and thus may provide an extra dimension to the control of trafficking. Furthermore, what is the physiological relevance of a

molecule such as cholesterol acting in the control of late endosomal trafficking? The implication of the ER membrane proteins VAP-A and VAP-B in the control of late endosomal cholesterol-dependent processes could hint at a role played by the Rab7-RILP-ORP1L in intercompartmental communication between late endosomal compartments and the ER and thus a possible function in integrating late endosomal trafficking and cellular cholesterol homeostasis (reviewed in [11]). In the ER, cholesterol is enzymatically processed to generate a variety of oxygenated derivatives known as oxysterols. Furthermore, what is the relevance of particular oxysterols, if any, as signaling molecules in the endocytic pathway?

Future studies will certainly address these and other questions and may reveal novel targets for therapeutic intervention in pathologies ascribed to malfunctioning of the endolysosomal system which can include immunity- and pigmentation-related syndromes (reviewed in [1]), as well as those associated to dysfunctions in cholesterol or oxysterol homeostasis (reviewed in [2] and [12]).

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