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

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# Chapter 1

## **Introduction:**

Scope of the thesis

MHC class II molecules on the move for successful antigen presentation

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## Scope of the thesis

Antigen presentation by major histocompatibility complex class II (MHC II) molecules to CD4<sup>+</sup> T-cells is crucial for the adaptive immune system to mount defensive reactions against pathogens. In addition, it is also implicated in the control of cytotoxic T-cell activation, maintenance of self-tolerance, autoimmune responses and other immune responses to pathogens or the environment.

MHC II molecules en route to the cell surface intersect the endocytic pathway where they acquire, in a notably unique and complex series of reactions, immunogenic peptides derived from internalized exogenous proteins. The primary site for antigen loading of MHC II molecules is the specialized lysosomal-related organelle (LRO) known as MHC II-containing compartment or MIIC. Ultimately, the MHC II-peptide complexes are transported for display at the cell surface.

Despite our advanced understanding of many of the mechanisms involved in the control of MHC II antigen presentation, some are still poorly understood. Studying the cell biology of antigen presentation is of crucial importance to reveal novel modes for the manipulation of MHC II-restricted immune responses, particularly those implicated in the pathogenesis of autoimmune diseases. This thesis focuses on the study of the mechanisms governing intracellular transport of MHC II-containing compartments.

**Chapter 1** serves as a general introduction on the role of MHC II antigen presentation in the immune system and on the cell biology of antigen presentation by MHC class II molecules, with an emphasis on the control of intracellular motor-based transport of MIICs.

In **Chapter 2**, we propose a model that aims at explaining how a molecular switch, such as the late endosomal small GTPase Rab7 lies at the heart of the control of microtubular transport of MIICs, late endosomes, lysosomes and LROs, such as early melanosomes, cytolytic granules, and phagosomes. A cascade of linked events, initiated by the activation of Rab7, leads to the assembly of a tripartite specific receptor for the minus end-directed dynein-dynactin motor on the cytosolic face of LROs. Upon activation, Rab7 becomes membrane-associated and recruits its effectors Rab7-interacting lysosomal protein (RILP) and OSBP-related protein 1L (ORP1L) to form the Rab7-RILP-ORP1L tripartite complex. We show that by interacting directly and simultaneously with GTP-Rab7 and a subunit of the dynein-dynactin motor (p150<sup>Glued</sup>), RILP establishes the molecular link between the small GTPase and the dynein motor. However, this appears to not suffice for active transport of LROs toward the minus end of microtubules. Instead, full activation of minus end-directed transport requires the parallel activity of a second receptor for the dynein-dynactin motor on the surface of LROs— $\beta$ III spectrin. In this way, the concerted action of two receptors on the surface of LROs is used to achieve control of minus end-directed transport: firstly, Rab7-RILP acts to specify the target membrane for dynein-dynactin recruitment; subsequently, in a process dependent on ORP1L,  $\beta$ III spectrin functions as a general receptor for the dynein motor.

Whereas this model explains how the small GTPase Rab7 controls minus end-directed transport, it does not suffice to explain the characteristic pattern of motility exhibited by LROs. These subcellular compartments move bidirectionally along microtubules by the alternating actions of kinesin and dynein motor proteins. In **Chapter 3**, we propose that the observed swift mechanism operating as directional switch in microtubular transport of LROs is, surprisingly, not based on the GTPase state of Rab7. Instead, we show that the cholesterol content of LROs determines the conformation of ORP1L which acts as a switch that controls binding of dynein-

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dynactin to its receptor Rab7-RILP. This mechanism regulates the direction of transport and late endosomal positioning. In addition, it may explain how cholesterol accumulation leads to lysosomal clustering at the minus end of microtubules, as observed in Niemann-Pick type C disease.

**Chapter 4** describes the identification and characterization of a naturally occurring splice variant of RILP (RILPsv). RILPsv lacks 27 amino acid residues encoded by exon VII and, although it binds to active GTP-bound Rab7 slowing down its GTPase activity and induces clustering of late endosomal compartments, unlike RILP, it does this independently of efficient direct dynein-dynactin recruitment.

In **Chapter 5**, we look at how the tripartite Rab7-RILP-ORP1L may constitute the mechanistic link that integrates the spatio-temporal control of transport and docking/tethering of LROs, two consecutive processes within the endocytic pathway.

**Chapter 7** summarizes the findings described in this thesis as well as their possible implications.

# MHC class II molecules on the move for successful antigen presentation

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**Major histocompatibility complex class II molecules (MHC II) are targeted to endocytic compartments, known as MIIC, by the invariant chain (Ii) that is degraded upon arrival in these compartments. MHC II acquire antigenic fragments from endocytosed proteins for presentation at the cell surface. In a unique and complex series of reactions, MHC II succeed in exchanging a remaining fragment of Ii for other protein fragments in subdomains of MIIC before transport to the cell surface. Here, the mechanisms regulating loading and intracellular trafficking of MHC II are discussed.**

## Role of MHC II in the immune system

Major histocompatibility complex class II molecules (MHC II) are expressed by immune cells like B cells, dendritic cells (DC), and monocytes/macrophages and designed to stably bind and present fragments from exogenous proteins to the immune system. MHC II present antigens to CD4<sup>+</sup> T helper cells and then control differentiation of B cells in antibody-producing B cell blasts. Patients or mice failing to produce proper MHC II-peptide complexes will not produce efficient antibody-responses to infection (Viville et al., 1993). MHC II are also important to control cytotoxic T cell activation, autoimmune responses and other responses to pathogens or the environment.

MHC II are polymorphic and various MHC II alleles show linkage disequilibrium to a variety of autoimmune diseases. These cannot be linked entirely to the MHC II allele implying further involvement of genetic and/or environmental factors. For example, 95%

of patients with Celiac Disease express an MHC II molecule, HLA-DQ2, present in 25% of the population. The gliadin peptide (a gluten fragment) is selectively presented by HLA-DQ2, which, in addition to unknown factors, causes this disorder ([www.enabling.org/ia/celiac](http://www.enabling.org/ia/celiac)). Studying the cell biology of antigen presentation by MHC II is of crucial importance to identify these factors or reveal modes for controlling MHC II antigen presentation.

## How MHC II acquire peptides in the endocytic route?

Antigen loading of MHC II occurs in the endocytic pathway at a site that is commonly known as MIIC (for 'MHC class II-containing compartment') (Neefjes et al., 1990). MHC II assemble as heterodimers in the endoplasmic reticulum (ER) to form a peptide-binding groove (Brown et al., 1993). Efficient ER egress of MHC II is assisted by the invariant chain (Ii) (Bikoff et al., 1993; Viville et al., 1993). An

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Ii region called CLIP occupies the peptide-binding groove, thereby preventing premature peptide binding (Roche and Cresswell, 1990). Ii also contains a cytosolic di-leucine targeting motif that directs MHC II complexes into the endocytic pathway, either directly from the trans-Golgi network or—if this fails—via rapid internalization (Bakke and Dobberstein, 1990; Roche et al., 1993). After having guided MHC II to MIIC, Ii is degraded by various late endosomal proteases, including cathepsin S and L, to prepare MHC II for peptide loading. Inhibition of these proteases will prevent MHC II antigen presentation, immune responses (Riese and Chapman, 2000) but also cell surface expression (Neeftjes and Ploegh, 1992). Consequently, inhibitors for cathepsin S are currently developed for the treatment of autoimmune diseases (Vasiljeva et al., 2007). The proteases degrade Ii in a stepwise fashion leaving the CLIP fragment occupying the peptide-binding groove. The resulting MHC II complex does not contain relevant antigenic information for the immune system. Exchange of CLIP for such antigenic fragments is facilitated by low pH, proteolytic trimming of the CLIP peptide, and by a unique chaperone called HLA-DM, which is surprisingly an MHC II look-alike (Mosyak et al., 1998). HLA-DM is a dedicated chaperone (only target known: MHC II) in a compartment where other proteins are usually degraded. HLA-DM stabilizes MHC II devoid of peptides, preventing aggregation and supporting peptide exchange until a high-affinity-binding peptide is acquired (Denzin et al., 1996; Sloan et al., 1995). HLA-DM is thus editing the MHC II peptide repertoire (Kropshofer et al., 1996). But the reaction is more complicated. The interaction between MHC II and HLA-DM occurs in subdomains of the MIIC (the intraluminal vesicles) and not at the limiting membrane as determined by FRET studies (Zwart et al., 2005). Consequently, MHC II fails to acquire antigenic peptides in phagosomes containing intracellular bacteria

as these lack intraluminal vesicles (Zwart et al., 2005). Possibly, microdomains like those formed by members of the tetraspanin family of proteins (the tetraspanin web) residing in the intraluminal vesicles of the MIIC and interacting with MHC II, HLA-DM, and other proteins (Hammond et al., 1998) play an additional role in efficient peptide loading of MHC II.

Whether loading of MHC II with high-affinity peptides is a prerequisite for transport from MIIC to the plasma membrane is unlikely. Endosomes may not have a sophisticated 'quality control system' like the ER that allows the egress of properly folded proteins only, since CLIP exchange by HLA-DM is not required for cell surface expression of MHC II (Fung-Leung et al., 1996; Martin et al., 1996). Proper expression levels of HLA-DM, transport of MHC II and HLA-DM to internal vesicles in MVB, transit time of MHC II through the MIIC, proteolysis of antigen and Ii, and delivery of antigenic fragments (by diffusion?) to MHC II probably ensure that the system suffices to efficiently load MHC II in transit through the MIIC.

### Definition of the MIIC

The exact definition of THE MIIC as the site of MHC II peptide loading has been a matter of debate. Originally, the MIIC was defined based on immuno-electronmicroscopy studies as a late endosome (LE) with multilamellar morphology containing MHC II (Peters et al., 1991). MHC II was subsequently found in many different compartments with distinct morphologies and its expression in HEK 293 cells even induced the multilamellar morphology (Calafat et al., 1994). Thus, neither morphology nor the presence of MHC II can define THE MIIC. Other factors required for efficient loading of MHC II include acidic pH (Ziegler and Unanue, 1982), HLA-DM and proteases like cathepsin S and L (Honey and

Rudensky, 2003). Electronmicroscopy showed that these locate in LEs that label for the conventional markers Lamp-1 and CD63.

Is the MIIC then a unique compartment or a LE expressing additional proteins for MHC II antigen presentation? Eliminating MHC II, cathepsin S or HLA-DM still shows LEs labeling for the conventional markers, indicating that MHC II-related proteins are not critical in this compartment. In addition, LEs lacking MHC II are difficult to detect in cells expressing MHC II. MIIC appears to be a LE with the components for efficient MHC II loading. Still, loading of MHC II at nearly every location of the endocytic route is reported. Since HLA-DM is transported in the MIIC to the plasma membrane along with MHC II (Wubbolts et al., 1996), loading may even be supported by HLA-DM at the plasma membrane (Moss et al., 2007), albeit at neutral pH and without proteases for antigen preparation. Moreover, HLA-DM contains a classical tyrosine-based internalization motif and will be internalized, thus entering early endosomal compartments in transit to MIIC. In principle, HLA-DM support in MHC II loading can occur whenever protein fragments are present, although the late endosomal MIIC likely is the primary site for antigen loading of MHC II, since it congregates all known components for efficient peptide loading.

### **Further control of MHC II antigen presentation**

The complex process of MHC II antigen presentation is further complicated by additional factors. Immature B cells express an HLA-DM homologue called HLA-DO (Liljedahl et al., 1996). This non-polymorphic MHC II-like molecule stably interacts with HLA-DM and acts as a pH sensor to preferentially stimulate presentation of antigens entering the more acidic LEs at the cost of normal HLA-DM functioning, paradoxically resulting in MHC II-CLIP complexes and reduced immune responses

(Denzin et al., 1997; van Ham et al., 1997). Other factors involved in MHC II presentation are more related to the control of protein targeting to MIIC or the control of proteolysis. Antibody-bound proteins can be recognized by Fc receptors for uptake, transfer to MIIC and degradation. Analogously, surface Ig receptors on B cells can specifically recognize and target antigens to LEs for degradation, which also affects the specificity of antigen proteolysis (Davidson and Watts, 1989). Alterations in proteolytic conditions contribute to the success of MHC II antigen presentation as well. In classic experiments, neutralization of acidic compartments inhibited MHC II antigen presentation, implying lysosomal proteases in antigen presentation (Ziegler and Unanue, 1982).

Some late endosomal proteases are critical in MHC II antigen presentation. Cathepsin-S- and -L-deficient mice have reduced Ii degradation and antigen presentation (Nakagawa et al., 1998; Shi et al., 1999). To complicate matters, naturally occurring inhibitors of lysosomal proteases, called cystatins, can also exert a regulatory role. Overexpression of cystatin C inhibits the activity of cathepsin S, and consequently, Ii degradation and MHC II cell surface expression in DC (Pierre and Mellman, 1998).

Finally, control of MHC II antigen presentation by interleukins and Toll-like receptors (Blander and Medzhitov, 2006) occurs in particular cell types. The 'immunosuppressive' interleukin IL-10 prevents MHC II cell surface expression in human monocytes (Koppelman et al., 1997) whereas interferon- $\gamma$  enhances MHC II expression and presentation.

Proteases, protease inhibitors, protease conditions and substrate delivery are all factors contributing to the efficiency and specificity of MHC II antigen presentation and therefore represent attractive targets for manipulating immune responses. In addition, motor proteins, kinases, GTPases and possibly other



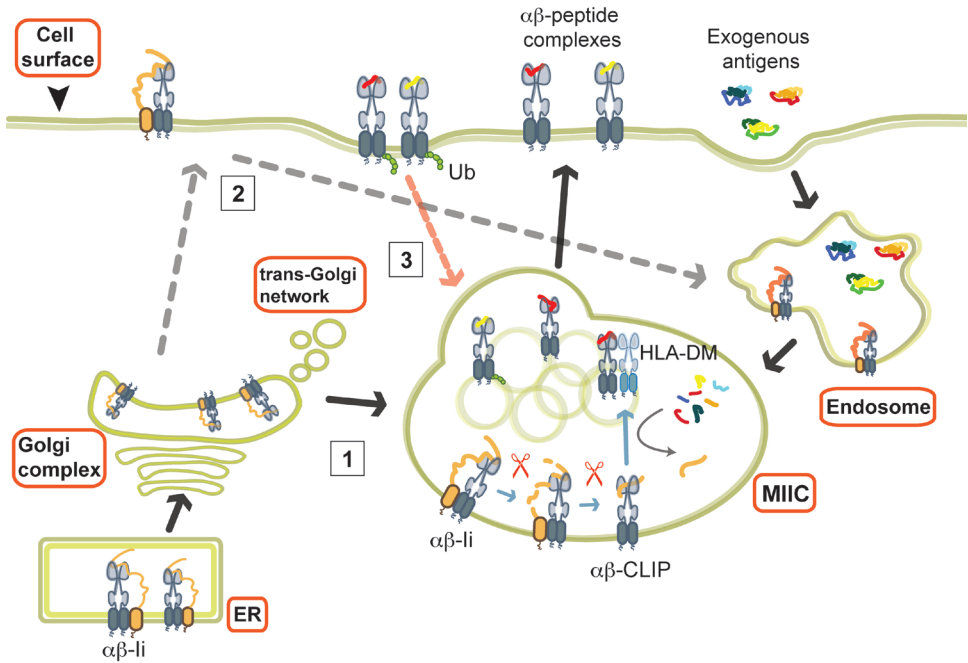


Figure 1. The cell biology of antigen presentation by MHC II. MHC II  $\alpha\beta$  heterodimers are assembled in the endoplasmic reticulum (ER) and form a peptide-binding groove that is occupied by Ii. Ii chaperones MHC II often directly (route 1; black solid arrows) and sometimes indirectly after internalization from the cell surface (route 2; grey dashed arrows) into MIIC where Ii is degraded by a series of endosomal proteases with the CLIP fragment remaining (orange). HLA-DM assists exchange of CLIP for relevant exogenous antigenic fragments (red or yellow) in subdomains of MIIC (the internal vesicles) prior to transport for stable integration in the plasma membrane (blue arrows in MIIC) unless internalization is induced by processes like ubiquitination (Ub) of the MHC II  $\beta$ -chain cytoplasmic tail (route 3; pink dashed arrow).

signaling systems control MHC II presentation. These include the actin-based motor protein myosin II that interacts with Ii following B cell receptor activation and is essential for antigen presentation (Vascotto et al., 2007), and GTPases of the families Rab and Rho (Ghittoni et al., 2006). We are only beginning to grasp the complexity of regulating MHC II antigen presentation.

### How to move MHC II to the plasma membrane?

Trafficking of late endosomal proteins, including MHC II, to the plasma membrane

is poorly understood. LEs may not have the machinery for the selective sorting of molecules and the appearance at the plasma membrane of many late endosomal proteins is followed by efficient internalization and transport back to LEs. Ii contains the targeting motif for MHC II. Since degradation of this motif occurs in the MIIC, MHC II remains stable at the plasma membrane upon delivery, unless internalization is supported for example by its ubiquitination (Shin et al., 2006; van Niel et al., 2006).

Transport of GFP-tagged MHC II has been studied in tissue culture cells (Wubbolts et al., 1996) B cells and mouse DC (Boes et al., 2002; Chow et al., 2002). We visualized MIIC with GFP-

tagged MHC II exhibiting the canonical motility of LEs. These two similar compartments move in a so-called bidirectional manner and in a stop-and-go fashion along microtubules to the plasma membrane (Wubbolts et al., 1996). This required the activities of oppositely directed motor proteins; dynein (powers transport to the microtubule-organizing center) and kinesin (powers outward transport) (Wubbolts et al., 1999). Ultimately, MIIC fuses to the plasma membrane (Raposo et al., 1996; Wubbolts et al., 1996).

An additional route for the transport of MHC II to the plasma membrane has been observed in activated DC. Upon activation, DC upregulate surface expression of MHC II from intracellular storages and tubular structures emanating from the MIIC and containing MHC II are formed (Boes et al., 2002; Chow et al., 2002; Kleijmeer et al., 2001). Live-imaging revealed that these tubules exhibit dynamics similar to MIIC, including bidirectional microtubule-based movement in a stop-and-go fashion (Vyas et al., 2007). Since immature DC, B cells and melanoma do not show these tubules but do express MHC II at the plasma membrane, tubules may be an activated DC-selective route for the transport of MHC II to the cell surface.

How MIIC (and possibly tubules) fuse to the plasma membrane is unclear. It probably requires the activities of Rab GTPases, actin-based motor proteins and actin depolymerizing factors, analogously to the situation for other specialized lysosome-related organelles such as cytolytic granules and melanosomes (Jordens et al., 2006; Raposo et al., 2007).

### Two collaborating receptors for one or more motor proteins on MIIC

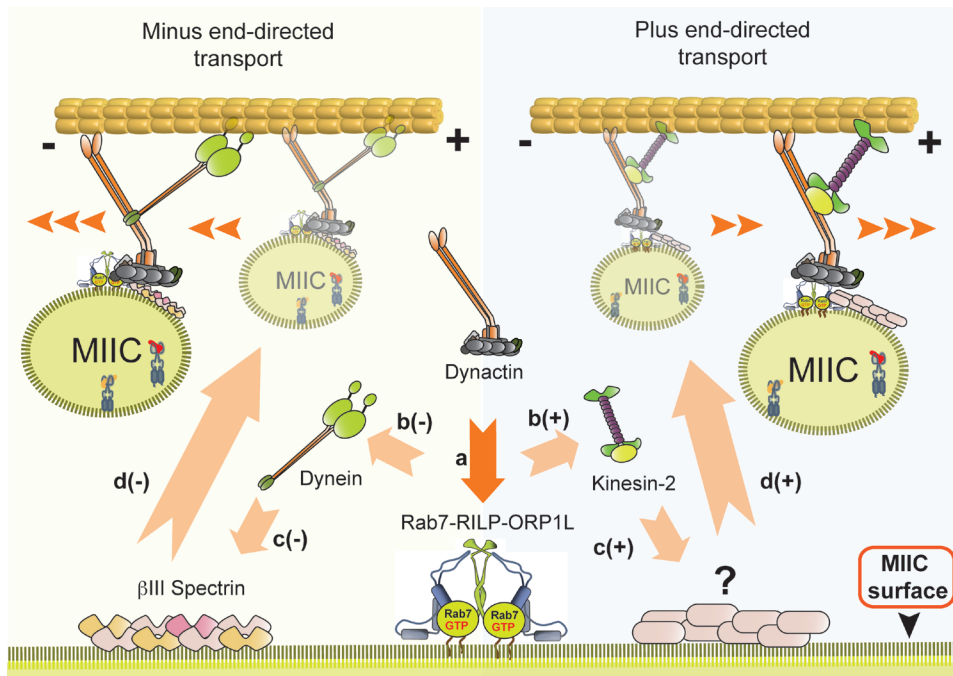
Rab7 is a small Rab GTPase decorating membranes of MIIC and other late endocytic structures (Chavrier et al., 1990; Meresse et al., 1995; Wubbolts et al., 1996). Activated

Rab7 specifies the target membrane for dynein recruitment through an interaction of its effector Rab7-interacting lysosomal protein (RILP) with the p150<sup>Glued</sup> subunit of dynactin, a critical component of the dynein motor complex (Johansson et al., 2007). RILP expression promotes inward-directed dynein-mediated transport of MIIC/LEs to the microtubule minus-end (Jordens et al., 2001).

The Rab7-RILP complex interacts with a second effector protein—OSBP-related protein 1L (ORP1L)—to form a tripartite complex on lysosomal membranes. ORP1L is required to transfer the dynein/dynactin motor complex from the specific lysosomal receptor Rab7-RILP to a general receptor termed  $\beta$ III spectrin (Johansson et al., 2007).  $\beta$ III spectrin is located on the cytosolic side of multiple compartments and can interact, via its actin-binding domain (ABD), with actin-related protein 1 (Arp1) at the base of dynactin (Karki and Holzbaur, 1999). The dynein motor only becomes active after consecutive interactions with these two membrane-associated receptors: the LE-specific receptor Rab7-RILP and the general receptor  $\beta$ III spectrin (Johansson et al., 2007) (Figure 2).

The bidirectional nature of vesicle movement implies that, in addition to the inward-directed dynein motor, at least one outward-directed motor is involved. Two members of the kinesin superfamily of motors may be involved in outward-directed motility of LEs along microtubules. Kinesin-1 (conventional kinesin or KIF5) but also kinesin-2 (heterotrimeric kinesin or KIF3) have been implicated (Hollenbeck and Swanson, 1990; Wubbolts et al., 1999).

How do motors of opposite polarity cooperate to achieve bidirectional motility? They may be reciprocally coordinated and not act simultaneously on one individual vesicle. *Xenopus* melanophores as well as *Drosophila* fast axonal cargoes and lipid droplets use dynactin (or its subunit p150<sup>Glued</sup>) to interact with dynein



**Figure 2.** Reciprocal coordination of motor proteins for bidirectional microtubule-based MIIC transport. Left: control of inward transport of MIIC toward the microtubule minus-end. Right: control of plus end-directed transport of MIIC to the cell periphery. Activation of Rab7 precedes formation of the tripartite Rab7-RILP-ORP1L complex. RILP interacts with the dynactin subunit p150Glued (a). Dynactin then interacts either with dynein [b(-)] or kinesin-2 (KIF3) [b(+)] motor proteins, specifying the direction of vesicle transport. Motor activity requires binding to a second LE membrane receptor,  $\beta$ III spectrin [c(-)]. Full activation of kinesin-2 may require a similar interaction with a general receptor on MIIC [c(+)]. In this model, the p150<sup>Glued</sup>-associated type of motor specifies the direction of MIIC transport (d).

and KIF3 motors in a mutually exclusive manner (Deacon et al., 2003). Furthermore, disruption of the dynactin complex by overexpressing p50<sup>dynamitin</sup> (Burkhardt et al., 1997) inhibits both minus- and plus-end motility (Deacon et al., 2003). The dynactin subunit p150<sup>Glued</sup> may be the adaptor for KIF3 and dynein on LEs (Brown et al., 2005; Deacon et al., 2003). Thus, the bidirectionality of MIIC movement may be accomplished by alternating interactions of p150<sup>Glued</sup>-dynein and p150<sup>Glued</sup>-KIF3 motor complexes with a single Rab7-RILP receptor on MIIC that likely employs  $\beta$ III spectrin in both cases (Figure 2). The interaction of Rab7-RILP with p150<sup>Glued</sup> (the common motor adaptor for

dynein and kinesin) would then be at the heart of the bidirectionality of MIIC motility.

The control of motor activities and motor-receptor binding may involve kinases, lipids, the Rab7 GTPase cycle, IL-10 signaling, JNK-interacting proteins (JIPs), and undoubtedly many other factors. How these factors control the motility of MIIC and how these factors are subsequently controlled remains to be determined.

Antigen presentation by MHC II incorporates activities like late endosomal proteolysis of Ii and antigen, regulation of late endosomal morphology and pH, and intracellular transport. Further identification

of molecules involved in controlling these processes should provide targets for further manipulation of MHC II-restricted immune responses, particularly those resulting in autoimmune responses.

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