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MHC class II molecules on the move for successful antigen presentation

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Major histocompatibility complex class II (MHC II) molecules 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.

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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 (DCs), and monocytes/macrophages and designed to stably bind and present fragments from exogenous proteins to the immune system. MHC II present antigens to $CD4^+$ 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). 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.

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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 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) and also cell surface expression (Neefjes 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 is 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 (Sloan *et al*, 1995; Denzin *et al*, 1996). 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 (Figure 1).

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 an 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 an 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 homolog 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).

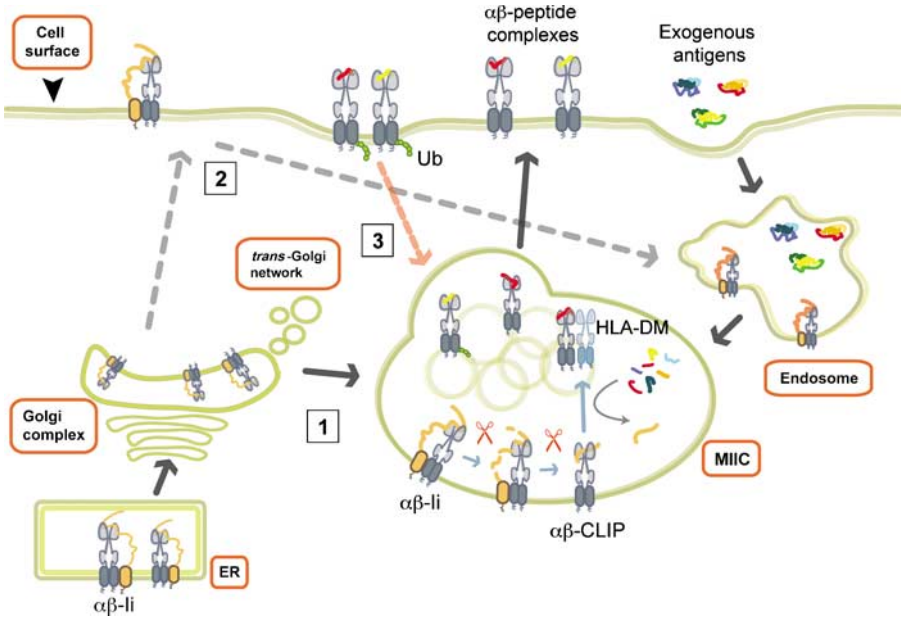

Figure 1 The cell biology of antigen presentation by MHC II. MHC II αβ heterodimers are assembled in the endoplasmic reticulum (ER) and form a peptide-binding groove that is occupied by Ii. Ii chaperones MHC II often directly (route 1; black solid arrows) and sometimes indirectly after internalization from the cell surface (route 2; gray 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 β-chain cytoplasmic tail (route 3; pink dashed arrow).

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-γ 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 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 of many late endosomal proteins at the plasma membrane 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 DCs (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, DCs upregulate surface expression of MHC II from intracellular storages and tubular structures emanating from the MIIC and containing MHC II are formed (Kleijmeer *et al*, 2001; Boes *et al*, 2002; Chow *et al*, 2002). 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 DCs, 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) fuses 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 $p150^{Glued}$ 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 βIII spectrin (Johansson *et al*, 2007). βIII spectrin is located on the cytosolic side of multiple compartments and can interact, via its actin-binding domain, 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 βIII 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 $p150^{Glued}$) to interact

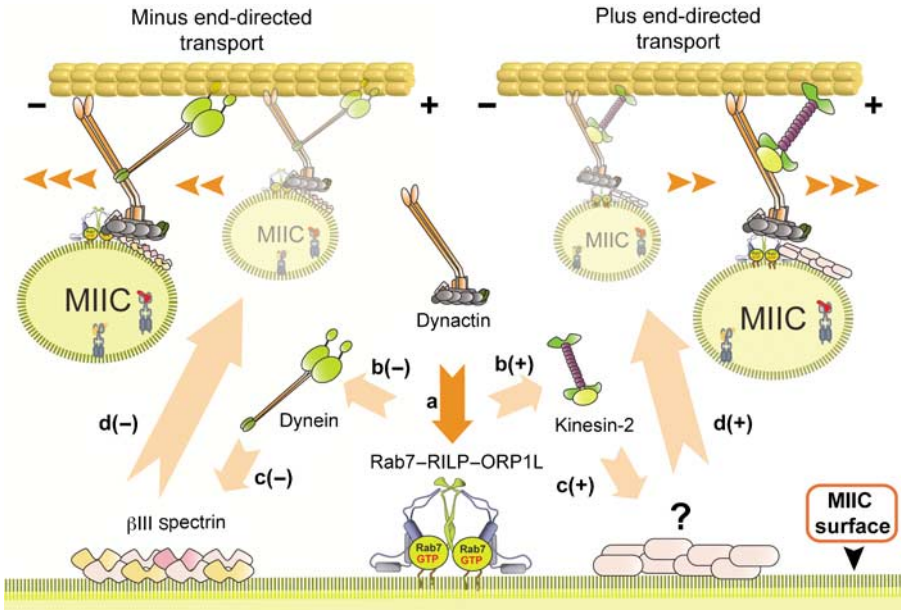

Figure 2 Reciprocal coordination of motor proteins for bidirectional microtubule-based MIIC transport. Left: control of inward transport of MIIC toward the microtubule minus-end. Right: control of plus end-directed transport of MIIC to the cell periphery. Activation of Rab7 precedes formation of the tripartite Rab7-RILP-ORP1L complex. RILP interacts with the dynactin subunit $p150^{Glued}$ (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, βIII spectrin (c(−)). Full activation of kinesin-2 may require a similar interaction with a general receptor on MIIC (c(+)). In this model, the $p150^{Glued}$-associated type of motor specifies the direction of MIIC transport (d).

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