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Invariant chain–MHC class II complexes: always odd and never invariant

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Major histocompatibility complex (MHC) class II trafficking begins with the association in the endoplasmic reticulum (ER) of the class II α- and β-subunits with the invariant chain (I_i) (reviewed in Blum *et al.*[1]). The $\alpha\beta I_i$ complex then travels via the trans Golgi network (TGN) or plasma membrane to the endocytic pathway. Here a combination of low pH and lysosomal proteinases causes I_i degradation, leaving CLIP (*CL*ass II-associated *I*nvariant chain *P*eptide) in the MHC class II peptide binding groove. CLIP is exchanged for locally generated high-affinity peptides by the action of the non-classical MHC class II molecule, DM. Subsequent surface expression of MHC class II complexes with peptides from foreign antigens induces CD4-positive T-cell responses.

Four isoforms of I_i are present in humans.[2] Two, p41 and p43, are expressed at relatively low levels and contain a protease inhibitory domain encoded by an alternatively spliced exon. The other two are p33, the shortest form, and p35, which like p43 is absent from the mouse. p35 and p43 have an extended N-terminal cytoplasmic domain, generated by alternative initiation of translation, that contains an ER retention motif consisting of three successive arginine residues. I_i assembles into trimers, and the four I_i isoforms can form mixed trimers.[3] Despite the fact that p35 expression is normally only about 20% that of p33, the high probability that a trimer will contain at least one I_i subunit containing the ER retention motif results in poor release from the ER unless it associates with MHC class II molecules, which inhibit the retention motif and allow egress.

More than 20 years ago we showed that the MHC class II–I_i complex that exits the ER is a nonamer, consisting of the I_i trimer associated with three class II αβ-dimers, and suggested that formation of a complete nonamer is essential for ER export. These conclusions were based on substantial evidence, including characterization of the nonamer by chemical cross-linking, size exclusion and sedimentation velocity analysis,[4] as well as pulse-chase analyses.[5] The work was sufficiently convincing that the principle was incorporated into many textbook illustrations of the MHC class II trafficking pathway.

A contradictory study recently argued that only one MHC class II αβ-dimer associates with an I_i trimer and that this pentameric complex is capable of ER export.[6] A hypothetical structural model suggested that the binding of one αβ-dimer ‘bends’ the putative pentamer towards the cell membrane, sterically preventing association with additional MHC class II

heterodimers. This concept is clearly incompatible with our original data, and the experimental techniques used were quite different. One method involved looking for the association of epitope-tagged MHC class II subunits and non-tagged subunits with the same Ii trimer, a second asked whether different human class II isotypes (DR, DQ and DP) could be found in the same 'mixed nonamer'. Both approaches were unsuccessful, and the model that complexes are pentameric was largely based on these negative data. Parenthetically, it was previously shown that in a transfected human B cell line, endogenous HLA-DR7 αβ-dimers and introduced mouse I-A^k αβ-dimers could associate with the same I_i trimer,[7] indicating that mixed species can exist.

In this issue of *Immunology and Cell Biology*, Thibodeau and colleagues have creatively re-addressed this issue.[8] Their work suggests that MHC class II association regulates transport from the ER by neutralizing the ER retention signals in the p35 form of I_i, allowing ER egress only when all retention motifs present in the trimer are 'cancelled'. Either p33 trimers or p35 trimers were expressed in HEK293T cells along with DR α- and a mixture of DR β-subunits, one of which was equipped with its own cytoplasmic ER retention signal (a di-lysine motif that functions similarly to the arginine motif present in p35). In cells solely expressing p33 trimers, the DR αβ-dimer lacking an ER retention signal was expressed on the cell surface even when co-expressed with the ER-retained form. In contrast, in cells expressing p35 trimers, the ER-retained dimer inhibited surface expression of the version lacking the retention signal, indicating that association of an ER-retained DR molecule with the same p35 trimer as the 'normal' DR molecule reduces ER exit of the latter.

These authors also asked whether different class II isotypes could interact with the same I_i trimer. When DQ αβ-dimers were introduced into cells along with ER-retained DR αβ-dimers, DQ expression on the surface was reduced in the presence of p35 trimers but not in the presence of p33 trimers. Curiously, some DQ was surface expressed in the presence of p35 but these molecules did not contain the I_i-derived CLIP peptide, suggesting that this subset was transported to the surface in an I_i-independent manner. Thus, its presence at the cell surface was irrelevant to the conclusion that ER-retained DR molecules could restrain DQ surface expression in the presence of p35 trimers. Again, the interpretation is that DR and DQ molecules can 'share' an I_i trimer.

Overall, the data are compatible with the following model (schematized in Figure 1). If I_i trimers only contain p33 (or potentially the analogous p41 alternatively spliced form), a pentameric structure consisting of an I_i trimer and an MHC class II heterodimer can leave the ER. If the trimers are exclusively composed of p35 (or p43), only MHC class II–I_i nonamers can escape. One can extrapolate and suggest that pentamers can also leave the ER if the trimer contains only one p35 or p43 molecule, whereas heptamers (containing two class II αβ-dimers) can leave the ER if the I_i trimer has two of them. Thibodeau and colleagues suggest that the first MHC class II heterodimer to bind may neutralize an ER retention signal or not, depending on which of the three I_i molecules it associates with in the mixed trimer. This could explain the results of our original studies, which indicated that nonamer formation was essentially complete even though the amount of p33 significantly exceeded that of p35. At a minimum, it seems likely that MHC class II–I_i complex stoichiometry varies depending on the ratio of p33/p35 expressed. It would, of course, be

very informative to examine the stoichiometry of the complex in mouse cells, in which p35 and p43 do not exist.

What does this mean functionally? I_i facilitates the assembly of MHC class II molecules, and its ER retention ensures that αβ-dimers generally do not leave home without it. I_i trimer formation improves ER retention, at least in the human system, because each trimer is likely to contain an ER retention signal. The cytoplasmic domain of all the I_i forms also contains an essential di-leucine motif that targets MHC class II–I_i complexes to the endocytic pathway, and the presence of three such targeting signals in an MHC class II–I_i complex may improve its association with clathrin-associated adaptor proteins, enhancing delivery to appropriate antigen-processing compartments. Furthermore, MHC class II–I_i complexes containing p35 follow a different intracellular trafficking pathway to antigen-processing compartments than those that do not,[9] potentially affecting the repertoire of bound antigenic peptides. Although these issues have faded into the rear-view mirror for most immunologists, Thibodeau and colleagues are to be commended for continuing to shine light upon this intriguing problem.

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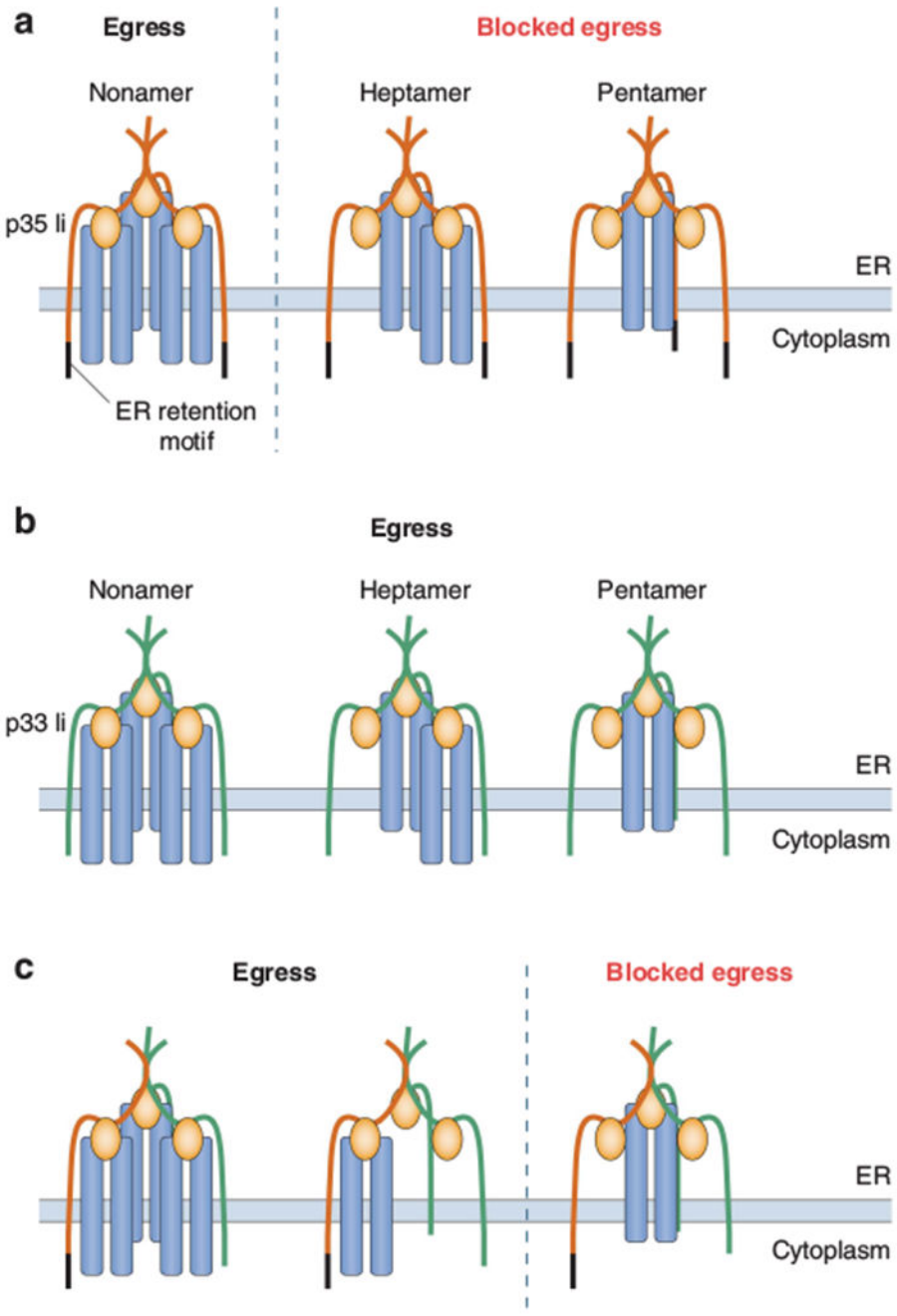

Figure 1.

Model for the regulation of MHC class II release from the ER by the composition of the I_i trimer. (**a**) Trimers containing three p35 forms (orange), shown with the N-terminal-extended cytoplasmic domain containing an ER retention signal (black), remain in the ER unless they associate with three MHC class II αβ-dimers (blue). If only one or two αβ-dimers associate, the 'unblocked' ER retention sequence(s) remain functional and transport of the complex from the ER is prevented. (**b**) Trimers containing one, two or three αβ-dimers can leave the ER if all three I_i species are the p33 form (green) that lacks the ER

retention signal. (**c**) A mixed I_i, trimer that contains only one p35 molecule may leave the ER with a single αβ-dimer if the latter associates with p35 and not one of the two p33 molecules also present in the trimer.