Time Course of Intracellular Associations, Processing, and Cleavages of Ii Forms and Class II Major Histocompatibility Complex Molecules*

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To determine how changing forms of class II major histocompatibility complex proteins and associated Ii molecules in intracellular compartments of human B lymphocytes might regulate or catalyze antigen processing or presentation, we analyzed immunoprecipitates of such molecules from subcellular fractions of [35S]methionine pulse-chase-labeled, 3-day-activated B lymphocytes after homogenization and distribution in Percoll density gradients. Two-dimensional gel electrophoresis of immunoprecipitates of subcellular fractions demonstrated: 1) progressive sialic acid addition to class II major histocompatibility complex β chains and Ii, but not to γ2, γ2', γ3, γ3' (p35), or p41 and its satellites; 2) association of p35 and p41 with class II complexes at 30-60 min after pulse labeling; 3) cleavage of an immature form of Ii, without sialic acid at 15-30 min after pulse labeling to a COOH-terminal, 25,000-dalton fragment, p25, with a 60-90-min half-life; 4) the presence of Ii-related p29 at only 30-min chase times; 5) an effect of chloroquine or monensin, at maximal nontoxic doses, to increase (a) the time for associations of p35 and p41 with class II complexes and (b) the half-life of p25, which was then formed from Ii, at reduced levels. In addition, while the half-lives of class II α and β chains and Ii were comparable within intracellular fractions of any one density, in intracellular fractions of intermediate densities the complexes appeared to be longer lived (much greater than 6 h) than in lighter fractions (2-3-h half-lives).

A detailed structural analysis of the changing forms of class II major histocompatibility complex (MHC)† molecules and associated proteins as a function of pulse-chase radiolabeling and of the separation of intracellular compartments might help us to understand how foreign antigens are processed, associated with class II MHC molecules, and brought to the cell surface for presentation to T cell receptors. The processing of antigenic proteins for presentation to CD4+ T helper cells was found to be a time- and energy-dependent process (1). Only some peptides, digested from a foreign protein, were T cell-presented (1, 2), and frequently those had sequences which might coil as amphipathic α helices, i.e. with opposing hydrophilic and hydrophobic surfaces (3). High affinity binding of such T cell-presented peptides to immunopurified class II molecules was restricted to the alleles which presented the peptides in vivo (4). However, the kinetics of in vitro association of foreign peptides with presenting class II MHC proteins were very slow (1 M⁻¹ s⁻¹) (5). In whole cells, regulatory or catalytic proteins might accelerate peptide binding to class II molecules, within the 1-h lag time required for antigen presentation (6).

A principal candidate to regulate class II MHC molecules' function in binding and presenting amphipathic peptides is the Ii molecule which associates with class II α and β chains from the time of their synthesis (7, 8). Multiple forms of Ii have been defined with either genomic or posttranslational bases for their structural differences: Ii, itself (7); Ip, which is the complex sugar form with variable sialic acids (9); γ2 and γ3 (also identified as p35b and p35a, respectively), which arise from an alternative initiation site for transcription (10, 11); p41, which has a 64-residue insert derived from expression of an additional exon placed after the 6th exon of Ii (11, 12); p25, which is cleaved 20-60 min after synthesis from the COOH-terminal, proteolytically released, exomembranal fragment of an "immature," high mannose form of Ii (13); and p21 and p10, which are seen only after treatment of cells with leupeptin or antipain 2-4 h after synthesis and are derived from complex sugar-sialic acid-containing forms of Ii, which are associated with fully processed class II α and β chains (14, 15). The Ii proteins could regulate class II molecules' functions by several methods. For example, Ii could block the class II desetope until release by a proteolytic process which might also serve to digest foreign proteins (16). Removal of Ii, could actually catalyze charging of the desetope with peptides structurally similar to the amphipathic helix I (Phe⁴⁵-Met¹⁹⁹) (17), or Ii might solely retard the class II complex from surface transport until "completed" with a foreign peptide, in a manner similar to heavy chain binding protein binding of IgM heavy chain until the λ or κ chains are attached (18). Ii does not appear to be essential for the surface expression of class II MHC molecules, since fibroblast transfectants without Ii are capable of cell surface expression of class II molecules and of class II-restricted antigen presentation (19-21). Toward an understanding of possible roles of these accessory molecules in the regulation of functions of class II molecules, we have examined the time course of association and/or cleavage of these class II accessory proteins in subcellular fractions.

MATERIALS AND METHODS

B Cells—Spleens were obtained from consenting, immunocompetent patients undergoing splenectomy for surgical or medical indica-
tions. Spleen pieces were pushed through a stainless steel mesh and suspended in RPMI 1640 medium with 10% fetal calf serum (FCS). Mononuclear cells were separated on a Ficoll-Hypaque gradient (density 1.078), washed twice with RPMI 1640 medium with 10% FCS, and resuspended at 40–100 × 10^6 cells/ml in ice-cold RPMI 1640 medium with 10% FCS and 10% dimethyl sulfoxide. Cells were frozen and stored in liquid nitrogen.

Frozen splenocytes were thawed quickly and washed twice in RPMI 1640 medium with 10% FCS. Adherent cells were depleted by incubation of the cell suspension (5 × 10^9 cells/ml) in sterile 100-mm polystyrene dishes (Corning) for 1 h at 37 °C in a 5% CO₂ atmosphere. After incubation, nonadherent cells were removed and added to washed sheep red blood cells (22). The cell mixture was centrifuged at 200 × g for 5 min at 4 °C and incubated for 1 h at 4 °C. The cells were gently resuspended and layered on a Ficoll-Hypaque gradient (density 1.078) and centrifuged at 1000 × g for 20 min at room temperature. Unrosetted lymphocytes in the buoyant cell halo were collected and washed twice in RPMI 1640 medium with 10% FCS. Fluorescence-activated cell sorter analysis recorded the composition of these cells, usually ranging: 88-94% leu 12+ (B cell), 2-4% leu 4+ (T cell), 0-2% leu M3+ (macrophage), and 0-1% leu 11A+ (NK cell).

Antibodies—A rabbit anti-peptide serum was prepared against the I{\textsubscript}{\textalpha} peptide sequence Ghu{\textsuperscript{166}-Glu}{\textsuperscript{169}}, with the addition of Tyr at the NH₂ terminus (15). Rabbit anti-p25,30 serum was prepared to the papain-solubilized, exomembrane portion of class II MHC antigen (23) and did not react with I{\textalpha} in a class II-, I{\textalpha}+ cell line (24). VIC-Y1 was a mAb to I{\textalpha} (25).

Cell Activation—Human B lymphocytes were activated with a 1/1250 dilution of 10% formalinized Staphylococcus aureus (Chemicon), added to 2 × 10^6 B cells/ml in RPMI 1640 medium with 10% FCS (26). The cells were incubated at 37 °C in a 5% CO₂ atmosphere. Activation was followed by forward light scattering analysis and fluorescence-activated cell sorting in a FACSSORT instrument (Becton-Dickinson), by cell sizing (Coulter channelizer), and by [3H] thymidine uptake.

Metabolic Radiolabeling—Cells were labeled with [35S]methionine (at 0.2 mCi/5 × 10^9 cells) (Du Pont-New England Nuclear) for 10 min after a 15-min period for methionine starvation except where specifically noted. After pulse labeling, cells were washed with ice-cold Hanks’ balanced salt solution, pH 6.0, containing 4 mM L-methionine (Sigma) and 10 mM Hepes, and were incubated at 37 °C in a 5% CO₂ atmosphere in complete RPMI 1640 medium with 10% FCS. Cells (5 × 10^6/sample) were lysed at the end of the chase time by vortex mixing in 1 ml of ice-cold lysis buffer (10 mM Tris-HCl, pH 7.2, 150 mM NaCl, 0.02% NaN₃, 0.5% (w/v) Triton X-100, 2 mM phenylmethylsulfonyl fluoride, and 10 mM N-ethylmaleimide). The lysate was centrifuged at 100,000 × g for 25 min at 4 °C and the supernate used in immunoprecipitation.

Cell Fractionation—Three-day-activated, [35S]methionine-labeled human B cells were fractionated in a Percoll gradient by the procedure of Ukkonen et al. (27), with some modifications. Briefly, 3 × 10^9 cells were resuspended in TES buffer (10 mM triethanolamine-HCl, pH 6.7, 1 mM EDTA, 0.25 M sucrose, 0.01% NaN₃, and 1% (v/v) aprotonin) and homogenized by 100 strokes in a stainless steel Dounce homogenizer (Kontes). The homogenate was centrifuged at 740 × g for 10 min at 4 °C, and the post-nuclear supernatant was layered on 20% Percoll (Pharmacia LKB Biotechnology Inc.) in 0.25 M sucrose. Centrifugation was performed using 37-ml Quick-Seal tubes (Beckman) in a Beckman VTi-50 rotor at 18,000 rpm for 1 h. Fractions (1 ml) were collected from the bottom of the tube, and immunoprecipitations were performed on each fraction with appreciable counts/min in the presence of 0.5% Triton X-100. Acid phosphatase and 5'-nucleotidase activities were assayed using a Sigma Acid Phosphatase Kit and according to Möré (28), respectively. The presence of [35S]labeled transferrin was determined (29).

Chloroquine and Monensin Treatment—Three-day-activated B cells were incubated in RPMI 1640 medium without methionine but with 3% dialyzed FCS, at 37 °C in a 5% CO₂ atmosphere. Activation was followed by forward light scattering analysis and fluorescence-activated cell sorting in a FACSSORT instrument (Becton-Dickinson), by cell sizing (Coulter channelizer), and by [3H] thymidine uptake.

Fig. 1. Pulse-chase analysis of class II MHC molecules and I{\textalpha} in B lymphocytes. Immuno-precipitates were formed with antibodies to class II MHC molecules, and to I{\textalpha}, after a 10-min pulse radiolabeling and subsequent chase. VIC-Y1 mAb recognizes all forms of I{\textalpha}, and anti-I{\beta}(183–193) serum recognizes forms without sialic acids. α and β chains of class II molecules, I{\textalpha}, sialic acid-derivatized I{\beta}, p41, and the γ2, γ2', γ3, γ3' series of proteins (marked collectively as p55) appear to associate with class II MHC proteins at chase times from 30 to 120 min. A is actin. The gels separate proteins in the horizontal direction by their isoelectric points (more basic proteins to the left), and by molecular weight in the vertical dimension.
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**RESULTS**

**Posttranslational Modifications of Class II β Chains and Ii—** [35S]Methionine labeling for 10 min, followed by unlabeled methionine chased from 0 to 120 min, revealed processing of Ii to acidic, sialic acid-derivatized forms (Ip) and of two series of β chains (Fig. 1), as observed previously (9, 34). VIC-Y1 recognized Ip and sialic acid-bearing forms of class β chains, while anti-I-(183–193) serum did not (Fig. 1). We have observed previously that anti-I-(183–193) do not recognize Ip or Ii-chondroitin sulfate and concluded that some carbohydrate additions block the epitope recognized with that antisem (13). This observation that the sialic acid-containing forms of Ii and associated class II β chains, are not present in immunoprecipitates with anti-I-(183–193) serum led to the view that only Ii, and its complexes in the cis-Golgi and endoplasmic reticulum can be recognized with that antibody. On the other hand, with VIC-Y1 one recognizes all forms of Ii, and coprecipitated class II molecules.

**p41 Association with Class II MHC Molecules from 30 to 60 Min after Synthesis—** Three p41 proteins, which were seen with each of the anti-class II, VIC-Y1, and anti-I-(183–193) antibodies, associated with class II α and β chains from 30 to 60 min after synthesis (Fig. 1). A large amount of p41 was synthesized, as revealed with VIC-Y1 serum (at 0 and 15 min chase times, Fig. 1), but was not immediately associated with class II MHC molecules. Proteins appearing just below each of the p41 spots could have been precursors to p41. Since p41 was well recognized with anti-I-(183–193) serum and was not processed to more acidic forms, we believed that p41 was not processed to complex sugar/sialic acid-derivatized forms and was either expressed only in the ER/cis-Golgi, or could not undergo such carbohydrate modifications.

**Association of the γ2, γ2’, γ3, γ3’ Series with Class II MHC Complexes from 15 to 60 Min after Synthesis in Parallel to the Kinetics of Association of p41 with Class II Complexes—** The appearance of γ2’ and γ3’, in addition to γ2 and γ3, was noted previously, although the proteins were not specifically so designated (11). While the γ2, γ2’, γ3, γ3’ proteins (labeled collectively as p35 in the illustrations) were synthesized abundantly (cf. VIC-Y1 and anti-I-(183–193) immunoprecipitates at 0 min chase, Fig. 1), they were not well represented in anti-
Formation of I-derived p25 at 15- to 120-Min Chase Times—I-derived p25 first appeared at 15- and 30-min chase times (VIC-Y1 and anti-I(183-193) immunoprecipitates, Fig. 1) and persisted up to 60-120-min chase times (anti-I(183-193) of Fig. 1). p25 was demonstrated previously to be derived from the COOH-terminal portion of I, by proteolysis, since it was immunoprecipitated by two antibodies to COOH-terminal peptides and not with an antibody to an NH2-terminal peptide and did not contain Cys81 (13). Since, during the time course of Ip formation, p25 did not demonstrate progressive acidic spots, and because p25 was recognized with anti-I(183-193) serum which did not recognize Ip, we concluded that p25 was derived from a high mannose or even nonglycosylated, early form of I, in the ER or cis-Golgi. In other experiments, p25 was more clearly seen in anti-class II immunoprecipitates than here (anti-class II immunoprecipitates at 30 and 60 min, Fig. 1). As a result of that observation and since the total amount of p25 was large and relatively stable (anti-I(183-193) immunoprecipitates at 30-60 min, Fig. 1), we hypothesized that at least some p25 was generated in the ER or cis-Golgi by proteolytic cleavage of I, bound to class II complexes, leading to the dissociation of p25 from I.

A Formation of p29 Only at 30-Min Chase Times—Immuno precipitates with all three antibodies demonstrated [35S]methionine-labeled p29 only at 30 min of chase time. The very sharply defined window in time for the appearance of p29 contrasted with the relatively broader kinetics for the association of p41 and p35 proteins with class II molecules and for the appearance of p25. p29 was not long-lived when not associated with class II molecules because its intensities in immunoprecipitates with VIC-Y1 and anti-I(183-193) antibodies were comparable to that with anti-class II antibody, and the anti-class II antibody did not recognize I, which was not associated with class II complexes (24). One might propose that p29 could be an intermediate in the cleavage of p25. However, the broader time course for creation of p25, with significant levels being expressed at 15-min chase times in immunoprecipitates with VIC-Y1 and anti-I(183-193), was not compatible with that idea. Also, it might be proposed that p29 is an intermediate in the creation of p21 and p10, both of which have been identified in leupeptin- or antipain-treated cells (14, 15). However, in those studies, p21 and p10 were found only after 2 h of chase time (maximally at 2-4 h) and had electrophoretic heterogeneity consistent with being derived from sialic acid-derivatedized forms of I. p29 could also represent some nonclass II-, non-I-derived protein which associates with class II molecules only at that point in time. The origin of p29 remains unclear.

Chloroquine or Monensin Extend the Expression and Processing of Class II and Accessory Molecules—Chloroquine and monensin can inhibit functions within intracellular compartments and trafficking among those compartments by blocking acidification (30, 31), although other mechanisms are possible. Machamer and Cresswell (35) and Nowell and Quaranta (36) demonstrated that these drugs promote the accumulation of I, some of which is associated with class II complexes. We confirmed these findings and found in addition that in drug-treated cells at 60-min chase time there is less processing of Ip to acidic forms. p41 and the p35 series were also formed, but had longer half-lives in association with class II molecules (anti-class II immunoprecipitates at 120 min, Fig. 2). p25 was formed at the same rate without the drugs, but to a lesser extent, and it had a longer half-life (anti-I(183-193) immunoprecipitates, Fig. 2). p29 was not observed in the presence of chloroquine or monensin. Results with chloroquine were very similar to those with monensin and are not illustrated.

Intracellular Compartments in Which Processing, Associations, and Dissociations Occurred—Three-day-activated B lymphocytes were labeled with [35S]methionine, and subcellular fractions were separated in a Percoll density gradient. Acid phosphatase and 5′-nucleotidase marked lysosomal and plasma membrane fractions, respectively, and after 30 min at 37 °C 5′Pi-transferrin marked an internalized endosome at the same density as the class II MHC and I, molecules (Fig. 3). Since preliminary studies had demonstrated stability of class II and I, forms at 1 h, we chose in this experiment to examine cells at 0, 90, 180, and 360 min after 1-h pulse labeling.

The Percoll density gradient resolved subcellular compartments which had distinct patterns of expression and life spans of class II accessory molecules. Negligible amounts of radio-labeled molecules were immunoprecipitated from lysosomal fractions 1-4. Proteins transferred to that compartment might have been degraded and rendered nonreactive with the antibodies. The proteins in the denser fractions 30-31 of this separation were long-lived, with a slight fall over a 6-h period in densitometer-traced densities. In contrast, radioactivity of proteins immunoprecipitated from fractions 34-35 and 36-37
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**Fig. 4.** Resolution of complexes of accessory proteins and class II MHC molecules in Percoll density gradients of subcellular fractions, as a function of 1-h pulse [35S]methionine label and various chase times. Subcellular fractions, separated by density, were solubilized and immunoprecipitated with anti-class II antibody. Complexes were much longer-lived in fractions 30–31 and 32–33 than in fractions 34–35 and 36–37. p41 and p25 were present in fractions 32–33 and 34–35 but not in 30–31, p25 was absent by 90-min chase times.

**Fig. 5.** Anti-Ii immunoprecipitates of pulse-chase [35S]methionine-labeled, Percoll density gradient-separated subcellular fractions. Samples analyzed in Fig. 4 were also subjected to immunoprecipitation with VIC-Y1 anti-Ii antibody. Complexes were longer lived in fractions 30–31 than in fractions 34–35 and 36–37. p41 and p25 were present in fractions 32–33 and 34–35 but not in fractions 30–31. More acidic proteins in the region of the β chain series (β) were seen in fractions 32–33 and 34–35 than in 30–31. p25 was absent by 90-min chase times.

**TABLE I**
**Composition of class II MHC accessory protein complexes in Percoll gradient-separated subcellular fractions**

|                  | Denser fractions | Intermediate fractions |
|------------------|------------------|------------------------|
|                  | 30–31            | 32–33 and 34–35        | 36-37                  |
| Half-life class II| 6+ h             | 3–6 h                  | 3 h                    |
| I → p25          | No               | Yes                    | No                     |
| p41 present      | No               | Yes                    | Yes                    |
| p35 present      | Yes              | Yes                    | Yes                    |

of Figs. 4 and 5, fell steadily with a t½ = 120 min (as judged from a semilogarithmic plot of density as a function of time). Additionally, p25 was observed at 0 chase time (after a 1-h labeling) in fractions 32–33 and 34–35 but not in fractions 30–31 (Figs. 4 and 5). p41 was likewise barely present in fractions 30–31 but was prominent in fractions 32–33 and 34–35 (Figs. 4 and 5). Fractions 32–33 demonstrated p25 and p41 as did fractions 34–35. The densities with respect to compartment markers (acid phosphatase, 5'-nucleotidase, transferrin) and time course for creation of p25, and associations of p41, p29, p35 proteins with class II MHC molecules, indicated that these associations, and the p25 dissociation, occurred in some nonlysosomal, intracellular compartment rather than at the plasma membrane. Different patterns of class II β chain and of p55 proteins were observed with the second spleen specimen which was used for the experiments of Figs. 3–5.

**DISCUSSION**

Since a primary function of class II molecules is to receive digested fragments of foreign proteins for presentation to antigen-specific receptors of T cells, the associations, cleav-
ages, dissociations, and potential traffickings which we have described for class II molecules and Ii-derived accessory proteins reflect processes which might regulate both foreign peptide binding to the desetope and other functions of class II molecules.

At least two density-separable compartments contained class II MHC, α and β chain complexes with different apparent life spans and patterns of accessory molecule expression (Figs. 4 and 5). Such class II molecules in denser fractions 30-31 had a half-life greater than 6 h and could survive to 18 h (as found in another experiment with chase times of 6, 12, and 18 h, not illustrated). The molecules in lighter fractions 34-35 had a half-life of 90-180 min. Functional differences between the compartments of the intermediate density fractions 32-33 and 34-35 and heavier fractions 36-37 were indicated by the appearance of p25, a proteolytic fragment of Ii, and associations of p41 with class II complexes only in the intermediate density fractions. Generally, lighter fractions had more mature molecules, e.g. sialic acid-derivatized Ii, and β chains, and could have reflected a compartment with molecules moving to the plasma membrane, while class II molecules in the denser compartment could have represented an intracellular reservoir or had another function.

Ii-derived p41 and the γ2, γ2', γ3, γ3' series (for convenience referred to as p35 (10, 11)) of accessory proteins were synthesized concurrently with class II α and β chains, but associated with class II molecules at a later time in the compartments of intermediate density (fractions 32-33 and 34-35). The p35 series of accessory proteins is derived from alternate initiation site mRNA transcripts of the Ii gene, and p41 includes an extra exon (10). These additional accessory proteins might associate with class II molecules for functions indirectly related to the binding of antigenic peptides or to antigen presentation. The p35 molecules have an extra 16 amino acids at the cytoplasmic NH₂ terminus of Ii, including Ser⁶ and Cys⁸, and the positive groups His²-Arg⁴-Arg⁶-Arg⁸-Arg₁₀-Lys₁⁴ (10, 11), which could be involved in regulating intracellular functions in response to exomembranal, or intravascular, interactions with the class II MHC molecules or other receptors. Expression of I, does not appear to be essential for cell surface expression of class II molecules or for presentation of measles virus determinants which are synthesized within the presenting cell (19-21). p41 is derived from an Ii gene with an insert for 64 amino acids which may encode specific functions (11, 12). We did not address the class II molecule association of the chondroitin sulfate-derivatized form of Ii, which is seen only after very long [³⁵S]methionine-labeling times or with [³⁵S]sulfate (37).

Finding p25 in only intermediate density fractions supports the view that the compartment participated in antigen processing and peptide binding to class II molecules. One function theorized for Ii has been to retard the class II MHC proteins until they are charged with some amphipathic peptide (38). Finding the α helix Ii[Pha₁₄-Val₅₄] with a high strip-of-helix hydrophobicity index, also led to the proposal that helix may be bound in the desetope about the time of synthesis (16, 17). Cleavage of Ii to COOH-terminal p25 at 20-60 min after synthesis could reflect a catalytic event in which that helix is replaced with structurally and functionally different foreign peptides. In this study, p25 was recorded only in fractions 32-33 and 34-35 of intermediate density and was not seen in denser fractions 30-31 with long-lived class II complexes, or in the lighter fractions 36-37. We conclude that p25 is generated in a nonlysosomal, intracellular compartment of the same density as that of internalized transferrin. Since Cresswell (39) showed that neuraminidase-conjugated transferrin could lead to removal of sialic acid residues from intracellular class II molecules, transferrin might well have entered class II MHC-containing vesicles in which p25 appears, although we only showed it to be present at the same density as were class II complexes and p25. p25 was expressed over a relatively short time period from 15- to 60-min chase times after a 10-min pulse labeling and was not present at the 90-, 180-, and 360-min chase times after a 1-h pulse labeling, at which times ample radiolabeled Ii was present (Figs. 4 and 5). Clearly, the bulk of Ii, is not cleaved to p25 but remains either for 6 or more hours in denser fractions 30-31 and 32-33, or for 90 to 180 min in lighter fractions 34-35 and 36-37. We also have not analyzed here the compartments and time course for induction of p21 and p10 which are derived from Ii, by proteolytic cleavages in leupeptin- or antipain-treated cells (14, 15). This entirely separate pathway for digestion of Ii occurs 2-4 h after pulse labeling and involves cleavage of fully processed, sialic acid-derivatized Ii, in contrast to nascent Ii, being cleaved to p25 at 15–60 min after synthesis.

In summary, we have demonstrated additional complexities in the association of class II molecules with Ii-derived accessory proteins in intracellular compartments, as separated by both density and time after pulse metabolic radiolabeling (Table I). The life spans of class II molecules ranged from 6 or more hours in denser fractions to 90-180 min in fractions of intermediate density. The patterns of accessory molecule associated with class II MHC molecules also differed, showing no p41 and no p25 in the denser fractions, while Ii, Ip, and p35 (γ2, γ2', γ3, γ3') were present in intermediate density fractions. p41 and p35 became associated with class II molecules at 30–60 min after pulse labeling. In this and related studies (13, 15), we have observed two pathways of Ii cleavage: (a) to p25, at 20–60 min after synthesis from a high mannose (ER or cis-Golgi) form, and (b) to p21/p10, about 2 h after synthesis from a sialic acid complex sugar (trans- or post-Golgi) form. Whether either (or both) of these cleavage events relates to the process of class II desetope charging with foreign peptides in some intracellular compartment and the function of class II MHC antigen associations with p41, p35, and Ii-chondroitin sulfate, remain objectives of future study.

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