Posttranslational Modifications of the Ia-Associated Invariant Protein p41 after Gene Transfer†

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ABSTRACT: Biochemical analysis of a rat fibroblast cell clone, transfected with the murine Ia associated invariant chain gene, demonstrates the expression of a family of proteins. This indicates that all members of the invariant protein family are derived from the same gene. The proteins p41, Ii, p27, p25, and p10, synthesized in the transfectant cell line, are identical with the invariant proteins previously shown to associate noncovalently with Ia antigens. These proteins are identified by immunoprecipitation and western blotting with a monoclonal antibody against the N-terminus and with an antiserum against the C-terminal part of the invariant chain. One protein, p41, has recently been shown to contain a thyroglobulin repeat (TgR) element. It was suggested that p41 might use the TgR element as a signal sequence which guides its intracellular transport to endosomes or lysosomes. Here, I demonstrate that p41 binds four N-linked carbohydrates and is heavily sialylated. During transport through trans Golgi compartments p41 binds palmitic acid, presumably at the same cytoplasmic cysteine as previously shown for Ii. A consensus sequence surrounding the palmitylated cysteine of the invariant chains (Ii, p41) and the human transferrin receptor was found. The transferrin receptor is known to follow an endocytic pathway, for which its cytoplasmic domain is essential. It is conceivable that the palmitylated domain of the invariant chains is a guiding structure for the membrane fusion process with transport vesicles. A role of the invariant proteins for antigen processing/presentation is discussed.

The Ia antigens consist of two polypeptides, which are associated intracellularly with an invariant polypeptide (Ii) of M_r 31 000. Early work suggested that Ia antigens are associated with a core glycosylated invariant chain located in the endoplasmic reticulum (Jones et al., 1978). Recently, it has been shown that during maturation fatty acid is bound to the Ii chain (Koch & Hämmerling, 1985), its two high-mannose carbohydrates are processed, and sialic acids are bound to N- and O-linked glycans (Claesson-Welsh et al., 1986a; Machamer & Cresswell, 1982; Machamer & Cresswell, 1984; Claesson & Peterson, 1983; Rudd et al., 1985). Moreover, the Ii chain is modified to a chondroitin sulfate proteoglycan (Sant et al., 1983). These posttranslational modifications take place concomitantly with the transport of the Ii chain through endoplasmic reticulum and Golgi compartments. Although it is known that Ia is expressed at the cell surface, the fate of the Ii chain is not clear. It is possible that part of Ii is expressed at the cell surface (Claesson & Peterson, 1983; Holdt et al., 1985; Claesson-Welsh et al., 1986b), possibly as a proteoglycan (Sant et al., 1985).

Since Ia antigens associate intracellularly with Ii, it was suggested that Ii may serve as a transport protein or may be essential for the assembly of Ia (Kvist et al., 1982). However, the transfection of Ia genes into invariant chain negative cells leads to the expression of Ia antigens on the cell surface (Sekaly et al., 1986; Miller & Germain, 1986). Although the presence of the Ii chain is not a requirement for cell surface expression of Ia antigens, it may nevertheless be important for Ia antigen function. This is supported by the observed coregulation of Ia antigens and invariant chain by interferon γ (IFNγ) (Koch et al., 1984; Momburg et al., 1986). This regulation appears to take place at the transcriptional level (Rahmsdorf et al., 1986; Collins et al., 1984; Paulnak-King et al., 1985). Ia and invariant chain genes have common consensus sequences at the 5′ flanking region (O'Sullivan et al., 1986; N. Koch et al., 1987), and those sequences may be responsible for transcriptional control.

The Ii chain is the most abundant member of an invariant protein family recently described by us and others (Zecher et al., 1984; Koch & Hämmerling, 1982; Quaranta et al., 1984). In this paper I demonstrate that the invariant protein family, which consists of the proteins p41, Ii, p27, p25, and p10, is expressed and regulated in a rat fibroblast cell line transfected with the murine invariant chain gene. This indicates that all of its members are derived from the same gene. The primary structure of the invariant chain gene has been described recently (N. Koch et al., 1987). One additional exon, encoding 64 amino acids, is used in the p41 protein compared to the Ii chain. This exon has high homology to an element in thyroglobulin. It was suggested that this thyroglobulin repeat element is a signal sequence for transport to endosomes/lysosomes (N. Koch et al., 1987). I addressed the question of whether p41 is posttranslationally modified, indicating its intracellular transport. Here I show that the p41 protein binds four N-linked carbohydrates, becomes fatty acylated, and travels through trans Golgi compartments where sialic acids are bound. The importance of these findings for Ia expression is discussed.

Materials and Methods

Cell Lines and Antibodies. All cell lines were cultured in RPMI 1640 containing 10% FCS.[1] The transfection of the rat 2 cells was described recently (Yamamoto et al., 1985).

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[1] Abbreviations: TgR, thyroglobulin repeat element; rIFNγ, recombinant interferon γ; PMSF, phenylmethanesulfonyl fluoride; NP40, Nonidet P40; mAb, monoclonal antibodies; FCS, fetal calf serum; NEPHGE, nonequilibrium pH gradient gel electrophoresis; TFR, transferrin receptor; Tris, tris(hydroxymethyl)aminomethane; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline.

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Two transfected cell clones were used in this study: I-9312 was transfected with a cosmid clone containing the invariant chain structural gene and about 20 kb of 5′ flanking sequences and the neomycin-resistance gene and I-9410 was transfected with the neomycin-resistance gene alone.

Rat 2 cells were stimulated by incubation with 20 units/mL mouse recombinant IFNγ (rIFNγ) for 3 days. The murine rIFNγ was produced by Genentech Inc. and was provided by Drs. Adler and Swetly, Boehringer Institute, Vienna. The monoclonal rat anti-mouse antibody In1 was described to react with the N-terminal cytoplasmic part of the mouse Ia associated invariant chain (Lipp & Dobberstein, 1986) and with the p41 invariant protein (Yamamoto et al., 1985). Ab MAR 18.5 (Lanier et al., 1982) reacts with rat κ chain and was used as a protein A binding sandwich antibody. The mAb Vic-Y1, a gift from Dr. Knapp (Vienna), reacts with the human invariant chain (Quaranta et al., 1984). The serum against the recombinant invariant chain fusion protein has been described recently (S. Koch et al., 1987). In brief, a 0.79-kb cDNA fragment encoding 114 amino acids of the C-terminus of the invariant chain was fused to a bacterial expression vector. This plasmid directs the synthesis of the invariant chain as a fusion protein of the bacteriophage MS2 polymerase. The purified fusion protein was used to raise an antiserum in rabbits. The antiserum shows interspecies cross-reactivity (S. Koch et al., 1987).

Metabolic Labeling of Cells. A total of 10^7 cells were washed several times with methionine-free RPMI 1640. Methionine-free medium with 10% dialyzed FCS and 200 μCi of [^{35}S]methionine (600 Ci/mmol, Amersham) was added, and cells were incubated for 1 h at 37 °C. After being labeled, cells were washed and resuspended in 500 μL of Tris-buffered PBS, containing 10 mM PMSF and Trasylol (1:1000). Cells were lysed by the addition of 50 μL of 10% NP40, and DNA and debris were removed by centrifugation (5 min at 10000*g*).

Fatty acid labeling was performed as described (Koch & Hämmerling, 1985). Briefly, 500 μCi of [^{3}H]palmitic acid (NEN, 30 Ci/mmol) was added to medium containing 10% dialyzed FCS and 10^6 cells/mL for 1 h.

Western Blotting. Spleen cells of a mouse were suspended in PBS and lysed with 1% NP40. From these extracts glycoproteins were isolated by affinity chromatography using lens culinaris Sepharose. Glycoproteins obtained from 1.5×10^6 spleen cells were separated in SDS–PAGE and transferred to Immobilon filters (Millipore, Bedford, MA) [see Lutz and Cresswell (1987)]. The filters were blocked with 10% fetal calf serum and incubated with either culture supernatant containing the rat antibody In1 (anti-murine Ii) or the rabbit antiserum against the invariant chain fusion protein (dilution 1:100), followed by alkaline phosphatase conjugated goat anti-rat or anti-rabbit antibodies (Jackson Immuno Research, West Grove, PA). The alkaline phosphatase was detected as described by Blake et al. (1984). In brief, after being washed with PBS containing 0.05% Tween 20 the filters were washed twice in 10 mM Tris, pH 9.6, with 150 mM NaCl. The enzyme reaction was performed in 100 mM Tris, pH 9.6, 100 mM NaCl, 5 mM $MgCl_2$, and the substrates *p*-nitro-blue-tetrazolium-chloride (stock 10 mg/mL of dimethylformamide) and 5-brom-4-chlor-3-indylphosphate (1 mg/mL of reaction buffer) (both substrates are from Biomol, Ilvesheim, FRG). The relation of the buffer to substrates was 900:100:5.

Neuraminidase Treatment. Immunoprecipitates of invariant chains synthesized by IFNγ-treated invariant chain gene transfected cells were obtained after labeling with [^{35}S]-methionine. Then, the immunoprecipitates were incubated with 20 milliunits (20 μL) of neuraminidase (Boehringer) for 0, 30, 60, and 120 min with repeated addition of neuraminidase, washed twice with 0.25% NP40, and subjected to two-dimensional gel electrophoresis.

Tunicamycin Treatment. Invariant chain gene transfected rat 2 cells were stimulated with rIFNγ as described above and subsequently cultured in the presence of tunicamycin (Sigma) for 150 min. To achieve an incomplete inhibition of N-glycosylation, the tunicamycin concentration was varied between 1 and 5 μg/mL.

Immunoprecipitation and One- and Two-Dimensional Gel Electrophoresis. Immunoprecipitation was performed after preabsorption of the cell lysate with Protein A–Sepharose, by the addition of 50 μL of 10 × concentrated hybridoma culture supernatant or 10 μL of antiserum and 10 μL of Protein A–Sepharose (packed beads). This suspension was rotated overnight at 4 °C and then washed 3 times with 0.25% NP40 in PBS containing the protease inhibitors PMSF and Trasylol. Samples were boiled in SDS sample buffer and separated in one-dimensional SDS gels as described (Kvist et al., 1982). Two-dimensional electrophoresis was performed as described previously (Koch & Hämmerling, 1985).

Results

Synthesis of the Invariant Proteins after Gene Transfer. The biochemical analysis of invariant chains, particularly of their minor forms, was limited by the small amount of these proteins which is expressed in certain cell lines. Moreover, the genetic origin of several of the Ia-associated invariant chains was unclear. To overcome these problems invariant chains were studied in rat 2 cells transfected with the invariant chain gene including its 5′ regulatory region (see Materials and Methods). Rat 2 cells previously have been shown not to express the rat invariant chain and class II (Ia) antigens (Eccles et al., 1986). Cells transfected with the murine invariant chain gene as well as nontransfected rat 2 cells were cultured in the absence or presence of 20 units/mL recombinant IFNγ and subsequently labeled with [^{35}S]methionine for 1 h. The immunochemical characterization (shown in Figure 1) was performed with a monoclonal antibody (In1) against the N-terminus of the invariant chain (Lipp & Dobberstein, 1986) and with an antiserum against a recombinant fusion protein consisting of 114 amino acids of the C-terminus of the invariant chain and of 98 amino acids of MS-2 polymerase (S. Koch et al., 1987). Both In1 (lanes 3 and 4) and the antiserum (lanes 5 and 6) immunoprecipitate the invariant chain (Ii) as the main band from invariant chain gene transfected rat 2 cells. Treatment of the cells with IFNγ strongly enhances the synthesis of invariant chains (compare lanes 3 and 4 and lanes 5 and 6). This allows the detection of additional bands marked as p41, p27, p25, and p10 (lanes 3 and 5). In contrast to the antiserum, the In1 antibody immunoprecipitates a p10 polypeptide (lane 3). This band previously has been shown to contain the sole cysteine of the invariant chain (Koch & Hämmerling, 1982) which is in the cytoplasmic part of the invariant chain. Therefore, p10 presumably represents the N-terminal part of the invariant chain. Untransfected rat 2 cells were negative for all of the invariant chains as shown in lanes 7–10. To demonstrate the specificity of the antibodies which were used for immunoprecipitation, western blots with glycoproteins obtained from resting spleen cells were performed (lanes 1 and 2). The antibody In1 binds to p41, Ii, and p10 but not to p25 and p27. Since p25 and p27 are immunoprecipitated by In1 (lane 3), it is likely that these polypeptides are coprecipitated because of their association to other invariant proteins. The antiserum against the

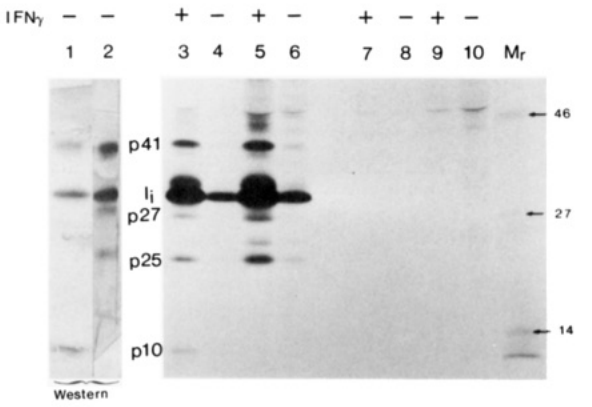

FIGURE 1: Immunochemical characterization of invariant proteins. Rat 2 cell clone I-9312 transfected with the invariant chain gene (lanes 3–6) or normal rat 2 cells (lanes 7–10) were cultured for 3 days in the presence of 20 units/mL recombinant IFNγ (lanes 3, 5, 7, and 9) or in medium alone (lanes 4, 6, 8, and 10). Cells were labeled with [^{35}S]methionine for 1 h and immunoprecipitated with mAb against invariant chain (In1, specific for the N-terminus of invariant chain) or an antiserum against the C-terminal end of the invariant chain. p41, Ii, p27, p25, and p10 indicate the position of the invariant proteins. Bands above p25, Ii, and p41 (see lane 5) correspond to sialylated forms of invariant proteins as will be shown in Figure 2. At the far right, protein standards are separated with M_r of 46 000, 27 000, and 14 000. In lanes 1 and 2 western blots with resting spleen cells are shown (see Materials and Methods). Ab In1 (lane 1) reacts with p41, Ii, and p10. The antiserum against the invariant chain fusion protein (lane 2) binds to p41, Ii, p27, and p25.

C-terminus of the invariant chain binds to p27, p25, Ii, and p41, indicating that all of them are members of the invariant protein family. The antiserum does not bind to p10, which is consistent with the assumption that p10 represents the N-terminus of the invariant chain.

Since in the rat 2 transfectant p27 and p10 are very faintly expressed, only proteins p41, Ii, and p25 are further considered in this paper. Figure 1 shows additional bands above p25, Ii, and p41 (lanes 3 and 5) which might be maturation products of p25, p41, and Ii.

Invariant Proteins Ii, p41, and p25 Are Transported from the Endoplasmic Reticulum to Trans Golgi Compartments. To achieve a better resolution of the putative maturation forms of the invariant chains, a two-dimensional separation of immunoprecipitates of IFNγ-stimulated (Figure 2C) or -non-stimulated (Figure 2A) Ii transfectants was performed. Three proteins, p41, Ii, and p25, were immunoprecipitated from nonstimulated cells (Figure 2A). A control immunoprecipitate of rat 2 cells, transfected with the neomycin gene alone, is negative (Figure 2B). In their physical parameters, size and charge, the proteins p41, Ii, and p25 are identical with those of the invariant protein family found associated with Ia antigens. Obviously, the common genetic origin of p41, Ii, and p25 explains their high homology which was described recently (Zecher et al., 1984; Yamamoto et al., 1985). The processed forms of p41, Ii, and p25 derived from the IFNγ-stimulated cells resolve into several distinct acidic spots (Figure 2C marked with brackets). Rat Ia antigens were not detected in stimulated rat 2 cells (Figure 2C).

Recently, it was shown that during maturation the Ii chain binds sialic acid (Koch & Hämmerling, 1985). Treatment of immunoprecipitates of IFNγ-stimulated Ii transfected cells with neuraminidase (Figure 2D) removes the acidic spots, indicating that besides Ii also p41 and p25 are sialylated. Sialylation of membrane proteins is evidence for transport through the trans Golgi, where sialyl transferases are located. The data presented in Figure 2C,D suggest that the invariant proteins pass through the Golgi, although part of the invariant

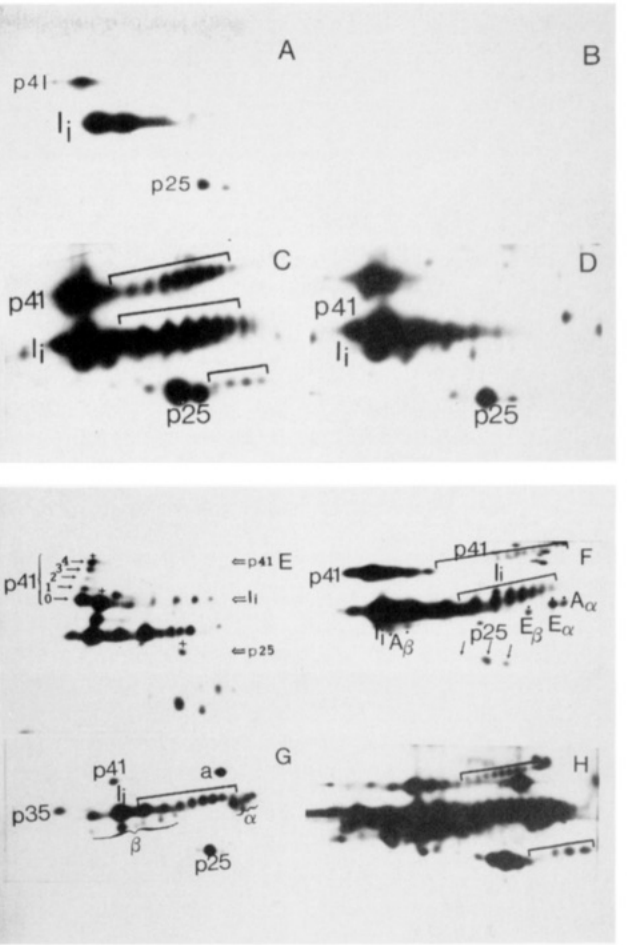

FIGURE 2: Two-dimensional analysis of the invariant protein family expressed after gene transfer. Transfectant rat 2 cells: (A) clone I-9312 transfected with the invariant chain gene; (B) clone I-9410 transfected with the neomycin-resistance gene; (C) clone I-9312 (invariant chain gene transfected) stimulated with IFNγ; (D) same as (C) but treated with neuraminidase; (E) same as (C) but incomplete inhibition of N-glycosylation with tunicamycin. (F) P388D$_1$ mouse macrophage cells were stimulated for 3 days with 20 units/mL IFNγ. (G and H) Human B lymphoma (Raji). (G) is a 10-h exposure and (H) a 120-h exposure of the same gel. Cells were labeled with [^{35}S]methionine (1 h) and immunoprecipitated with the rat mAb In1 (anti-mouse Ii, A–F) or Vic-Y1 (anti-human Ii, G and H). The immunoprecipitates were separated in the first dimension in nonequilibrated pH gradient gel electrophoresis and in the second dimension in SDS–polyacrylamide gel electrophoresis. Sialylated invariant chains were marked with brackets. Basic p27 is not separated in this gel, and p10 runs off the front of this gel. The acidic end of the gel is to the right. The arrows at the left in (E) indicate the number of N-linked glycans bound to p41. The arrows at the right in (E) indicate the size of the fully N-glycosylated invariant chains, which are labeled with a (+). Dots in (F) indicate the position of Ia α and β chains. The brackets in (H) correspond to sialylated p41 and p25. In (G), the heavy and light chains of HLA-D antigens are marked with α and β. The position of actin is indicated with an "a".

chains remains in the ER in precursor form. Analysis of the in vitro cell lines P388D$_1$ (mouse) (Figure 2F) and Raji (human) (Figure 2G,H) demonstrates sialylation of mouse and human p41 (Figure 2F,H) and of p25 (Figure 2H) and excludes that this modification is an artifact of the transfected cell line.

Two Asp-X-Thr motifs in exon 4 serve as acceptor sites for N-glycosylation in both Ii and p41. The primary structure of the p41, deduced from the nucleotide sequence of a genomic clone, contains in exon 6b two additional potential N-glycosylation sites. The number of N-linked glycans was determined. To distinguish intermediate glycosylation forms of p41, an incomplete inhibition of N-glycosylation by

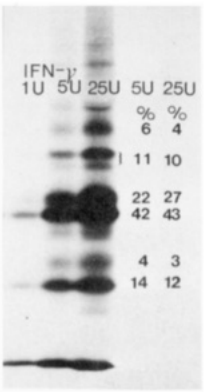

FIGURE 3: Enhancement of invariant chain synthesis after treatment with rIFNγ. The Ii-transfectant cell line was treated with either 1, 5, or 25 units/mL rIFNγ and labeled with [^{35}S]methionine for 1 h. The invariant proteins were immunoprecipitated with mAb In1. The gels were fluorographed, and additionally, the radioactivity in lanes 2 (5 units) and 3 (25 units) was scanned by a Berthold gel scanner. The normalized values of the radioactivity are listed at the right.

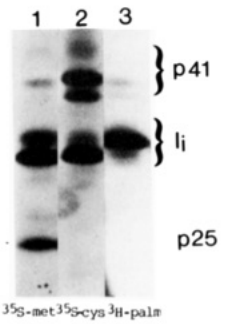

FIGURE 4: Palmitylation of the invariant p41. The invariant chain gene transfectant cell line was cultured for 48 h in the presence of 50 units of rIFNγ. Then the cells were labeled for 1 h either with [^{35}S]methionine (lane 1), with [^{35}S]cysteine (lane 2), or with [^{3}H]-palmitic acid (lane 3). Invariant proteins were immunoprecipitated with mAb In1. Ii and p41, but not p25, were labeled with [^{3}H]palmitic acid. The strong label of p41 with cysteine (lane 2) is due to the high number of cysteines (eight Cys) in p41 compared to only one cysteine in Ii.

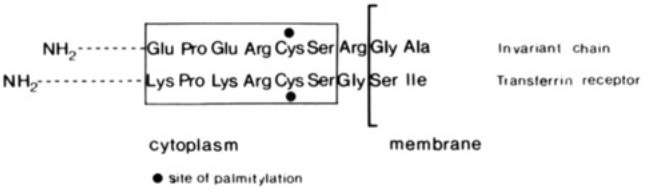

FIGURE 5: Partial sequence comparison of the cytoplasmic domains of the invariant chains (Ii and p41) and the human transferrin receptor. The cytoplasmic part of the invariant chains (N. Koch et al., 1987) and the transferrin receptor (Schneider et al., 1984), respectively, consist of 28 or 64 amino acids. A six amino acid residue containing motif surrounding the palmitylated cysteine is shown.

treatment of cells with tunicamycin was performed. Figure 2E exhibits five different forms of p41 (marked with arrows). The numbers at the left in Figure 2E correspond to the four N-linked carbohydrate side chains bound to p41 and to its nonglycosylated form. N-Glycosylation of Ii and p25 is not indicated in Figure 2E but has been described previously (Koch & Hämmerling, 1985).

Expression and Transport of Invariant Proteins Is Synchronized. To examine the dose influence of IFNγ on the expression of the various invariant proteins, transfected rat 2 cells were cultured in the presence of 1, 5, or 25 units of IFNγ (Figure 3). With 5 and 25 units a strong enhancement of synthesis of invariant chains was observed, compared to 1 unit of IFNγ. The radioactivity of the gels was quantified by gel scanning, and the normalized values (%) are listed at the right of Figure 3. The Ii chain (including the sialylated forms) represents 60–70% and p25 or p41 about 15% of the total radioactivity. However, 5 or 25 units of IFNγ did not change the relative amounts of the invariant chains, nor the percent of their sialylated forms.

p41 Protein Is Fatty Acylated. Recently, we have described that the Ii chain is fatty acylated at a sole cysteine adjacent to the cytoplasmic part of the membrane (Koch & Hämmerling, 1985). The low efficiency of incorporation of fatty acid and the low expression of p41 and p25 proteins in most eucaryotic cells make it difficult to examine binding of fatty acid to these molecules. The strong enhancement of the invariant proteins in the transfectant cell line after stimulation makes it possible to study fatty acylation. The transfected cell line was cultured in the presence of rIFNγ and labeled with either [^{35}S]methionine, [^{35}S]cysteine, or [^{3}H]palmitic acid. Immunoprecipitates of the invariant proteins are shown in Figure 4. Cysteine and palmitic acid are only bound to p41 and Ii but not to p25. This suggests that p25 lacks the N-terminal cysteine and therefore is not fatty acylated. Since p41 and Ii have the same cytoplasmic part with a single cysteine, it is likely that this cysteine is fatty acylated in both Ii and p41. We have previously shown that under these labeling conditions no radioactivity is interconverted into amino acids (Koch & Hämmerling, 1986). This is in agreement with the observation that p41 but not p25 is labeled with palmitic acid (Figure 4).

Up to now no consensus sequence for the palmitylation site of proteins has been described. However, a sequence surrounding the palmitylated cysteine of the invariant proteins (p41 and Ii) and the human transferrin receptor (TFR) shows homology (Figure 5). In this sequence four out of six amino acids are identical and two changes between charged amino acids (Glu to Lys) are present. However, we could not provide evidence for a consensus motif for palmitylation, because no other fatty acylated proteins contain this sequence (Pessara and Koch unpublished results). TFR, Ii, and p41 are type II membrane proteins with their N-terminus at the cytoplasmic site. It has been suggested that the transferrin receptor and Ii follow a similar intracellular pathway (Koch & Hämmerling, 1985). Recently it was shown that the cytoplasmic tail of the transferrin receptor is important for endocytosis of the receptor–ligand complex (Rothenberger et al., 1987). Therefore, it is conceivable that a signal sequence composed of the palmitic acid and the motif shown in Figure 5 permits interaction with other membranes and thereby initiates membrane fusion.

Discussion

In this paper I demonstrate that the complete invariant protein family is expressed in a rat fibroblast cell line after transfer of the invariant chain gene. Several mechanisms can account for the generation of a protein family. First, a common pre-mRNA can be spliced differently. In a recent paper we identified two mRNAs encoding the proteins p41 and the invariant chain (Yamamoto et al., 1985). Moreover, we and others have shown that in both mouse and human a sequence between the sixth and seventh exon of the invariant chain gene encodes part of the p41 protein (N. Koch et al., 1987; Strubin et al., 1986a).

A second possibility of producing two proteins from one mRNA is the use of different initiation sites. A the 5′ end of the human Ii mRNA there are several AUGs in frame with coding sequences. By site-directed mutagenesis it was shown that the translation of the human p35 and of the invariant chain is initiated differently (Strubin et al., 1986b). However,

the mouse gene lacks this additional ATG and therefore does not encode a p35 analogue (N. Koch et al., 1987).

The appearance of some members of the invariant protein family could also be explained by posttranslational modifications. Ii and p25 both have two N-glycosylation sites and have protein backbones of M_r 25 000 or 19 000, respectively (Koch & Hämmerling, 1985). Ii and p41, but not p25 (this paper), have an N-terminal cysteine, which becomes palmitylated. It is possible that cleavage of the invariant chain or of a common protein precursor could account for the appearance of p25. However, p25 is already observed after 5 min of pulse labeling (Koch & Hämmerling, 1982). Therefore, the cleavage must occur soon after synthesis. An artificial degradation of the invariant chain due to the isolation procedure is unlikely because no precursor/product relationship could be observed (Zecher et al., 1984).

The expression of the invariant proteins is enhanced after treatment of cells with IFNγ. Under these activating conditions it is possible to detect maturation products of the invariant proteins Ii, p41, and p25 which remain undetected in noninduced cells. This is due to the quantity of the invariant proteins which is synthesized in stimulated cells. Under activating conditions with IFNγ, Ia antigens are induced in macrophages. Since under these conditions also the amount of sialylated invariant proteins is increased, it is suggestive that Ia antigens which associate with invariant chains soon after synthesis travel with Ii to trans Golgi compartments.

Recently the murine invariant chain gene was sequenced revealing that Ii is encoded by eight exons (N. Koch et al., 1987). One additional exon between exon 6 and exon 7 encodes part of the p41 protein. This exon (6b) has a cysteine-rich motif which shows high homology to an element which is repeated 10 times in the thyroglobulin molecule (Malthiery et al., 1985). This motif in exon 6b is as homologus to the TgR elements as they are to each other. The TgR element in p41 could be a sorting signal which guides intracellular transport of antigen to the lysosomes or endosomes. In these cellular compartments proteolytic degradation of antigen may take place. For such a function it is necessary that this molecule is intracellularly transported either to endosomes/lysosomes or to the cell surface.

During transport, either in late endoplasmic reticulum or in early Golgi compartments, palmitic acid is bound to the sole cysteine of the invariant chains Ii and p41 (this paper). This fatty acylation seems to be a prerequisite for further transport, because no sialylation of Ii was observed when fatty acylation was inhibited (Koch & Hämmerling, 1985).

Similarities of the invariant chains with the transferrin receptor, such as fatty acylation at a cysteine residue close to the membrane (Omary & Trowbridge, 1981; Jing & Trowbridge, 1987; Koch & Hämmerling, 1985), an asymmetric membrane orientation with the N-terminus inside (McClelland et al., 1984; Lipp & Dobberstein, 1986), formation of homodimers (Koch & Hämmerling, 1982), and expression as proteoglycans (Sant et al., 1983; Fransson et al., 1984), were observed. The common features of invariant chain and transferrin receptor lead to the suggestion that the invariant chain may be involved in intracellular transport and recycling which possibly is linked to antigen processing and presentation (Koch & Hämmerling, 1985). This model is supported by experiments demonstrating that, during internalization of the transferrin receptor and a transferrin–neuraminidase conjugate, the ligand interacts with Ia antigens and results in desialylation of the DR β and the invariant chain by the neuraminidase (Cresswell, 1985). This experiment suggests that the DR and invariant chain polypeptides and the transferrin receptor have part of their intracellular pathway in common. Moreover, a recent report by Rothenberger et al. (1987) demonstrated that a deletion of the cytoplasmic part of the transferrin receptor prevents the receptor from endocytosis. It is conceivable that the fatty acid residue which is linked to the cytoplasmic part of Ii and the transferrin receptor act as an acceptor site for the endocytosis and for a transport-mediated fusion of intracellular vesicles.

The sequence homology at the fatty acylation site in the invariant chains and the transferrin receptor supports a similar role of their cytoplasmic tails. Therefore, it is possible that the fatty acylated invariant chains and the transferrin receptor are endocytozed and travel to specialized membrane compartments, their direction being guided by the fusion process and the use of the specific acceptor site. Indeed, recent reports by Lanzavecchia et al. (1988) indicate that antibodies bound to the transferrin receptor are internalized, intracellularly processed, and presented by class II molecules in an antigen-specific fashion to T lymphocytes. Therefore, the function of the invariant chain could be to internalize antigen and to guide its intracellular transport to digestive compartments where the antigen is processed.

In the context of the function of Ia antigens it is interesting that an Ia-transfected L cell is able to present lysosyme in the form of a peptide but not as the native protein (Shastri et al., 1985). It was suggested that this may be due to inadequate processing of the antigen or the lack of target structures for T cell accessory molecules. It could be that the low amounts of the invariant chains, which are synthesized in L cells (Koch & Harris, 1984), are not sufficient for a proper processing of antigen.

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