The EMBO Journal Vol.1 No.5 pp.649-654, 1982

Translation and assembly of HLA-DR antigens in *Xenopus* oocytes injected with mRNA from a human B-cell line

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Communicated by B. Mach
Received on 3 June 1982

HLA-DR antigens are polymorphic cell surface glycoproteins, expressed primarily in B lymphocytes and macrophages, which are thought to play an important role in the immune response. Two polypeptide chains, α and β, are associated at the cell surface, and a third chain associates with α and β intracellularly. RNA isolated from the human B-cell line Raji was injected in *Xenopus laevis* oocytes. Immunoprecipitates of translation products with several monoclonal antibodies revealed the presence of HLA-DR antigens similar to those synthesized in Raji cells. One monoclonal antibody was able to bind the β chain after dissociation of the three polypeptide chains with detergent. The presence of all three chains was confirmed by two-dimensional gel electrophoresis. The glycosylation pattern of the three chains was identical to that observed *in vivo*, as evidenced in studies using tunicamycin, an inhibitor of N-linked glycosylation. The presence of α chains assembled with β chains in equimolar ratio was further demonstrated by amino-terminal sequencing. An RNA fraction enriched for the three mRNAs, encoding α, β, and intracellular chains, was isolated. This translation-assembly system and the availability of monoclonal antibodies make it possible to assay for mRNA encoding specific molecules among the multiple human Ia-like antigens.

Key words: HLA-DR assembly/oocyte injection

Introduction

HLA-DR antigens constitute a family of highly polymorphic cell surface glycoproteins made up of two non-covalently linked subunits, the α chain of ~35 000 daltons, and the β chain of ~29 000 daltons (Strominger *et al.*, 1981). Their tissue distribution as well as their biochemical structure suggest that they are the equivalent of the mouse Ia antigens, encoded in the I region of the H-2 complex (Klein, 1979). They are, therefore, often referred to as human Ia antigens. They are involved in a number of biological functions like the interaction between macrophages, T cells, and B cells (Benacerraf, 1981). They are important in the stimulation of the mixed lymphocyte reaction, in allograft rejection, and in their linkage to disease susceptibility (Bach and van Rood, 1976; Ryder *et al.*, 1981).

A third subunit, associated intracellularly with Ia antigens, was first observed in mouse and called the invariant chain (Jones *et al.*, 1979). A human equivalent to the mouse invariant chain, with a mol. wt. of ~32 000, was also found in DR antigens (Charron and McDevitt, 1979; Shackelford and Strominger, 1980). This third chain cannot be detected at the cell surface and is thought to be involved in assembly and/or transport of DR antigens to the cell surface (Owen *et al.*, 1981; Kvist *et al.*, 1982).

The polymorphism in DR antigens is restricted to the β chain (Silver and Ferrone, 1979; Charron and McDevitt, 1980; Walker *et al.*, 1980; Shackelford and Strominger, 1980; Accolla *et al.*, 1981; Corte *et al.*, 1981b). The complexity of DR antigens is not only limited to the allelic polymorphism. Monoclonal antibodies have defined subsets of DR antigens within homozygous cell lines (Quaranta *et al.*, 1980; Lampson and Levy, 1980; Carrel *et al.*, 1981). Two DR β chains can be distinguished in several homozygous lines by peptide analysis (Accolla *et al.*, 1981). Several other loci exist, encoding polymorphic Ia-like antigens, that are closely linked to HLA-DR (Tosi *et al.*, 1978; Corte *et al.*, 1981a; Shackelford *et al.*, 1981; Nadler *et al.*, 1981).

In view of this complexity, and as a first step towards cloning cDNA copies of DR antigens, it is important to develop a mRNA translation assay that would enable distinction between the various Ia-like antigens. Cell-free translation systems, like the rabbit reticulocyte lysate, will not process or assemble multimeric proteins, and are not suited for this purpose. On the other hand, injection of mRNA in oocytes of the clawed toad *Xenopus laevis* has been used as a translation system for a variety of proteins. Specific post-translational modifications are taking place and, in some cases, assembly of multimeric proteins has been observed (reviewed by Asselbergs, 1979). We chose to investigate this translation system to assay for mRNA encoding DR antigens. We show that the three polypeptide chains assemble in the oocytes and can be immunoprecipitated with anti-DR monoclonal antibodies. This assembly system proved to be of great value in the subsequent cloning and identification of cDNA molecules encoding DR antigens.

Results

HLA-DR antigens present in Raji cells after 16 h of internal labeling with [^{35}S]methionine were immunoprecipitated with different monoclonal antibodies. The three polypeptide chains were revealed in an SDS-polyacrylamide gel as bands of apparent mol. wt. 35 000-36 000 for the α chain, ~33 000 for the intermediate chain, and 31 000 and 29 000 for the β chains (Figure 1). [It might be confusing to call the 33 000-dalton intracellular chain "invariant", as in the mouse, because the human DR α chain is also invariant. We prefer the term "intermediate" because this chain is intermediate in size, between the α and β chains, and is also an intermediate in the biosynthesis of DR antigens]. The two monoclonal antibodies D1-12 and D4-22 recognize different subsets of DR antigens. The relative intensity of the bands varies between the immunoprecipitates obtained with these two monoclonal antibodies. After denaturation of the antigen by boiling in SDS the third monoclonal antibody, BT 2.2, was still able to bind the two chains of 31 000 and 29 000 daltons (Figure 1, lane 5).

Xenopus oocytes were injected with cytoplasmic poly(A)$^+$

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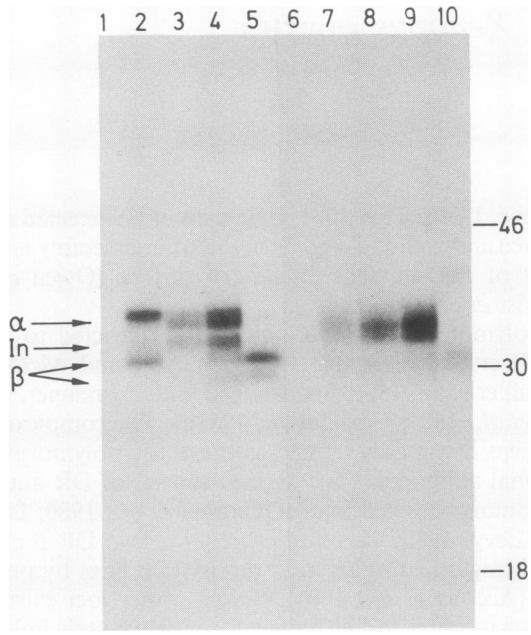

Fig. 1. SDS-PAGE with HLA-DR antigens immunoprecipitated from Raji cells and from *Xenopus* oocytes injected with Raji mRNA. Raji cells (**lanes 1 – 5**) and injected oocytes (**lanes 6 – 10**) were labeled with [^{35}S]methionine. Cell lysates were passed over a lentil lectin column. The material bound to the column was eluted and immunoprecipitated with the following ascites fluids: (**1, 6**) PX63; (**2, 7**) monoclonal D1-12; (**3, 8**) monoclonal D4-22; (**4, 5, 9, 10**) monoclonal BT 2.2. Samples in **lanes 5** and **10** were denatured by boiling in SDS prior to immunoprecipitation. The positions of the α, intermediate (In), and β chains are indicated by arrows. Size markers are indicated in kilodaltons.

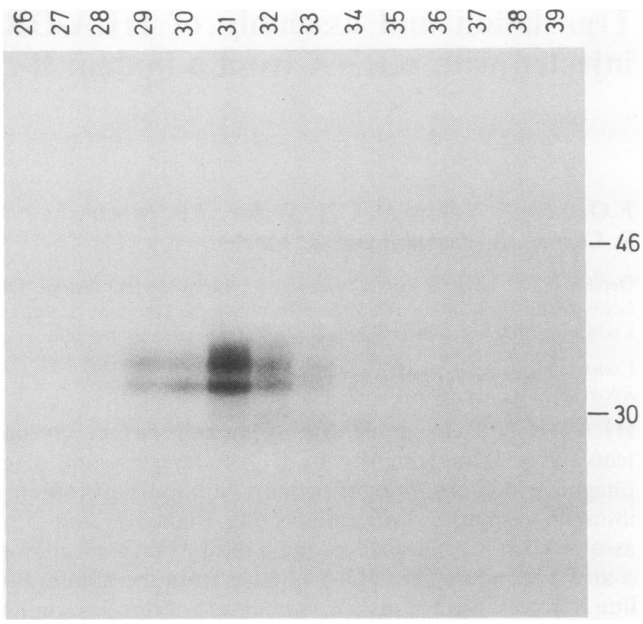

Fig. 2. SDS-PAGE of immunoprecipitates from *Xenopus* oocytes injected with fractionated Raji mRNA. RNA extracted from slices of a preparative agarose-urea gel was injected in oocytes. The [^{35}S]methionine-labeled translation products were immunoprecipitated with a mixture of monoclonal antibodies D1-12, D4-22, and BT 2.2. Lanes are numbered according to the RNA gel slice number. 18S rRNA was in slice 24. Slices 26 – 39 correspond to RNA between 1600 and 800 nucleotides in length. Protein size markers are indicated in kilodaltons.

RNA from Raji cells, and incubated for 24 h. The translation products were immunoprecipitated with the same three monoclonal antibodies. The oocyte homogenate was first passed over a column of lentil lectin and only retained material was analyzed. Each monoclonal antibody bound material with the same mol. wt. range as the DR antigens made in Raji cells (Figure 1). After denaturation of the oocyte homogenate by boiling in SDS, only the monoclonal antibody BT 2.2 was still able to bind material with a mol. wt. between 31 000 and 29 000 (Figure 1, lane 10). This result provides strong evidence that DR β chains were synthesized in oocytes. Four additional anti-DR monoclonal antibodies (D1-11, D1-17, 2-72, PTF 29) were used to immunoprecipitate material synthesized in oocytes (not shown). Each one bound material very similar, by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), to that obtained with D1-12, D4-22, and BT 2.2 All of these monoclonal antibodies, as well as additional ones, were tested with the translation products of Raji mRNA in the rabbit reticulocyte lysate. No specific immunoprecipitation was observed with any of them.

Total cytoplasmic poly (A)$^+$ RNA from Raji cells was size-fractionated on a preparative agarose-urea gel. Each size fraction of RNA molecules between 700 and 1600 nucleotides in length was tested in the oocyte translation assay. Fraction 31 appeared highly enriched in DR mRNA (Figure 2). The nature of the translation products obtained with enriched mRNA was analyzed further.

DR antigens of Raji cells gave a complex pattern of spots on two-dimensional gel electrophoresis (Figure 3). The α chain appeared as a set of acidic spots. The β chain represented a group of more basic spots. The use of monoclonal antibody BT 2.2, after SDS-denaturation of the DR subunits, allowed unambiguous definition of each β spot. The intermediate chain was a basic spot just above the β spots. Additional spots, namely a string of minor spots extending between the intermediate and α spots, as well as two very basic spots of ~34 000 daltons, have been shown, by tryptic peptide analysis, to be related to the intermediate chain (D. Charron, personal communication).

DR antigens synthesized in *Xenopus* oocytes injected with enriched Raji mRNA had a very similar two-dimensional gel pattern (Figure 3). The α chain appeared as two rows of three and four spots. The intermediate and related spots were also present, although the relative intensity of the related spots was quite different from that in Raji cells. The pattern of β spots was less complex than in Raji cells. Again, the use of monoclonal BT 2.2 after SDS denaturation allowed β chain spots to be identified. Since this monoclonal antibody is directed against the β chain, the presence of α and intermediate chains in the non-denaturated translation product is most likely due to assembly of all three chains in the oocyte.

The DR antigens synthesized in oocytes injected with Raji mRNA are glycosylated, since they bound to a lentil lectin column (see Figure 1). The glycosylation pattern was analyzed further by treating the oocytes with tunicamycin, an inhibitor of N-linked glycosylation, using a protocol derived from the one first described by Colman *et al.* (1981). Unglycosylated DR antigens made in oocytes were very similar to those made in Raji cells (Figure 4). The samples shown in Figure 4 were also analyzed by two-dimensional gel electrophoresis (not shown). From this analysis it was clear that the unglycosylated α chain appeared as two bands with apparent mol. wts. of 30 000 and 29 000. The intermediate chain appeared as a strong band of 27 000 daltons, indicating that two

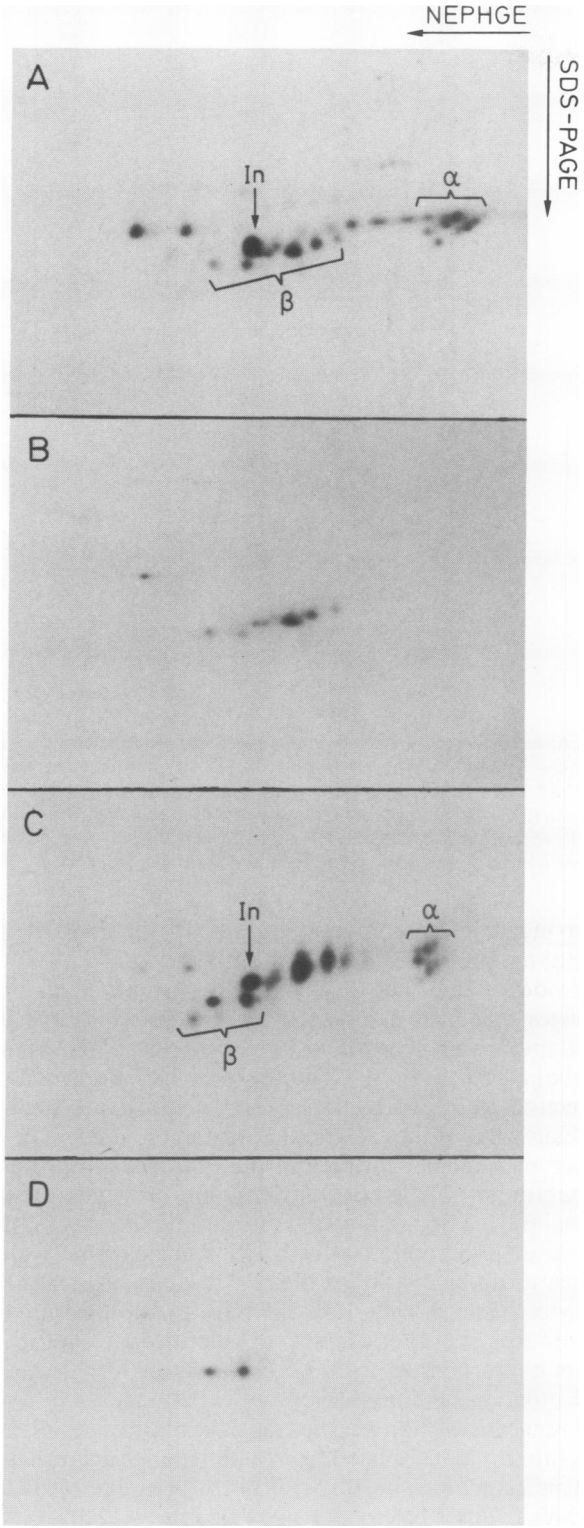

Fig. 3. Two-dimensional gel analysis of HLA-DR antigens immunoprecipitated from Raji cells and from *Xenopus* oocytes injected with enriched Raji mRNA. Raji cells (**A, B**) and injected oocytes (**C, D**) were labeled with [^{35}S]methionine. All immunoprecipitations were with the monoclonal antibody BT 2.2. Samples in **B** and **D** were denatured by boiling in SDS prior to immunoprecipitation. The position of α spots and of β spots is indicated by brackets. The position of the intermediate chain (In) is indicated by an arrow. The original gels were longer in the vertical dimension.

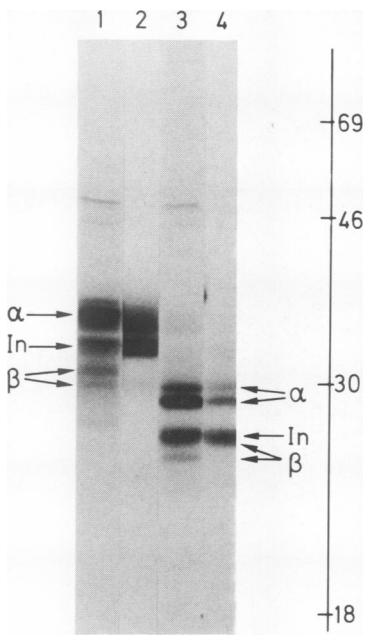

Fig. 4. Effect of tunicamycin on HLA-DR antigens synthesized in Raji cells and in *Xenopus* oocytes injected with enriched Raji mRNA. The [^{35}S]-methionine-labeled translation products were immunoprecipitated with a mixture of monoclonal antibodies D1-12, D4-22, and BT 2.2. **1**, Raji cells without tunicamycin; **2**, injected oocytes without tunicamycin; **3**, Raji cells treated with tunicamycin; **4**, injected oocytes treated with tunicamycin. The positions of the α, intermediate (In), and β chains are indicated by arrows. Protein size markers are indicated in kilodaltons.

N-linked oligosaccharides are present on the mature chain. The unglycosylated β chain in Raji cells appeared as two bands of 27 000 and 26 000 daltons. In oocytes, only the 27 000-dalton band was found. Since the β chain overlapped with the intermediate chain in a SDS-polyacrylamide gel, the combined use of monoclonal BT 2.2 after SDS denaturation and of two-dimensional gel electrophoresis was necessary to discriminate between these two unglycosylated chains.

It is known that under non-reducing conditions DR β chains migrate faster than reduced β chains in SDS-polyacrylamide gels (Allison *et al.*, 1978). The same observation was made with DR β chain synthesized in oocytes injected with enriched Raji mRNA (Figure 5). However, the β chains synthesized in oocytes were slightly slower migrating than β chains synthesized in Raji cells. In both cases they appeared as two sharp bands. Non-reducing conditions allow a much clearer identification of β chains (compare Figure 4, lane 2, with Figure 5, lane 3).

In order to confirm that α chains were present in the translation products, amino-terminal sequencing was carried out. It has been reported by several groups that isoleucine is present at positions 1, 7, and 8, and phenylalanine at position 12, of DR α chain (reviewed in Strominger *et al.*, 1981). There are multiple DR β chains and published amino-terminal sequences diverge (Allison *et al.*, 1978; Strominger *et al.*, 1981; Kratzin *et al.*, 1981; Wiman *et al.*, 1982). However, isoleucine was in no instance found in the first 10 amino acids of the DR β chain, and phenylalanine was always found in position 7. Oocytes injected with enriched Raji mRNA were labeled with either [^{3}H]isoleucine or [^{3}H]phenylalanine. The translation products were immunoprecipitated with a pool of anti-DR monoclonal antibodies (Figure 5). The entire immunoprecipitate was used for sequencing without gel purification of the bands (Figure 6). Isoleucine residues in equimolar amounts were observed at positions 1, 7, and 8. Phenylalanine residues

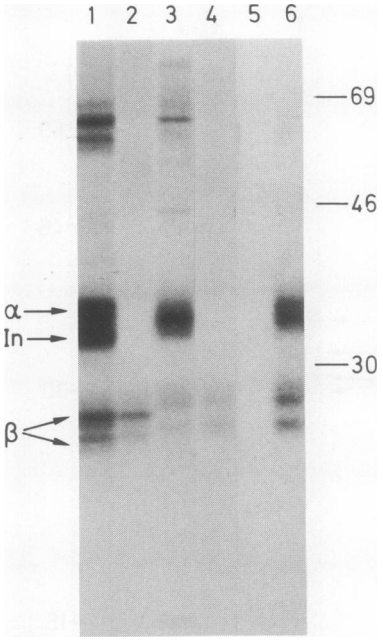

Fig. 5. SDS-PAGE and HLA-DR antigens under non-reducing conditions. Samples were denatured in SDS in the absence of reducing agent before loading on the gel. The positions of the α, intermediate (In), and β chains are indicated by arrows. Protein size markers are indicated in kilodlatons. **1**, [^{35}S]methionine-labeled Raji cells, immunoprecipitations with monoclonal antibody BT 2.2; **2**, as in **1** but the sample was denatured in SDS prior to immunoprecipitation; **3**, [^{35}S]methionine-labeled oocytes injected with enriched Raji RNA, immunoprecipitation with monoclonal antibody BT 2.2; **4**, as in **3** but the sample was denatured in SDS prior to immunoprecipitation; **5**, [^{3}H]phenylalanine-labeled oocytes injected with enriched Raji mRNA, immunoprecipitation with PX63 ascites; **6**, same as **5** but immunoprecipitation with a mixture of monoclonal antibodies D1-12, D4-22, and BT 2.2. The autoradiographic exposure of **lanes 5** and **6** was one month, as opposed to 5 days for the rest of the gel.

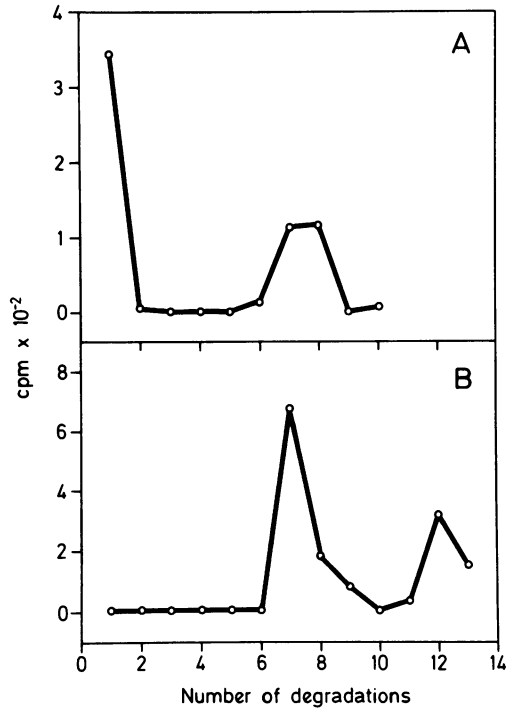

Fig. 6. Amino-terminal isoleucine and phenylalanine residues in HLA-DR antigens synthesized by *Xenopus* oocytes. Oocytes were injected with enriched Raji mRNA and labeled with [^{3}H]isoleucine (**A**) or [^{3}H]phenylalanine (**B**). Translation products were immunoprecipitated with a mixture of the monoclonal antibodies D1-12, D4-22, and BT 2.2, and the position of the labeled amino acids determined by Edman-degradation.

in equimolar amounts were observed at positions 7 and 12. Possible minor phenylalanine residues were observed at positions 8, 9, and 13. These results are in full agreement with the presence of assembled equimolar α and β chains in the injected oocytes. They also suggest that the intermediate chain either does not contain these amino acids at its amino-terminus, or that its amino-terminus is blocked.

Discussion

Recently, evidence has accumulated for the existence in homozygous B-cell lines of multiple HLA-DR antigens (Quaranta *et al.*, 1980; Lampson and Levy, 1980; Carrel *et al.*, 1981; Accolla *et al.*, 1981) and other human Ia-like antigens (Tosi *et al.*, 1978; Corte *et al.*, 1981a; Shackelford *et al.*, 1981; Nadler *et al.*, 1981). Monoclonal antibodies directed against specific human Ia antigens are now available. The aim of this study was to develop a mRNA translation system for DR antigens that would allow the use of monoclonal antibodies in the identification of the products. Previous studies using cell-free translation in the rabbit reticulocyte lysate system had to rely on antisera raised against denatured Ia antigens for the identification of the translation products (Korman *et al.*, 1980; Wiman *et al.*, 1982; Kvist *et al.*, 1982). Such xenoantisera will not discriminate between the multiple human Ia-like antigens. Here, we present evidence that DR antigens are synthesized and assembled in *Xenopus* oocytes injected with mRNA from the human B-cell line Raji, and that they can be immunoprecipitated with anti-DR monoclonal antibodies.

Evidence that DR β chains are synthesized in injected oocytes relied on monoclonal antibodies which specifically bind the β chain. The monoclonal antibody BT 2.2 bound the β chain even after the DR antigens had been denatured. Therefore, BT 2.2 provides a powerful tool to analyze the DR β chain without the presence in the immunoprecipitate of the other two chains, the α and the intermediate. After SDS denaturation of the oocyte translation products, the monoclonal BT 2.2 bound material that co-migrated in two-dimensional gel electrophoresis with DR β chains from Raji cells. Thus, it can be concluded that DR β chains are translated in oocytes injected with Raji mRNA. In the absence of denaturation, all three chains, α, intermediate, and β, were immunoprecipitated from injected oocytes with several anti-DR monoclonal antibodies.

Using this highly specific translation assay, a mRNA size fraction could be isolated from a preparative agarose-urea gel that still contained the three mRNAS for α, intermediate, and β chains. The RNA in this size fraction, ~1200–1300 nucleotides long, was enriched ~20-fold over total mRNA. The availability of such an enriched fraction, besides its usefulness for deriving cDNA clones, facilitated the analysis of translation products from injected oocytes.

Having established that DR β chains were synthesized in injected oocytes, evidence for assembly must rely on the unambiguous identification of α chains and intermediate chains in immunoprecipitates obtained with monoclonal antibodies directed against the β chain. Immunoprecipitation of apparently complete DR antigens from injected oocytes was observed with seven different monoclonal antibodies. This

strongly suggests that the three chains were immunoprecipitated together because of assembly rather than because of cross-reaction of each monoclonal antibody with all three chains. Two additional monoclonal anti-DR antibodies, L203 and L227, have been shown in another report to bind DR antigens synthesized in *Xenopus* oocytes injected with mRNA from Raji cells (Finn and Levy, 1982).

The presence of the intermediate chain in immunoprecipitates with antibody BT 2.2 is evident from two-dimensional gel analysis. In addition to the intermediate chain spot, a set of spots known to be related to the intermediate chain (D. Charron, personal communication) was present in the oocyte translation products. The intensity of the intermediate chain spots observed in oocytes labeled with [^{35}S]methionine was much stronger than α chain and β chain spots. It has been shown that the murine equivalent, the invariant spot found in Ia antigens, is unusually rich in methionine but poor in phenylalanine (McMillan *et al.*, 1981). Human intermediate chain and murine invariant chain are homologous as determined by tryptic peptide analysis (Charron *et al.*, 1982). In accordance with these results, the relative intensity of the intermediate chain synthesized in oocytes was much weaker after [^{3}H]phenylalanine labeling. The intensity of the intermediate chain spots observed in oocytes labeled with [^{35}S]-methionine was also stronger compared with the intermediate chain immunoprecipitated from Raji cells that had been labeled for 16 h. This may be due to the fact that the intermediate chain is released from DR antigens before it reaches the cell surface (Owen *et al.*, 1981). If the DR antigens made in injected oocytes remain intracellularly, it is possible that the intermediate chain does not dissociate from the α and β chains, thus being present in a higher molar ratio than in Raji cells. In the presence of tunicamycin, the intermediate chain synthesized in oocytes was 6000 daltons smaller than its glycosylated counterpart. This mol. wt. difference is expected since two N-linked glycosylations take place *in vivo* (Owen *et al.*, 1981).

The DR α chains synthesized in oocytes appeared virtually identical to those synthesized in Raji cells as determined by two-dimensional gel analysis. Furthermore, α chains synthesized in oocytes in the presence of tunicamycin were 6000 daltons smaller than their glycosylated counterparts, in accordance with the presence of two N-linked glycosyl groups on DR α chains (Strominger *et al.*, 1981). Finally, the identity of α chains made in injected oocytes was confirmed by sequencing amino-terminal ends in the immunoprecipitated material. Isoleucine and phenylalanine were found at the expected positions. Furthermore, the phenylalanine at positions 7 and 12 were equimolar as expected from a 1:1 ratio of α and β chain in the assembled DR antigens.

The data presented here provide evidence that the three chains observed intracellularly for DR antigens, namely the α, intermediate and β chains, assemble in oocytes injected with mRNA from Raji cells. Post-translational modifications, such as glycosylation, that are specific to the DR antigen also take place in oocytes. The use of this faithful translation and assembly system has allowed the isolation of cDNA clones encoding the DR α chain (Wake *et al.*, in preparation) and two distinct DR β chains (Long *et al.*, in preparation). The availability of these cDNA probes and of the oocyte assembly system should make it possible to analyze the steps involved in the biosynthesis of DR antigens.

Materials and methods

Growth and labeling of cells

Raji cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum, glutamine, and gentamicin as previously described (Carrel *et al.*, 1981). Cells were metabolically labeled by incubation for 16 h at 37°C at a concentration of 2 x 10^6 cells/ml in complete methionine-free medium supplemented with 1 mCi of [^{35}S]methionine per 50 x 10^6 cells. To study the unglycosylated DR molecules, tunicamycin was added at 2 μg/ml 2 h before the addition of [^{35}S]methionine.

Preparation and enrichment of RNA

Frozen cell pellets were lysed in ice-cold 10 mM Tris-HCl (pH 7.6), 0.1 M NaCl, 1% Nonidet P40 (1 ml lysis buffer/10^8 Raji cells) by vortexing 4 times for 15 s, at 1-min intervals. Lysed cells were centrifuged at 4°C for 4 min at 4000 r.p.m. in the Beckman J-6 centrifuge (4500 *g*). 4 ml of cytoplasmic supernatant was loaded over the following gradient, from bottom to top in an SW41 polyallomer tube: 2 ml 5.7 M CsCl in 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 4.2 ml of a linear gradient of 40–20% (w/v) CsCl in 20 mM Tris-HCl (pH 7.4), 2 mM EDTA, 0.8 ml 5% (w/v) sucrose, 20 mM Tris-HCl (pH 7.4), 0.1 M NaCl, 4 mM EDTA. Gradients were prepared and equilibrated at 14°C before lysing the cells. RNA was pelleted at 37 000 r.p.m. for 14 h at 14°C. Larger RNA preparations can be spun in SW27 tubes at 26 000 r.p.m. for 16 h at 14°C. Tubes were inverted and the bottom cut with a blade. RNA was dissolved in 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, adjusted to 0.3 M sodium acetate (pH 5.0), and precipitated with two volumes of ethanol. RNA was dissolved in 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 1% SDS, heated at 100°C for 2 min and cooled to room temperature. After addition of 1 volume of 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 1 M NaCl, it was loaded on an oligo(dT) cellulose column (Collaborative Research). Poly $(A)^+$ RNA was eluted with H_2O and precipitated twice with ethanol in the absence of EDTA.

Poly $(A)^+$ RNA was size-fractionated in an agarose-urea gel. The buffer system of Rosen *et al.* (1975), 6 M urea in 25 mM sodium citrate (pH 3.8), is well suited for high capacity and high resolution. It is also fully denaturing (Lehrach *et al.*, 1977). In this buffer, a 2.5% agarose gel gives good resolution of RNA molecules smaller than 18S. 500 μg poly $(A)^+$ RNA was dissolved in 100 μl 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 0.5% SDS. 200 μl of 99% dimethyl sulphoxide (adjusted to 1 mM EDTA and pH 8.0) were added. The RNA was heated at 45°C for 5 min and loaded on a 4 x 0.5 cm slot. Electrophoresis was carried out in the cold for 36 h, until the bromophenol-blue reached the bottom of the gel. 2-mm slices were cut along the gel and dispersed with an Ultra-Turrax in 4 ml of 10 mM Tris-HCl (pH 7.4), 10 mM EDTA, 0.5 M NaCl, 0.1 mg/ml *Escherichia coli* tRNA. The dispersed suspension was adjusted to 0.5% SDS and shaken overnight. mRNA was isolated from the supernatant by chromatography over small oligo(dT)-cellulose columns and precipitated twice in the absence of EDTA. Recoveries throughout the procedure were monitored by including 3′ end-labeled Raji mRNA in the sample before the preparative gel electrophoresis.

Oocyte injections

Stage 6 oocytes were manually isolated from *X. laevis* ovaries after a 90–120 min incubation at room temperature with agitation in 0.2% crude collagenase (Sigma C-0130) in Ca^{2+}-free OR2 medium (Wallace *et al.*, 1973). Oocytes were injected with 20 ng mRNA in a volume of 50 nl (Moar *et al.*, 1971) and incubated for 24 h in OR2 medium containing 0.5 mCi/ml of the indicated radio-labeled amino acid, and 50 units/ml of penicilin and streptomycin. Tunicamycin treatment was modified from Colman *et al.* (1981). Oocytes were incubated in the presence of 5 μg/ml tunicamycin for 12 h, injected with RNA in 50 nl containing 40 μg/ml tunicamycin and incubated for 24 h in medium containing 5 μg/ml tunicamycin. Oocytes were homogenized as described by Rungger and Türler (1978), except that 1 ml of buffer was used per 20 oocytes.

Immunoprecipitation

The reactivity of the various monoclonal antibodies used in this study has been described in previous publications (D1-12, D4-22, D1-11, and D1-17 in Carrel *et al.*, 1981; PTF 29.12 in Corte *et al.*, 1981b; BT 2.2 and 2.72 in Accolla *et al.*, 1982).

The supernatant of the oocyte homogenate was adjusted to pH 8.0 with Tris-HCl (pH 7.0) and to 1% Aprotinin (Sigma). 20 μl PX63 ascites were added per ml, incubated >2 h in the cold, incubated another 2 h in the presence of protein A-Sepharose (Pharmacia). 20 μl anti-DR monoclonal antibody under the form of ascites fluid were added per ml supernatant (this corresponds to 1 μl ascites/injected oocyte) and incubated overnight at 4°C. Samples were spun for 3 min in the Eppendorf microfuge and the pellets discarded. This step is crucial to avoid high background due to aggregated material. Protein A-Sepharose was added to the supernatant and incubation

continued for 4 h. Immunoprecipitates were washed twice in 50 mM Tris-HCl (pH 7.4), 5 mM EDTA, 0.15 M NaCl, 1% Nonidet P40, 10 mM methionine, 1% Aprotinin, three times in the same buffer without Aprotinin and with 0.5 M NaCl, and twice in 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 0.15 M NaCl, 0.5% Nonidet P40. Denaturation of the antigens prior to immunoprecipitation was carried out as follows. Oocyte homogenates were adjusted to 1% SDS, incubated for 3 min at 100°C, and diluted 5-fold in 50 mM Tris-HCl (pH 7.4), 5 mM EDTA, 0.15 M NaCl, 0.5% Nonidet P40, 1% Aprotinin. Lentil lectin columns were run as follows: 50 injected oocytes were homogenized in 1 ml of buffer as described above. The supernatant was adjusted to 2 ml in 0.15 M NaCl, 0.25% Nonidet P40 and loaded over a 1-ml column of lentil lectin-Sepharose 4B (Pharmacia). After extensive washing with loading buffer, glycosylated material was eluted with the same buffer containing 0.1 M α-methyl mannoside. 1.3% of [^{35}S]methionine counts in the oocyte homogenate were eluted in the bound fraction. With [^{35}S]methionine-labeled Raji cells, 2.8% of the counts were eluted in the bound fraction. SDS denaturation and immunoprecipitations of Raji cell lysates were as described for injected oocytes. 1 μl ascites fluid was added per lysate of 1 x 10^6 cells.

Gel electrophoresis

Immunoprecipitates were eluted in 0.5 M Tris-HCl (pH 8.8), 1 M sucrose, 5 mM EDTA, 0.01% bromophenol-blue, 3% SDS, 8.3 mM dithiothreitol (when present) by heating at 100°C for 3 min, and loaded on 12% SDS-polyacrylamide gels. Two-dimensional gel electrophoresis, with non-equilibrium pH gradient electrophoresis in the first dimension, was run according to O'Farrell *et al.* (1977). The second dimension was in 12% polyacrylamide gels. Gels were fixed in 10% trichloroacetic acid, treated with Enhance (New England Nuclear), washed in 20% methanol, 3% glycerol and dried. Dried gels were exposed to preflashed Kodak X-AR film, with intensifying screens (Cawo) at −70°C.

Amino acid sequence determination

Immunoprecipitates were washed with distilled water and subjected to automatic amino acid sequence determination in a Beckman 890 sequencer.

Acknowledgements

We thank G. Corte for a gift of monoclonal antibody BT 2.2, D. Rungger for teaching us the oocyte injection method, M. Crippa and M. Fischberg who provided laboratory space for the injections, J.-C. Jaton for performing the amino acid sequencing, and M. Strubin for help with some of the experiments. This research was supported by the Swiss National Fund for Scientific Research.

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