β_2-Microglobulin Induces Intracellular Transport of Human Class I Transplantation Antigen Heavy Chains in *Xenopus laevis* Oocytes

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ABSTRACT Human class I transplantation antigens are cell-surface-expressed molecules composed of one glycosylated, membrane-integrated heavy chain and one nonglycosylated, water-soluble subunit, β_2-microglobulin (β_2m). We have examined the intracellular transport of the two subunits by microinjecting mRNA into *Xenopus laevis* oocytes.

β_2m, translated in oocytes, was transported and secreted into the medium in the absence of heavy chains whereas heavy chains were retained in the endoplasmic reticulum if not co-translated with β_2m. In the presence of β_2m, heavy chains resisted digestion by endoglycosidase H (Endo H), suggesting that β_2m promotes the transport of heavy chains from endoplasmic reticulum to the Golgi compartment. Pulse-chase experiments confirmed this notion.

The possibility that heavy chains aggregate irreversibly when synthesized in the absence of β_2m was ruled out and it is demonstrated that preformed heavy chains will become transported once β_2m is available. It is suggested that intracellular transport is controlled by structural features that are part of the transported polypeptide. If so, β_2m but not heavy chains may possess such features.

Class I transplantation antigens serve as restricting elements that permit cytotoxic T-cells to recognize viral and tumor antigens on the surface of infected and transformed cells (1). The two subunits of class I antigens, i.e., the glycosylated, genetically polymorphic heavy chain and the nonglycosylated, invariant β_2-microglobulin (β_2m)[1] are held together by non-covalent bonds only (2). Studies on the biosynthesis and intracellular transport of class I antigens have shown that the two subunits associate in the endoplasmic reticulum and that they are transported as heterodimers via the Golgi complex to the cell surface (3).

The lymphoblastoid cell line Daudi does not produce β_2m (4) due to a point mutation in the initiation codon of the mRNA (5). Synthesis of class I antigen heavy chains occur in Daudi cells (6, 7). However, the heavy chains are not expressed at the cell surface (8). These observations led to the suggestion that β_2m controls the intracellular transport and, thus, the cell surface expression of the heavy chain (9, 10).

To analyze the role of β_2m in the intracellular transport of human heavy chains we have used the *Xenopus laevis* oocyte translation system. By microinjecting fractions enriched for β_2m and heavy chain mRNA, respectively, we here demonstrate that the presence of β_2m is a prerequisite for the transport of the heavy chains from the endoplasmic reticulum to the Golgi compartment.

[1] *Abbreviations used in this paper:* β_2m, β_2-microglobulin; Endo H, endoglycosidase H.

MATERIALS AND METHODS

Antisera: A rabbit anti-human class I antigen serum was rendered specific for heavy chains by extensive absorptions on a β_2m column (11). A previously described rat monoclonal antibody against human β_2m was used (12). The rabbit antiserum against human class II antigens has also been described (13).

Labeling of Cells: Raji cells were labeled with [^{35}S]methionine as previously described (9). Approximately 4×10^6 cells/ml were incubated with 150 μCi/ml of [^{35}S]methionine (Amersham Corp., Amersham, Buckinghamshire, England) for 3.5 h. The labeled cells were kept for an additional hour in the presence of 20 mM unlabeled methionine.

Isolation of mRNA and Cell-free Translation: mRNA was obtained from Raji cells essentially as described (3). Briefly, microsomes were isolated from lysed cells and total microsomal RNA was extracted by phenol/chloroform isoamylalcohol (49:49:2 vol/vol). Poly A-containing mRNA was

THE JOURNAL OF CELL BIOLOGY · VOLUME 99 JULY 1984 226-232

isolated by two cycles of oligo dT column chromatography, and subsequently size fractionated by sucrose-density gradient centrifugation. The distribution in the sucrose gradient fractions of mRNA coding for class I and II antigen subunits was monitored by subjecting aliquots of each fraction to cell-free translation. A previously described rabbit reticulocyte lysate system containing dog pancreas microsomes was used (3). Fractions containing mRNA coding for only one of the two class I antigen subunits were used for microinjection (see Results and Fig. 1).

Purification of mRNA by Hybridization Selection: Highly purified mRNA coding for β_2m was isolated by hybridization selection. A β_2m cDNA clone, kindly supplied by Dr. M. Fellous, was used to transform *Escherichia coli* strain 294 (14). Plasmid DNA was isolated from tetracycline-resistant colonies and immobilized onto nitrocellulose filters (15). To one-half filter 2.5 μg of mRNA was hybridized (16). Messenger RNA eluted from the filters was analyzed by in vitro translation and by microinjection into oocytes.

Microinjection and Translation of mRNA in Oocytes: Large *Xenopus laevis* females were injected with 1,000 IU of human chorionic gonadotropin (Sigma Chemical Co., St. Louis, MO) 1–2 wk prior to sacrifice. In a typical experiment, 30 to 40 oocytes were microinjected with 50 nl each of an mRNA fraction containing 200 to 500 μg of mRNA per ml of distilled water. If not stated otherwise the oocytes were incubated in 150 μl of Bart's medium (17) containing 0.2 mCi of [^{35}S]methionine at 19°C for 20 to 24 h. The oocytes were homogenized in 0.5 ml of ice-cold, 20 mM Tris-Cl buffer, pH 8.0, containing 1% Triton X-100, 0.15 M NaCl, and 1% Trasylol (Bayer AG, Leverkusen, Federal Republic of Germany) in a small Dounce homogenizer. After solubilization, the suspension was centrifuged for 15 min at 10,000 *g*. The supernatants were usually enriched for glycoproteins on Lens Culinaris hemagglutinin–Sepharose 4B columns (18). Glycoproteins were eluted by the addition of 10% α-methyl-mannoside in the lysis buffer. The eluates from the lectin columns were subjected to indirect immunoprecipitation.

Immunoprecipitations and Electrophoretic Analyses: Indirect immunoprecipitations were carried out according to a standard protocol (9). Immunoprecipitated proteins were separated by gradient slab SDS PAGE essentially as described by Blobel and Dobberstein (19), or by two-dimensional isoelectric focusing and SDS PAGE (20). Gels were fixed in 10% trichloroacetic acid for 15 min at 4°C, treated with Enhancer (New England Nuclear, Boston, MA) for 60 min, and finally soaked in water containing 1% glycerol for 15–30 min. After drying fluorography of the gels was performed as described (21). Kodak XAR films were used throughout.

In accordance with previous studies, terminally glycosylated heavy chains have been assigned an apparent molecular weight of 45,000, while core-glycosylated and Endo-H-digested heavy chains display apparent molecular weights of 43,000 and 41,000, respectively (9). Although the values may vary slightly from one gel to another, the relative positions of the three molecular forms of the heavy chains were always the same.

The immunoprecipitates of exogeneous proteins translated in the oocyte system often contained varying amounts of contaminants. These contaminants were reproducible and are described below. The occurrence of large amounts of yolk proteins that stick nonspecifically to the immunoprecipitates, gives rise to a substantial background (see Fig. 2, lanes *A* and *B*). However, most of the background could be eliminated provided a lectin-purified fraction of occyte glycoproteins was used for the immunoprecipitations (Fig. 2, lanes *E* and *F*). Nonetheless, an endogeneous oocyte protein with an apparent molecular weight of ~53,000 was frequently encountered (see Figs. 4 and 8). In addition, rabbit (Fig. 3) and monoclonal antibodies against β_2m (e.g., Fig. 6) reacted with an oocyte protein with an apparent molecular weight of ~75,000. The possible relationship between this protein and β_2m is unknown. Finally, most fractions enriched for β_2m mRNA and translated in the oocytes gave rise to two contaminating proteins, both with apparent molecular weights of ~35,000, present in most immunoprecipitates (cf. Fig. 6, lanes *A–D* with lanes *E* and *F*). Different batches of oocytes vary in translation efficiency. Consequently, the level of background visualized after fluorography differed somewhat with the particular batch of oocytes used. Despite the occurrence of the contaminants listed above, the interpretation of the data was unambiguous, since contaminants with apparent molecular weights similar to those of the class I antigen subunits did not occur.

Enzyme Digestions: Asn-linked, core-sugar moieties were removed from immunoprecipitated proteins using Endo H (Seikagaku Kogyoco, Tokyo, Japan) and sialic acid was digested by neuraminidase (*Vibrio cholerae*, 1 IU/ml, Calbiochem-Behring Corp., Lucerne, Switzerland) according to the protocols previously used (9).

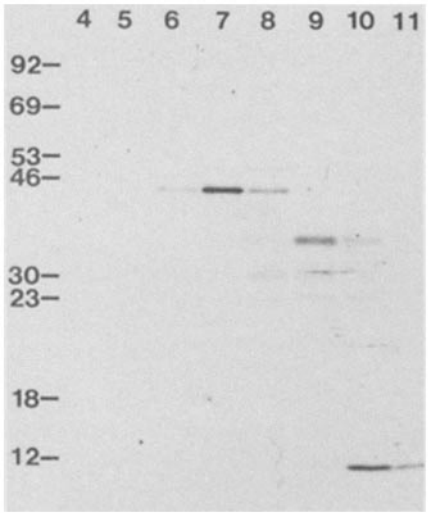

FIGURE 1 In vitro translation of mRNA size fractionated by sucrose density gradient centrifugation. Microsomal, poly A-containing human mRNA from the cell line Raji was size fractionated by sucrose density gradient centrifugation. Aliquots from sucrose gradient fractions *4* to *11* were translated in vitro in a rabbit reticulocyte lysate cell-free system containing dog pancreas microsomes. Lysed microsomes were immunoprecipitated with a mixture of antibodies against class I and class II transplantation antigens. The immunoprecipitates were analyzed by SDS PAGE. The numbers of the fractions are denoted on the top of the figure. Figures on the vertical axis denote apparent molecular weights in kilodaltons as determined by a mixture of marker proteins run in parallel.

RESULTS

Translation of Human Class I Antigen Heavy Chains and β_2m in Xenopus laevis Oocytes

To ascertain that sucrose gradient fractions enriched for mRNA coding for class I antigen heavy chains and β_2m, respectively, were free from cross-contamination, aliquots from all fractions were subjected to in vitro translation, indirect immunoprecipitation, and SDS PAGE. To localize fractions enriched for the class I antigen subunits a rabbit antiserum reactive with both polypeptide chains was used. This antiserum was mixed with a rabbit antiserum against class II antigen α and β chains to ascertain that adequate separation of the mRNA for the different subunits had occurred. Fig. 1 shows a typical result. While class I antigen heavy chain mRNA was enriched in fractions 6 to 8, β_2m mRNA occurred predominantly in fractions 10 and 11. As expected, class II antigen-coding mRNA occurred in between the fractions containing the mRNA for class I antigen subunits. Thus, mRNA for β- and α-chains were enriched in fractions 8 and 9 and 9 and 10, respectively. Fig. 1 shows that there is no detectable cross-contamination of mRNA for the two class I antigen subunits in any fraction. Therefore, fractions 7 and 10, respectively, were used for the oocyte microinjection experiments.

The mRNA fractions selected (see above) were separately microinjected into *Xenopus* oocytes that were subsequently labeled with [^{35}S]methionine. β_2m from the oocyte homogenate and from the incubation medium, respectively, was immunoprecipitated with a monoclonal antibody and analyzed by SDS PAGE. As can be seen in Fig. 2 a distinct 12-

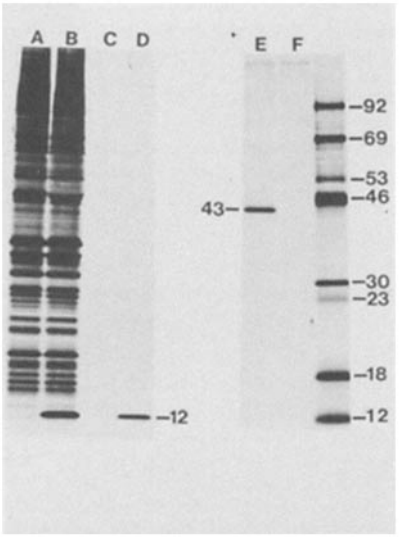

FIGURE 2 Translation in *Xenopus laevis* oocytes of human mRNA fracations coding for β_2m and class I antigen heavy chains. *Xenopus laevis* oocytes, microinjected with mRNA fractions coding for β_2m (*A–D*) and heavy chains (*E* and *F*), respectively, were labeled with [^{35}S]methionine. Translation products from homogenized oocytes injected with β_2m mRNA were immunoprecipitated with a monoclonal antibody against human β_2m (*B*) and normal rat immunoglobulin (*A*), respectively, prior to SDS PAGE. Secreted translation products from oocytes injected with β_2m mRNA were also subjected to immunoprecipitation using the monoclonal antibody (*D*) and normal rat immunoglobulin (*C*) prior to SDS PAGE. An oocyte glycoprotein fraction, isolated on a Lens culinaris hemagglutinin Sepharose 4B column, from oocytes injected with heavy chain mRNA, was subjected to immunoprecipitation using a rabbit antiserum against heavy chains (*E*) and normal rabbit serum (*F*), respectively. Figures denote apparent molecular weights in kilodaltons of the marker proteins.

kd component is visualized despite the presence of all contaminating proteins (lane *B*). An apparently identical component was immunoprecipitated also from the incubation medium (lane *D*). Normal rat serum did not precipitate this protein (Fig. 2, lanes *A* and *C*).

The fraction enriched for heavy chain mRNA was also translated in oocytes. To reduce the substantial amount of nonspecifically precipitated proteins (see Fig. 2, lanes *A* and *B*) we restricted all our immunoprecipitations of class I antigen heavy chains to oocyte glycoprotein fractions enriched by affinity chromatography on Lens culinaris hemmaglutinin Sepharose 4B columns (18). A rabbit antiserum specific for heavy chains precipitated a single component with an apparent molecular weight of 43,000 (Fig. 2, lane *E*) from such a glycoprotein fraction. Normal rabbit serum did not react with this molecule (Fig. 2, lane *F*). In the absence of microinjected heterologous mRNA the anti-heavy chain serum did not precipitate the 43,000-dalton chain (not shown).

Intracellular Transport of Human Class I Antigens in Xenopus laevis Oocytes

To examine whether the class I antigen heavy chains were transported intracellularly the glycosylation state of the oocyte-translated heavy chains was determined (2 and 22). Thus, the sensitivity to digestion by the enzyme Endo H was used as a marker for the subcellular distribution of the heavy chains, since the enzyme digests core sugar moieties but not trimmed or terminally glycosylated oligosaccharides (23).

Human class I antigen heavy chains translated in oocytes were immunoprecipitated from a [^{35}S]methionine-labeled glycoprotein fraction with an anti-class I antigen serum. One portion of the immunoprecipitate served as the control (Fig. 3, lane *A*) while the remainder was digested with Endo H (Fig. 3, lane *B*). As expected, the untreated sample gave rise to a single radioactive, heavy chain band following SDS PAGE (for discussion of contaminating proteins, see Materials and Methods). The Endo H-digested material also gave rise to a single, sharp heavy chain band, whose apparent molecular weight was 41,000 as compared with 43,000 for the untreated control (cf. Fig. 3, lanes *A* and *B*). These data show that class I antigen heavy chains synthesized in oocytes in the absence of β_2m do not become terminally glycosylated. The absence of such carbohydrate processing that confers Endo H resistance may be due to heterologous heavy chains that are poor substrates for oocyte trimming enzymes and/or glycosyl transferases. Alternatively, the transport of heavy chains to the Golgi complex may be impeded by the absence of β_2m. The latter possibility was examined.

Fractions enriched for β_2m and heavy chain mRNA, respectively, were mixed and translated in oocytes. A glycoprotein fraction was immunoprecipitated with a mixture of antibodies against heavy chains and β_2m. Prior to SDS PAGE, part of the immunoprecipitated material was treated with Endo H. Fig. 3 (lane *C*) demonstrates that upon co-translation of β_2m and heavy chains, two partially overlapping, electrophoretic bands of heavy chains were visible. All or the majority of the heavy chains with an apparent molecular weight of 45,000 were resistant to Endo H digestion while the chains with an apparent molecular weight of 43,000 were sensitive to the enzyme (Fig. 3, lane *D*). These data strongly suggest that human class I antigen heavy chains are transported from the endoplasmic reticulum to the Golgi complex in oocytes but only provided β_2m associates with the heavy chains.

To ascertain the latter conclusion pulse-chase experiments were performed. The fraction enriched for heavy chain

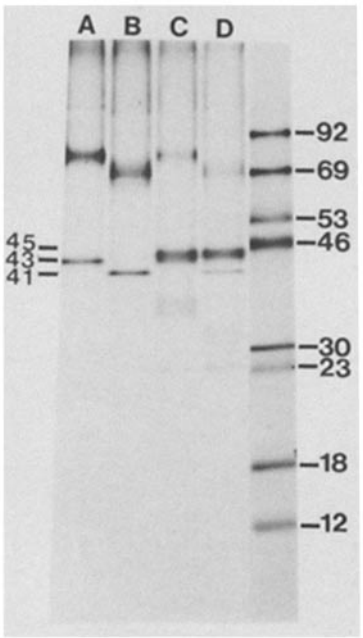

FIGURE 3 Intracellular transport of human class I antigen heavy chains after co-translation with β_2m. The fraction enriched for class I antigen heavy chain mRNA was translated alone (*A* and *B*) and together with the fraction enriched for β_2m mRNA (*C* and *D*) in *Xenopus* oocytes. [^{35}S]Methionine-labeled glycoproteins were immunoprecipitated using a mixture of rabbit antibodies against β_2m and class I antigen heavy chains and subjected to SDS PAGE. Prior to the electrophoresis part of the immunoprecipitates were treated with Endo H (*B* and *D*). Figures denote apparent molecular weights in kilodaltons of the marker proteins.

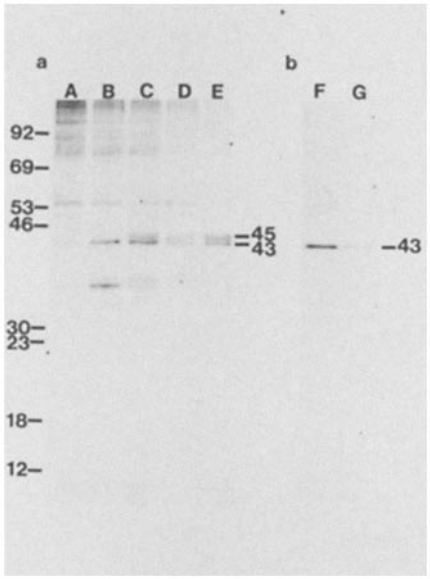

FIGURE 4 Pulse-chase experiments of class I antigen heavy chains translated in oocytes in the presence and absence of β_2m. Oocytes were microinjected with the class I antigen heavy chain mRNA fraction alone (lanes *F* and *G*) and together with the β_2m mRNA fraction (lanes *A–E*). The oocytes were labeled with [^{35}S]methionine for 1 h and then chased in the presence of 20 mM methionine for up to 24 h. Samples were withdrawn after the labeling period (lanes *A* and *F*) and after chase periods of 4 h (lane *B*), 8 h (lane *C*), 12 h (lane *D*), and 24 h (lanes *E* and *G*). Glycoproteins were isolated and immunoprecipitated with an anti-heavy chain serum. Lanes *F* and *G* are from a different gel than lanes *A–E*. Figures denote apparent molecular weights in kilodaltons of the marker proteins run in parallel.

mRNA was injected alone and together with the fraction enriched for β_2m mRNA, respectively. Oocytes were labeled with [^{35}S]methionine for 1 h, washed, and then chased in the presence of 20 mM methionine for varying periods of time. Glycoproteins with affinity for the Lens culinaris hemagglutinin were isolated and immunoprecipitated with anti-heavy chain serum and analyzed by SDS PAGE. Fig. 4*b*, lanes *F* and *G*, shows that heavy chain mRNA injected alone gave rise to the 43,000-dalton species even after a period of chase of 24 h (Fig. 4*b*, lane *G*). When fractions enriched for heavy chain mRNA and β_2m mRNA, respectively, were injected together a single heavy chain band with an apparent molecular weight of 43,000 was visible after the pulse period (Fig. 4*a*, lane *A*). After 4 h of chase (lane *B*), this heavy chain species was still predominating. However, after 8 h of chase a second heavy chain band with an apparent molecular weight of 45,000 was visualized (Fig. 4*a*, lane *C*). This band increased in relative intensity during the remaining period of chase (Fig. 4*a*, lanes *D* and *E*).

These data reinforce the notion that human class I antigen heavy chains are transported from the endoplasmic reticulum to the Golgi complex in oocytes only in the presence of β_2m.

Terminal Glycosylation of Class I Antigen Heavy Chains

The addition of sialic acid to oocyte-translated heavy chains was examined. Fractions enriched for heavy chain and β_2m mRNA, respectively, were injected together into oocytes. These oocytes, mock-injected oocytes, and Raji cells, which were the source of the mRNA, were separately labeled with [^{35}S]methionine. Glycoproteins with affinity for Lens culinaris hemagglutinin were isolated and part of the glycoprotein fractions were subjected to neuraminidase treatment. Class I antigens were immunoprecipitated with an antiserum against heavy chains. Endogeneous oocyte glycoproteins were precipitated with trichloroacetic acid. All samples were subsequently analyzed by two-dimensional electrophoresis (Fig. 5). Since Raji cells are heterozygous at the major histocompatibility complex locus (J. Bodmer, personal communication), at least six different types of heavy chains should be expressed. On two-dimensional gel electrophoresis the heavy chains gave rise to approximately twice as many spots (Fig. 5*a*). Previous results suggest that at least part of this heterogeneity is due to varying contents of sialic acid among the heavy chains (24, 25). After neuraminidase digestion the array of Raji heavy chain spots appeared at more basic pI values, consistent with a diminished content of sialic acid (Fig. 5*b*). Fig. 5*c* shows that Endo H-resistant heavy chains, co-translated with β_2m in oocytes (see Fig. 3), gave rise to fewer spots than heavy chains of Raji cells (cf. Fig. 5*a*). Neuraminidase digestion of

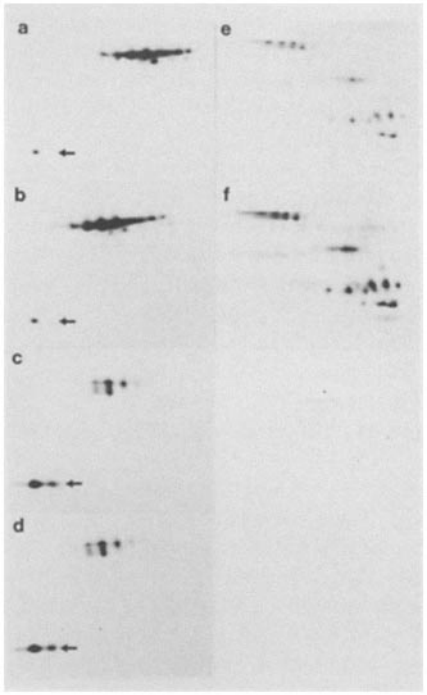

FIGURE 5 Effect of neuraminidase on class I antigen heavy chains translated in oocytes and in Raji cells. The heavy chain and β_2m mRNA fractions were combined and microinjected into oocytes (*c* and *d*), which were labeled with [^{35}S]methionine for 20 h. Mock-injected oocytes were treated identically (*e* and *f*). Raji cells, which were the source of the microinjected mRNA, were also labeled with [^{35}S]methionine but only for 3 h (*a* and *b*). Glycoproteins were separately isolated from the two types of oocytes and from the Raji cells. Part of the glycoprotein fractions were digested with *Vibrio cholerae* neuraminidase (*b*, *d*, and *f*). Class I antigens were isolated from the microinjected oocytes (*c* and *d*) and Raji glycoprotein fractions (*a* and *b*) using an anti-heavy chain serum. Endogeneous glycoproteins from mock-injected oocytes were concentrated by trichloroacetic acid precipitation (*e–f*). Precipitated proteins were solubilized and subjected to isoelectric focusing (*horizontal*, anode to the left) and SDS PAGE (*vertical*). Gels were aligned by including an internal marker protein (*arrow*).

oocyte translated heavy chains did not measurably change their apparent pI (Fig. 5 *d*). Endogeneous oocyte glycoproteins also seemed resistant to the *Vibrio cholerae* neuraminidase (cf. Fig. 5, *e* and *f*). These data suggest that oocytes do not add sialic acid to glycoproteins in such a manner that the sialic acid can be digested with the *Vibrio cholerae* neuraminidase.

Binding of β_2m to Preformed Heavy Chains Induces Intracellular Transport

To examine whether the β_2m-induced intracellular transport of heavy chains required simultaneous translation of the two subunits, oocytes were microinjected with a heavy chain mRNA fraction. After incubation in the presence of [^{35}S]-methionine for 20 h, the oocytes were washed and injected with a mixture of a β_2m mRNA fraction and unlabeled methionine. Subsequently, the oocytes were incubated in the presence of unlabeled methionine for another 20 h. In control experiments oocytes were either injected with the heavy chain mRNA fraction or with the combined heavy chain and β_2m mRNA fractions, respectively. Such oocytes were incubated in the presence of [^{35}S]methionine for 20 h.

As expected, oocytes injected with a fraction containing heavy chain but not β_2m mRNA translated a 43,000-dalton protein, which reacted with the antiserum against heavy chains (Fig. 6, lane *B*). This component, did not react with the monoclonal antibody against β_2m (Fig. 6, lane *A*). However, a contaminant with an apparent molecular weight of ~75,000 seemed to react with the monoclonal antibody (see Materials and Methods). Heavy chains translated in oocytes injected first with an mRNA fraction coding for heavy chains and 20 h later with a fraction of mRNA coding for β_2m resolved into two electrophoretic bands with apparent molecular weights of 45,000 and 43,000, respectively, (Fig. 6, lane *D*). When the antiserum against heavy chains was replaced by the monoclonal antibody against β_2m, most if not all heavy chains exhibited an apparent molecular weight of 45,000 (Fig. 6, lane *C*).

In the experiments described above the incorporation of [^{35}S]methionine had ceased at the time of the β_2m translation, as shown by the absence of labeled β_2m in Fig. 6, lanes *C* and *D*. [^{35}S]Methionine is indeed incorporated into β_2m when the radioactive amino acid is available (Fig. 6, lanes *E* and *F*). Thus, these data show that nascent, β_2m can bind to previously synthesized, heavy chains and promote their intracellular transport.

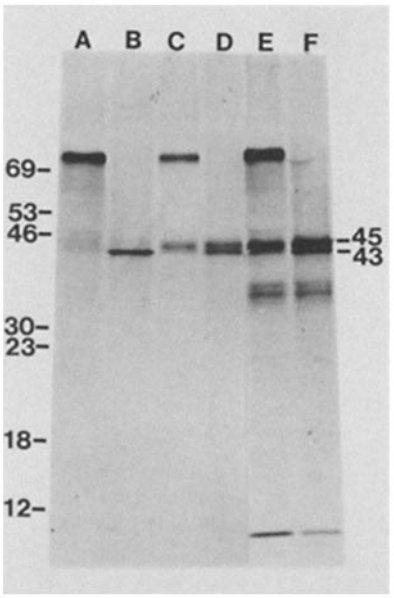

FIGURE 6 Effect of β_2m on the intracellular transport of previously synthesized class I antigen heavy chains. Oocytes were microinjected with the heavy chain mRNA fraction alone (*A–D*) and together with the β_2m mRNA fraction (*E* and *F*). After labeling for 20 h with [^{35}S]methionine, a glycoprotein fraction from oocytes injected with both types of mRNA (*E* and *F*) was subjected to immunoprecipitation using the monoclonal antibody against β_2m (*E*) and the antiserum against the heavy chains (*F*). Oocytes that had been injected with heavy chain mRNA alone and incubated with [^{35}S]-methionine for 20 h were divided into two portions. One portion was cultivated for another 20 h in fresh medium containing 20 mM unlabeled methionine (*A* and *B*). The other portion was microinjected with the β_2m mRNA fraction containing 20 mM methionine and was subsequently cultivated for another 20 h in fresh medium containing 20 mM methionine (*C* and *D*). Oocyte glycoproteins were isolated and immunoprecipitated using the monoclonal antibody against β_2m (*A* and *C*) and the antiserum against heavy chains (*B* and *D*). The figures denote apparent molecular weights in kilodaltons of marker proteins run in parallel (not shown).

Hybridization Selected β_2m mRNA Induces Intracellular Transport of Class I Antigen Heavy Chains

An alternative explanation to the data described above would have to be entertained should the β_2m mRNA fraction contain some unidentified mRNA species whose translation product directly or indirectly promoted the intracellular transport of heavy chains. To rule this out β_2m mRNA was isolated by hybridization selection to a cDNA clone immobilized onto nitrocellulose filters. The purity of the hybridization-selected β_2m mRNA was assessed by cell-free translation in a reticulocyte lysate system containing dog pancreas microsomes. Prior to hybridization-selection the fraction enriched for β_2m mRNA gave rise to several translation products (Fig. 7, lane *C*), but after hybridization-selection only a single band with an apparent molecular weight of 12,000 could be visualized (Fig. 7, lane *B*). That the mRNA coding for this translation product had specifically hybridized to the immobilized cDNA clone was apparent, since in the absence of added exogeneous mRNA no endogeneous translation product with the migration behavior of β_2m could be observed (Fig. 7, lane *A*). Immunoprecipitation of translated hybridization-selected β_2m mRNA gave rise to β_2m only (Fig. 7, lane *E*), while several contaminants were observed when the translation products of the enriched β_2m mRNA fraction were subjected to the same treatment (Fig. 7, lane *F*). In the absence of exogeneous mRNA the monoclonal antibodies against β_2m did not precipitate any labeled protein (Fig. 7, lane *D*).

To examine the effect of hybridization-selected β_2m mRNA on the intracellular transport of class I antigen heavy chains, such mRNA was injected into oocytes at two concentrations together with the fraction enriched for heavy chain mRNA. Glycoproteins, labeled with [^{35}S]methionine and isolated on a Lens Culinaris hemagglutinin-Sepharose column, were immunoprecipitated with an anti-heavy chain serum and subjected to SDS PAGE. In the absence of hybridization-selected

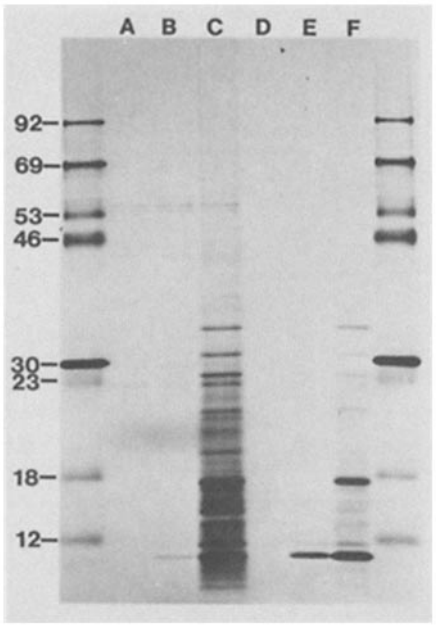

FIGURE 7 In vitro translation of β_2m mRNA purified by hybridization to a β_2m cDNA clone. A fraction enriched for β_2m mRNA was purified by hybridization to a β_2m cDNA clone immobilized onto a nitrocellulose filter. The mRNA eluted from the filter after extensive washings, and the mRNA fraction prior to hybridization, were separately translated in vitro in the presence of dog pancreas microsomes. The microsomal fractions were isolated, lysed, and subjected to SDS PAGE directly (*A–C*) or after immunoprecipitation with a monoclonal antibody against β_2m (*D–F*). Translation products generated in the absence of exogeneously added mRNA are shown in lanes *A* and *D*. Translation products of β_2m mRNA obtained after hybridization-selection (*B* and *E*) and of the mRNA fraction enriched for β_2m mRNA (C and *F*) are also shown. Figures denote apparent molecular weights of the marker proteins.

β_2m mRNA only heavy chains with an apparent molecular weight of 43,000 were observed (Fig. 8, lane *A*). Although the 43,000-dalton component was the dominating form also when hybridization-selected β_2m mRNA had been injected into the oocytes together with heavy chain mRNA, the 45,000-dalton species of the heavy chain did emerge (Fig. 8, lane *B*). On increasing the amount of hybridization-selected β_2m mRNA, the 45,000-dalton form of the heavy chain became more pronounced (Fig. 8, lane *C*). Thus, these data ascertain that it is the β_2m mRNA that promotes the intracellular transport of the heavy chains.

DISCUSSION

By microinjecting into oocytes fractions enriched for human β_2m and class I antigen heavy chain mRNA, respectively, we could demonstrate that (*a*) β_2m is efficiently translated, transported intracellularly, and secreted into the medium; (*b*) heavy chains in the absence of β_2m are translated but do not leave the endoplasmic reticulum (see below); (*c*) heavy chains in the presence of β_2m are transported from the endoplasmic reticulum to the Golgi complex.

To monitor the intracellular transport of the heavy chains we took advantage of the fact that Asn-linked core-sugar moieties, apart from the *N*-acetylglucosamine attached to the amino acid, are completely removed by the enzyme Endo H (23) provided trimming and terminal glycosylation have not occurred (26). Thus, sensitivity to Endo H digestion indicates that heavy chains remain in the endoplasmic reticulum while resistance to the enzyme suggests that the heavy chains have become transported, at least to the *cis*-Golgi compartment. Since it proved difficult to establish cell surface expression of oocyte-translated class I antigens, we tried to examine the intracellular transport beyond the *cis*-Golgi compartment by analyzing the content of sialic acid of the heavy chains translated in the presence of β_2m. This was accomplished by digesting heavy chains with *Vibrio cholerae* neuraminidase. While this enzyme removed sialic acid from class I antigen heavy chains of Raji cells it failed to do so with oocyte-translated heavy chains. Of course, this does not exclude that heavy chains occurred in the *trans*-Golgi compartment of the oocyte, since the neuramianidase also failed to reveal the presence of sialic acid on endogeneous oocyte glycoproteins. In fact, Endo H-resistant, oocyte-translated heavy chains exhibited an apparent molecular weight consistent with terminal glycosylation. However, further studies are obviously required to determine the precise glycosylation state of heavy chains manufactured by oocytes.

The present study demonstrates that class I antigen heavy chains in free form are not transported from the endoplasmic reticulum to the *cis*-Golgi compartment. In contrast, free β_2m is transported and even secreted by the oocytes. However, following association with β_2m heavy chains become transported. The possibility that mRNA species other than that coding for β_2m promoted the transport of the heavy chains was ruled out since highly purified β_2m mRNA, isolated by hybridization-selection induced the intracellular transport. This conclusion is fully consistent with the observation that the human lymphoblastoid cell line Daudi, which does not produce β_2m (4), fails to transport class I antigen heavy chain to the cell surface (8, 9). Thus, the translation of β_2m seems to regulate the cell surface expression of class I antigens. Examinations of the β_2m and heavy chain translation during early stages of embryogenesis (27, 28) may, therefore, prove rewarding, since β_2m may have a role similar to that of light immunoglobulin chains in the fetal liver, inasmuch as the latter control the cell surface expression of IgM heavy chains (29).

It is intriguing that free, human class I antigen heavy chains

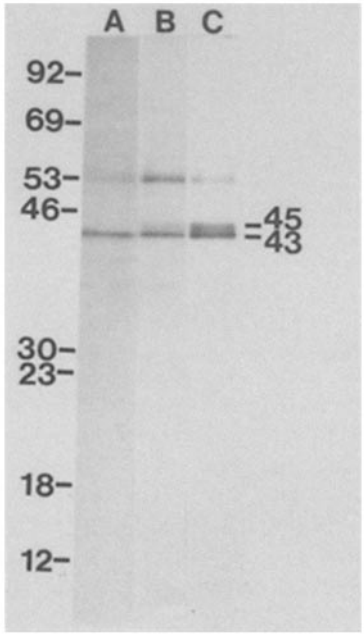

FIGURE 8 Class I antigen heavy chains translated in oocytes in the absence and presence of hybridization-selected β_2m mRNA. Oocytes were microinjected with the fraction enriched for heavy chain mRNA alone (lane *A*) or together with different concentrations of hybridization-selected β_2m mRNA (lanes *B* and C). The amount of microinjected β_2m mRNA in C was twice that in *B*. Glycoprotein fractions were isolated and immunoprecipitated with an anti-heavy chain serum. Figures denote apparent molecular weights in kilodaltons.

become confined to the endoplasmic reticulum regardless of whether they are translated in syngeneic or xenogeneic cells. A trivial explanation to this finding is that the heavy chains, in the absence of β_2m, become irreversibly aggregated. Previous studies have shown that highly purified class I antigens, separated into free heavy chains and β_2m, are difficult or impossible to reconstitute (30). This suggests that one or both polypeptides may display different conformations when free and when interacting with the other subunit. However, the present study shows that free heavy chains, translated in oocytes prior to β_2m, do not attain a conformational state that irreversibly prevents binding of β_2m. Whether, a particular conformation of the heavy chain, which can only be obtained by the binding of β_2m, is a prerequisite for the intracellular transport is a matter of conjecture only.

In procaryotes the information for export of proteins seems to be endowed in the NH_2-terminal two-thirds of the amino acid sequence (31). Whether a similar structural requirement prevails also for intracellular transport of eucaryotic proteins is unknown. Recently, it was reported that the secretion of a murine λ_2 myeloma light immunoglobulin chain is abrogated in a mutant that produces a variant λ_2 light chain. The only difference between the two light chains is at position 15 where the secreted form displays glycine and the nonsecreted form arginine (32). Thus, intracellular transport in eucaryotic cells may also be governed by discrete segments of the polypeptide chain. Obviously the signal sequence represents such a segment (33) and other segments like stop-transfer and sorting sequences may also exist. Should this turn out to be the case, it is conceivable that such segments are recognized by "receptors" (34, 35). The ease with which the intracellular transport of human class I antigens in oocytes can be examined renders this an attractive model system to examine these possibilities.

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