Proc. Natl. Acad. Sci. USA
Vol. 76, No. 5, pp. 2273–2277, May 1979
Biochemistry

Cell-free translation of the mRNAs for the heavy and light chains of HLA-A and HLA-B antigens

(histocompatibility antigens/membrane protein synthesis/Daudi cells)

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ABSTRACT **Cell-free translation of poly(A)-containing RNA from the JY lymphoblastoid cell line followed by immunoprecipitation has indicated the presence of mRNAs for both the heavy and light chains of HLA-A and HLA-B antigens in these preparations. Both chains are synthesized with an NH_2-terminal extension, approximately 20 residues in length for the light chain, and 20 or 24 residues for the heavy chains. The precursors can be processed by dog pancreatic microsomes to products similar to those obtained *in vivo*. Immunoprecipitation of the cell-free products has been employed as an assay for partial purification of the mRNAs. Investigation of the Daudi cell line, which cannot synthesize the small subunit, β_2-microglobulin, has indicated that the heavy chains of HLA-A and HLA-B antigens are synthesized intracellularly *in vivo* and can also be translated from their cognate RNAs *in vitro*. The implications of these findings for biosynthesis of membrane proteins in general and multimeric membrane proteins in particular, as well as the role of β_2-microglobulin in expression of HLA-A and HLA-B antigens, are discussed.**

The products of the major histocompatibility complex (MHC) have attracted considerable attention because of their role in various aspects of the immune response, their polymorphism, and their association with certain diseases in man. The HLA-A and HLA-B antigens consist of a two-chain structure: a MHC-encoded heavy chain of 44,000 daltons that is glycosylated and anchored in the membrane in noncovalent association with a light chain of 12,000 daltons identical to β_2-microglobulin (β_2m) (1). The NH_2-terminal 280 residues of the heavy chain are found outside the cell, associated with β_2m. Serologic activity of the HLA-A and -B antigens appears to depend on the association of the two subunits.

A full understanding of the genetics and regulation of expression of the MHC products will require an analysis of the DNA encoding the genes for these antigens. β_2m (2) and at least a portion of the structure of the heavy chains of HLA-A and -B antigens are thought to be structurally homologous to immunoglobulin domains (3, ‡). The organization of the genes encoding these molecules is, therefore, of considerable interest.

In order to approach this problem, an assay for the nucleic acid sequences encoding the two polypeptides of the HLA-A and -B antigens is a prerequisite. All such assays ultimately depend upon translation *in vitro* of the mRNAs for these polypeptides as a means of identification of the gene product. In the present paper, the cell-free translation of mRNAs encoding each of the polypeptides and partial purification of these mRNAs from the JY lymphoblastoid cell line (HLA-A2, HLA-B7 homozygous) are described. In addition, similar experiments have been carried out with RNA preparations from the Daudi cell line, which does not express HLA-A and -B antigens because it cannot synthesize light chain, β_2m (4).

MATERIALS AND METHODS

Materials. The lymphoblastoid cell lines JY (homozygous HLA-A2 and HLA-B7) and Daudi [haplotypes: A10 (Aw26) Bw17 and A1 Bw16 (Bw38) (5)] were used for RNA isolations and labeling experiments *in vivo*. The lymphoblastoid cell line 23.1 (homozygous HLA-A2 and HLA-B27) was used as a source of standards. Tunicamycin was a gift from Eli Lilly. Phenylmethylsulfonyl fluoride and heparin (grade I) were from Sigma. [^{35}S]Methionine (400–600 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) was purchased from New England Nuclear. Papain, tosylphenylalanyl chloromethyl ketone-treated trypsin, and chymotrypsin were from Worthington. Nonidet P-40 was from Particle Data Laboratories (Elmhurst, IL). Purification of papain-solubilized HLA-B7 antigen and subsequent separation of the chains were achieved as described (6). All reagents for sodium dodecyl sulfate ($NaDodSO_4$)/polyacrylamide gel electrophoresis were from Bio-Rad. All other chemicals were of reagent grade.

Labeling of Cells. Cells (10^6 per ml) were labeled in methionine-free RPMI 1640 medium (GIBCO) supplemented with 15% (vol/vol) fetal calf serum amd 100–500 μCi of [^{35}S]methionine per ml, for periods of 5–16 hr. Labeling of cells in the presence of tunicamycin (1 μg/ml) was initiated after a 30-min preincubation with the antibiotic at this concentration. Cell lysates were prepared by incubation of cells for 30 min on ice at 5×10^6 cells per ml in 0.5% Nonidet P-40/10 mM Tris·HCl, pH 7.3/1 mM $MgCl_2$/1 mM, phenylmethylsulfonyl fluoride followed by centrifugation for 15 min at $16,000 \times g$. The supernatants were stored at −70°C.

Isolation of RNA. RNA was isolated as described by Efstratiadis and Kafatos (7), with the substitution of chloroform/isoamyl alcohol (24:1) extractions for steps requiring phenol extractions. Enrichment for poly(A)-containing RNA was achieved by chromatography on oligo(dT)-cellulose (Collaborative Research, Waltham, MA; T-3). Further fractionation of RNA by electrophoresis on 1.5% agarose gels, elution of gel slices, and recovery of RNA were as described (8).

Cell-Free Translation. Cell-free translation was carried out in the rabbit reticulocyte lysate system made mRNA dependent as described by Pelham and Jackson (9). RNA and [^{35}S]methionine were added at concentrations of 20–60 μg and 1–2 mCi per ml, respectively. Assay mixtures were incubated at 37°C for 60 min. Translation in the presence of dog pancreatic microsomes (a gift of J. Majzoub and H. Kronenberg) and subsequent proteolysis were carried out as described (10).

Antisera. The antisera used were: (*i*) W6/32, a mouse monoclonal antibody that reacts with all HLA-A, -B, and -C allospecificities examined so far (11); (*ii*) rabbit anti-human β_2m;

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Abbreviations: MHC, major histocompatibility complex; β_2m, β_2-microglobulin; $NaDodSO_4$, sodium dodecyl sulfate.

‡ H. T. Orr, D. Lancet, R. Robb, and J. L. Strominger, unpublished data.

(*iii*) alloantisera specific for HLA-A2 (Starkenburg), HLA-B7 (Jackson), HLA-A1, HLA-A10, and HLA-Bw16 (Sidney Farber Cancer Institute numbers 166, 92, and 230, respectively); and (*iv*) anti-heavy chain (anti-H) serum, a serum raised by A. Fuks in a rabbit against papain-solubilized, highly purified HLA-B7 heavy chain, obtained after gel filtration in guanidine·HCl (6). Although this antiserum reacts to a slight extent with intact HLA-A and -B antigens, it is highly specific for the isolated HLA-A and -B heavy chains (M. Krangel, personal communication). This antiserum is probably very similar to that described by Tanigaki *et al.*(12).

Immunoprecipitation. Immunoprecipitation was performed essentially as described (13), with formalin-fixed, heat-killed *Staphylococcus aureus* (Cowan I strain). Samples to be precipitated were pretreated with normal rabbit serum and the immunoadsorbent. Bound materials were eluted by boiling in $NaDodSO_4$ sample buffer or 2% $NaDodSO_4$ for 5 min.

Sequencing. NH_2-Terminal sequencing was performed in a Beckman 890 C instrument employing a 0.1 M Quadrol program (14). Immune precipitates were loaded directly in the cup after elution from the immunoadsorbent with 2% $NaDodSO_4$. Sperm whale apomyoglobin (50 nmol) was added as carrier to monitor degradation efficiency.

$NaDodSO_4$/Polyacrylamide Gel Electrophoresis. Electrophoresis was carried out according to the method of Laemmli (15) on 7–15% linear gradient slab gels. Protein bands were detected by fluorography (16) on Kodak SB-5 x-ray film. Radioactive bacteriophage ϕX174 proteins (a gift of Kirston Koths) were used as molecular weight standards.

RESULTS

Cell-Free Translation of mRNA for the Heavy and Light Chains of HLA-A and HLA-B Antigens from the JY Cell Line. Cytoplasmic RNA was obtained from the JY cell line by Mg^{2+} precipitation (7) in good yields (3 mg per 10^9 cells; average of nine isolations). About 2–3% of this RNA could be bound to and eluted from an oligo(dT)-cellulose column. This polyadenylylated RNA was translated *in vitro* in the rabbit reticulocyte lysate system. A complex pattern of products was observed on $NaDodSO_4$/polyacrylamide gel electrophoresis (see Fig. 3 below), similar to that obtained by labeling of intact JY cells.

Immunoprecipitation from the products programmed by JY RNA was carried out with a variety of antisera recognizing HLA-A and -B antigens, including rabbit anti-β_2m serum; human HLA-A and -B alloantisera; the monoclonal mouse antibody W6/32, which recognizes all HLA-A, -B, and -C specificities examined so far (11); and anti-heavy chain serum (anti-H serum), a serum raised in a rabbit against the denatured separated HLA-B7 chain. Only two of these antisera, the anti-β_2m and the anti-H serum, were capable of precipitating appreciable amounts of radioactivity (Fig. 1, lanes c and d). These two products were independently precipitated, indicating that they were not associated at this stage of their biosynthesis. The precipitation of the cell-free products by anti-β_2m and anti-H serum could be inhibited by the addition of highly purified β_2m (Fig. 1, lane g) and HLA-B7 heavy chain (Fig. 1, lane f), respectively. The HLA-A2- (Fig. 1, lane a) and HLA-B7-specific (not shown) alloantisera and the mouse monoclonal antibody W6/32 (Fig. 1, lane b) all failed to precipitate significant amounts of radioactivity. This is not surprising because it has been established that the W6/32 determinant is only present on complexes consisting of an HLA-A, -B, or -C chain and β_2m, as is found in alloantigenically active HLA-A, -B, and -C molecules (17).

Presence of NH_2-Terminal Extensions in the Heavy and Light Chains Synthesized by *In Vitro* Translation. Because glycosylation does not occur under the conditions of cell-free translation employed, a comparison of the apparent molecular weights (M_rs) of nonglycosylated HLA-A and -B heavy chains obtained from tunicamycin-treated cells was made with those of the immunoprecipitated products of cell-free translation. Tunicamycin inhibits N-linked glycosylation of eukaryotic glycoproteins (18), including HLA-A and -B antigens (H. Ploegh, H. Orr, and J. L. Strominger, unpublished data). Under the conditions of the experiments with tunicamycin, the heavy chains of the HLA-A and -B antigens were a mixture of fully glycosylated (apparent M_r 44,000) and nonglycosylated (apparent M_r 40,000) materials, whereas the light chain, β_2m, was unaffected (Fig. 1, lanes h and o).

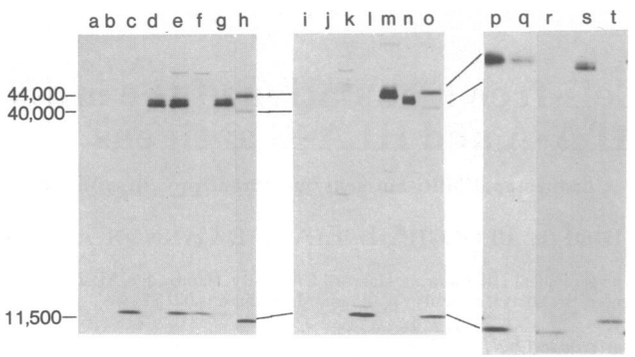

FIG. 1. Immunoprecipitation from cell-free translation products synthesized with RNA from JY cells. Lanes h and o: standards of glycosylated (M_r 44,000) and nonglycosylated (M_r 40,000) HLA-A and -B heavy chains and β_2m (M_r 11,500) obtained from tunicamycin-treated 23.1 cells by immunoprecipitation by W6/32 antibody. Other lanes: cell-free products immunoprecipitated with the following antisera and additions. Lane a, Starkenburg (anti-HLA-A2) serum; lane b, W6/32 antibody; lane c, anti-β_2m serum; lanes d and n, anti-H serum; lane e, anti-H and anti-β_2m serum; lane f, as lane e, plus HLA-B7 heavy chain; lane g, as lane e, plus β_2m. Lanes i–m and p, q, and r, microsomal membranes present during translation, immunoprecipitations with the following sera. Lane i, W6/32 antibody; lane j, Starkenburg (anti HLA-A2) serum; lane k, Jackson (anti HLA-B7) serum; lane l, anti-β_2m serum; lane m, anti-H serum; lane p, anti-H serum plus anti-β_2m serum; lane q, proteolysis after translation, anti-H serum; lane r, proteolysis after translation, anti-β_2m serum; lane s, microsomal membranes added after translation, anti H-serum; lane t, microsomal membranes added after translation, anti-β_2m serum. Lanes p–t are not on the same scale as lanes a–o.

The anti-H serum precipitated a cell-free product with an apparent M_r of 42,000 (Fig. 1, lane d). Usually a doublet could be observed, probably representing the HLA-A2 and -B7 heavy chain precursors, because the anti-H serum does not distinguish between heavy chains of different specificities. The comparison with nonglycosylated heavy chains of HLA-A and -B antigens obtained from tunicamycin-treated cells (M_r 40,000) suggested the presence of approximately 20 additional residues in the cell-free product.

The presence of an NH_2-terminal extension was confirmed by sequence analysis of the HLA-A and -B heavy chain precursors (Fig. 2). Methionines were found at positions 1, 4, 25, and 29. The interpretation of this experiment is complicated by the fact that the precursors of both the HLA-A2 and -B7 heavy chains, whose NH_2-terminal extensions may differ in size, are presumably present in the material whose sequence is being determined. Each of these antigens contains a methionine at position 5 in the mature product. It is assumed that the methionines observed at positions 25 and 29 are the two methionines at position 5 in the mature products (19). This assumption is based on the following calculation. The repetitive yield obtained for myoglobin (uncorrected for background) was 97.2%. If the peak values for cycles 25 and 29 are extrapolated to cycle 1 (broken lines in Fig. 2), both lines yield values lower than those

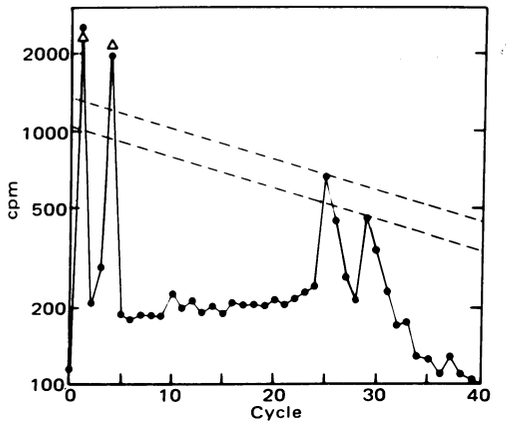

FIG. 2. [^{35}S]Methionine in the NH_2-terminal region of the HLA-A and -B heavy chains. A semilogarithmic plot of cpm obtained by liquid scintillation counting of half the *n*-chlorobutane extract at each cycle of the sequencer degradation of [^{35}S]methionine-labeled anti-H immune precipitate is shown. The counts are not corrected for background. The broken lines represent extrapolations of the counts obtained at cycles 25 and 29, assuming an average repetitive yield of 97.2%, uncorrected for background, calculated for the carrier apomyoglobin degradation as quantitated by gas/liquid chromatography and high-pressure liquid chromatography. The Δs at cycles 1 and 4 are the sums of the cpm that would be expected at cycles 1 and 4 from the repetitive yield extrapolation of the cpm at cycles 25 and 29. After conversion the radioactivity was identified as the phenylthiohydantoin of [^{35}S]methionine by autoradiography of two-dimensional polyamide thin-layer chromatographs and by liquid scintillation counting of effluent from high-pressure liquid chromatography. A conditioning cycle, without coupling reagent, was conducted prior to commencing Edman degradation, and the *n*-chlorobutane extract at this cycle contained 116 cpm (cycle 0).

observed for the methionines at cycles 1 and 4, but their sum is equal to the experimentally observed values at these cycles. The most likely interpretation is the presence of a methionine residue at positions 1 and 4 for both the HLA-A2 and -B7 heavy chain precursors. The HLA-A2 and -B7 heavy chain precursors would be of different size: i.e., 20 and 24 amino acid residues for the NH_2-terminal extensions. This interpretation is in good agreement with the apparent M_r of the precursors.

Alternatively, it might be argued that both the HLA-A2 and -B7 heavy chain precursors have methionines at positions 1, 4, 25, and 29 and that the methionine at position 5 in the sequence of the mature products (19) has not been reached yet. Because no additional methionines were found up to cycle 40, the minimum size of the NH_2-terminal extension compatible with this interpretation would be 36 residues, hardly in agreement with the observed gel mobility. In addition, the repetitive yield actually observed for the cell-free product would have to differ significantly from that for the carrier myoglobin; i.e., the yield would have to be about 91%.

The material precipitated from the cell-free products with the anti-β_2m serum was found to have an apparent M_r of 13,500 compared to 11,500 observed for β_2m in HLA-A and -B antigens labeled *in vivo* (Fig. 1, lane c). This gel mobility suggested the presence of approximately 20 additional residues in the cell-free product. NH_2-Terminal sequence analysis after labeling with [^{35}S]methionine showed the presence of methionine at position 1 of the cell-free product, indicating the presence of at least one additional residue at the NH_2-terminus. No methionine residues were positively identified for an additional 24 cycles. This result is compatible with the fact that the only methionine residue that occurs in the sequence of human β_2m is at the COOH-terminal (100th) residue.

Processing of Heavy and Light Chain Precursors *In Vitro*. When translation was carried out in the presence of dog pancreatic microsomes, the apparent M_r of the β_2m precursor was reduced to that of authentic β_2m (Fig. 1, lane l). However, the anti-H-reactive material increased slightly in apparent M_r (Fig. 1, lane m). This result is presumably due to removal of the NH_2-terminal extension concomitant with the addition of the glycan chain. Glycosylation reactions are known to be supported by the dog pancreatic microsome preparation (20). This question may be answered by determining the NH_2-terminal sequence of products of cell-free translation in the presence and absence of dog pancreatic microsomes.

These results were not affected by the removal of extramicrosomal proteins by proteolysis after translation in the presence of microsomes (Fig. 1, lanes q and r). Anti-H serum precipitated the processed heavy chain (Fig. 1, lanes m and p), but W6/32 antibody and alloantisera did not precipitate it (Fig. 1, lanes i, j and k). No processing of either heavy or light chain precursors occurred when microsomes were added after translation (Fig. 1, lanes s and t), nor could products be specifically immunoprecipitated when proteolysis was carried out in the presence of 0.5% Nonidet P-40/0.1% sodium deoxycholate (not shown). Thus, when HLA-A and -B heavy chain and β_2m mRNAs were translated in the presence of microsomes, segregation of the protein products occurred.

Partial Purification of the mRNAs for Heavy and Light Chains of HLA-A and HLA-B Antigens. Cell-free translation in combination with immunoprecipitation has been used as an assay for partial purification of the mRNAs. Polyadenylylated RNA was fractionated on a 1.5% agarose gel under nondenaturing conditions. After recovery of the RNA from the gel slices and translation (Fig. 3*A*), mRNA for the heavy chain of HLA-A and -B antigens was detected at the leading edge of the 18S ribosomal RNA still present in this preparation (Fig. 3*B*). β_2m mRNA showed a somewhat broader distribution and migrated faster in the gel (Fig. 3*B*). These experiments make it possible to obtain mRNA preparations sufficiently pure for use in molecular cloning of these sequences.

Cell-Free Translation of RNA from the Daudi Cell Line. Intact Daudi cells *in vivo* and RNA from these cells *in vitro* synthesized products nearly identical to those synthesized by the JY cells and RNA. Although no alloantigenically active HLA-A and -B antigens are synthesized by Daudi cells (6), the RNA from this cell line directed the synthesis *in vitro* of HLA-A and -B heavy chain precursors (Fig. 4). Neither β_2m nor its precursor could be detected *in vivo* and *in vitro*, in accordance with the observation that this cell line does not synthesize β_2m (5).

Immune precipitations from whole cell lysates labeled *in vivo* with [^{35}S]methionine confirmed the absence of any material reactive with anti-β_2m serum. However, the anti-H serum precipitated material that comigrated with mature, fully glycosylated HLA-A and -B heavy chains. This chain was not precipitated by W6/32 antibody or by the relevant alloantisera (Fig. 4). When cell lystates of radiolabeled Daudi cells were incubated at 0 or 37°C in the presence of β_2m in concentrations up to 1 mg/ml, again, no material was precipitated with alloantisera or W6/32 serum, indicating that proper association of the subunits of HLA-A and -B antigens had not occurred *in vitro*. However, analysis of somatic cell hybrids has established that the HLA-A and -B heavy chains from Daudi are capable of interacting with both murine and human β_2m *in vivo* (5). The failure of β_2m to combine *in vitro* with HLA-A and -B heavy chains, therefore, is unlikely to be due to a genetic defect in these chains.

When Daudi cells were labeled during a 3-min pulse, followed by a chase, the amount of heavy chains detectable by immunoprecipitation with anti-H serum remained constant for at least 1 hr, showing that it is relatively stable *in vivo*. In

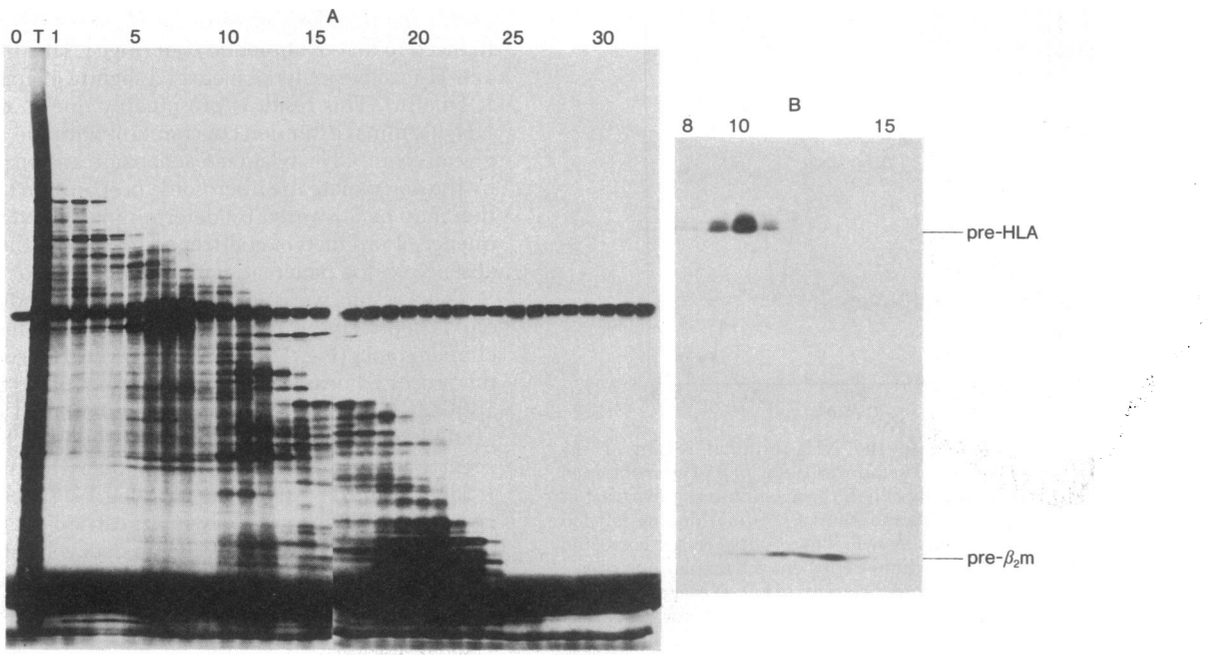

FIG. 3. Fractionation of poly(A)-RNA from JY cells and detection of translation of heavy and light chains of HLA-A and -B directed by these RNA fractions. (*A*) Fractionation and translation of RNA. Poly(A)-containing RNA (200 μg) was electrophoresed on a 1.5% agarose gel. The gel was cut into 2-mm slices and the slices were eluted. Translation of 10% of the eluate and $NaDodSO_4$/polyacrylamide gel electrophoresis of the products resulted in the pattern shown. The migration position of the 18S ribosomal RNA, identified by ethidium bromide staining of a guide strip, was fractions 8–9. Migration of RNA was greater in higher-numbered fractions. Fractions were taken from about 22 S to the dye front (fraction 32). 0, No RNA; T, unfractionated RNA; and fractions 1–32 from the agarose gel. (*B*) Immunoprecipitation from translation products. The products of the cell-free translation were specifically immunoprecipitated with a mixture of anti-H and anti-β_2m serum and subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis. The peak of HLA-A and -B heavy chain mRNA was found in fraction 10, the peak of β_2m mRNA was found in fraction 14.

marked contrast, papain, trypsin, and chymotrypsin degraded the anti-H-reactive material rapidly *in vitro*, and no protection from proteolysis was afforded by the addition of β_2m. The degradation of this material was in contrast to the limited proteolysis of the mature intact HLA-A and -B antigens by these enzymes (1).

Further experiments have established that none of the anti-H-reactive material is found at the cell surface. These experiments have included the failure of Daudi cells to react with anti-H serum in microcytotoxicity assays, in ^{125}I-labeled staphylococcal protein A binding, and in precipitation experiments with ^{35}S-labeled intact cells. Confirming earlier results, Daudi cells failed to react with the W6/32 antibody and relevant alloantisera in these assays. These results conflict with the report of Ostberg *et al.* (21) that Daudi cells express on their surface HLA-A and -B heavy chains that are relatively resistant to proteolysis, capable of associating with β_2m *in vitro*, and precipitable with a rabbit anti-HLA-A, -B heteroantiserum.

DISCUSSION

The HLA-A and -B antigens comprise only 0.05–0.1% of the total cellular protein of JY and other lymphoblastoid cell lines. The experiments described here demonstrate that a cytoplasmic RNA fraction from relatively modest quantities of cells contains enough HLA-A and -B heavy and light chain mRNA to be readily detected by *in vitro* translation and immunoprecipitation of the products. With this assay, the mRNAs have been partially purified from this preparation for possible use in molecular cloning experiments.

The heavy and light chains synthesized *in vitro* from these mRNAs are approximately 2000 daltons larger than their respective nonglycosylated counterparts obtained *in vivo*. Partial amino acid sequencing of [^{35}S]methionine-labeled cell-free products has shown that both have an NH_2-terminal extension, and, in the case of the heavy chain, these experiments also suggest this extra peptide to be 20 or 24 residues long, in agreement with molecular weight estimates and the sizes of NH_2-terminal extensions found previously for a number of secretory proteins (22, 23), including immunoglobulins (24) and a viral membrane glycoprotein (25). These NH_2-terminal extensions, "signal sequences" (26), are characteristically hydrophobic. Preliminary experiments using [^{3}H]leucine-labeled cell-free products have shown that the heavy chain precursor is leucine-rich in the 20 or 24 residues of the NH_2-terminal extension. Thus it appears that an endogenous eukaryotic membrane glycoprotein (as distinct from a viral membrane glycoprotein) is also synthesized with a signal sequence. The observation that processing and segregation of the HLA-A and -B heavy and light chains occur when translation is carried out in the presence of dog pancreatic microsomes strongly suggests that the biosynthesis of these eukaryotic membrane glycoproteins utilizes a mechanism similar to that postulated for secretory proteins. In the case of secretory proteins and viral membrane glycoproteins, it is thought that after association of the nascent chain with the microsomal membrane, segregation occurs as translation proceeds. At some stage during this process, the NH_2-terminal extension, the signal peptide, is removed (26). At least in the case of viral membrane glycoproteins and secreted glycoproteins, it has been shown that glycosylation occurs on the nascent chain (20). A similar process may occur in the case of the HLA-A and -B heavy chains, in which the final translation products remain anchored in the lipid bilayer, however, by a hydrophobic region close to the COOH terminus (27). The features of structure that result in retention of this hydrophobic sequence, which is approximately the same size (25 residues) as the signal sequence, as contrasted to the removal

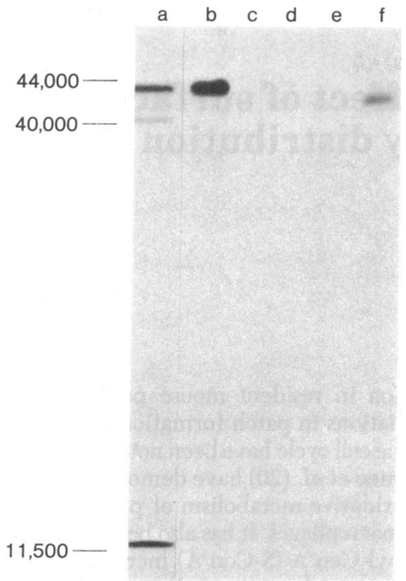

FIG. 4. Synthesis of HLA-A and HLA-B heavy chains by Daudi cells *in vivo* and *in vitro*. Daudi cells synthesize HLA-A and -B heavy chains *in vivo*. Shown are immunoprecipitates of: lane a, whole cell lysate from tunicamycin-treated 23.1 cells precipitated with W6/32 antibody (HLA-A and -B standards, as in Fig. 1); lane b, Daudi whole cell lysate precipitated with anti-H serum; lane c, Daudi whole cell lysate precipitated with anti-β_2m serum; lane d, Daudi whole cell lysate precipitated with W6/32 antibody; lane e, Daudi whole cell lysate precipitated with anti HLA-A10 serum. Daudi RNA directs the synthesis of HLA-A and -B heavy chain precursor *in vitro*. Lane f, cell-free translation products of Daudi RNA, immunoprecipitated with anti-H serum.

of the latter, are not presently known. The results obtained here for HLA-A and -B antigens provide strong support for use of viral membrane proteins as a model system.

The heavy and light chain precursors synthesized *in vitro* in the presence or absence of dog pancreatic microsomes are not associated. The mechanism of chain association and folding after penetration of both chains through the membrane for expression at the surface of the cell is of considerable interest. Experiments with the Daudi cell line shed some light on these questions. This cell line does not synthesize β_2m and no functional mRNA for this chain was detected in RNA preparations from these cells. On the other hand, they do contain mRNAs that code for the precursors of the heavy chains of HLA-A and -B antigens. *In vivo*, it appears that these precursors can be processed and glycosylated in the absence of β_2m or its precursor. Subsequent addition of β_2m to whole cell lysates did not result in chain association, although the HLA-A and -B heavy chains of the Daudi cell line are capable of interacting with β_2m *in vivo*, as seen in somatic cell hybrids. Possibly, the association occurs *in vivo* at a step prior to completion of the HLA-A and -B heavy chains, or some further enzymatic modification may be required for chain association to occur. The lack of expression of the heavy chains of the Daudi cell line at the cell surface indicates that β_2m is essential for expression of HLA-A and -B antigens at the cell surface. Synthesis of the HLA-A and -B heavy chains does occur, however, in the absence of β_2m. That the glycosylated HLA-A and -B heavy chains of the Daudi cell line synthesized *in vivo* react with anti-heavy chain serum, but not with W6/32 antibody or relevant alloantisera, suggests an important role for β_2m in the folding of the heavy chain into the alloantigenically active conformation.

The helpful advice of Robert Freund and the technical assistance of Jeffrey Peirce are gratefully acknowledged. This research was supported by a research grant to J.L.S. from the National Institutes of Health (AM 13230). L.E.C. was supported by a grant-in-aid from the National Cancer Institute (CA 25049-02).

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