Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 1844–1848, March 1982
Biochemistry

cDNA clones for the heavy chain of HLA-DR antigens obtained after immunopurification of polysomes by monoclonal antibody

(mRNA purification/cDNA cloning/immune response genes/major histocompatibility complex/membrane protein)

ALAN J. KORMAN, PETER J. KNUDSEN, JAMES F. KAUFMAN, AND JACK L. STROMINGER

Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge, Massachusetts 02138

Contributed by Jack L. Strominger, December 31, 1981

ABSTRACT **A monoclonal antibody (HC 2.1) directed against the separated heavy chain of HLA-DR has been prepared. By binding HC 2.1 to polysomes from human B lymphoblastoid cells followed by the use of a protein A-Sepharose column as an immunoadsorbent, we have purified the mRNA coding for the HLA-DR heavy chain nearly to homogeneity. The immunopurified mRNA has been used to prepare labeled cDNA with which to probe cDNA libraries. Double-stranded cDNA was also made from the immunopurified mRNA and cloned directly into pBR322. Two clones, one from each of the above procedures, positively selected DR heavy chain message as assayed by cell-free translation and immunoprecipitation. One clone, pDRH-2 [500 base pairs plus 75 base pairs of poly(A)] contains the entire 3′ untranslated region as well as coding information for the carboxy-terminal hydrophilic intracellular domain and part of the hydrophobic transmembrane region. Results of carboxypeptidase digestion of the heavy chains from detergent-solubilized (p34) and papain-treated (p33) HLA-DR antigen were consistent with the predicted protein sequence. Specific immunopurification of polysomes by defined monoclonal antibodies followed by direct cloning of cDNA to the highly purified mRNA is a powerful method for obtaining identified cDNA clones.**

Purification of a single species of mRNA has relied on the combined use of numerous procedures, of which the first step is finding the cell type that produces the desired mRNA in the largest amounts. For the purpose of cloning cDNAs corresponding to mRNAs that have no outstanding features of size, base composition, or abundance, partially purified mRNA is used to create a bank of cDNA clones which is then screened by various methods such as positive selection, expression following microinjection into oocytes, or differential screening (1, 2). An early idea for mRNA purification, proposed in 1961 (3), was to allow the nascent polypeptides on polyribosomes to react with antibody and to precipitate the specific polysomes along with its mRNA. Immunoprecipitation of polysomes has been widely investigated, but its use has been limited to abundant proteins such as ovalbumin (4) and immunoglobulin (5). When this method has been applied to proteins of lesser abundance, the purification has not been very effective (6).

A recent improvement of this technique used a protein A-Sepharose column as an immunoadsorbent together with heteroantiserum to purify the relatively abundant trypanosome surface antigen mRNA (initially 7–10% of total mRNA) (7). We have used this procedure with a monoclonal antibody directed against the heavy chain of HLA-DR antigen to achieve purification of its low-abundance mRNA (estimated, 0.01–0.05%) to near-homogeneity in a single step. This mRNA has been used to obtain cDNA clones coding for part of the DR heavy chain.

HLA-DR antigens are membrane glycoproteins composed of two chains: an invariant heavy chain (34,000 daltons) and a polymorphic light chain(s) (29,000 daltons) (8). Both chains span the membrane bilayer. The HLA-DR antigens were first described functionally as lymphocyte-activating determinants and are currently recognized as restricting elements in antigen presentation to T cells. The DR heavy and light chains are homologous, by NH_2-terminal amino acid sequence, to the corresponding chains of the murine I-E alloantigen. These *I* region antigens are generally believed to be the products of the immune response genes (9, 10). Thus, the cDNA clones described here provide a probe into a critical region of both human and murine histocompatibility complexes.

MATERIALS AND METHODS

Cell Lines. Human B lymphoblastoid cell lines JY (DR 4,6) and LB (DR 6,6) were grown in RPMI-1640 containing 5% fetal calf serum. JY was grown at the Cell Culture Facility of the Massachusetts Institute of Technology (Cambridge, MA); LB was grown at the Tissue Bank, National Naval Medical Center (Bethesda, MD). Cells were harvested and used for polysome isolation on the same day.

Polysome Isolation and Immunopurification. JY or LB cells were washed in RPMI-1640 containing cycloheximide (1 μg/ml) or trichodermin (1 μg/ml; a generous gift of Leo Pharmaceuticals) and pelleted. The cells were lysed by homogenization in a Potter–Elvehjem homogenizer, the nuclei were pelleted, and the polysomes were precipitated by the addition of buffer containing $MgCl_2$ (100 mM, final concentration) as described (11). The polysomes were pelleted over a cushion of sucrose and resuspended in polysome buffer Na300 (300 mM NaCl/5 mM $MgCl_2$/50 mM Tris·HCl, pH 7.5/0.1% Nonidet P-40) at 2.5 ml of Na300 per 1 g of cells (12). All solutions contained 100 μg of heparin (Sigma) and 1 μg of cycloheximide or trichodermin per ml. The polysomes generally were used immediately; however, they could be frozen at −70°C for several months.

The immunopurification of polysomes followed the procedure of Shapiro and Young (7) and is outlined below. The murine monoclonal antibody used, HC 2.1, was prepared after immunization with the separated heavy chain from papain-treated detergent-solubilized HLA-DR antigen. An ammonium sulfate fraction of HC 2.1 was made RNase free by passage over protein A-Sepharose Cl-4B (Pharmacia), elution with 0.1 M glycine (pH 2.5), and dialysis against phosphate-buffered saline (13). The polysome suspension was centrifuged for 10 min at 12,000 × *g* to remove aggregates, and HC 2.1 was added (0.5 mg/g of initial weight of cells, usually 10 g). After incubation for several hours at 4°C, the polysomes were passed over a 5-ml column of protein A-Sepharose, and the flow-through was passed over once again. The column was washed with 20 column vol of polysome buffer supplemented with heparin and either cyclohex-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: ds, double-stranded; kb, kilobase(s).

amide or trichodermin. The polysomes were dissociated by elution of the column with 20 ml of 25 mM Tris·HCl, pH 7.5/20 mM EDTA. For reuse, the column was washed in 1 M acetic acid to dissociate the protein A-Sepharose–antigen complex and then reequilibrated with polysome buffer. RNA was isolated from the eluate without deproteinization by passage over oligo(dT)-cellulose. The Tris/EDTA eluate was incubated for 5 min at 65°C, placed on ice, made 0.5 M in NaCl, 10 mM in Tris·HCl (pH 7.5), and 0.2% in $NaDodSO_4$, and passed over 0.2 ml of oligo(dT)-cellulose (Collaborative Research, type T-3) in a 1-ml syringe. The column was washed with 20 ml of 0.5 M NaCl/10 mM Tris·HCl, pH 7.5/0.2% $NaDodSO_4$, and the mRNA was eluted with 1 ml of H_2O. The mRNA was precipitated by the addition of sodium acetate (pH 4.5) to 0.3 M and 2 vol of ethanol and stored at −20°C. Isolation of total poly(A)$^+$ mRNA and cell-free translations in a rabbit reticulocyte lysate were performed as described (14, 15). Immunoprecipitations with formalin-fixed *Staphylococcus aureus* by the method of Kessler (16) were performed as described (15).

cDNA Synthesis and Selection of Recombinants. cDNA synthesis was performed by standard procedures with avian myeloblastosis virus reverse transcriptase (obtained from J. Beard, Life Sciences, St. Petersburg, FL). For large amounts of mRNA, the first-strand reaction was boiled prior to the addition of DNA polymerase I (Bethesda Research Laboratories) for second-strand synthesis (17). For small amounts, the mRNA was hydrolyzed by incubation in 0.3 M NaOH for 30 min at 70°C. The ds cDNA was treated with S1 nuclease and tailed with terminal deoxynucleotidyltransferase (Bethesda Research Laboratories) and CTP. All phenol extractions and column separations for removal of labeled nucleotides after each of the above reactions were replaced by two spermine precipitations of the DNA (18). The reaction mixtures were made 10 mM in spermine, incubated 10 min at 4°C, and pelleted in an Eppendorf Microfuge. The hard whitish pellet was resuspended in a small amount of 0.4 M sodium acetate/10 mM magnesium acetate, diluted 1:10 with H_2O, and reprecipitated with 10 mM spermine. The second precipitate was resuspended in 0.4 M Na acetate/10 mM Mg acetate and ethanol precipitated to remove spermine.

dC-tailed double-stranded cDNA was hybridized with *Pst* I-digested, dG-tailed pBR322 and used to transform *Escherichia coli* MM294 or C600 incubated overnight in $CaCl_2$ (19). Transformants were plated or transferred to Millipore filters and amplified on chloramphenicol plates (20). Filters were screened by hybridization (21) with [^{32}P]cDNA made to HC 2.1-immunopurified mRNA. Hybridizing clones were grown (10 ml) and plasmid DNA was prepared as described (22), with the addition of one phenol extraction. Positive selections with total poly(A)$^+$-mRNA (200–400 μg/ml) were performed as described (23) except that DNA was spotted onto nitrocellulose filters (24, 25).

Carboxypeptidase Digestion. Treatment of the heavy chains of native detergent-solubilized DR antigen (p34) and of the DR antigen obtained after detergent solubilization of papain-treated membranes (p33) (27) with carboxypeptidases A and B (Worthington) was carried out as described (28).

RESULTS

Immunopurification of Polysomes by Using an Anti-DR Heavy Chain Monoclonal Antibody. We previously identified a precursor of the heavy chain of HLA-DR (p34) in cell-free translations of poly(A)$^+$mRNA from human B lymphoblastoid cell lines by immunoprecipitation with a heteroserum raised against the separated, denatured heavy chain (anti-p34) (15). Monoclonal antibodies were prepared against the separated heavy and light chains of papain-treated detergent-solubilized HLA-DR (p23, 30). One such monoclonal antibody, HC 2.1, reacted with the separated glycosylated DR heavy chain synthesized *in vivo* (p34) and showed no reactivity with the native DR complex, as was shown for anti-p34 (15). As expected, it also precipitated the nonglycosylated precursor to the heavy chain, p31, synthesized in cell-free translation (e.g., Fig. 2, lane b).

Milligram amounts of HC 2.1 were prepared from ascites and made RNase free by passage over protein A-Sepharose columns. This antibody was added to polysomes prepared by Mg^{2+} precipitation from Triton X-100 lysates of JY cells. The polysomes bound by HC 2.1 were purified by passage over protein A-Sepharose. After extensive washing, the polysomes were eluted with Tris/EDTA, and the mRNA was purified by oligo(dT)-cellulose chromatography. The translations of total mRNA and mRNA from HC 2.1 immunoaffinity-purified polysomes are presented in Fig. 1A. Note the ratio of anti-p34 reactive material to anti-H reactive material from total translate (about 1:5 by densitometry, lane b to lane c). Anti-H is a heteroserum prepared against the heavy chain of HLA-A,B,C antigens (30). In the absence of mRNA (lane d) only globin and an artifact of the system were seen, and nothing was precipitated with the antibodies used (lanes e and f).

When HC 2.1-immunopurified mRNA was translated, the pattern of total translation (lane g) revealed a dramatic increase in a band migrating at the position of the precursor of the DR heavy chain. There was a concomitant decrease in almost all other proteins except for those proteins already making up a large percentage of the total protein—i.e., actin (the band below the [^{35}S]methionine-labeled artifact of cell-free translation), and a doublet below the major product. The major product (but not the doublet below it) was precipitable by the anti-p34 heteroserum (lane h). The doublet was also not precipitable by HC 2.1 and thus is not likely to represent material corresponding to antigenically related products of another DR locus (32–34). Lane i shows the immunoprecipitation of the supernatant of lane h by an additional aliquot of anti-p34 and anti-H. Comparison of the ratio of the densities of anti-p34 precipitable material and anti-H precipitable material from translation of HC 2.1-immunopurified mRNA (150:1) to that ratio for translation of total mRNA (1:5) is an indication of the level of purification obtained relative to the mRNA for the heavy chain of HLA-A,B,C antigens. In this case the ratio was approximately 750. By comparing the ratios of two immunoprecipitable products, the extent of purification can be estimated; differences in efficiencies of translation due to varying mRNA concentrations or unequal numbers of methionines in each of the molecules can be neglected. However, faulty estimations due to differences in the intrinsic rate of translation of DR heavy chain mRNA and HLA-A,B,C heavy chain mRNA *in vitro* cannot be excluded.

Fig. 1*B* presents another example of immunopurification of polysomes by HC 2.1 In this case, the purification was much greater and the mRNA had been purified nearly to homogeneity. The purity of the mRNA shown in lane g of Fig. 1A was estimated to be about 30% by densitometry of the cell-free translation products, and therefore purification to homogeneity required a 2000- to 3000-fold purification from total mRNA (750/0.3). These polysomes were prepared in the continuous presence of trichodermin at 1 μg/ml [a fungal antibiotic that is thought to inhibit termination of translation (35, 36)]. It is believed that polysomes prepared in the presence of trichodermin might have longer nascent chains (and therefore more and greater exposure of antigenic sites per polysome) than polysomes isolated in the presence of chain-elongation inhibitors such as cycloheximide. Alternatively, the yield of polysomes may be greater. A 2-fold increase in specific immunoprecipi-

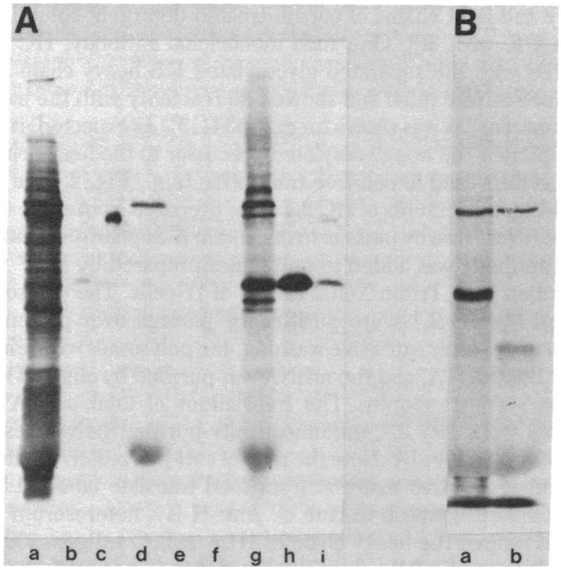

FIG. 1. Cell-free translation of total and of immunopurified mRNA analyzed by immunoprecipitation. (*A*) Cell-free translation of total JY mRNA and of mRNA immunopurified with anti-DR heavy chain monoclonal antibody (HC 2.1), analyzed by immunoprecipitation and $NaDodSO_4$/polyacrylamide (10%) gel electrophoresis (29). Lanes: a, cell-free translation of total poly(A)$^+$mRNA (1 μg/25 μl of reaction volume); b, immunoprecipitation of cell-free translation products from total mRNA by anti-p34 heteroserum (10-fold the amount in lane a); c, immunoprecipitation of the supernatant of lane b by anti-H heteroserum (30) and an additional aliquot of anti-p34; d, cell-free translation with no added mRNA (note the artifact of cell-free translation with [^{35}S]methionine and the reticulocyte proteins synthesized from endogenous mRNA); e and f, immunoprecipitations of cell-free translations with no added mRNA as in lanes b and c, respectively; g, cell-free translation of poly(A)$^+$mRNA isolated from HC 2.1-immunopurified polysomes (represents the poly(A)$^+$mRNA from 0.4 g of JY cells); h, immunoprecipitation by anti-p34 of cell-free translation of HC 2.1-immunopurified mRNA (10-fold the amount in lane g); i, immunoprecipitation of supernatant of lane h by anti-H and an additional aliquot of anti-p34. Fluorographic exposure time was 10 hr (31). (*B*) Cell-free translation of poly(A)$^+$mRNA from HC 2.1-immunopurified polysomes (lane a) and translation with no added mRNA (lane b). See text for details. Fluorographic exposure time was 5 hr.

tation of chicken histone polysomes in the presence of trichodermin has been reported (37). A systematic analysis of the effect of this drug was not performed here.

Isolation of cDNA Clones. With mRNA of this purity, the cloning and selection of cDNAs complementary to DR heavy chain was straightforward. Two approaches were simultaneously taken to obtain these cDNA clones: (*i*) ds cDNA copies were prepared directly from immunopurified mRNA and cloned into pBR322, and (*ii*) a cDNA library was probed with labeled single-stranded cDNA made from the immunopurified mRNA.

Cloning cDNA directly has several advantages (see *Discussion*) but it was not clear that there was enough DR heavy chain mRNA to produce sufficient amounts of ds cDNA. Standard procedures were used for preparing ds cDNA from the immunopurified mRNA but all phenol extractions and column separations were avoided by precipitating DNA with spermine. The ds cDNA was tailed with dCTP and hybridized to *Pst* I-digested dG-tailed pBR322. The hybrids were used to transform competent *E. coli* C600. ^{32}P-Labeled cDNA from HC 2.1-immunopurified mRNA was also made and used to probe 2000–3000 cDNA clones prepared from total poly(A)$^+$mRNA from the LB cell line (DR 6,6), in addition to the clones prepared directly from immunopurified mRNA.

From the small number of clones prepared directly from immunopurified mRNA, one clone that hybridized very strongly with labeled cDNA was analyzed by positive selection. This clone, pDRH-1 positively selected mRNA which, when translated, was immunoprecipitable by both HC 2.1 and anti-p34 (data not shown). Five colonies from the LB library that hybridized to the cDNA probe were also screened by positive selection. One of these clones, pDRH-2, was also found to select the DR heavy chain mRNA (Fig. 2).

Nucleotide Sequence of pDRH-1 and pDRH-2. The sequences of pDRH-1, cloned directly from ds cDNA synthesized from HC 2.1-immunopurified mRNA from the JY cell line (DR 4,6), and pDRH-2, isolated from a cDNA library from the LB cell line by using single-stranded cDNA as a probe, are presented in Fig. 3. pDRH-2, which is 500 nucleotides long [not including the poly(A) and C·G tails], contains the 3′ end of the mRNA followed by a succession of about 75 adenosines. Twenty-seven nucleotides upstream from the poly(A), the polyadenylylation signal A-A-T-A-A-A begins. Another A-A-T-A-A-A sequence occurs 130 bases from the poly(A) tail (and also on the complementary strand at position 343). The existence of two such sequences might indicate the presence of two separate messengers differing by 100 nucleotides. Alternatively, the existence of two A-A-T-A-A-A sequences could indicate that this sequence alone is not sufficient to serve as the signal for transcription termination and polyadenylylation (38).

At the 5′ end of pDRH-2, one reading frame encodes 31 amino acids followed by a stop codon and then, after 12 base pairs, a second stop codon in the same reading frame. These amino acid residues bear the general properties expected at the COOH terminus of a transmembrane protein such as the DR heavy chain. First, a stretch of 14 hydrophobic amino acids with no charged residues occurs, although two threonines are present. Similar contiguous hydrophobic sequences have been observed for transmembrane proteins such as the heavy chains of HLA-A,B,C and H-2K,D antigens, glycophorin, and membrane IgM. Threonines have been reported in the transmembrane segment of H-2K as well as in glycophorin (39, 40). Following the hydrophobic region is a stretch of 15 amino acids, predominantly hydrophilic, that comprise the cytoplasmic COOH terminus. It begins with a cluster of positively charged residues (Lys-Gly-Leu-Arg-Lys). This feature has also been observed in transmembrane proteins and has been postulated to stabilize interactions with the negatively charged phospholipid. Within the hydrophilic COOH terminus is a single serine residue which is the presumed site of phosphorylation of the heavy chain (27). This serine is located two residues from an arginine, a characteristic shared by many cAMP-dependent protein ki-

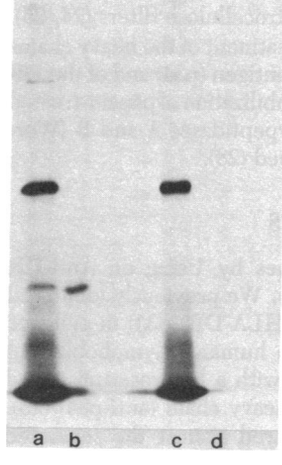

FIG. 2. Cell-free translation of mRNA hybridizing to filter-bound pDRH-2 analyzed by immunoprecipitation. Lanes: a, translation of pDRH-2 selected mRNA; b, immunoprecipitation of equal aliquot of cell-free translation products of lane a by HC 2.1; c, translation of mRNA bound to another plasmid (pBR322 + insert) that was negative in hybridization with [^{32}P]cDNA to HC 2.1-immunopurified mRNA; d, immunoprecipitation of cell-free products of lane c by HC 2.1.

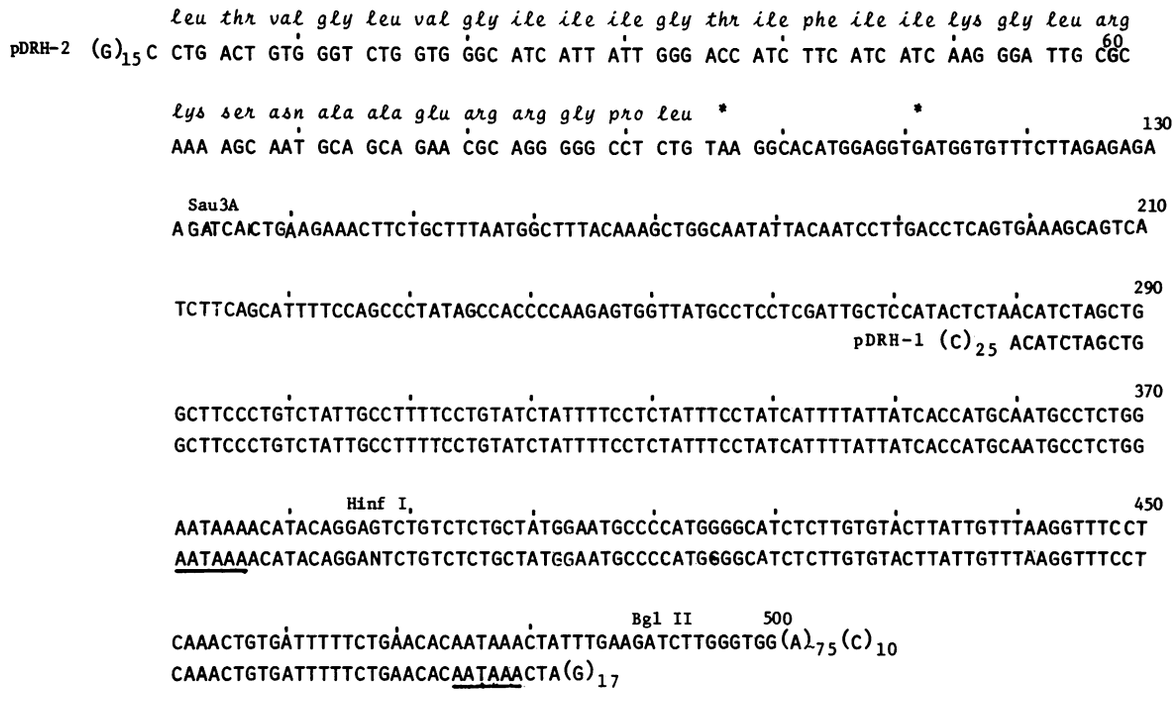

FIG. 3. The nucleotide sequences of pDRH-1 and pDRH-2. The restriction enzyme sites shown are those that were end-labeled at 3′ ends with the Klenow fragment of DNA polymerase I or with terminal deoxynucleotidyltransferase and [α-^{32}P]cordycepin (Amersham) for sequence analysis. A single *Pst* I site at the 5′ end of pDRH-2 was also labeled. Sequences were determined by the method of Maxam and Gilbert (26) but 60% formic acid was used for the A+G specific base modification reaction. *, Stop codon.

nase acceptor sites (41). Finally, papain cleavage removes the COOH-terminal hydrophilic region, decreasing the apparent M_r from 34,000 to 33,000 (a difference of about 10 amino acid residues). Although the exact site of papain cleavage is not known, the size of the hydrophilic sequence in the reading frame of pDRH-2, 15 residues, is consistent with these data and is considerably smaller than the corresponding region in HLA-A,B antigens. Papain actually removes only nine residues of the hydrophilic region (see below).

The sequence of pDRH-1 (204 base pairs) is wholly contained within the 3′ untranslated region of pDRH-2 and appears to be identical with it (Fig. 3). These two cDNA clones come from mRNA from cell lines established from two different individuals.

Digestion of DR Antigen Heavy Chain by Carboxypeptidases. Confirmation of the deduced sequence was sought by carboxypeptidase digestion of purified DR heavy chains. Detergent-solubilized DR heavy chains (p34) and papain-treated detergent-solubilized DR heavy chains (p33) were isolated from cell lines JY and LB as described (27). Carboxypeptidase A digestion of p34 from JY cells yielded only leucine (0.42 mol of Leu per mol of p34); addition of carboxypeptidase B at this point released no amino acids in comparable amounts. This result is expected from the sequence of pDRH-2 because carboxypeptidases A and B do not release proline. Papain treatment of B lymphoblastoid membranes removes the COOH-terminal hydrophilic peptide from DR heavy chains to yield p33, which can then be solubilized by detergent. Carboxypeptidase A digestion of p33 from both JY and LB cells yielded mostly serine (0.47 mol per mol of p33); addition of carboxypeptidase B at this point yielded predominantly arginine (0.33 mol/mol) and a small amount of leucine (0.10 mol/mol). The lysine position was obscured by a contaminant. These data are consistent with papain cleavage after the serine in the putative hydrophilic sequence of pDRH-2. Thus, these data are consistent with pDRH-2 being a clone of authentic DR heavy chain.

DISCUSSION

The purification of mRNA by specific immunoprecipitation of polysomes is a highly specific method of mRNA isolation. Its technical difficulties include impurity of antibodies and nonspecific trapping of polysomes; the latter has been partly circumvented by the use of antibodies coupled to a solid support (42) and by using either protein A-containing bacteria (12) or protein A itself (7) as an immunoadsorbent. By combining the use of protein A-Sepharose column chromatography and a monoclonal antibody against the heavy chain of HLA-DR, we have purified to near homogeneity the mRNA for the heavy chain of HLA-DR. This procedure has been successful for purification of a message whose estimated abundance is 0.01–0.05% of total mRNA and could be useful for even scarcer messages. The increasing number of monoclonal antibodies currently available for proteins for which there are little structural data potentially could be exploited in this manner. The initial purification of the mRNA by a highly selective antibody eliminates the need for positive selection as an initial screening procedure.

Another advantage of this technique is that, once a polysome preparation is made, two or more different antibodies can be sequentially added to the polysomes and removed by protein A-Sepharose columns. This has been done with HC 2.1 antibody followed by anti-H, the heteroserum recognizing the HLA-A,B heavy chain precursor (30). Although the yield of the anti-H reactive polysomes was not as good as that for DR heavy chain, the purity nevertheless was high (unpublished data). An antibody capable of recognizing a nascent NH_2-terminal epitope could be critical for the success of this procedure.

The most signficant aspect of the power of specific immunopurification of polysomes is its potential use in the study of the structure and genetic organization of a multigene family. By cloning full-length cDNAs directly from mRNA isolated from immunopurified polysomes, it is possible to relate a DNA sequence directly to one of a number of similar antigens, without

requiring extensive protein information—i.e., the identification is provided by the specificity of the monoclonal antibody. The direct correlation of a DNA sequence with a specific antigen circumvents those difficulties that arise in subsequent analyses as a result of the numbers of homologous sequences detected by nucleic acid hybridization. For example, hybridization with HLA-A,B or H-2K,D probes detects 10–15 fragments in genomic DNA digests (43–45). To determine what these fragments are one must resort either to a detailed analysis of each sequence or to cell transformation, which will ultimately depend upon serological methods for detection of transformants and a knowledge of the requirements for cell surface expression as well. The light chains of human DR antigens and the antibodies that recognize subpopulations of these antigens (e.g., 32–34) will provide a test of the efficacy of this idea as applied to the analysis of major histocompatibility complex genes.

The availability of cDNA probes for HLA-DR will permit further analysis of other areas of the human major histocompatibility complex. Of particular interest is the question of the number of *HLA-DR* and *DR*-like genes and their relationship to the nature of the immune response. It has been argued that, to serve as antigen recognition molecules, the current number of known DR or Ia antigens is insufficent to present the large number of protein antigens (10, 46). Southern blot analysis of genomic DNA using either [^{32}P]cDNA prepared to HC 2.1-immunopurified mRNA or nick-translated pDRH-2 revealed only a single 18-kilobase (kb) *Bam*HI fragment hybridizing to these probes even at low stringency. In addition, four different genomic clones have been isolated from a human library in phage λ by hybridization with pDRH-2. All of these clones share an internal *Eco*RI fragment of 3.5-kb that hybridizes with pDRH-2. These data suggest that the DR antigen heavy chain (the constant chain of the complex) may be encoded only once in the genome. The gene for β_2-microglobulin, also a constant chain, is also thought to be a single-copy gene (23). The cDNAs made from mRNA and pDRH-2 do not appear to be sufficiently related to other DR heavy chains (32–34) to cross-hybridize with them. Differences in peptide maps for the heavy chains of DR and DC1 (a second DR-like molecule) have recently been observed (32). It should also be noted that, in the mouse, I-A α chains and I-E α chains have little homology by peptide mapping (47) and might not be expected to cross-hybridize by virtue of nucleic acid homology.

We thank Dr. M. Strong and T. Williams (Naval Medical Research Institute) and D. Giard (Cell Culture Facility, Massachusetts Institute of Technology) for their efforts to provide live cells. For their encouragement, reagents, and countless conversations, we wish to thank the members of the Strominger, Gilbert, Losick, and Pero laboratories. This work was supported by research grants from the National Institutes of Health (AM-13230) and the Kroc Foundation and a Damon Runyon–Walter Winchell Fellowship (to P.J.K.).

1. Ricciardi, R. P., Miller, J. S. & Roberts, B. E. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4927–4931.
2. Derynck, R., Remaut, E., Saman, E., Stanssens, P., De Clerq, E., Content, J. & Fiers, W. (1980) *Nature (London)* **287**, 193–196.
3. Cowie, D. B., Spiegelman, S., Roberts, R. B. & Duerksen, J. D. (1961) *Proc. Natl. Acad. Sci. USA* **47**, 114–118.
4. Palacios, R., Palmiter, R. D. & Schimke, R. T. (1972) *J. Biol. Chem.* **247**, 2316–2321.
5. Schechter, I. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 2256–2260.
6. Flick, P. K., Chen, J., Alberts, A. W. & Vagelos, P. R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 730–734.
7. Shapiro, S. Z. & Young, J. R. (1981) *J. Biol. Chem.* **256**, 1495–1498.
8. Strominger, J. L., Engelhard, V. H., Fuks, A., Guild, B. C., Hyafil, F., Kaufman, J. F., Korman, A. J., Kostyk, T. G., Krangel, M. S., Lancet, D., Lopez de Castro, J. A., Mann, D. L., Orr, H. T., Parham, P., Parker, K. C., Ploegh, H. L., Pober, J. S., Robb, R. J. & Shackelford, D. A. (1981) in *The Role of the Major Histocompatibility Complex in Immunobiology*, ed. Dorf, M. E. (Garland, New York), pp. 115–172.
9. Klein, J. (1979) *Science* **203**, 516–521.
10. Benaccerraf, B. (1981) *Science* **212**, 1229–1238.
11. Efstratiadis, A. & Kafatos, F. C. (1976) in *Methods in Molecular Biology*, ed. Last, J. (Dekker, New York), Vol. 8, pp. 1–124.
12. Gough, N. M. & Adams, J. M. (1978) *Biochemistry* **17**, 5560–5566.
13. Goudswaard, J., Van Der Donk, J. A., Noordzij, A., Van Dam, R. H. & Vaerman, J. P. (1978) *Scand. J. Immunol.* **8**, 21–28.
14. Pelham, H. R. B. & Jackson, R. J. (1976) *Eur. J. Biochem.* **67**, 247–256.
15. Korman, A. J., Ploegh, H. L., Kaufman, J. F., Owen, M. J. & Strominger, J. L. (1980) *J. Exp. Med.* **152**, 65s–82s.
16. Kessler, S. W. (1975) *J. Immunol.* **115**, 1617–1624.
17. Wickens, M. P., Buell, G. N. & Schimke, R. T. (1978) *J. Biol. Chem.* **253**, 2483–2495.
18. Hoopes, B. C. & McClure, W. R. (1981) *Nucleic Acids Res.* **9**, 5493–5504.
19. Dagert, M. & Erlich, S. D. (1979) *Gene* **6**, 23–28.
20. Hanahan, D. & Meselson, M. (1980) *Gene* **10**, 63–67.
21. Thayer, R. E. (1979) *Anal. Biochem.* **98**, 60–63.
22. Holmes, D. S. & Quigley, M. (1981) *Anal. Biochem.* **114**, 193–197.
23. Parnes, J. R., Velan, B., Felsenfeld, A., Ramanathan, L., Ferrini, U., Appella, E. & Seidman, J. G. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2253–2257.
24. Kafatos, F. C., Jones, C. W. & Efstratiadis, A. (1979) *Nucleic Acids Res.* **7**, 1541–1552.
25. Ploegh, H. L., Orr, H. T. & Strominger, J. L. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6081–6085.
26. Maxam, A. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560.
27. Kaufman, J. F. & Strominger, J. L. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 6304–6308.
28. Waxman, D. J. & Strominger, J. L. (1981) *J. Biol. Chem.* **256**, 2067–2077.
29. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
30. Ploegh, H. L., Cannon, L. E. & Strominger, J. L. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2273–2277.
31. Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83–88.
32. Corte, G., Calabi, F., Damiani, G., Bargellesi, A., Tosi, R. & Sorrentino, R. (1981) *Nature (London)* **292**, 357–360.
33. Shackelford, D. A., Mann, D. L., Van Rood, J. J., Ferrara, G. B. & Strominger, J. L. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4566–4570.
34. Shackelford, D. A., Lampson, L. A. & Strominger, J. L. (1981) *J. Immunol.* **127**, 1403–1410.
35. Cundliffe, E., Cannon, M. & Davies, J. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 30–34.
36. Wei, C. M., Hansen, B., Vaughan, M. & McLaughlin, C. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 713–717.
37. Scott, A. C. & Wells, J. R. E. (1975) *Biochem. Biophys. Res. Commun.* **64**, 448–455.
38. Woo, S. L. C., Beattie, W. G., Catterall, J. F., Dugaiczyk, A., Staden, R., Brownlee, G. G. & O'Malley, B. W. (1981) *Biochemistry* **20**, 6437–6446.
39. Coligan, J. E., Kindt, T. J., Uehara, H., Martinko, J. & Nathenson, S. G. (1981) *Nature (London)* **297**, 35–39.
40. Tomita, M., Furthmayr, H. & Marchesi, V. T. (1978) *Biochemistry* **17**, 4756–4770.
41. Kemp, D. E., Graves, D. J., Benjamini, E. & Krebs, E. G. (1977) *J. Biol. Chem.* **252**, 4888–4894.
42. Schutz, G., Kieval, S., Groner, B., Sippel, A. E., Kurtz, D. T. & Feigelson, P. (1977) *Nucleic Acids Res.* **4**, 71–84.
43. Orr, H. T., Bach, F., Ploegh, H. L., Strominger, J. L., Kavathos, P. & DeMars, R. (1982) *Nature (London)*, in press.
44. Steinmetz, M., Frelinger, J. G., Fisher, D., Hunkapiller, J., Pereira, D., Weissman, S. M., Uehara, H., Nathenson, S. & Hood, L. (1981) *Cell* **24**, 125–134.
45. Cami, B., Brégègére, F., Abastado, J. P. & Kourilsky, P. (1981) *Nature (London)* **291**, 673–675.
46. McDevitt, H. O. (1980) in *Immunology 80*, eds. Fougereau, M. & Dausset, J. (Academic, London), pp. 503–512.
47. Uhr, J. W., Capra, J. D., Vitetta, E. & Cook, R. G. (1979) *Science* **206**, 292–297.