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Immunology

cDNA cloning of the immunoglobulin heavy chain binding protein

(hybrid-selected mRNA translation/RNase A protection assay/cDNA sequence/heat shock proteins/endoplasmic reticulum)

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ABSTRACT **A cDNA library was constructed from size-fractionated poly(A)$^+$ RNA prepared from a murine pre-B-cell hybridoma expressing high levels of immunoglobulin heavy chain binding protein (BiP) and μ heavy chains. Transformed bacterial colonies were screened for recombinant plasmids containing cDNA coding for BiP by hybrid-selected mRNA translation. A clone, pMBiP, containing a 736-base-pair insert was shown to encode the protein. Translation *in vitro* of hybridoma mRNA selected by hybridization to the pMBiP cDNA yielded a single polypeptide of BiP-like size. The authenticity of this mRNA was verified by comparing the peptides obtained by the limited proteolysis of its *in vitro* translation product with those obtained from the *in vivo* produced BiP. Likewise, the authenticity of the cDNA insert was verified by an RNase A protection assay of heteroduplex molecules obtained by annealing a uniformly labeled single-strand copy of the cDNA clone with the same mRNA selected by hybridization and tested by translation. The nucleotide sequence of this clone enabled us to deduce the carboxyl-terminal 142 amino acids of BiP and to establish its kinship with the 70-kDa heat shock protein family. The finding of a single copy of the BiP gene in DNA blots of mouse and rat implies that the BiP-related RNA transcripts constitutively expressed in various murine tissues and cell lines are indeed products of the same gene. These findings imply that BiP plays a more general role than previously anticipated on the basis of the discovery of its association with immunoglobulin heavy chains.**

The heavy (H) chain subunit of immunoglobulins, unlike the light (L) chains, are very rarely exported as unassembled polypeptides from their site of synthesis in the endoplasmic reticulum (ER). As best studied in the mouse, intracytoplasmic H chains are associated either with L chains or with a 78-kDa polypeptide that, owing to its peculiar property of binding different H chain isotypes, was designated immunoglobulin H chain binding protein (BiP) (1). H chains and BiP are localized in the lumen of the ER, where their complexes seem to be specifically retained (2). The failure to detect H chain–BiP complexes in murine plasmacytomas that express defective molecules lacking the C_H1 domain indicates that this region of the H chain is implicated in the association with BiP as well as with L chains (3). Surprisingly, in these cells, the H chain is produced and secreted in the absence of L chains. It is interesting to note that the defective immunoglobulin molecules found in human H chain diseases also characteristically lack L chains and carry deletions encompassing the C_H1 domain of the H chain (4).

A theory advanced to explain the allelic and isotypic exclusion of immunoglobulin gene expression proposed that the BiP–H chain complex might act as a signal for inducing L chain gene rearrangement (5). Furthermore, cells expressing higher amounts of H chains due to productive rearrangements of both H chain alleles would not undergo clonal expansion because of "H chain toxicity." Indeed, a growth inhibition effect had been attributed to free H chains by Köhler (6), who noted that myelomas or plasma cell-derived hybridomas very rarely yield subclones that express H chain alone or in excess over L chains, except when cells are mutagenized (7) or happen to synthesize a defective H chain (8). In contrast, clones that express only L chain can easily be isolated (9).

At odds with the concept of H chain toxicity, the precursors of B lymphocytes, pre-B cells, are characterized by the production, in the absence of L chain synthesis, of intact μ H chains that are neither exported to the cell surface nor secreted (10). This pre-B cell property is transferable to plasma cells by cell fusion (11).

As we proposed that BiP might play a critical role as the physiological antidote for the toxic effect of immunoglobulin chains, we resolved to undertake its molecular cloning to gain insights into the nature of the protein.

MATERIALS AND METHODS

Biosynthetic Labeling, *in Vitro* Translation and Immunoprecipitation, Immunoblotting, and Peptide Mapping. The characteristics of hybridoma TKH1.22 have been reported (12). [^{35}S]Methionine labeling and $NaDodSO_4$/polyacrylamide gel electrophoresis were performed as described (12). Rabbit reticulocyte lysate was prepared and used as previously described (13), and immunoprecipitations were performed (14) with commercial anti-μ antiserum (Litton Bionetics).

Radioactively labeled total cellular proteins were separated on $NaDodSO_4$/10% polyacrylamide gels, transferred to nitrocellulose filters (15), and analyzed with our preparation of goat anti-BiP antiserum. The labeled polypeptide bands were excised from the blot or from dried gels and subjected to limited proteolysis (100 pg of papain per slot) according to Cleveland *et al.* (16).

Isolation and Size Fractionation of Nucleic Acids. DNA was isolated according to standard procedures (ref. 17, pp. 280–281). RNA preparations were essentially according to Schibler *et al.* (18). DNA and RNA blotting was done according to Maniatis *et al.* (ref. 17, pp. 203 and 383–385). Double-stranded cDNA was labeled by random priming (19).

Size fractionation of poly(A)$^+$ RNA was performed by horizontal electrophoresis (250 μg of RNA per cm^2) in 1% ultra-low-melting agarose (FMC, Rockland, ME) containing 15 mM methylmercuric hydroxide according to Lonberg and Gilbert (20). Individually remelted fractions (about 1.5 mm) were tested directly as agarose solutions (1:25 final dilution in the translation assay) by *in vitro* translation (Fig. 1). Poly(A)$^+$ RNA used for cDNA synthesis was reextracted from the remelted fractions by means of poly(U)-Sephadex G-10 (Bethesda Research Laboratories).

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Abbreviations: H chain, heavy chain; L chain, light chain; ER, endoplasmic reticulum; BiP, immunoglobulin H chain binding protein; HSP, heat shock protein; nt, nucleotide(s).

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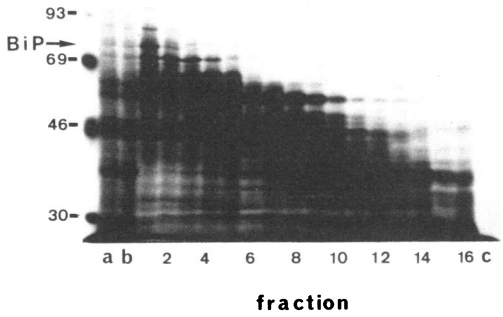

FIG. 1. Cell-free translations of TKH1.22 poly(A)$^+$ RNA [1–3 kilobases (kb)] fractionated on agarose gels. The same amount of incorporated radioactivity was applied to each slot of a 10% polyacrylamide gel to evaluate the enrichment of specific bands in the various fractions (lanes 1–16; note only alternate lanes are numbered) as compared to unfractionated cytoplasmic RNA (lane a) or poly(A)$^+$ RNA (lane b). The endogenous mRNA activity of the reticulocyte lysate was estimated by a control of *in vitro* translation without exogenous RNA (lane c). Marker proteins and their molecular masses (kDa) are on the left.

Construction and Screening of a cDNA Library. Double-stranded cDNA was synthesized from 1 μg of purified size-enriched poly(A)$^+$ RNA according to a modification of the RNase H method (21). Standard procedures (ref. 17, pp. 239–242) were used for poly(dC)-tailing of cDNA and annealing with poly(dG)-tailed *Pst* I-cut vector pUC9 (Pharmacia P-L Biochemicals). The equivalent of 20 ng of cDNA was used to transform *Escherichia coli* 5K rendered competent by the method of Hanahan (22), yielding about 20,000 clones. A ^{32}P-labeled cDNA probe was obtained by reverse transcription of the same size-enriched purified poly(A)$^+$ RNA fractions (17).

Translation and RNase Protection of Hybrid-Selected mRNA. *In vitro* translation was performed with the total amount of messenger selected by hybridization (23) of cellulose powder-immobilized BL95 cDNA [50 μg of pUC9 containing the 736-base-pair (bp) insert] with 400 μg of total TKH1.22 RNA. A portion of the BL95 cDNA [nucleotides (nt) 1–380; cf. Fig. 5] was cloned in the Bluescribe M13$^+$ vector (Stratagene, San Diego, CA). Uniformly labeled RNA was obtained by *in vitro* transcription of the linearized vector (24) using [α-^{32}P]CTP (Amersham, 111 TBq/mmol) as radioactive source. The DNase-treated anti-sense RNA probe was precipitated with the test RNA and dissolved in 20 μl of 20 mM Tris·HCl, pH 7.1, containing 75% formamide, 0.5 M NaCl, and 1 mM EDTA. The nucleic acids were denatured by heating for 10 min at 70°C and allowed to hybridize at 55°C for 16 hr. Nuclease treatment was performed, after dilution of the hybridization mixture with 300 μl of 10 mM Tris·HCl, pH 7.5, containing 400 mM NaCl, 5 mM EDTA, and 60 μg of RNase A per ml, at 25°C for 30 min, followed by the proteinase K treatment and analyses on 5% polyacrylamide sequencing gels (ref. 17, p. 478).

Sequencing. The 736-bp BL95 cDNA insert was cleaved into two fragments (nt 1–380 and nt 381–736 in Fig. 5), which were inserted into M13 mp18 and M13 mp19 vectors and sequenced by using the dideoxy method of Sanger *et al.* (25). The same fragments were also cloned in Bluescribe M13$^+$, allowing the sequencing of over 80% of the nucleotides by an RNA-sequencing technique improved in our laboratory (26).

RESULTS

Primary *in Vitro* Translation Product of BiP-Encoding mRNA. Due to lack of information on the primary structure of BiP, the cloning strategy depended in a crucial way on the ability to identify the primary *in vitro* translation product of BiP mRNA. An antiserum we had previously raised against BiP was able to detect the protein in immunoblots of cell lysates, but it failed to react with the protein in its native conformation or as an *in vitro* translated polypeptide. As originally described (1), BiP can readily be identified by coprecipitation with immunoglobulin H chains in, for example, μ-synthesizing pre-B-cell-derived hybridomas (Fig. 2*A*, lane 3). Surprisingly, no coprecipitating polypeptide could be found in association with the μ chains synthesized in a cell-free translation assay directed with mRNA isolated from the same hybridomas (lane 4), even when the proteins were subjected to post-translational modifications prior to immunoprecipitation (lane 4*). The failure to detect H chain–BiP complexes in the *in vitro* translation assays shown in Fig. 2*A* is not due to the poor glycosylating activity of the microsomal membranes used in this experiment, because the same results were obtained with more active preparations. Moreover, it is known that *in vivo* inhibition of glycosylation does not prevent H chain/BiP association (2, 27, and our unpublished results).

Protein blots and two-dimensional gel electrophoreses of cell lysates (data not shown) led us to conclude that BiP is an abundant cytoplasmic component in pre-B-cell-derived hybridomas. When RNA isolated from these cells was translated *in vitro*, a major band of BiP-like size was observed that did not undergo notable modifications after post-translational processing *in vitro* (cf., e.g., Fig. 2*A*, lanes 2 and 2*). As BiP is not glycosylated *in vivo* (unpublished results), we tested whether the prominent BiP-sized band would contain BiP-precursor molecules by comparing the size of its proteolytic cleavage products with the sizes of those obtained from authentic BiP, synthesized *in vivo*. Fig. 2*B* shows that the papain peptides of *in vivo* labeled BiP isolated by coprecipitation with H chains (lane a) or by using the anti-BiP antiserum on blots of total cellular proteins (lane b) are clearly related in structure to those derived from the BiP-sized translation products (lane c). Thus, the BiP precursor molecules exhibit an electrophoretic mobility identical or very close to that of *in vivo* synthesized BiP and represent a major component of the BiP-sized RNA translation products from a

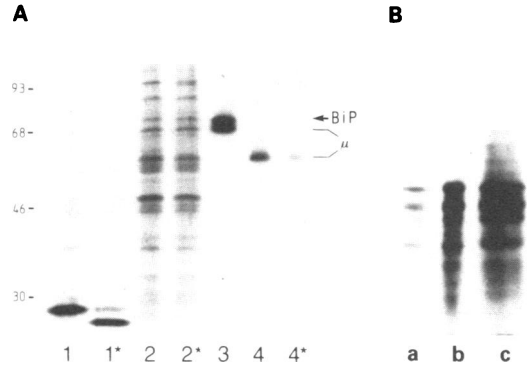

FIG. 2. (*A*) NaDodSO$_4$/PAGE analysis of proteins translated from the TKH1.22 pre-B-cell hybridoma. *In vitro* translation was performed in the absence or presence (lanes marked with *) of dog pancreas microsomal membranes, whose activity was tested by using human placental lactogen mRNA (lane 1). Total or anti-μ-precipitated products were analyzed in lanes 2 and 4, respectively. Anti-μ antiserum was also used to immunoprecipitate the BiP (arrow)–μ chain complex (lane 3) from *in vivo* labeled proteins of TKH1.22. Note the size difference between the μ chains synthesized *in vivo* (lane 3) and *in vitro* (lane 4). (*B*) Identification of BiP translated in a cell-free system by peptide map comparison of *in vivo* and *in vitro* synthesized forms. Lane a, *in vivo* labeled papain-derived peptides of BiP obtained by coprecipitation with immunoglobulin H chain. Lane b, papain digestions of *in vitro* translated polypeptides constituting the putative BiP band (*A*, lane 2). Lane c, *in vivo* labeled BiP purified by using a goat anti-BiP on nitrocellulose blots of metabolically labeled TKH1.22 cells.

cell-free system. More importantly, these data provided us with a reliable method for identifying BiP mRNA in spite of the lack of information on the BiP primary structure and an antiserum able to recognize its precursor.

Construction and Screening of a cDNA Library. Poly(A)$^+$ RNA from the BiP mRNA-enriched (about 7-fold) fractions was used to construct a plasmid cDNA library. About 1000 transformant colonies were individually transferred from ampicillin-containing agar plates into the wells of tissue culture plates, to obtain multiple sets of clone replicates for hybridization screenings.

Part of the mRNA preparation selected for the synthesis of double-stranded cDNA was used as template for synthesizing a radioactive probe enriched in BiP cDNA. Under conditions in which most of the colonies did not give any hybridization signal, the remaining recombinant clones could be divided according to the intensity of their hybridization signal into three groups each containing about 50–100 members. The 50 clones giving the strongest signal were tested for their capacity to hybrid-select BiP mRNA.

Within this first group, a number of recombinant clones did represent copious RNA transcripts but failed to select mRNAs able to direct the synthesis of a single polypeptide chain. These cDNAs hybridized with two highly represented transcripts of large size (>>28 S) that translated *in vitro* with poor efficiency, giving rise to a complex pattern of polypeptides. Out of the group of very strongly hybridizing clones, those that selected for a messenger able to translate into a single polypeptide chain hybridized with the same 3-kb messenger encoding a very abundant 58-kDa protein and were not further characterized.

Finally, analyzing the second most intensely hybridizing group of recombinant clones, we found a likely BiP-encoding candidate in the cDNA clone BL95. This clone hybridized with a mRNA of about 2.6 kb, which, as expected for BiP mRNA, is abundant in pre-B-cell-derived hybridomas (Fig. 3*A*, lanes a and b) and is clearly enriched in the fraction used for cloning (lane c). In fact, the mRNA selected by hybridization with this clone translated *in vitro* into a single BiP-sized polypeptide (Fig. 3*B*, lane 3). To verify that this protein indeed represents a BiP-precursor molecule, we subjected it to a peptide map comparison with authentic BiP. Thus, Fig. 3*C* shows that most of its papain peptides (lane a) migrate with the same electrophoretic mobilities as the peptides obtained from papain digestion of *in vivo* labeled BiP (lane b) isolated by coprecipitation with immunoglobulin H chains. From these results we concluded that the cDNA clone BL95 recognizes BiP-encoding mRNA transcripts, which represent about 1% of the mRNA of TKH1.22 cells as estimated from translation efficiency.

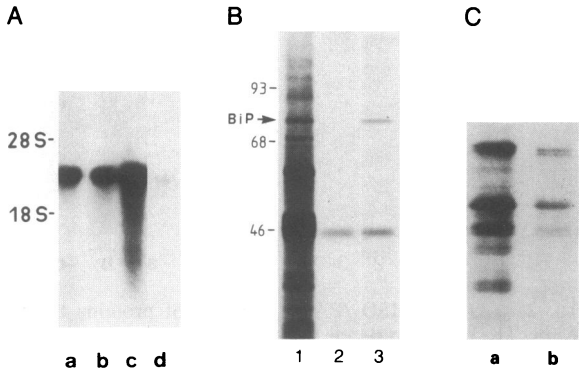

FIG. 3. Hybridization of cDNA clone BL95 with BiP transcripts. (*A*) Blot of total TKH1.22 RNA (15 μg, lane a), unfractionated poly(A)$^+$ RNA (0.3 μg, lane b), size-fractionated poly(A)$^+$ RNA (0.3 μg, lane c), or total mouse liver RNA (15 μg, lane d), hybridized to the insert of BL95 labeled by random priming. (*B*) Identification of the putative BiP mRNA. Cell-free translations were performed with TKH1.22 total RNA (lane 1), in the absence of exogenous RNA (lane 2), or with the transcripts selected from the hybridoma RNA by hybridization to cDNA clone BL95 (lane 3). (*C*) Peptide map comparison of the BiP precursor with authentic BiP: papain-derived peptides from the protein obtained by cell-free translation of the hybrid-selected mRNA (lane a) and of the *in vivo* labeled BiP obtained by coprecipitation with immunoglobulin H chains (lane b).

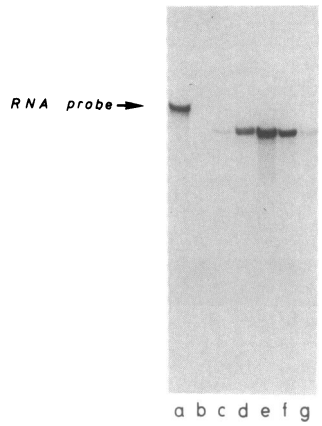

FIG. 4. RNase A protection assay on hybrid molecules composed of BiP-encoding messenger and a synthetic RNA transcript derived from clone BL95. A uniformly labeled anti-sense RNA probe (439 nt) was obtained by transcription of 380 bp of the BL95 insert and 59 additional nucleotides from the vector (lane a). RNase A treatment was performed after hybridization of the probe with 20 μg of *E. coli* tRNA (lane b), with 4 and 20 μg of TKH1.22 total RNA (lanes c and d, respectively), with two different amounts (5/12 or 1/12, lanes e and f, respectively) of the mRNA selected with clone BL95 from 400 μg of total TKH1.22 RNA, or with 20 μg of total mouse liver RNA (lane g).

Sequence Identity of the cDNA Clone BL95 with BiP mRNA. To rule out the possibility that the cDNA clone BL95 is not perfectly complementary to the BiP sequence but is able to select a relatively abundant messenger such as BiP by cross-hybridization, we assessed the degree of nucleotide identity of the cloned sequence with that of the hybridized messenger by an RNase A protection assay. Mispairings in the hybrid molecules composed of BiP-encoding mRNA and a radioactive copy of the cDNA under test would be detected as fragmentation of the probe by the single-strand-specific nuclease. A uniformly labeled RNA probe was synthesized from a 380-bp fragment of BL95 containing 59 additional vector nucleotides (Fig. 4, lane a, 439 nt). The efficiency of the digestion is shown by the failure of tRNA to protect the probe from RNase A attack (lane b). In contrast, hybridization to total cytoplasmic RNA (lanes c and d) or to the hybrid-selected, BiP-encoding RNA (lanes e and f) allowed the quantitative protection of a single fragment of the expected size (380 nt). Thus, a single messenger species hybridizes to cDNA BL95 and the cloned sequence is indistinguishable from that of the natural BiP mRNA.

As shown in Fig. 4 (lane g), BiP-encoding RNA is also present in liver cells, albeit at 1/5th as much as in pre-B-cell-derived hybridomas.

BiP Is Encoded by a Messenger Similar in Sequence to Messengers of the Heat Shock Protein (HSP) Family. As determined by DNA sequencing, the insert of BL95, hereafter referred to as pMBiP (mouse BiP-encoding plasmid), has a length of 736 bp (Fig. 5), representing approximately one-third of the BiP mRNA.‡ Computer analysis of the deduced amino acid sequences and similarity searches in the

‡This sequence is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03629).

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   1
 A  E  D  K  G  T  G  N  K  N  K  I  T  I  T  N  D  Q  N  R  L  T  P  E  E  I  E  R  M  V
GCT GAA GAC AAA GGT ACA GGA AAC AAA AAC AAA ATC ACA ATT ACC AAT GAC CAA AAC CGC CTG ACA CCT GAA GAA ATT GAA AGG ATG GTT
  91
 N  D  A  E  K  F  A  E  E  D  K  K  L  K  E  R  I  D  T  R  N  E  L  E  S  Y  A  Y  S  L
AAT GAT GCT GAG AAG TTT GCT GAG GAA GAC AAA AAG CTC AAA GAG CGC ATT GAC ACC AGG AAT GAA TTG GAA AGC TAT GCT TAT TCT CTC
 181
 K  N  Q  I  G  D  K  E  K  L  G  G  K  L  S  S  D  D  K  E  T  M  E  K  A  V  E  E  K  I
AAG AAC CAG ATT GGA GAT AAA GAA AAG CTG GGA GGT AAA CTT TCT TCT GAC GAT AAA GAA ACC ATG GAA AAA GCT GTA GAG GAA AAG ATT
 271
 E  W  L  E  S  H  Q  D  A  D  I  E  D  F  K  A  K  K  K  E  L  E  E  I  V  Q  P  I  I  S
GAA TGG CTG GAG AGC CAC CAG GAT GCG GAC ATT GAA GAC TTT AAA GCC AAA AAG AAG GAA CTA GAA GAA ATT GTT CAG CCA ATT ATC AGC
 361
 K  L  Y  G  S  G  G  P  P  P  T  G  E  E  D  T  S  E  K  D  E  L
AAA CTC TAT GGT AGT GGT GGC CCT CCC CCA ACT GGT GAA GAG GAT ACA TCA GAA AAA GAT GAG TTG TAG GTG CAC TGA TCT GCT AGA GCT
 451
GTA ATA TTG TAA ATA CTG GAC TCA GGA ACT TTC GTT GGA AGA AAA TTG AGA GAA CTT AAG TCT CGA ATG TAA TTG GAA TCT TCA CCT CAG
 541
AGT GGA GTT GAA AAT GCT ATA GCC CAA GTG GCT GTT TAC TGC TTT TGA TTA GCA GTT GCT CAC ATG TCT TGG GGT CAG GGA GAG GAG GAA
 631
TTG GCT ATT TTA AAA ATT GAG AAA AAG GTG GGT CAG GGT GTG TGT TCA CCT TGG ATA TGG TCT ATT TAA CAA TTG GGT CAT CCA CAT GTG
 721
GTG TAG GAA CTT TTT T
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FIG. 5. Sequence of the cDNA insert of pMBiP. The predicted amino acid sequence of the protein is displayed in the one-letter code above the nucleotide sequence and corresponds to the longest open reading frame of the insert. Note the carboxyl-terminal tetrapeptide K D E L, which is characteristically found in three other soluble proteins retained in the lumen of the ER.

PseqIP sequence collections (28) revealed that the longest open reading frame of pMBiP matches the carboxyl-terminal portion of proteins related to the 70-kDa heat shock protein (HSP 70) family. In fact, an about equal degree of similarity (50–52%) is found between the first 50 amino acids deduced from pMBiP and the 70-kDa HSPs of species as different as chicken, *Drosophila melanogaster*, or *Xenopus laevis* (29–32). The highest degree of similarity, however, is found with p72, a protein of the ER recently cloned by Munro and Pelham (33) from a rat liver cDNA library by cross-hybridization with a *Drosophila* HSP 70 genomic probe. Twenty-nine nucleotide substitutions (i.e., about 4%) distinguish pMBiP from p72; they are equally distributed in the noncoding and coding portion and give rise to two amino acid changes.

BiP Is Encoded by a Single-Copy Gene in the Mouse. The RNase assay showed that our BiP probe detected only a single species of mRNA, which was identical in pre-B-cell-derived hybridomas and liver. Nonetheless, in view of the phylogenetic variability in the size of the 70-kDa HSP gene family (32), it was of interest to determine the number of BiP-related genes in the mouse. Fig. 6 shows a Southern blot analysis of DNA digested by four different restriction enzymes. Regardless of the enzyme used, only one restriction fragment of mouse or rat genomic DNA hybridized with the relatively short probe, indicating the presence of only one copy of the BiP gene in this species.

DISCUSSION

We present here the molecular cloning of BiP mRNA from mouse pre-B cells, in which the protein was originally detected and biochemically defined (1). The sequence deduced from a 736-bp cDNA clone identified by translation of hybrid-selected mRNA shows that BiP is a member of the 70-kDa HSP family. The extraordinary sequence conservation and antigenic crossreactivity among HSP 70 proteins even from distant taxa (34), combined with the variability of their biological properties and sequence complexity in different species (32), are a source of confusion in the literature and render the identification of individual members of the family somewhat hazardous. Therefore, it was crucial in our clone identification to be able to demonstrate that the cDNA

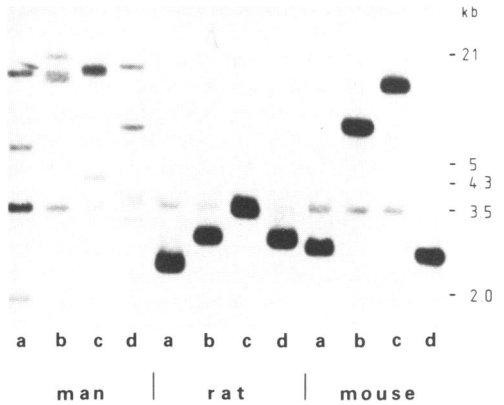

FIG. 6. Southern blot analysis of BiP-related genes in mouse, rat, and man. Genomic DNA was completely digested with *Eco*RI (lanes a), *Hin*dIII (lanes b), *Bam*HI (lanes c), or *Bgl* II (lanes d). After separation on 0.8% agarose gels, the restriction fragments were blotted on GeneScreen membranes and hybridized to the ^{32}P-labeled 736-bp insert of pMBiP. (The less intense hybridizing band of about 3.5 kb present in most of the lanes is from contaminating plasmid DNA.) Hybridization was in 30% (vol/vol) formamide/5× SSC at 42°C, and final washes were done in 0.5× SSC/0.1% $NaDodSO_4$ at 65°C (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7). Note the presence of probably three BiP-like genes in man.

insert is identical in sequence and not just cross-hybridizing with the natural BiP mRNA. Furthermore, while the human genome seems to contain three BiP-like genes, under the conditions allowing the hybrid-selection of natural BiP mRNA, only a single copy of the BiP gene is detected in mouse and rat. Thus, the availability of a BiP cDNA clone now allows a straightforward identification of this member of the HSP 70 family in mouse and rat tissues.

Using a *Drosophila* HSP 70 genomic probe, Munro and Pelham (33) recently isolated two clones from a rat liver cDNA library, one of which encodes a nonglycosylated protein, p72, whose properties suggested to them that it is very similar to if not identical with GRP 78, a glucose-regulated protein described in hamster fibroblasts (35), as well as with BiP. Hamster GRP 78 has been cloned by means of hybrid-selected mRNA translation (36); the cDNA sequence, however, has not yet been reported. Moreover, it is not clear if one or several copies of GRP 78-like genes are present in the hamster genome (37). From immunoprecipitation studies of folding variants of the influenza hemagglutinin found associated with a BiP-like protein in the ER of monkey CV-1 cells, Gething and co-workers (38) concluded that this 77-kDa polypeptide may be identical with BiP, whereas GRP 78 is not, for it differs from BiP in the apparent molecular mass.

Compared to the rat p72 cDNA sequence, the BiP-encoding clone exhibits about 4%, mainly silent, nucleotide substitutions. Since BiP is encoded by a single-copy gene in these rodent species, our cloning studies conclusively demonstrate that the HSP 70 p72 is the rat BiP.

The relatedness of BiP to a group of ancient proteins implicated in the recovery of cells from physically or metabolically induced stresses is significant in several respects. Its immunoglobulin H chain binding activity provides the long-sought paradigm of a physiological activity for the HSP 70 protein family. Likewise, the constitutive presence of BiP in liver and other nonlymphoid tissues (data not shown) suggests that its role is broader than the one anticipated on the basis of its association with immunoglobulin H chains. However, the multiple functions attributed to the HSP 70-like proteins, such as intervening in the assembly and disassembly of proteins and protein-containing structures (39), is fully consistent with our early proposition that BiP is the antidote to immunoglobulin H chain toxicity (11).

The localization of BiP in the ER (2) is apparently ensured by the carboxyl-terminal tetrapeptide Lys-Asp-Glu-Leu (K D E L) shared with other proteins retained in the ER [endoplasmin, protein disulfide isomerase, and a 55-kDa protein (reviewed in ref. 40)]. In our view, the very strong association (data not shown) of nascent H chains with BiP has two effects. On one hand, the H chains are preserved from aggregation in the ER. On the other hand, they are provided with the opportunity to undergo conformational changes possibly required for promoting L chain binding while losing the tertiary structure responsible for polymerization and BiP binding. Hence, the function of BiP would not be to mask a putative secretory signal carried on the H chain or to ensure that only correctly assembled immunoglobulin molecules leave the ER (41). In a more general way, any subunit of proteins destined for export that, by nature or accident, assumes a folding incompatible with assembly will require a transition to an assembly-competent conformation. By its dual property of binding to various proteins (38, 42) and of being a constitutive component of the ER, BiP seems well adapted to assist in this conformational transition by retaining the complex in the subcellular compartment where such putative modifications might take place. Since pre-B-cell hybridomas synthesize large amounts both of BiP and of nonsecreted H chains, it is likely that the level of BiP is correlated with the amount of intracytoplasmic H chain rather than with the secretory state of the cells *per se*, as suggested by others (33). The mechanism by which BiP-bound H chains would be able to refold to acquire an L chain binding competence remains intriguing despite the evidence that BiP complexes can be dissociated by ATP (33).

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