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Immunology

Analysis of a cDNA clone expressing a human autoimmune antigen: Full-length sequence of the U2 small nuclear RNA-associated B″ antigen

(autoimmunity/phage λgt11 expression library/recombinant DNA/rheumatic diseases)

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ABSTRACT **A U2 small nuclear RNA-associated protein, designated B″, was recently identified as the target antigen for autoimmune sera from certain patients with systemic lupus erythematosus and other rheumatic diseases. Such antibodies enabled us to isolate cDNA clone λHB″-1 from a phage λgt11 expression library. This clone appeared to code for the B″ protein as established by *in vitro* translation of hybrid-selected mRNA. The identity of clone λHB″-1 was further confirmed by partial peptide mapping and analysis of the reactivity of the recombinant antigen with monospecific and monoclonal antibodies. Analysis of the nucleotide sequence of the 1015-base-pair cDNA insert of clone λHB″-1 revealed a large open reading frame of 800 nucleotides containing the coding sequence for a polypeptide of 25,457 daltons. *In vitro* transcription of the λHB″-1 cDNA insert and subsequent translation resulted in a protein product with the molecular size of the B″ protein. These data demonstrate that clone λHB″-1 contains the complete coding sequence of this antigen. The deduced polypeptide sequence contains three very hydrophilic regions that might constitute RNA binding sites and/or antigenic determinants. These findings might have implications both for the understanding of the pathogenesis of rheumatic diseases as well as for the elucidation of the biological function of autoimmune antigens.**

Sera from certain patients with a connective tissue disease contain antibodies against a variety of small nuclear ribonucleoprotein particles (snRNPs; refs. 1 and 2). In particular, sera containing antibodies directed against proteins unique for U1 RNP [anti-(U1)RNP] and those against common determinants on U1, U2, U4, U5, and U6 snRNPs (anti-Sm) have been extensively used to study assembly, structure, and function of U snRNPs (reviewed in refs. 3 and 4).

Recently we described an autoantibody specificity, termed anti-(U1,U2)RNP, reactive with polypeptides present in U1 and U2 RNPs (5). These sera greatly facilitated investigations towards the fine structure of U2 RNP (6, 7). Data obtained from such immunological studies, together with the results of earlier nuclease-digestion experiments (8) revealed that U1 and U2 RNPs both contain a common protein core consisting of polypeptides B′/B, D, E, F, and G as well as several unique proteins; U1 RNPs have been described to contain additional polypeptides called "70K" (70-kDa), A, and C (9–14), whereas U2 RNPs are characterized by the presence of at least two unique polypeptides, A′ and B″ (5, 9, 10, 13, 15).

Both U1 and U2 RNP particles are involved in the splicing process of mRNA precursors. U1 RNP has been shown to bind to the 5′ splice site of precursor mRNAs *in vivo* and *in vitro* (16–21), whereas U2 RNP was shown to associate with the so-called branch point of the lariat-shaped intron, an intermediate in the cascade of splicing events (21). Whether snRNA-associated proteins play an active (enzymatic) role in this process or are merely essential for the structural integrity of the snRNP particles has not yet been established.

Recently, successful attempts have been described to partially characterize cDNA clones of autoimmune antigens or their associated proteins by making use of patients' sera (22, 23). Here we describe the isolation and characterization of a cDNA clone containing the complete coding sequence for the U2 RNP-specific B″ protein.

MATERIALS AND METHODS

Bacterial Strains and Phage λgt11 Expression Library. *Escherichia coli* strains Y1089 and Y1090 were purchased from Promega Biotec (Madison, WI). A phage λgt11 expression library (24) was constructed from human KG1 cells as described (25).

Antibody Screening. Antibody screening was performed by using the human anti-(U1,U2)RNP serum V26 (5) essentially as described by Huynh *et al.* (26).

For immunoblotting, bacterial lysates were prepared by quick-freezing isopropyl β-D-thiogalactoside-induced lysogenic bacteria carrying the recombinant phage (26). Protein lysates were fractionated on a $NaDodSO_4$/10% acrylamide gel and then electroblotted onto nitrocellulose. Immunoblots were probed with antisera as described (27).

Partial Peptide Mapping. Slices containing A or B″ antigens were excised from preparative $NaDodSO_4$/15% acrylamide gels, and proteins were recovered by electroelution (28). Cross-contamination of both samples was checked by immunoblotting with serum V26 and monoclonal anti-A and anti-B″ antibodies (29, 30). These samples and the recombinant antigen, affinity-purified by using anti-β-galactosidase columns (Promega Biotec), were transferred into sample wells of a $NaDodSO_4$/18% acrylamide gel together with various amounts of *Staphylococcus aureus* V8 protease. Digestion was allowed to proceed by shutting off the power for 30 min after the samples had concentrated in the stacking gel (31). After electrophoresis, the gel was blotted onto nitrocellulose and epitope-bearing peptides were detected with serum V26.

RESULTS

Isolation of Clone λHB″-1. A cDNA library in the phage λgt11 expression vector was screened by using the human

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Abbreviation: snRNP, small nuclear ribonucleoprotein particle.
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anti-(U1,U2)RNP serum V26, which contains antibodies directed against the U1 RNP-specific 70-kDa protein as well as two distinct antibody specificities directed against U2 RNP-specific proteins A′ and B″. The latter specificity crossreacts with the U1 RNP-specific A protein (5). Screening of about 600,000 plaques resulted in a positive recombinant carrying a 1.0-kilobase (kb) cDNA insert in the *Eco*RI cleavage site of the *lacZ* gene of the λgt11 vector. This clone will be referred to as clone λHB″-1 hereafter.

λHB″-1 was introduced as a lysogen in *E. coli* strain Y1089. Phage production was induced at 45°C, and isopropyl β-D-thiogalactoside was added to induce the production of fusion protein. Bacterial lysates were analyzed by immunoblotting for reactivity with murine anti-β-galactosidase antiserum and the anti-(U1,U2)RNP serum V26 used for the screening procedure. Both sera positively identified a fusion protein with an apparent molecular mass of 145 kDa (Fig. 1 *Left*), ≈30 kDa larger than β-galactosidase, next to some major degradation products (indicated by an asterisk).

Immunological Identification. To establish the identity of clone λHB″-1, nitrocellulose filters containing plaques with the expressed recombinant antigen were prepared. The filter shown in Fig. 1 *Right* was cut into 12 pieces and analyzed with 11 different human autoimmune sera and a normal human serum control. The four sera that reacted positive (P21, G18, B25, and V26) were previously identified as anti-(U1,U2)RNP sera, and all four were then shown to have only one specificity in common—namely, antibodies against the U2 RNA associated B″ antigen (5). None of the control sera containing anti-(U1)RNP, -Sm, -Ro, -La, Jo-1, -Scl-86, or -centromere antibodies showed any specific staining. It also should be noted that it was the serum with the highest anti-B″ titer on immunoblots (V26) that produced the strongest signal in this plaque-screening assay.

Additional evidence for the identity of cDNA clone λHB″-1 was obtained from experiments performed with monoclonal and monospecific antibodies. Therefore, the three autoantibody specificities contained in serum V26 (see above) were separated by affinity purification from stained bands on HeLa nuclear immunoblots as described (5, 34). After their monospecificity was confirmed (not shown), these antibodies were used as probes on immunoblots of bacterial lysates containing the β-galactosidase fusion protein. Only monospecific anti-B″ antibodies identified the recombinant antigen, regardless of whether they were purified from A or B″ polypeptide bands (Fig. 2, lanes 5 and 7). Anti-A antibodies as contained in anti-(U1)RNP sera showed no reaction with the fusion protein (Fig. 2, lane 2). When these experiments were repeated with monoclonal anti-A and monoclonal anti-B″ antibodies (15, 30), again, only anti-B″ antibodies reacted with the fusion protein (not shown). The reverse was also found to be true; i.e., when immunoblots of bacterial lysates were probed with serum V26 and antibodies were eluted from the recombinant antigen, they exclusively identified A and B″ antigens on HeLa nuclear blots (Fig. 2, lane 10). These antibodies were able to precipitate exclusively U1 and U2 RNPs from HeLa nuclear extracts (not shown).

In conclusion, the data described above indicate that only two possibilities for the identity of clone λHB″-1 have to be considered: (*i*) λHB″-1 contains coding capacity for part of the A antigen, including the epitope this antigen shares with the B″ antigen but excluding the epitope reactive with anti-(U1)RNP sera; and (*ii*) λHB″-1 contains genetic sequences encoding the B″ antigen. The experiments described below will show the latter possibility to be true.

Partial Peptide Mapping. In a first attempt to discriminate between these two possibilities, λHB″-1 was identified as a B″ clone by partial peptide mapping. Therefore, protein fractions containing A or B″ antigens were digested with various amounts of *S. aureus* V8 protease, and immunoreactive

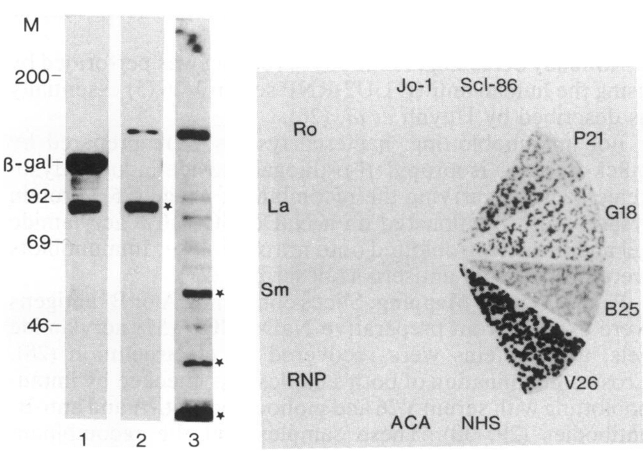

FIG. 1. Partial identification and characterization of clone λHB″-1. (*Left*) Immunoblots of proteins isolated from lysogenic bacteria carrying either wild-type phage λgt11 (BNN 97, lane 1), or the λHB″-1 recombinant (lanes 2 and 3) were probed with antibodies to β-galactosidase (lanes 1 and 2) or with serum V26 (lane 3). Asterisks indicate the major degradation products of the fusion protein. Other faint bands are considered to be nonspecific background, as they were occasionally observed when normal control sera were used. Lane M shows molecular mass markers in kDa run in an adjacent lane. Position of the β-galactosidase marker is indicated. (*Right*) Clone λHB″-1 expressed as λgt11 plaques on nitrocellulose filters was probed with 11 different sera from patients with connective tissue diseases and 1 normal serum control as indicated. Sera V26, B25, G18, and P21 are anti-(U1,U2)RNP sera and have been described elsewhere (5). The identity of the other sera was established on immunoblots containing HeLa nuclear and cytoplasmic proteins (33, 34).

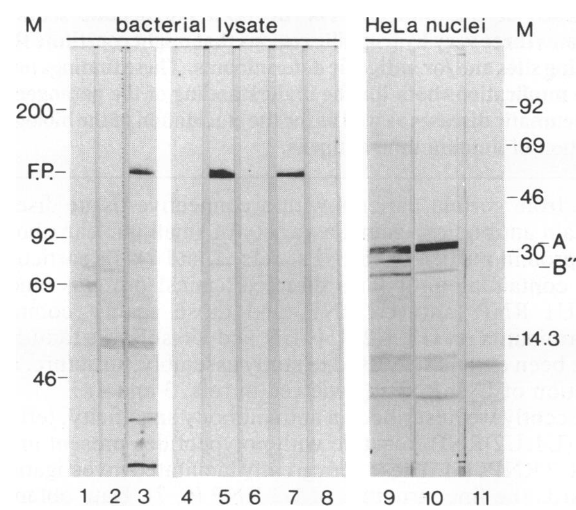

FIG. 2. Immunological identification of λHB″-1-encoded recombinant antigen. (*Left*) Immunoblot strips containing bacterial lysate from a λHB″-1 lysogen were probed with normal human serum (lane 1), an anti-(U1)RNP serum (lane 2), and with serum V26 (lane 3). Strips from the same blot were also incubated with monospecific antibodies affinity-purified from HeLa nuclear blots probed with serum V26: monospecific antibodies affinity-purified from the 70-kDa antigen (lane 4), from the A antigen (lane 5), from the A′ antigen (lane 6), from the B″ antigen (lane 7), and from a nonstaining region on a HeLa nuclear blot (lane 8). (*Right*) Hela nuclear blots incubated with serum V26 (lane 9) or with antibodies affinity-purified from serum V26, eluted either from the β-galactosidase fusion protein (lane 10) or from native β-galactosidase (lane 11). Lane M shows positions of molecular mass markers (kDa) run in an adjacent lane. Position of the fusion protein (F.P.) and A and B″ antigens are indicated.

peptides in their digestion patterns were compared with those obtained with immunopurified fusion protein.

Even without addition of V8 protease, some degradation products were found reproducibly in the fraction containing the A antigen, possibly as a result of some endogenous proteolytic activity (Fig. 3*A*). The B″ fraction did not show such a phenomenon, but here (Fig. 3*B*) a minor band just above B″ was present. This band was not always found and certainly is not the A antigen, since it showed no reaction when the same blot was reprobed with monoclonal anti-A antibodies (not shown). The digestion pattern of the fusion protein (Fig. 3*C*) remarkably resembled that of the B″ protein. In the lanes containing 1000 ng of V8 protease, three identical, epitope-containing peptides could be seen (indicated by arrows in Fig. 3) that were absent in the digestion patterns of the A antigen.

Hybrid Selection and *in Vitro* Translation. Two micrograms of the cDNA insert of clone λHB″-1, subcloned into a pUC18 vector, was immobilized on nitrocellulose and hybridized with 100-μg of oligo(dT)-selected poly(A)$^+$ RNA from HeLa cells (32, 35). Hybrid-selected mRNA was eluted and translated *in vitro* in an exogenous message-dependent rabbit reticulocyte lysate in the presence of [^{35}S]methionine. As a control, translations were performed with total poly(A)$^+$ RNA. Upon immunoprecipitation with V26 serum and fractionation on NaDodSO$_4$/13% acrylamide gels, the A and B″ polypeptides could easily be identified in the translation products of the lysate programmed with total poly(A)$^+$ RNA (Fig. 4, lane 4). Only the B″ band appeared in the immunoprecipitate of the lysate programmed with mRNA, hybrid-selected by clone λHB″-1 (Fig. 4, lane 6). This polypeptide comigrated with *in vivo* labeled B″ protein—i.e., in between polypeptides B′ and B (Fig. 4, lanes 5 and 8). From these data it may be concluded that the cDNA insert of clone λHB″-1 contains coding sequences for the U2 RNP-specific B″ protein.

Nucleotide Sequence of Clone λHB″-1 and Identification of λHB″-1-Specific mRNA. Each *Sau*3A fragment of the λHB″-1 cDNA insert (restriction sites indicated in Fig. 5) was subcloned into M13mp8 and M13mp10, and its nucleotide sequence (Fig. 5) was determined by the Sanger dideoxynucleotide chain termination method (36, 37).

Because clone λHB″-1 was isolated by virtue of its ability to produce a fusion protein, the reading frame of the cDNA insert must be in phase with that of the *lacZ* gene of the λgt11 vector. In good agreement with this consideration, clone

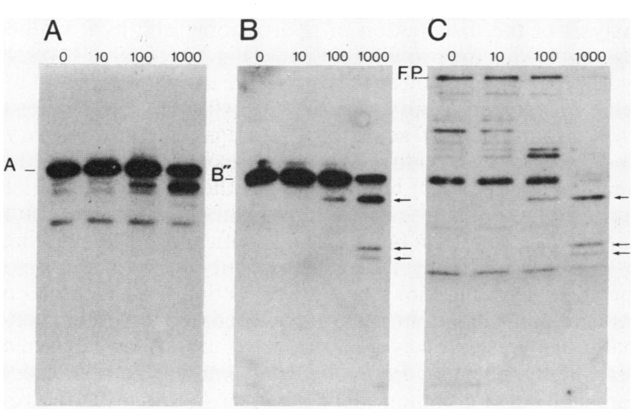

FIG. 3. Partial peptide mapping of λHB″-1-encoded recombinant antigen. Samples containing eukaryotic A (*A*) or B″ antigen (*B*) or the recombinant fusion protein (*C*) were digested with various amounts (in ng) of *S. aureus* V8 protease as indicated. After electrophoresis and blotting, immunoreactive peptides were visualized by immunodetection with serum V26. Arrows indicate identical peptides in B″ and fusion protein digests.

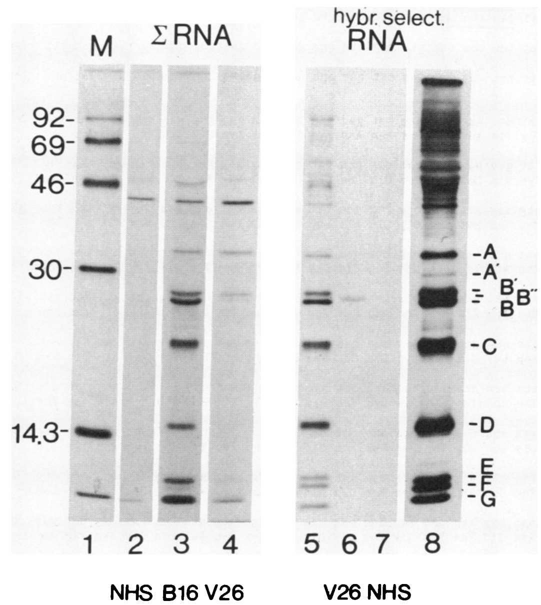

FIG. 4. *In vitro* translation of hybrid-selected mRNA. Lanes: 1, molecular mass markers (kDa); 2–4, NaDodSO$_4$/15% acrylamide gel electrophoresis of immunoprecipitates from a rabbit reticulocyte lysate programmed with total HeLa poly(A)$^+$ RNA (Σ RNA) in the presence of [^{35}S]methionine; 2, immunoprecipitate obtained with normal human serum (NHS); 3, same with an anti-Sm serum; 4, same with serum V26; 6 and 7, clone λHB″-1 hybrid-selected (hybr. select.) mRNA translated *in vitro* and immunoprecipitated with serum V26 (lane 6) or normal human serum (lane 7); 5 and 8, immunoprecipitate from an *in vivo* ^{35}S-labeled HeLa nuclear extract obtained with an anti-Sm serum. Lane 8 is a 6 times longer exposure of the same sample as shown in lane 5 to visualize the A′ antigen. Positions of the snRNA-associated polypeptides are indicated at the right of lane 8.

λHB″-1 was found to contain only one large open reading frame which starts immediately after the *Eco*RI cleavage site at the 5′ end of the insert and is terminated by a TAA codon at positions 801–803. Polyadenylylation signal AATAAA is present at positions 992–997 but the poly(A) tail was not present in this cDNA clone. The length of the B″ encoding mRNA was determined by blot-hybridization analysis. RNA isolated from HeLa and KG1 cells was glyoxylated and subjected to gel electrophoresis on a 1% agarose gel. After blotting and hybridization, the ^{32}P-labeled λHB″-1 insert identified a single transcript of 1.3 kb in both cell lines (not shown).

Deduced Complete Amino Acid Sequence of B″. Because clone λHB″-1 comprised about 90% of the B″ mRNA excluding the poly(A) tail, the possibility that it contains the complete coding sequence was investigated. Therefore, the *Eco*RI insert was recloned into a pSP65 vector and transcribed *in vitro*. The resulting RNA was purified by gel electrophoresis and subsequently was translated *in vitro* in an exogenous message-dependent rabbit reticulocyte lysate supplemented with [^{35}S]methionine. Three major products appeared in the lysate programmed with λHB″-1-encoded RNA (Fig. 6). These polypeptides were immunoprecipitable with serum V26 (Fig. 6, lane 4), indicating that they originate from the same reading frame that constitutes the B″ epitope(s). The presence of other polypeptides than the full-length translation product could be the result of *in vitro* proteolytic degradation or alternatively might represent initiation at different ATG codons. The latter explanation is the more favorable one, since no such degradation occurs when a reticulocyte lysate is programmed with genuine B″ mRNA (Fig. 3).

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λgt11-gaa ttc cgc gcc ttc tac ctc gct gtt tcg gtt ttc ctg gct cct cgg   41

ccc ttt tct ccc ctg ttg cag ctg gga gcg gac gaa gcg cga agc tgg gat ttt   95

tta ctg tct cct gaa gaa ttt aac aca aac atg gat atc aga cca aat cat aca  149
                                        Met Asp Ile Arg Pro Asn His Thr
                                                                Sau3A
att tat atc aac aat atg aat gac aaa att aaa aag gaa gaa ttg aag aga tcc  203
Ile Tyr Ile Asn Asn Met Asn Asp Lys Ile Lys Lys Glu Glu Leu Lys Arg Ser

cta tat gcc ctg ttt tct cag ttt ggt cat gtg gtg gac att gtg gct tta aag  257
Leu Tyr Ala Leu Phe Ser Gln Phe Gly His Val Val Asp Ile Val Ala Leu Lys

acc atg aag atg agg ggg cag gcc ttt gtc ata ttt aag gaa ctg ggc tca tcc  311
Thr Met Lys Met Arg Gly Gln Ala Phe Val Ile Phe Lys Glu Leu Gly Ser Ser

aca aat gcc ttg aga cag cta caa gga ttt cca ttt tat ggt aaa cca atg cga  365
Thr Asn Ala Leu Arg Gln Leu Gln Gly Phe Pro Phe Tyr Gly Lys Pro Met Arg

ata cag tat gca aaa aca gat tcg gat ata ata tca aaa atg cgt gga act ttt  419
Ile Gln Tyr Ala Lys Thr Asp Ser Asp Ile Ile Ser Lys Met Arg Gly Thr Phe

gct gac aaa gaa aag aaa aaa gaa aag aaa aaa gcc aaa act gtg gaa cag act  473
Ala Asp Lys Glu Lys Lys Lys Glu Lys Lys Lys Ala Lys Thr Val Glu Gln Thr

gca aca acc aca aac aaa aag cct ggc cag gga act cca aat tca gct aat acc  527
Ala Thr Thr Thr Asn Lys Lys Pro Gly Gln Gly Thr Pro Asn Ser Ala Asn Thr

caa gga aat tca aca cca aat cct cag gtc cct gat tac cct cca aac tat att  581
Gln Gly Asn Ser Thr Pro Asn Pro Gln Val Pro Asp Tyr Pro Pro Asn Tyr Ile

tta ttc ctt aat aac tta cca gaa gag act aat gag atg atg tta tcc atg ctg  635
Leu Phe Leu Asn Asn Leu Pro Glu Glu Thr Asn Glu Met Met Leu Ser Met Leu

ttt aat cag ttc cct ggc ttc aag gaa gta cgt ctg gta cca ggg agg cat gac  689
Phe Asn Gln Phe Pro Gly Phe Lys Glu Val Arg Leu Val Pro Gly Arg His Asp

att gct ttt gtt gaa ttt gaa aat gat ggg cag gct gga gct gcc agg gat gct  743
Ile Ala Phe Val Glu Phe Glu Asn Asp Gly Gln Ala Gly Ala Ala Arg Asp Ala
                 Sau3A                           Sau3A
tta cag gga ttt aag atc aca ccg tcc cat gct atg aag atc acc tat gcc aag  797
Leu Gln Gly Phe Lys Ile Thr Pro Ser His Ala Met Lys Ile Thr Tyr Ala Lys

aaa taa cat ttg gga tag tcg tct tta aaa gac ttg gtg tta ttt aca gtg ttt  851
Lys ***             ***

gtt ttg ata aca ttt ggc tgg gtc att tta ata gtt aga gat gag gag gag taa  905
                                                                    ***
aag tga aat ttt tgt gaa gga ctt aaa tta tcc agt gtt tct tta gcc ttg gtg  959
    ***
aac tat gaa ata cga agg cct taa ttt tgt aca ata aac ttt tat ttg tat tct 1013
                            ***
gtg gaa ttc-λgt11
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FIG. 5. Nucleic acid sequence of the cDNA insert of clone λHB″-1 and the predicted amino acid sequence of B″ protein. Numbers at the right refer to the last nucleotide of every line starting with the first nucleotide of cDNA clone λHB″-1 after the *Eco*RI linker sequences. *Sau*3A restriction sites are indicated. *** indicates a termination signal; the polyadenylylation signal is underlined.

Since the largest of these three products has the same electrophoretic mobility as *in vivo* labeled B″, it can be concluded that the cDNA of clone λHB″-1 harbors the complete coding sequence for the B″ protein. The isoelectric point of the deduced polypeptide (Fig. 5) was estimated (39) to be ≈8.0, a value in good agreement with the experimental data described earlier for the B″ protein (5). The deduced polypeptide contains several hydrophilic regions, and computer analysis revealed that three of these regions (around amino acids 20, 105, and 200) have a high probability of being exposed at the surface of the protein (40, 41) and, therefore, may represent RNA-binding sites or antigenic sites, or both.

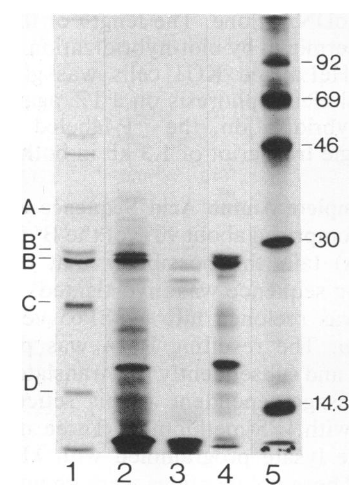

FIG. 6. *In vitro* translation of λHB″-1-encoded RNA. The cDNA insert of clone λHB″-1 was recloned into a pSP65 vector and transcribed *in vitro* (38). The resulting RNA was purified by gel electrophoresis and after electroelution was translated *in vitro* in a rabbit reticulocyte lysate. Lanes: 1, anti-Sm immunoprecipitate of an *in vivo* ^{35}S-labeled HeLa nuclear extract; 2, *in vitro* translation of λHB″-encoded RNA; 3, *in vitro* translation without λHB″-encoded RNA; 4, immunoprecipitate of translation products in lane 2 with IgG from anti-(U1,U2)RNP serum V26; 5, molecular mass markers (kDa).

DISCUSSION

Several lines of evidence support our conclusion that we have isolated a cDNA clone expressing the human U2 snRNA-associated B″ protein. Because this protein shares an epitope with the U1 RNP-specific A antigen, the immunological evidence left room for the alternative possibility that λHB″-1 encodes part of the A antigen. However, further characterization by hybrid selection and partial peptide mapping eliminated this alternative. Translation of RNA transcribed from a pSP65 vector carrying the λHB″-1 cDNA insert revealed that this insert contained the complete coding sequence for the B″ protein.

Characteristics of Clone λHB″-1 cDNA. The molecular mass of the native B″ polypeptide was originally estimated to be ≈28.5 kDa (5), but recalculation using the molecular mass markers shown in Fig. 2 revealed a molecular mass of 26.5 kDa. Considering this and the finding that λHB″-1 cDNA contains the complete coding sequence for B″ terminating at nucleotides 801–803, and given an average of 115 Da per amino acid, synthesis of the B″ protein theoretically may be expected to start somewhere around position 110. The first ATG codon at positions 126–128 is, therefore, the most likely candidate, use of which would result in a 25,457-Da protein. However, the *in vitro* translation of λHB″-1-encoded RNA (Fig. 6) also revealed the presence of an additional, slightly smaller polypeptide. If this is also a primary translation product, it possibly initiated at the ATG codon at positions 165–167. Both ATG codons are in the correct reading frame, but comparison with the consensus translation initiation sequence $^{A}_{G}$NNATGG (42) reveals that the first ATG codon has a higher probability to be used *in vivo*. Use of such a first ATG is commonly found for eukaryotic genes (43). Definite proof for the *in vivo* use of this initiation codon, however, has to await direct biochemical characterization of the NH_2-terminus of the cellular B″ protein.

Deduced Amino Acid Sequence of the B″ Protein. Computer analysis of the distribution of hydrophobic and hydrophilic segments along the protein sequence (Fig. 5) revealed several hydrophilic regions, three of which have a high probability of being recognized as an antigenic site (40, 41). One of these sites (around amino acid 105) contains an extraordinary amount of lysine residues and shows extensive (11 from 13 identical amino acids) homology with the charged region of the circumsporozoite protein of the malaria parasite *Plasmodium knowlesi* (44, §). The significance of this finding is presently difficult to evaluate, but it is tempting to speculate about a link between infection with a malaria parasite and the autoimmune phenomena observed in rheumatic diseases. However, a direct relation does not seem very likely because *P. knowlesi* is known as a monkey parasite, but a common mechanism of (auto)antibody production in both diseases seems a testable hypothesis. Remarkable in this respect is the observation that anti-nuclear antibodies of the anti-RNP and -Sm type are frequently found in patients with malaria (45, 46).

§Protein Identification Resource (1985) Protein Sequence Database (Natl. Biomed. Res. Found., Washington, DC), Release 8.0.

Only a very small number of anti-(U1,U2)RNP sera have been described (5, 13, 47). Therefore, it has not yet been possible to establish clearly whether presence of anti-B″ antibodies in sera from patients with autoimmune diseases is associated with a particular clinical syndrome. Preliminary data obtained from a routine immunoblotting screening performed in our laboratory indicate that anti-(U1,U2)RNP antibodies are not as rare as originally assumed, but that they occur in about 15% of autoimmune sera containing anti-(U1)RNP and/or anti-Sm antibodies (unpublished data). The main reason why this has not been recognized earlier probably lies within the fact that interpretation of the characteristic staining of A′ and B″ bands on immunoblots is rather difficult, especially when additional antibody specificities are present in the serum. Therefore, the recombinant fusion protein might be particularly useful as antigen in a specific ELISA to screen a large number of sera from patients with rheumatic diseases.

The mechanisms by which an autoimmune response is induced and maintained are not yet understood. Some theories presuppose an invading microorganism to account for the production of antibodies able to crossreact with host-specific antigens. Studies as the one presented here may address such questions and contribute to the understanding of the pathogenesis of rheumatic diseases.

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