Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 2115–2119, April 1985
Immunology

Isolation and analysis of cDNA clones expressing human lupus La antigen

(systemic lupus erythematosus/small nuclear ribonucleoprotein/ELISA/cDNA expression libraries/autoimmunity)

JASEMINE CHOY CHAMBERS AND JACK D. KEENE

Department of Microbiology and Immunology, Duke University Medical Center, Durham, NC 27710

Communicated by Wolfgang K. Joklik, October 9, 1984

ABSTRACT **Several cDNA clones of the La antigen recognized by certain lupus autoantibodies were isolated from λgt11 expression libraries made from human liver. Recombinant clones were used to hybrid-select HeLa cell mRNA that was subsequently translated *in vitro* into a single protein species that comigrated with HeLa cell La protein. The *in vitro* translated protein was reactive with anti-La patient sera and was identical to the authentic La protein by peptide mapping. By analyzing overlapping cDNA clones, we mapped an antigenic site of La protein at the terminal 12% of the carboxyl end of the molecule. Within this region we identified a unique decapeptide of high hydrophilicity that may constitute a La antigenic determinant. We further demonstrated that the La antigen expressed from the recombinant clones can be used in a definitive enzyme-linked assay (ELISA) for the classification of sera from patients with systemic lupus erythematosus.**

Sera from patients with humoral autoimmune diseases often contain antibodies that react with various normal cellular components as if these antigens were foreign. In trying to identify the antigenic proteins that a given patient contains, one often finds more than a single antigenic specificity. Current clinical assays of rheumatological autoimmune diseases, such as systemic lupus erythematosus (SLE) and Sjogren's syndrome, allow only approximate assignment of these antigenic specificities against standard "prototype" antisera such as the La, Ro, Sm, and RNP (ribonucleoprotein) autoantibodies (1). To select autoantibodies of unique antigenic specificity, we screened sera from hundreds of autoimmune patients by identifying the proteins and nucleic acids that are precipitable in the presence of *Staphylococcus aureus* protein A (2, 3). We identified patient sera unique to the lupus La specificity and have used these autoantibodies to identify recombinant cDNA clones made from human liver that express the La protein.

The mammalian cell La protein was found to be associated with precursor forms of RNA polymerase III transcripts including tRNA and 4.5S, 5S, 7S, and 7-2 RNAs (3–6). Some small viral transcripts, such as VAI RNA of adenovirus (7, 8), EBER RNAs of Epstein–Barr virus (9), and the leader RNAs of vesicular stomatitis virus (2, 10) and rabies virus (11), also were shown to be complexed with the La protein. By immunoprecipitation of complexes using La antibodies, the site of La protein binding to several of these RNA species was shown to reside near the 3′ end (4, 8, 12). The presence of uridylate residues at the 3′ ends of these RNAs may be required for binding. Furthermore, the addition of extra 3′ terminal uridylate residues was found to enhance the binding of La protein to VAI RNA (13) and to tRNA (14). Because La protein preferentially binds to unprocessed transcripts, Steitz and co-workers (4, 5) have proposed that La protein is a transcription factor for RNA polymerase III. Despite uncertainty regarding its exact function in RNA metabolism, it is clear that the La protein is biologically important in the regulation of gene expression because it associates with a variety of cellular and viral RNAs, many of which have known functions.

To further characterize the biochemical functions of the La protein, attempts have been made to purify it from mammalian cells (14). Recoveries from these purification procedures have been <7%, and the La protein showed biochemical heterogeneity apparently due to interactions with RNA components. To produce large amounts of highly purified La protein, we derived recombinant cDNA clones that code for La protein. We report the identification of La cDNA clones from λgt11 expression libraries (15), using sera from selected lupus patients as antibody probes. Thus, the expressed cDNA clones are appropriate for the production of purified antigens, or the antigenic peptides may be directly synthesized from the amino acid sequences for use in biochemical and diagnostic procedures.

MATERIALS AND METHODS

Enzymes. Enzymes were purchased from Bethesda Research Laboratories and New England Biolabs. Rabbit reticulocyte lysate system for *in vitro* translation was from New England Nuclear.

Antisera. Antisera were obtained from the Duke University Medical Center Fluorescent Antinuclear Antibody Laboratory.

Cells and Immunoprecipitation. HeLa cells were labeled in methionine-free medium for 5 hr with [^{35}S]methionine (New England Nuclear) at 0.2–0.5 mCi/ml (1 Ci = 37 GBq) for antibody precipitations. Protein extracts were prepared, and immunoprecipitations were carried out as described (8) with Pansorbin (Calbiochem) as a source of protein A. Immunoprecipitated proteins were analyzed by $NaDodSO_4$/15% polyacrylamide gel electrophoresis (16), followed by fluorography.

Bacterial Strains and λgt11 Expression Libraries. *Escherichia coli* strains Y1088 and Y1090 were obtained from D. Stafford (University of North Carolina at Chapel Hill). Human liver cDNA libraries constructed with λgt11 were provided by D. Stafford and P. Modrich (Duke University Medical Center) and by R. A. Lazzarini (National Institutes of Health).

Screening of Expression Libraries with Antibody Probes. As described by Young and Davis (15), λgt11 recombinant phages were plated on *E. coli* Y1090 at 10^5 plaque-forming units (pfu) per 576-cm^2 plate (Nunc Inter Med). Expression of β-galactosidase fusion proteins was induced by overlaying

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Abbreviations: RNP, ribonucleoprotein; SLE, systemic lupus erythematosus; pfu, plaque-forming units; iPrSGal, isopropyl thiogalactopyranoside.

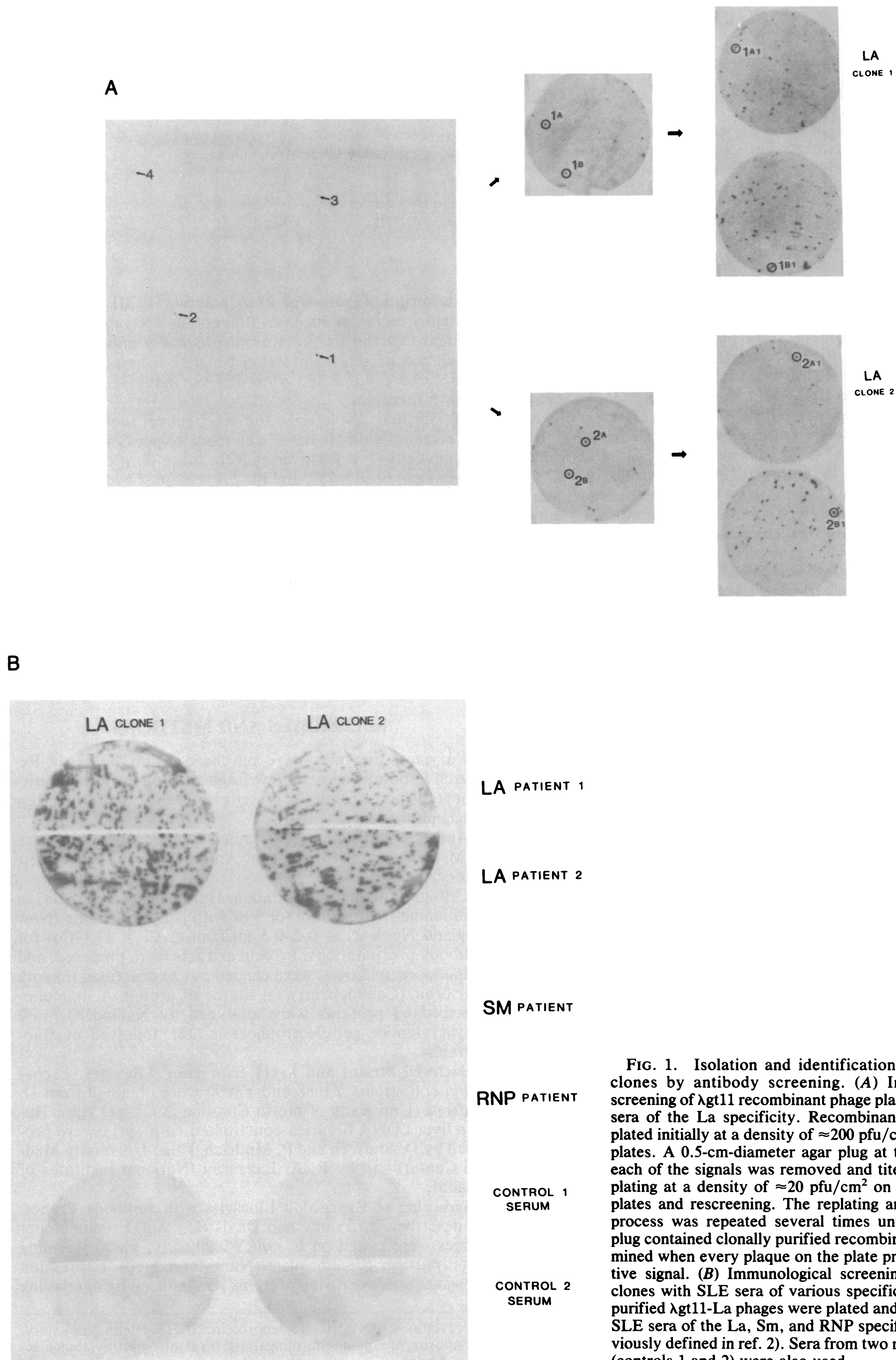

FIG. 1. Isolation and identification of La cDNA clones by antibody screening. (*A*) Immunological screening of λgt11 recombinant phage plaques with SLE sera of the La specificity. Recombinant phages were plated initially at a density of ≈200 pfu/cm^2 on 576-cm^2 plates. A 0.5-cm-diameter agar plug at the position of each of the signals was removed and titered before replating at a density of ≈20 pfu/cm^2 on 9-cm-diameter plates and rescreening. The replating and rescreening process was repeated several times until each phage plug contained clonally purified recombinants, as determined when every plaque on the plate produced a positive signal. (*B*) Immunological screening of λgt11-La clones with SLE sera of various specificities. Clonally purified λgt11-La phages were plated and screened with SLE sera of the La, Sm, and RNP specificities (as previously defined in ref. 2). Sera from two normal humans (controls 1 and 2) were also used.

with nitrocellulose filters (Schleicher & Schuell) that were saturated with 10 mM isopropyl thiogalactopyranoside (iPrSGal) (Boehringer Mannheim). Sera from SLE patients were screened by immunoprecipitation of [^{35}S]methionine and tritiated amino acid-labeled HeLa cell extracts to confirm the unique specificity of the autoantibodies. The La antisera were determined to be free of Ro, Sm, RNP, and other SLE autoantibodies prior to use in screening expression libraries. After transfer of induced proteins to nitrocellulose, the filters were blocked with fetal calf serum, screened with 1:100 dilutions of SLE antisera, and probed with ^{125}I-labeled protein A (ICN).

For immunoblotting, bacterial lysates were prepared from plate cultures infected with either λgt11 or clone La-6 and induced by iPrSGal. Proteins from lysates were concentrated by precipitation with ammonium sulfate (final concentration, 80%) and fractionated on a NaDodSO$_4$/6% acrylamide gel. The proteins were then electroblotted onto nitrocellulose (17) and probed with antibodies to either β-galactosidase or La protein, followed by ^{125}I-labeled protein A.

Hybrid Selection and *in Vitro* Translation. The *Eco*RI-cut DNA inserts, recloned into pBR322, were immobilized on nitrocellulose filters and used for hybrid selection. Total HeLa cell cytoplasmic RNA was used in the hybrid selections as described by Maniatis *et al.* (18). Hybrid-selected mRNAs were translated *in vitro* by using rabbit reticulocyte lysates in the presence of [^{35}S]methionine, and the products were analyzed by NaDodSO$_4$/15% polyacrylamide gel electrophoresis. Gel slices identified by autoradiography and containing the La protein were transferred to sample wells of a second gel. Various amounts of *Staphylococcus aureus* V8 protease (Sigma) were added to each well, and digestion was allowed to proceed in the stacking gel by the method of Cleveland *et al.* (19). The peptides generated by limited proteolysis were fractionated in the same gel and identified by fluorography.

DNA Sequencing. *Eco*RI inserts isolated from λgt11-La clones were fragmented by using restriction enzymes and subcloned into M13 mp18 and mp19 vectors. Dideoxy DNA sequencing (20) was carried out by using ^{35}S-labeled adenosine 5′-[γ-thio]triphosphate (Amersham) and buffer gradient gels (21).

ELISA. Sheets of nitrocellulose soaked in 10 mM iPrSGal were coated with La antigen by induction of confluent phage plaques containing the clonally purified La cDNA. For more sensitive assays, the partially purified β-galactosidase–La fusion protein was used to coat nitrocellulose sheets. Antigen-coated nitrocellulose sheets were treated with a bovine serum albumin-containing blocking solution (Kirkegaard and Perry, Gaithersburg, MD), and dilutions of human sera were placed in contact with the antigen for 1 hr. The sera were removed, and the filters were washed thoroughly with buffer containing 0.02 M imidazole-buffered saline and 0.02% Tween 20 (Kirkegaard and Perry). The nitrocellulose sheets were washed for 10 min each in the Tween 20 buffer and twice in phosphate-buffered saline. The antibody-treated sheets were incubated with lactoperoxidase-conjugated protein A at a concentration of 1 μg/ml for 30 min. After three cycles of washing, filters were treated with 4-chloro-1-naphthol in a peroxidase substrate system (Kirkegaard and Perry) for 10 min.

RESULTS

Isolation and Identification of La cDNA Clones. We have screened human cDNA libraries constructed with the phage expression vector, λgt11, using anti-La sera from SLE patients as antibody probes. Initial plaque screening of 500,000 recombinants identified 20 putative La clones. Upon several rounds of purification and rescreening with various La-specific autoantibodies, we isolated and confirmed three positive La clones (Fig. 1*A*). Demonstration of their clonal purity was evident because every plaque on a plate was reactive with La antisera from several different patients. When two of the La clones were expressed as λgt11 plaques and were analyzed for reactivity with normal human sera and with a panel of sera from patients with SLE that did not contain La-specific autoantibodies [as assayed by immunoprecipitation of RNA and protein, cell fluorescence, and counterimmunoelectrophoresis], the data of Fig. 1*B* were obtained. It is clear that clones that expressed La antigen were only reactive with sera from SLE patients of the La specificity. These clonally expressed La antigens were further identified by ELISA. Results of a typical dot ELISA with one of the La antigen clones are shown in Fig. 2. Sera from 18 SLE patients were analyzed for reactivity with the expressed La antigen. Sera from three SLE-La patients showed a clear positive response, while patients with other lupus specificities (Sm, Ro, RNP, To, and unclassified reactivities) and normals showed only a low background of reactivity. In some cases, sera of unknown specificity by cell fluorescence and counterimmunoelectrophoresis were found by the ELISA to be weakly positive for La antibodies. Subsequent analysis by RNA and protein precipitations confirmed the presence of La antibodies in these samples (data not shown).

Data from DNA·DNA hybridization (not shown) and restriction enzyme analysis indicated that we had isolated overlapping La clones, all of which contained the carboxyl-terminal portion of the coding sequence. When these clones were tested for inducibility of antigen production by iPrSGal, only two-thirds of them were inducible (data not shown). Upon DNA sequencing of the junctions of the *lacZ*

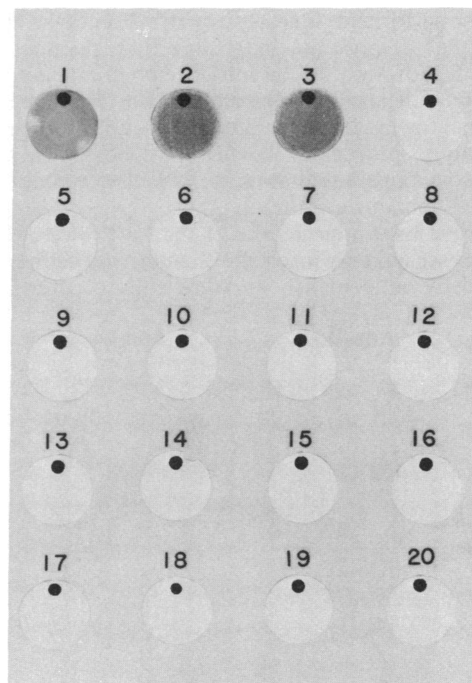

FIG. 2. ELISA using expressed La antigen bound to nitrocellulose and treated with sera from 18 patients with autoimmune diseases and 2 normal sera (19 and 20). Filters were prepared and processed as described and were analyzed by lactoperoxidase-linked *S. aureus* protein A. Wells and specificity: 1 and 2, La; 3, La/Ro; 4, Ro; 5, Sm; 6, RNP; 7, Sm/RNP; 8, Sm/RNP; 9, unknown SLE; 10, To; 11, RNP; 12, RNP + unknown; 13, Sm/RNP; 14, RNP; 15, unknown SLE; 16, Sm/RNP; 17, unknown SLE (La); 18, unknown SLE; 19, normal; 20, normal.

gene insertion, the noninducible clones were found to be in the opposite orientation in the λgt11 genome as the inducible ones. It is likely that a late phage λ promoter in the vector, whose direction of transcription is opposite to that of β-galactosidase, was utilized in the noninducible La clones (22). One of the inducible clones was then examined for production of β-galactosidase–La fusion protein. Fig. 3 shows an immunoblot of the proteins isolated from the clone La-6-infected bacteria. An insert of 390 base pairs produced a fusion protein of 129,000 daltons (about 13,000 daltons larger than β-galactosidase) that was reactive with antibodies to both La protein and β-galactosidase.

Protein Identity by Hybrid Selection and *in Vitro* Translation. To confirm the identity of the cDNA clones, we used the techniques of hybrid selection and *in vitro* translation. DNA inserts from the λgt11-La clones, subcloned into pBR322, were used for hybrid selection of mRNA from total HeLa cytoplasmic RNA. The selected filter-bound mRNA was eluted and translated *in vitro* by using rabbit reticulocyte lysates. Fig. 4 shows a fluorograph of the [^{35}S]methionine-labeled products synthesized *in vitro* and fractionated by $NaDodSO_4$/polyacrylamide gel electrophoresis. A single protein of ≈50,000 daltons was the only protein species unique to the La cDNA-selected mRNA (Fig. 4 *Left*, lanes 2 and 3; Fig. 4 *Right*, lane 2). This protein comigrated with the *in vivo* labeled La protein immunoprecipitated by anti-La antiserum (Fig. 4 *Left* and *Right*, lane 1). The *in vitro* synthesized species was found to be reactive with the same antiserum (Fig. 4 *Right*, lane 3). To further demonstrate that the *in vitro* translated protein was indeed the authentic cellular La protein, we performed hybrid selection and translation and fractionated the products on preparative gels. Wet preparative gels were autoradiographed, and the gel slices containing both *in vitro* and *in vivo* labeled La protein were transferred to sample wells of a second gel. Using the method of Cleveland *et al.* (19), we performed limited proteolysis on these samples with *S. aureus* V8 protease and analyzed the peptides generated. Fig. 5 shows a fluorograph of peptide mapping of the La protein synthesized *in vitro* and *in vivo*. These data demonstrate that the *in vitro* translated product has the same partial proteolytic profile as the *in vivo* labeled La protein. Therefore, we conclude that the cDNA clones code for and express the human La protein.

Mapping of an Antigenic Site of the La Protein. Because λgt11 expresses cDNA inserts in the correct reading frame, as defined by antigenicity, we were able to determine the amino acid sequence from the DNA sequence starting immediately after the 5′ *Eco*RI cleavage site. The cDNA insert of the fusion protein-producing clone, La-6 (Fig. 3), was 390 nucleotides long, which included 366 bases coding for the carboxyl-terminal 122 amino acids of La protein in addition to 24 nucleotides of noncoding information. The coding sequence of another cDNA insert (La-8) was found to include only the carboxyl-terminal 55 amino acids and a 1200-base-pair-long untranslated region. Because both cDNA clones were reactive with La antibodies, we conclude that at least one antigenic site reactive with La antisera resides in this 55-amino acid overlap region (terminal 12% of the protein) (Fig. 6). This sequence has a high content of hydrophobic amino

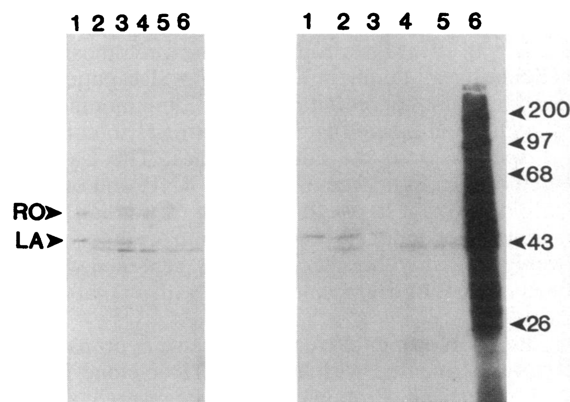

FIG. 4. *In vitro* translation of hybrid-selected HeLa cell mRNA with cDNA for La protein. Hybrid-selected HeLa mRNAs were translated *in vitro* by using rabbit reticulocyte lysates, and the products were analyzed on $NaDodSO_4$/15% acrylamide gels. (*Left*) Lanes: 1, *in vivo* ^{35}S-labeled HeLa cell proteins immunoprecipitated with SLE sera of the La and Ro specificities; 2, *in vitro* translated products from HeLa mRNA hybrid-selected with the La cDNA insert; 3, *in vitro* translated products from HeLa mRNA hybrid-selected with La cDNA plasmid; 4 and 5, *in vitro* translated products from HeLa mRNA hybrid-selected with the plasmid pBR322; 6, *in vitro* translated products with 10 μg of carrier tRNA. (*Right*) Lanes: 1, *in vivo* ^{35}S-labeled HeLa cell proteins immunoprecipitated with SLE sera of the La specificity; 2, same as lane 3 in *Left*; 3, translated material from lane 2 immunoprecipitated with anti-La antiserum; 4, same as lane 4 in *Left*; 5, same as lane 6 in *Left*; 6, *in vitro* translated products with 0.1 μg of total HeLa cell RNA.

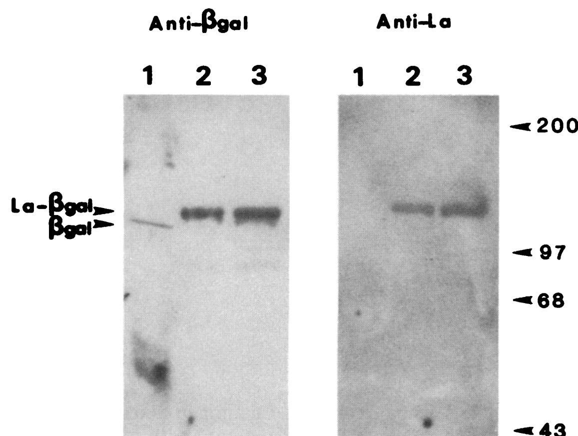

FIG. 3. Characterization of β-galactosidase–La fusion protein. Immunoblots of proteins isolated from phage-infected bacteria were probed with antibodies to either β-galactosidase (*Left*) or La protein (*Right*) as described. Lanes: 1, total lysate from λgt11-infected cells; 2 and 3, 30 μl and 50 μl of lysate from clone La-6-infected cells, respectively.

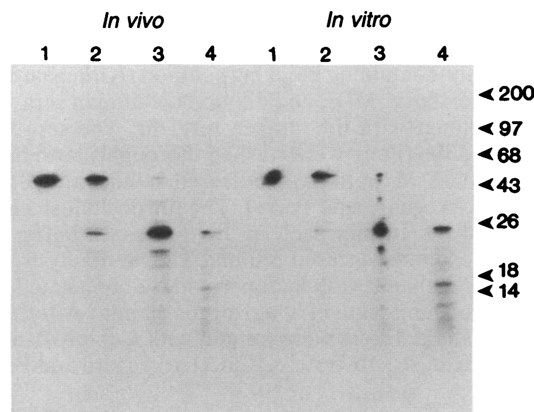

FIG. 5. Partial peptide mapping of the La protein. *In vivo* ^{35}S-labeled HeLa cell La protein and the comigrating protein band synthesized *in vitro* with rabbit reticulocyte lysates and La cDNA hybrid-selected mRNA were gel purified, digested with various amounts of *S. aureus* V8 protease, and analyzed on a $NaDodSO_4$/15% acrylamide gel. Samples were incubated for 30 min in the stacking gel with lanes 1–4 representing 0, 10, 100, and 1000 ng of protease, respectively.

GGC	TGG	GTA	CCT	TTG	GAG	ATA	ATG	ATA	AAA	30
gly	trp	val	pro	leu	glu	ile	met	ile	lys	
TTC	AAC	AGG	TTG	AAC	CGT	CTA	ACA	ACA	GAC	60
phe	asn	arg	leu	asn	arg	leu	thr	thr	asp	
TTT	AAT	GTA	ATT	GTG	GAA	GCA	TTG	AGC	AAA	90
phe	asn	val	ile	val	glu	ala	leu	ser	lys	
TCC	AAG	GCA	GAA	CTC	ATG	GAA	ATC	AGT	GAA	120
ser	lys	ala	glu	leu	met	glu	ile	ser	glu	
GAT	AAA	ACT	AAA	ATC	AGA	AGG	TCT	CCA	AGC	150
asp	lys	thr	lys	ile	arg	arg	ser	pro	ser	
AAA	CCC	CTA	CTG	AAG	TGA	168				
lys	pro	leu	leu	lys	TERM					

FIG. 6. Nucleic acid and amino acid sequences of the carboxyl-terminal 12% of the human La protein. At least one antigenic site resides in this 55-amino acid region. A strongly hydrophilic decapeptide (blocked sequence) is a predicted antigenic determinant.

acids. Hydrophilicity analysis by the method of Hopp and Woods (23) identified a decapeptide from amino acid 40 to amino acid 49 with a value of +1.61 (blocked region of Fig. 6). The average hydrophilicity of the 55-amino acid antigenic portion was calculated to be +0.3. Based on this method, we predict that this decapeptide region has a high probability of being exposed on the surface of the protein and may overlap with an antigenic determinant for La protein.

DISCUSSION

We have demonstrated that autoantibodies from patients with SLE, when properly assessed for antibody specificity, will react with and identify the corresponding human antigens expressed in cDNA clones from λgt11 expression libraries. The interaction between the La-specific autoantibodies and the phage-produced antigen reported here is highly specific, since SLE sera with other antigenic specificities did not react with the λgt11-La clones (Fig. 1B). Furthermore, the genetically pure and abundant antigen expressed by these recombinant phage clones was used to detect La antibodies in sera from a panel of SLE patients by ELISAs (Fig. 2).

The availability of large amounts of lupus antigens will allow studies into the origins of autoimmunity in rheumatological diseases and the elucidation of the biochemical functions of these proteins in gene expression. La protein is bound to a variety of transcripts made by RNA polymerase III and has been suggested to function in transcription, processing, or transport of these RNA species.

The processes by which the lupus proteins or other cross-reacting materials are presented as antigens in the generation of an autoimmune response are not understood. In this report we have delimited an antigenic site by the analysis and expression of overlapping cDNA clones. Further, we have predicted the precise location of a La antigenic determinant by identifying a strongly hydrophilic decapeptide (Glu-Asp-Lys-Thr-Lys-Ile-Arg-Arg-Ser-Pro) within this delimited region of the molecule. The antigenic nature of this region can be confirmed by testing the immunogenic properties of a synthetic decapeptide.

We thank D. Stafford, P. Modrich, and R. A. Lazzarini for providing λgt11 expression libraries and M. B. Mathews and P. Reichel for advice with hybrid selection. We thank our close associates, M. Kurilla, J. Wilusz, and S. Chambers, for helpful suggestions during the course of this work. This work was supported by grants from the National Institutes of Health. J.C.C. is the recipient of U.S. Public Health Service Viral Oncology Postdoctoral Training Grant T32 CA 09 111, and J.D.K. is the recipient of a Faculty Research Award from the American Cancer Society.

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