

# Molecular Definition and Sequence Motifs of the 52-kD Component of Human SS-A/Ro Autoantigen

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## Abstract

Serum SS-A/Ro autoantibodies are commonly found in patients with Sjogren's syndrome, systemic lupus erythematosus, neonatal lupus, and subacute cutaneous lupus. Two proteins of 60 and 52 kD have been described as targets for these autoantibodies. To define the 52-kD component unambiguously, cDNA clones were isolated from human HepG2 and MOLT-4 cell cDNA libraries. The identity of cDNA was established by (a) the specificity of the antibody affinity purified from the recombinant protein, (b) the reactivity of the purified recombinant protein with prototype SS-A/Ro sera in immunoblot and ELISA, and (c) two-dimensional gel comigration of MOLT-4 cell 52-kD protein and the recombinant protein. A 1.9-kb cDNA encoded the complete 52-kD protein containing 475 amino acids (M, 54,082). Putative zinc-finger domains and a leucine zipper motif were identified in the amino-terminal half of the 52-kD protein, implicating its possible association with DNA/RNA. Sequence homology detected between the 52-kD protein and human *ret* transforming protein, and mouse T cell gene expression down-regulatory protein *rpt-1*, may provide leads to the functional role of the 52-kD protein in addition to the possibility that these proteins might constitute members of a subfamily of finger proteins. (*J. Clin. Invest.* 1991, 87:68–76.) **Key words:** autoantibody • Sjogren's syndrome • zinc finger protein • systemic lupus erythematosus • autoimmunity

## Introduction

The molecular characteristics of SS-A/Ro<sup>1</sup> antigens are of special clinical interest because of the relatively high frequency of

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The original nucleotide and amino acid sequence data have been submitted to EMBL/Genbank under accession No. M35041.

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1. *Abbreviations used in this paper:* CD<sup>4</sup>, cluster designation 4; IPTG, isopropyl- $\beta$ -thiogalactopyranoside; PBS-T, phosphate buffered saline with 0.05% Tween-20; SS-A/Ro, nuclear antigen A of Sjogren's Syndrome; SS-B/La, nuclear antigen B of Sjogren's syndrome.

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the SS-A/Ro autoantibody in patients with several rheumatic diseases including systemic lupus erythematosus, Sjogren's syndrome, neonatal lupus syndrome with congenital heart block, and subacute cutaneous lupus (for current reviews, see references 1–3). Various molecular species ranging from 50 to 150 kD have been described for the antigenic component(s) of SS-A/Ro (summarized in reference 4). In 1984 Wolin and Steitz (5) showed that autoantibodies to SS-A/Ro immunoprecipitated small ribonucleoprotein particles composed of hY-RNAs and a 60-kD protein component. The 60-kD protein was defined as the major autoimmune target because SS-A/Ro autoimmune sera did not recognize hY-RNAs alone. Recent work of Boire and Craft (6) reported a subpopulation of autoantibodies recognizing an antigenic epitope restricted to the intact ribonucleoprotein particle composed of hY5 RNA and the 60-kD SS-A/Ro protein. Besides the 60-kD protein, Ben-Chetrit et al. (4) found that most SS-A/Ro-positive sera as defined by immunoprecipitation in Ouchterlony double diffusion assay also recognized another protein of 52 kD. It was not clear whether the 52-kD protein interacted with hY-RNAs directly or via the association with the 60-kD protein that was known to have in vitro RNA-binding activity (5, 7). Indirect immunofluorescence using these specific anti-52-kD reagents gave nuclear punctate staining similar to those observed for antibodies specific to the 60-kD SS-A/Ro component (4). It was also shown by antibody affinity purification that patient sera contained distinct, noncross-reacting antibodies directed to the 60- and 52-kD components (4). Autoantibodies to the 52-kD component were in fact very common in SS-A/Ro autoimmune sera analyzed (> 80%) and the reason the 52-kD component had not been detected earlier was probably a result of coexisting autoantibodies to SS-B/La and to the incomplete separation of the 47-kD SS-B/La antigen from the 52-kD component in the usual gel separation system (8). These two SS-A/Ro components were found in all human cell lines tested including HeLa, MOLT-4, Raji, and Wil-2 (4). Rader et al. (9) also described two human SS-A/Ro species of 60 and 52 kD from lymphocytes and, in addition, two red cell-specific SS-A/Ro components of 60 and 54 kD that were immunologically related to the lymphocyte 60- and 52-kD species, respectively. To define the 52-kD component of SS-A/Ro protein unambiguously, we have cloned the full-length cDNA encoding the 52-kD protein. This report describes the isolation and characterization of cDNA clones using a human autoimmune serum with specific autoantibody to the 52-kD SS-A/Ro protein and the special features of the deduced polypeptide that might provide insights into the functional role of the protein.

## Methods

*Cell extracts.* MOLT-4 cells (human T cell lymphoblastic leukemia, ATCC CRL 1582) were cultured in DMEM containing 10% calf serum

at 37°C in an 8% CO<sub>2</sub> incubator. Cultures were supplemented with 2.5 µg/ml gentamicin sulfate and maintained at 10<sup>6</sup> cells per ml. Cells were harvested and extracted in buffer A (150 mM NaCl, 10 mM Tris-HCl, pH 7.2, 0.5% Nonidet P-40) and freed of cell nuclei by centrifugation at 12,000 g for 15 min.

**Immunoblotting.** For high-resolution separation of the 60-kD, 52-kD, and 47-kD SS-A/Ro-SS-B/La proteins, cell extracts were separated by PAGE as described by Laemmli (10) using 15% gel slabs (20 × 13 × 0.1 cm) and the ratio of acrylamide:bis-acrylamide was changed to 172.4:1 in the separating gel (8). It was shown recently that the standard gel composition used by many laboratories was not optimal for the separation of the 52-kD SS-A/Ro and 47-kD SS-B/La proteins (8). Proteins were transferred from gel onto nitrocellulose using a constant voltage of 60 V for 2 h (11). The nitrocellulose sheet was air-dried, cut into strips, and incubated for 30 min in 3% nonfat milk diluted in PBS to block nonspecific binding sites. The nitrocellulose strips were incubated with 1 ml of a 1:100 dilution of sera for 1 h and then washed in PBS with 0.05% Tween 20 (PBS-T). <sup>125</sup>I-protein A (ICN Biochemicals, Irvine, CA) was used to detect bound human antibodies. The following protein standards (Bio-Rad Laboratories, Richmond, CA) were used to determine molecular weights: phosphorylase B, 92,500; BSA, 66,200; ovalbumin, 45,000; carbonic anhydrase, 31,000; soybean trypsin inhibitor, 21,500; and lysozyme, 14,400.

**Screening of phage libraries for SS-A/Ro cDNA.** A HepG2 cell λZap cDNA library was a kind gift from Dr. Frank R. Jirik, University of British Columbia, Vancouver, BC, Canada. A high titer SS-A/Ro human serum Bo was used for immunoscreening of 10<sup>6</sup> recombinants. <sup>125</sup>I-protein A was used to detect bound human antibodies. One positive clone Cl was identified after multiple screenings and was subcloned in vivo into pBluescript plasmid pCl using R408 helper phage (Stratagene, La Jolla, CA) as recommended in the manufacturers' instructions.

Human T cell lymphoblastoma (MOLT-4) λgt-II cDNA library was constructed by Dr. K. Ogata and Dr. D. J. Noonan, Scripps Clinic and Research Foundation. The MOLT-4 library was screened for full-length SS-A/Ro cDNA clones by DNA hybridization. Two partially complementary synthetic oligonucleotides (5'-TGTGCAGTGCATGGAGAGAGACTTCACCTG-3' and 5'-TCTTTCTCACAGAACAGGTGAAGTCTCTCT-3') were designed based on the 5' sequence of pCl cDNA insert. They were mixed and labeled with [<sup>α</sup><sup>32</sup>P]-ATP using the standard fill-in reaction of Klenow polymerase (12). All screenings were carried out with duplicate filters and positive phages were plaque purified. The resulting cDNAs were subcloned into pBluescript vectors (Stratagene) for further analysis.

**Cloning of rfp protein.** Two partially complementary synthetic oligonucleotides (5'-TGGCTCCGGAGTGTGGCCGAGTGCC-3' and 5'-GGTGGTCTCCTGCCTGCAGGACTCGGCC-3') were designed based on the 5' sequence of the rfp cDNA reported (13). They were mixed and labeled with [<sup>α</sup><sup>32</sup>P]-ATP using the standard fill-in reaction of Klenow polymerase (12) and used to screen 10<sup>6</sup> recombinant from the MOLT-4 cell cDNA library as described above. Two initial positives were detected and one of these, 2rl, was purified and the cDNA insert subcloned to pBluescript SK plasmid for further analysis. A rabbit anti-rfp reference serum was kindly provided by Dr. M. Takahashi, Aichi Cancer Center Research Institute, Nagoya, Japan.

**Affinity-purified antibodies.** λZap clone Cl phage plaques were induced to produce recombinant protein by overlaying with isopropyl-β-thiogalactopyranoside (IPTG, Sigma Chemical Co., St. Louis, MO) saturated nitrocellulose filters and grown overnight at 37°C. These nitrocellulose filters were washed with PBS-T, incubated with diluted serum for 1 h, washed again with PBS-T before elution of bound antibodies with 0.1% BSA in 0.1 M phosphate buffer, pH 2.2. Affinity-purified antibodies were immediately neutralized by the addition of 1 M Tris-HCl, pH 8.8. The antibodies were concentrated with Centricon 100 microconcentrators (Amicon Corp., Danvers, MA).

**Purification of recombinant protein.** Plasmid pCl was transformed in *Escherichia coli* strain XL1-Blue (Stratagene). A 200-ml culture of

the recombinant cells was grown to OD<sub>600</sub> = 0.6 at 37°C and IPTG was added to a final concentration of 10 mM. The culture was allowed to resume growth overnight before harvesting by centrifugation. Purification of recombinant protein was performed as described by Adam et al. (14). The final pellet was extracted in 8 M urea for 15 min and the supernatant was stored at -70°C in aliquots.

**ELISA.** Standard protocol for ELISA was employed as described by Rubin (15). Purified recombinant proteins in 8 M urea were diluted 1,000–64,000-fold in PBS for coating Immulon 2 microtiter plates (Dynatech Laboratories, Inc., Alexandria, VA). Peroxidase-conjugated goat anti-human IgG + M reagents (Caltag Laboratories, So. San Francisco, CA) and the substrate 2,2'-azinobis(3-ethylbenzthiazoline sulfonic acid) (Boehringer Mannheim GmbH, Mannheim, FRG) were used as detecting agents.

**Southern analysis.** For determining the complexity of the gene encoding the 52-kD SS-A/Ro protein, human genomic DNA samples (10 µg/lane) from HeLa cells and normal peripheral blood were digested with EcoRI or HindIII restriction enzyme to completion and analyzed essentially according to Southern (16) using labeled pCl cDNA insert.

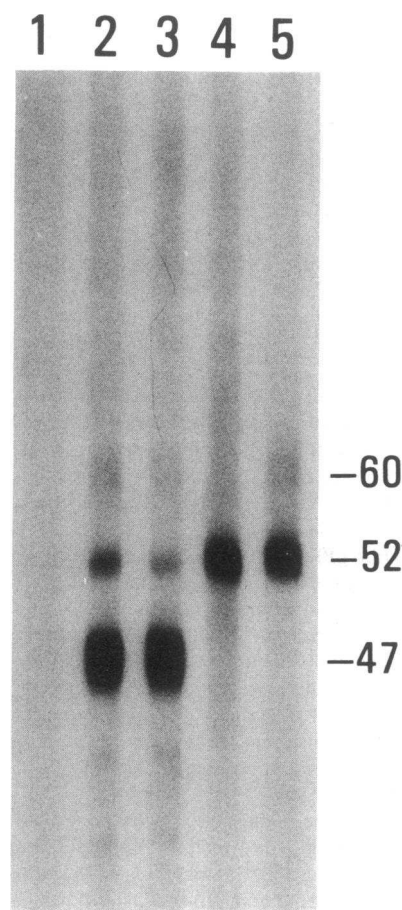
**In vitro RNA transcription and translation.** The EcoRI λgtII cDNA insert of 52FL was subcloned into pBluescript SK vector. The resulting plasmid p52FL with the cDNA insert in the same orientation as β-galactosidase was linearized by restriction enzyme ClaI at the 3' end of the insert. RNA was transcribed in vitro from the linearized plasmid using T3 RNA polymerase and was translated in vitro in a rabbit reticulocyte lysate (Promega, Madison, WI) in the presence of [<sup>35</sup>S]-methionine (Tran<sup>35</sup>S-label, 70% methionine and 15% cysteine, ICN Biochemicals) as described in the manufacturers' instructions. Products of in vitro translation were stored in aliquots for immunoprecipitation (17, 18) and two-dimensional gel analysis (19).

**DNA sequencing and analysis.** The sequence strategy was to combine data derived from restriction fragments subcloned in pBluescript vector and the use of synthetic oligonucleotides for primers. Plasmids were purified by the Chen and Seeburg method (20) and DNA sequencing was performed by the dideoxy chain termination method of Sanger et al. (21). DNA and protein sequences were analyzed by the Genetics Computer Group Sequence Analysis Software Package for VAX computers (22). Alignment of protein sequences was initially achieved with the GAP program that employed the algorithm of Needleman and Wunsch (23). Multiple sequence alignments were performed with CLUSTAL programs (24, 25).

## Results

**Cloning and characterization of 52-kD SS-A/Ro cDNAs.** In the screening of 10<sup>6</sup> recombinant phages from the λZap HepG2 cell cDNA library, clone Cl was selected by human serum Bo and was purified with three rounds of plaque purification. To show that Cl encoded the 52-kD SS-A/Ro protein, purification of affinity antibody was performed with Cl phage protein and a different human autoimmune serum Ca that recognized both SS-A/Ro and SS-B/La proteins in MOLT-4 cell extracts (Fig. 1, lane 2). Affinity purified antibody from the Cl phage protein was specific in recognizing the 52-kD SS-A/Ro protein alone (lane 4). This specific reactivity was consistent with our previous finding that human affinity purified anti-52-kD antibodies did not recognize either the 60-kD SS-A/Ro or SS-B/La proteins (4).

The λZap clone Cl was subcloned in vivo into pBluescript plasmid pCl. Recombinant protein derived from pCl grown in XL1-Blue bacteria had a molecular mass of ≈ 46,000 as detected by immunoblotting and was purified as described in Methods. The final preparation was a highly antigenic substrate for the detection of anti-52-kD SS-A/Ro antibody in an



**Figure 1.** Immunoblotting analysis of affinity purified antibodies from  $\lambda$ Zap CI-derived fusion protein. Molt-4 cell extracts were separated on SDS-PAGE using a 20-cm 15% separating gel as described in Methods. After transfer to nitrocellulose filter, strips were cut and probed with different human antibodies: lane 1, a normal human serum; lane 2, human serum Ca with weak reactivity to the 60-kD SS-A/Ro protein and strong reactivities to both the 52-kD SS-A/Ro and 47-kD SS-B/La proteins; lane 3, Ca serum absorbed with CI phage proteins shows a drop in intensity of the 52-kD band but no effect on the reactivity to SS-B/La; lane 4, antibody from Ca serum affinity purified from CI phage protein shows very strong reactivity with 52-kD

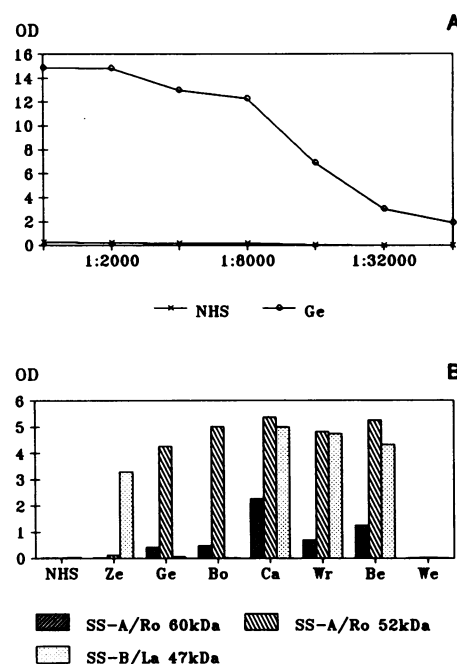
SS-A/Ro alone; lane 5, serum Bo, the original serum used in the cloning of CI. The results show that affinity purified antibody from CI recombinant protein recognized only the 52-kD SS-A/Ro protein.

ELISA format (Fig. 2 A). Even with the highest dilution of 1:64,000 for coating microtiter wells, an OD<sub>410</sub> reading of 2 was obtained 1 h after the addition of detecting reagents. Fig. 2 B illustrates the specificity of the ELISA when a 1:30,000 dilution of the purified recombinant antigen was used for the detection of SS-A/Ro antibodies. Recombinant 60-kD SS-A/Ro and SS-B/La proteins derived from cDNA clones previously reported (17, 26) were purified similarly and are included in this figure for comparison. High OD readings for the 52-kD SS-A/Ro recombinant protein were only seen in sera with detectable antibodies by immunoblotting. Our data indicated that in most anti-SS-A/Ro sera tested, the reactivities as determined by ELISA were higher for the 52-kD than the 60-kD recombinant antigens. Besides the negative controls shown in Fig. 2 B, 20 normal human sera and standard prototype autoimmune sera were also negative including antibodies to PCNA, Sm, U1-RNP, ribosomal RNP, Jo-1, Scl-70, SL/Ki, and Ku specificities (data not shown).

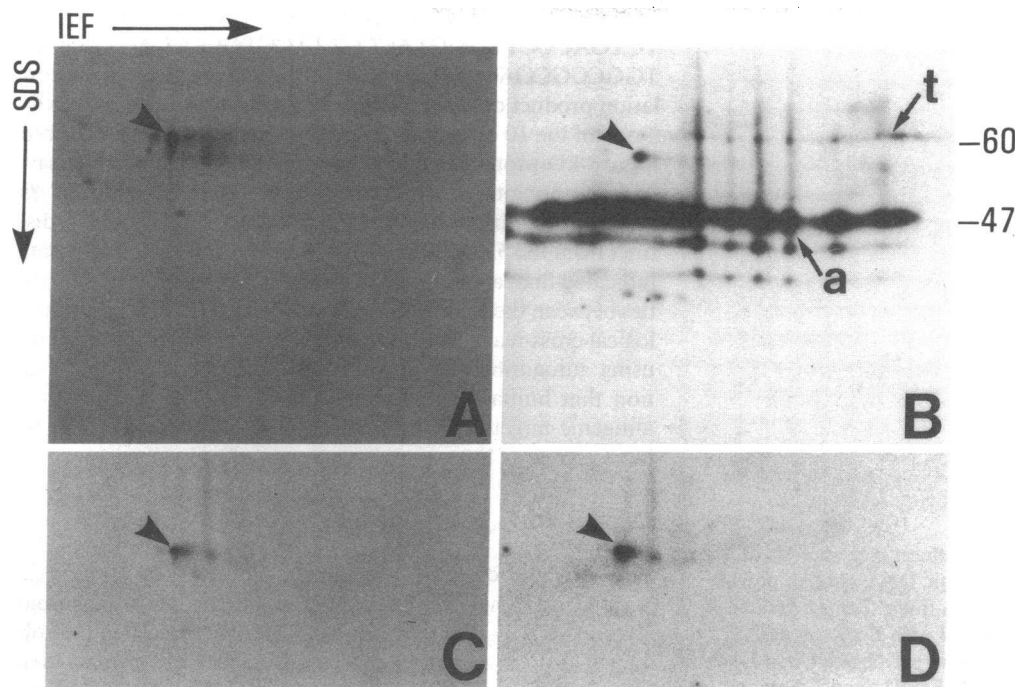
The immunological data above suggested that pCI encoded at least one immunoreactive region of the 52-kD SS-A/Ro protein and the next step was to determine if the cDNA insert of pCI encoded the entire 52-kD protein. Plasmid pCI was digested with EcoRI restriction enzyme and a cDNA insert of 1.5 kb was detected after electrophoresis in a standard agarose gel.

Northern blot analysis of the mRNA from MOLT-4 and HeLa cells using radiolabeled 1.5-kb fragment showed that there was a single band of  $\sim 1.9$  kb. This indicated that pCI probably contained only a fraction of the complete cDNA for the 52-kD SS-A/Ro protein. To obtain a full-length cDNA,  $10^6$  recombinant phages from the MOLT-4 cell cDNA library were screened with a radiolabeled DNA probe corresponding to the 5' sequence of pCI cDNA insert. Of the two clones selected, clone 52FL had the longest insert of  $\sim 1.9$  kb and was subcloned into pBluescript plasmid p52FL for further analysis.

To determine if p52FL cDNA insert encoded the complete 52-kD protein, comparison was made between the in vitro transcription and translation products of p52FL and the 52-kD SS-A/Ro protein from MOLT-4 cells. Preliminary data showed that they comigrated in standard one-dimensional gel SDS-PAGE, and therefore two dimensional gels were used for more discriminating analysis (Fig. 3). In vitro [<sup>35</sup>S]-methionine labeled translation products of p52FL RNA gave a major spot (arrow) and several minor spots that were observed only in an overexposed autoradiogram (Fig. 3 A). Immunoprecipitation of these translation products with human SS-A/Ro sera showed that the major 52-kD product was immunoreactive. Immunoblotting of MOLT-4 cell proteins with serum Ca detected multiple SS-B/La isospecies of different pI migrating at



**Figure 2.** Reactivity of purified recombinant protein in ELISA. Recombinant protein derived from pCI plasmid was purified as described in Methods. (A) Titration curve of the reactivities (OD<sub>410</sub>) of a prototype SS-A/Ro serum Ge and a normal human serum with recombinant protein diluted from 1:1,000 to 1:64,000. (B) Reactivities of human sera with respect to different purified recombinant proteins. Recombinant 60-kD SS-A/Ro and 47-kD SS-B/La proteins were purified from cDNA clones previously reported (17, 26). Serum Ze is the CDC prototype anti-SS-B/La, sera Ge and Bo are mostly reactive with 52-kD SS-A/Ro, sera Ca, Wr, Be recognized both SS-A/Ro and SS-B/La, and serum We is the CDC prototype anti-Sm.



**Figure 3.** Autoradiograms of two-dimensional gels separating MOLT-4 cell proteins and in vitro translation products of p52FL RNA transcript. (A) Separation of [<sup>35</sup>S]-methionine labeled in vitro translation product of p52FL RNA. An overexposed autoradiogram of the dried gel is shown to indicate the major 52-kD spot (arrowhead) and other minor species. (B) The immunoblot of MOLT-4 cell proteins with serum Ca, showing strong reactivity with SS-B/La isospecies migrating at 47 kD and isoforms of the 60-kD SS-A/Ro proteins. In contrast the reactivity with 52-kD SS-A/Ro protein is restricted to a major spot (arrowhead) with a pI of  $\approx 6.5$ . The pI estimated by the ISOELECTRIC program (22) based on the protein sequence is 6.35. Arrows marked *a* and *t*

correspond to the positions of major cell proteins actin and tubulin, respectively. To determine unambiguously the gel migration positions of the 52-kD SS-A/Ro protein and the recombinant protein, a mixture of MOLT-4 cell proteins and the [<sup>35</sup>S]-methionine labeled in vitro translation product of p52FL RNA was separated on a single gel and then transferred to nitrocellulose filter. (C) 4-d exposure of the labeled [<sup>35</sup>S]-methionine on the nitrocellulose filter. (D) 12-h exposure with intensifying screen for the same nitrocellulose after immunoblotting with human serum Ge, which is specific for 52-kD SS-A/Ro protein, and [<sup>125</sup>I]-protein A. The major spots (arrowhead) in C and D match to the same position showing that p52FL encoded the entire 52-kD protein of SS-A/Ro.

47 kD and several isoforms of the 60-kD SS-A/Ro proteins (Fig. 3 B). On the other hand, the 52-kD SS-A/Ro protein was restricted to a major spot with an estimated pI  $\approx 6.5$  (Fig. 3 B, arrowhead). The colocalization of the p52FL encoded protein and the 52-kD SS-A/Ro protein was determined unambiguously by running both samples in the same gel (Fig. 3, C and D). These independent results provided further confirmation that p52FL cDNA encoded the entire 52-kD protein of SS-A/Ro.

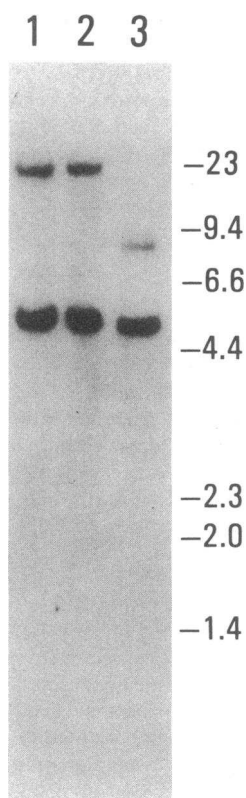
**Southern analysis.** Human genomic DNA samples from HeLa cells and normal peripheral blood were digested with EcoRI or HindIII restriction enzyme to completion. Either restriction site was not found in the complete 52FL sequence. Results of the genomic blot probed with C1 cDNA insert showed a major band of  $\approx 5$  kb and a minor band of  $\approx 8$  kb (HindIII) and  $\approx 20$  kb (EcoRI) (Fig. 4). These data indicated that there was probably only one and at most two genes for the 52-kD SS-A/Ro protein in the human genome.

**Sequence analysis.** The nucleotide sequences of the cDNA inserts of C1 and 52FL were determined by sequencing both DNA strands as outlined in Fig. 5. Clone C1 represented the 3' 80% of clone 52FL and there was no difference detected between C1 and 52FL clones in the corresponding regions. The DNA sequence of 52FL and the deduced amino acid sequence are shown in Fig. 6. There is only one large open reading frame composed of 475 amino acids corresponding to a calculated molecular mass of 54,082. The sequence flanking the first ATG codon is in close agreement with the eukaryotic translation

initiation consensus sequence (27). In the 5' noncoding region, there are three stop codons that are in-frame with the ATG start codon (Fig. 6). Two polyadenylation signal sequences are found at the end of the 3' noncoding sequence. The consensus sequence for nuclear localization signal has not been detected.

Special features of the 52-kD SS-A/Ro protein are summarized in Fig. 7. The interesting features are the amino-terminal zinc finger domains, a central domain with a leucine zipper motif, and a carboxy-terminal domain that is highly similar to human protein *rfp*. Fig. 8 shows the putative zinc-finger structures in the 52-kD protein. The arrangement of the Cys residues for the chelation of metal such as zinc has been found in many DNA/RNA-binding proteins (28, 29) and these zinc finger structures are generally thought to be responsible for the interaction with either DNA or RNA. The proposed finger structures in Fig. 8 are atypical as compared to most known zinc finger protein sequences (28, 29). There is no published data at the present to indicate whether the SS-A/Ro protein binds zinc or other metal ions. However, a single zinc finger motif has also been described in the 60-kD SS-A/Ro protein (7, 17). A leucine zipper motif (residues 211–232) was detected in the center domain of the 52-kD protein. Leucine zippers were originally described in DNA-binding proteins (30) and now these motifs are known to participate in protein–protein interaction or dimer formation that is important for DNA-binding (31–33). This motif in 52-kD SS-A/Ro may be important for interaction with other proteins or for homo-dimer formation.

When the DNA and protein sequences were determined for



**Figure 4.** Southern blot analysis of human genomic DNA samples probed with C1 cDNA insert. Lane 1, HeLa cell DNA digested with EcoRI; lane 2, normal human peripheral blood DNA digested with EcoRI; lane 3, human peripheral blood DNA digested with HindIII restriction enzyme. The results suggest only one or two genes encoding the 52-kD SS-A/Ro protein.

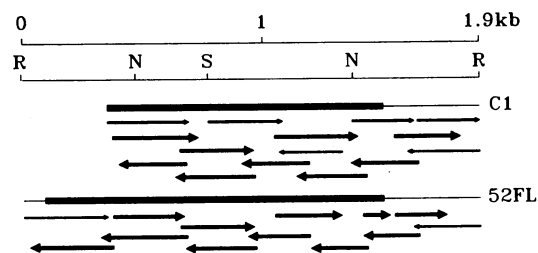
the 52-kD protein, homology search with other known proteins was performed with the WORDSEARCH program available from the Genetics Computer Group Sequence Analysis Software Package (22). Three proteins were identified with high degree of homology to the 52-kD protein and they were the mouse *rpt-1* protein (34), human *ret* transforming protein (35), and its related protein *rfp* (13). Fig. 9 A shows the alignments among the three protein sequences. Note that the least number of gaps has been introduced to optimize the alignment. There is over 30% identity and 50–60% similarity in sequences at the corresponding amino-terminal halves of these proteins. The cysteine residues of the zinc finger motifs are conserved among all three sequences. The 52-kD protein is more similar to either *rfp* or *rpt-1* than *rfp* is to *rpt-1* (Fig. 9 A). The data suggest that these three protein domains might be related as a subfamily of finger proteins. In addition, the carboxy-terminal halves of the 52-kD SS-A/Ro and *rfp* proteins are highly similar with > 50% of identity and 65% similarity (Fig. 9 B).

***rfp* cDNA isolation and analysis for antigenic reactivity.** Because up to 50% identity in amino acid residues was found between the carboxy-terminal halves of the 52-kD SS-A/Ro and *rfp* proteins, it was of interest to determine whether (a) anti-52-kD SS-A/Ro autoantibodies could recognize *rfp* protein, and (b) *rfp* might be a member of SS-A/Ro proteins. A cDNA clone 2rl was isolated from the same MOLT-4 library and restriction enzyme analysis confirmed its identity to the published *rfp* cDNA clone (13). This was supported by DNA sequencing data which showed that 2rl was an authentic *rfp* protein cDNA with 104 extra nucleotides at the 5' end as compared to the original clone described by Takahashi et al. (13). The extra 5' sequence is TCCGCTCGGACGCGCCACGT-

TGTCTTGCGCGCTTTGCCCGCCTGGCCCTGGGACTCTGACCCTCGGCTACCCTTTCCTGCCCCACTAGCGTGGCCGCGAGCCT. In vitro [<sup>35</sup>S]-methionine labeled translation product of 2rl-encoded RNA was a protein of ~ 58 kD. None of the 10 different human anti-SS-A/Ro sera recognized this 58-kD protein in a standard immunoprecipitation assay. In immunoblotting, the rabbit antiserum to recombinant *rfp* protein recognized a 58-kD MOLT-4 cell protein that was distinct from the 52-kD and 60-kD SS-A/Ro proteins. These data indicated that, although there are significant sequence similarities between the 52-kD SS-A/Ro and *rfp* proteins, no immunological cross-reaction could be observed between these proteins using autoantibodies. It also reconfirms the general observation that human autoantibodies are highly specific for their antigenic targets and are generally noncross-reactive with related proteins (1).

## Discussion

A cDNA encoding the complete 52-kD SS-A/Ro protein described by Ben-Chetrit et al. (4) was cloned from a human T-cell (MOLT-4) cDNA library. A partial clone was also obtained from a human liver (HepG2) cDNA library and there was no difference detected between their overlapping regions. The nucleotide and deduced amino acid sequences for the 52-kD protein showed no homology with the corresponding sequences for the related 60-kD SS-A/Ro (7, 17) and 47-kD SS-B/La antigens (26). DNA hybridization experiments using the cDNAs from all three antigens above also showed no cross-hybridization (data not shown). Human SS-A/Ro autoantibodies recognized epitope(s) expressed on the recombinant proteins derived from the initial λZap phage and the subcloned pBlue-script plasmid. The partially purified recombinant protein was a specific substrate for the sensitive detection of anti-SS-A/Ro antibodies in an ELISA format. In addition, in vitro translation product derived from cDNA clone could be immunoprecipitated by anti-SS-A/Ro sera and was found to comigrate with the cellular 52-kD protein as determined by immunoblotting and two-dimensional gel electrophoresis. Because data from mRNA blotting indicated that there was only one band of ~ 1.9 kb in MOLT-4 cells, the 1.9-kb cDNA insert of p52FL probably represented the full-length mRNA. The presence of putative zinc fingers and a leucine zipper in the 52-kD protein



**Figure 5.** Schematic representation of the cDNA clones C1 and 52FL derived from HepG2 and MOLT-4 cell libraries, respectively. Coding regions are indicated by solid bars and the 5' and 3' untranslated regions are represented by thin lines. Thin and thick arrows represent DNA sequence derived from restriction fragment subcloning and sequencing with synthetic oligonucleotides, respectively. Restriction enzyme sites R, N, and S are EcoRI, NcoI, and SacI, respectively.





mRNA level was highest in 11.5-d mouse embryos as compared to other stages (13). The fact that *rfp* is part of a transforming gene *ret* raises the question whether the 52-kD protein might have similar transforming potential. The second protein that the 52-kD SS-A/Ro protein was found to share similarity with was *rpt-1* (for regulatory protein of T-lymphocyte [1]). *Rpt-1* was first described by Patarca et al. (34) as a protein selectively expressed by resting but not activated CD4<sup>+</sup> inducer T cells. *Rpt-1* is a 41-kD nuclear protein that down-regulates gene expression of IL-2 receptor  $\alpha$ -chain gene and human immunodeficiency virus type 1 genes. Sequence similarities among the 52-kD SS-A/Ro, *rfp*, and *rpt-1* proteins can be useful when the function of an index protein such as the 52-kD SS-A/Ro is unknown, but it is well acknowledged that such interpretations should be taken with caution. Nevertheless, it provides new avenues for future investigations into the possible functional roles of the 52-kD SS-A/Ro protein.

Because the report of Ben-Chetrit et al. (4) showing that SS-A/Ro autoantibody targets consisted of at least two components of 52 and 60 kD, the results of many studies based on the assumption that the 60-kD protein was the only SS-A/Ro autoantigen would have to be reevaluated. Buyon et al. (41) recently showed that in neonatal lupus syndrome with congenital heart block, all 20 mothers of permanently affected infants had antibodies to either SS-A/Ro or SS-B/La antigens. The predominant antibody response was to the 52-kD SS-A/Ro antigen (41). In another report by Ben-Chetrit et al. (42), it was shown that although most SS-A/Ro-positive autoimmune sera had reactivity to both the 52- and 60-kD antigens in immunoblotting, antibody to the 52-kD antigen without concomitant antibody to the 60-kD antigen was seen only in patients with primary Sjogren's syndrome whereas antibody to the 60-kD antigen without concomitant antibody to the 52-kD antigen was seen only in patients with systemic lupus erythematosus (42). The latter observation that there was a dissociation of immune responses to the two SS-A/Ro components might suggest different events stimulating the autoimmune process in these diseases. The current report on the sequence and molecular characteristics of the 52-kD protein establishes its identity and might be useful in elucidating its function and that of the multi-component SS-A/Ro complex. Such information could contribute to understanding some features of this autoimmune reaction.

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*Note added in proof:* Recently we have detected protein sequence similarity between the 52-kD SS-A/Ro protein and the 55-kD RAD18 gene product of *Saccharomyces cerevisiae* (Chanet et al. 1988. *Gene (Amst.)* 74:543-547). The region of similarity is restricted to NH<sub>2</sub>-terminal 54 amino acid residues, and the Cys residues of the zinc finger domain are conserved.

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