Molecular Characterization of an Autoantigen of PM-Scl in the Polymyositis/Scleroderma Overlap Syndrome: A Unique and Complete Human cDNA Encoding an Apparent 75-kD Acidic Protein of the Nucleolar Complex

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Summary

About 50% of patients with the polymyositis/scleroderma (PM-Scl) overlap syndrome are reported to have autoantibodies to a nuclear/nucleolar particle termed PM-Scl. The particle is composed of several polypeptides of which two have been identified as autoantigens. In this report, human cDNA clone coding for the entire 75-kD autoantigen of the PM-Scl particle (PM-Scl 75) was isolated from a MOLT-4 λgt-11 library. The deduced amino acid sequence of the cDNA clone represented a protein of 355 amino acids and 39.2 kD; the in vitro translation product of this cDNA migrated in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) at ~70 kD. The aberrant migration of the polypeptide in SDS-PAGE was shown to be related to the COOH half that was rich in acidic residues. Authenticity of the cDNA coding for PM-Scl 75 was shown by immunoreactivity of PM-Scl sera with in vitro translation products and recombinant fusion proteins encoded by the cDNA. In addition, rabbit antibodies raised to recombinant fusion protein reacted in immunofluorescence, immunoblotting, and immunoprecipitation with the characteristic features displayed by human anti–PM-Scl sera.

Autoantibodies in systemic autoimmune diseases can be highly specific and can serve as diagnostic markers for autoimmune diseases. Examples of this include the Sm system of SLE, the nRNP system of mixed connective tissue disease, the centromere, RNA polymerase I, and U3 RNP systems of scleroderma, and the transfer RNA synthetases in myositis (for review see reference 1). More recently, autoantibodies have been used in the molecular cloning of many autoantigens in an attempt to further define these molecules and their association with the autoimmune response.

Autoantibodies have also become useful reagents in the study of basic cellular functions. Anti-Sm/nRNP autoantibodies were important in elucidating the involvement of the Sm/nRNP particle in pre-mRNA splicing (2–4). Autoantibodies to proliferating cell nuclear antigen (PCNA)[1] played an important role in elucidating DNA replication involving DNA polymerase delta (5, 6). The function of SS-B/La in the processing of RNA polymerase III transcripts and tran-

scription termination have been greatly aided by using human autoantibodies (7, 8).

Autoantibodies to the PM-Scl antigen system are produced predominantly by patients with features of scleroderma and polymyositis. The incidence of anti–PM-Scl has been reported to be 8% in polymyositis patients, 3% in scleroderma patients, and 50% in patients with polymyositis and scleroderma overlap (9). Indirect immunofluorescence studies have shown that PM-Scl autoantibodies stain predominantly the cell nucleolus (10–12) with some weaker nucleoplasmic staining (11). Studies to localize the nucleolar staining pattern employed the property of actinomycin D (Act D) to segregate nucleoli into the fibrillar and granular components (13). In Act D–treated Vero cells, indirect immunofluorescence with PM-Scl antibodies specifically stained the granular component of the nucleolus (11). The location of PM-Scl in the granular component was also confirmed by immunoelectron microscopy using regenerating rat liver (11).

At the molecular level, PM-Scl sera have been shown to immunoprecipitate a complex of 11–16 polypeptides ranging in apparent molecular mass from 20 to 110 kD (11, 12, 14). Of these polypeptides, several were reported to be phosphorylated (Reimer et al. [11] reports 80 and 20 kD; Gelpi et al. [12] reports 68, 39, and 20 kD). No RNA was found

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[1] *Abbreviations used in this paper:* Act D, actinomycin D; IVTP, in vitro translation product; NBRF, National Biomedical Research Foundation; PCNA, proliferating cell nuclear antigen; PM-Scl, polymyositis scleroderma; RT, room temperature.

to be precipitated with the particle (14). In immunoblotting studies, reactivity with a 110-kD antigen was reported in all PM-Scl sera examined (12), while only some recognized an 80-kD antigen (11). The structure and function of the PM-Scl complex is not known, but its localization within the granular component of the nucleolus suggests that it may have some role in ribosome maturation and/or transport.

In this study, we report the use of human autoantibodies in the cloning of human cDNA encoding the 75-kD autoantigen (previously called 80 kD [11]) of the PM-Scl particle. We show that the 1.6-kb cDNA encodes a 39.2-kD polypeptide that migrates aberrantly in SDS-PAGE and that the COOH region of the molecule was responsible for this phenomenon. Rabbit antibodies raised against recombinant fusion protein displayed immunoreactivity similar to human PM-Scl 75 autoantibodies.

Materials and Methods

Human Serum. Eight PM-Scl sera defined by double immunodiffusion and indirect immunofluorescence staining pattern and derived from patients with overlapping PM-Scl, as described previously (9), were obtained from our laboratory serum bank. Five disease control sera were also from the laboratory serum bank and obtained from patients with autoantibody profiles of other specificities. Normal control sera were from laboratory personnel.

Cell Lines. HeLa cells (ATCC CCL 2.2; American Type Culture Collection, Rockville, MD), MOLT-4 (human T cell lymphoblastic leukemia; ATCC CRL 1582), HepG2 (human hepatocellular carcinoma, ATCC HB 8065), HL60 (human promyelocytic leukemia, ATCC CCL 240), and HEp-2 (human epidermoid carcinoma) cells were maintained in RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine, and 5 μg/ml gentamicin sulfate in a 5% CO_2/95% air incubator.

SDS-PAGE and Immunoblotting. Proteins were solubilized in SDS sample buffer (625 mM Tris-HCl, pH 6.8, 5% β-ME, 2.3% SDS, 10% glycerol) and separated by discontinuous SDS-PAGE according to the method of Laemmli (15) employing various percentage resolving gels, pH 8.8, and 4% stacking gels, pH 6.6. Electrophoresis was carried out at 10–15 mA/gel through the stacking and 20–25 mA/gel through the resolving gel. Bromophenol blue (EM Science, Gibbstown, NJ) was included in the sample buffer or added to the top reservoir to visualize buffer fronts.

Proteins were transferred to nitrocellulose essentially as described by Towbin et al. (16) in transfer buffer (12.5 mM Tris, 96 mM glycine, 20% methanol) overnight at 4°C with a constant voltage of 7 V/cm. After transfer, nitrocellulose sheets were stained with Ponceau S (Sigma Chemical Co., St. Louis, MO) to monitor transfer efficiency. Unbound sites on the nitrocellulose were blocked with 3% nonfat milk in PBS with 0.02% thimerosol for 1 h at room temperature (RT). Diluted sera were incubated with the nitrocellulose for 1 h at RT followed by successive 10-min washes with PBS, twice with PBS containing 0.05% Tween-20 (PBS-T), and again with PBS to remove unbound antibody. Bound antibody was traced by ^{125}I-protein A (2-4 × 10^5 cpm; ICN Radiochemicals, Irvine, CA) at RT for 1 h and unbound ^{125}I-protein A removed by washing as described above. Bound antibodies were visualized by exposure of air-dried nitrocellulose to X-OMAT AR film (Eastman Kodak, Co., Rochester, NY).

Immunoprecipitation. Immunoprecipitation of [^{35}S]methionine-labeled cell extract or in vitro translation products were performed

using protein A–bound Sepharose, essentially as described (17). Briefly, 100 μl of 10% (wt/vol) protein A-Sepharose was incubated with 10 μl of human sera and 2-5 μl in vitro translation product at 4°C for 60–90 min. After incubation, Sepharose beads were washed five times with 1 ml of buffer C (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.5% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) and resuspended in 40 μl SDS sample buffer. Samples were analyzed by SDS-PAGE followed by autoradiography.

cDNA Library Screening. Clones from a MOLT-4 λgt-11 cDNA library were initially selected by immunological screening as described by Young and Davis (18). All screening was performed on duplicate filters and positive bacteriophages were subsequently purified to 100% purity. Before screening the cDNA library, anti-PM-Scl serum was extensively adsorbed against bacterial and wild-type λgt-11 phage to reduce background binding. Bound antibodies were detected as described above for immunoblotting.

To obtain the full-length cDNA clone, the MOLT-4 library was rescreened using the method of Benton and Davis (19). Partially overlapping 30-mer synthetic DNA oligonucleotides (5'-TTTT-GCAGAGTCTATAGCAAATCAAAGGAT-3' and 5'-ATTTTAA-ATGCTGTGATCCTTTGATTTGCT-3') were designed based on the 5' sequence of λM9.12 cDNA insert (see Fig. 3 *A*) using a DNA synthesizer (model 380B; Applied Biosystems Inc., Foster City, CA) and radiolabeled with [α^{32}P]-ATP using the standard fill-in reaction of Klenow polymerase (20). Duplicate filters of λ gt-11 plaques were prehybridized at 42°C overnight and hybridized with 10^6 cpm/ml [^{32}P]-oligonucleotides at 42°C, overnight. Filters were washed at 51°C with three successive 20-min washes in 0.1× SSC, 0.1% SDS, and exposed to X-OMAT AR film.

DNA Subcloning and Sequence Determination. Recombinant DNA samples were isolated from 100-ml cultures of λ phage–infected *E. coli* LE392 cells as described by Maniatis et al. (21). cDNA inserts were excised from the λ DNA using EcoRI restriction sites and subcloned into pBluescript SK- vector (pBST; Stratagene Inc., La Jolla, CA). Plasmid DNA was transformed into DH5α cells as described by Hanahan (22). Nucleotide sequence was obtained by the dideoxy method of Sanger et al. (23) and double-stranded DNA sequencing by the procedure of Chen and Seeburg (24) using T7 DNA polymerase (Pharmacia Fine Chemicals, Piscataway, NJ). T3, T7, and additional oligonucleotide primers were synthesized with a DNA synthesizer.

Polymerase Chain Reaction. PCR was used to determine the size and orientation of cDNA inserts using methods essentially as described in the GeneAmp DNA Amplification Reagent Kit (Perkin Elmer Corp., Norwalk, CT). 50- or 100-μl reaction volumes were used and were composed of PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.0 mM MgCl₂, 0.01% [wt/vol] gelatin), dATP, dCTP, dGTP, dTTP to a final concentration of 200 μM each, and PCR oligonucleotide primers each to a final concentration of 0.2 μM. Sample DNA was added and solutions heated to 95°C for 5 min before brief centrifugation, addition of 2.5 U amplitaq (rTaq DNA polymerase; Perkin Elmer Corp.), and one drop paraffin oil (Perkin Elmer Corp.). DNA amplification was performed using a microcycler (Eppendorf Inc., Fremont, CA) with 35 cycles of 94°C, 30 s; 55°C, 30 s; 72°C, 90 s; and a final 7-min elongation step at 72°C. Typically, 10–40% of reaction volumes were separated on 1% agarose gels and stained with ethidium bromide to visualize PCR products. DNA samples for PCR were obtained from phage suspensions or bacterial colonies (25).

Affinity-Purified Antibody. Bacterial cell extract expressing IPTG-induced fusion protein was separated by SDS-PAGE and transferred to nitrocellulose as described above. After incubation with primary

antibody, thin vertical strips were taken from the edges and center of nitrocellulose sheet and processed with ^{125}I–protein A as described above for immunoblotting. Areas on the remaining nitrocellulose corresponding to the reactive sites on the strips were excised and used for antibody elution. Antibodies were affinity purified from nitrocellulose filter by elution at pH 2.55. For affinity purification of antibody from λgt-11 clones, confluent plates of λgt-11 clone M9.12 were induced to produce fusion protein with isopropyl-β-thiogalacto-pyranoside (IPTG; Fisher Scientific, Fair Lawn, NJ)-impregnated nitrocellulose filters. After overnight incubation on the plates, the filters were blocked and probed with primary antibody and washed as described above for immunoblotting. Bound antibody was eluted with 3 ml of elution buffer (50 mM KH2PO4, pH 2.55, 0.3% nonfat milk) by rocking at RT for 5 min. The eluate was collected and the filter washed with 1 ml elution buffer before neutralizing with 225 μl of 1 M Tris, pH 8.8. Antibodies were concentrated with Centricon 30 microconcentrators (Amicon Corp., Danvers, MA) and used for immunofluorescence and immunoblotting analysis.

Immunofluorescence. Indirect immunofluorescence was performed on cells grown on glass coverslips or commercial HEp-2 slides (BION, Park Ridge, IL) with sera at 1:50 dilution in PBS for 30 min at RT. Unbound antibody was removed with successive 5-min washes of PBS, PBS-T, and PBS. Human antibody was traced with FITC conjugated goat anti–human Igs and mouse antibody with rhodamine conjugated goat anti–mouse Igs (Caltag Laboratories, So. San Francisco, CA) at 1:50 dilution in PBS. Second antibody was applied for 30 min at RT and unbound antibody removed with washes as stated above. Coverslips were rinsed in water, mounted with Fluorsave (Calbiochem-Behring Corp.) and allowed to set at RT for 1 h before viewing by fluorescence microscopy.

Northern Analysis. Total RNA were isolated from MOLT-4 and HL60 cells using a 340A nucleic acid extractor (Applied Biosystems). Approximately 10 μg of RNA was separated by 1% agarose in 2.2 M formaldehyde and transferred overnight to a nylon filter (ONCOR, Gaithersburg, MD). The filter was prehybridized at 42°C for 8 h and hybridized with 6 × 10⁶ cpm/ml ³²P-labeled cDNA fragment that was gel-purified using the Geneclean kit (Bio 101, La Jolla, CA). After overnight hybridization, the filter was washed three times for 20 min in 0.1× SSC and 0.1% SDS at 60°C, followed by exposure to X-OMAT AR film.

RNA Transcription and In Vitro Translation. Clones pA3.212 and pPMSCL75 were used for in vitro transcription and translation experiments. Both clones had identical cDNA inserts but had opposite insert orientation. Clone pA3.212 utilized the T7 promoter site of the Bluescript vector, and pPMSCL75 being in the opposite orientation, utilized the T3 promoter site of Bluescript vector. Full-length transcription was achieved by linearizing pA3.212 with SacI or pPMSCL75 with XhoI and transcribing with T7 or T3 RNA polymerase (Stratagene Inc.), respectively. Truncated transcripts were obtained by using the internal restriction sites. Truncated clones encoding residues 1-212 used DraI-linearized pPMSCL75 and T3 RNA polymerase, while clones encoding residues 1-123 used BamHI-linearized pA3.212 and T7 RNA polymerase. Typically, 1 μg of linearized plasmid DNA was used in each transcription reaction.

RNA transcripts were analyzed in a 0.8% agarose gel containing 2.2 M formaldehyde. Before in vitro translation, RNA samples were extracted with phenol/chloroform and precipitated with ethanol. Typically, 1 μg of RNA was added in a 50 μl translation reaction containing rabbit reticulocyte lysate (Promega, Madison, WI), ³⁵S-methionine (Trans-³⁵S-label, 70% methionine, 15% cysteine; ICN Biochemicals), and RNase block II (Stratagene Inc.) as per manufac-

turer's instructions. Translation were carried out at 30°C for 1 h followed by 10% SDS-PAGE of a 5–10 μl aliquot to confirm presence of translation products. Samples were stored at −80°C until required.

Fusion Protein Production. Expression plasmid ORF75 containing full-length PM-Scl 75 was constructed using PCR amplification of the open reading frame of pA3.212. Synthetic oligonucleotide primers spanning the initiation and termination sequences of pA3.212 were constructed. The primer spanning the start codon was 5′-GGGAATTCACAGATGGCCGCTCCAGCT-3′, which contained an EcoRI restriction site (underlined), and the 3′ primer was 5′-CCCTCGAGCTTTAATTGGAGCTCTC-3′, with a XhoI restriction site (underlined). The PCR product was purified from a 0.8% low melting agarose gel, digested with EcoRI and XhoI restriction enzymes, subcloned into the EcoRI and XhoI sites of pBST, and transformed into DH5α cells. Resulting clones were screened for insert size and orientation using PCR and the selected clones sequenced across the polylinker arms to confirm sequence identity with parental pA3.212. Thus, pORF75 fusion protein consisted of 38 residues of β-galactosidase from the Bluescript vector, an extra serine and glutamine introduced during the subcloning step and the 355 amino acids that encoded for PM-Scl 75. Fusion protein was prepared from pORF75/DH5α cultures grown to $OD_{600} = 0.6$ and induced with IPTG added at a final concentration of 10 mM. Bacteria were harvested by centrifugation and directly solubilized in SDS sample buffer. Proteins were separated by SDS-PAGE and stained with Coomassie blue or transferred to nitrocellulose for subsequent immunoblotting experiments. For immunization of experimental rabbits, recombinant fusion protein was enriched by the procedure of Adam et al. (26). The final pellet of inclusion bodies was extracted with 8 M urea on ice for 15 min and the supernatant stored at −70°C.

Rabbit Immunization. Two female New Zealand White rabbits were immunized with 2.5 mg of fusion protein in CFA. Rabbits were boosted twice with a further 2.5 mg fusion protein in IFA at 30-d intervals. Serum samples were collected and stored at −20°C.

Computer Analysis of Nucleic Acid and Protein Sequences. Nucleic acid and protein sequences were analyzed by the University of Wisconsin Genetic Computer Group Sequence Analysis Software Package (version 6.2) (27). Data bases searched were Genebank (release 65.0), EMBL (release 24.0), National Biomedical Research Foundation (NBRF), Nucleic acid (release 36.0), and NBRF Protein (release 25.0).

Results

Immunoblotting. Screening of eight PM-Scl sera by immunoblotting of MOLT-4 cell extract revealed reactivity with two antigens of 75 and 110 kD (Fig. 1). Reactivity with the 75-kD antigen was observed in two of eight sera (lanes *1* and *2*) and with the 110-kD antigen in four of eight sera (lanes *2, 4, 6,* and *8*). One serum displayed clear preference for the 75-kD antigen with a weak 110-kD reactivity (lane *1*), a single serum displayed equal reactivity with the 75- and 110-kD antigens (lane *2*), and all other positive reactivities were with the 110-kD antigen. Similar reactivities with the 75- and 110-kD antigens were also observed in HeLa and HepG2 cellular extracts.

Reactivity with an additional 80-kD antigen of MOLT-4 cells was occasionally observed with PM-Scl sera positive for antibody to the 75-kD antigen (not reproduced in Fig. *1,* but see Fig. *2*). The 80-kD antigen appeared to be cell growth
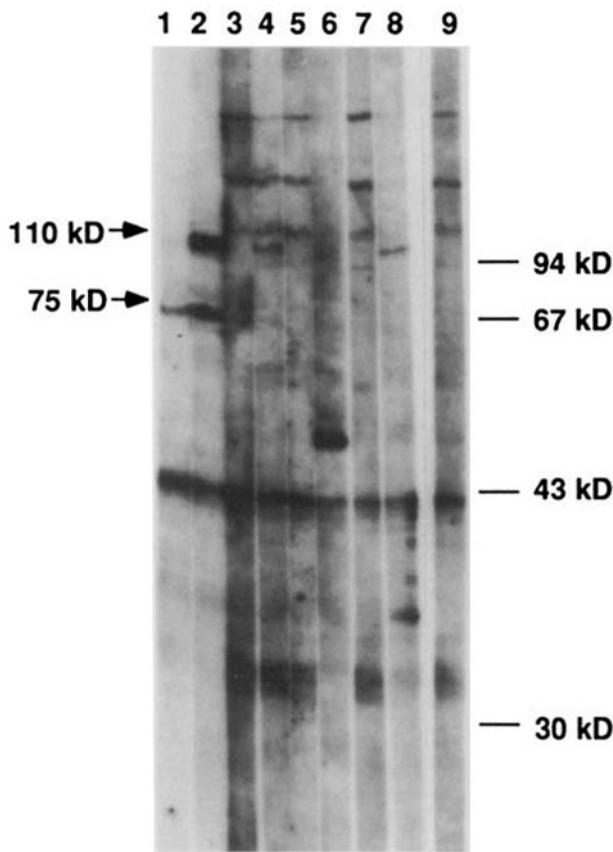
Figure 1. Immunoblotting profile of PM-Scl sera. MOLT-4 whole cell extract was separated by SDS-PAGE, transferred to nitrocellulose, and probed with sera at a dilution of 1:100. Lanes *1–8* show reactivity with PM-Scl sera, and lane *9* shows reactivity with normal control serum. Reactive bands common to normal control serum and PM-Scl sera were nonspecific reactivity. Arrows at left hand edge indicate position of 110- and 75-kD PM-Scl antigens. Molecular mass markers are indicated at right hand edge.

tive signals were obtained, four of which retained specificity after secondary and tertiary screening to 100% purity. Insert size of λM4.23 was found to be 600 bp and those of clones λM4.62, λM9.12, and λM10.61, were all 700 bp. The cDNA inserts were subcloned into Bluescript plasmid, sequenced across the polylinker arms, and found to represent one sequence, with all containing a poly-A tail. The sequence was used to search the Genebank, EMBL, and NBRF data banks for homologous sequences, with no close similarity found with any reported sequences, implying our clone was new and unique.

The cDNA inserts in λ clones were shown to code for a portion of the 75-kD antigen by affinity purification of antibody from IPTG-induced fusion protein of λM9.12. Using a selected PM-Scl serum containing reactivity with both the 75 and 100-kD antigens (Fig. 2 *A*, lane *1*), adsorption against λM9.12 fusion protein selectively removed the 75-kD reactivity from the serum but not the 100-kD reactivity (Fig. 2 *A*, lane *2*). The complete adsorption of 75-kD reactivity from the serum suggested that the major autoepitope(s) was located within the sequence of the polypeptide encoded by λM9.12. Reactivity with the 75-kD antigen was subsequently found in the affinity-purified antibodies from the λM9.12 fusion protein (Fig. 2 *A*, lane *3*). The fact that the eluted antibodies from the recombinant fusion protein were also able to identify the 80-kD antigen suggested that the 75- and 80-kD polypeptides may share antigenic determinants. These eluted antibodies were further shown to recognize the nucleolus and nucleoplasm of HEp-2 cells by indirect immunofluorescence (Fig. 2 *C*) in a pattern similar to that produced by whole PM-Scl serum (Fig. 2 *B*).

Further screening of 100,000 plaques from the MOLT-4 λgt-11 library with [^{32}P]-labeled synthetic oligonucleotide corresponding to the 5' end of pM9.12 cDNA resulted in isolation of 12 positive plaques of which nine remained positive through subsequent screening. From this group, λA3.21 with an insert of ~1.6 kb was subcloned into Bluescript (pA3.212) and its sequence was subsequently determined in both directions. The sequence strategy is illustrated in Fig. 3 *A*. Clone pA3.212 was composed of a 419-nucleotide 5' untranslated region, 1,065 nucleotides of coding sequence, and 78 nucleotides of 3' untranslated sequence. The complete nucleotide and deduced amino acid sequence is shown in Fig. 3 *B*. The sequence contained a Kozak consensus sequence (28, 29) at the ATG start codon and an ATTAAA polyadenylation signal 18 nucleotides upstream from a string of 20 As at the 3' end. The deduced amino acid sequence consisted of 355 residues with a predicted molecular weight of 39,190 and calculated pI of 4.8 as determined by the protein analysis program ISOELECTRIC (27). From the deduced amino acid sequence, the protein displayed a high proportion of charged residues at its COOH end, with a string of seven basic amino acids (KRRKKKR) between residues 346 and 352 that could be a nuclear or nucleolar localization signal for the molecule (30, 31).

Northern Blot Analysis. Clone pA3.212 appeared to be bona fide clone with a 5' untranslated region, a long open reading frame and a poly-A tail. Due to the large discrepancy between
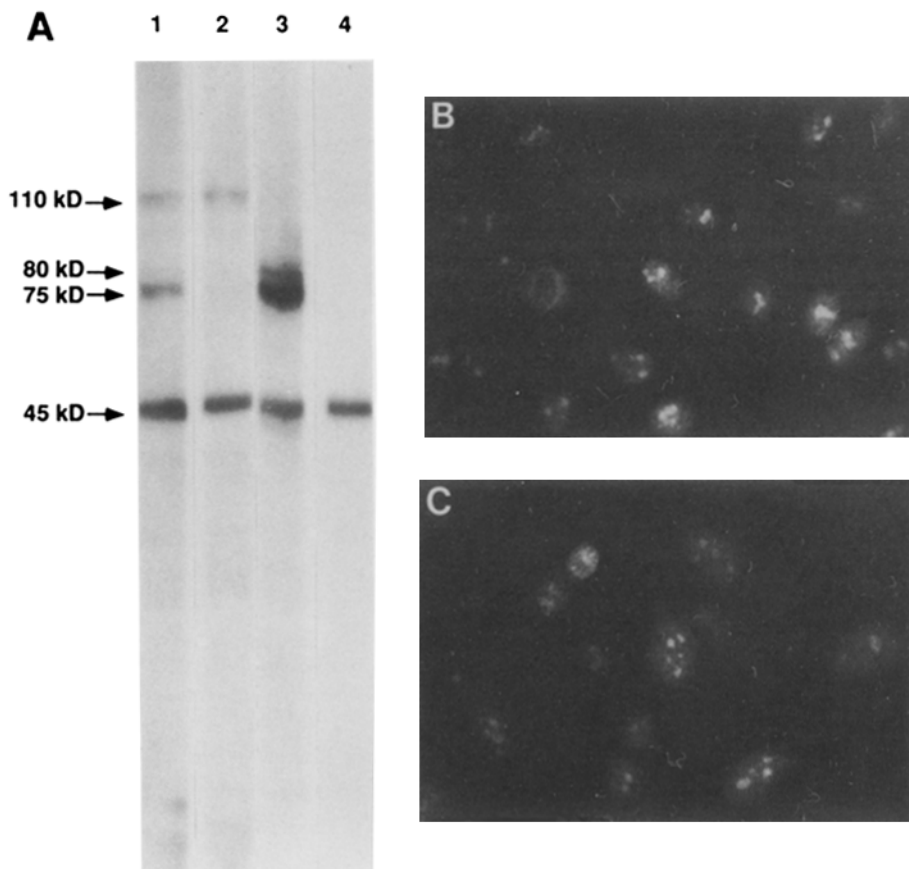
associated, in that MOLT-4 cells examined in log phase of growth displayed the 80-kD antigen, with much weaker reactivity observed when cells were not dividing. Reactivity with the 80-kD antigen was always weaker than the 75-kD reactivity and never observed alone. The association or relationship of the 75- and 80-kD antigens was not addressed from these data and will be the subject of future study.

Earlier immunoblotting studies from this laboratory initially assigned 80-kD to an antigenic polypeptide of PM-Scl (11). From our present immunoblotting data, we believe the molecular mass of the two antigens of PM-Scl are more accurately described as 110 and 75 kD. The 110-kD molecular mass estimate is in agreement with the recent observations of Gelpi et al. (12). It is possible that the previously reported 80-kD reactivity (11) is identical to the 80-kD reactivity we observed with log phase MOLT-4 cells, but this seems unlikely since the 80-kD band was never observed without 75-kD reactivity and was not the major reactivity in this region.

Cloning and Sequencing of PM-Scl cDNA. Human serum Lea (Fig. 1, lane *1*), reactive predominantly with the 75-kD antigen of PM-Scl, was chosen to screen a MOLT-4 λgt-11 expression library. From screening 970,000 plaques, 11 posi-

A

110 kD→
80 kD→
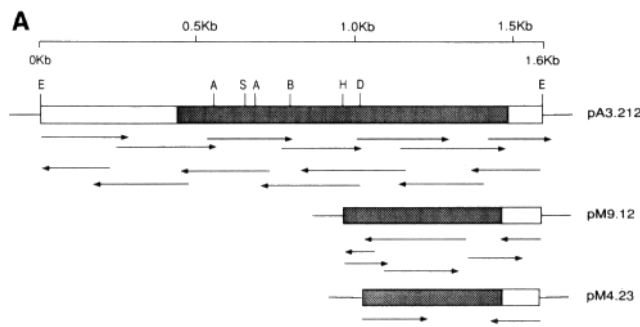75 kD→
45 kD→

1 2 3 4

Figure 2. Immunoblotting and indirect immunofluorescence of affinity-purified antibodies. Antibodies were purified from λM9.12 fusion protein. (*A*) Immunoblotting of PM-Scl serum containing reactivity with 75- and 110-kD antigens in MOLT-4 whole cell extract (lane *1*), immunoblotting reactivity remaining in serum after adsorption against λM9.12 fusion protein (lane *2*), immunoblotting reactivity eluted from λM9.12 fusion protein (lane *3*), and reactivity of the blocking solution used for antibody dilution (lane *4*). Reactive band at ∼45 kD is nonspecific reactivity. (*B*) Indirect immunofluorescence of whole PM-Scl serum, and (*C*) antibody eluted from λM9.12 fusion protein.

the predicted molecular weight and the observed molecular mass by immunoblotting of cellular extracts, the size of the mRNA encoding the 75-kD antigen was determined. Whole RNA from MOLT-4 and HL60 cells were probed with [³²P]-labeled cDNA insert of pA3.212. Fig. 4 shows a single mRNA of ∼1.6 kb hybridized by the labeled cDNA, suggesting that pA3.212 could be encoding the full-length clone for the "75-kD" PM-Scl antigen.

In Vitro Transcription and Translation Products. Having established that the cDNA of pA3.212 might probably encode the full-length sequence for the 75-kD antigen, the in vitro transcribed and translated products of pA3.212 were further analyzed (Fig. 5 *A*). Three prominent products were seen, with the largest migrating at ∼70 kD, and two lower molecular mass products migrating at 45 kD (possibly a doublet) and 30 kD (Fig. 5 *A*, lane *1*). The nature of the lower molecular mass products was unclear and might be the result of degradation, internal start sites, or premature termination of translation. The cellular PM-Scl 75 from MOLT4 cell extract was composed to the full-length in vitro translation product and bacterial pORF75 fusion protein. SDS-PAGE of MOLT-4 cell extract, recombinant fusion protein and [³⁵S]methionine labeled translation product followed by immunoblotting with PM-Scl sera confirmed the translation product and fusion protein migrated with a lower molecular mass than the native molecule (Fig. 5 *B*). It is interesting to note that the fusion protein encoding PM-Scl 75 (Fig. 5

B, lane *2*) did not migrate at a higher molecular mass than the in vitro translation product (Fig. 5 *B*, lane *3*) even though it coded an extra 30 amino acids (∼3.4 kD) derived from β-galactosidase. The possibility of electrophoretic artifact causing the mol wt difference between native and recombinant proteins was eliminated by immunoblotting experiments involving mixing of [³⁵S]methionine-labeled translation product and MOLT-4 cell extract. It was found that the signal produced by [³⁵S]methionine-labeled translation product was slightly lower than the signal produced with the cell extract after immunoblotting with PM-Scl serum (data not shown). The nature of the difference in molecular mass of the translation product and fusion protein with the native 75-kD antigen is not known at this point. It might imply post-translational modification of the antigen. Using the glycosylation system provided with the in vitro translation kit, we found that the full-length translation product was not glycosylated to a higher molecular mass (data not shown).

The [³⁵S]labeled in vitro translation product was immunoprecipitated with a panel of PM-Scl sera (Fig. 5 *C*, lanes *2–9*), disease control sera (Fig. 5 *C*, lanes *10–14*) and healthy control sera (Fig. 5 *C*, lanes *15* and *16*). Reactivity with the 70-kD translation product was prominent with two PM-Scl sera (Fig. 5 *C*, lanes *2* and *3*, the same two that immunoblotted the 75-kD antigen in cell extracts), while the remaining sera displaying weaker or no reactivity (Fig. 5 *C*). The lower 45- and 30-kD translation products also appeared to be

A

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        0.5Kb           1.0Kb           1.5Kb
0Kb                                              1.6Kb
  E           A  S A    B      H D            E
                                                    ___ pA3.212


                                                    ___ pM9.12


                                                    ___ pM4.23
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B

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-419 CGACCGGCACGTTCACCCCATCCCTCAGGCTTTATTTATTTTTTTCGACAGGTTCTTTTCAAGG
     CTCCAGTCACCGCAGCAGTTGTCCATGCTGTAGTTTCCACTTTCCTGTATGGGCGGGCTGGTTAGG
     ATTCCACTTTCCCCCAAGTGCTTAGCCCAGGGCCAGACAAAAAGTAGTTGCTTAAGAAATACTTGT
     TGAAGGAATAAATTAATGAATGAATTTGTGCTTACAGCGGCTGGATGGCAGACAAACCTATGATTA
     TAGGAACATCAGGATCTCATTTGGAACAGATTACGGATGCTGCATTGTGGAACTTGGAAAAACAAG
     AGTTCTTGGACAGGTTTCCTGTGAACTTGTGTCTCCAAAACTCAATCGGGCAACAGAAGGTATTCT
     TTTTTTAACCTTGAACTCTCTCAG
   1 ATGGCCGCTCCAGCTTTCGAACCTGGCAGGCAGTCAGATCTCTTGGTGAAGTTGAATCGACTCATG  66
   1  M  A  A  P  A  F  E  P  G  R  Q  S  D  L  L  V  K  L  N  R  L  M   22

  67 GAAAGATGTCTAAGAAATTCGAAGTGTATAGACACTGAGTCTCTCTGTGTTGTTGCTGGTGAAAAG  132
  23  E  R  C  L  R  N  S  K  C  I  D  T  E  S  L  C  V  V  A  G  E  K   44

 133 GTTTGGCAAATACGTGTAGACCTACATTTATTAAATCATGATGGAAATATTATTGATGCTGCCAGC  198
  45  V  W  Q  I  R  V  D  L  H  L  L  N  H  D  G  N  I  I  D  A  A  S   66

 199 ATTGCTGCAATCGTGGCCTTATGTCATTTCCGAAGACCTGATGTCTCTGTCCAAGGAGATGAAGTA  264
  67  I  A  A  I  V  A  L  C  H  F  R  R  P  D  V  S  V  Q  G  D  E  V   88

 265 ACACTGTATACACCTGAAGAGCGTGATCCTGTACCATTAAGTATCCACCACATGCCCATTTGTGTC  330
  89  T  L  Y  T  P  E  E  R  D  P  V  P  L  S  I  H  H  M  P  I  C  V  110

 331 AGTTTTGCCTTTTTCCAGCAAGGAACATATTTATTGGTGGATCCCAATGAACGAGAAGAACGTGTG  396
 111  S  F  A  F  F  Q  Q  G  T  Y  L  L  V  D  P  N  E  R  E  E  R  V  132

 397 ATGGATGGCTTGCTGGTGATTGCCATGAACAAACATCGAGAGATTTGTACTATCCAGTCCAGTGGT  462
 133  M  D  G  L  L  V  I  A  M  N  K  H  R  E  I  C  T  I  Q  S  S  G  154

 463 GGGATAATGCTACTAAAAGATCAAGTTCTGAGATGCAGTAAAATCGCTGGGGTGAAAGTAGCAGAA  528
 155  G  I  M  L  L  K  D  Q  V  L  R  C  S  K  I  A  G  V  K  V  A  E  176

 529 ATTACAGAGCTAATATTGAAAGCTTTGGAGAATGACCAAAAGTAAGGAAAGAAGGTGGAAAGTTT  594
 177  I  T  E  L  I  L  K  A  L  E  N  D  Q  K  V  R  K  E  G  G  K  F  198

 595 GGTTTTGCAGAGTCTATAGCAAATCAAAGGATCACAGCATTTAAAATGGAAAAGGCCCCTATTGAT  660
 199  G  F  A  E  S  I  A  N  Q  R  I  T  A  F  K  M  E  K  A  P  I  D  220

 661 ACCTCGGATGTAGAAGAAAAAGCAGAAGAAATCATTGCTGAAGCAGAACCTCCTTCAGAAGTTGTT  726
 221  T  S  D  V  E  E  K  A  E  E  I  I  A  E  A  E  P  P  S  E  V  V  242

 727 TCTACACCTGTGCTATGGACTCCTGGAACTGCCCAAATTGGAGAGGGAGTAGAAAACTCCTGGGGT  792
 243  S  T  P  V  L  W  T  P  G  T  A  Q  I  G  E  G  V  E  N  S  W  G  264

 793 GATCTTGAAGACTCTGAGAAGGAAGATGATGAAGGCGGTGGTGATCAAGCTATCATTCTTGATGGT  858
 265  D  L  E  D  S  E  K  E  D  D  E  G  G  G  D  Q  A  I  I  L  D  G  286

 859 ATAAAAATGGACACTGGAGTAGAAGTCTCTGATATTGGAAGCCAAGATGCTCCCATAATACTCTCA  924
 287  I  K  M  D  T  G  V  E  V  S  D  I  G  S  Q  D  A  P  I  I  L  S  308

 925 GATAGTGAAGAAGAAGAAATGATCATTTTGGAACCAGACAAGAATCCAAAGAAAATAAGAACACAG  990
 309  D  S  E  E  E  E  M  I  I  L  E  P  D  K  N  P  K  K  I  R  T  Q  330

 991 ACCACCAGTGCAAAACAAGAAAAAGCACCAAGTAAAAAGCCAGTGAAAAGAAGAAAAAAGAAGAGA 1056
 331  T  T  S  A  K  Q  E  K  A  P  S  K  K  P  V  K  R  R  K  K  K  R  352

1056 GCTGCCAATTAA 1068
 353  A  A  N  *  355
     GCTACAGTTGTATATCTGTATATATAACTATTAAAAGGGATATTTATTCCATTAAAAAAAAAAAA
     AAAAAAAAA 1143
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Figure 3. Sequence of cDNA encoding PM-Scl 75. (*A*) Restriction map and strategy for sequencing cDNA encoding PM-Scl 75 polypeptide. Sequencing was performed with synthetic oligonucleotides primers, and direction of sequencing is indicated by arrows. Restriction sites are *A*, AccI; *B*, BamHI; *D*, DraI; *E*, EcoRI; *H*, HindIII; *S*, StyI. (*B*) Complete nucleotide and deduced amino acid sequence of PM-Scl 75 polypeptide. Underlined are (*a*) the first upstream in frame stop codon (TGA) at nucleotide −69; (*b*) putative nuclear or nucleolar localization signal KRRKKKRA starting at amino acid 346; (*c*) polyadenylation signal ATTAAA at nucleotide 1099. Also indicated by arrowhead and arrow are positions of the 5' ends of λ clones M9.12 and M4.23, respectively. These sequence data are available from EMBL/Genbank/DDBJ under accession number M58460.
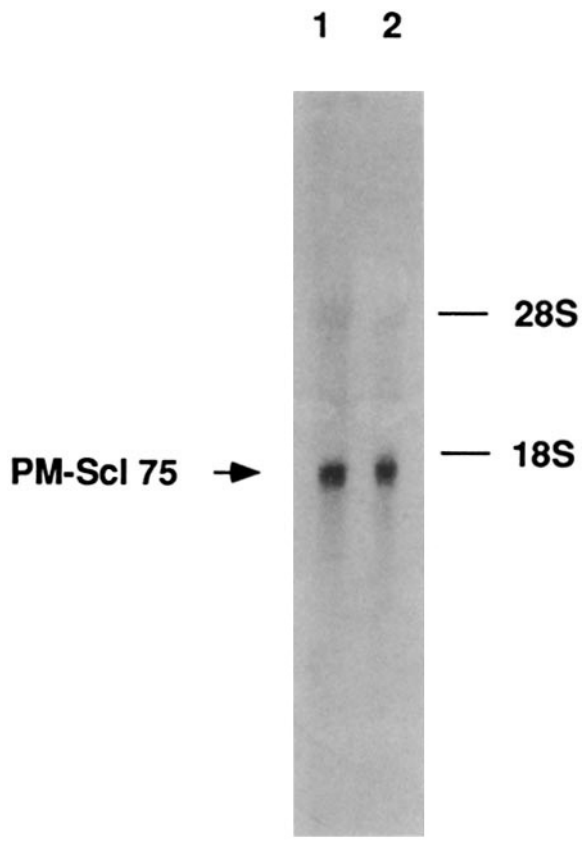

Figure 4. Northern blot analysis. Total RNA from MOLT-4 (1) and HL60 (2) cells were isolated and separated by agarose gel electrophoresis, transferred to Nylon paper, and probed with ^{32}P-labeled pA3.212. Position of PM-Scl 75 mRNA, and 28S and 18S rRNA markers are indicated.

specifically immunoprecipitated, indicating the presence of at least one epitope in these smaller fragments. This data supported the immunoblotting results in which only two sera were found to react with the cellular 75-kD antigen. The fact that the majority of the sera did not immunoprecipitate or reacted very poorly with the in vitro translation product suggested the absence or very low titer of antibodies to the 75-kD antigen.

Analysis of Aberrant Protein Migration in SDS-PAGE. The predicted molecular mass of 39.2 kD for the protein encoded by the full-length clone and the observed molecular mass of the in vitro translation product of 70 kD, suggested that the molecule had some characteristics that caused it to migrate aberrantly by SDS-PAGE. This phenomenon has been observed with other cloned polypeptides in which the highly charged nature of these molecules are implicated as the probable cause of the aberrant migration (see Discussion). The following describes the expression of truncated cDNA to localize the region of the protein that was responsible for the migration discrepancy.

In vitro translation product (IVTP) of truncated PM-Scl 75 cDNA encoding residues 1–212 (IVTP2) migrated at 24 kD (Fig. 6 *C*, lane *3*), which was close to the predicted 23.5
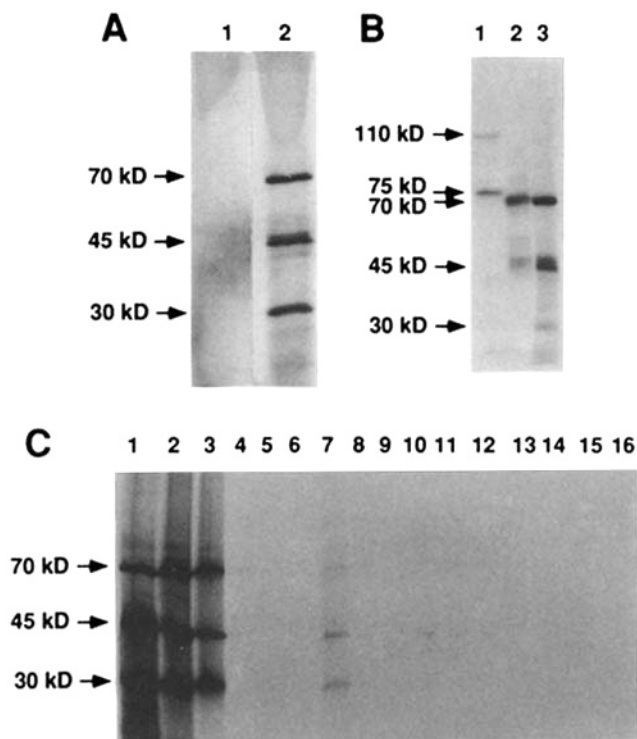
Figure 5. In vitro transcription and translation of pA3.212, and immunoprecipitation and comparison of apparent molecular mass with native PM-Scl 75 and pORF75 fusion protein. (*A*) In vitro translation product of pA3.212 linearized with SacI restriction enzyme, transcribed with T7 RNA polymerase, and translated in vitro in a rabbit reticulocyte system (lane *2*); and the no RNA control translation is shown in lane *1*. The largest translation product migrated at 70 kD with two smaller translation products also observed, migrating at 45 kD (doublet) and 30 kD. (*B*) Comparison of apparent molecular mass of native PM-Scl 75 (lane *1*), bacterial fusion protein ORF75 (lane *2*), and in vitro translation of full-length PM-Scl 75 cDNA (lane *3*). The samples were separated by SDS-PAGE and immunoblotted with PM-Scl serum. Reactivity seen in lanes *1* and *2* represented the signal from ^{125}I–protein A used to trace the bound IgG in immunoblotting, and the signal in lane *3* is from the [^{35}S]methionine-labeled translation product. Note the similarity in molecular mass of the reactive bands in the fusion protein extract and the in vitro translation products. (*C*) Immunoprecipitation of in vitro translation product with PM-Scl sera and control sera. Lane *1* represents the in vitro translation product used in the immunoprecipitation. Immunoprecipitation of PM-Scl sera are shown in lanes *2–9* and control immunoprecipitation were with sera containing auto-antibodies to U3 RNP (lane *10*), Ku (lane *11*), rRNP (lane *12*), SS-B/La (lane *13*), Sm (lane *14*), and with healthy control sera (lanes *15* and *16*). Note the gel was overexposed to detect weaker signals.

kD; similarly, truncation to yield IVTP3 (residues 1–123) produced a fragment that migrated at 14 kD (Fig. 6 *C*, lane *4*), which was also very close to the predicted 13.6 kD. The initial clone λM9.12 (residues 196–355) was expressed as a β-galactosidase fusion protein, FP1 (Fig. 6 *A*). The predicted molecular mass of FP1 was 133 kD, but the observed molecular mass was 160 kD in SDS-PAGE (Fig. 6 *B*, lane *3*), a difference of 27 kD. Identity of FP1 as coding for the carboxy region of PM-Scl 75 was confirmed by immunoblotting with PM-Scl sera (Fig. 6 *B*, lanes *4–6*) and mouse mAb

to β-galactosidase (Fig. 6 *B*, lanes *7–9*). The latter antibody also identified β-galactosidase present in BioRad high molecular mass standards (Fig. 6 *B*, lane *7*). Thus, a discrepancy of 27 kD was observed, which to a major extent could account for the difference in molecular mass between the deduced molecular mass of full-length PM-Scl 75 (39.2 kD) and the apparent molecular mass of recombinant fusion protein encoding PM-Scl 75 (70 kD) observed in SDS-PAGE. A summary of the results along with the deduced pI for the truncated in vitro products are outlined in Table 1, and indicates that the NH$_2$ half of the polypeptide migrated as predicted and the COOH half of the molecule was the cause of the aberrant migration in SDS-PAGE. The possible nature of the aberrant migration could be associated in part with the highly acidic nature of the COOH half of the molecule. Comparison of the pI values of IVTP2 and FP1, from Table 1, shows that the COOH half of PM-Scl 75 is over 2 pH units more than the NH$_2$ half of the molecule.

Immunoblotting of Fusion Protein. Fusion proteins derived from full-length cDNA subcloned in plasmid pORF75 and control plasmids were analyzed by immunoblotting using PM-Scl and control sera. The fusion protein produced by pORF75 was reactive with PM-Scl sera previously shown to immunoblot the 75-kD antigen in whole cell extracts and not with control disease serum or normal human serum (Fig. 7 *A*). As with the in vitro translation product, the fusion protein also migrated with a molecular mass lower than the native molecule (Fig. 7 *A*, lane *2*). In addition, reactivity was also seen with lower molecular mass bands that may correspond to the lower molecular mass products of the full-length cDNA in vitro translation. Specificity was shown using Bluescript vector control and ABN9 clone encoding a COOH fragment of the 60-kD SS-A/Ro autoantigen. These fusion products were not identified by the PM-Scl serum (Fig. 7 *A*, lanes *3* and *4*). The SS-A/SS-B serum did not react with pORF75 fusion protein (Fig. 7 *A*, lane *6*), but it did identify the native SS-A/SS-B polypeptides (Fig. 7 *A*, lane *5*) and partial SS-A/Ro fusion protein (Fig. 7 *A*, lane *7*). The normal human control serum did not show specific reactivity with cell extract or fusion proteins (Fig. 7 *A*, lanes *9–12*). The panel of eight PM-Scl sera used in MOLT-4 immunoblotting were subsequently used for immunoblotting of pORF75 fusion protein. The 2 PM-Scl sera which reacted with MOLT-4 cellular PM-Scl 75 (Fig. 1, lanes *1* and *2*), displayed strong reactivity with the 70-kD fusion protein (Fig. 7 *B*, lanes *1* and *2*). Three additional PM-Scl sera also displayed weaker anti–70-kD fusion protein reactivity (Fig. 7 *B*, lanes *3–5*). In addition, antibody eluted from the 70-kD recombinant fusion protein was shown to immunoblot a 75-kD antigen of MOLT-4 cell extract (Fig. 7 *C*, lane *2*), corresponding to the same molecular mass as the antigen recognized by PM-Scl serum (Fig. 7 *C*, lane *1*). This further strengthened the argument that the recombinant fusion protein and thus cDNA pA3.212 encoded PM-Scl 75 polypeptide.

Rabbit Antibody to Recombinant Fusion Protein. Further support for the authenticity of our cloned cDNA as coding for PM-Scl 75 was obtained by analyzing rabbit antisera raised
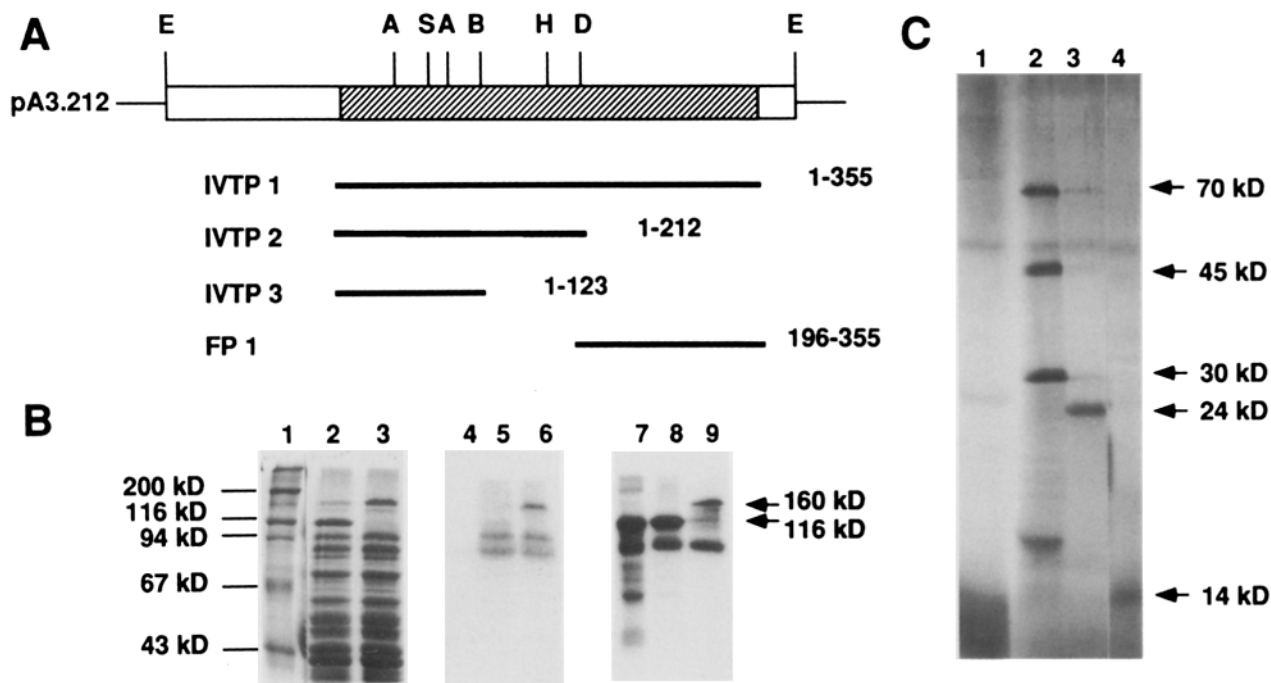
Figure 6. Analysis of aberrant protein migration in SDS-PAGE. (*A*) Diagrammatic illustration of PM-Scl cDNA employed for in vitro translation products or fusion protein. (*B*) Immunoblotting analysis of FP1. Lanes *1, 4,* and *7* were BioRad high molecular mass standards: myosin (200 kD), β-galactosidase (116 kD), phosphorylase B (94 kD), BSA (67 kD), and OVA (43 kD). Lanes *2, 5,* and *8* were wild-type λgt-11 β-galactosidase and lanes *3, 6,* and *9* were λM9.12 (FP1) fusion protein. Lanes *1-3* were stained by Coomassie blue. Lanes *4-6* and *7-9* were probed with PM-Scl serum and mouse mAb to β-galactosidase, respectively. Positions of the 160-kD λM9.12 fusion protein and the 116-kD β-galactosidase are indicated at right hand edge. (*C*) In vitro translation products of no RNA control (lane *1*), IVTP1 (lane *2*), IVTP2 (lane *3*), and IVTP3 (lane *4*) were separated by 10–25% gradient SDS-PAGE, dried, and exposed to autoradiographic film. Arrows at right hand edge indicate position of the 70-, 45-, 30-, 24-, and 14-kD translation products. Higher molecular mass translation products observed in lanes *3* and *4* were due to incomplete restriction enzyme digestion to generate the truncated transcripts.

against pORF75 fusion protein. The resulting antibodies were shown to react in a similar manner to PM-Scl sera by indirect immunofluorescence, immunoblotting, and immunoprecipitation. Fig. 8 *A* illustrates the immunofluorescence staining

Table 1. *Deduced and Observed Molecular Mass of Defined Regions of PM-Scl 75 Protein*

cDNA constructs	Amino acids represented	Deduced molecular mass	SDS-PAGE molecular mass	Deduced pI*
		(kD)	*(kD)*	
IVTP1‡	1–355	39.1	70	4.80
IVTP2	1–212	23.5	24	6.50
IVTP3	1–123	13.6	14	5.99
FP1§	196–355	17.4	44	4.37

* Deduced molecular mass and pI were calculated using the sequence analysis software package (49).
‡ In vitro translation products were separated by SDS-PAGE and molecular mass determined from autoradiograph.
§ λgt-11 fusion protein was separated by SDS-PAGE and molecular mass determined directly from Coomassie blue stained gel.

pattern of PM-Scl serum (Fig. 8 *A*), showing the characteristic nucleolar and nucleoplasmic staining, and the induced nucleolar reactivity in rabbit sera (Fig. 8 *C*) after immunization with recombinant fusion protein. It was difficult to assess if nucleoplasmic staining was present due to the inherent background staining even observed with rabbit pre-immune serum (Fig. 8 *B*). Immunoblotting of MOLT-4 cellular extract with rabbit antisera showed reactivities similar to that produced by human serum (Fig. 8 *D*). The rabbit antiserum reacted specifically with the 75-kD antigen in MOLT-4 extract (Fig. 8 *D*, lane *3*). Prebleed serum showed no reactivity with MOLT-4 protein (Fig. 8 *D*, lane *2*). Finally, rabbit antiserum was able to immunoprecipitate in vitro translation product in an identical manner to human PM-Scl sera (Fig. 8 *E*, compare lanes *3* and *4* with lane *6*).

Discussion

This work is aimed at extending the knowledge of the autoantigen system PM-Scl. To begin analysis of this nucleolar complex, we have cloned the full-length cDNA of the 75-kD autoantigen identified by PM-Scl sera and named this PM-Scl 75. The 1.6-kb cDNA coded for a protein of 355 amino acids with aberrant SDS-PAGE migration, which was found to be associated with the highly charged COOH half of the
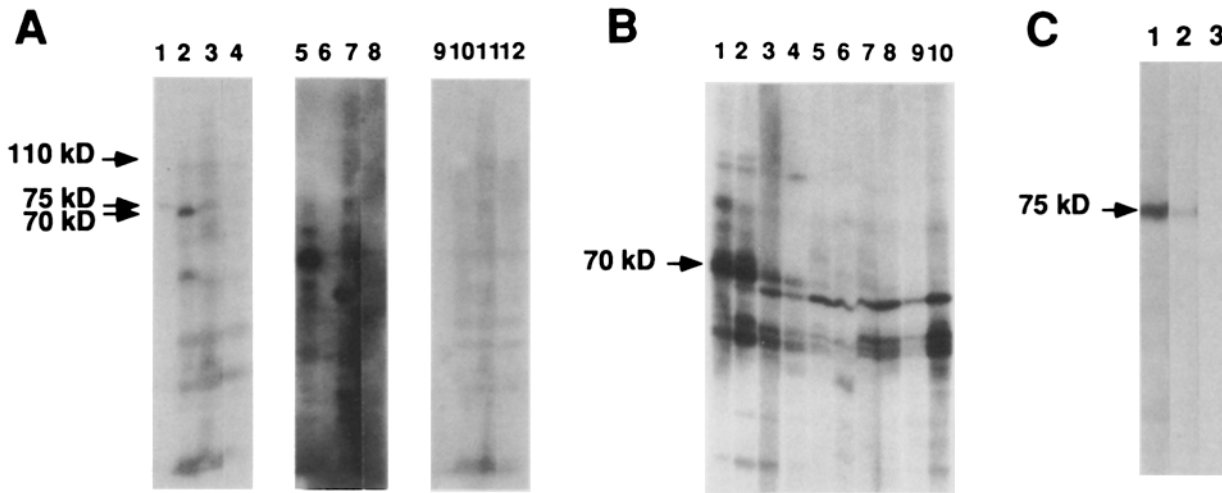
Figure 7. Immunoblotting analysis of fusion proteins and PM-Scl sera. (A) MOLT-4 whole cell extract (lanes 1, 5, and 9), PM-Scl 75 full-length fusion protein derived from pORF75 (lanes 2, 6, and 10), partial SS-A/Ro fusion protein ABN9 (lanes 3, 7, and 11), and proteins from Bluescript vector alone (lanes 4, 8, and 12) were probed with a PM-Scl serum (lanes 1–4), an anti–SS-A/SS-B serum (lanes 5–8), and a normal human serum (lanes 9–12). PM-Scl autoantibodies reacted with the 75- and 110-kD antigens of MOLT-4 extract (lane 1) and fusion protein products of 70 and 45 kD (lane 2). No specific reactivity was seen with the control fusion products (lanes 3 and 4). Control serum with SS-A/SS-B autoantibodies reacted specifically with the MOLT-4 SS-A/SS-B proteins (lane 5) and the fusion protein SS-A/Ro (lane 7). The normal serum showed no specific reactivity with MOLT-4 extract or fusion proteins (lanes 9–12). (B) full-length fusion protein from pORF75 was immunoblotted with the panel of eight PM-Scl sera (lanes 1–8) or normal control sera (lanes 9 and 10). Reactivity with the 70-kD fusion protein is seen in lanes 1–5. (C) Immunoblotting analysis of affinity-purified antibodies from the 70-kD fusion protein. MOLT-4 whole cell extract was probed with a PM-Scl serum with 75-kD reactivity (lane 1), antibody eluted from the 70-kD fusion protein (lane 2), and antibody eluted from an unrelated area of nitrocellulose (lane 3).
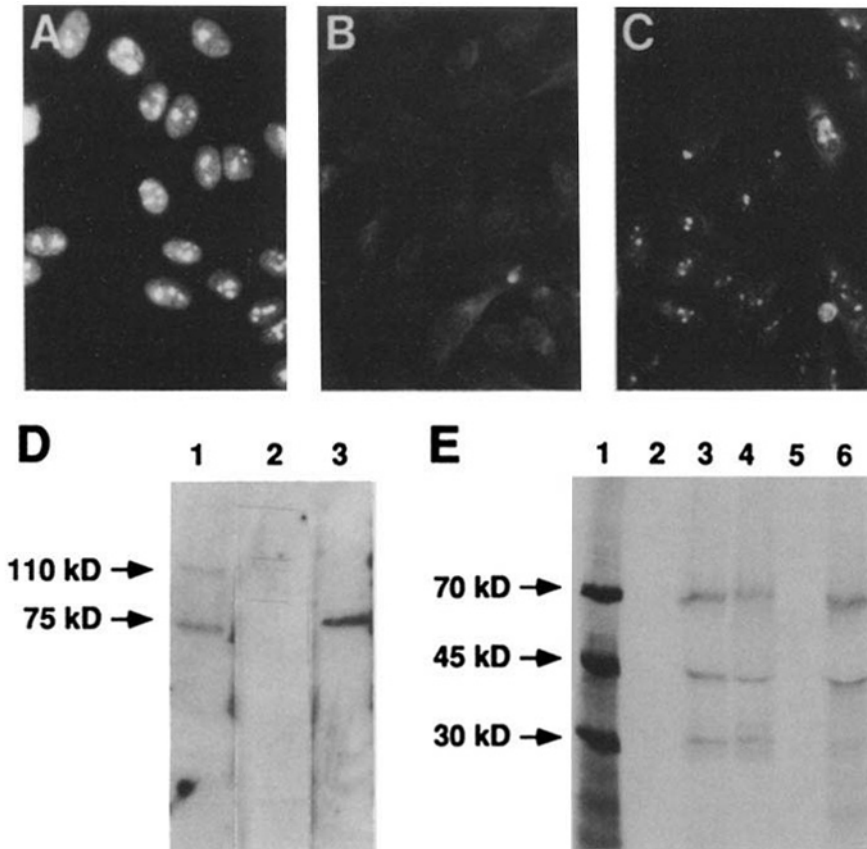

Figure 8. Analysis of rabbit antibodies raised against full-length recombinant fusion protein. Indirect immunofluorescence on HEp-2 cells of human PM-Scl serum displaying characteristic nucleolar/nucleoplasmic staining (A), rabbit serum before immunization showing no reaction (B), and rabbit serum after immunization clearly showing nucleolar reactivity (C). The presence of nucleoplasmic associated staining could not be confirmed due to background staining. (D) Immunoblotting of MOLT-4 cellular extract with human PM-Scl and rabbit sera. Samples were separated by 10% SDS-PAGE and immunoblotted with PM-Scl serum reactive with 110- and 75-kD antigens (lanes 1), rabbit serum before immunization (lane 2) and after immunization (lane 3). Positions of 110- and 75-kD antigens are indicated by arrows. (E) Immunoprecipitation of in vitro translation products with human PM-Scl sera (lanes 3 and 4), rabbit serum before immunization (lane 5) and after immunization (lane 6). Lanes 1 and 2 represent translation product alone and a no serum control immunoprecipitation, respectively. Positions of translation products are indicated by arrows.

molecule. Although this study has not attempted to define the antigenic determinants of PM-Scl 75, a region of 138 amino acids that must contain an antigenic determinant was identified. This was the coding region of λM4.23 initially isolated by antibody screening of the MOLT-4 cDNA library. Whether other antigenic sites are present on the molecule will be examined in future studies.

Features of PM-Scl 75. Clone pA3.212 has many characteristics of a full-length clone. It contains a 5' untranslated region, a long open reading frame, and a 3' untranslated region with a poly-A tail. Northern blot analysis provided independent evidence that the mRNA and thus full-length clone for PM-Scl 75 was ∼1.6 kb. Analysis of the predicted primary structure of pA3.212 shows an area of basic residues beginning at position 346 that may encode a nuclear/nucleolar localization signal. The string of residues KRRKKKRA between 346 and 353 resembles the nuclear localization signal KKKRK of SV-40 large T antigen (30), and also displays similarities with the molecular signal RKKRRQRRRA found in the HIV tat protein (31). Whether this region of PM-Scl 75 is actually responsible for nuclear/nucleolar localization of PM-Scl 75 will need to be confirmed experimentally.

The discrepancy between the deduced molecular mass of the amino acid sequence of pA3.212 at 39.2 kD and the observed molecular mass of the 70-kD in vitro translation product and fusion protein in SDS-PAGE is an interesting phenomenon. Differences between observed and predicted molecular mass have been documented for a number of cloned polypeptides including the 70-kD autoantigen of U1-snRNP (32), the centromere autoantigen CENP-B (33), SS-A/Ro (34), amphibian N1/N2 histone binding protein (35), and amphibian nucleoplasmin (36). It is still unclear why these proteins have an aberrant migration in SDS-PAGE, but the highly charged nature of these proteins, especially regions of high negative charge, are thought to interfere with SDS binding and thus influence migration in SDS gels (33, 34, 36). Analysis of the primary structure of PM-Scl 75 reveals that the COOH half contains a high proportion of acidic residues. In this region, there is a single stretch of 101 residues beginning at position

220 in which the charged amino acids are almost exclusively acidic. The deduced pI for the COOH half of PM-Scl 75 is > 2 pH units more acidic than the NH₂ half. We have been able to separate and express the NH₂ and COOH regions of PM-Scl 75 and have shown that the highly acidic carboxy half had the ability to directly cause aberrant gel migration.

An obvious goal in the study of PM-Scl would be the elucidation of its structure and function. The available data on the complex, such as its localization to the granular region of the nucleolus (11), its presence in the nucleoplasm, its absence from the cytoplasm, and the apparent migration of nucleolar staining to the nucleoplasm after actinomycin D treatment (12), suggest that it may be associated with ribosomal biogenesis. This however must remain speculation since no direct evidence associating PM-Scl with ribosomes has been described. Previously described proteins found or thought to be associated with pre-ribosomes include the 40-kD protein ribocharin, which was associated with the precursor of the large ribosomal subunit (37), a 100-kD protein with protease activity (38), a 94-kD protein localized to the dense fibrillar component and granular region of the nucleolus (39), and protein B23 associated with the 55S rRNA subunit (40). Superficially, these polypeptides appear distinct from the PM-Scl polypeptides but until directly examined, this must remain assumption.

In summary, we describe the cloning of a 1.6-kb cDNA encoding the 75-kD polypeptide of the PM-Scl complex. The molecule was found to migrate aberrantly by SDS-PAGE due to the charged residues in its COOH portion. The recombinant polypeptide was found to elicit antibodies in rabbits with characteristics of human PM-Scl 75 autoantibody. The role of PM-Scl 75 in the autoimmune response of PM-Scl patients is unclear, but can now be examined in more detail through the study and use of this cloned polypeptide. In addition, PM-Scl 75 is the first polypeptide cloned from the PM-Scl particle and may aid in characterizing other components of PM-Scl and in elucidating the structure and function of this complex.

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