Antifibrillarin Autoantibodies Present in Systemic Sclerosis and Other Connective Tissue Diseases Interact with Similar Epitopes

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Summary

Autoantibodies specific against fibrillarin, a 34-kD nucleolar protein associated with U3-snRNP, are present in patients with systemic sclerosis (SSc). To understand the mechanisms involved in the induction of these autoantibodies, we prepared a series of human fibrillarin recombinant proteins covering the entire molecule and analyzed their interaction with the autoantibodies present in various connective tissue diseases. Our results showed that antifibrillarin autoantibodies are present not only in SSc, as previously reported, but also in a variety of other connective tissue diseases. Patients with SSc (58%), mixed connective tissue diseases (60%), CREST syndrome (calcinosis, Raynaud phenomenon, esophageal dismotility, sclerodactyly, and telangiectasia syndrome) (58%), systemic lupus erythematosus (39%), rheumatoid arthritis (60%), and Sjogern’s syndrome (84%) showed presence of antifibrillarin autoantibodies. Results obtained from competitive inhibition radioimmunoassay and Western blot analyses with purified recombinant fusion proteins revealed that these autoantibodies react primarily with epitope(s) present in the NH2- (AA 1-80) and COOH-terminal (AA 276-321) domains of fibrillarin. Autoantibodies reacting with internal regions of fibrillarin are less frequent. Analysis of the hydrophilicity profiles of reactive peptides showed presence of three potential antigenic sites in the NH2- and two in the COOH-terminal regions. While a hexapeptide sequence NH2 terminus of fibrillarin is shared with an Epstein-Barr virus-encoded nuclear antigen, the COOH-terminal region shares sequence homology with P40, the capsid protein encoded by herpes virus type 1. Interestingly, these two regions of fibrillarin also contain the most immunodominant sequences, as predicted by surface probability and the Jameson and Wolf antigenic index. These observations suggest that molecular mimicry might play an important role in the induction of antifibrillarin autoantibodies.
bodies present in scleroderma patients and then to determine
SS reacted equally well with the epitopes present at the
drome, which reacted primarily with the epitopes located at
autoantibodies present in SSc, MCTD, SLE, and CREST syn-
or similar epitopes are recognized. However, in contrast to
a similar immunochemical specificity suggesting that the same
are directed against a common epitope(s) or a characteristic
preparing a panel of six overlapping fibrillarin fusion pro-

Materials and Methods.

Sera. Sera were obtained from patients with various connec-
tive tissue diseases such as SSc, CREST syndrome, MCTD, RA,
SS, and SLE and control healthy subjects were stored at −20°C
until use (the patients were classified by the criteria described by
the American Association of Rheumatism). A total of 237 serum
samples (56 SSc, 81 SLE, 22 MCTD, 11 CREST syndrome, 38
RA, 15 SS, and 14 healthy subjects) were used in this study.

Antigens. Recombinant human fibrillarin fusion proteins, hu-
man Sm antigen (kindly provided by Dr. H. Dang, University of
Texas, San Antonio, TX), rat collagen I (from Dr. R. Holmdahl,
University of Uppsala, Uppsala, Sweden), human myelin basic pro-
tein (MBP) (from G. Hashin, Saint Lukes Hospital, New York),
and dsDNA were used as antigens in the direct-binding and com-
petitive inhibition RIA. Recombinant fusion proteins containing
five contiguous moieties of human fibrillarin were obtained by
cloning the corresponding cDNA sequences into pGEX-2T expres-
sion vector (28).

Expression Cloning of Human Fibrillarin cDNA. The coding re-
gion of human fibrillarin cDNA (cDNA clone kindly provided by
Dr. J. Aris, University of Florida, Gainesville, FL) and the DNA
sequences corresponding to amino acid residues 1-321, 1-85, 86-
148, 149-229, 230-275, and 276-321 of fibrillarin were amplified
with oligonucleotide primers by PCR (29). The amplified DNA
fragments were subcloned into pGEX-2T expression vector, which
expresses glutathione-S-transferase (GST). The cloned cDNAs were
expressed as GST fusion proteins. Schematic representation of the
fibrillarin regions that were cloned is shown in Fig. 1. The fol-
lowing primers were used for PCR amplification: (a) primer 01:
5'-atgaagccaggattcatgcctccc-3'; primer 321: 5'-gttcttcaccttggtgggt-
3'; (b) primer 01: 5'-atgaagccaggattcatgcctccc-3'; primer 85: 5'-aat-
ggattcatgcctccc-3'; (c) primer 86: 5'-atccttttctcctgctgctccc-3';
primer 148: 5'-gattctgctgctgctgctgctccc-3'; (d) primer 149: 5'-atc-
ttgctgctgctgctgctccc-3'; primer 229: 5'-cattgcgatgagcatgcggta-
3'; primer 230: 5'-atgctggatctgatctttgctg-3'; primer 275: 5'-ggctga-
atctgatctttgctg-3'; primer 321: 5'-gttcttcaccttggtgggt-3'. The 5' and 3'
end primers contained BamHI and EcoRI sites, respectively, at the 5'
end of the sequence. The amplified DNAs were digested with restric-
tion enzymes, ligated to BamHI–EcoRI–cut pGEX-2T plasmid,
and the BL-21 competent cells were transformed. The bacteria ex-
pressing the recombinant plasmids were selected on ampicillin plates.
The recombinants were grown in 1 ml Luria broth (LB) medium
and induced with 1 mM isopropyl β-D-thiogalactopyranoside (IPTG)
for 5 h at 37°C. The bacterial lysates were run on SDS–polyacryl-
amide gels, and the recombinants expressing appropriate molecu-
lar size fusion proteins were selected. Plasmid DNAs were se-
genated by the dideoxy chain termination method (30), with an
oligonucleotide primer 5'-gttcttcaccttggtgggtg-3', corresponding to
855-871 of the pGEX sequence, to confirm that the inserts were
cloned in frame.

![Schematic diagram showing regions of human fibrillarin cloned as fusion proteins.](image-url)

Figure 1. Schematic diagram showing regions of human fibrillarin expressed as fusion proteins. DNA fragments coding for defined regions of fibrillarin A-F generated by PCR were cloned into pGEX-2T expression vector to produce six in-frame fusion proteins as described in Materials and Methods. Fibrillarin proteins containing 13 hydrophilic regions were kept intact to preserve the potential antigenic sites formed by linear amino acid sequences.
Antigen-binding Studies. Direct-binding assay. Microtiter plates were coated with recombinant fusion proteins or GST (as control) at 5 μg/ml in carbonate buffer, pH 9.0, at 4°C, overnight. After washing, the plates were blocked with 5% BSA in PBS for 1 h at room temperature, washed three times, and incubated with 1:400 dilution of sera for 2 h. After thorough washing, 5 × 10^4 cpm of 125I-labeled rabbit F(ab')2 anti-human γ antibody in 50 μl was added and incubated for a further 2 h. After washing, the bound radioactivity was measured in a gamma counter. Cpm bound to GST coated plates were subtracted from the cpm bound to fibrillarin fusion protein-coated plates and expressed as specific cpm bound.

Competitive Inhibition Assay. 10 μg/ml of serum IgG, purified by use of protein A-Sepharose or 1 μg/ml of affinity-purified antifibrillarin antibodies, was incubated with various amounts of collagen I, Sm, dsDNA, MBP, or fibrillarin in 50 μl for 1 h at room temperature, and then transferred to fibrillarin-coated plates following the procedure described previously (31). The cpm bound to fibrillarin was measured as above. The inhibition was expressed as a percentage.

Western Blotting. Purified fibrillarin fusion proteins were run on 10% polyacrylamide gels in the presence of SDS (32). Separated proteins were transferred to nitrocellulose filters (33) at 100 V for 4 h at 4°C, blocked with 5% BSA in PBS, and then incubated for 1.5 h with a 1:500 dilution of human sera or 0.1 μg/ml of mouse anti-human fibrillarin mAb in PBS containing 5% BSA and 0.05% Tween 20. After washing with PBS containing Tween 20, filters were incubated for 1.5 h at room temperature with 125I-rabbit F(ab')2 anti-human IgG or 125I-labeled rat anti-mouse &K mAbs at 2 × 10^5 cpm/ml. Filters were washed, dried, and autoradiographed as previously described (31).

Indirect Immunofluorescence. NIH 3T3 cells were grown on glass coverslips in 35-mm dishes. After 2 d in culture at 60% confluence, the cells were washed with PBS and then fixed with 2% paraformaldehyde in PBS, pH 7.4, for 15 min at room temperature. The fixed cells were permeabilized by exposure to 0.2% Triton X-100 in PBS for 5 min on ice. After permeabilization, cells were rinsed three times for 10 min with PBS and incubated with 50 μg/ml of IgG antibodies or 5 μg/ml of affinity-purified antibodies diluted in 1% goat serum for 1 h at room temperature. The unbound antibody was removed by rinsing three times for 10 min with PBS and then incubated with affinity-purified goat F(ab')2 anti-human IgG antibody conjugated with FITC at a dilution of 20 μg/ml for 1 h. The unbound second antibody was removed by washing with PBS, and the cells were mounted in 90% glycerol, 10% PBS (pH 8.0) containing 4% n-propyl gallate (34). The cells were examined under fluorescence illumination or laser light in a microscope (Carl Zeiss, Inc., Thornwood, NY).

Results

Expression and Purification of Recombinant Fibrillarin Proteins. To identify the immunodominant B cell epitopes of fibrillarin, the PCR-amplified DNA fragments coding for various moieties of fibrillarin (Fig. 1), each containing one to three hydrophilic regions, were cloned into pGEX-2T expression plasmid. Thus, a panel of six in-frame contiguous fibrillarin fusion proteins were generated as described in Materials and Methods. Bacterial cultures expressing recombinant proteins were induced with IPTG, and the fusion proteins were purified by affinity chromatography on a glutathione-S-Sepharose gel column as previously described (28).

Total bacterial lysates and purified recombinant proteins run on SDS-PAGE and stained with Coomassie blue R stain are shown in Fig. 2. The level of induction of recombinant fusion proteins varied depending on the insert sequence. The expected molecular mass of fibrillarin fusion proteins FP-A, FP-B, FP-C, FP-D, FP-E, and FP-F were 60.0, 34.5, 32.5, 34.1, 30.6, and 30.6 kD, respectively. The observed sizes of fusion proteins correspond well to this (Fig. 2). All recombinant proteins were full-length products. Only FP-C fusion protein showed lower molecular mass derivatives, indicating proteolytic cleavage in specific regions of fibrillarin. Sequence analysis of recombinant plasmid DNAs confirmed that the insert DNAs were inframe with the gene coding for GST protein.

Frequency of Antifibrillarin Antibodies in Sera of Patients with Connective Tissue Diseases. The presence of antifibrillarin antibodies in the sera of individuals with various connective tissue diseases was studied by solid-phase RIA with purified fibrillarin fusion protein FP-A. Background binding to GST was determined by parallel assays on GST-coated plates. Cpm bound to GST protein was subtracted from total cpm bound to fibrillarin fusion protein. The specific binding activities of sera from patients with various connective tissue diseases are presented as cpm bound in a scatter diagram (Fig. 3). The sera showing three- to fourfold higher binding than that of the control healthy subjects were considered positive. To confirm the results obtained by RIA and exclude any false positives, we tested all available positive sera on Western blots containing purified fibrillarin fusion protein and GST. The sera were preabsorbed with GST-coupled Sepharose 4B to remove the antibodies that might react with GST. We also included a mouse antifibrillarin mAb and four sera from healthy humans as positive and negative controls, respectively. Representative Western blots are shown in Fig. 4. The results showed a strong positive correlation between RIA and Western blot assay methods. The high sensitivity of the immunoblot analysis method is clearly demonstrated by the strong fibrillarin band signal in the blot incubated with mouse antifibrillarin mAb at 0.1 μg/ml.

The data summarized in the histogram (Fig. 5) show that ~58% of SSc patients have significant titers of antibodies that bind to fibrillarin fusion proteins. A similar percentage of patients with CREST syndrome (58%), MCTD (60%), RA (59%), and SLE (39%) also exhibited the presence of these antibodies. To our surprise, >84% of patients with SS showed high titers of antifibrillarin antibodies. Thus, our data clearly show that antifibrillarin autoantibodies are present at high frequency not only in patients with SSc but also a variety of other connective tissue diseases.

Epitope Specificity of Antifibrillarin Antibodies. RIA was performed by use of fibrillarin fusion proteins FP-B, FP-C, FP-D, FP-E, and FP-F to determine the domains of fibrillarin that interacted with the autoantibodies. The sera exhibiting significant titers of antifibrillarin antibodies were further tested by direct binding assay with purified fibrillarin fusion proteins FP-B to FP-F. Out of 56 SSc sera, 31 showed significant binding to fibrillarin fusion protein FP-A, 30 to FP-B, 5 to
FP-D, and 8 to FP-F (Table 1). Only 1 sample failed to bind to any fusion protein other than the complete fibrillarin molecule. The titer of antibodies against individual epitopes present in this sera may be low and is not quite sufficient to show above the background when individual fusion proteins were used, or it is a conformational site that was lost as individual fusion proteins were prepared. Sera from MCTD, SLE, CREST, RA, and SS showed high frequency of binding activity to the fusion protein, FP-B, containing the NH₂-terminal region. Comparison of binding profiles of sera from SSc, MCTD, SLE, and CREST disease groups revealed no specific patterns of antibody reactivity. The frequency of antibody reacting with the immunodominant epitope present on FP-B and FP-F did not show significant difference between the above disease groups. However, sera from RA and SS showed a high frequency of binding to the COOH-terminal portion corresponding to amino acid residues 276-321 (FP-F). This is comparable to the binding activity to the immunodominant epitope present on FP-B. This suggests that anti-FP-F antibody may be an additional marker to discriminate RA and SS from other connective tissue disorders. The binding specificity and the pattern of reactivity of autoantibodies were confirmed by Western blot analysis. Samples of positive sera, selected randomly, were tested at 1:500 dilution on Western blots. The binding profiles of several sera were examined in Western blots containing fusion proteins FP-A to FP-F. Representative Western blots are shown in Fig. 6. Distinct patterns of binding by different sera demonstrate that a number of epitopes are recognized by the autoantibodies, confirming the results obtained by direct-binding RIA. This assay also shows that the antibodies demonstrated are specific and do not react with GST. The amount of antibodies bound to the fusion protein FP-B band in Western blots appears to be somewhat lower than that detectable in RIA (data not shown). This may be due to reduced availability of some conformational epitopes present on FP-B after SDS denaturation.

Specificity of Antifibrillarin Antibodies. To determine whether the autoantibodies present in these disease groups are specific to fibrillarin, we tested the binding activity of autoantibodies by competitive inhibition RIA by use of several autoantigens as competitive inhibitors (collagen I, Sm, dsDNA, and MBP are target autoantigens in SSc, SLE, and multiple sclerosis [MS]). Results of inhibition RIA of four antibodies are shown in Fig. 7. The specificity of affinity-purified antibodies obtained from a SSc serum that was also used in Western blot analysis is shown in Fig. 7A. Preincubation of purified antibodies with chromatographically purified soluble recombinant fibrillarin inhibited the binding of autoantibodies to fibrillarin-coated plates, while other autoantigens used as inhibitors did not show any significant inhibition. Results of inhibition assays
of sera from SSc, MCTD, and SLE shown in Fig. 7, B–D, further demonstrate that the autoantibodies detected by RIA are specific to fibrillarin and are devoid of polyreactivity. Similar results were obtained with sera from patients with RA and SS (data not shown).

**Indirect Immunofluorescence Analysis.** Total serum IgG from SSc patients, and affinity-purified antifibrillarin antibodies, from the fusion protein FP-A affinity column were used for determining the pattern of binding to nuclear components on freshly cultured 3T3 cells by immunofluorescence and confocal microscopy. The number of nucleoli per cell vary depending on the cell type used. The 3T3 fibroblasts show two to several nucleoli (10). IgG from SSc patients showed bright nucleolar staining with very weak or no staining of nucleoplasm (Fig. 8B). On the other hand, affinity-purified antifibrillarin antibodies, in addition to brightly staining nucleolar material, also stain the nucleoplasm feebly but consistently. The weaker staining of nucleoplasm by total IgG may be due to the polyclonal nature of the antibodies present. In other words, in these sera, antibodies that are specific for other nucleolar proteins are probably more abundant than antifibrillarin antibodies. Staining of fibrillar regions of the nucleolus was more evident when affinity-purified antibodies were used. Confocal microscopy confirmed that the staining of the fibrillar and granular regions of the nucleolus is characteristic of antifibrillarin antibodies (Fig. 8G).

**Discussion**

Autoantibodies reacting with nucleolar proteins are produced in connective tissue diseases such as SSc, SLE, and SS (6). Autoantibodies present in SSc show diverse binding patterns in immunofluorescence staining, suggesting that a variety of autoantigens might be recognized. The molecular nature of some of these autoantigens has been described recently. A distinct nucleolar protein, fibrillarin, associated with U3-snRNP, has been reported as a target autoantigen recognized by autoantibodies present in diffuse scleroderma (10). Immunofluorescence staining with antifibrillarin antibodies shows staining of fibrillar and granular regions of the nucleoli.

**Table 1. Epitope Specificity of Anti-Fibrillarin Autoantibodies**

| Disease | Fibrillarin | FP-B | FP-C | FP-D | FP-E | FP-F |
|---------|-------------|------|------|------|------|------|
| PSS     | 31/56       | 30   | 0    | 5    | 0    | 8    |
| MCTD    | 13/22       | 10   | 1    | 6    | 0    | 2    |
| RA      | 22/38       | 14   | 0    | 6    | 0    | 17   |
| SS      | 13/15       | 8    | 0    | 3    | 0    | 11   |
| CREST   | 6/11        | 4    | 0    | 2    | 0    | 4    |
| SLE     | 30/80       | 22   | 0    | 5    | 0    | 10   |
Figure 6. Western blot analysis of autoimmune sera from connective tissue diseases. Selected examples of sera reactive with fibrillarin fusion proteins are shown. Western blots containing recombinant fusion proteins A–F were incubated with a 1:500 dilution of autoimmune sera and developed with 125I-labeled rabbit F(ab')2 antibody specific for human IgG chain. (A) SSc serum. (B) MCTD serum. (C) SSc serum. (D) SLE serum.

Figure 7. Demonstration of the specificity of antifibrillarin autoantibodies by competitive inhibition RIA. Various concentrations of antigen, 1–20 μg/ml, were mixed with 1 μg/ml of affinity purified antibody or 10 μg/ml of IgG from different sera, preincubated, and then transferred to fibrillarin-coated plates, and the inhibition was calculated as described in Materials and Methods. (A) Affinity-purified antibody isolated from an SSc serum. (B) SSc serum IgG. (C) MCTD serum IgG. (D) SLE serum IgG.
of antifibrillarin autoantibodies in the sera of patients with other connective tissue diseases. It is of interest that the presence of antifibrillarin antibodies has been reported in certain malignant diseases such as hepatocellular carcinoma (35).

Antifibrillarin antibodies present in connective tissue diseases are highly specific to fibrillarin antigen. Results from competitive inhibition RIA demonstrate that binding of affinity-purified antifibrillarin antibodies and autoantibodies present in the sera to fibrillarin antigen-coated plates is inhibited only by soluble fibrillarin and not by other autoantigens such as DNA, Sm, collagen type III, and MBP, which are target antigens in SLE, SSc, and MS. These data, as well as low binding to BSA or GST, suggest that antifibrillarin autoantibodies are not endowed with polyspecific properties. The variation in the degree of inhibition between different sera might be due to differences in the concentration, affinity, and epitope specificity of antibodies present in different sera. The data obtained from RIA and immunoblotting of fibrillarin fusion proteins, encompassing the entire fibrillarin polypeptide sequence, show that the immunodominant B cell epitopes are located in the NH$_2$-terminal glycine–arginine–rich region, since most of the SSc sera, as well as sera from MCTD, SLE, and CREST syndrome, react with FP-A, the complete fibrillarin, and the fusion protein FP-B, which contains the NH$_2$-terminal moiety. About 15–30% of SSc patients also had antibodies that bound to the COOH-terminal region of fibrillarin, FP-F, while a small percentage of sera also bound to FP-D, the internal helical region. The autoantibodies that bound to the NH$_2$-terminal portion are distinct from those that bind to the epitopes present in the COOH-terminal region because all the sera that showed binding to FP-B did not bind to FP-F. Western blot analysis confirmed RIA results. In Western blots, the antibodies present in some sera recognized only NH$_2$-terminal fusion protein, while others recognized only COOH-terminal peptide (Fig. 6). Antibody profiles obtained for RA and SS, by use of recombinant fusion proteins, showed higher correlation of the presence of anti-FP-F antibody with the disease. The variation in the epitope reactivity and specificity of autoantibodies may be influenced by factors such as the duration or severity of the disease, HLA haplotype of the patient, and nature of the therapy.

Swiss-Protein database search revealed the presence of homology between the NH$_2$-terminal portion of fibrillarin and RNA binding proteins from various sources and EBV-encoded nuclear protein (Fig. 9). The NH$_2$-terminal portion of fibrill-
chicken myosin heavy chain and microtubule associated protein contains two hydrophilic regions at aa residues 281-306 and EBV-encoded nuclear antigen (36). The next most commonly majority of autoimmune sera. Interestingly, this region contains one in this region. This fusion protein is recognized by a analysis (Genetic Computer Group, Madison, WI), the aa residue sequence 66-84 appears to be the most immunodominant one in this region. This fusion protein is recognized by a majority of autoimmune sera. Interestingly, this region contains a hexapeptide sequence, GRGRGG, which is shared with EBV-encoded nuclear antigen (36). The next most commonly recognized fusion protein, FP-F, by autoimmune sera, contains the COOH-terminal portion of fibrillarin. This region contains two hydrophilic regions at aa residues 281-306 and 314-321. The first hydrophilic region bears high homology with yeast fibrillarin as well as some self-proteins such as chicken myosin heavy chain and microtubule associated protein 1A (37). The most antigenic peptide in this region is aa sequence 311-319, GYRPPPK (Fig. 9), bearing sequence homology with capsid protein P40 encoded by HSV type 1 (38).

One may ask how antifibrillarin autoantibodies are induced in patients with various connective tissue diseases. The first possibility is that the peptides that were generated from the nucleolar protein fibrillarin, derived from the endogenous pathway, are transported to the surface, where they are recognized by fibrillarin-specific lymphocytes. In this mechanism, it remains unclear why antifibrillarin antibodies are produced only in patients with connective tissue diseases and not in healthy subjects or in patients afflicted with other diseases. Whether MHC or analogous molecules play a role in presentation of the peptides derived from fibrillarin remains to be determined. The second possibility is that environmental factors, chemicals, or drugs might play a role in presentation of these self-peptides to T lymphocytes, leading to their amplification, and thereby help in the activation of autoreactive B cells. The presence of elevated levels of antifibrillarin antibodies in mercuric chloride–treated mice supports this possibility (13, 14). The third possibility is molecular mimicry, in which the autoantibody response is induced in the host by infectious agents. The classical example is the cross-reactivity of anti–Streptococcus A antibodies with cardiac myosin (39). There are increasing examples showing presence of shared antigenic peptide sequences between viral, bacterial, parasitic, and autoantigens (40). Sharing of peptide sequence homology between MBP and viral proteins (41), adenovirus-12 E1B protein and gliadin A (42), gp210 protein reacting with autoantibodies from patients with primary biliary cirrhosis (43), leucine zipper region of C-myc and V-myc with p70 KLu autoantigen (44), RAL-I antigen of river blindness pathogen Onchocerca volvulus and 60-kD RO/SS-A autoantigen (45), regions of topoisomerase I with CMV (26) and mammalian type C virus P30(36) (46) are well documented. Sharing hexapeptide sequence homologies between fibrillarin and EBV-encoded nucleoprotein and HSV type 1-encoded P40 suggests that molecular mimicry might play a significant role in the induction of autoimmunity and production of autoantibodies reacting with fibrillarin.

The data presented here have twofold importance. First is the demonstration that autoantibodies specific for fibrillarin epitopes are produced in a number of connective tissue diseases in addition to SSc. Second is the demonstration of the presence of autoantibodies against immunodominant epitopes located at the NH2- and COOH-terminal regions of fibrillarin. In addition, presence of homology between the NH2-terminal region of fibrillarin and the nuclear protein encoded by EBV on the one hand and capsid protein P40 encoded by HSV and the COOH-terminal region of fibrillarin on the other hand suggests that common latent viruses might play a role in the induction of autoantibodies. Furthermore, correlation between the high incidence of antifibrillarin antibodies and the presence of EBV infection in SS patients supports this. Studies are in progress to isolate mAbs against various fibrillarin epitopes and determine whether the antibodies specific for EBV-encoded nuclear protein or P40 interact with fibrillarin peptides. Such experiments might shed light on the possible role of an antigen mimicry mechanism in the production of autoantibodies against autoantigens.

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