Human Autoantibody to a Novel Protein of the Nuclear Coiled Body: Immunological Characterization and cDNA Cloning of p80-coilin

By Luis E. C. Andrade, Edward K. L. Chan, Ivan Raska, Carol L. Peebles, Goran Roos, and Eng M. Tan

From the W. M. Keck Autoimmune Disease Center, Department of Molecular and Experimental Medicine, Scripps Clinic and Research Foundation, La Jolla, California 92037

Summary

Antibodies producing an unusual immunofluorescent pattern were identified in the sera of patients with diverse autoimmune features. This pattern was characterized by the presence of up to six round discrete nuclear bodies in interphase cell nuclei. Immunoblotting analysis showed that these sera recognized an 80-kD nuclear protein, and affinity-purified anti-p80 antibody from the protein band reproduced the fluorescent staining of nuclear bodies. Colloidal gold immunoelectron microscopy showed that the affinity-purified anti-p80 antibody recognized the coiled body, an ultramicroscopic nuclear structure probably first described by the Spanish cytologist Ramon y Cajal. Five cDNA clones were isolated from a MOLT-4 cell λgt-11 expression library using human antibody and oligonucleotide probes. The longest cDNA insert was 2.1 kb and had an open reading frame of 405 amino acids. A clone encoding a 14-kD COOH-terminal region of the protein was used for expression of a β-galactosidase fusion protein. An epitope was present in this COOH-terminal 14-kD region, which was recognized by 18 of 20 sera with anti-p80 reactivity, and affinity-purified antibody from the recombinant protein also reacted in immunofluorescence to show specific staining of the coiled body. This is the first demonstration and molecular cloning of a protein that appears to have particular identification with the coiled body, and it was designated p80-coilin. Autoantibody to p80-coilin may be useful for the elucidation of the structure and function of the coiled body, and the availability of a cDNA sequence could be helpful in further studies to clarify the clinical significance of this autoantibody response.

The presence of antibodies specifically directed against self nuclear constituents in the serum of patients with connective tissue diseases is a well known and extensively studied phenomenon (for review, see reference 1). Some of these autoantibodies, such as anti-Sm and anti-native DNA, are disease restricted and have become useful clinical markers, since they are found almost exclusively in systemic lupus erythematosus. Most autoantibodies in systemic rheumatic diseases have not been shown to be directly involved in disease pathogenesis. However, anti-DNA and anti-SS-A/Ro autoantibodies are examples of pathogenic antibodies that may play significant roles in lupus nephritis and neonatal lupus erythematosus, respectively.

Antinuclear autoantibodies (ANA)[1] have also been potent reagents in the elucidation of the nature and function of nuclear proteins and subcellular structures. Several autoantibodies are known to recognize specific cellular organelles, such as the kinetochore/centromere (2), mitotic spindles (3), and centrosomes (4). Antibody probes can be used to further characterize these structures and to screen cDNA libraries in order to determine the primary sequence of their target antigens. Autoantibodies were useful in the characterization of the structure and function of small nuclear ribonucleoprotein particles (5), in the cloning of the centromere antigen CENP-B (6), and several proteins associated with small nuclear RNAs (7–16).

This study reports on a novel human autoantibody that was used to characterize a specific nuclear domain known as the coiled body, an ultramicroscopic nuclear structure whose nature and function are still unknown. The target autoantigen was identified as an 80-kD protein, which we named p80-coilin. Electron microscopic studies using anti-p80-coilin antibodies suggested that the antigen is localized predominantly in the coiled body. Molecular information on the nature of the 80-kD autoantigen was also provided by the cloning of a partial cDNA sequence.

---

[1] *Abbreviations used in this paper:* ANA, antinuclear antibodies; IIF, indirect immunofluorescence; IPTG, isopropyl-β-thiogalactopyranoside; KM, Kern-Matrix; VLS, variable large speckles.

Materials and Methods

Patients and Sera. Sera were collected either from patients seen at Scripps Clinic and Research Foundation or from patients referred to our laboratory for ANA testing. In all cases the diagnoses were based on the review of clinical charts or information from the attending physician. Normal sera were collected from laboratory personnel. The initial criterion for the selection of sera was based on a characteristic immunofluorescent pattern that consisted of up to six discrete nuclear dots per interphase cell nucleus. This pattern was clearly distinguishable from the speckled nuclear staining produced by antibodies to ribonucleoprotein peptides such as Sm and SS-B/La or from the more numerous dot pattern of antibodies to kinetochore/centromere. Sera characterized immunologically and clinically in previous studies (1, 2, 13, 16) and known to contain antibodies to other nuclear antigens were used in ELISA to establish specificity and prevalence.

Cell Extracts. All cell lines were obtained from the American Type Culture Collection (Rockville, MD). Human HeLa, MOLT-4, WI38, HEp-2, HepG-2, mouse 3T3, and marsupial PtK2 cells were cultured in DMEM containing 10% FCS and 2.5 μg/ml gentamicin sulfate at 37°C and 8% CO_2. Cells were harvested and were either solubilized immediately in Laemmli sample buffer (17) or extracted with buffer A, B, or C. Extractions were carried out at 4°C for 30 min with either low ionic strength buffer A (150 mM NaCl, 10 mM Tris-HCl, pH 7.2, 0.5% NP-40), high ionic strength buffer B (500 mM NaCl, 50 mM Hepes, pH 7.0, 1% NP-40), or triple detergent buffer C (150 mM NaCl, 50 mM Tris, pH 8.0, 0.1% SDS, 1.0% NP-40, 0.5% sodium deoxycholate). All extraction buffers contained the following protease inhibitors: 1 μg/ml leupeptin, 1 μg/ml pepstatin, 2 μg/ml aprotinin (Sigma Chemical Co., St. Louis, MO), and 1 mM PMSF (Calbiochem-Behring Corp., La Jolla, CA). After centrifugation at 12,000 g for 15 min, supernatant and pellet were separately mixed with Laemmli sample buffer to equal final volume.

Indirect Immunofluorescence (IIF). IIF cell substrate included commercial HEp-2 cell slides (Bion, Park Ridge, IL), mouse kidney/stomach slides (Kallestad Lab, Austin, TX), and slides prepared from cell lines mentioned above. Cultures were grown to subconfluency on coverslips in the conditions described above. Cells were washed with PBS, pH 7.4, fixed in cold methanol (−20°C) for 10 min, followed by cold acetone (−20°C) for 20 s, and air dried. Autoimmune and control sera, as well as affinity-purified antibodies, were diluted in PBS and used as primary reagent. The secondary detecting reagent was fluorescein-labeled goat antibody to total human Ig, human IgG, or IgM heavy chain (Tago Inc., Burlingame, CA).

For extraction and digestion procedures, subconfluent HeLa cells grown on coverslips were washed for 10 min on ice with Kern-Matrix (KM) buffer (10 mM N-morpholinoethanesulfonic acid, pH 6.3, 10 mM NaCl, 1.5 mM $MgCl_2$, 10% glycerol; reference 18). This buffer appeared to prevent detachment of the cells from the coverslips during successive incubations and washings (18). Cells were permeabilized with 0.1% Triton X in KM buffer on ice for 10 min twice and then washed with KM buffer on ice five times for a total of 20 min. Cells were then incubated with one of the following solutions: (*a*) 50 μg/ml DNase I (Boehringer Mannheim Biochemicals, GmbH., FRG) in KM buffer at 37°C for 30 min; (*b*) 5 μg/ml RNase A (Sigma Chemical Co.) in 10 mM phosphate, pH 7.3, 3 mM $MgCl_2$, 100 mM NaCl at 37°C for 30 min; (*c*) 0.5, 1.0, or 2.0 M urea in KM buffer at room temperature for 30 min; (*d*) 0.5, 1.0, or 2.0 M NaCl in KM buffer at room temperature for 30 min; (*e*) 0.5 μg/ml proteinase K (Boehringer Mannheim Biochemicals) in KM buffer at room temperature for 10 min.

Control cells were incubated with KM buffer alone at 37°C for 30 min. These steps were carried out with gentle shaking. Except for proteinase K digestion, all other solutions were supplemented with protease inhibitors, to the same concentrations as described for the extraction buffers above.

Electron Microscopy. For conventional electron microscopy, cultured cells were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer for 1 h at 4°C, post-fixed in 1% osmium tetroxide for 1 h, and embedded in epon. Thin sections were stained with uranyl acetate and lead citrate. For immunoelectron microscopy, cells were processed according to Reimer et al. (19). Briefly, cells were fixed with paraformaldehyde, infused with sucrose, frozen, and cut. Thin sections were incubated for 30 min with primary antibody diluted in PBS. After washing with PBS, the sections were incubated with 10 nm gold particle-labeled goat anti-human IgG and IgM (Janssen Pharmaceutica, Beerse, Belgium) for 25 min, washed in PBS, and post-embedded in methylcellulose.

Immunoblotting. Cell extracts were separated in SDS-PAGE using 12.5% gel slabs (15 × 10 × 0.1 cm) according to Laemmli (17). Separated proteins were electrotransferred to nitrocellulose at a constant voltage of 60 V for 150 min at 8°C. Nitrocellulose sheets were cut into strips, blocked in 3% nonfat milk in PBS containing 0.05% Tween 20 (PBS-T) for 30 min, and then exposed for 1 h to serum diluted 100-fold in the same blocking solution. After extensive washing with PBS-T, bound antibody was detected with [^{125}I]-labeled protein A (ICN Biochemicals, Irvine, CA) followed by autoradiography at −70°C. Protein standards for molecular weight determination were obtained either from Bio-Rad Laboratories, Richmond, CA, or from Bethesda Research Laboratories, Gaithersburg, MD.

Isolation of cDNA Clones. A human leukemia T cell (MOLT-4) λgt11 cDNA library was constructed by Drs. K. Ogata and D. J. Noonan (Scripps Clinics and Research Foundation). 10^6 recombinants were screened with a high titer serum Sh and [^{125}I]-labeled protein A according to Young and Davis (20). All screenings were carried out with duplicate filters. One positive clone λ213 was selected after multiple re-screening and was grown in *Escherichia coli* LE 392 for phage DNA isolation. The cDNA insert released by digestion with EcoRI was 1.25 kb and was subcloned into plasmid Bluescript (Stratagene, La Jolla, CA). The plasmid obtained, designated pGR14, was used for large-scale DNA preparation and sequence determination by the dideoxy method (21). Overlapping restriction fragments were subcloned into plasmid Bluescript for determination of DNA sequence in both strands.

To obtain longer cDNA clones, the same cDNA library was re-screened by DNA hybridization using α[^{32}P]-labeled synthetic oligonucleotides. Two partially complementary synthetic oligonucleotides (TCTGTTACCACTGTTAGCAGCTGCCCCTCA and TCTTTTCTCCAACTTGAGGGGCAGCTGCTA) were designed corresponding to the 5′ sequence of pGR14 cDNA insert. They were mixed and labeled with α[^{32}P]-ATP using the standard fill-in reaction of Klenow polymerase (22).

Affinity Purification of Antibodies. Affinity purification of antibodies from nitrocellulose-bound antigen was performed according to the method of Olmsted (23). The antigen source was either the 80-kD band from HeLa cell extract resolved by SDS-PAGE and transferred to nitrocellulose, or cDNA-encoded antigen from λ213 lysogen adsorbed onto nitrocellulose filters. The expression of the λ213 recombinant antigen was induced with isopropyl-β-thio-galactopyranoside (IPTG; Sigma Chemical Co., St. Louis, MO). After blocking with 3% nonfat milk in PBS-T for 30 min, nitrocellulose filters were incubated for 1 h with serum diluted 1:50 in the same blocking buffer. Bound antibodies were eluted by brief

incubation with 0.1 M phosphate buffer, pH 2.5, in 0.1% BSA, 150 mM NaCl. The eluted antibody was immediately neutralized by addition of 1 M Tris-HCl, pH 8.8, and concentrated with Centricon 30 microconcentrators (Amicon, Danvers, MA), and tested in IIF and immunoblotting.

Purification of Recombinant Protein. Plasmid pGR14 was used for cDNA expression of p80-coilin as a β-galactosidase fusion protein. A 200-ml culture of the recombinant cells was grown to $OD_{600} = 0.6$ at 37°C, and IPTG was added to a final concentration of 10 mM. The culture was further grown overnight at 37°C before harvest. The inclusion bodies were purified according to the method of Adam et al. (24). Aliquots of the supernatant in the final 8 M urea extraction of the inclusion bodies were stored at −70°C and subsequently used for ELISA and immunoblotting.

ELISA. Standard ELISA was performed according to the method described by Rubin (25). Immulon 2 microtiter plates (Dynatech Laboratories, Inc., Alexandria, VA) were coated overnight at 4°C with serial dilutions of the purified recombinant protein in PBS with 1 $\mu g/ml$ leupeptin. The detecting reagents were peroxidase-conjugated goat anti–human IgG+M (Caltag Laboratories, So. San Francisco, CA) and the substrate 2,2'-azinobis (3-ethylbenzthiazoline sulfonic acid) (Boehringer Mannheim Biochemicals).

Results

Characterization of Patients and Sera. From a total of 25 sera defined to contain antibodies reacting with the special nuclear structures described below, 21 were identified on the basis of the immunofluorescent pattern, and four additional sera were later detected by screening in ELISA with the recombinant protein (see below). Clinical data were available for 20 patients, 16 of whom were female; the mean age was 40.4 yr, ranging from 15 to 70 yr. Table 1 depicts the principal clinical features as well as the titer of the nuclear body staining and of other immunofluorescent patterns. Although the clinical diagnoses appeared to be highly diverse, the majority could be grouped into certain categories. There were three patients with primary biliary cirrhosis, 12 patients with some form of rheumatic disease, such as Sjögren's syndrome (including Tt who also had PBC), scleroderma, and systemic lupus erythematosus, and three patients with neurological involvement. It needs to be stated that the clinical information was inadequate and was obtained from retrospective chart review or personal information from physicians, and the completeness of the clinical data is uncertain. In all sera, the immunofluorescent staining of nuclear bodies was found to be due to IgG antibodies. No precipitin line on standard Ouchterlony immunodiffusion was observed when tested against rabbit thymus extract.

Indirect Immunofluorescent Detection of Autoantibodies to Nuclear Bodies. All sera shared a distinct immunofluorescent pattern, consisting of bright, discrete dots distributed in interphase nuclei (Fig. 1), which from observations described

Table 1. *Clinical and Serological Synopsis of Patients with Antibodies to p80-coilin*

Patient	Sex	Age	Clinical presentation	Nuclear body IIF titer	Concomitant IIF pattern[*] and titer
Tk	M	40	Primary biliary cirrhosis	1:40,960	CS/1:5,120
Km[‡]	F	70	Primary biliary cirrhosis	1:1,280	CS/1:320
Tt[‡]	F	55	Primary biliary cirrhosis and Sjögren's syndrome	1:10,240	CS/1:2,560
Ab[‡]	F	33	Primary Sjögren's syndrome	1:1,280	NS/1:1,280
Gm[‡]	M	42	Primary Sjögren's syndrome	1:10,240	NS/1:2,560
Sf	F	45	Progressive systemic sclerosis	1:20,480	CS/1:640
Pz	F	31	Systemic lupus erythematosus	1:640	NH/1:640
Wo	F	47	Raynaud's phenomenon	1:640	NH/1:160
Mo	F	16	Dermatomyositis	1:640	NS/1:1,280
Nu	F	68	Polymyalgia rheumatica	1:5,120	NH/1:640
Tu	F	27	Skin rashes/arthralgia	1:640	NH/1:160
El	F	59	Osteoarthritis/fibromyalgia	1:640	NS/1:160
Wi	F	60	Osteoarthritis	1:160	NS/1:160
Bu	F	63	Soft tissue rheumatism	1:1,280	NH/1:160
Wk	F	47	Amyotrophic lateral sclerosis	1:2,560	NH/1:640
Cl	F	24	Sixth nerve palsy/photosensitivity	1:1,280	NH/1:640
Pk	F	15	Severe right brachial plexopathy	1:1,280	NH/1:640
Du	F	30	Hashimoto's disease	1:640	NH/1:640
Sh	M	20	Respiratory infection/rash	1:2,560	NH/1:320
Op	M	16	Cold urticaria	1:10,240	NS/1:640

[*] Concomitant staining patterns: CS, cytoplasmic speckles; NH, nucleoplasmic homogeneous; NS, nucleoplasmic fine speckles.
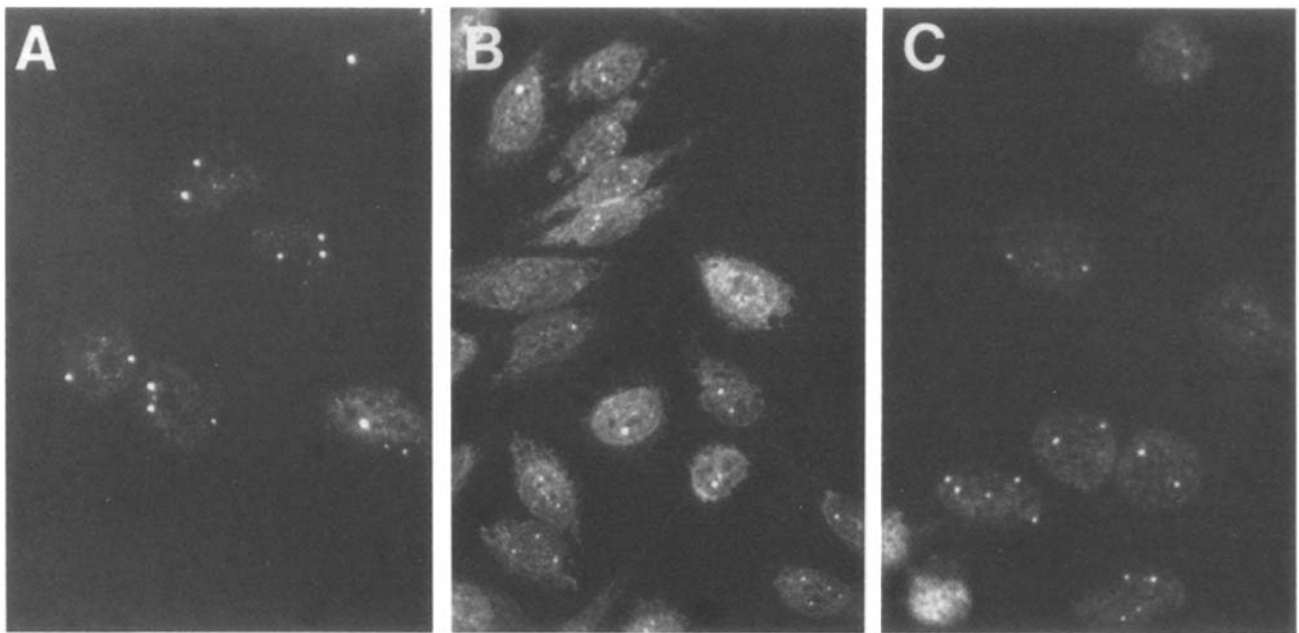[‡] Sera identified by ELISA screening (see also Table 3).

Figure 1. Immunofluorescent pattern characteristic of antibodies to nuclear coiled bodies. Commercial HEp-2 cells were stained by different selected human sera. (*A*) serum Sh; (*B*) serum Tk; and (*c*) serum Nu. Discrete round bodies were stained in the nucleoplasm of interphase cells. Most sera showed an additional fine grainy nucleoplasmic staining. In *B*, a speckled cytoplasmic staining was also observed.

below will be called nuclear bodies. The number of stained nuclear bodies varied from zero to six per cell, the most frequent being two per cell (Fig. 2). Although most nuclear bodies were located randomly in the nucleoplasm, they were often seen in close proximity to the nucleolus. At low dilution, all sera presented other concomitant fluorescent staining patterns, such as homogeneous nuclear, fine speckled nucleoplasmic, or cytoplasmic speckled patterns, which disappeared at higher dilutions (see Table 1). These concomitant staining patterns were probably related to the presence of other autoantibodies, but partially because of their lower titers and heterogeneity, this feature was not further examined.

The nuclear bodies were observed in IIF on a number of human substrates (peripheral lymphocytes, HEp-2, HepG-2, W138, HeLa, and MOLT-4 cell lines), mouse tissues (brain, liver, kidney, stomach, and smooth muscle), and marsupial PtK2 cells. The range in the number of nuclear bodies and their sizes appeared to be the same among these substrates, except for a lower frequency of nuclear bodies in PtK2 cells.

To obtain some insights into the nature of the target antigen, HeLa cells were subjected to treatment with various reagents before standard immunofluorescence. The staining of nuclear bodies with human autoantibodies was not altered when the substrate was pre-treated with either 2 M NaCl, 2 M urea, or 0.1 N HCl. Staining was completely abolished after digestion with proteinase K and barely visible after digestion with RNase A, but was unaffected with DNase I. These observations suggested that the antigen recognized by human autoantibodies was a protein and it could be complexed in some form with RNA.

Immunoblotting Analysis. Sera selected on the basis of the

characteristic immunofluorescent pattern showed common reactivity with an 80-kD protein in Western blotting with HeLa whole cell extract (Fig. 3 *A*). Other bands were observed in some lanes but they were not uniformly present. Affinity-purified antibodies from the 80-kD band did not cross-react with other protein bands (Fig. 3 *A*, lane *8*). The affinity-purified antibody preparations were also shown to reproduce the characteristic nuclear staining pattern, providing further
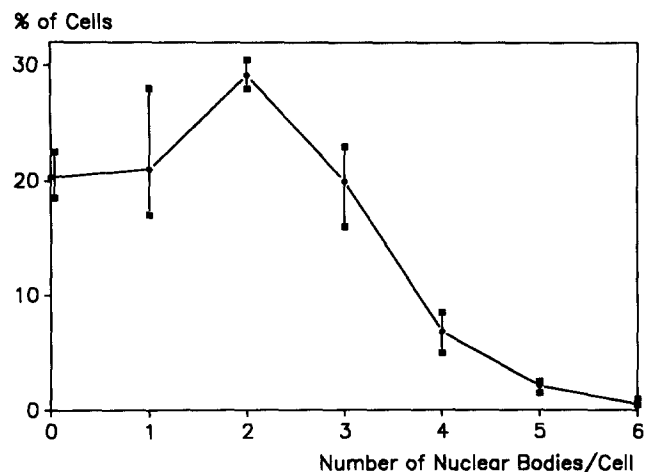

Figure 2. Distribution of cells with different number of nuclear bodies. The frequency of nuclear bodies per cell was determined by immunofluorescence using Hep-2 cell substrate. The curve represented the average, and the bar at each data point represented the range for three sera, Wo, Op, and Sh. 200 cells were analyzed for each serum.
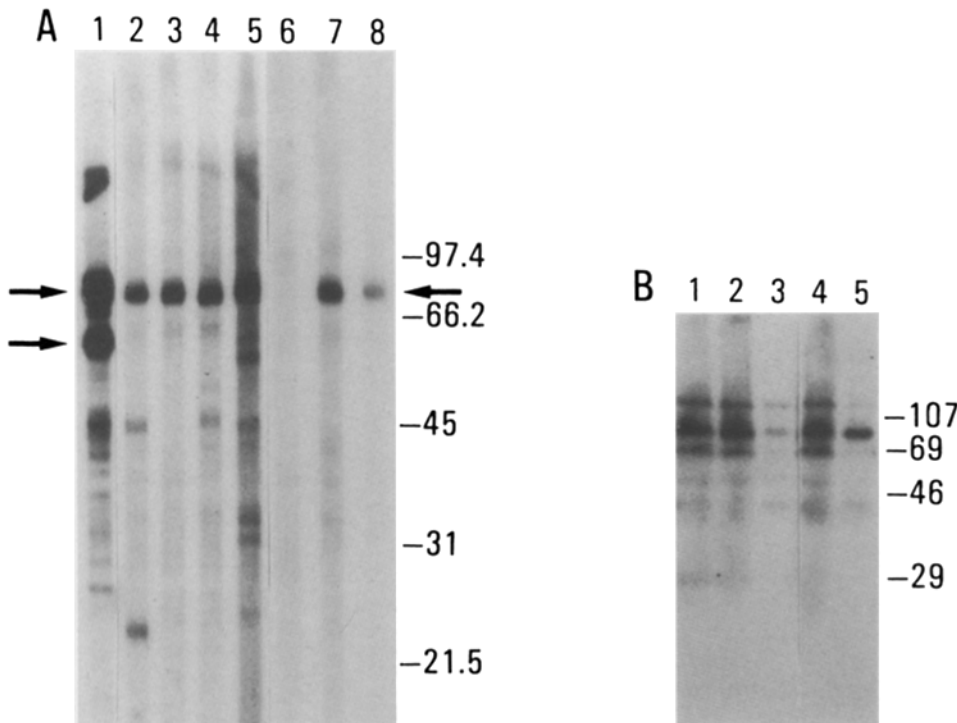
Figure 3. Immunoblotting analysis. (A) HeLa whole-cell extract resolved in 12.5% SDS gel electrophoresis was used as a substrate for immunoblotting. Lane 1, prototype human anti-Ku serum AF showing strong reactivity at 70 and 82 kD (left border arrows); lanes 2–5 and 7, sera from patients Tk, Sh, Pk, Du, and Op, respectively, recognizing a common protein of 80 kD (right border arrow); lane 6, normal human serum; lane 8, antibodies from serum Op affinity purified from the 80-kD band. The affinity-purified antibodies in lane 8 did not show reactivity with other protein bands. (B) In an attempt to solubilize the 80-kD antigen, MOLT-4 cells were extracted and fractionated as described in Materials and Methods using either high ionic strength buffer B or triple detergent buffer C. The fractions were separated in a 12.5% gel and transferred to nitrocellulose, which was probed with serum Nu diluted 1:100. Lane 1, whole cell extract; lanes 2 and 3, insoluble and soluble fractions, respectively, after extraction with triple detergent buffer C; the bulk of 80-kD protein was present in the insoluble fraction and only traces were detected in the soluble fraction; lanes 4 and 5, insoluble and soluble fractions, respectively, after extraction with high ionic strength buffer B, when approximately half of the 80-kD protein was present in the soluble fraction. Other bands displayed by serum Nu are given by unrelated antibodies and behave differently from the 80-kD band.

evidence of the association of the 80-kD band with the IIF pattern (Fig. 4).

When MOLT-4 cells were extracted with low ionic strength buffer A before solubilization in Laemmli buffer, the bulk of the 80-kD reactivity remained in the nuclear, insoluble fraction (data not shown). Even the triple detergent buffer C did not extract the 80-kD protein. However, when harsher conditions of extraction were used, as with high ionic strength
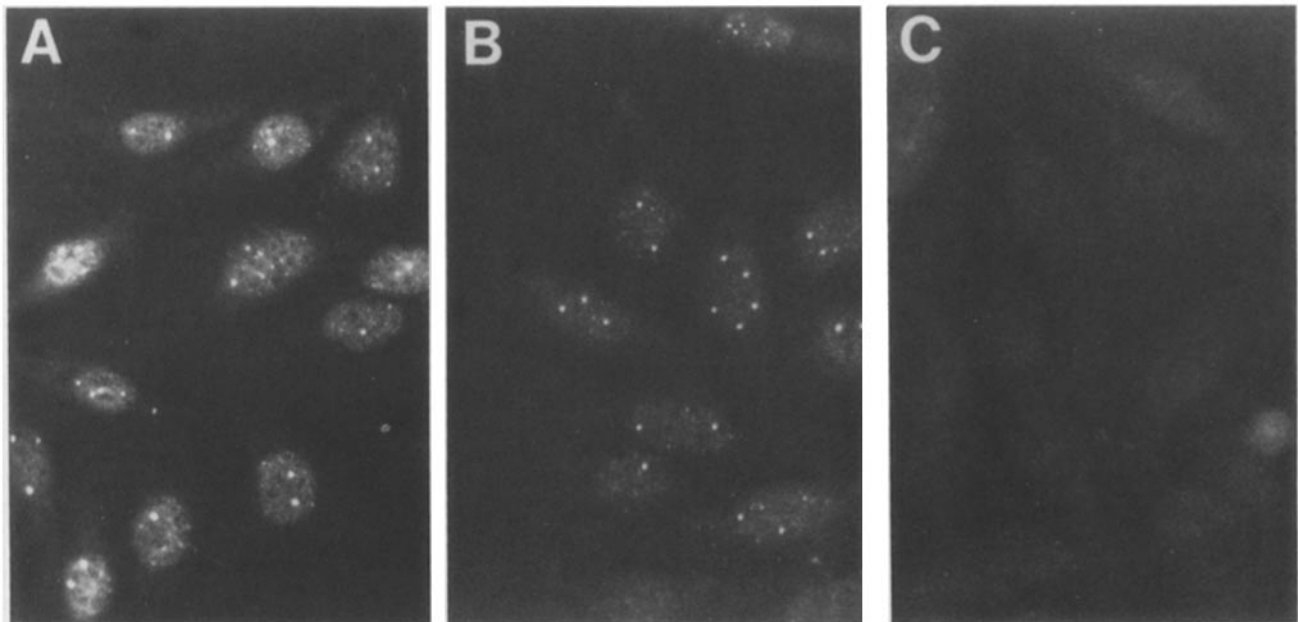

Figure 4. IIF staining using affinity-purified antibody to the 80-kD protein. HEp-2 cells were stained with serum Op (A), antibody from serum Op affinity purified from the 80-kD band (B, same antibody preparation as used in Fig. 3 A, lane 8), and normal human serum (C). The affinity-purified antibody preparation recognized the characteristic nuclear bodies with slight nucleoplasmic background staining.
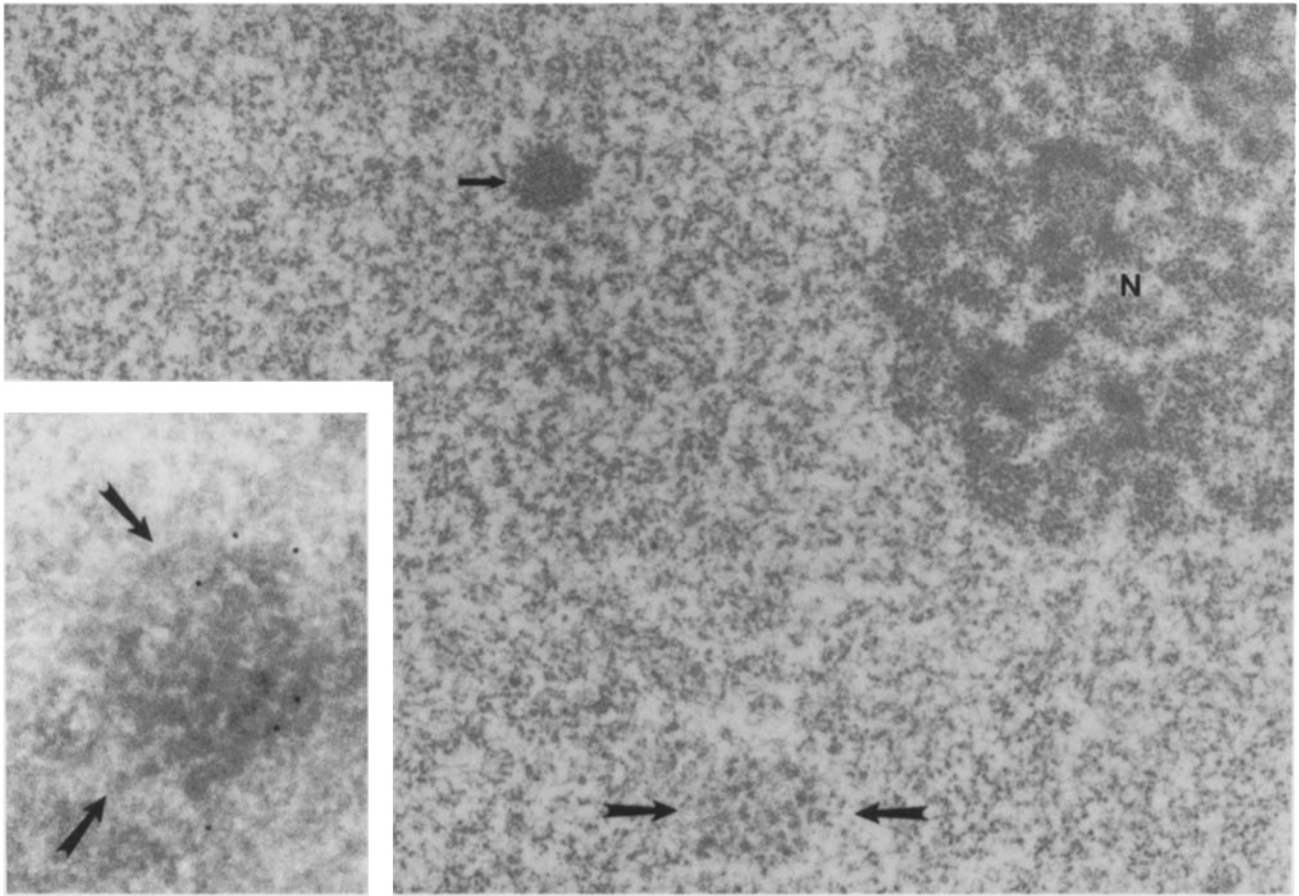
Figure 5. Immunolocalization of nuclear bodies in electron microscopy. Conventional epon section of a HeLa cell. A coiled body (*large arrows*) and a simple nuclear body (*small arrow*) are present in the nucleoplasm. N, Nucleolus (×36,800). The inset shows the immunolocalization of a coiled body (*arrows*) in a cryosection of a HeLa cell using human antibodies affinity purified from the 80-kD protein and 10 nm gold particles conjugated to goat anti–human IgG and IgM. The localization of gold particles was restricted to the coiled body (×90,000).
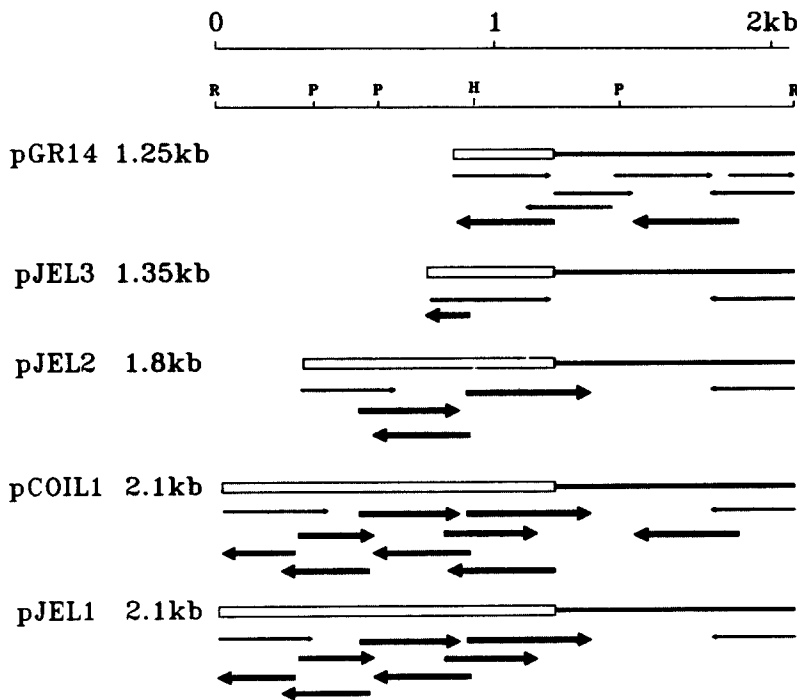

Figure 6. Schematic representation of cDNA clones derived from the MOLT-4 library. Open boxes represent the open reading frame and heavy lines represent the 3' untranslated regions. Partial nucleotide sequencing showed identity among overlapping regions of the five clones. Thin and thick arrows represent DNA sequences derived from restriction fragment subcloning and sequencing with synthetic oligonucleotide primers, respectively. Restriction enzyme sites R, P, and H are EcoRI, PstI, and HindIII, respectively.

buffer B, which contains 0.5 M NaCl, the antigen appeared to be equally distributed between the soluble and insoluble fractions (Fig. 3 B).

Immunoelectron Microscopy. Using an electron microscopic technique in which ultra-thin sections of HeLa cells were first incubated with whole serum or affinity-purified antibodies and then with 10 nm gold-labeled detecting reagent, it was possible to define the ultrastructure of the nuclear bodies. The gold particles were strikingly enriched in round bodies of 0.5–0.8 μm in diameter, which correspond to what has been described as the coiled body (26–28). Fig. 5 shows a

HeLa cell nucleus, depicting three morphologically distinct structures: the nucleolus, a simple nuclear body, and a coiled body. The latter structure possesses certain distinctive aspects consisting of high density meandering threads of 30–60 nm in cross-section interspersed with areas of density similar to the rest of the nucleoplasm. The coiled body has been described in the interchromatin space, usually in no more than one per ultra-thin section. The inset of Fig. 5 shows a coiled body stained with antibodies affinity purified from the 80-kD band. In all sections examined, the coiled bodies were positively labeled, suggesting that this antigen is a constant

```
   1  CACAGTGGGTGATGATAACGAAGAGGCCAAAAGAAAATCACCAAAGAAAAAGGAGAAATGTGAATATAAAAAAAAGGCTAAGAATCCCAAG
   1   T  V  G  D  D  N  E  E  A  K  R  K  S  P  K  K  K  E  K  C  E  Y  K  K  K  A  K  N  P  K

  92  TCTCCGAAAGTACAGGCAGTGAAAGACTGGGCCAATCAGAGATGTAGTTCTCCAAAAGGTTCTGCTAGAAACAGCCTTGTTAAAGCCAAA
  31   S  P  K  V  Q  A  V  K  D  W  A  N  Q  R  C  S  S  P  K  G  S  A  R  N  S  L  V  K  A  K

 182  AGGAAAGGTAGTGTAAGCGTTTGCTCAAAAGAGAGTCCCAGTTCCTCCTCGGAGTCTGAGTCTTGTGATGAATCTATCAGTGATGGTCCC
  61   R  K  G  S  V  S  V  C  S  K  E  S  P  S  S  S  S  E  S  E  S  C  D  E  S  I  S  D  G  P

 272  AGCAAAGTCACTTTGGAGGCCAGAAATTCCTCAGAGAAATTACCAACTGAGTTATCAAAGGAAGAACCCTCTACCAAAAATACAACTGCA
  91   S  K  V  T  L  E  A  R  N  S  S  E  K  L  P  T  E  L  S  K  E  E  P  S  T  K  N  T  T  A

 362  GACAAACTGGCTATAAAACTTGGCTTTAGCCTTACCCCCAGCAAGGGCAAGACCTCTGGAACAACATCTTCCAGTTCAGACTCTAGTGCA
 121   D  K  L  A  I  K  L  G  F  S  L  T  P  S  K  G  K  T  S  G  T  T  S  S  S  D  S  S  A

 452  GAGTCAGACGACCAATGCTTGATGTCATCGAGCACCCCGGAGTGTGCTGCGGGTTTCTTAAAGACAGTAGGCCTTTTTGCAGGAAGAGGT
 151   E  S  D  D  Q  C  L  M  S  S  S  T  P  E  C  A  A  G  F  L  K  T  V  G  L  F  A  G  R  G

 542  CGTCCAGGCCCAGGGCTGTCATCACAGACTGCAGGTGCTGCTGGATGGAGGCGTTCTGGCTCAAATGGTGGTGGACAGGCTCCTGGTGCT
 181   R  P  G  P  G  L  S  S  Q  T  A  G  A  A  G  W  R  R  S  G  S  N  G  G  G  Q  A  P  G  A

 632  TCTCCCAGTGTGTCTCTCCCTGCTAGTTTAGGAAGAGGATGGGGTAGAGAAGAGAACCTTTTTTCTTGGAAGGGAGCTAAGGGACGGGGC
 211   S  P  S  V  S  L  P  A  S  L  G  R  G  W  G  R  E  E  N  L  F  S  W  K  G  A  K  G  R  G

 722  ATGCGGGGGAGAGGTCGAGGACGAGGGCATCCTGTTTCCTGTGTTGTAAATAGAAGCACTGACAACCAGAGGCAACAGCAATTAAATGAC
 241   M  R  G  R  G  R  G  R  G  H  P  V  S  C  V  V  N  R  S  T  D  N  Q  R  Q  Q  L  N  D

 812  GTGGTAAAAAATTCATCTACTATTATCCAGAATCCAGTAGAGACACCCAAGAAGGACTATAGTCTGTTACCACTGTTAGCAGCTGCCCCT
 271   V  V  K  N  S  S  T  I  I  Q  N  P  V  E  T  P  K  K  D  Y  S  L  L  P  L  L  A  A  A  P

 902  CAAGTTGGAGAAAAGATTGCATTTAAGCTTTTGGAGCTAACATCCAGTTACTCTCCTGATGTCTCTGACTACAAGGAAGGAAGAATATTA
 301   Q  V  G  E  K  I  A  F  K  L  L  E  L  T  S  S  Y  S  P  D  V  S  D  Y  K  E  G  R  I  L

 992  AGCCACAATCCAGAGACCCAGCAAGTAGATATAGAAATTCTTTCATCCTTACCTGCCTTGAGAGAACCTGGGAAATTTGATTTAGTTTAT
 331   S  H  N  P  E  T  Q  Q  V  D  I  E  I  L  S  S  L  P  A  L  R  E  P  G  K  F  D  L  V  Y

1082  CACAATGAAAATGGAGCCGAGGTAGTGGAGTACGCTGTGACACAGGAGAGCAAGATCACTGTATTTTGGAAAGAGTTGATTGACCCAAGA
 361   H  N  E  N  G  A  E  V  V  E  Y  A  V  T  Q  E  S  K  I  T  V  F  W  K  E  L  I  D  P  R

1172  CTGATTATTGAATCTCCAAGTAACACATCAAGTACAGAACCTGCCTGAGTATGACCTCTCCACCTTATAGTTTATGAATGTCTTGTTTGT
 391   L  I  I  E  S  P  S  N  T  S  S  T  E  P  A  *  405

1262  GAAAGTGACTATAACCCAAACTTTTTTTTTTTTTAAAGAGGATTTGGAAGTTGTATGGATTTTTTTGTTATCTTCACTTTACTGCATAGG
1352  AAACAATCTACCTCATCATTTAAAATGACATGGGTGTCGGTTTTGTAGATCTTTGGTTTTTTTGTCAGGTTTAATTACCATTAACAAATG
1442  TAAAACATGACATTCCCTGCAGATATTGTTGTATACCAGTATGGTTTATTATCTTTCTTTAAATCTTATTGGCCATCAAGTATGCAGTCG
1532  TCAGTATGTGATGTTTATAATACCAATGAATGTGCTGCGTATCTTGTCTCAATAAGTTTTAAGTAACATTTAAAAATATTAAAGCATGTT
1622  ATTTGACCTAATTTTTTAGCATTTGAGTTGTTCCATTAAATGGAGCATCTTGTAAATTTCAAGTATTTTATACTTGCAATTGTTAAGAGT
1712  TAACAGGTAGTTGGATTTGTCGCAGACAATGAGTTAAGGAATCCTTTCACGTTTTTCCCAACTTTAAAATTAAGGATTCTCAGGTCCCTG
1802  TGTAGAGCAGTGAAAATAAGATGTGCGTATGTGTGTGTATGCCTGGAGATTGGTGTTTCACTTCAGTGAGAGGATTGGCTGTGAGCTTCA
1892  GACCAGAAATGTGTCATCTTGCCAGCCCCTGGCTGAGTGTGCTGGAGTGAGGATCTTGAACAGAAACTTCCTTTTCTGTTATTATTCACT
1982  ACGAAGCTAAAATGGCCAAATATATACCGTGAAAATTGGTTTCATTTAACAAAAGATCAGATCCCTCCTTCAGCTGTACACATTTTTAAA
2072  TAAAATCATATTGAACT-2088  poly-A
```

Figure 7. Nucleotide sequence and deduced amino acid sequence of p80-coilin. Nucleotide sequence of 2,088 bases was derived from analysis of the overlapping cDNA clones outlined in Fig. 6. Shown below the DNA sequence is the deduced protein sequence, representing 405 amino acids of p80-coilin with a predicted molecular mass of 43 kD. Ser- and Thr-rich regions (*boxed areas*) and Lys- and Arg-rich regions (*underscored*) of p80-coilin sequence are indicated. The predicted polypeptide has relatively high percent of Ser (15.3%) and basic amino acid residues (Arg + Lys + His = 15.3%). The pI estimated by the ISOELECTRIC program (49) is 9.72. The occurrence of short peptide repeats Ala-Arg-Asn-Ser and Ser-Leu-Pro-Ala and the four direct repeats of Arg-Gly are underscored by two lines. The significance of the amino acid repeats is not known. The sequence data are available from EMBL/Genbank/DDBJ under accession number M58411.
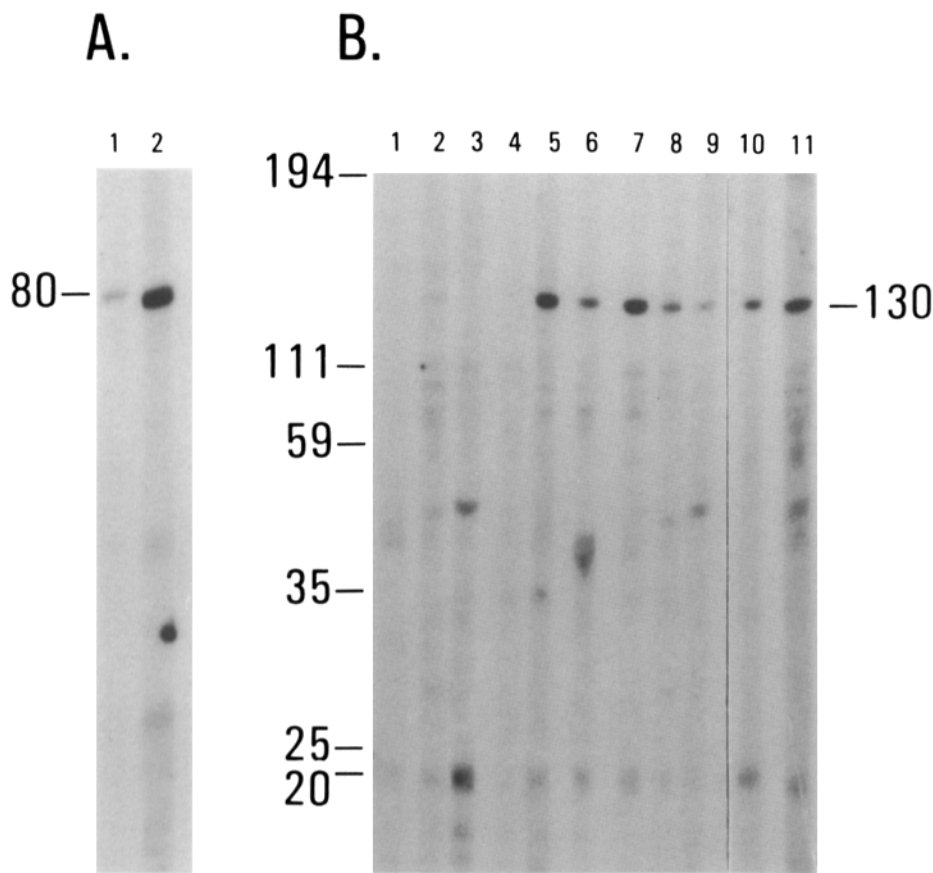
Figure 8. Immunoblotting analysis of recombinant protein derived from λ213. Immunological relatedness between the λ213 recombinant protein and p80-coilin was examined by Western blotting. Extracts were resolved on SDS-PAGE using 12.5% separating gels and transferred to nitrocellulose filters. Individual strips were probed with sera or affinity-purified antibody. (A) Reactivity of affinity-purified antibody in MOLT-4 whole cell extract. IPTG-induced plaques of phage λ213 were adsorbed to nitrocellulose filters and were used for the affinity purification of antibody from serum Sh. Lane 1, affinity-purified antibody from serum Sh; lane 2, serum Sh. (B) Reactivity of human sera to bacteria lysate expressing the recombinant protein of λ213. Lane 1, normal human serum; lane 2, serum with antibodies to Sm/RNP and SS-A/SS-B; lane 3, serum with antibody to Ku and ribosomal RNP; lane 4, serum with antibody to Scl-70 and PCNA; lanes 5–11, seven anti-p80-coilin sera, Sh, Pk, Nu, Bu, Tu, Du, and Ve, respectively. The 130-kD band blotted in common by anti-p80-coilin sera represented the fusion protein of β-galactosidase and the 14-kD fragment of p80-coilin encoded in λ213. The 130-kD band was not recognized by normal human serum or other autoimmune antibodies.

component of this ultrastructure. On the other hand, no consistent accumulation of the gold-labeled probe was observed elsewhere in the nucleus or cytoplasm. Accordingly, this 80-kD antigen was provisionally designated p80-coilin.

Cloning of p80-coilin cDNA. Fig. 6 depicts the cDNA clones obtained from a MOLT-4 λgt11 expression library. After screening 10^6 recombinant colonies with serum Sh, clone λ213 was obtained and subcloned into plasmid Bluescript as pGR14. Clones JEL1, JEL2, and JEL3 were obtained from screening another lot of 10^6 recombinant colonies from the same library, using synthetic oligonucleotides corresponding to the 5' end of the cDNA insert of pGR14. Clone COIL1 was obtained from a third round of screening of the MOLT-4 library using synthetic oligonucleotides corresponding to the 5' region of JEL1 cDNA insert. COIL1 cDNA was in fact 30 nucleotides shorter than that of JEL1. The overlapping regions of these clones were identical. The longest clone JEL1 had an open reading frame of 1,215 nucleotides, which would encode a polypeptide with a predicted molecular mass of 43 kD. The partial cDNA sequence for p80-coilin antigen and the deduced amino acid sequence are shown in Fig. 7. No consensus sequence motif or homology with other published sequences was identified. Special features of the amino acid sequence include a high percentage of serine (15.3%), the presence of short peptide repeats (Ala-Arg-Asn-Ser and Ser-Leu-Pro-Ala), and four direct repeats of Arg-Gly. In an attempt to obtain full-length cDNA, three other libraries de-

rived from HL60, HepG2, and WI38 cells were analyzed. After screening 10^6 phages from each library, we obtained clones that confirmed the sequence obtained from the MOLT-4 library, but did not uncover the lacking 5' sequence.

A recombinant protein was obtained from clone λ213 by inducing the expression of the phage lac gene with IPTG. This fusion protein contains the deduced 14-kD COOH-terminal region of p80-coilin. Affinity-purified antibody was able to reproduce both the 80-kD band in Western blotting (Fig. 8 A) and the IIF nuclear body staining pattern (Fig. 9). The homogeneous nucleoplasmic staining observed with the original serum (Fig. 9 A) was not detected by antibodies affinity purified from the recombinant protein (Fig. 9 B).

A lysate of *E. coli* expressing the recombinant protein from λ213 was used as substrate for immunoblotting with different sera (Fig. 8 B). The reactivity with the 130-kD (β-galactosidase–p80-coilin fusion protein) was observed in 18 of 20 sera with anti-p80-coilin specificity, and not detected by normal human serum or sera with other known autoantibody specificities. Such a specific reactivity with most of the prototype sera suggests that at least one immunodominant autoepitope is present in the COOH-terminal region of the molecule represented by clone λ213.

A purified preparation of the recombinant protein derived from pGR14 (the same cDNA fragment in λ213, subcloned into plasmid Bluescript) was used to coat microtiter plates for ELISA. Fig. 10 shows the titration curves of the antigen
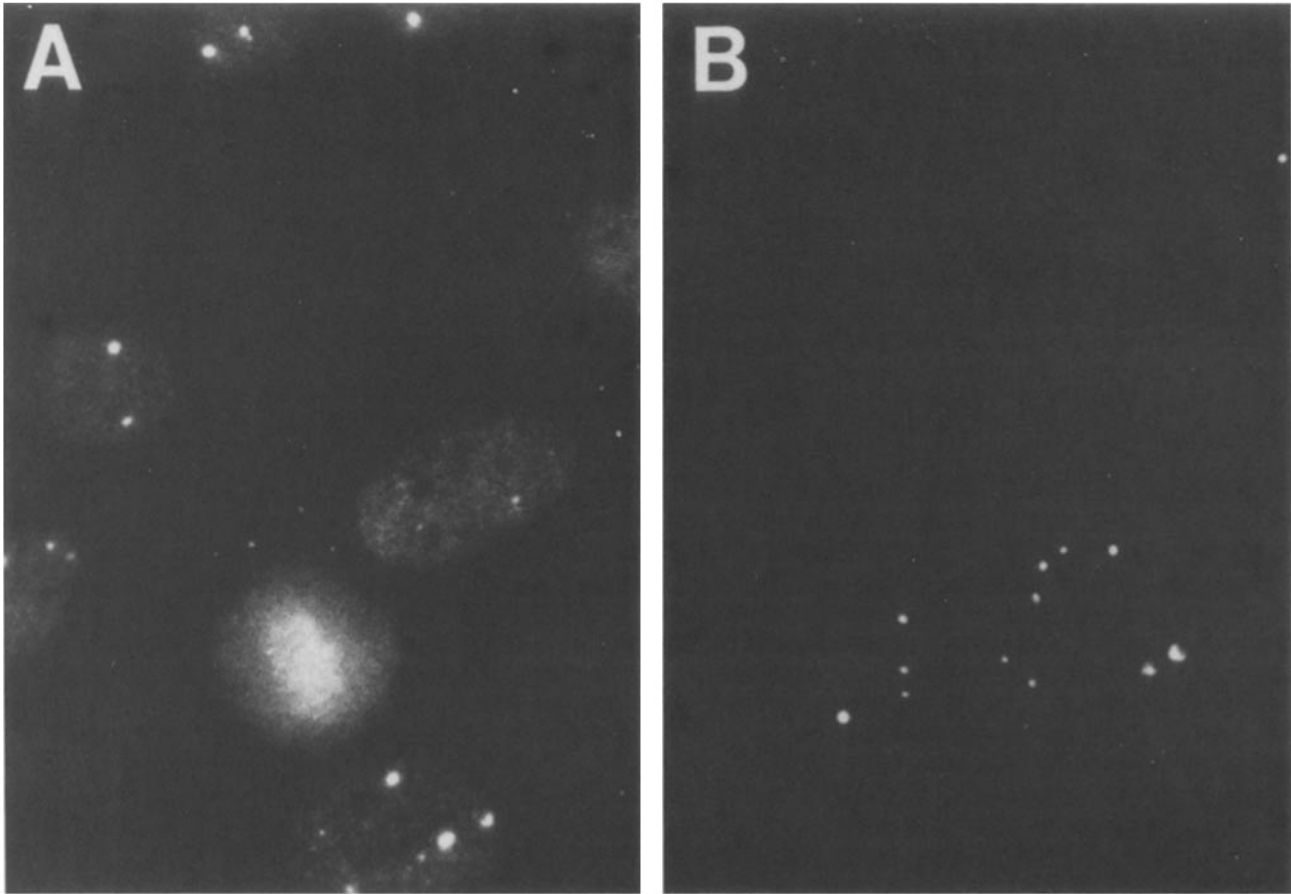
Figure 9. IIF staining using affinity-purified antibody from recombinant protein encoded by phage λ213. HEp-2 cells were immunostained either with serum Sh (*A*) or affinity-purified antibody, the same preparation as in Fig. 8 *A*, lane *1* (*B*). The characteristic staining of nuclear coiled bodies was exhibited by both preparations, but the weak nucleoplasmic staining exhibited by the whole serum was not detected with the affinity-purified antibody.
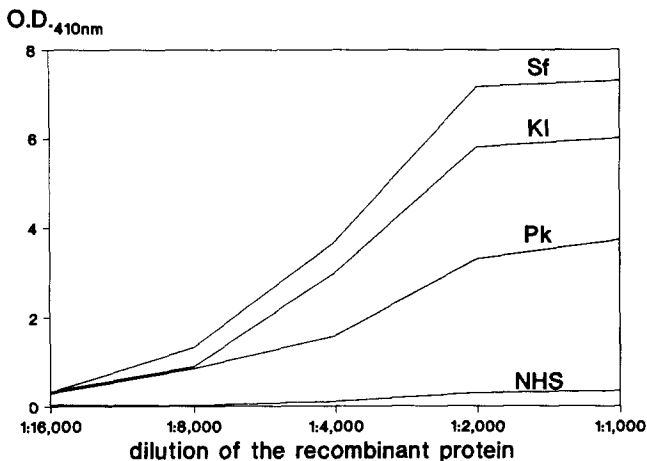

Figure 10. ELISA reactivity of the recombinant protein derived from pGR-14. A partially purified preparation of recombinant pGR-14 was prepared as described in Materials and Methods, diluted from 1:1,000 to 1:16,000, and used to coat microtiter wells. Titration curves for the recombinant protein were obtained with three prototype anti-p80-coilin sera and a normal human serum all used at 1:200 dilutions.

against three prototype anti-p80-coilin sera and a normal human serum. Table 2 depicts the ELISA readings obtained with normal human sera, different autoimmune sera, and anti-p80-coilin sera after 1 h of incubation with the detecting reagents, confirming the sensitivity and specificity of the assay.

This ELISA system was used to screen sera from several different diseases (Table 3). Sera with OD_{410} reading three times above the normal range were further screened by Western blotting and IIF. None of the patients with rheumatoid arthritis, chronic active hepatitis, multiple sclerosis, scleroderma, or systemic lupus erythematosus were found to have antibodies to p80-coilin. 2 of 59 sera from primary biliary cirrhosis (3.4%) and 2 of 54 sera from primary Sjögren's syndrome (3.7%) contained antibodies to p80-coilin.

Discussion

The coiled body is a nuclear ultrastructure defined as round-to-oval bodies averaging 0.5–0.8 μm in diameter, composed of coiled and fibrillar strands. It is located in the interchromatin space at various distances between the nucleolus and the nu-

1415   Andrade et al.

Table 2. *Reactivity of Recombinant Protein pGR-14 in ELISA*

Sera	Patient	Reactivity
Normal human	Ru	0.002
	Sa	0.006
	Pe	0.045
	La	0.029
	To	0.012
Autoimmune	Anti-dsDNA	0.010
	Anti-ssDNA	0.016
	Anti-Sm	0.000
	Anti-SSA/SSB	0.004
	Anti-Ku	0.004
Anti-p80-coilin	Mo	0.220
	Tu	0.261
	Wi	0.293
	El	0.381
	St	0.401
	Pz	0.402
	Cl	0.407
	Du	0.407
	Wk	0.465
	Ve	0.481
	Wo	0.490
	Bu	0.500
	Ab	0.574
	Km	0.630
	Nu	0.649
	Cq	0.670
	Pk	0.758
	Sh	0.768
	Op	0.872
	Kl	1.405
	Sf	1.526
	Tt	1.607
	Gm	1.666
	Kr	2.419
	Tk	3.108

Recombinant protein substrate was used at the dilution of 1:8,000 (see Fig. 10). All sera were diluted 1:200. ELISA OD_{410} readings were 1 h after addition of detecting reagent.

Table 3. *Antibodies to P80-coilin*

Disease	Patients tested	Positive patients
Rheumatoid arthritis	31	0
Scleroderma	44	0
Systemic lupus erythematosus	44	0
Sjögren's syndrome	54	2
Primary biliary cirrhosis	59	2
Chronic active hepatitis	32	0
Multiple sclerosis	26	0

Sera above were first screened by ELISA against the recombinant protein derived from pGR14. Those with OD_{410} three times above the normal range were further tested and confirmed by immunofluorescence and Western blotting. Those sera identified on basis of immunofluorescence alone (see also Table 1) are not included.

clear envelope. It was first described at the light microscopic level in 1903, by the Spanish cytologist Ramon y Cajal (26). Using special silver staining techniques, Cajal detected heavily stained inclusions in nuclei of neurons and designated them "accessory bodies". The ultrastructural identity of bodies defined as the coiled body and the accessory body of Cajal was suggested by Hardin et al. (29) and confirmed by Seite et al. (30), and Lafarga et al. (27). The coiled body is a highly conserved cell component, being observed in several eukaryotic cells (31), and although it has been the subject of many morphological studies, no major insight into its function has been achieved.

Human sera with a distinct immunofluorescent pattern, consisting of discrete round intranuclear bodies, were shown to recognize a cellular protein with an electrophoretic mobility of 80 kD in Western blotting. This association was confirmed by the reproduction of the nuclear body staining pattern using antibodies affinity purified from the 80-kD band. By immunoelectron microscopy, the antigen was shown to be predominantly located in the coiled body, thus being designated p80-coilin. Human autoantibodies to p80-coilin and synthetic oligonucleotides were used as probes to isolate cDNA clones. The recombinant protein λ213 representing a 14-kD region from the COOH terminus was recognized by most of the prototype anti-p80-coilin sera in Western blotting and in ELISA, suggesting that at least one epitope is present at the COOH-terminal end of the molecule.

The literature on indirect immunofluorescence of antinuclear antibodies describes a variety of nuclear speckled patterns. The IIF pattern observed with anti-p80-coilin antibody, which will be called nuclear coilin pattern, consisted of discrete round bodies randomly distributed in the interphase nucleoplasm, ranging from zero to six per cell. Characteristically, these nuclear bodies were not evident in the metaphase plate of mitotic cells. The nuclear coilin pattern can be easily distinguished from the one related to anticentromere antibodies, since the latter presents a higher number and smaller size of speckles and a characteristic distribution at the metaphase plate (2). Another readily distinguishable immunofluorescent pattern is the one called variable large speckles (VLS), which is represented by 3–10 nuclear dots of variable size, and is caused by antihistone H3 antibodies (32). In contrast to the nuclear coilin pattern, VLS staining is primarily observed in tissue substrates and not detected in lymphocytes and cultured cells (32).

Reports of discrete speckled nuclear IIF patterns are found in the literature under various designations, such as "multiple nuclear dots" (33–35), "atypical discrete speckled nuclear staining" (36), "nuclear dots" (33, 37), and "NSpI" (nuclear speckled I) (38). Since these studies did not provide further characterization of the target antigens, it is not possible to make retrospective comparisons with p80-coilin. By means of an exchange of sera with Dr. M. J. Fritzler (Calgary, Canada), it was possible to rule out the identity of "NSpI" and coiled bodies. Recently, a patient with a paraneoplastic syndrome resembling systemic lupus erythematosus was reported to have serum antibodies reacting with three bands ∼80 kD in Western blotting and also yielding an immunofluorescent pattern of two to three nuclear dots (39). It is conceivable that one of the antigens targeted by this serum was p80-coilin, although other autoantibodies may yield a similar IIF pattern.

The biochemical composition and function of the coiled body are largely unknown since heretofore it has been identified only by light and electron microscopy. In preliminary studies in this laboratory, we have used immunoelectron microscopy and double label immunofluorescence, with anti-p80-coilin as the reference marker and other specific antinuclear antibodies as detecting reagents, in order to determine which nuclear antigens of known structure and function might colocalize with p80-coilin. The most striking finding was the close association of p80-coilin with fibrillarin, a 34-kD protein component of the U3 ribonucleoprotein particle (40), which has been shown to be involved in 5' ribosomal RNA processing (41). Also identified in the coiled body were Sm and U1 RNP antigens and m_3G capped small RNA, all of which are components of small nuclear ribonucleoproteins involved in pre-mRNA splicing (42, 43). The presence of Sm antigens in the coiled body had also been demonstrated previously (44, 45). These preliminary findings might suggest a role for the coiled body in splicing or other RNA processing functions, but further studies need to be performed. Also of interest are some electron microscopic studies that suggest a relationship between the coiled body and the nucleolus (29, 30). Recently, we were able to obtain some evidence supporting these observations (46). Using double immunofluorescence with human autoantibodies to p80-coilin and mouse mAb to fibrillarin (40), we showed that in rat

neurons the coiled body is frequently in close association with the nucleolus. Furthermore, when HeLa cells were treated with actinomycin D or 5,6-dichloro-1-β-D-ribofuranosyl-benzimidazole, agents that promote nucleolar segregation into granular and fibrillar components, p80-coilin immunostaining was detectable in the fibrillarin-positive regions of the segregated nucleoli (46). Although the sensitivity of p80-coilin immunofluorescence to RNase might suggest an association of the protein with RNA, the sequence of the partial cDNA for p80-coilin did not exhibit any of the traditional RNA-recognition motifs (14, 47). However, it should be noted that in the primary structure of the available sequence, there are two stretches rich in basic amino acids arginine and lysine that could be involved in RNA binding (reviewed in reference 48).

Autoantibodies to p80-coilin were not associated with a single disease entity but were detected in diverse disease conditions. In spite of this apparent diversity, 12 of 20 patients, in whom clinical information was available, fell into the broad category of rheumatic diseases, including Sjögren's syndrome, systemic lupus erythematosus, scleroderma, and Raynaud's phenomenon, as well as other rheumatic diseases usually thought to be nonautoimmune in nature. The apparent association with rheumatic diseases, however, may need to be taken with reservation since this study was initiated from the observation of an unusual signal in ANA immunofluorescence and it is highly likely that the patients from which these sera were derived were being seen predominantly in rheumatology clinics. Although not extensive, analysis in ELISA using recombinant p80-coilin (Table 3) showed that the antibody was detected in a few patients with Sjögren's syndrome and primary biliary cirrhosis. It is intriguing to consider the possibility that this autoimmune response might be related to some special clinical feature in diverse diseases, but at the present time, the limited clinical information and retrospective nature of the study have not helped in providing any leading clues to this question. It is possible that prospective studies and analysis of other diseases might be more revealing. At the present time, the identification of a distinctive antibody marker for the nuclear coiled body and the availability of cDNA clones encoding a related protein component might be of help in elucidating the function of this nuclear organelle.

Address correspondence to Eng M. Tan, W. M. Keck Autoimmune Disease Center, Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, CA 92037.

References

1. Tan, E.M. 1989. Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. *Adv. Immunol.* 44:93.

2. Moroi, Y., C. Peebles, M.J. Fritzler, J. Steigerwald, and E.M. Tan. 1980. Autoantibody to centromere (kinetochore) in scleroderma sera. *Proc. Natl. Acad. Sci. USA.* 77:1627.

3. Price, C.M., G.A. McCarty, and D.E. Pettijohn. 1984. NuMA protein is a human autoantigen. *Arthritis Rheum.* 27:774.

4. Callarco-Gillan, P.D., M.C. Siebert, R. Hubble, T. Mitchison, and M. Kirschner. 1983. Centrosome development in early mouse embryos as defined by an autoantibody against pericentriolar material. *Cell.* 35:621.

5. Lerner, M.R., and J.A. Steitz. 1979. Antibodies to small nuclear RNAs complexed with proteins are produced by patients with systemic lupus erythematosus. *Proc. Natl. Acad. Sci. USA.* 76:5495.

6. Earnshaw, W.C., K.F. Sullivan, P.S. Macklin, C.A. Cooke, D.A. Kaiser, T.D. Pollard, N.F. Rothfield, and D.W. Cleveland. 1987. Molecular cloning of cDNA for CENP-B, the major human centromere autoantigen. *J. Cell Biol.* 104:817.

7. Theissen, H., M. Etzerodt, R. Reuter, C. Schneider, F. Lottspeich, P. Argos, R. Lurhmann, and L. Philipson. 1986. Cloning of the human cDNA for the U1 RNA-associated 70 K protein. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:3209.

8. Spritz, R.A., K. Strunk, C.S. Surowy, S.O. Hoch, D.E. Barton, and U. Francke. 1987. The human U1-70K protein: cDNA cloning, chromosomal localization, expression, alternative splicing, and RNA-binding. *Nucleic Acids Res.* 15:10373.

9. Sillekens, P.T.G., R.P. Beijer, W.J. Habets, and W.J. Van Venrooij. 1987. Molecular cloning of the human U1 snRNA-associated A protein: extensive homology between U1 and U2 snRNP-specific proteins. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:3841.

10. Habets, W.J., P.T.G. Sillekens, M.H. Hoet, J.A. Schalken, A.J.M. Roebroek, J.A.M. Lennissen, W.J.M. Van de Ven, and W.J. Van Venrooij. 1987. Analysis of a cDNA clone expressing a human autoimmune antigen: full-length sequence of the U2 small nuclear RNA-associated B" antigen. *Proc. Natl. Acad. Sci. USA.* 84:2421.

11. Rokeach, L.A., J.A. Haselby, and S.O. Hoch. 1988. Molecular cloning of a cDNA encoding the human Sm-D autoantigen. *Proc. Natl. Acad. Sci. USA.* 85:4832.

12. Stanford, D.A., M. Kehl, C.A. Perry, E.L. Holicky, S.E. Harvey, A.M. Rohleder, K. Rehder Jr., R. Luhrmann, and E.D. Wieben. 1988. The complete primary structure of the human snRNP E protein. *Nucleic Acids Res.* 16:10593.

13. Chan, E.K.L., K.F. Sullivan, R.L. Fox, and E.M. Tan. 1989. Sjögren's syndrome nuclear antigen B(La): cDNA cloning, structural domains, and autoepitopes. *J. Autoimmunity.* 2:321.

14. Chan, E.K.L., K.F. Sullivan, and E.M. Tan. 1989. Ribonucleoprotein SS-B/La belongs to a protein family with consensus sequences for RNA-binding. *Nucleic Acids Res.* 17:2233.

15. Deutscher, S.L., J.B. Harley, and J.D. Keene. 1988. Molecular analysis of the 60-kDa human ribonucleoprotein. *Proc. Natl. Acad. Sci. USA.* 85:9479.

16. Ben-Chetrit, E., B.J. Gandy, E.M. Tan, and K.F. Sullivan. 1989. Isolation and characterization of a cDNA clone encoding the 60-kDa component of the human SS-A/Ro ribonucleoprotein autoantigen. *J. Clin. Invest.* 83:1284.

17. Laemmli, U.K. 1970. Cleavage of structural protein during assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680.

18. Staufenbiel, M., and W. Deppert. 1984. Preparation of nuclear matrices from cultured cells: subfractionation of nuclei in situ. *J. Cell Biol.* 98:1886.

19. Reimer, G., I. Raska, U. Scheer, and E.M. Tan. 1988. Immunolocalization of the 7-2 ribonucleoprotein in the granular component of the nucleolus. *Exp. Cell Res.* 176:117.

20. Young, R.A., and R.W. Davis. 1983. Efficient isolation of genes by using antibody probes. *Proc. Natl. Acad. Sci. USA.* 80:1194.

21. Sanger, F. 1981. Determination of nucleotide sequence in DNA. *Science (Wash. DC).* 214:1205.

22. Feinberg, A.P., and B. Vogelstein. 1983. A technique for radio-labelling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 132:6.

23. Olmsted, J.B. 1981. Affinity purification of antibodies from diazotized paper blots of heterogeneous protein samples. *J. Biol. Chem.* 256:11955.

24. Adam, S.A., T. Nakagawa, M.S. Swanson, T.K. Woodruff, and G. Dreyfuss. 1986. mRNA polyadenylate-binding protein: gene isolation and sequencing and identification of a ribonucleoprotein consensus sequence. *Mol. Cell. Biol.* 6:2932.

25. Rubin, R.L. 1986. Enzyme-linked immunosorbent assay for anti-DNA and anti-histone antibodies. *In* Manual of Clinical Laboratory Immunology. 3rd ed. N.R. Rose, H. Friedman, and J.L. Fahey, editors. American Society for Microbiology, Washington, D.C. 744–748.

26. Ramon Y Cajal, S. 1903. Un sencillo metodo de coloracion seletiva del reticulo protoplasmatico y sus efectos en los diversos organos nerviosos de vertebrados e invertebrados. *Tra. Lab. Invest. Biol.* 2:129.

27. Lafarga, M., J.P. Hervas, M.C. Santa-Cruz, J. Villegas, and D. Crespo. 1983. The "accessory body" of Cajal in the neuronal nucleus. A light and electron microscopic approach. *Anat. Embriol.* 166:19.

28. Moreno Diaz de la Espina, S., M.A. Sanchez-Pina, and M.C. Risueno. 1982. Localization of acid phosphatasic activity, phosphate ions and inorganic cations in plant nuclear coiled bodies. *Cell Biol. Int. Rep.* 6:601.

29. Hardin, J.H., S.S. Spicer, and W.B. Greene. 1969. The para-nucleolar structure, accessory body of Cajal, sex chromatin, and related structures in nuclei of rat trigeminal neurons: a cytochemical and ultrastructural study. *Anat. Rec.* 164:403.

30. Seite, R., M-J. Pebusque, and M. Vio-Cigna. 1982. Argyrophilic proteins on coiled bodies in sympathetic neurons identified by Ag-NOR procedure. *Biol. Cell.* 46:97.

31. Moreno Diaz de la Espina, S., A. Sanchez Pina, M.C. Risueno, F.J. Medina, and M.E. Fernandez-Gomez. 1980. The role of plant coiled bodies in the nuclear RNA metabolism. *Electr. Microscopy.* 2:240.

32. Molden, D.P., G.L. Klipple, C.L. Peebles, R.L. Rubin, R.M. Nakamura, and E.M. Tan. 1986. IgM anti-histone H-3 antibody associated with undifferentiated rheumatic syndromes. *Arthritis Rheum.* 29:39.

33. Bernstein, R.M., J.M. Neuberger, C.C. Bunn, M.E. Callender, G.R.V. Hughes, and R. Williams. 1984. Diversity of autoantibodies in primary biliary cirrhosis and chronic active hepatitis. *Clin. Exp. Immunol.* 55:553.

34. French, M.A.H., and R.M. Bernstein. 1987. Immunoglobulin G subclass distribution of autoantibodies in systemic sclerosis, primary biliary cirrhosis, and overlap syndromes. *Ann. Rheum. Dis.* 46:436.

35. Cassani, F., F.B. Bianchi, M. Lenzi, U. Volta, and E. Pisi. 1985. Immunomorphological characterization of antinuclear anti-

bodies in chronic liver disease. *J. Clin. Pathol.* 38:801.

36. Powell, F., A.L. Schroeter, and E.R. Dickson. 1984. Antinuclear antibodies in primary biliary cirrhosis. *Lancet.* i:288.

37. Bernstein, R.M., J.C. Steigerwald, and E.M. Tan. 1982. Association of antinuclear and antinucleolar antibodies in progressive systemic sclerosis. *Clin. Exp. Immunol.* 48:43.

38. Fritzler, M.J., D.W. Valencia, and G.A. McCarty. 1984. Speckled pattern antinuclear antibodies resembling anticentromere antibodies. *Arthritis Rheum.* 27:92.

39. Freundlich, B., D. Makover, and G.G. Maul. 1988. A novel antinuclear antibody associated with a lupus-like paraneoplastic syndrome. *Ann. Intern. Med.* 109:295.

40. Reimer, G., K.M. Pollard, C.A. Penning, R.L. Ochs, M.A. Lischwe, H. Busch, and E.M. Tan. 1987. Monoclonal antibody from a (New Zealand Black × New Zealand White)F1 mouse and some human scleroderma sera target an Mr 34,000 nucleolar protein of the U3 RNP particle. *Arthritis Rheum.* 30:793.

41. Parker, K.A., and J.A. Steitz. 1987. Structural analysis of the human U3 ribonucleoprotein particle reveal a conserved sequence available for base pairing with pre-rRNA. *Mol. Cell. Biol.* 7:2899.

42. Yang, V.W., M.R. Lerner, J.A. Steitz, and S.J. Flint. 1981. A small nuclear ribonucleoprotein is required for splicing of adenoviral early RNA sequences. *Proc. Natl. Acad. Sci. USA.* 78:1371.

43. Sharp, P.A. 1987. Splicing of messenger RNA precursors. *Science (Wash. DC).* 235:766.

44. Fakan, S., G. Leser, and T.E. Martin. 1984. Ultrastructural distribution of a nuclear ribonucleoprotein as visualized by immunocytochemistry on thin sections. *J. Cell Biol.* 98:358.

45. Eliceiri, G.L., and J.S. Ryerse. 1984. Detection of intranuclear clusters of Sm antigens with monoclonal anti-Sm antibodies by immunoelectron microscopy. *J. Cell. Physiol.* 121:449.

46. Raska, I., R.L. Ochs, L.E.C. Andrade, E.K.L. Chan, R. Burlingame, C. Peebles, D. Gruol, and E.M. Tan. 1991. Association between the nucleolus and the coiled body. *J. Structural Biol.* 104:120.

47. Dreyfuss, G., M. Swanson, and S. Pinōl-Roma. 1988. Heterogeneous nuclear ribonucleoprotein particles and the pathway of mRNA formation. *Trends Biochem. Sci.* 13:86.

48. Zamore, P.D., M.L. Zapp, and M.R. Green. 1990. RNA bindings: βs and basics. *Nature (Lond.).* 348:485.

49. Deveraux, J., P. Haeberli, and O. Smithies. 1984. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* 12:387.