Intranuclear Localization of snRNP Antigens

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Abstract. Anti–Sm antibodies recognize a group of small, nuclear RNA-protein complexes (snRNPs) containing U1, U2, U4, U5, and U6 snRNAs. Anti–RNP antibodies only react with U1 snRNA-containing complexes.

The intranuclear distribution of snRNP particles was studied by double immunofluorescence staining of human fibroblasts. Mouse monoclonal anti–Sm antibodies and polyclonal patient sera reacting with different peptides in the snRNP complexes were used. The immunofluorescence patterns obtained with fluorescein isothiocyanate-conjugated anti-mouse Ig and tetramethylrhodamine isothiocyanate–conjugated anti–human Ig second antibodies were examined using computer analysis of digitized images. With this approach the similarity of different patterns could be visualized and estimated with mathematical methods. It was found that human anti–Sm serum as well as three different anti–RNP sera produced speckled patterns overlapping with the anti–Sm monoclonal pattern. Thus, Sm antigenic intranuclear domains also reacted with anti–RNP antibodies, suggesting a high degree of co-localization of the antigenic structures. A partial overlap was found between speckles detected by mouse anti–Sm antibodies and a human La-antiserum. No significant co-localization occurred between speckles detected by mouse anti–Sm antibodies and speckles detected by human antisera reacting with Scl-70 and centromeric antigens.

As the U1 snRNP complex is believed to play a role in the splicing of RNA polymerase II transcripts, it appears that the speckles detected by Sm and RNP antibodies may be regions of hnRNA synthesis and mRNA processing. Although no function has been demonstrated for the U2, U4, U5, and U6 snRNPs, the co-localization with the U1 RNA complexes shown in this report indicate that they too participate in some aspect of mRNA processing. The results suggest that computer-assisted analysis of nuclear immunofluorescence patterns will be a useful tool in studies of the spatial and functional organization of the interphase nucleus.

IMMUNE staining of mammalian cells with autoimmune sera frequently results in a speckled nuclear pattern. The two major specificities that produce speckled patterns are known as Sm and RNP. These autoantibodies occur primarily in systemic lupus erythematosus, mixed connective tissue disease, and less frequently in other autoimmune diseases (3, 31, 35).

The Sm and RNP antigenic structures are thus concentrated in certain nuclear domains. Immunochemical and biochemical studies have elucidated the nature of the antigens (1, 11–13, 16, 18, 20, 25, 33, 34, 37, 38). Sm-antibodies were shown to immunoprecipitate RNA-protein complexes containing a class of small nuclear RNAs designated U1, U2, U4, U5, and U6 snRNAs.[1] RNP-antibodies, on the other hand, immunoprecipitated only U1 RNA-containing complexes (18). Proteins were required for antigenicity (18). The fact that small, nuclear RNA–protein complex (snRNP)[2] particles containing only U1 RNA can be immunoprecipitated separately by RNP antibodies, suggest that the U1 snRNP particles are not strongly associated with the other snRNPs. The report of an anti–U2 snRNP antiserum allows the same conclusion to be made concerning the U2 snRNPs (20). In the case of U4 and U6, it has been shown that, most likely, they form a distinct complex (4, 9). Furthermore, it is possible to separate the snRNP particles by gradient centrifugation of cell extracts, indicating that the U1, U2, U4–U6, and U5 RNAs are present as distinct, stable RNA-protein complexes and not as firmly integrated subunits of a large multi-snRNA complex (16).

However, using low ionic strength buffers they can be found to co-migrate with hnRNP particles in gradients (30, 32, 39). By immunoprecipitation of ^{35}S-labeled cell extracts it has

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[1] U3 is nucleolar and not precipitated by Sm/RNP antibodies.

[2] *Abbreviations used in this paper:* FITC, fluorescein isothiocyanate; snRNP, small, nuclear RNA-protein complexes; TRITC, tetramethylrhodamine isothiocyanate.

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been demonstrated that the snRNPs contain at least seven to nine polypeptides denoted A–G (18). Two additional proteins were subsequently identified and named A′ and 68K (11). Some seem to be common to all the particles, like B/B′ and D. Others are restricted to individual complexes. The 68K, A, and C proteins are specific for U1 RNA-containing particles. At least one U2-specific polypeptide, A′, has been found (13, 20). Proteins uniquely associated with the other particles have not been reproducibly demonstrated. In summary, judging from studies of cell extracts, RNP-sera recognize one distinct U1 snRNP complex, and Sm sera recognize four different complexes, the U1, U2, U4/U6, and U5 snRNPs. In situ in the nucleus, they are restricted to certain regions, giving rise to the speckled immunofluorescence seen with Sm and RNP sera.

A distinguishing feature of the snRNA family is the presence of a 5′-terminal 2, 2, 7-trimethylguanosine cap structure. Antibodies prepared against this structure produced the same type of speckled nuclear immunofluorescence patterns as did the Sm and RNP antibodies (26).

Evidence concerning the biological function of snRNPs is more circumstantial. Consistent observations indicate that some members of this class play a role in the processing of polymerase II transcripts. U1-snRNPs are thought to participate in hnRNA splicing (14, 16, 23, 24), and U4 snRNPs could be involved in polyadenylation (2, 21). Taken together these observations suggest that the speckles seen with Sm and RNP antibodies represent transcript-processing nuclear domains, possibly in connection with hnRNA transcription. Such a connection has been demonstrated in the *Chironomus tentans* system by Sass et al. (29). Transcriptionally active regions in the salivary gland polytene chromosomes of *Chironomus tentans* larvae were shown to react strongly with Sm and RNP antibodies.

As several different snRNP complexes exist, interesting questions are whether all the snRNA species and all the antigens detected by Sm and RNP antisera are present in the same intranuclear domains, and what the fate of these structures is during the cell cycle. To approach such questions we have combined the use of well-characterized antisera with computer-aided image analysis of immunofluorescence patterns. The results obtained by immune staining with Sm and RNP antibodies have been compared with those obtained with antisera directed against the La (3, 31, 35), the Scl-70 (5), and centromeric antigens (22, 35).

Materials and Methods

Cells

Human fibroblasts of the 253/79 strain were kindly provided by Dr Stefan Söderhäll, Department of Clinical Genetics, Karolinska Hospital, Stockholm, and cultured on Dulbecco's modified Eagle's medium containing 10% fetal calf serum, streptomycin, and penicillin. The cells were seeded on Bürker hemocytometer slides, grown until subconfluent, and then used for immunofluorescence studies. Jurkat cells, a human T-cell leukemia line, were cultured on RPMI 1640 medium containing 5% fetal calf serum, streptomycin, and penicillin.

Antisera and Monoclonal Antibodies

All but one of the human sera used in this study were selected among a large number of autoimmune sera from the University of Missouri, Columbia (UMC). The patients had had serial clinical and serological evaluations. The UMC-ANA Laboratory profile included hemagglutination tests for antibodies to extractable nuclear antigen and DNA, immunodiffusion test for antibodies to RNP, Sm (Table I), La (SS-B), Ro (SS-A), Scl 70, and Pm-1; and *Crithidia luciliae* testing for antibodies to native DNA. Only Sm antibodies were detected in serum TA; sera CL, RE, and AL had only RNP antibodies; serum HA was positive only for antibodies to La-antigen; serum PE was positive only for antibodies to Scl-70. Analyses of snRNAs in immunoprecipitates and immunoblotting were finally used to rigorously define these sera as reacting specifically with certain RNAs and peptides as summarized in Table II. Serum LU was from a Stockholm CREST patient who had anti-centromere antibodies as judged by the fluorescent ANA test. The mouse anti-Sm hybridoma Y12 was kindly provided by Dr Joan Steitz, Yale University, New Haven, CT (17).

Analysis of Immunoprecipitated RNA

Immunoprecipitation, RNA extraction, and electrophoresis were carried out essentially as described (11, 18). The modifications involve the amounts of serum and cell extract as unlabeled cells were used in these experiments. For each immunoprecipitate, 20 μl of serum and a Jurkat cell extract corresponding to 5×10^7 cells was used.

Detection of Peptide Antigens by Immunoblotting

Jurkat cells were dissolved in SDS gel sample buffer, boiled for 10 min, and then electrophoresed on 15% SDS polyacrylamide gels according to Laemmli (15). The amount loaded corresponds to 1×10^6 cells/mm slot width. After separation, the peptides were transferred to Trans-Blot nitrocellulose sheets (Bio-Rad Laboratories, Richmond, CA) by electroblotting for 2 h at 225 mA in a buffer containing 25 mM Tris, 192 mM glycine, 0.1% SDS, and 25% methanol (20, 36).

The nitrocellulose filters were then blocked by incubating overnight in a 0.1% ovalbumin solution. After rinsing in PBS, 0.05% Tween-20, the filters were probed for the presence of antigenic peptides by incubating with patient sera diluted 1/250 or 1/400 (LU serum) in PBS-Tween. The anti-Sm monoclonal was diluted 1/100. The binding of these antibodies was detected using horseradish peroxidase conjugated anti-mouse Ig or anti-human IgG second antibodies (Dakopatts AS, Glostrup, Denmark) with 4-chloro-1-naphtol as the substrate (8).

Double Immunofluorescence Staining

The human fibroblasts were chosen because of their extreme flatness, a fact that greatly facilitated fluorescence microscopy. Slides with exponentially growing human fibroblasts of the 253/79 strain were rinsed in PBS and fixed in 1:1 acetone/ethanol at room temperature. Before staining, the slides were rehydrated in PBS, blocked with a 1% solution of ovalbumin, and rinsed three times in PBS. The preparations were then reacted with a mixture of mouse monoclonal anti-Sm antibodies, diluted 1:100, and human antisera. The latter were diluted 1:250 except the LU anticentromeric antiserum which was diluted 1:400. Staining was carried out for 40 min at 37°C, followed by three washes in PBS at room temperature. Incubation with second antibodies was carried out in two successive 20-min steps at room temperature. First the preparations were incubated with fluorescein isothiocyanate (FITC)-conjugated anti-mouse Ig antibodies (Dakopatts) diluted 1:20. After washing three times in PBS, the cells were then stained with tetramethylrhodamine isothiocyanate (TRITC)-conjugated antihuman-IgG antibodies (Dakopatts) diluted 1:20. This antibody had been absorbed with normal mouse serum. The slides were finally mounted in *p*-phenylendiamin in 9:1 glycerol/PBS.

Controls were done to detect cross-reactivities and nonspecific staining. To exclude the possibility of competitive antibody binding, the first antibodies were added in either order or mixed together, no detectable differences could be seen. After reacting with mouse or human first antibodies the preparations were incubated with inappropriate second antibodies. Other tests were made by omitting the first antibody and only staining with second antibodies. The results of these control experiments showed that the procedure used allowed accurate and simultaneous identification of the binding of mouse and human antibodies.

Digital Image Analysis

A detailed description of this procedure is given by Hallman et al. (manuscript in preparation). In short, the immune stained preparations were examined in a Zeiss fluorescence microscope (Carl Zeiss, Inc., Thornwood, NY) using a 100× oil immersion objective. A silicon intensifier target video camera was used to register the image and to transfer it to a Zeiss/Kontron IBAS computer. Using filters, two images, one representing the FITC and one representing the TRITC-conjugated antibody, were recorded. Control experiments established that the filters did not allow leakage of emitted light from one fluorochrome into the image recorded for the other. After recording a binary image representing the distribution of the first fluorochrome, the procedure was repeated for the second fluorochrome. Lateral displacements between the first and the second image due to the switch of filters on the microscope were corrected for. Subsequently, the two binary patterns were assigned different gray levels and added together. Overlapping image components would then have a different gray level from the original patterns. The visualization of the differences in gray levels was facilitated by the use of the pseudocolor feature of the instrument. The size and position of speckles as well as the degree of overlap between FITC and TRITC speckles was then recorded.

The number of nuclei and nuclear speckles analyzed per sample is indicated in Figs. 7 and 8.

Results

Specificity of the Antisera

Fig. 1 shows the RNAs found in immunoprecipitates using the mouse monoclonal Sm antibody Y12 and a number ofhuman autoantisera. Serum TA is a polyclonal patient anti-Sm serum, and CL, AL, and RE are human anti-RNP sera as defined by RNase digestions, hemagglutination, and immunodiffusion tests (Table I). The mouse and human anti-Sm immune precipitates (lanes *1* and *2*) contain all five snRNAs, i.e., U1, U2, U4, U5, and U6, whereas the anti-RNP precipitates contain only U1 RNA (lanes *3–5*). The band seen just below U1 is a slightly shorter breakdown product designated U1* in previous publications (16). The anti-La serum (lane *6*) immunoprecipitated a heterogenous mixture of small RNAs, which have been shown to consist of early RNA polymerase III transcripts (10, 27). La immunoprecipitates also contain a small amount of U1 RNA (10, 19).

The PE antiserum, lane *7*, directed against the Scl-70 (5) antigen and normal human control sera, lane *9*, bring down traces of U1 RNA and 5S RNA which represent nonspecific binding to the protein–A Sepharose immunoadsorbent. The LU immunoprecipitate shown in lane *8* contains some U1 RNA, the two small ribosomal RNA species 5S and 5.8S, and a smear with some clear bands included in the upper part of the gel. This pattern indicates that the anti–centromere sera also contain antibodies to ribosomes (17).

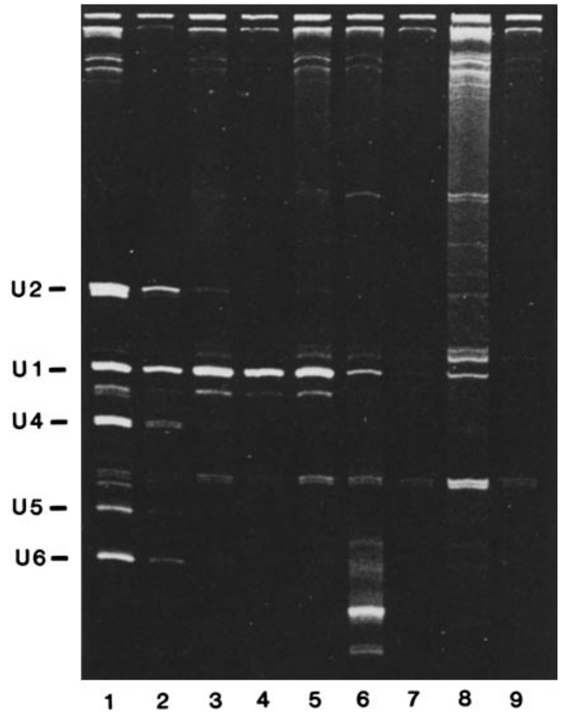


Figure 1. Polyacrylamide gel fractionation of immunoprecipitated RNAs. Precipitates were obtained using an extract from a human cell line, Jurkat, and antibodies from a mouse anti–SM hybridoma Y12 (lane *1*), human autoimmune sera (lanes *2–8*), and a normal human serum (lane *9*). The following autoimmune specificities were represented: anti–Sm serum, TA (lane *2*), anti–RNP sera, CL (lane *3*), RE (lane *4*), AL (lane *5*), anti–La serum, HA (lane *6*), anti–scl 70 serum, PE (lane *7*), and anti–centromere serum, LU (lane *8*).

Table I. Results of Hemagglutination and Immune Diffusion Assays

	Extractable nuclear antigen		Immunodiffusion	
Sera	HA titer	RNase result	RNP	Sm
RE	1:10,000	Sensitive	+	0
AL	1:10,000	Sensitive	+	0
CL	1:100,000	Sensitive	+	0
TA	1:10,000	Resistant	0	+

For a discussion of these techniques, see reference 31.

Fig. 2 illustrates the results obtained in immunoblotting experiments with the antisera listed in Table II. The Sm monoclonal and the anti–Sm serum react with snRNP polypeptides B/B′ and D (lanes *1* and *2*) in accordance with previously published results (20, 25). The three anti–RNP sera react with different combinations of the U1-associated snRNP polypeptides: CL with a 68K protein (lane *3*), RE with B/B′ and faintly with C (lane *4*), and AL with A (lane *5*) (20, 25). The La antiserum (lane *6*) identifies a 50K polypeptide (6, 7) which penetrates the nitrocellulose and thus stains more strongly on the backside of the sheet. The front side is shown here to demonstrate the absence of reactivity against the Sm or RNP specific peptides.

The scl-70 antiserum (lane *7*) did not react with any of the peptides normally present in snRNP complexes. The LU antiserum (lane *8*) reacted strongly with an 18K polypeptide associated with centromere regions (Hadlaczky, G., and N. R. Ringertz, manuscript in preparation). The normal human control serum (lane *9*) gives a weak background staining with histones. This background reactivity is present to a varying extent in different patient antisera.

The use of both immunoblotting and immunoprecipitation has confirmed the specificities assigned by other methods. It has also extended the previous analysis in two aspects important for this study: Firstly, most patient sera designated anti-Sm are actually mixed anti-Sm/anti-RNP (25). The B/B′ + D blotting pattern of serum TA demonstrates that it is a pure anti-Sm antisera. No reactivity is detected against peptides 68K, A, or C which are typically seen with RNP or Sm/RNP sera. Secondly, although all RNP sera by definition immunoprecipitate complexes containing U1 RNA, they contain antibodies reacting with different polypeptides in the U1 RNA–protein complex.

Immunofluorescence Patterns

Figs. 3–6 illustrate the immunofluorescence patterns obtained with the anti–Sm and anti–RNP antisera and with antisera to other nuclear antigens that do not involve snRNA–protein complexes. For each human autoantibody an internal standard was provided by the mouse monoclonal anti–Sm antibody using double immune staining as described in the Materials and Methods section.

To obtain the pictures, the fluorescent patterns were con-

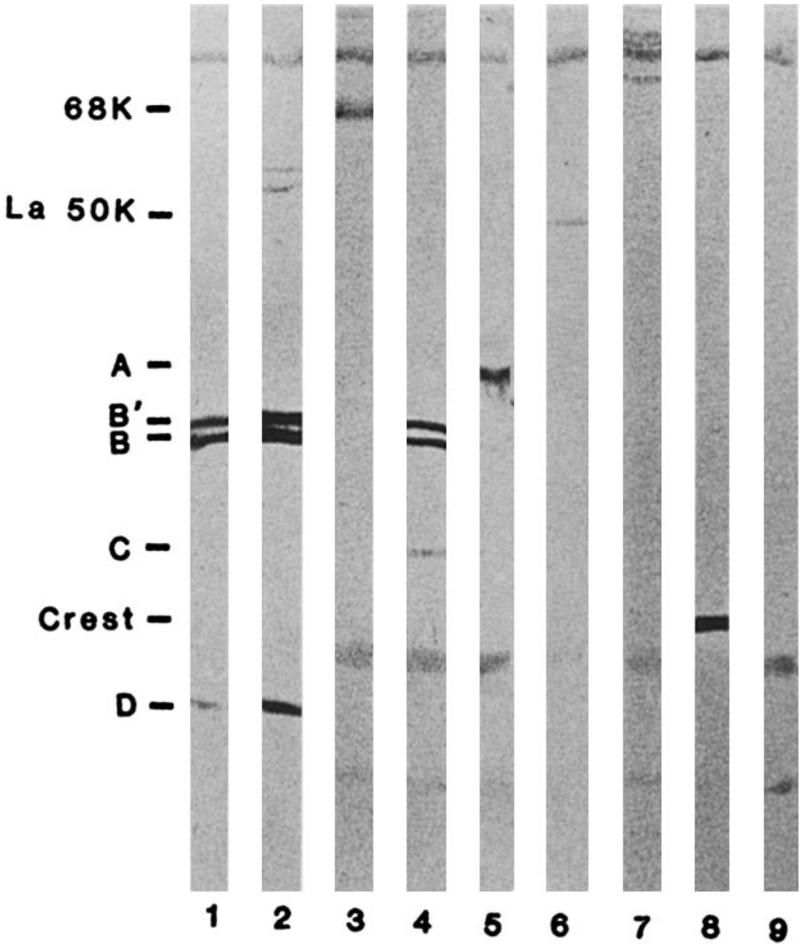


Figure 2. Immunoblotting of SDS polyacrylamide gel-fractionated Jurkat proteins. A mouse monoclonal, human autoimmune sera, and normal human sera were used. The snRNP proteins are denoted with the nomenclature of reference 25. The nitrocellulose strips with the transferred proteins were reacted with the following antibodies: The mouse anti-Sm monoclonal Y12 (strip *1*), anti-Sm serum TA (strip *2*), anti-RNP sera CL (strip *3*), RE (strip *4*), AL (strip *5*), anti-La serum HA (strip *6*), anti-Scl-70 sera PE (strip *7*), anti-centromere sera LU (strip *8*), and normal human serum (strip *9*).

Table II. Summary of Antibody Specificities

Specificity/antibody	Disease or strain	Major RNA in immunoprecipitation	Immunoblot peptide reactivity
Anti-Sm			
Y12 mouse monoclonal	MRL/1pr strain	U1, U2, U4, U5, U6	B/B', D
TA	SLE	U1, U2, U4, U5, U6	B/B', D
Anti-RNP (68K+)			
CL	MCTD	U1	68K
Anti-RNP (68K−)			
RE	MCTD	U1	B/B', C
AL	SLE	U1	A
Anti-La			
HA	Sjögrens syndrome	U1, 5S, and 4.5S smear	50K
Anti-Scl-70			
PE	PSS	—	—
Anti-centromere			
LU	CREST	5.8S, U1, 5S	18K

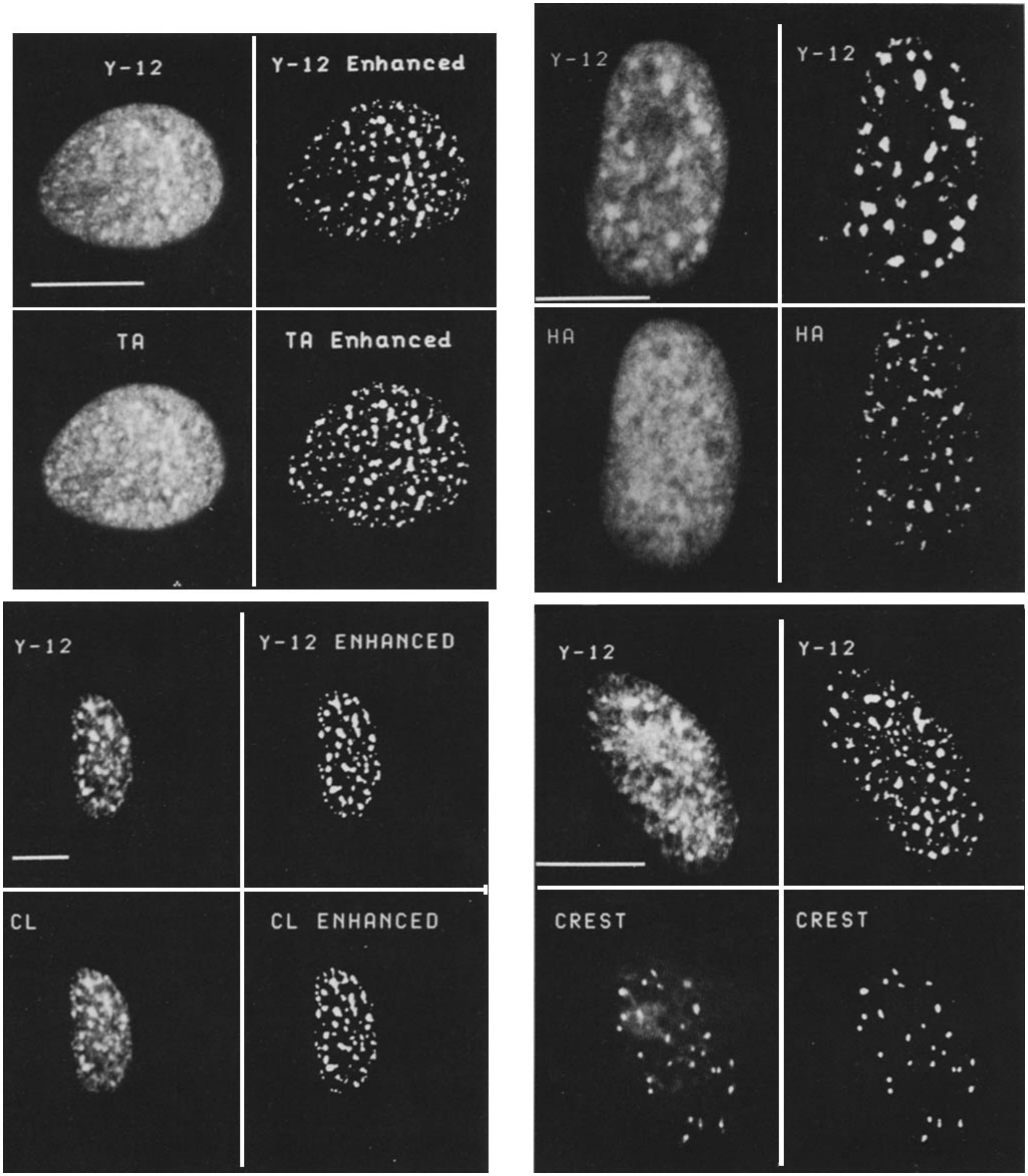
SLE, systemic lupus erythematosus; MCTD, mixed connective tissue disease; PSS, progressive systemic sclerosis or scleroderma; CREST, form of scleroderma characterized by calcinosis, Raynaud's phenomenon, esophageal dysmotility sclerodactyly and teleangiectasia (3, 31, 35). Patient sera are indicated by two capital letters.

verted to a digital image, displayed on a TV-monitor, and photographed. Note that the quality of the photographed image is limited by the monitor. In the left hand panels, the nuclear fluorescence is shown as recorded. The speckles are seen with diffuse borders embedded in a haze.

The images shown in the right hand panels are generated in the computer by a background-dependent gray level discrimination followed by a filtering procedure. As a result the haze and very small speckles are removed and in the subsequent image the speckles are presented as sharp bright spots against a dark background.

Fig. 3 shows the patterns obtained with the human anti-Sm serum and the mouse monoclonal anti-Sm. The similarity is obvious. The monoclonal anti-Sm versus human anti-Sm comparison provides the reference for overlapping patterns that are interpreted as co-localization of the antigens. As anti-RNP sera only immunoprecipitate one of the anti-Sm reactive complexes it was conceivable that correspondingly only a subgroup of Sm speckles would also contain the RNP antigen. On the contrary, the speckles seen with anti-RNP serum CL also agreed very well with those obtained with the mouse anti-Sm monoclonal (Fig. 4). The same results were obtained with the two other RNP sera (images not shown). Thus all Sm-reactive regions also contained the RNP antigen. In terms of the intranuclear distribution of the snRNP complexes this implies that the Sm-reactive U2, U4, U5, and U6 RNA-protein complexes are localized in the same nuclear domains as the Sm- and RNP-reactive U1 RNA-protein complexes.

Striking differences in the distribution of speckles were found when the La-antiserum (Fig. 5) and the anticentromeric serum (Fig. 6) were compared with the mouse anti-Sm monoclonal. Although the former sera also produced speckled



Figures 3–6. Nuclear immunofluorescence patterns after double immune staining of human fibroblasts. The mouse and human primary antibodies were identified with FITC- and TRITC-conjugated second antibodies, respectively. The fluorescence was recorded for both fluorochromes and converted into digital images by an IBAS computer. To obtain the pictures, the images were displayed on a TV-monitor and photographed. Left panel, unedited image. Right panel, image after gray level discrimination and filtering. Bar, 10 μm. (Fig. 3, upper left) Anti–Sm antiserum TA and mouse anti–Sm monoclonal; (Fig. 4, lower left) anti–RNP antiserum CL and mouse anti–Sm monoclonal; (Fig. 5, upper right) anti–La antiserum HA and mouse anti–Sm monoclonal; (Fig. 6, lower right) anti–centromere antiserum LU and mouse anti–Sm monoclonal.

nuclear immunofluorescence patterns, the speckles were differently distributed from those observed with anti–Sm and anti–RNP sera. It should be pointed out, however, that a certain overlap exists between the patterns produced by the La antiserum and the mouse anti–Sm monoclonal. This is partly due to the fact that the La antigen is more evenly distributed, whereas the mouse anti-Sm antibodies produce relatively well-defined speckles. The anti-centromeric serum produced very distinct speckles that showed a lower degree of overlap with Sm-speckles than did any of the other non-snRNP antisera.

The degree of overlap between anti-Sm and anti-RNP sera on the one hand, and the non-overlap of mouse anti-Sm with the other patient antisera on the other hand is quantified in Figs. 7 and 8. For each Y12 speckle its percent overlap with a speckle (or speckles) generated by a certain serum was calculated. The distribution of the speckles into different overlap categories was then plotted. The anti-Sm TA, anti-RNP sera, CL, RE, and AL have most of their speckles clustered around 80–100% overlap (Fig. 7). The controls (Fig. 8) with one exception show the opposite distribution: Most speckles are found in the 0% overlap region. The La antiserum HA gives a slightly different distribution: Like the other control sera it has no speckles in the high overlap region, but ~25% of the speckles are found in the intermediate overlap, 30–50% category. This parallels the observation that La sera immunoprecipitate U1 snRNPs to some extent (Fig. 1 and reference 19). Thus partial overlap can also be recognized with this method.

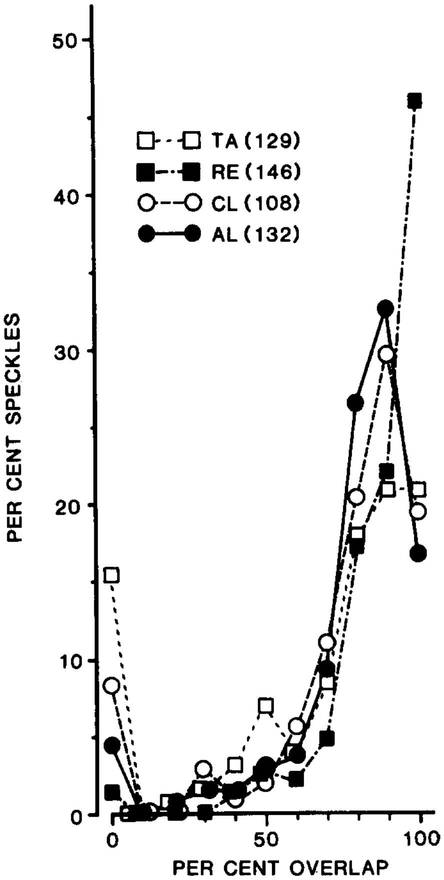


Figure 7. Degree of overlap between immunofluorescence patterns generated by a mouse anti-Sm monoclonal and human anti-Sm and anti-RNP antisera. For each speckle generated by the monoclonal the degree of overlap with a human Sm or RNP speckle was determined as described in Materials and Methods (and by Hallman, H., U. Nyman, I. Pettersson, G. Sharp, and N. R. Ringertz, manuscript in preparation). On the X-axis the percentage of overlap is divided into ten percentiles with zero being no overlap of the two immunofluorescence patterns and 100% being complete agreement. The Y-axis represents the proportions of speckles in each percentile to the number of speckles investigated (number in parentheses). Four to six nuclei were examined for each pair of antisera. □, TA human anti-Sm (129); O, CL human anti-RNP (108); ■, RE human anti-RNP (146); ●, AL human anti-RNP (132).

Discussion

In the present study we have attempted to analyze if certain nuclear antigens are located in the same nuclear region, within the resolving power of light microscopy. To achieve this we have combined the use of well-characterized antisera and computer-aided image analysis.

Examination of the double immunofluorescence images reported here showed that the pattern obtained with the mouse and the human Sm antibodies in one and the same cell and in the same focal plane is almost identical (Fig. 7). The identity is perfect if the comparison is restricted to the larger speckles. The fact that not all the small speckles coincide may be due to limitations in the technique as one approaches limits set by the wavelength of light or to imperfections in the alignment of the FITC and TRITC images relative to each other. As pointed out in the Results section, the immunoflu-

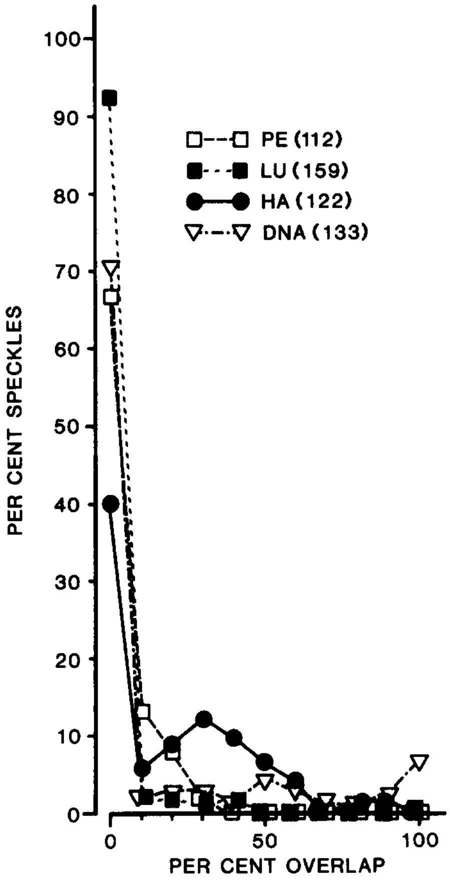


Figure 8. Degree of overlap between immunofluorescence patterns generated by mouse anti-Sm and anti-DNA monoclonals and human autoantisera. The curves were generated as described in Fig. 7. □, PE human anti-Scl-70 overlap with mouse anti-Sm monoclonal (112); ■, LU human anti-centromere overlap with mouse anti-Sm monoclonal (159); ●, HA human anti-La overlap with mouse anti-Sm monoclonal (122); ▽, mouse anti-DNA monoclonal overlap with human anti-Sm TA (133).

orescence pattern obtained with the three different patient anti–RNP sera overlaps with the monoclonal Sm pattern to the same extent as the patient Sm sera. Hypothetically it would be possible to obtain fluorescent speckles consisting of a pure Sm-antigen, a pure RNP-antigen, or a combination. The results show that all speckles, as far as can be judged, contain the RNP–antigen and nothing indicates that any Sm–antigenic complexes, i.e, U2, U4, U5, and U6 snRNPs, are located outside the RNP antigenic speckles. Naturally the Sm-antigenicity could mirror the sole presence of the U1 snRNP. However, to consider the possible interpretations the number of each snRNP in the cell nucleus must be taken into account. Estimations done by Ro-Choi and Busch (28), concerning the amount of snRNA, results in the following figures: U1 and U2 snRNAs are present at $\sim 1 \times 10^6$ copies each, per cell nucleus, U5 and U6 snRNA at $\sim 1 \times 10^5$ copies per cell nucleus. All snRNA molecules are present complexed to proteins, indicating that the number of snRNP particles should equal the number of snRNAs (18).

Therefore it is unlikely that co-localizing speckles, on the one hand visualized by antibodies specific to an antigen common to all snRNPs and on the other hand visualized by antibodies specific for U1 snRNP antigens, should reflect only the presence of the U1 snRNP in situ. Eventually the question whether all speckles in the fluorescence pattern contain all snRNP antigens, i.e., all speckles harbor U1, U2, U4, U5, and U6 snRNP or just U1 snRNPs and some number of the other snRNPs cannot be settled from these studies. The presence of speckles without U1 snRNPs is, however, ruled out by the overlap in patterns.

The La immunofluorescence pattern is quite different from that produced by the Sm/RNP antibodies. Still, as demonstrated in Fig. 8, there is a partial overlap between the two. Although the La antigen is primarily associated with RNA polymerase III transcripts (10, 27), the results of Madore et al. (19) support the finding of an overlap by showing that La antisera immunoprecipitate U1 snRNPs. This is also observed in the La immunoprecipitate in Fig. 1. Objections can be raised to these results, claiming the presence of anti–RNP antibodies in the La antiserum. However, Madore et al. (19) showed by using a La antigen free cell system that the U1 snRNP precipitation depends on the anti–La specificity.

Comparisons of the Sm and RNP immunofluorescence patterns with some other non-Sm/RNP specificities did not result in co-localization. A monoclonal DNA antibody detected epitopes concentrated along the nuclear periphery, a region that normally is occupied by dense heterochromatin. If anything, the anti-DNA immunofluorescence of the central portion of the nucleus showed an inverse relationship to the speckles detected with Sm/RNP antisera (image not shown). Neither did the speckles detected with the LU, anticentromeric, or the PE, anti Scl-70, antisera show any co-localization with Sm and RNP epitopes. The nonoverlap is clearly demonstrated in the plots of Fig. 8, but is not so readily appreciated by a look on the immunofluorescence patterns (Figs. 5 and 6).

The use of the double immunofluorescence technique combined with digital image analyses has made it possible to compare the distribution of different Sm/RNP antigens with considerable precision. The results so far, indicating that all the different snRNPs are present in the same nuclear regions, together with the fact that the La antigen co-localizes to some extent with the Sm/RNP complex, is of interest in the analysis of the intranuclear organization. It will be of great interest to compare the localization of these antigens with other antigens, such as the cap-antigen or RNA polymerases II and III, associated with transcriptional activity.

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