MINIREVIEW

Autoantibodies as Probes in Cell and Molecular Biology (43645)

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Sera obtained from human autoimmune patients contain autoantibodies that react with many important cellular antigens. These human sera have proven to be remarkably powerful investigative tools for cell and molecular biologists. In addition to allowing for the identification of many critical cellular proteins, human autoantisera also have been used to elucidate the details of many important intracellular events, for the purification and characterization of intracellular particles and organelles, for the cloning of the cDNA encoding several key cellular proteins, and for the characterization of the autoantigens that drive autoimmune reactions. In the future, it is hoped that molecular investigations of autoantigens will begin to provide clues as to the nature of the clinical abnormalities that result in the onset of autoimmune responses and, perhaps, give rise to new and novel immunosuppressive therapies that can be used to treat the individual autoimmune conditions.

The use of human autoantisera as research probes originated with the pioneering work of Hargraves *et al.* (1), Robbins *et al.* (2), and Holman and Deicher (3). Hargraves *et al.* (1) developed the lupus erythematosus cell test, a clinical laboratory procedure that is positive in a majority of systemic lupus erythematosus (SLE) patients. Subsequent investigations by Robbins *et al.* (2) and Holman and Deicher (3) demonstrated that the basis for the lupus erythematosus test was the presence of circulating antibodies in SLE patient sera that were directed against both DNA and deoxyribonucleoprotein. Since that time, numerous investigators have identified several different types of human autoantisera that contain useful autoantibodies and utilized these human sera for various research applications. These laboratory uses have covered almost all aspects of cell biology, ranging from studies of cell surface molecules to investigations of intranuclear events such as RNA processing and DNA synthesis (4, 5). Moreover, these research investigations have resulted in the molecular characterization of the autoantibody profiles that are expressed during the various autoimmune reactions, and this has proven to be a useful diagnostic tool for the clinician.

In this Minireview, the utility of human autoantisera as probes in studies of various cellular phenomena will be detailed. This will be achieved by first providing a brief background description of the particular cellular event that is about to be discussed, and then describing how human autoantisera have been used to contribute to our understanding of the workings of the particular cellular structures that are involved in that process. However, due to space limitations, it will not be possible to provide all of the details concerning many of the cellular pathways that are to be considered. Therefore, the reader will be directed to more comprehensive reviews covering each process when appropriate. In addition, it will not be possible to cite every instance when a human autoantiserum has been used in a cellular or molecular investigation. Instead, emphasis will be placed on the specific instances when human autoantisera have been instrumental in defining a cellular phenomenon. Moreover, this review will be limited to a description of autoantibodies that have been used to study general cellular processes and will not include antibody probes that are specific for one cell type. For example, myasthenia gravis autoantisera have been useful for investigating the acetylcholine receptor on muscle cells (6). Likewise, sera obtained from patients with

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various forms of pemphigus are being used to investigate the junctional complexes that allow for epithelial cells to adhere both to one another and to the basement membrane (7). Because these autoantisera are limited in their utility for investigating cellular phenomena exclusive to specific cell types, they will not be included in this review.

The Processing of Heterogenous Nuclear RNA to Messenger RNA

The most impressive example of the utility of human autoantisera as probes for cell and molecular biology has been the elucidation of the mechanisms involved in the intranuclear processing of heterogenous nuclear RNA to mRNA (8). Various investigators have used sera obtained from patients with SLE, mixed connective tissue disease, and scleroderma for experimental studies that resulted in both the identification of the proteins and RNA that are involved in the splicing reactions as well as for the characterization of the spliceosome and the splicing pathway. In addition, human autoantisera also are being used in studies concerning other forms of RNA processing and maturation, such as polyadenylation reactions. These investigations will be summarized in a later section.

Mammalian genes are composed of protein coding regions, called exons, and noncoding regions, termed introns. The primary transcript that is produced during transcription is an exact copy of the gene including both introns and exons, and because of the size heterogeneity of primary transcripts, these immature RNA are called heterogenous nuclear RNA (hnRNA). Before an RNA exits the nucleus to be translated into protein, the intron sequences are cut out of the hnRNA transcript to produce a mature mRNA that then is ready for translation. Because the coding sequences on either side of the intron sequence are joined together after the intron has been cut out, this series of events is termed RNA splicing. This hnRNA splicing reaction is thought to be catalyzed by a complex apparatus called the spliceosome (9, 10).

As RNA is synthesized, the hnRNA rapidly becomes associated with protein to form heterogenous nuclear ribonucleoprotein (hnRNP). Soon after, a group of small nuclear ribonucleoproteins (snRNP) bind to the hnRNP and the spliceosome complex assembles. Experimental evidence has demonstrated that the snRNP play a critical role in the excision of intron sequences. This is achieved by the binding of specific snRNP to conserved consensus sequences in introns (8) and then the removal of the introns from the pre-mRNA in a series of splicing and re-annealing reactions that occur under the direction of the snRNP. During the cleavage of the intron, a novel RNA structure called a lariat is formed, and the intron is released from the pre-mRNA as a lariat. The accuracy of the proper splicing is of paramount importance to the functioning of the cell. In fact, mutations in the splicing machinery have been shown to be the molecular abnormalities that result in the thalassemia syndromes in humans. For more in-depth discussions of the splicing of hnRNA, readers are directed to several recent reviews on the subject (11–14).

Much of what is known about mRNA precursor splicing is the result of two key experimental developments. The first break that assisted in the elucidation of these pathways was the identification of anti-snRNP autoantibodies in the sera of certain autoimmune patients, and the second major advancement was the development of systems that allowed for accurate splicing reactions to occur *in vitro* (15, 16). As a result of these developments, rapid progress was made in the understanding of spliceosome assembly, snRNP composition, and hnRNP processing.

Human antisera containing autoantibodies against either RNP or the Sm autoantigens have been used to characterize the molecular organization of snRNP. This was achieved by using the autoimmune sera to immunoprecipitate the reactive autoantigens from cell lysates and then determining the makeup of the precipitated substances (17). Analysis of the material that was immunoprecipitated using either anti-Sm or anti-RNP antisera demonstrated that the isolated cellular substances were composed of both protein and RNA. Moreover, the RNA was found to be of the small, nuclear, uracil-rich subtype (hence the name U RNA). To date, 13 different snRNP have been identified, and these snRNP are designated as U1–U13 (14, 17–21). The U RNA are small, approximately 50–200 nucleotides in length, and the major species, U1, U2, U4, U5, and U6, are present at up to 1 million copies per cell (12). All U RNA, except U6, contain a trimethylguanosine cap structure at their 5′ ends and most of the U RNA are nucleoplasmic, although U3, U8, and U13 are localized to the nucleolus (21–23). With the exception of U4 and U6, which occur together as a particle, all of the snRNP have been isolated as monomeric entities (12, 24, 25). In addition to containing U RNA, snRNP also are composed of up to nine different proteins and it is the proteinaceous components of the snRNP that are recognized by the human autoantibodies.

The Sm protein antigens are fundamental components of the U1, U2, U4, U5, and U6 snRNP. The Sm antigens, which form the core proteins of the snRNP, are six proteins with molecular masses of 11, 12, 13, 16, 28, and 29 kDa (5) that are bound to the U RNA at an AU(4–6)G consensus sequence (26). In addition to these proteins that are constant in all of the major snRNP, the individual snRNP also contain unique protein components. For example, sera obtained from lupus patients, designated as anti-RNP antisera, contain

circulating antibodies that recognize a set of protein antigens that are limited to the U1 snRNP. Experimental analysis has demonstrated that the U1-specific snRNP antigens are proteins with molecular masses of 22, 33, and 70 kDa (15, 18). In addition, it has been reported that certain patient sera recognize protein components that are present on the U2 snRNP alone (27). Finally, another snRNP, U3, is precipitated by sera obtained from a subclass of scleroderma patients whose sera contain circulating antibodies that recognize a 34-kDa U3-specific protein called fibrillarin (28, 29). The fact that patient sera have been identified that are reactive toward individual snRNP has allowed for precise experimental analysis of the roles that the individual snRNP play in the splicing process. For example, U1-specific human sera have been added to an *in vitro* splicing reaction to disrupt the functioning of the U1 snRNP, thereby allowing for an investigation of the role of the U1 snRNP in the hnRNP processing reactions.

The first hint that snRNP might be involved in hnRNA splicing was the observation that the sequence of the 5′ end of the U1 RNA was complimentary to the consensus sequence at the 5′ sites of introns (30, 31). For the most part, the information contained in introns encodes genetic nonsense. It is only the sequences at the 3′ and 5′ splice sites, as well as sequences within the lariat RNA, that are pivotal for the splicing process. Careful analyses of the RNA sequence of U1 RNA and the sequences at the 5′ splice site of introns led Lerner *et al.* (30) and Rogers and Wall (31) to hypothesize that U1 RNA could bind to the 5′ splice site of an intron through complimentary base pairing and, via some unknown mechanism, align the two ends of the intron during splicing. Although this model has been altered somewhat, the basics of this proposal have been verified both genetically and biochemically (8).

Antibody inhibition experiments provided the first evidence supporting the notion that snRNP, and particularly U1, played a role in the processing of hnRNA (15). In these experiments, either isolated nuclei or whole cell extracts were treated with patient sera that contained either anti-Sm antibodies or antisera that were specific for either U1 or U2 snRNP, and the level of spliced pre-mRNA was analyzed. From these studies it was observed that all sera that contained antibodies that reacted with the U1 snRNP inhibited splicing. Neither anti-U2 snRNP sera nor control human sera showed any inhibition of splicing under the conditions that were used. These results, which suggested that the snRNP were important in splicing, were verified subsequently by experiments in which the RNA component of snRNP was selectively inactivated by digestion with nucleases (32–34).

An RNase protection/immunoprecipitation assay has been used to determine the roles of the individual snRNP in the splicing reaction and to identify the RNA sequences that are bound by the individual U RNA. In this procedure, hnRNA is added to an incubation mixture that is composed of snRNP, antibodies that tag the snRNP, and RNase. During this procedure, the region of the hnRNA that is bound by a snRNP is protected from RNase degradation. The protected region then is immunoprecipitated using human autoantiserum and the sequence of the residual RNA fragment is determined. Using this experimental strategy, Mount *et al.* (35) demonstrated that U1 snRNP is bound by a 17-nucleotide stretch near the 5′ intron splice site and Chabot *et al.* (36) determined that U5 snRNP interacted with a 15-nucleotide long region near the 3′ end of the intron. In addition, Black *et al.* (34) were able to identify a U2 snRNP binding site within the lariat RNA. This region of the lariat is called the branch point because it is the site at which the spliced 5′ end of the intron is joined via a 2′-5′-phosphodiester bond to an adenosine residue near the 3′ splice region to produce a lariat structure. The U2 snRNP binds to a 40-nucleotide stretch of RNA in this region during spliceosome assembly. After formation of the lariat structure, the intron splicing reaction is completed by cutting off the RNA at the 3′ splice site, release of the intact lariat RNA, and ligation of 5′ and 3′ exon regions.

Pulse-chase experiments have determined the pathways of both snRNP and spliceosome assembly. In these studies, pulse-labeled cellular extracts were immunoprecipitated with either Sm or anti-U1 snRNP antisera at various times after labeling, and the composition of the immunoprecipitated complexes was characterized (14). These experiments have demonstrated that snRNP are assembled in the cytoplasm and then the intact particles are transported into the interphase nucleus (37). Once inside the nucleus, the spliceosome assembles on the hnRNP and intron excision occurs. Recent evidence suggests that spliceosome assembly and intron removal begin while the pre-mRNA transcript is still associated with the DNA template (38). After intron removal, the mature mRNA then pass through the nuclear pore complexes to the cytoplasm where translation occurs.

Assembly of the snRNP particles occurs in the cytoplasm. The core Sm proteins begin the assembly process in the absence of snRNA. Each of the major snRNP proteins is encoded by an individual mRNA, and the snRNP core proteins are stored in cytoplasmic pools (41, 42). Kinetic studies, coupled with anti-Sm immunoprecipitation, have demonstrated that several snRNA-free intermediates composed of snRNP core proteins exist in the cytoplasm (43, 44). These preassembled intermediates then are able to associate with the cytoplasmic U RNA via binding to the Sm concensus sequence. The final step in snRNP assembly occurs when the snRNP-specific proteins are added (45). After

completion of the snRNP assembly process, the mature snRNP are transported into the nucleus. It is believed that the snRNP core proteins contain the signals responsible for directing the intact snRNP to the nucleus (46).

Kinetic experiments also have been performed in an effort to elucidate the mechanisms of spliceosome assembly. U1 snRNP binds to pre-mRNA almost immediately after the addition of an RNA to an *in vitro* splicing extract (48). Soon after, U2 snRNP binds to the branch point of the intron (49), with binding of U2 snRNP being dependent upon the presence of an accessory protein called U2 auxiliary factor. U2 auxiliary factor binds specifically to the 3′ splice site (50) and, by some unknown mechanism, primes the branch point for binding by U2 snRNP. Finally, kinetic studies suggest that the U4, U5, and U6 snRNP then bind to the 3′ splice site as a single entity (51, 52). The mature spliceosome is a 50 S complex containing the five major snRNP as well as the pre-mRNA and associated accessory proteins (Fig. 1). At the conclusion of the splicing reaction, the excised intron is released in the form of a lariat. Associated with the lariat are U2, U5, and U6 snRNP (52, 53). The U4 snRNP is not attached to the lariat, and present data suggest that the U4 snRNP is not involved in the actual splicing reactions. Instead, the sole function of the U4 snRNP may be to deliver

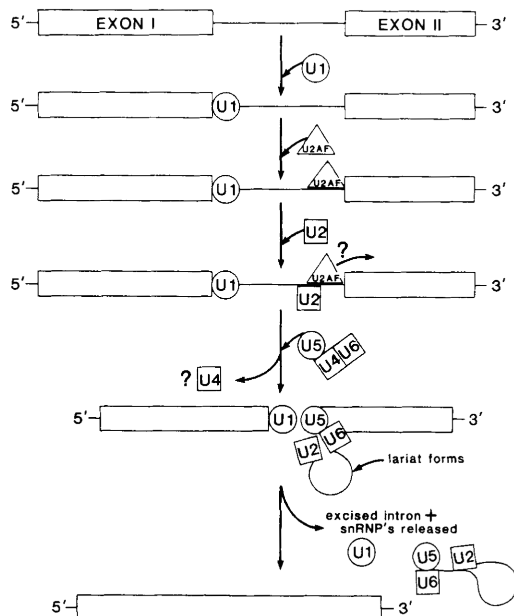

Figure 1. A schematic summarizing the events of RNA splicing. The U1 snRNP binds to an hnRNA molecule near the 5′ splice site of an intron. The U2 accessory factor attaches and this primes the hnRNA for U2 snRNP binding near the branch point site. U4, U5, and U6 then bind near the 3′ splice site, and the U4 snRNP apparently dissociates from the complex, suggesting that the function of U4 is to deliver the U6 snRNP to the growing spliceosome complex. The intron is spliced out and the two exons are joined. The excised intron and the snRNP are released and the snRNP then are re-united in additional splicing reactions. Modified from Zieve and Sauterer (14).

U6 to the assembling spliceosome (54). The snRNP then are recycled for further splicing reactions.

The mechanism by which snRNP direct the cleavage process still is not known, although several possible explanations have been proposed. It is conceivable that the snRNP may actually catalyze the splicing reactions. Alternatively, the snRNP may not be involved in the cleavage events themselves, but may simply hold the pre-mRNA in the appropriate configuration so that self-splicing of the mRNA precursor occurs (8, 12). Finally, it is possible that the snRNP may contain binding sites for as yet unidentified accessory proteins that would be responsible for catalyzing the intron splicing reactions. For instance, it is possible that the snRNP bind to the appropriate concensus sequences within introns and then the necessary putative enzymes bind to the snRNP and carry out the excision reactions. Further experimentation will be needed in order to elucidate the details of the splicing process.

In addition, other key questions still need to be addressed concerning hnRNA processing. For example, it is not known how the two exon-containing regions of the pre-mRNA are held together after the initial cleavage of the intron at its 5′ splice site. Also, the exact composition of the spliceosome has not been determined. Clearly, hnRNP and snRNP are present in spliceosomes, but it seems likely that other components also are involved in splicing. As was mentioned in the previous paragraph, it is possible that important enzymes, such as endonucleases and ligases, may be part of the spliceosome complex. However, these enzymes may not be necessary for splicing since it is well established that certain forms of RNA are self-splicing (55) and it is possible that the enzymatic activity required for splicing is contained within the RNA itself. Once the exact composition of the spliceosome is detailed, it should be possible to fill in some of the gaps in our understanding of the intron splicing pathway. Finally, one of the major unanswered questions regarding intron splicing is how differential splicing of pre-mRNA is regulated. During embryonic development, a single pre-mRNA can be spliced in different ways or in different tissues at various stages of development to produce closely related isoforms of a protein (56). The involvement of the snRNP in these events is unknown presently. The use of human autoantisera, together with mutant analysis in yeast and Drosophila, should allow for these questions regarding snRNP, splicing, and spliceosomes to be answered.

Other RNA Processing Reactions

In addition to splicing, several other events occur during the intranuclear maturation of RNA. Recently, several minor snRNP have been identified by immunoprecipitation using autoimmune anti-Sm antiserum. Experimental evidence suggests that some of these less

abundant snRNP may be involved in other pre-mRNA processing events. Specifically, U11 has been implicated as participating in the polyadenylation reactions that occur at the 3′ end of the pre-mRNA. Also, U7 has been reported to be an essential participant in the processing of histone pre-mRNA, and U3, U8, and U13 may be involved in the specialized events that result in the production of rRNA.

Unlike most eukaryotic RNA, histone mRNA do not contain poly-A tails. Instead, the 3′ end of histone mRNA is produced by a simple cleavage reaction (57, 58). U7, a minor snRNP that originally was immunoprecipitated from sea urchin extracts using Sm serum, plays an essential role in histone mRNA processing (19, 59). U7 fulfills its function by binding to a sequence near the 3′ end of the histone mRNA that was identified using an *in vitro* nuclease protection assay similar to the one that was described in the previous section (59). In addition, a second RNA sequence also was precipitated in these experiments. This sequence, which contains a hairpin loop that is thought to be involved in regulating the stability of histone mRNA (60), is not bound by an snRNP. Instead, an uncharacterized protein factor that contains Sm determinants is bound to this region of the histone mRNA (19, 61). How the binding of U7 snRNP and the second Sm-precipitable protein results in cleavage of the 3′ end of the histone mRNA is not known yet.

Unlike histone mRNA, most RNA polymerase II transcripts are defined by the presence of a poly-A tail at the 3′ end (polymerase I and III transcripts do not contain a poly-A tail). After transcription, the pre-mRNA is cleaved at a specific site and the enzyme poly-A polymerase adds up to 200 adenylate residues to the 3′ end of the RNA transcript. Although many of the details concerning the cleavage and polyadenylation reactions are not known, it is clear that either snRNP or Sm-reactive polypeptides are involved. This conclusion is based on the reported observation that *in vitro* polyadenylation can be inhibited by the addition of Sm antiserum to the splicing extract (62). To date, the Sm-precipitable component responsible for inhibiting polyadenylation has not been identified.

In addition to Sm-precipitable products, recent evidence suggests that U11 snRNP also is involved in the polyadenylation process. Starting with HeLa cell nuclear extracts that were able to perform the cleavage and polyadenylation of pre-mRNA, Cristofori and Keller (20) were able to purify three protein factors that contributed to the polyadenylation event. In addition to poly-A polymerase, two other proteinaceous components were characterized. One of these components was a protein of 70–120 kDa that was involved in the cleavage events only, while the other factor was necessary for both cleavage and polyadenylation reactions and was termed cleavage and polyadenylation factor (CPF). Extensive purification of CPF determined that CPF activity co-purified with U11 snRNP, suggesting that the U11 snRNP was a component of the CPF activity. However, sequence analysis has demonstrated that U11 snRNA does not contain a sequence that is complementary to the AAUAAA stretch that serves as the polyadenylation signal of pre-mRNA. Therefore, the binding of U11 snRNP to the polyadenylation signal most probably does not occur through RNA-RNA base pairing. Clearly much more needs to be learned regarding polyadenylation reactions.

Finally, U3, U8, and U13 are thought to be involved in the processing of pre-rRNA. rRNA is initially transcribed into a 45 S precursor, and this large precursor molecule subsequently is cleaved into the 28, 18, and 5.8 S rRNA. These RNA, along with the 5 S rRNA (the 5 S rRNA is transcribed separately), then complex with proteins to form the ribosomes. Due to their localization to the nucleolus by immunofluorescence microscopy (21, 23), it has been suggested that these snRNP may be involved in the cleavage of the 45 S precursor. Although U3, U8, and U13 snRNP were immunoprecipitated using scleroderma sera, these snRNP are not of the Sm class (21, 63). The detailed composition of these three snRNP is not known, but experimentation has demonstrated that the U3 snRNP comprises at least six other proteins, two of which are phosphoproteins, in addition to the scleroderma autoantigens (64). The non-scleroderma-reactive components that comprise the U8 and U13 snRNP remain unknown.

The evidence that supports the notion that U3, U8, and U13 are involved in rRNA processing is largely circumstantial. Specifically, U3 and U8 have been localized to the nucleolus by immunocytochemistry (28, 29) and U3 has been crosslinked to high molecular weight rRNA precursors using chemical crosslinking reagents (65). However, the specific sequence that binds U3 has not been identified yet, and, as a result, the roles of U3, U8, and U13 in rRNA processing remain largely unknown.

In summary, human autoantisera have allowed for the identification of a family of intranuclear particles called snRNP that play critical roles in the processing of RNA. The function of the snRNP in the splicing of the hnRNA is quite well understood, although some key gaps still exist in our knowledge of these cellular events. Minor snRNP also appear to be involved in other forms of RNA processing, such as polyadenylation, rRNA cleavage, and histone pre-mRNA processing. The exact roles of the snRNP in these cellular events remain a relative mystery, although the next few years should shed considerable light on the workings of the minor snRNP.

The Nucleolus

The nucleolus is the region of the nucleus that is specialized for the production of ribosomes from rRNA and proteins. In the previous section, the processing of rRNA was considered and a description of the nucleolus is a logical extension of these preliminary discussions. The nucleolus is the region of the nucleus that contains the tandemly arranged rRNA genes. As stated earlier, mammalian rRNA is transcribed as a 45 S precursor that is rapidly cleaved and processed, events that occur within the nucleolus, to yield the mature 28 S, 18 S, and 5.8 S rRNA species. The rRNA then associate with numerous proteins to form mature 40 S and 60 S ribosomal subunits, which then exit the nucleus to the cytoplasm to participate in the events of protein synthesis. For more in-depth discussions of ribosome assembly, readers are directed to more comprehensive reviews of ribosome biochemistry (66–69).

Three different regions can be resolved when the nucleolus is examined by electron microscopy: a granular region, dense fibrillar regions, and fibrillar centers (66, 67). Presumably each of these subregions of the nucleolus contains the molecular machinery responsible for carrying out various steps in the ribosome synthesis and maturation pathways. To date, relatively little information exists that would explain how the nucleolus is able to achieve the events of ribosome assembly, although many of the probes that will be necessary for carrying out studies of ribosome formation have been identified (67). Autoantibodies directed against the nucleolus are prevalent in sera obtained from autoimmune patients, mostly from patients with systemic sclerosis (70). In addition to autoantibodies reactive with the U3, U8, and U13 snRNP, various other nucleolar autoantigens have been characterized, including antibodies against various proteins and RNP particles (5, 67).

Ribosomal genes are transcribed specifically by RNA polymerase (pol) I. Autoantibodies against RNA pol I have been used in an immunoelectron microscopic study to localize RNA pol I to the fibrillar centers of the nucleolus (71). The localization of RNA pol I specifically to the fibrillar centers, and not to either the granular region or fibrillar regions, suggests that the transcription of the rRNA genes occurs exclusively in the fibrillar centers of nucleoli. Moreover, fibrillarin, the 34-kDa autoantigen that is associated with the nucleolar snRNP, also is localized to the fibrillar centers of the nucleolus (29). As detailed in the previous section, the U3 snRNP is thought to be involved in processing of the 45 S rRNA primary transcript (72). Presumably, then, the initial steps in rRNA maturation also occur in the fibrillar centers. The cDNA encoding human fibrillarin has been cloned and sequenced, and the protein sequence contains a putative RNA-binding region (73). The determination of the primary structure of human fibrillarin offers an opportunity for investigating the events of rRNA processing in great detail.

A novel autoantibody has been identified in sera obtained from certain patients with polymyositis/scleroderma (Pm-Scl) overlap syndromes that recognizes an antigen called the PM-Scl antigen (67, 74). Immunoelectron microscopic analysis has localized the PM-Scl antigen to the granular region of nucleoli (75), the proposed site of ribosomal assembly. To date, the role played by the PM-Scl antigen in preribosome assembly is unknown. Moreover, the antigen recognized by the PM-Scl autoantibodies has been neither cloned nor characterized. Cloning of the cDNA encoding this autoantigen will be essential for elucidating the role of this protein in ribosome maturation. The final nucleolus-specific autoantigen is a small RNP complex called the 7-2 ribonucleoprotein (75, 76). Based on its localization to the granular nucleolar region (76) one might speculate that 7-2 RNP is involved in one of the stages of ribosome biogenesis and assembly that are thought to occur in the granular zone. Much more experimentation will be required before the role of the 7-2 RNP can be established, however.

Human autoantibodies have been used to investigate nucleolar reformation following mitosis (77, 78). Extreme morphologic and biochemical changes occur in a cell during mitotic division, and one of these modifications is that ribosome biogenesis ceases and the nucleolus break down. The fate of the nucleolar components during mitosis is relatively unknown due to the fact that the nucleolar subunits cannot be resolved at the electron microscopic level during cell division. The nucleolus then reforms at the telophase/G_1 boundary in association with chromosomal regions termed the nucleolus organizing regions (NOR).

The fate of various nucleolar autoantigens has been investigated by immunofluorescence and immunoelectron microscopy. Fibrillarin (29, 78) was shown to bind to all regions of the chromosome surface, except for the centromere, during mitosis, while PM-Scl antigen was demonstrated to be scattered diffusely throughout the spindle region during cell division (74). A novel autoantigen termed NOR 90 has been identified using serum obtained from a scleroderma patient (77). Antibodies present in this patient's serum specifically recognized an antigen that was localized to the NOR of human chromosomes 13, 14, 15, 21, and 22. At telophase, the nucleolar fragments reform around the NOR of each of these chromosomes and the prenucleoli then coalesce to form the intact interphase nucleolus. Presently, the function of NOR 90 is not known, but it is tempting to speculate that this protein autoantigen has a role in nucleolar reformation following mitosis.

Mitosis and the Microtubule Organizing Centers

Human autoantisera have been especially useful for studies of mitosis. In particular, autoantibodies that are present in sera obtained from a subclass of scleroderma patients have been instrumental in investigations of the kinetochore/centromere domains of mitotic chromosomes and, to a lesser degree, for studies of the centrosome/spindle pole region. Sera obtained from patients with the calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia variation (CREST variation) of scleroderma contain autoantibodies that specifically recognize the centromere/kinetochore regions of mitotic chromosomes when cells are fixed and processed for immunofluorescence microscopy (79–81). In addition, sera obtained from a small number of patients exhibiting scleroderma and/or Raynaud's phenomenon contain circulating antibodies that react specifically with the centriole/centrosome region of cells (82–86). These antisera have allowed for both the biochemical characterization and functional analysis of kinetochores and centrosomes. In addition, scleroderma sera also have allowed for the cloning and characterization of the cDNA encoding some of the protein constituents that make up these important organelles. In the future, it is hoped that human autoantisera will allow for the purification and detailed analysis of kinetochores and centrosomes.

For decades, the terms centromere and kinetochore were used interchangeably by cell biologists and geneticists. We now know that the indiscriminate use of these two terms should be avoided. Scleroderma sera have been used to demonstrate that important biochemical differences exist between these two regions of mitotic chromosomes (80). In addition, electron microscopy has shown that the kinetochore is a specialized subregion of the centromere to which the spindle microtubules attach (87). Therefore, in this Minireview the term centromere will be used to describe the entire primary constriction region of mitotic chromosomes, including the regions of alphoid satellite DNA repeats and the microtubule attachment sites, while the term kinetochore will be used specifically to define the region of the chromosomes to which spindle microtubules attach and, presumably, the area through which mitotic forces are generated.

The kinetochore originally was described by Brinkley and Stubblefield (87). These investigators examined mitotic, cultured mammalian cells by electron microscopy and reported the presence of an elaborate structure on the surface of the mitotic chromosomes where the spindle fibers terminated. The kinetochore was described as being a trilaminar plate-like structure composed of an inner plate that rested on the subjacent centromeric heterochromatin, an electron lucent middle layer, and an electron-dense outer plate where the spindle microtubules attached (see Ref. 88 for review). This is largely where our knowledge of the kinetochore stood until the early 1980s. At that time, a serendipitous discovery was made in a rheumatology clinic that had a remarkable impact on studies of the centromere/kinetochore. Tan and co-workers (79, 80) reported that sera obtained from scleroderma CREST patients contained antibodies that recognized the mammalian kinetochore (Fig. 2). The identification of antikinetochore antibodies provided the molecular tools that have allowed for in-depth molecular and biochemical analysis of the mammalian centromere.

The three-dimensional organization of the kinetochore changes during the cell cycle. In interphase, the kinetochores can be identified in the nucleus by immunofluorescence microscopy using CREST serum as amorphous fluorescent foci. Not surprisingly, the number of fluorescent speckles that can be identified in the interphase nucleus corresponds exactly with chromosome numbers in most species (80). Near the S/G_2 boundary of the cell cycle, the number of fluorescent speckles observed in cell nuclei doubles (80). This is an apparent indication that chromosome duplication has occurred. As the cell enters prophase, the daughter kinetochores can be resolved on opposite sides of the primary constriction, and by metaphase, the typical trilaminar kinetochore morphology that was described in the previous paragraph is observed (88).

Western blot analysis of isolated mammalian chromosomes using CREST antisera has allowed for the identification of a family of kinetochore/centromere proteins ranging in molecular masses from 14 to 140 kDa (89–93). In the most comprehensive study of the circulating antibodies present in CREST patient sera, Earnshaw and Rothfield (90) consistently identified three proteins of molecular masses 18 kDa (CENP-A), 80 kDa (CENP-B), and 140 kDa (CENP-C) on immunoblots of human chromosomes. In addition to these proteins, other investigators have identified additional CREST-reactive proteins by immunoblotting (89, 93). The reasons for these discrepancies in the antibody repertoire in the sera of various CREST patients are not known at this time.

CREST antisera have been used to investigate the roles of the kinetochore in spindle formation and chromosome segregation. In these studies, autoimmune CREST antibodies were microinjected into either dividing mouse eggs (94) or cultured mammalian cells (95), and the effects of the injected antibodies on both the assembly of the mitotic apparatus and chromosome segregation events were analyzed. These experiments convincingly demonstrated that the CREST antigens play an essential role in the events of prometaphase. In cells that were injected before nuclear envelope breakdown, prometaphase chromosome congression events

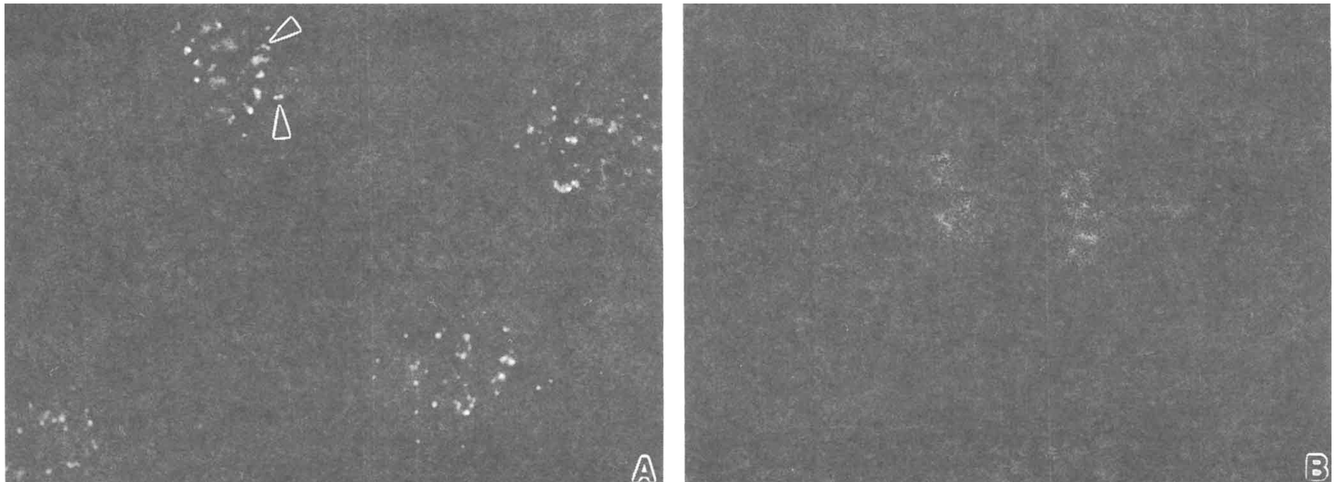

Figure 2. Immunofluorescent staining of cultured mammalian cells using human autoimmune scleroderma CREST antiserum. (A) The kinetochores can be identified as amorphous intranuclear speckles during interphase. Late during the cell cycle, the kinetochores are duplicated and appear as closely spaced pairs of fluorescent foci (arrows). (B) During mitosis, CREST sera stain the microtubule attachment sites of chromosomes. In this anaphase cell, the two sets of segregating chromosomes can be identified by the CREST-reactive kinetochores.

were disrupted. Chromosomes that were unable to align on the metaphase plate subsequently were segregated aberrantly, resulting in aneuploidy in daughter cells (94). Moreover, the fact that anaphase segregation events were not disrupted when CREST antibodies were injected into anaphase cells suggests that the kinetochore contains other, non-CREST proteins that are responsible for anaphase chromosome movements.

Chemical crosslinking experiments have been performed in an effort to determine which of the CREST antigens might be involved in the microtubule interactions that were discussed in the preceding paragraph. As was mentioned earlier, CREST sera contain autoantibodies that recognized several different kinetochore proteins on immunoblots. To characterize which of these proteins was involved in kinetochore-microtubule interactions, tubulin, the major protein subunit of microtubules, was covalently attached to kinetochores using nearest-neighbor crosslinking reagents (96, 97). The crosslinked complexes subsequently were purified by antitubulin affinity chromatography and then characterized biochemically. Analysis of the crosslinked complexes demonstrated that the 80-kDa CREST antigen was contiguous to kinetochore-bound tubulin, suggesting that the 80-kDa antigen is involved in the attachment of chromosomes to microtubules during mitosis. However, this conclusion has not been supported by immunoelectron microscopic localization of CENP-B (81). Monospecific antibodies against CENP-B were produced using peptide fragments of the protein that were generated using the cloned cDNA that encoded the protein (98). By immunoelectron microscopy, the monospecific anti-CENP-B antibodies were localized to deeper regions of the centromere, and were not observed to be associated with the kinetochore plates (81). Several possible explanations exist that might explain the discrepancies that exist between the results obtained using crosslinking reagents and those reported using immunolocalization. First, the differences might be due to variability in the organization of centromeres. The crosslinking experiments that were described were performed using CHO chromosomes, whereas the immunolocalization was performed using cultured human cells. This possibility has received recent support from Zinkowski *et al.* (99), who demonstrated that fundamental differences exist in the distribution of the 80-kDa CENP-B protein in the centromeres of CHO and HeLa chromosomes. Alternatively, it is conceivable that the crosslinking of tubulin to the 80-kDa protein might be due to the fact that the crosslinking experiments were performed using chromosomes that were isolated from drug-arrested cells. It has been shown previously that treatment of cells with mitotic inhibitors, such as colchicine, results in minor alterations in kinetochore morphology (88). These drug-induced changes in kinetochore ultrastructure may have resulted in the placement of the 80-kDa protein in an orientation that would have allowed for crosslinking of tubulin to occur. Clearly, much more experimentation will be required in order to understand how the CREST antigens are involved in kinetochore-microtubule interactions.

cDNA expression libraries have been screened using CREST sera and the cDNA encoding CENP-B and CENP-C have been cloned and sequenced (98, 100). Analysis of the cDNA sequence has determined that CENP-B is a very acidic protein with a P_i of near 5.0. It is an appealing hypothesis to envision the acidic CENP-B interacting with the basic histone molecules during chromosome condensation, with the result being

the formation of the mitotic centromere/kinetochore region (101, 102). This hypothesis is supported by recent evidence demonstrating that CENP-B may have the ability to bind directly to alphoid DNA through a 17-bp motif within CENP-B called the CENP-B box (103, 104). How this association contributes to centromere organization is not clear at this time. Additional sequence analysis of CENP-B has identified a small acidic domain that shows direct homology to a subregion of tubulin (105). This acidic region of tubulin is involved in the binding of a subclass of proteins called microtubule-associated proteins (MAP) to microtubules (106). The MAP-binding domain of CENP-B may provide a possible explanation for how kinetochores bind to microtubules. According to this scheme, CENP-B would not bind directly to tubulin, but instead would interact with an MAP (105). This MAP, in turn, then could bind to microtubules.

Brinkley and co-workers (107, 108) have utilized CREST sera to demonstrate the importance of the kinetochore in mitotic events. In these studies, kinetochores were experimentally detached from chromosomes by treating cultured CHO cells with millimolar concentrations of caffeine. When the treated cells entered mitosis the cells were fixed and processed for immunofluorescence microscopy using CREST serum. Immunofluorescence demonstrated that the detached kinetochores were able to bind to spindle microtubules and to undergo the entire repertoire of mitotic movements including prometaphase congression and anaphase segregation events. Moreover, the chromosome arms, which lacked microtubule binding sites, were unable to attach to the spindle fibers and were displaced to the cell periphery, demonstrating that the chromosome arms contribute little to the mitotic process. Whether the kinetochore contains the force-generating molecules that drive chromosome movements or whether specific kinetochore proteins exist that allow for kinetochore association with cellular microtubule ATPases has not been determined.

Human autoantisera also are being utilized for studying the centrosomes. Unlike microfilaments, which can assemble at any location inside the cytoplasm, microtubules always are nucleated from and associated with specific intracellular structures termed microtubule organizing centers (MTOC). The principal MTOC in the mammalian cell is the centriole/centrosome complex, although other MTOC have been described.

The centrosome has been well-defined at the ultrastructural level (139). In animal cells, centrosomes are composed of a pair of centrioles arrayed at right angles to each other that are surrounded by an amorphous, electron-dense cloud called the pericentriolar material. As a cell prepares for mitosis, the centrosome duplicates and the daughter centrosomes then serve as the two spindle poles during the ensuing mitosis (109). Along with centrosome duplication, the microtubule nucleating capacity of the centrosomes undergoes a dramatic change as the cell enters mitosis. As the cell enters prophase, the interphase microtubule cytoskeleton is disassembled and replaced by the mitotic apparatus. Along with this change in microtubule cytoskeletal architecture, the microtubule nucleating ability of the centrosomes is increased substantially. Presumably, this increase in the nucleation capacity of the centrosome ensures the proper formation of the mitotic spindle. The molecular changes that occur in the centrosome that give rise to the heightened nucleating ability of the centrosome during mitosis may be driven by the p34 cdc2 kinase of M-phase promoting factor (MPF). MPF is the protein complex that is involved in driving a cell through mitosis. MPF achieves this by phosphorylating various cellular substrates at the G_2/M transition (110), and potential cellular targets of MPF kinase are thought to be proteins within the centrosome complex. This supposition is based both on the finding that the phosphorylation state of unknown centrosome proteins changes during the interphase to mitosis transition (111) and by the localization of p34 to mitotic centrosomes by immunofluorescence microscopy (112).

Two distinct types of immunofluorescent staining patterns have been identified when cultured cells were fixed and stained for anticentrosome immunofluorescence microscopy using human autoantisera obtained from patients exhibiting either scleroderma and/or Raynaud's phenomenon (Fig. 3). One set of patient sera recognized epitopes that were present in both interphase and mitotic centrosomes (82, 83, 86, 114, 115), while the other set of patient antisera contained autoantibodies that recognized epitopes that were present only in mitotic centrosomes (85, 86). These human anticentrosome autoantisera have been used in Western blot studies to identify several centrosome proteins. Rattner *et al.* (115) demonstrated that certain human autoimmune anticentrosome antisera reacted with a 48-kDa protein that was identified either as the glycolytic enzyme enolase or as a protein that shared antigenic epitopes with enolase. Balczon and West (86) have identified centrosome-specific antigens of 39, 185, and 220 kDa, although the possibility that the 185-kDa polypeptide is a proteolytic breakdown product of the 220-kDa protein was not eliminated. Mitosis-specific centrosome antigens have been identified with molecular masses of 110–115 kDa (85) and 190 kDa (86) by immunoblot analysis using antisera that react only with mitotic centrosomes. Recent evidence (116, 117) suggests that these mitosis-specific, centrosome-associated antigens may actually be nuclear proteins that segregate to the spindle poles along spindle microtubules during mitosis. If this is true, then this would be the first demonstration of a cellular component besides chro-

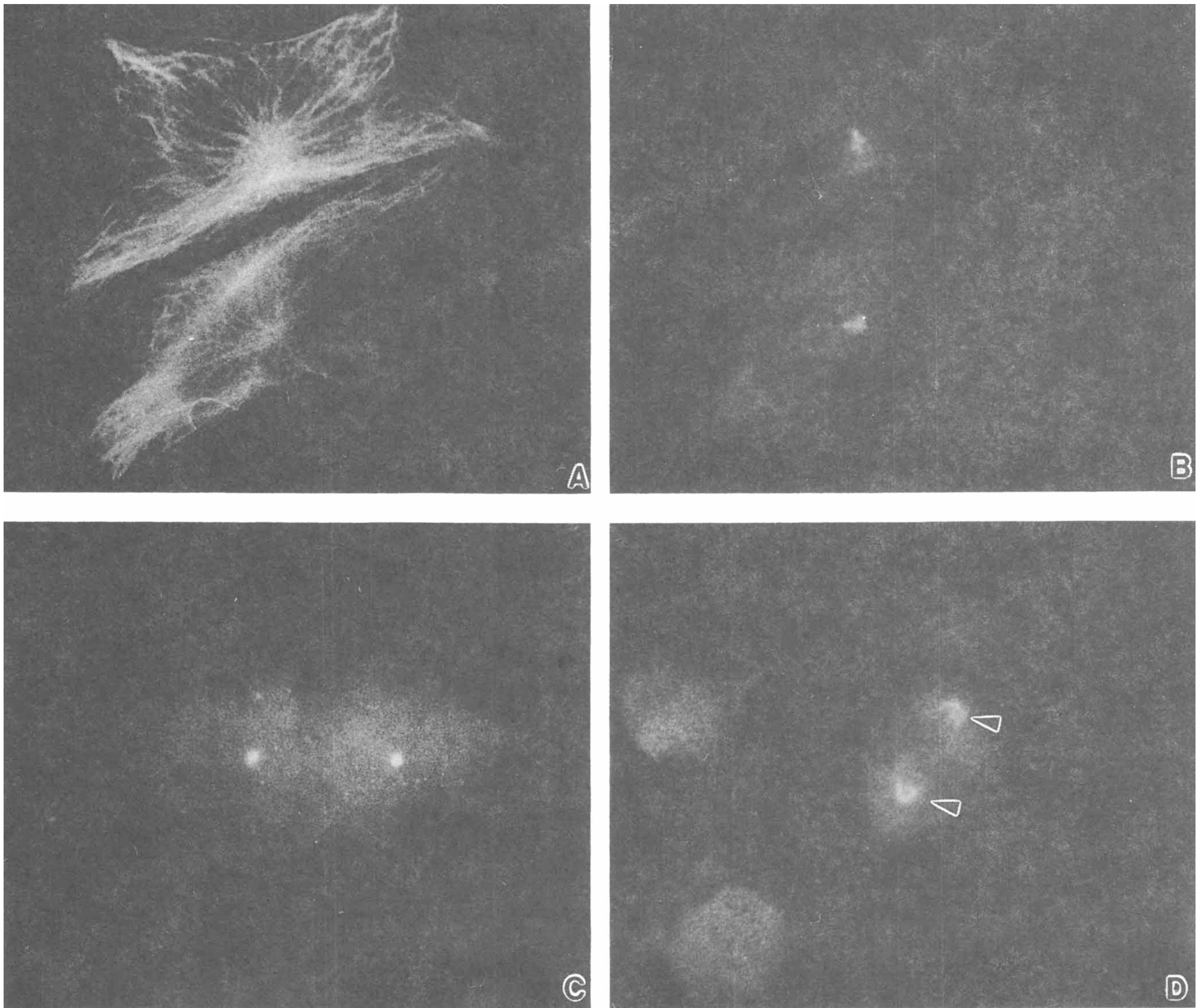

Figure 3. Immunofluorescent staining of cultured mammalian cells using antitubulin and human autoimmune centrosome antisera. (A) and (B) are a double label image of the same field of cells. (A) Antitubulin staining. (B) Human autoimmune anticentrosome staining. Notice that the anticentrosome-reactive material is at the focal point of the microtubule cytoskeleton. (C) Staining of a mitotic cell using the same serum as in (B). Note that both spindle poles are recognized. (D) Staining of cells using a second human autoimmune serum that recognizes only mitotic centrosomes. The spindle poles on the mitotic cell (arrowhead) are stained while the centrosomes in the numerous surrounding interphase cells are nonreactive.

mosomes that utilizes spindle fibers for segregation during mitosis.

To date, a function has not been assigned to any of the centrosome autoantigens. Using *in vitro* microtubule assays, Balczon and West (86) were unable to inhibit microtubule assembly from centrosomes in a lysed cell system using antisera that reacted with either mitotic centrosomes specifically or sera that recognized antigens present in both interphase and mitotic centrosomes. The most obvious conclusion from those experiments is that the centrosome autoantigens played no role in microtubule organization. However, because the experiments were performed using colcemid-arrested cells, other possibilities, such as the fact that small microtubule fragments may still have been associated with the pericentriolar material, exist that may explain these results. Therefore, more precise experimentation, such as microinjection of autoantibodies, will be required to elucidate the functions of those antigens.

Several other mitotic spindle autoantigens have been identified. As mentioned previously, an antigen has been characterized that reacts only with mitotic spindle fibers and mitotic centrosomes. This protein, which has been called either prophase-originating polar antigen (85), nuclear mitotic antigen (116, 117), or centrophilin (118), probably arises from the nucleus and is transported along mitotic spindle fibers to the spindle pole region during mitosis. Other mitotic spin-

dle antigens also have described. One of these autoantigens, called INCENP, is a 38-kDa protein that associates with the centromere region during early mitosis, but then dissociates from the chromosomes as anaphase segregation begins (119, 120). Another spindle autoantigen, MSA-36, is a 36-kDa protein that associates with nuclei during the G_2 stage of interphase, with chromosomes during early mitosis, focuses at the centromeres at metaphase, and then becomes associated with midbodies at telophase (121). To date, a function has not been attributed to any of these autoantigens.

In summary, human autoantisera have provided the first specific probes for MTOC. Sera obtained from scleroderma CREST patients have been used for the characterization of the kinetochore at the cellular, molecular, and biochemical levels. Although numerous questions still remain concerning the organization, composition, and functioning of the centromere/kinetochore regions of chromosomes, it is clear that human autoantisera have allowed for the first detailed investigations of this region of the mammalian chromosome. Most certainly, future studies of the centromere/kinetochore will rely heavily on sera obtained from human patients. In addition, human autoantisera recently have been identified that are being used to study the biochemical and molecular biology of the centrosome complex. Although these studies are just beginning to unravel the complexities of the centrosome region, human sera are providing the first reliable probes for studies of the centrosome/spindle pole. Clearly, these probes have allowed for many important advances to be made in our understanding of the process of mitosis, and human autoantisera should play a key role in future studies of cell division.

Nucleic Acid Synthesis

Until recently, our concepts concerning the events of DNA synthesis in eukaryotic cells were simple. DNA synthesis and repair events were thought to be the result of the activity of three separate enzymes. DNA polymerase α was thought to be the replicative polymerase, polymerase β was presumed to be the DNA repair enzyme, and polymerase γ was thought to be involved in duplicating mitochondrial DNA (122). However, this neat and clean scheme has unraveled. Recently, additional polymerases and their associated cofactors have been defined. Now, it is neither clear what the function of each of these polymerases is nor certain how many different polymerases are necessary to replicate DNA.

Sera obtained from human autoimmune patients have been instrumental in the identification and characterization of one of these recently discovered polymerase-associated factors. Between 3% and 5% of patients with SLE produce autoantibodies that are reactive toward a 36-kDa nuclear antigen called proliferating cell nuclear antigen (PCNA; 123, 124). PCNA is necessary for the efficient replication of DNA *in vitro* by a fairly recently discovered polymerase called polymerase δ (see 125 for review).

PCNA originally was identified by immunofluorescent staining of tissues and cultured cells using a subclass of scleroderma sera. These types of studies identified an antigen that was present only in the nuclei of rapidly dividing cells, hence the name proliferating cell nuclear antigen was coined (4, 5). Moreover, immunofluorescence studies using autoantisera demonstrated that PCNA synthesis was induced in cells at the G_1/S boundary (126, 127), suggesting that PCNA functioned in DNA synthesis. In addition, it was noted that the physical characteristics of PCNA closely resembled those of an accessory protein that was able to regulate the synthetic activity of purified pol δ (128). These observations led two different laboratories to investigate whether PCNA and the pol δ accessory protein were, in fact, identical (129, 130). Various techniques, including co-reactivity with human autoantibodies and the demonstration that the proteins were functionally equivalent using *in vitro* assays, demonstrated that PCNA was the pol δ accessory factor.

Human anti-PCNA autoantibodies were able to disrupt the activity of the accessory protein of pol δ when used in both *in vitro* assays (131) and microinjection studies (132). In the *in vitro* studies, the ability of pols α and δ to replicate primed DNA was tested by measuring the incorporation of [^{3}H]dTMP either in the presence or absence of PCNA. As expected, the activity of pol δ was dependent upon the addition of PCNA to the reaction mixture, but pol α activity was unaffected by the addition of exogenous PCNA (131). When these experiments were repeated in the presence of anti-PCNA human autoantibody, pol δ catalytic activity was abolished, whereas polymerase α function was unhindered (131). Microinjection of anti-PCNA autoantibodies into Xenopus eggs allowed for the relative contributions of pols α and δ in DNA synthesis to be investigated, and it was determined that these two polymerases were involved to varying degrees in the synthesis of both plasmid and genomic DNA (132).

The exact role of the PCNA autoantigen in DNA synthesis is not clear at this time. Prelich and Stillman (133) have demonstrated that PCNA plays a key role in the synthesis of the leading strand of DNA, but is not required for lagging strand synthesis. Lee *et al.* (134) suggested that PCNA may exert its effects by inactivating a cellular inhibitor of pol δ. These investigators used a reconstituted *in vitro* DNA synthesis system to demonstrate that PCNA was able to stimulate polymerase-directed DNA synthesis when crude cellular extracts were used in the reaction system. However, PCNA had no effect on activity of pol δ when purified protein components were used in the reaction mixture. To explain these results, Lee *et al.* (134) postulated that

an inhibitor of DNA pol δ must exist in the crude homogenates that is not present in the purified protein preparations. PCNA then could bind to and inactivate this putative pol δ inhibitor. To date, this inhibitor of pol δ activity has not been isolated.

Other human autoantigens also are involved in DNA synthesis events. The principal autoantigen that is recognized by autoantibodies that are present in sera obtained from scleroderma patients is called Scl-70 (135). Three independent laboratories have demonstrated that Scl-70 is the well-characterized nuclear enzyme topoisomerase I (136–138). In addition, antibodies to poly(ADP-ribose) polymerase have been identified in sera of certain patients with Sjogren's syndrome (139). Finally, numerous autoantisera have been identified that contain autoantibodies that specifically recognize single- and double-stranded DNA and histones (4, 5).

Earlier, the roles of various autoantigens in RNA processing events were outlined. In addition to processing functions, several autoantigens also appear to play key roles in the events of RNA synthesis. Some of these autoantigens, such as the various tRNA synthetases and RNA polymerase I, had been fairly well characterized. However, autoantisera have been used for the discovery and partial characterization of additional novel proteins that appear to be involved in RNA synthesis. These proteins will be the focus of the remainder of this section.

Two novel RNA-associated antigens have been identified using sera obtained from patients with Sjogren's syndrome. These antigens, termed SS-A/Ro and SS-B/La, show completely distinct characteristics. Immunoblot analysis using human sera has demonstrated that the La autoantigen is a 50-kDa phosphoprotein that is highly conserved through evolution (140). The La antigen is characterized by its association with recently synthesized RNA pol III transcripts. Immunoprecipitation of the La antigen using anti-La autoantisera has demonstrated that SS-B/La is associated with tRNA precursors, 5 S rRNA, 7 S RNA, Y RNA, and several other small RNA species (75, 76, 141, 142). Experimentation has demonstrated that RNA pol III products are organized into RNA-protein complexes with the La antigen immediately after synthesis (140, 141), and binding of the La protein seems to be dependent upon the presence of an intact poly-U region that is present on pol III transcripts (142, 143). These observations led to a proposal that the La antigen is an RNA pol III transcription factor.

The role of the La antigen in pol III transcription events was examined directly by Gottlieb and Steitz (144, 145). These investigators specifically depleted HeLa cell extracts of the 50-kDa La antigen by immunoprecipitation using human autoimmune anti-La antiserum and then investigated the types of pol III transcripts that were produced. La-immunodepletion resulted in a significant decrease in the number of RNA pol III transcripts that were produced. Moreover, the few transcripts that were produced had considerably fewer U-residues at their 3′ ends. These results suggest that the La antigen is a pol III transcription factor, and that La is required specifically for transcription termination. The details by which La achieves termination of transcription are unknown.

SS-A/Ro autoantibodies specifically recognize a 60-kDa antigen that is a component of a subclass of small ribonucleoprotein complexes (146). Circumstantial evidence suggests that, like the La antigen, the Ro protein also may be involved in RNA pol III activity (147). Although the exact role of the Ro autoantigen in pol III activity is unknown, it has been proposed that it may serve as a cofactor to regulate the activity of the La protein in the termination of RNA polymerase III transcription (147). If this is the case, then this regulatory activity must be highly specific since the Ro antigen has not been identified in association with the vast majority of RNA polymerase III transcripts. To date, the Ro autoantigen has only been found in association with a small subclass of RNA called the Y RNA (148). If the Ro autoantigen is a cofactor in the activity of polymerase III, then it should be possible to test this by specifically immunodepleting the Ro autoantigen from HeLa cell extracts, as has been done for the La protein (144, 145), and assaying for effects on the synthesis of class III genes.

In summary, autoantisera have been used to identify several novel proteins that appear to play essential roles in the synthesis of nucleic acids in cells. Autoantibodies that specifically react with PCNA are present in sera obtained from a small percentage of SLE patients. Anti-PCNA autoantibodies have been instrumental in demonstrating that PCNA is an essential accessory factor for the synthesis of the leading strand of DNA by pol δ in eukaryotic cells. How PCNA fulfills this role is not clear, but evidence suggests that PCNA may exert its effects by inactivating a cellular inhibitor of pol δ, although this putative inhibitor has never been identified. SS-B/La is an autoantigen that has been identified using sera obtained from patients with Sjogren's syndrome. Experimentation has demonstrated that the La autoantigen binds to a polyuridylate tail on RNA polymerase III transcripts, and it has been proposed that La is involved in the termination of transcription by RNA pol III, although a detailed description of how this might occur is still lacking. Finally, a second autoantigen that is recognized by certain Sjogren's syndrome sera, SS-A/Ro, copurifies with a class of RNA pol III transcripts called the Y RNA. The function of this protein is not known at this time. However, since anti-Ro autoantisera are the only probes presently available to study the Ro autoantigen, it is

clear that sera of Sjogren's syndrome patients will play a key role in elucidating the function of this protein.

Conclusions

Human sera containing autoantibodies that specifically recognize important cellular antigens are a key tool being used for studying cellular processes (summarized in Table I). Human autoantisera have been used as essential research probes that allowed for the identification of many important cellular proteins and for the elucidation of several biochemical processes. As has been shown in this Minireview, in certain instances, such as the processing of hnRNA to mRNA, human autoantisera have allowed for cellular events to be investigated in incredible detail and an exhaustive literature exists that summarizes these experimental studies. Human autoantibodies have been used for the identification and purification of the snRNP particles and for investigations into the assembly and workings of spliceosomes. However, studies of mRNA processing are more the exception than the rule. In most instances, human autoantibodies are only beginning to be realized as powerful experimental tools. Human autoantisera have provided the first experimental probes for the microtubule organizing centers and for important nucleolar antigens, and have allowed for the identification of several enzymes and cofactors that are essential for RNA and DNA synthesis. In addition, although it was not described in this Minireview, human autoantibodies have been identified that react with cell type-specific molecules, such as the acetylcholine receptor antibodies that are present in sera from patients with myasthenia gravis (4). Autoantibodies that are limited in reactivity to a single cell type also are being used by numerous investigators to study the functioning of specific cells.

In the future, human autoantibodies probably will be used to a greater extent than they have been to date as the potential of autoimmune antisera as experimental probes is realized. Granted, there are certain limitations and drawbacks that must be overcome when working with complex sera instead of affinity-purified antibody probes. However, when Mother Nature presents an investigator with a powerful experimental tool, it most certainly will be utilized in a creative and clever

Table I. Human Sera Used for Studies of Cellular Processes

Location	Designation of autoantigens	M_r of the autoantigens	Functions of the autoantigens	Ref.
Nucleus	ANA, Sm, or SnRNP	13, 16, 28, and 29 kDa	1. Involved in the splicing reactions	14
			2. U7 involved in histone mRNA processing	19
			3. U3, U8, and U13 involved in rRNA production	67
			4. U11 involved in polyadenylation	20
Nucleus	U1 snRNP	70 kDa (22 and 33 kDa rare)	Binds to 5′ splice site	35
Nucleus	U2 snRNP	29 and 32 kDa	Binds to branch point of introns	34
Nucleolus	U3 snRNP or fibrillarin	34 kDa	Processing of pre-rRNA	67
Nucleolus	RNA pol I	190 kDa (?)	Transcription of rRNA genes	71
Nucleolus	PM-Sc1	11 peptides from 20–110 kDa identified by immunoprecipitation	Ribosomal assembly (?)	74
Nucleolus	7-2 RNP	40 kDa	Ribosome biogenesis (?)	76
Nucleolus organizing region	NOR-90	90 kDa	Nucleolus reformation after mitosis (?)	77
Centromere/kinetochore	ACA or CREST antigens	18, 50, 80, and 140 kDa	Centromere organization and microtubule binding (?)	105
Centriole	Centrosome or centriole	39, 185, and 220 kDa	Unknown	86
Nucleus/Spindle pole	NuMA, POPA, or centrophilin[a]	110–115, 185, and 220 kDa	Unknown	85, 116, 117
Nucleus	PCNA, cyclin, or pol δ accessory factor	29 kDa	DNA synthesis; pol δ accessory factor	130
Nucleus	Topoisomerase I	100 kDa	DNA synthesis	136–138
Nucleus	SS-A or Ro	60 kDa	Termination of RNA pol III transcripts (?)	146
Nucleus	SS-B or La	50 kDa	RNA pol III transcription factor	144

[a] NuMa, nuclear mitotic antigen; POPA, prophase-originating polar antigen.

manner. Clearly, autoantisera will continue to be important for studies of basic cell biology. Additionally, it seems likely that in the future autoantisera will be essential for studies that begin to result in clinical treatments for rheumatoid conditions. As more is learned about the immune response and as the characteristics of autoantigens that are responsible for driving the autoimmune reactions are understood, it should be possible to develop novel immunomodulatory and immunosuppressive therapies to treat the autoimmune diseases and to ease the suffering of patients afflicted with these conditions.

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