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Histone acetylation: chromatin in action

Paul A. Wade, Dmitry Pruss and Alan P. Wolffe

Histone acetylation acts as a landmark and determinant for chromatin function. Active roles in the transcription and assembly of chromatin have been discovered for histone acetyltransferases and deacetylases. This review highlights these roles and discusses their significance for the maintenance of cell differentiation.

CHROMATIN is not a static entity where DNA is packaged up and forgotten by the molecular machines controlling transcription, replication, recombination and repair. Instead, chromatin structure is dynamic, accommodating the need for DNA to partake in the various functions that require it as a template.

In the past, changes in chromatin conformation were usually viewed as a consequence of DNA being used for some purpose, but recently, a remarkable series of observations challenge this view. Here, we discuss the exciting progress in the identification and characterization of chromatin-modifying proteins, particularly acetyltransferases and deacetylases. The precise roles of these histone-modifying enzymes in transcription are not yet known and this review includes speculative ideas about their possible functions. For example, components of the basal transcriptional machinery, transcriptional coactivators and co-repressors have been found to directly modify histones[1–3]. Histone acetyltransferases and deacetylases might exert regulatory functions at the level of promoter recognition, activation by upstream activators or in the process of initiation or elongation by RNA polymerase. This review focuses on only some of these possibilities, in particular, the establishment and the maintenance of a transcriptionally competent chromatin environment. These biochemical observations complement existing structural and genetic data that establish chromatin as an integral regulatory component of the transcription process.

Chromosomal replication and repair has been found to require a molecular chaperone that shares a common subunit with particular histone acetyltransferases and deacetylases, and that also interacts with a tumor suppressor[4–7]. Other links between histone acetylation and tumorigenesis exist: for example, the adenovirus oncoprotein E1A has been shown to modulate the interactions between acetyltransferases[8,9], and fusions between the genes that encode two such putative histone acetyltransferases are associated with certain cancers[10].

Recent studies also support an essential role for histone acetylation in the establishment and maintenance of a differentiated phenotype. This surge of new information implicates histone acetylation and the associated chromatin conformational transitions as having a central role in cell and developmental biology.

This review attempts to integrate the phenomenon of histone acetylation with the molecular events that it appears to regulate. We will describe: (1) some of the potential mechanisms by which histone acetyltransferases regulate transcription; (2) the potential roles of components of histone acetyltransferases and deacetylases used in chromatin assembly in monitoring chromosomal integrity during the cell cycle; and (3) we will speculate on the significance of histone acetylation for the maintenance of the differentiated state and of aberrant acetylation for cell transformation.

What does histone acetylation do to chromatin?

Before discussing the various proteins that acetylate and deacetylate the core histones, it is useful to know what structural changes might occur within chromatin as a consequence of acetylation. Each core histone has two domains: a histone fold domain, which is involved in histone–histone interactions and in wrapping DNA in nucleosomes, and an amino-terminal tail domain that lies on the outside of the nucleosome, where it can interact with other regulatory proteins and with DNA. The amino-terminal tail domains are lysine-rich and are targets for acetylation. Acetylation greatly reduces the affinity of the histone H4 tail for DNA[11]. The physical consequences for nucleosomal integrity of acetylating all of the histone tails in the absence of any other proteins are relatively minor. However, there is a modest reduction in the wrapping of DNA around the histone octamer and nucleosomes pack together less efficiently in arrays (for reviews, see Refs 12, 13).

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More dramatic transitions are revealed through the use of other DNA-binding proteins as probes of nucleosomal integrity. Transcription factor TFIIIA does not bind efficiently to a 5S rRNA gene within a nucleosome if the core histones are not acetylated, but it does bind following acetylation of the histones[14]. Likewise, Gal4 binding to nucleosomal DNA is facilitated by acetylation of histone H4 (Ref. 15). These observations demonstrate that acetylation of the amino-terminal tails substantially weakens the constraints on DNA imposed by the core histones. Other *in vivo* events or forces might further destabilize acetylated chromatin.

Histone acetylation provides a molecular mechanism by which DNA can be rendered generally accessible to *trans*-acting factors while still maintaining a nucleosomal architecture. Exactly how this is achieved is currently unknown.

Table 1. Histone acetyltransferases[a]

Histone acetyltransferase	Organism	Interactions	Histones modified
Gcn5p	*Saccharomyces cerevisiae*	ADA2p, ADA3p	H3/H4
P/CAF	Human	p300/CBP	H3/H4
p300/CBP	Human	Nuclear receptors, steroid receptors, c-Jun/v-Jun, c-Fos, c-Myb/v-Myb, MyoD, CREB	H2A/H2B, H3/H4
$TAF_{II}230/250$	Human, *Drosophila*	TAFs in TFIID	H3, H4

[a]Histones discovered in 1996 are shown together with the organism from which they were characterized, the proteins with which they interact and their histone targets. Abbreviations used: CAF, CBP-associated factor; CBP, CREB-binding protein; CREB, cAMP response element-binding protein; $TAF_{II}230/250$, 230 kDa/250 kDa RNA polymerase II TATA-binding protein-associated factor.

Transcription acetyltransferases

The synthesis of mRNA requires coordination of the activity of many transcription factors and enzymes. Specificity in transcriptional control relies upon the combinatorial binding of sequence-selective transcription factors to regulatory elements flanking the transcription start site. Activation domains within these factors recruit transcriptional coactivators, which, in turn, facilitate the activity of the basal transcriptional machinery. The role of coactivators is to integrate the signals from the various sequence-selective factors so that a final level of transcription can be determined. The simplest mechanism by which this might be achieved is for the coactivators to act as a scaffold between sequence-selective factors and the basal machinery[16]. However, the unexpected finding that at least three different coactivators function as histone acetyltransferases (Table I) introduces an alternative and potentially more dynamic model.

The Gcn5p–Ada2p–Ada3p complex in yeast acts as a transcriptional coactivator targeted by transcription factors with acidic activation domains; this coactivator is also the archetypical transcription acetyltransferase[1]. The Gcn5p component of the complex has the capacity to acetylate specific lysine residues in histones H3 and H4, which are known to be associated with transcriptional activity[17]. Similar enzymatic activities have now been found for P/CAF (a human homolog of Gcn5p); a factor that associates with the p300/CBP coactivator[8] (CBP, CREB-binding protein; CREB, cAMP response element-binding protein), and for p300/CBP itself[9]. p300/CBP interacts with a variety of sequence-selective DNA-binding transcription factors including nuclear hormone receptors, c-Jun/v-Jun, c-Myb/v-Myb, c-Fos and MyoD (for review, see Ref. 9).

Recently, a component of the DNA-binding basal transcription factor TFIID has also been shown to have histone acetyltransferase activity[2]. The 250 kDa RNA polymerase II TATA-binding protein (TBP)-associated factor ($TAF_{II}250$) serves as the core subunit of TFIID and interacts with a variety of other TAFs as well as TBP. $TAF_{II}250$ is required for the activation of particular genes indicative of coactivator function, and associates with components of the basal transcriptional machinery such as TFIIA, TFIIE and TFIIF (for review, see Ref. 18). In addition, $TAF_{II}250$ functions as both a kinase and a histone acetyltransferase[2,18]. Although Gcn5p and P/CAF are related proteins, there is no significant sequence identity or known structural similarity with p300/CBP or $TAF_{II}250$. Thus, diverse proteins possess histone acetyltransferase activity (Table I).

The facts that: (1) core histone acetylation greatly facilitates the access

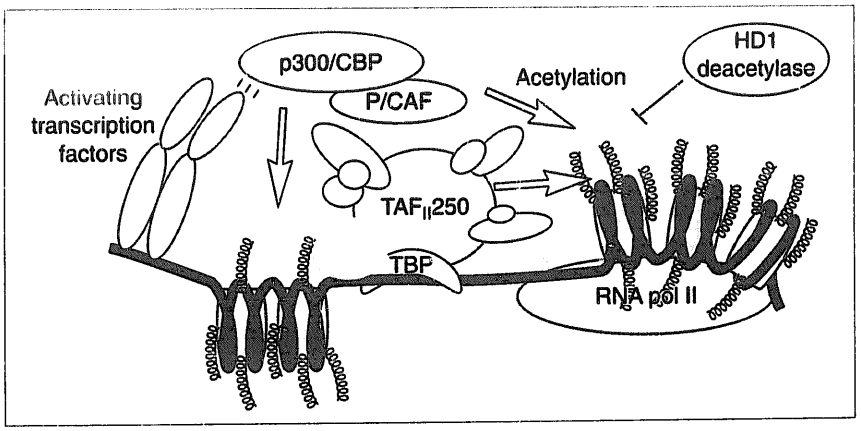

Figure 1

A model for the role of coactivators/histone acetyltransferases and histone deacetylase in the continual regulation of transcription. 'Activating' transcription factors recruit the coactivator/histone acetyltransferase (p300/CBP–P/CAF) (CBP, CREB-binding protein; CREB, cAMP response element-binding protein; CAF, p300/CBP-associated factor), which derepresses nucleosomes such that the transcription factor complex TFIID histone acetyltransferase (shaded), including the 250 kDa RNA polymerase II TATA-binding protein (TBP)-associated factor ($TAF_{II}250$), can facilitate the activity of the basal transcriptional machinery (RNA polymerase II). The grey ellipsoids attached to $TAF_{II}250$ represent other TAFs within TFIID and those attached to RNA pol II represent other components of the basal transcriptional machinery (TFIIA, TFIIE, TFIIF, TFIIH). The histone deacetylase (HD1) continually deacetylates histones in chromatin, therefore, persistent activity of the coactivator/histone acetyltransferase is required to maintain gene activity (see text for details).

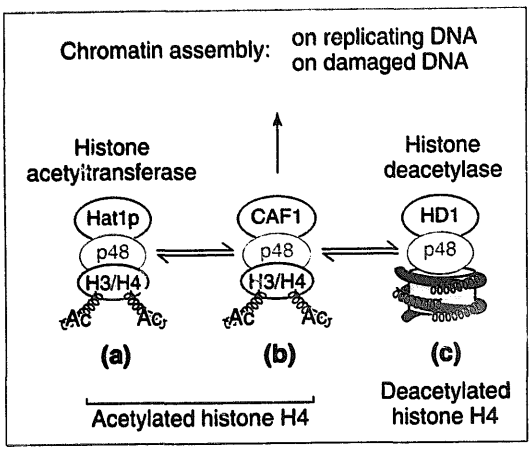

Figure 2

A model for the roles of p48-associated proteins. p48 is a component of: **(a)** a cytoplasmic histone acetyltransferase with Hat1p; **(b)** a chromatin-assembly factor with CAF1; and **(c)** a histone deacetylase with HD1. Dependent on the subunit composition, this protein will be variously equipped to contribute to all these diverse functions in which the modification state of H4, its cellular localization and deposition in a nucleosome will change as indicated. Hypotheses discussed in the text propose that transitions in histone acetylation might determine the distribution of p48 within these different complexes and their availability for these diverse functions.

of transcription factors to DNA in a nucleosome[14,15]; and (2) that transcriptional coactivators are histone acetyltransferases, leads to a model for transcriptional regulation in which the recruitment of coactivators could direct the local destabilization of repressive histone–DNA interactions. Repressive nucleosomes might prevent either the association or function of the basal transcriptional machinery on a particular promoter. Targeted acetylation provides a means of allowing the basal transcriptional machinery to displace nucleosomes, to assemble a functional transcription complex and to never have to deal with chromatin again.

However, a more interesting possibility follows from the discovery that transcriptional regulators exist that also deacetylate the histones[3]. This finding provides a molecular mechanism whereby transcription might be continually controlled. Substantial evidence exists to suggest that core histone proteins remain associated with DNA in the vicinity of a promoter despite the recruitment of the basal transcriptional machinery[19]. Thus, the targeted or general activity of histone deacetylases will tend to return nucleosomes to their repressive configuration. The maintenance of gene activity would therefore require the continued activity of the coactivators as acetyltransferases. In this way, transcriptional activity could be continually modulated through variation in chromatin conformation (Fig. 1). These observations further emphasize that the eukaryotic transcriptional machinery is not only adapted to function in a chromatin environment, but has the potential to make use of the packaging of DNA to regulate genes.

Histone acetylation in cell-cycle progression

Replacement of the four lysines (acetylation targets) in the H4 tail domain in *Saccharomyces cerevisiae* with arginine, such that basic charge is maintained, leads to extremely slow growth. Substitution of these lysines with glutamines (which mimic acetylated lysines) leads to a delay in G2–M progression[20]. None of these mutations alters the eventual assembly of replicated DNA into nucleosomes. Therefore, both the acetylation and deacetylation of lysines in the H4 tail appear necessary for cell-cycle progression itself.

One explanation for these results is that structural changes in chromatin are necessary for the transcription of genes that regulate or drive the cell cycle (Fig. 1). However, the aberrant cell-cycle characteristics could also be related to other checkpoints that monitor chromosome integrity. For example, the mutations in histone H4 increase reliance on DNA-damage-sensitive cell-cycle checkpoint controls[20], suggesting that increased DNA damage occurs in the H4-mutant cells. How might histone H4 acetylation be involved in cell-cycle checkpoint control, DNA damage and chromosome repair?

Newly synthesized histones H3 and H4 are acetylated[21] and deacetylated shortly after their incorporation into the nascent chromatin, which is assembled immediately following replication[22]. Both the histone acetyltransferase and deacetylase involved in these modifications have been characterized[3,6]. These enzymes interact with H3 and H4, and appear to share a common subunit known in mammalian cells as p48/p46. The molecular chaperone involved in the assembly of chromatin on newly replicated DNA is CAF1, which also interacts with H3 and H4 and contains p48/p46 (Refs 4, 5). Dynamic alterations in H3 and H4 acetylation might be necessary to drive the exchange of p48/p46 between the acetyltransferase, deacetylase and CAF1. These dynamic transitions will not occur if the lysine residues in histone H4 are mutated[20]. A failure to mediate these transitions might, in turn, impact on the role of CAF1 in the repair of damaged chromatin. For instance, CAF1 might be irreversibly sequestered on nascent chromatin and might not be available to facilitate chromosomal repair on damaged DNA. Alternatively, if DNA damage occurs more readily, because of alterations in chromatin compaction following from the inability to appropriately acetylate or deacetylate histones, then inappropriate sequestration of CAF1 and p48/p46 on damaged DNA might interfere with cell-cycle progression as a result of a decrease in the rate of chromatin assembly (Fig. 2).

It is dangerous for a cell to synthesize naked DNA in the absence of chromatin assembly. This is because of the multiple roles of chromatin both in constraining inappropriate gene activity and in directing the appropriate packaging of DNA into chromosomes. Consequently, it might be anticipated that molecular mechanisms will exist to monitor chromosomal integrity. An additional role for p48/p46 might lie in its association with the retinoblastoma (RB) gene product[7]. RB has important functions both in cell-cycle control and as a tumor suppressor (for review, see Ref. 7). The significance of the RB–p48 association has not yet been explored. Again, the characterization of components of histone acetyltransferases and deacetylases reveals an unexpected regulatory potential.

Histone acetylation – an epigenetic imprint?

The association of histone acetylation with transcription and the maintenance of chromosomal integrity point to a central biological role for this regulatable modification within chromatin. A fusion protein generated by a recurrent chromosomal translocation associated with acute myeloid leukemia incorporates two putative acetyltransferase domains[9,10]. This suggests that aberrant histone acetylation might contribute to cellular transformation. The two genes fused in this translocation encode the coactivator/histone acetyltransferase CBP and MOZ (for monocytic-leukemia zinc-finger) protein. MOZ contains both the CBP acetyltransferase domain and a region of identity with a yeast protein

involved in transcriptional silencing known as SAS2, which shares homology with the Gcn5p protein in the acetyltransferase catalytic domain[23]. In addition, the P/CAF acetyltransferase interacts with the p300/CBP acetyltransferase at the same interface as does the adenovirus oncoprotein E1A, such that E1A can modulate both the association of these proteins[8,9] and, potentially, their function.

A connection between histone acetylation and cell differentiation has long been known. Histone deacetylase inhibitors such as sodium butyrate and Trichostatin A both promote cell lines to differentiate[24] and restrict cell transformation[25]. These drugs also induce defects during early vertebrate embryogenesis[26]. Clearly, inappropriate changes in acetylation patterns might contribute to loss of the differentiated phenotype and cell transformation. How might aberrant acetylation contribute to such events?

Many controls in early vertebrate development depend upon the capacity to establish the stable functional differentiation of chromosomal domains: for example, the imprinting of chromosomes dependent on parental origin. These epigenetic effects are known to contribute to control of growth and tumorigenesis[27]. Maintenance of histone acetylation states provide an excellent mechanism for the propagation of stable chromosomal imprints determining gene activity. This is because: (1) the distributive segregation of nucleosomes during DNA replication will ensure that the parental histone acetylation states are present on both daughter chromatids[28]; and (2) states of chromosomal acetylation are preserved through mitosis[29,30]. A speculative model for the maintenance of elements of chromatin structure through the cell cycle (Fig. 3) would involve a causal role either for histone acetylation states within the nucleosome itself, or for proteins that specifically recognize particular acetylation states and that might segregate in association with the core histones. Strong candidates for such regulatory molecules include the coactivators/histone acetyltransferases themselves. Once segregated, the histone acetyltransferases would spread the appropriate state of acetylation over a contiguous imprinted domain of chromatin. Disruption of these imprints by synthesis or localization of a dysfunctional histone acetyltransferase would therefore be expected to contribute to cellular transformation.

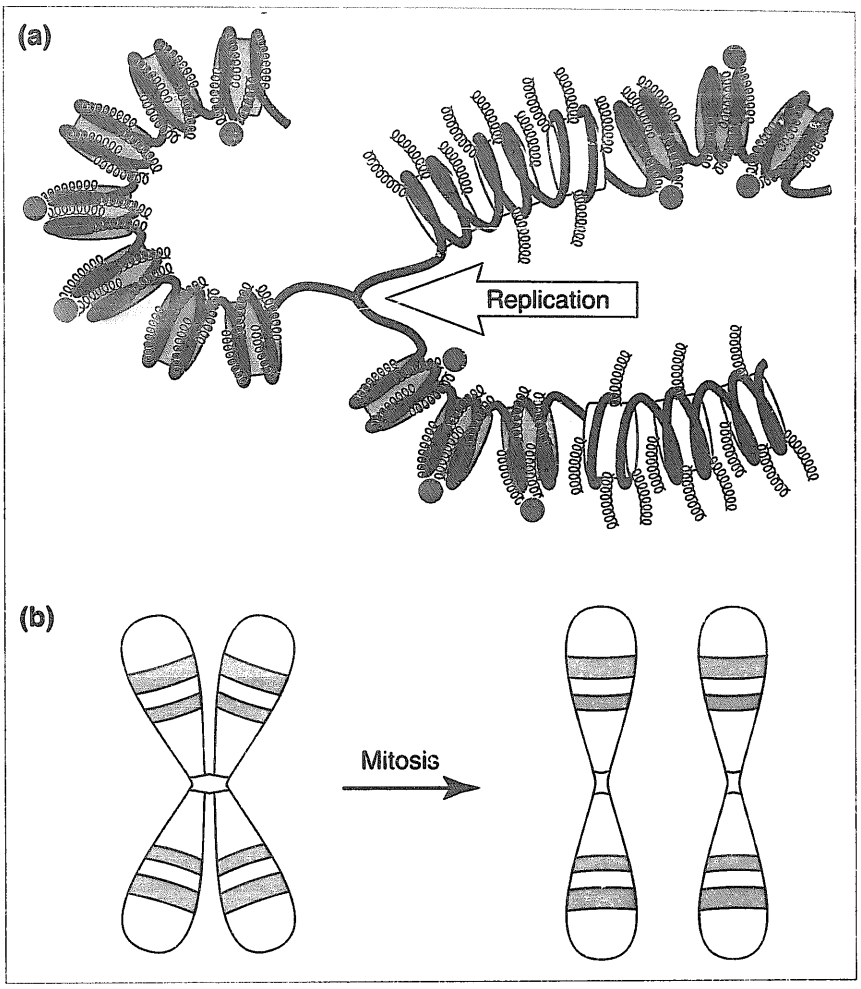

Figure 3

A speculative model for the maintenance of acetylation states within chromatin during the cell cycle. **(a)** Replication leads to the random distribution of parental nucleosomes (orange) in small groups to daughter chromatids. Acetylated tail specific histone-binding proteins including coactivators/histone acetyltransferases (red circles) might also be distributed to daughter chromatids. New nucleosomes (50% of total) contain diacetylated H4 (yellow) – it is possible that histone acetyltransferases segregated with parental nucleosomes will re-establish a predominant acetylation state. **(b)** Domains of chromatin with particular acetylation states are maintained through mitosis.

It is fortunate that excellent reagents exist to determine states of histone acetylation[29,30]. Examination of alterations in the histone acetylation status of chromosomal domains containing known imprinted genes in transformed cell lines will be informative[10,25].

Concluding remarks

Transcriptional regulation, cell-cycle checkpoint control and mechanisms of cellular transformation occupy the interests of many scientists. It is, at first sight, remarkable that chromatin modification would be relevant to all these important aspects of nuclear function. However, all of the molecular machines and processes that allow a cell to respond to external signals, and to propagate and maintain the differentiated state, make use of chromatin as a template. An abundance of new information directly implicates chromatin components in regulatory events. All of these observations lead to the hypothesis that the integrity of chromatin structure is essential for cell viability. If this is true, then the capacity to modulate that structure through histone modification acquires an important regulatory role.

Histone acetyltransferases and deacetylases are now implicated in transcription, the cell cycle and cell transformation. In many instances, the proteins with these activities have already been characterized, as having important regulatory functions. A focus for current research is to determine the exact consequence of histone acetylation for

these specific regulatory functions. It is also important to recognize that these functions are likely to find a close relationship with the role in histone acetylation in chromatin and chromosomal structure. Our knowledge of these issues is still far from complete, nevertheless, the study of regulated histone acetylation has opened a window for visualizing chromatin in action.

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Pre-mRNA splicing: the discovery of a new spliceosome doubles the challenge

Woan-Yuh Tarn and Joan A. Steitz

A rare class of pre-mRNA introns with non-canonical consensus sequences has been identified in metazoan genes. The novel, low-abundance spliceosome that excises these introns contains one small nuclear ribonucleoprotein (snRNP) in common with the major spliceosome (U5) and four snRNPs that are distinct from, but structurally and functionally analogous to U1, U2 and U4–U6. The architecture of RNA components at the presumptive core of the AT–AC spliceosome supports current models of the spliceosomal active center and raises tantalizing questions about spliceosome evolution.

THE PRECISE REMOVAL of introns from mRNA precursors is a critical step in gene expression in all eukaryotic cells. The appearance of highly conserved sequences near the ends of introns engendered the notion that a single splicing machinery accomplishes this task. The spliceosome, which has slowly unveiled its secrets over the 13 years since the splicing reaction was coaxed to proceed in the test tube, is remarkably similar in yeast and human cells[1]. From the beginning, small nuclear RNA–protein (snRNP) complexes were invoked as major players in the two-step splicing reaction[1] (Fig. 1).

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snRNPs in pre-mRNA splicing

First, it was realized that the U1 snRNP and the U2 snRNP base-pair with consensus sequences at the 5′ splice site and the branch point of the intron, respectively. Next, the participation of the U5 snRNP and U6, which enters the assembling spliceosome base-paired to the U4 snRNP, was established. More recently, there has been an appreciation of the dynamic rearrangements that swap base-pairing interactions within the spliceosome under the direction of ATP-hydrolysing protein factors[1–3]. For instance, U6 is released from U4 and replaces U1 at the 5′ splice site before the first reaction step[4–8], in which the 5′ splice site is cleaved and the lariat intermediate is formed. Such conformational rearrangements also precede the second step (exon ligation) of the splicing reaction[9]. Over the years, the remarkable versatility of this complex machine has also become apparent[1]. Not only can it splice introns of many different lengths and sequences, but it is also subject to regulation. *Trans*-acting factors may modulate the use of splice sites in different tissues or at different stages of development, thereby amplifying the genetic information via alternative splicing.

The idea that the spliceosome engages in protein-assisted RNA catalysis derives from both biochemical and genetic analyses[1–9], which have localized highly conserved snRNP RNA sequences close to the reacting sites on the pre-mRNA. Aside from these short (6–10 nt) invariant stretches, the RNA components of splicing snRNPs exhibit different lengths and secondary structure features across the eukaryotic kingdom. For dissecting both the structures and functions of snRNPs, auto-antibodies found in the sera of patients with a variety of

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