Base-specific ribonucleases potentially involved in heterogeneous nuclear RNA processing and poly(A) metabolism

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Received 23 March 1984

Polyadenylation and splicing of heterogeneous nuclear RNA, two crucial steps in mRNA processing, are apparently enzymically mediated processes. This contribution summarizes the properties and the presumed functions of the known poly(A) catabolic enzymes (endoribonuclease IV and V, 2',3'-exoribonuclease) as well as those of the pyrimidine-specific endoribonucleases associated with snRNP–hnRNP complexes (endoribonuclease VII, acidic p*I* 4.1 endoribonuclease and poly(U)-specific U1 snRNP-nuclease).

hnRNA processing *Poly(A)$^+$ mRNA* *Polyadenylation* *Splicing* *Ribonuclease* *snRNP*

1. INTRODUCTION

Internal membranes partition the eucaryotic cells into functionally distinct compartments. In eucaryotes the DNA is sequestered in the nucleus which is delimited by the nuclear envelope. This double membrane isolates DNA replication, RNA transcription and RNA processing from protein translation. In procaryotic organisms mRNAs are transcribed in most instances from polycistronic transcriptional units and are translated as such even before the transcription process has been completed. In contrast to procaryotic mRNA, the primary gene transcript of eucaryotes, which is termed heterogeneous nuclear RNA (hnRNA), must be modified by a series of posttranscriptional processing steps (capping, cleavage, polyadenylation, methylation and splicing) before it can leave the nucleus through the nuclear pore complex [1,2] as translationally active mRNA. Such steps provide eucaryotes with the opportunity for a regulation of mRNA synthesis at the level of post-transcription [3]. In most mature eucaryotic cell types less than 20% of the hnRNA molecules are processed to mRNAs [4]. Parallel with the processing of expressed genes, a reduction of sequence complexity of hnRNA to mRNA occurs [5]. The processing reactions proceed matrix associated [6] and are both structurally [7] and enzymatically controlled [8,9].

In this review, we provide a brief synopsis of those ribonucleases which are presumably involved in poly(A) metabolism of mRNA and in some events during the overall splicing reaction.

2. POLY(A) METABOLIC ENZYMES

Most eucaryotic mRNAs, from sponges [10] to humans, carry a polyadenylic acid sequence [poly(A)] of 50–250 AMP residues at the 3'-end [11]. This highly conserved sequence has also been detected during the initial stage of synthesis of some histone mRNAs [12]. In contrast, the bulk of procaryotic mRNAs lack poly(A) tracts (see [8]). The chain length of poly(A) tails is determined by the interplay between poly(A) anabolic and poly(A) catabolic enzymes which is under partial control of non-enzymic molecules (see below).

Poly(A) synthesis is mediated by poly(A) polymerase(s) (EC 2.7.7.19) (review [13]) and occurs at the 3'-OH terminus in pre-mRNA usually 10–30 nucleotides downstream from the

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00145793/84/$3.00

5′-AAUAAA-3′ sequence [14]. A specific endonuclease, perhaps complexed with the poly(A) polymerase, has been implicated in 3′-end formation [15].

2.1. *Poly(A)-specific ribonucleases*

It is well established that nuclear poly(A)$^+$ mRNA or its precursor are not quantitatively recovered in the cytoplasm [16], indicating that an intranuclear turnover of poly(A) occurs. Detailed analytical studies revealed that poly(A) shortening proceeds by endonucleolytic cleavage [17], during which oligo(A) fragments with less than 20 AMP residues are released [18]. In 1975 we discovered the first poly(A)-degrading nuclease [19] which was later purified from chick oviduct to homogeneity [20] and termed endoribonuclease IV (EC 3.1.26.6). This enzyme (M_r 45000) is highly specific for poly(A) [21], and displays no influence on the integrity of the polyribosomal complex or the translational capacity of cell-free protein-synthesizing systems. The enzyme forms oligonucleotides of an average chain length of 10 AMP units which are terminated by 3′-OH and 5′-P ends. The poly(A)-free part of the mRNA molecule remains unaffected. We could detect this enzyme in oviduct cells only in the nucleus [21], while some evidence has been presented that it is also localized in the cytoplasm from rat liver cells [22]. Similarly to the poly(A) polymerase, the activity of endoribonuclease IV is also under the control of actin and tubulin, if tested in vitro [23] and it is perhaps modulated by a posttranslational phosphorylation process [24]. A further peculiar property of the endoribonuclease IV, which could be of potential physiological importance, is its high affinity to bind to poly(A) polymerase [20,25].

The second endonuclease, termed endoribonuclease V (EC 3.1.27.8), which we have detected in calf thymus and purified to homogeneity (table 1) cleaves specifically single-stranded poly(A) and poly(U); none of the other single- as well as double-stranded synthetic polyribo- and polydeoxyribonucleotides tested were degraded [26]. In contrast to endoribonuclease IV, endoribonuclease V produces 3′-AMP as end product. The enzyme consists of one polypeptide chain with an M_r of 52300; its isoelectric point was determined to be 6.3.

In 1967, authors in [27] described an exoribonuclease with a high specificity for poly(A). We discovered a similar or identical poly(A)-specific exoribonuclease, termed 2′,3′-exoribonuclease (EC 3.1.13.4), first in crude extracts from L5178y cells [28] and later in calf thymus from which we purified the enzyme to homogeneity [29]. The exoribonuclease, which is composed of two subunits with M_r 58000 and 31000, cleaves specifically poly(A) in the single- or double-stranded form [poly(A)·poly(U)] under formation of 5′-AMP (3′-exonucleolytic activity). The exoribonuclease does not disintegrate polyribosomes isolated from quail oviduct. Interesting was the finding [30] that this enzyme degrades not only oligoadenylates with 3′–5′-linkages but also the 2′–5′-linked oligomers (2′-exonucleolytic activity) which are synthesized in interferon-treated cells [31,32].

2.2. *Potential regulatory function*

It has been shown by a series of studies that the poly(A)-metabolizing enzymes respond to altered physiological conditions and neoplasia. Pertinent data on the changes of poly(A) polymerase activity have been excellently reviewed [13,33].

Determinations of the levels of poly(A)-metabolizing enzymes, which are present in the nucleus of synchronized L5178y cells revealed [28] that the activity of poly(A) polymerase remains essentially constant during the transition from the G_1 to S phase. However, the extractable poly(A) catabolic enzyme activities change parallel with the extent of DNA synthesis. Endoribonuclease IV increases 4-fold during the transition from the G_1 to S phase, while 2′,3′-exoribonuclease activity increases 30-fold. This means that during the S phase a change of the balance between poly(A) anabolic and poly(A) catabolic enzymes occurs in favour of the latter. This result agrees well with the observations that during the S phase most of the isolated mRNAs which are formed are devoid of poly(A) [34]. A similar parallelism between the amount of DNA synthesis and the extent of extractable poly(A) catabolic enzyme has been observed in herpes simplex virus-infected cells [10,35]. The balance between the poly(A) metabolic enzymes also changes during the development of the organism. Studies with quail oviduct revealed [36] that the activity of the extractable poly(A) polymerase remains unchanged during the post-mature period, while the activity of endoribonuclease IV

increases 1.2-fold and that of the poly(A)-specific 2′,3′-exoribonuclease 3-fold during the transition from the mature to old stage. Simultaneously performed analytical studies showed [37] that the size of the poly(A) segment of mRNA from old oviducts is shorter [average size: poly(A)$_{70}$] compared to that measured in mRNA from mature oviducts [poly(A)$_{150}$].

These examples show that the activities of poly(A) metabolic enzymes are correlated with the physiological state of the organism and hence also depend on the developmental stage of the animal. The size of the poly(A) segment of mRNA is dependent firstly on the balance between the activities of poly(A) anabolic and poly(A) catabolic enzymes and secondly on the number of poly(A)-associated proteins present per poly(A) tail [37]. Previously it was established [38] that endoribonuclease IV does not hydrolyze poly(A) if this polymer is 'protected' by the associated proteins; after removal of these proteins, the poly(A) segment is rapidly degraded. In contrast, the 2′,3′-exoribonuclease destroys the poly(A) segment independently of the presence of poly(A)-associated proteins.

3. RNP-ASSOCIATED RIBONUCLEASES

The splicing process occurs in the nucleus and usually follows poly(A) addition. Compared to the polyadenylation process, which is completed within a few minutes [39], splicing of hnRNA is slow and requires approx. 20 min [40]. The splicing reaction is apparently enzymatically controlled [9] and presumably guided by small nuclear RNPs (snRNP) [41] and perhaps by the 3′-poly(A) sequence of mRNA [42].

3.1. *Poly(A)-modulated endoribonuclease VII and acidic pI 4.1 endoribonuclease*

Recently we succeeded in the isolation and purification of a novel pyrimidine-specific 3′-endoribonuclease (M_r 74000) from calf thymus that was termed endoribonuclease VII [43]. The enzyme cleaves specifically poly(U) and poly(C) while other single-stranded homopolyribo- as well as polydeoxyribonucleotides are not degraded; poly(A,C) is hydrolyzed to a smaller extent, while poly(U)·poly(A) is not degraded at all. During hydrolysis, oligo(U)$_{12}$ fragments with 3′-OH and 5′-P termini are formed. The enzyme has a pH optimum of 7.2, an isoelectric point at pH 8.5 and requires neither monovalent nor divalent cations. The enzyme was found to be bound to nuclear 45 S particles which are composed of RNA and 10 major proteins; one of these proteins (P74) is the enzyme itself. The most prominent property of the enzyme is that its poly(U)-degrading activity could be modulated by poly(A). Poly(A)$_{95}$ stimulates the hydrolysis of poly(U) by the enzyme by more than 100% within a limited concentration range; at a molar ratio (based on phosphate content) of approx. 1 [poly(A)]:10 [poly(U)] maximal stimulation of the enzyme activity was achieved; at lower ratios , 1:2 or 1:1, the hydrolysis of poly(U) was inhibited by 52 or 87%, respectively. Oligo(A)$_4$ fragments were found to have only a little modulating effect on the enzyme activity. On the other hand, poly(G) and poly(dA) strongly inhibited endoribonuclease VII. Another remarkable feature of the enzyme is its high affinity for poly(A) and poly(U); no other homo- or heteropolymeric nucleic acids were found to bind to the enzyme. Moreover, an RNA fragment that was found to be tightly bound to the enzyme was shown to be oligo(U) (unpublished).

During the final purification of endoribonuclease VII by preparative isoelectric focusing, a second endoribonuclease, called p*I* 4.1 acidic endonuclease, was separated from endoribonuclease VII (p*I* 8.5 [43]). The p*I* 4.1 endoribonuclease was found to be associated with 12 S particles [44] that can be characterized as snRNPs by the following criteria. Firstly, by a sedimentation coefficient of 12 S; secondly, by electrophoresis in SDS-polyacrylamide gels as shown in fig.1 (12 S particles containing the p*I* 4.1 acidic endoribonuclease show a protein pattern which is very similar to that of immunoaffinity purified snRNPs [45]); thirdly, by the fact that the purification procedure of these particles is closely related to that for snRNPs described in [46]; and fourthly, by their antigenicity to Sm antibodies as checked by counterimmunoelectrophoresis (fig.2). The latter finding is in good agreement with the results obtained by SDS gel electrophoresis showing the potential Sm antigens D, E and F [47] to be present in the 12 S particles (fig.1). In addition to these polypeptides some minor protein bands (A, A1, A2, A3, B, C) were found, which could be par-

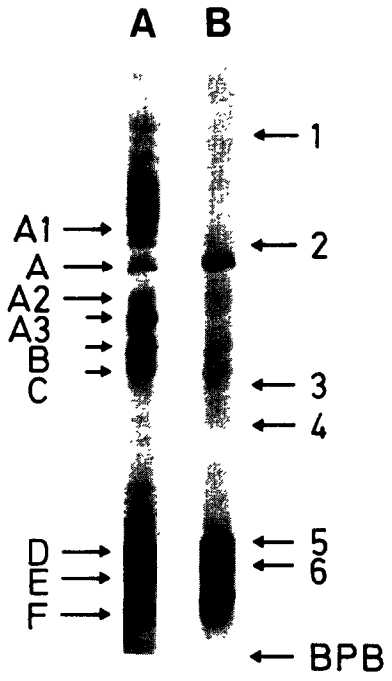

Fig.1. Polyacrylamide gel electrophoresis of the p*I* 4.1 acidic endoribonuclease-containing 12 S particle and of U snRNP. Electrophoresis was performed in SDS-polyacrylamide gels (10% separation gel, 6% spacer gel). (A) 12 S particle containing the p*I* 4.1 acidic endoribonuclease, (B) U snRNP purified by immunoaffinity chromatography. The following proteins were used as standards: (1) bovine serum albumin (M_r 68000), (2) egg white albumin (M_r 43000), (3) chymotrypsinogen A (M_r 25000), (4) trypsin inhibitor from soybean (M_r 21500), (5) ribonuclease A (M_r 13700) and (6) cytochrome *c* (M_r 12500). BPB, bromophenol blue. Protein bands are indicated by capital letters.

tially adsorbed to an anti-RNP column (A, A1, A2). This column was used for isolation of the poly(U)-specific U1 snRNP-nuclease (see below). In contrast to endoribonuclease VII, the pyrimidine-specific acidic p*I* 4.1 endoribonuclease requires Mg^{2+} for optimal activity and is not modulated by polyadenylate.

3.2. *Poly(U)-specific U1 snRNP-nuclease*

Guided by the assumption that U1 snRNA is essential for the splicing process we searched for nuclease activities associated with U1 snRNP particles. Previous reports have indicated the presence of poly(A) polymerase [48], a non-specific endoribonuclease [49] and an endoribonuclease

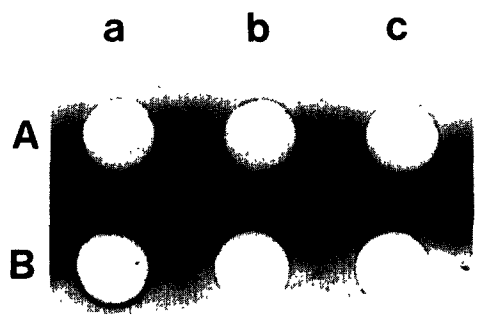

Fig.2. Counterimmunoelectrophoresis. SnRNPs obtained by DEAE–cellulose chromatography (100 mM NaCl eluate fraction [43]) were analyzed. Lane a, anti-Sm; lane b, control serum; lane c, anti-RNP. A, antigen; B, antibody. The electrophoresis was performed as in [56].

specific for double-stranded RNA [50] in the hnRNP complex. By chromatography on lupus erythematosus antibody affinity columns we succeeded in identifying a poly(U)-specific endoribonuclease [45]. In SDS-polyacrylamide gels the RNP preparation obtained showed a protein pattern typical for snRNP; 4 major polypeptides (M_r 42000, 13500, 11500, 10800) and two minor polypeptides (M_r 29000, 26000) could be identified (fig.1B). Moreover, the particles had the characteristic sedimentation coefficient of 12 S. Because the antibody contained a high titer of anti-U1 snRNP the isolated snRNP should represent primarily U1 snRNPs. The U1 snRNP-nuclease requires Mg^{2+} for optimal activity, has a pH optimum of 6.2 and degrades specifically poly(U) under formation of $(U)_{6-12}$ oligomers with 3′-OH and 5′-P termini.

3.3. *Hypothetical function*

Although the physiological function of endoribonuclease VII, p*I* 4.1 acidic endoribonuclease and poly(U)-specific U1 snRNP-nuclease is not yet known, from their presence in RNP particles and their characteristics it is likely that these enzymes could play a functional role in RNA processing.

Based on its properties, poly(A)-modulated and oligo(U)-recognizing endoribonuclease VII might be a 3′-processing enzyme removing the oligo(U) sequences from RNA polymerase III transcripts, such as 5 S RNA precursors [51] and Ro RNAs [52]; perhaps this enzyme may recognize the

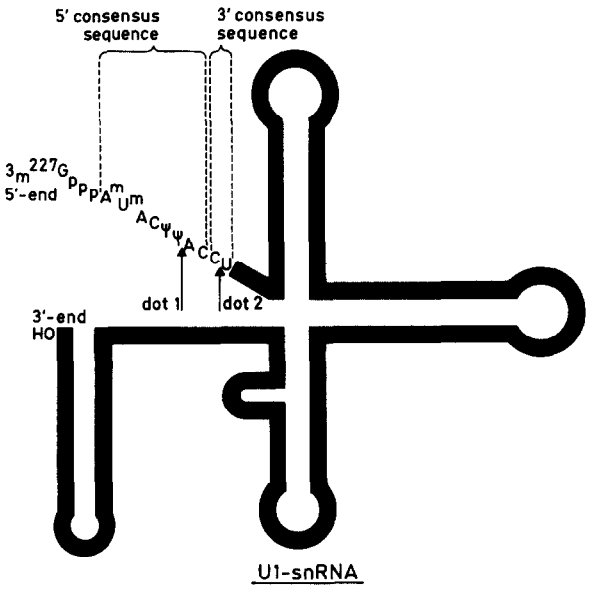

Fig.3. Possible cleavage points of the poly(U)-specific U1 snRNP-nuclease within the U1 snRNP particle. (▬) Part of the U1 snRNA which is protected against nuclease attack by proteins. The poly(U)-specific U1 snRNP-nuclease could split the U1 snRNA at dot 1 (U1→U1*) or dot 2 (the single unmodified uridylate residue within the protein unprotected part of the U2 snRNP particle).

pyrimidine-rich stretch in the intron loops of hnRNA. In addition, all of these reactions could be modulated by the poly(A) sequence and therefore be controlled by poly(A) anabolic and catabolic enzymes.

As shown in fig.3, in U1 snRNP the greater part of the RNA is protected by protein; only a short nucleotide sequence at the 5'-terminus (nucleotide residues 1–11) of the U1 snRNA seems to be protein free [53]. Because of its complementarity to the 3'- and 5'-donor and acceptor splicing sites, this nucleotide sequence is thought to comprise the recognition site for the hnRNA splicing apparatus [54,55]. In particular, all except one of the uridylate residues in this small RNA piece are modified by methylation or replaced by pseudouridines. It is not yet known whether the poly(U)-specific nuclease could cleave at pseudouridine residues; if so, one possible function of this enzyme might be the conversion of U1 to U1* (dot 1 in fig.3). Another uridylate residue at which the U1 snRNA might be cleaved by the poly(U)-specific nuclease is the eleventh nucleotide from the 5'-end (dot 2) which is the only unmodified nucleotide that was found to be protein unprotected. Because by both reactions the interaction of the U1 snRNA with the complementary splice sites of hnRNA could be prevented, poly(U)-specific and U1 snRNP-associated endoribonuclease might be attributed a modulating function during splicing.

4. THE FUTURE

This review shows the existence of novel ribonucleases present in RNP particles. Two different yet similar enzymes could be identified in 12 S snRNPs using homopolymeric nucleic acids as substrates. The elucidation of the function of these enzymes by the use of their natural substrates may be a task for the future.

ACKNOWLEDGEMENTS

This work has been supported by the Deutsche Forschungsgemeinschaft (Mu 348/7-4). H.C.S. was supported by a habilitation traineeship (Liebig Stipendium) of the Fonds der Chemischen Industrie.

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