Polyadenylate Polymerases from Normal and Cancer Cells and Their Potential Role in Messenger RNA Processing: A Review

Samson T. Jacob, Michael P. Terns, and Kathleen A. Maguire

Department of Pharmacology and Cell and Molecular Biology Center, The Pennsylvania State University, College of Medicine, Hershey, Pennsylvania 17033

Abstract

Two structurally and immunologically distinct species of nuclear polyadenylate (poly(A)) polymerases have been characterized. One of these enzymes is relatively absent in normal tissues but is predominant in primary and transplanted tumors and transformed cell lines. The presence of the tumor type enzyme in fetal liver, but not in regenerating liver, suggests that it is an oncofetal protein. Antibodies against the tumor-type poly(A) polymerases are present in the sera of rats bearing tumors and in some cancer patients. These antibodies are also found in the sera of rats fed hepatocarcinogen even before preneoplastic nodules were visible, which suggests that elicitation of these antibodies is an early event in neoplastic transformation. Autoantibodies against both liver-type and tumor-type poly(A) polymerase are also present in some rheumatic autoimmune sera. Polyclonal antibodies against purified enzyme from a rat hepatoma, which exhibit a single band upon immunoblot analysis, were used in cell-free extracts to study the role of poly(A) polymerase in the 3' -end processing of pre-mRNA. These studies showed that the antibodies blocked both endonucleolytic cleavage and poly(A) addition at the cleavage site and complex formation between factors in the extract and pre-mRNA. Independent studies in other laboratories have demonstrated that both the cleavage and poly(A) polymerase activities require the same component for their function. These observations suggest that both cleavage and polyadenylation reactions are tightly coupled in a functional complex.

I. Introduction

The vast majority of eukaryotic mRNAs contain poly(A)$^*$ tails at their 3' ends. Despite the significant progress made towards the identification and partial characterization of a few factors involved in the cleavage/polyadenylation of pre-mRNAs, the mechanism by which a properly polyadenylated mRNA 3' terminus is formed has not been fully elucidated. Current evidence indicates that RNA polymerase II-directed transcription proceeds well beyond the poly(A) site and terminates over a heterogeneous DNA stretch at considerable distances from the eventual 3' end. The 3' terminus of mRNA arises from a posttranscriptional reaction which involves an endonucleolytic cleavage of the larger precursor followed by the addition of an adenosine tract, approximately 200–250 nucleotides long, to this newly generated end (for extensive reviews, see refs. 1–3). It is presumed that the latter reaction is enzymatically accomplished by poly(A) polymerase (polynucleotide adenylyltransferase; ATP: polynucleotide adenylyltransferase, EC 2.7.7.19). This review focuses on the important characteristics of poly(A) polymerase and the recent studies which have provided direct evidence for the role of this well characterized enzyme in the 3' end processing of eukaryotic pre-mRNAs.

II. Summary of the Properties of Poly(A) Polymerase

For extensive discussions on the characteristics of poly(A) polymerase the reader is asked to refer to previous reviews (see Refs. 4 and 5). These reviews have covered in great detail intracellular distribution, size and subunits, other physical properties, substrate, primer and divalent metal ion requirements, inhibitors, phosphorylation of enzyme, and nucleases associated with enzyme preparations. Only a brief description of the properties of this enzyme from higher organisms that are relevant to the present discussion is presented here.

Poly(A) polymerase appears to be a ubiquitous enzyme and is present in all organisms ranging from prokaryotes to eukaryotes. In mammalian cells, the enzyme has been identified in the nucleus (6), RNPs (7), mitochondria (8), microsomes (9), ribosomes (10), and postmitochondrial fractions (11). The nuclear enzyme is found exclusively in the extranucleolar fraction, which is consistent with the lack of polyadenylation of rRNA in the nucleolus. The nuclear enzyme occurs as chromatin-bound and free forms (12–16). Following solubilization of the bound enzyme, it attains the characteristics of the "free" enzyme, which indicates that the two populations of the enzyme represent the same polypeptide in two functional states. The chromatin-bound enzyme is highly sensitive to low levels of cordycepin triphosphate (3'-dATP) whereas the free form of the enzyme is inhibited only by relatively high concentrations of the ATP analogue (17). The differential sensitivity of the two poly(A) polymerase populations to 3'-dATP is consistent with similar responses of the initial polyadenylation and poly(A) extension reactions to cordycepin in vivo (18, 19). Based on these data, we have concluded that the chromatin-bound and free forms of the enzyme are responsible for the initial polyadenylation and poly(A) elongation reactions, respectively (17, 20). More recent studies with a reconstitution system in vitro have further supported this contention (see Section VIII).

Purification of enzymes consisting of a single subunit has been accomplished in several animal systems (for review, see Refs. 4, 5, 21, and 22). Poly(A) polymerase is assayed usually by monitoring the incorporation of labeled AMP into synthetic primer. It is clear that purified poly(A) polymerase cannot catalyze the entire reaction which leads to mRNA 3' end formation. Poly(A) polymerase activity is only one of the activities required for this highly specific and complex reaction.

The size of the enzyme, as displayed after gel electrophoresis under denaturing conditions, ranges from 37,000 to 60,000 depending upon the source of the enzyme (see Refs. 4 and 5). The enzyme can occur as a dimer or tetramer (see Section III). Very high molecular weights (300,000) have been reported under certain conditions (see Section IV). The enzyme has displayed heterogeneity upon chromatography on phosphocellulose (23, 24) or carboxymethyl cellulose (25) columns. Al-
though multiple forms of the enzyme may correspond to structurally distinct species of the enzyme, such display of heterogeneity in, in most cases, the result of posttranslational modification of the enzyme such as phosphorylation which is known to alter its chromatographic elution characteristics (25).

The enzyme activity can also undergo rapid changes in response to a variety of physiological and pathological stimuli (4). The earliest and perhaps the most rapid change in the enzyme activity occurs in rabbit heart following treatment with noradrenaline or dibutyryl cyclic AMP (26, 27). The nuclear enzyme can also be stimulated by glucocorticoid (28), progesterone (16), and estradiol (29). The nuclear poly(A) polymerase activity can vary with age of the animal. Thus, the enzyme activities in the liver and brain nuclei derived from 25-day-old rats are 3- and 26-fold greater than the corresponding enzyme activities from 30-day-old animals (30). Mixing experiments have shown that the reduced poly(A) polymerase activity in tissues from the older rats is not due to high levels of inhibitors (30). The differences in the enzyme activity appear to be tissue and perhaps species specific (31). Poly(A) polymerase activity can also respond to mitogens (32) and is subject to diurnal variations (33). The enzyme is also known to respond to amino acid supply (34). Alterations in poly(A) polymerase are not confined to the mammalian enzyme, as wheat poly(A) polymerase has been shown to be stimulated by the plant hormone, gibberellic acid, a process that is dependent on de novo protein synthesis (35). Poly(A) polymerase activity can also be modulated by actin and tubulin (36). The poly(A) polymerase from calf thymus appears to be closely associated with a poly(A)-specific endoribonuclease (36). The enzyme activities in this complex are strongly inhibited by G-actin and tubulin dimer whereas the polymerized forms of this protein are less effective.

Changes in poly(A) polymerase activity were detected by assaying the solubilized or partially purified enzymes using eukaryotic primers. Since solubilized enzymes generally lose their specificity for primers (17, 20), the physiological relevance of the alterations in the enzyme is not evident. Further studies using reconstituted cell-free systems are required to understand the exact implications of these interesting findings. The advent of such in vitro systems for studying polyadenylation and the recent success in reconstituting partially purified poly(A) polymerase with other components in the polyadenylation of certain specific mRNA precursors (see Section VIII) have provided further impetus for exploring the potential alterations of the polyadenylation reaction by posttranslationally modified poly(A) polymerase.

III. Two Distinct Nuclear Species of Poly(A) Polymerase: Demonstration of a Liver-type and Tumor-type Enzyme

More than a decade ago, we (6) showed that the nuclear enzyme from rat hepatoma is distinct from the corresponding rat liver enzyme with respect to molecular weight and amino acid composition. Subsequent studies demonstrated that the two enzymes differ in zinc content (37) and degree of phosphorylation (23). The hepatoma enzyme is much more phosphorylated than the liver enzyme (23). Since phosphorylation of poly(A) polymerase causes a dramatic activation of the enzyme, it is likely that the higher activity of the hepatoma enzyme is, in part, due to its high degree of phosphorylation.

Later studies have shown that rat liver nuclei contain a predominant M, 38,000 enzyme and a minor M, 48,000 species. The M, 48,000 enzyme constitutes approximately 1% of the total liver nuclear enzyme. The corresponding enzyme from the rat tumor, Morris hepatoma 3924A, is composed of a single species of M, 48,000 enzyme which is identical to the minor liver nuclear poly(A) polymerase in every respect (38). Gel filtration studies indicate that the major liver nuclear enzyme is a tetramer of M, 38,000 polypeptide whereas the hepatoma enzyme is a dimer of a M, 48,000 polypeptide (38). Fractionation of liver poly(A) polymerase by Sephacryl S-200 resulted in complete loss of activity which could be restored by incubation with exogenous Nt-type protein kinase (38). The latter kinase was initially associated with poly(A) polymerase but could be separated and purified following extensive chromatographic fractionations (39). The rapid loss of the liver enzyme activity by dephosphorylation is consistent with the relatively reduced level of phosphorylation in the liver enzyme relative to the hepatoma enzyme (38).

IV. Structural and Immunological Differences between the Liver-type and Tumor-type Enzyme

Cleavage of liver (M, 38,000) and hepatoma (M, 48,000) poly(A) polymerases with cyanogen bromide followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and silver staining showed that the peptide maps of the two enzymes are distinct. The peptide map of the tumor enzyme was identical with that of minor (M, 48,000) liver enzyme. It is not evident why the liver nuclei contain a small proportion (<1%) of the hepatoma-type enzyme. The difference in the peptide maps indicates that the major liver poly(A) polymerase is not derived from the hepatoma enzyme by proteolytic processing. There is no evidence for the existence of a high molecular weight precursor for poly(A) polymerase, as demonstrated by cell-free translation (40) or by [35S]methionine labeling in vivo (see Ref. 69). It appears unlikely that the various molecular weights (35,000-120,000) for poly(A) polymerase derived from different sources are due to various degrees of proteolysis during purification (see Ref. 40). Very recently, molecular weights ranging from 50,000 to 60,000 to as high as 300,000 from the same source (HeLa) have been reported (see Section II). It is plausible that the high molecular weights in some instances resulted from enzyme aggregation.

Polyclonal antibodies have been raised against the hepatoma poly(A) polymerase (41). However, every attempt to produce antibodies against the liver enzyme has failed (41), which suggested that the two enzymes are immunologically distinct. Western blot analysis showed that the major liver (M, 38,000) enzyme did not react with the anti-hepatoma poly(A) polymerase antibodies whereas the minor liver (M, 48,000) nuclear enzyme formed a distinct immune complex with the above antibodies. The antibodies reacted strongly with five and weakly with two of the eight CNBr cleavage fragments of the hepatoma enzyme (38). Further immunoblot analysis using CNBr-cleaved minor liver (M, 48,000) and hepatoma enzymes revealed identical immune complex profile. These observations were consistent with the structural data.

The structural and immunological differences between the liver and tumor poly(A) polymerase have suggested that the two enzymes could be the products of two distinct genes. Cell-free translation experiments have lent further credence to this notion (40). When polyadenylated RNA from rat hepatoma was translated in the rabbit reticulocyte system and the product was immunoprecipitated with anti-hepatoma poly(A) polymerase antibodies, only one specific [35S]methionine-labeled band corresponding to poly(A) polymerase was visible in the autoradiograph. This band was not visible when liver polyadenylated
RNA was translated or when preimmune serum was used for precipitation.

The presence of structurally and immunologically distinct nuclear poly(A) polymerase in transplanted hepatoma raised the possibility that this enzyme may be produced at an early stage of tumorigenesis. To test this possibility, primary hepatomas were induced in male Fischer 344 rats by feeding an azo dye diet (42). Nuclear poly(A) polymerase purified from the primary hepatomas was identical to the corresponding enzyme from the transplanted hepatoma with respect to molecular weight and immunoreactivity with the anti-poly(A) polymerase antibodies (42). The production of a distinct poly(A) polymerase in the early stages of neoplastic process suggests that this enzyme may be involved in some way in tumor development. The enzymatic activity of this protein may not be crucial in tumorigenesis. Alternatively, the tumor-type poly(A) polymerase in concert with other factor(s) could polyadenylate a distinct class of mRNAs that are required for the transformation of normal cells to cancer cells.

V. The Tumor-type Poly(A) Polymerase Is an Oncofetal Antigen

The expression of fetal antigens in tumor has been well documented (43-45). To investigate whether the hepatoma poly(A) polymerase is related to the fetal enzyme, poly(A) polymerase was purified from fetal livers derived from pups at 18 days of gestation (46). Since the quantity of tissue was limited, the enzyme was only partially purified. When this enzyme was subjected to Western blot analysis, only one band corresponding to $M_0$ 48,000 was visible. There was no immune complex formation when anti-hepatoma RNA polymerase I antibodies were used for the immunoblot analysis. The adult liver-type poly(A) polymerase ($M_0$ 38,000) was either absent or present in minute quantity in the fetal liver (46). The immunological data were consistent with the structural data which showed that the CNBr cleavage patterns of the fetal enzyme and the hepatoma enzyme were identical. Further studies have shown that fetal poly(A) polymerase is not characteristic of actively growing tissue, because poly(A) polymerase from regenerating liver (18 h posthepatectomy) did not contain tumor-type poly(A) polymerase; rather, partial hepatectomy caused a 3-fold increase in the level of liver-type poly(A) polymerase.

These observations coupled with the cell-free translation data have concluded that tumor poly(A) polymerase gene is inactive in normal tissues postnatally but is reexpressed in tumors by reactivation at an early stage in carcinogenesis.

VI. Detection of Anti-Tumor-type Poly(A) Polymerase Antibodies in the Sera of Tumor-bearing Rats and Cancer Patients

The immunological difference between adult or regenerating liver poly(A) polymerase and the corresponding hepatoma enzyme raised the possibility that the tumor enzyme may be immunogenic to the host. The tumor poly(A) polymerase may be released by tumor necrosis which may then lead to elicitation of antibodies against the tumor enzyme in the sera of the tumor-bearing hosts. To test this possibility, sera from rats bearing transplanted Morris hepatomas with different growth rates (44, 7288C, 5123D, 28A, 9618A, 7787, 3924A) and mammary adenocarcinoma R3230AC were screened for anti-hepatoma poly(A) polymerase antibodies by immunoblot analysis (47). A distinct immune complex was observed when sera from all rats bearing tumors for more than 7 weeks were used for analysis.

Since rats carrying Morris hepatoma 3924A usually die prior to the minimal period required for significant immune response, antibodies were not found in the sera of these animals despite the high sensitivity of the technique used for Western blot analysis. Anti-tumor poly(A) polymerase antibodies were also detected in the sera of azo dye-fed rats (42). Interestingly, sera from rats fed the carcinogen for just 21 weeks contained antibodies against the hepatoma enzyme (42). The antibody titer of sera from rats that were fed carcinogens for 30 weeks was identical to that from rats bearing the transplanted Morris hepatoma 3924A. Poly(A) polymerase from adult rat liver did not react with the sera from carcinogen-fed rats, which indicate the specificity of the antibodies against the tumor enzyme. Since antibodies against RNA polymerase I or II were not detected in these sera, the elicitation of anti-poly(A) polymerase antibodies in these sera do not appear to be a random occurrence. The detection of the latter antibodies prior to the formation of preneoplastic nodules suggests that tumor-type poly(A) polymerase is produced at an early stage of carcinogenesis and may very well be a unique tumor marker. Further studies are required to validate this possibility.

The presence of anti-poly(A) polymerase antibodies in the sera of tumor-bearing rats provided an impetus to examine the sera of cancer patients for these antibodies. Studies in our laboratory (47) have demonstrated that sera from patients with acute lymphocytic leukemia and Wilms' tumor contain anti-poly(A) polymerase antibodies. Since the presenting antigen for these studies was purified rat hepatoma poly(A) polymerase, it appears that poly(A) polymers from rat and human are, at least, antigenically and probably structurally, identical. The most significant observation was a dramatic increase (as much as 15-fold) in the antibody titer following relapse of a patient with acute lymphocytic leukemia. Although this is an encouraging finding, it is important to screen several sera before meaningful conclusions can be made. One noteworthy finding was the elicitation of antibodies against tumor poly(A) polymerase in polycythemia vera, a condition marked by an abnormal number of erythrocytes with a potential relationship to myelocytic leukemia. The specificity of the anti-tumor poly(A) polymerase antibodies in the human cancer sera was evident by the absence of such antibodies in the sera of (a) healthy individuals, (b) cancer patients in remission for several months, (c) hypertensive patients, or (d) patients with certain inflammatory diseases such as Crohn's disease, Guillain-Barré syndrome or postneonatal meningitis (47). However, certain rheumatic autoimmune sera also contain anti-tumor-type poly(A) polymerase antibodies (see Section VII).

VII. Autoantibodies against Nuclear Poly(A) Polymerases in Rheumatic Autoimmune Diseases

The presence of circulating autoantibodies against a variety of nuclear proteins is one of the major characteristics of rheumatic autoimmune diseases, which has been used in the clinical diagnosis of these diseases (48). Recent studies in our laboratory have shown that poly(A) polymerase is an autoantigen to which antibodies are produced by patients with a variety of rheumatic autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, and scleroderma (49). Sera from 53 patients, 26 with systemic lupus erythematosus, 8 with rheumatoid arthritis, 9 with Sjögren's syndrome, and 10 with scleroderma were screened for anti-poly(A) polymerase antibodies using purified liver and hepatoma poly(A) polymerases as the presenting antigens. Interestingly, some
patients produced antibodies against liver poly(A) polymerase which has not been an effective antigen in rabbits (41). The presence of anti-liver poly(A) polymerase antibodies in these sera is consistent with the capacity of the autoimmune patients to elicit antibodies against highly conserved molecules (see Ref. 48). Sixty and 74% of the sera used for screening contained antibodies against liver poly(A) polymerase and tumor poly(A) polymerase, respectively, and 25% of the sera exhibited antibodies exclusively against the tumor poly(A) polymerase. IgG containing anti-liver and anti-hepatoma poly(A) polymerase antibodies inhibited liver and hepatoma poly(A) polymerase, respectively, whereas IgG against liver and tumor enzymes inhibited both enzymes. These observations confirmed the specificity of the antibodies present in the autoimmune sera. Unlike the very early appearance of poly(A) polymerase antibodies in the hepatocarcinogen-fed rats well before preneoplastic nodules were detected, these antibodies are produced only after the onset of the rheumatic diseases.

It was of interest to note that poly(A), the product of the poly(A) polymerase reaction, is also a major autoantigen (50). There are reports indicating inhibition of polyadenylation of adenovirus mRNA by sera classified as anti-sm, anti-(U)RNP, and anti-La (51, 52). Although these sera might contain antibodies against the endonuclease responsible for the specific cleavage of mRNA precursor, it is plausible that these sera affect the polyadenylation reaction via direct inhibition of poly(A) polymerase.

Antibodies against the liver-type poly(A) polymerase were recovered from the autoimmune sera by using an affinity purification procedure. These antibodies were used to study tissue and species distribution of the liver-type enzyme. These studies showed that the liver-type enzyme is present in rat brain, kidney, spleen, and heart whereas the tumor-type enzyme is predominant in a variety of tumors and transformed liver cell lines. The rat liver-type poly(A) polymerase was also found in pig and human tissues and cell lines.

VIII. Occurrence of Poly(A) Polymerase as a Complex and Its Functional Implications

Studies on the polyadenylation reactions in vivo as well as in cell-free systems suggest that the endonuclease and poly(A) polymerase activities may act in a coupled fashion whereby cleavage occurs at the poly(A) site and addition of adenylic tracts rapidly follows cleavage. This apparent linkage between cleavage and polyadenylation implies a physical association of the two enzymes involved in these reactions and has led to the concept that poly(A) polymerase may exist as part of a large multicomponent enzyme complex. The existence of a poly(A) polymerase complex could account for the efficiency of 3' editing. Interaction with such a multicomponent poly(A) polymerase complex could induce conformational changes in the pre-mRNA substrate and the resultant RNA structures would be readily and coordinately acted upon by intrinsic processing activities of this enzyme complex. Alternatively, selection of cleavage at the poly(A) site and poly(A) addition could be performed by separate factors which individually act on mRNA precursors.

Elucidation of the mechanisms by which poly(A) polymerase mediates the processing of eukaryotic pre-mRNAs requires identification and characterization of the molecules with which the enzyme specifically interacts. Despite efforts to elucidate the catalytic components required for 3' end processing, progress in the positive identification of the trans factors involved in these reactions has been relatively slow. Hashimoto and Steitz (53) showed that a factor with the properties of an snRNP associates with the hexanucleotide sequence upstream of the poly(A) site. A M, 64,000 polypeptide which can interact specifically with the AAUAAA sequence has been identified by UV cross-linking (54). This factor appears to be a component of the cleavage machinery. Zarkower and Wickens (55) have demonstrated the formation of a stable precleavage complex between a factor present in HeLa nuclear extracts and the AAUAAA sequence. This hexanucleotide is located 10–30 nucleotides upstream of the poly(A) site of almost all eukaryotic mRNAs (56) and is implicated in the cleavage/polyadenylation reactions (57–61). Zarkower and Wickens have postulated that this factor may indeed be poly(A) polymerase. Further studies are needed to prove that poly(A) polymerase can bind to the hexanucleotide sequence.

A gel mobility shift analysis of in vitro polyadenylation reactions has been used for the detection of factors involved in the cleavage and polyadenylation processes. These studies analyzed the RNA/protein complexes produced after addition of pre-mRNA precursors to HeLa nuclear extracts under conditions favorable for 3' end-processing. Electrophoretic separation on nondenaturing gels of the complexes formed with HSV1-TK (62), Adenovirus L3 (63, 64), herpes simplex virus (65), and SV40 late (66) pre-mRNA substrates revealed common characteristics. Similar complex formation has also been observed following glycerol (67) or sucrose density gradient (68) fractionation of the product formed with Adenovirus L3 mRNA. Distinct complexes were formed with pre-mRNAs over time and the association of these factors correlated with the requirements essential for the in vitro reactions. Humphrey et al. (64) have reported that mRNA precursors containing the Adenovirus L3 polyadenylation site can form specific complexes with HeLa nuclear extract and that the complex protects a 67-nucleotide region of the mRNA from RNase T1 digestion. This protected fragment contains both the AAUAAA- and GU-rich downstream cis element. On the basis of their findings, Humphrey et al. (64) have proposed that the assembly of specific complexes represents an essential step during the 3' end processing of mRNA.

Recent studies in our laboratory (69) suggest that newly synthesized poly(A) polymerase is associated with other polypeptides. Polyclonal antibodies raised against highly purified tumor-type poly(A) polymerase were used to immunoprecipitate poly(A) polymerase from [35S]methionine-labeled McA-RH 7777 cells. In addition to immunoprecipitating the expected poly(A) polymerase molecule, at least 4 other labeled proteins with approximate molecular weights of 70,000, 35,000, 30,000, and 23,000 were also consistently immunoprecipitated (69). The specificity of the anti-poly(A) polymerase antibodies was demonstrated by immunoblot analysis of the 7777 nuclear extract. A single band corresponding to M, 48,000 tumor-type enzyme was observed by this analysis. Further fractionation of the enzyme showed that the complex consisting of poly(A) polymerase and other polypeptides could be dissociated; only one [35S]-labeled band corresponding to poly(A) polymerase was visible after the fractionation. No polypeptide was precipitated following reaction with preimmune sera. The observation that an antibody directed against a single purified protein can precipitate additional components implies that nascent poly(A) polymerase is associated with four distinct polypeptides as a complex. It is conceivable these polypeptides may have a role...
in the polyadenylation of pre-mRNAs.

Another factor that appears to be part of the polyadenylation complex is small nuclear RNA and perhaps its associated proteins (51–53, 68, 70, 72, 73). U1 and U6 snRNAs have been implicated in the cleavage/polyadenylation reactions (52, 53, 71, 74). Interestingly, histone mRNA precursors that are not polyadenylated require U1-RNA for the cleavage reaction which generates the correct 3’ terminus of these transcripts (75, 76). Recently, our laboratory has been able to demonstrate a close association of U1-RNA in the polyadenylation complex by immunoprecipitating extract derived from 32P-labeled 7777 cells with anti-hepatoma poly(A) polymerase antibodies followed by extraction of RNA and gel electrophoresis under denaturing conditions. The only RNA immunoprecipitated under these conditions corresponded to U1-RNA (74). These studies do not establish that U1 is directly involved in the 3’ end processing of pre-mRNA. It is plausible that other snRNAs/RNP RNPs play a more direct role in the cleavage/polyadenylation reaction (see below).

Studies in our laboratory (77) have shown that anti-poly(A) polymerase antibodies raised against highly purified Mg, 48,000 poly(A) polymerase can inhibit polyadenylation of Adenovirus L3 pre-mRNA. Surprisingly, the same antibodies also inhibit the cleavage reaction when it was coupled or uncoupled with polyadenylation. The anti-poly(A) polymerase antibodies could also prevent formation of specific complexes between pre-mRNA and components of nuclear extract. These studies have concluded that previous characterized poly(A) polymerase is the enzyme responsible for the addition of poly(A) tract at the correct cleavage site and that coupling of cleavage and polyadenylation appears to result from the potential requirement of poly(A) polymerase for the cleavage reaction. These observations have prompted us to conclude that poly(A) polymerase is associated with the endonuclease and probably with other essential components in a functional complex.

Another interesting observation (77) has been with regard to Mg2+-dependent and Mn2+-dependent poly(A) polymerase activities which correspond to chromatin-bound and free form of the enzyme (also see Refs. 12 and 17). In the presence of Mg2+, 200–250-nucleotide-long poly(A) were added at the poly(A) site of Adenovirus L3 pre-mRNA whereas as much as 400–800 adenylic acid residues were added to the presence of Mn2+ (77). Both Mg2+– and Mn2+-activated poly(A) polymerases were inhibited by anti-poly(A) polymerase antibodies which supports the previously held notion (see Refs. 13 and 17) that the initial addition of poly(A) tract and poly(A) elongation reactions are directed by the same enzyme. Although the bound enzyme has been designated as “chromatin bound,” it is probably associated with the complex involved in the cleavage/polyadenylation (78).

Takagaki et al. (78) have succeeded in fractionating HeLa nuclear extract into fractions containing poly(A) polymerase and cleavage activity. Efficient and accurate cleavage of three different pre-mRNAs required reconstitution of the two fractions. Gilmartin et al. (72) have observed that an snRNP is required for cleavage at the poly(A) site, although the exact nature of the snRNP has not been investigated. However, snRNP was not essential for poly(A) addition to the cleavage site (79). Very recently, Christofori and Keller (73) have separated and purified three factors that are required for accurate cleavage and polyadenylation of Adenovirus L3 mRNA. One of the factors is a poly(A) polymerase with a molecular weight of approximately 50,000–60,000 which is in general agreement with the molecular weight (48,000) of the enzyme purified in our laboratory (38). The second factor is a cleavage factor with a native molecular weight in the range of 70,000–120,000. The third component is a 200,000 factor that is needed for the cleavage activity and also confers specificity to the poly(A) polymerase activity. Further purification has revealed that the M, 200,000 cleavage factor activity is associated with U11-snRNP, which suggest that U11-RNA may be involved in the 3’ end processing of pre-mRNA. The conclusion that both the cleavage and polyadenylation activities require the same component is consistent with our observation that anti-poly(A) polymerase antibodies can inhibit both cleavage and polyadenylation and further suggests that both these reactions are tightly coupled.

IX. Future Directions

Although significant advances were made earlier on the enzymology of poly(A) polymerase, characterization of the factors controlling cleavage at the poly(A) site and subsequent polyadenylation has progressed at a relatively slow pace. Recent success in the fractionation of poly(A) polymerase from the endonuclease and their reconstitution into a functional complex (73, 78) should facilitate further studies on the mechanism by which these two enzymes direct polyadenylation of specific mRNAs. The role of U1-RNA that has been found associated with poly(A) polymerase in a functional complex should be determined. For example, what has prevented detection of U1-RNA/RNP in the complex by other techniques? Is it involved at some stages of the processing reaction in a indirect manner and if so, at what stage of 3’ end processing is this RNA required? Is it essential for both endonucleolytic cleavage and poly(A) addition? Is U1-RNA simply involved in facilitating the function of U11-snRNA associated with the cleavage factor? Do the proteins associated with U11– or U1-RNA play a role in mRNA 3’ end processing?

The nature and functions of the four polypeptides associated with nascent poly(A) polymerase should be determined. Are these proteins related to endonuclease and other cleavage factors. Is any one of these polypeptides related to proteins associated with snRNAs?

Another interesting development is the discovery of two structurally and immunologically distinct species of nuclear poly(A) polymerase. Since the tumor-type enzyme is found in fetal liver, but not in regenerating liver, it seems unlikely that this enzyme is required for normal cellular proliferation. It would be of considerable interest to determine the exact function(s) of these two enzymes. Does the tumor-type enzyme play a key role in tumorigenesis? Could it be a viable marker for certain tumors? Do these enzymes polyadenylate specific classes of mRNA, and if so, what is the factor(s) regulating a shift from the liver-type to the tumor-type poly(A) polymerase? Are liver-type and tumor-type poly(A) polymerases recognized by specific cis-acting elements on mRNA precursors? Is the tumor-type poly(A) polymerase gene related to an oncogene?

Does posttranslational modification of poly(A) polymerase affect polyadenylation of pre-mRNAs. Finally, the kinetics of the cleavage/polyadenylation reaction should be examined in great detail. The various stages at which poly(A) polymerase and other key components participate in the processing should be determined.

Acknowledgments

The authors thank Doris Lineweaver for her assistance in the preparation of this article.
Polyadenylate Polymerases from Normal and Cancer Cells and Their Potential Role in Messenger RNA Processing: A Review

Samson T. Jacob, Michael P. Terns and Kathleen A. Maguire