MESSENGER RNA METABOLISM OF ANIMAL CELLS

Possible Involvement of Untranslated Sequences and mRNA-Associated Proteins

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The past several years have seen a virtual revolution in the study of eukaryotic mRNA.[1] Among the notable recent achievements are the positive identification of mRNA precursors in HnRNA, the enumeration of the DNA sequences from which mRNA is transcribed, and the finding that mRNA in cultured cells is much more stable than was previously believed. One of the most far-reaching discoveries has been the finding that mRNA in eukaryotes contains poly A. This discovery, aside from providing a powerful tool for mRNA isolation, has generated a large body of research into the properties and metabolism of poly A itself. In addition, the finding of a poly A-associated protein has given a renewed stimulus to the study of proteins associated with mRNA. This review is devoted to a discussion of these and related achievements, and some of their implications.

mRNA SEQUENCES IN HnRNA

It has long been recognized that the rate of synthesis of HnRNA greatly exceeds the rate of entrance of mRNA into the cytoplasm, and that much HnRNA must turn over within the nucleus. Furthermore, HnRNA molecules are much larger than mRNA molecules; HnRNA molecules may exceed 10^7 daltons, whereas mRNA molecules are generally smaller than 2×10^6 daltons (for references, see the review by Darnell [1]). Attempts to reconcile these facts with the idea that HnRNA is the precursor of mRNA have postulated that the formation of mRNA involves the selection of certain HnRNA sequences and their processing into mRNA in a series of events which includes a stepwise reduction in size. The production of mRNA from HnRNA would thus parallel the production of 28S and 18S rRNA from their 45S precursor (1). While this scheme is plausible, there have been few experimental data to sustain it. Recently, however, there has been a accumulation of evidence which seems to establish firmly that mRNA sequences are found in high molecular weight HnRNA. Thus, one aspect of the postulated scheme of HnRNA processing has been confirmed.

This evidence was first obtained with virus-infected cells, in which it was established by means of DNA-RNA hybridization that virus-specific RNA sequences are present both in mRNA and in the much larger HnRNA molecules. This has been found in the case of SV40 for both lytically infected and transformed cells (2, 3). In some cases it has been shown in competition experiments that the virus-specific sequences in mRNA are apparently identical with sequences found in HnRNA, e.g., herpes simplex virus-infected cells and adenovirus-infected cells (4, 5).

In addition, workers in several laboratories have

[1] *Abbreviations used in this paper:* AMV, avian myeloblastosis virus; dA, deoxyadenylic acid; dT, deoxythymidylic acid; EDTA, ethylenediaminetetraacetate; HnRNA, heterogeneous nuclear RNA; mRNA, messenger RNA; mRNP, messenger ribonucleoprotein; poly A, polyadenylic acid; poly U, polyuridylic acid; poly A(+)HnRNA, HnRNA which contains poly A; poly A(+)mRNA, mRNA which contains poly A; RNP, ribonucleoprotein; rRNA, ribosomal RNA; SDS, sodium dodecyl sulfate.

now carried out experiments demonstrating the existence of globin mRNA sequences in HnRNA of globin-synthesizing erythroblasts or fetal liver cells. Melli and Pemberton (6) made use of the ability of bacterial DNA-dependent RNA polymerase to transcribe an RNA template in order to synthesize a radioactive RNA complementary to globin mRNA. They found that this RNA hybridized efficiently with duck erythroblast HnRNA to form ribonuclease-resistant duplexes. More recently, Imaizumi et al. (7) made DNA complementary to duck globin mRNA purified by sucrose density gradient sedimentation with AMV reverse transcriptase, and showed that it hybridized with HnRNA. The use of reverse transcriptase has the advantage that the DNA copy has the size expected for a nearly complete copy of globin mRNA. In the case of RNA polymerase it is not known whether the entire nucleotide sequence of globin mRNA is represented in the complementary RNA. Also, Imaizumi et al. (7) were careful to isolate HnRNA under denaturing conditions so as to minimize the possibility of aggregation with mRNA.

Williamson et al. (8) have isolated high molecular weight RNA (greater than 30S) from fetal mouse liver, injected it into *Xenopus* oocytes, and demonstrated the synthesis of mouse globin in the oocytes. As a control for possible contamination of the high molecular weight RNA with 9S globin mRNA they showed that high molecular weight mouse brain RNA isolated in the presence of a reticulocyte lysate did not direct globin synthesis. A similar experiment has been done by Ruiz-Carillo et al. (9). They isolated RNA larger than 45S from duck erythroblasts by sedimentation in 99% dimethylsulfoxide gradients and found that it directed globin synthesis in an ascites cell-free system. Rabbit 9S globin mRNA added to the gradient was not detected in the high molecular weight RNA which directed duck globin synthesis.

Each of the above experimental approaches has drawbacks. In both cases, it is difficult completely to exclude the possible contamination of high molecular weight RNA by globin mRNA. In the case of the hybridization experiments it is impossible to be sure that the complementary RNA or DNA hybridized to bona fide globin mRNA sequences in HnRNA, rather than to sequences which are merely similar to globin mRNA. Nevertheless, these experiments taken together provide strong evidence for the occurrence of mRNA sequences in HnRNA. There is still no direct evidence for processing of these sequences into mRNA. Furthermore, there is no information as to whether all HnRNA molecules are precursors of mRNA, or whether some HnRNA molecules have a different function. As will be discussed below, it has been suggested that the presence of poly A in HnRNA is the basis for selection of those molecules to be processed into mRNA. However, the weight of evidence seems to be against this hypothesis.

POLY A

The existence of poly A in eukaryotic cells was first demonstrated by Hadjivasiliou and Brawerman in 1966 (10), who found it in rat liver microsomes. However, it was not recognized until later that the poly A is associated with mRNA. Kates (11) in 1970 reported that isolated virions of vaccinia virus synthesize poly A which can become associated with in vitro-synthesized viral mRNA, and furthermore, that vaccinia mRNA isolated from infected HeLa cells, and HeLa cell mRNA itself, contain poly A. Lim and Kanellakis also in 1970 reported the presence of poly A in mouse globin mRNA (12). In 1971 three laboratories confirmed that poly A is covalently bound to mammalian mRNA and also demonstrated its presence in HnRNA. Their papers appeared simultaneously (13–15). Since this dramatic event there have been a great many reports on the occurrence of poly A in the mRNA of mammals and birds, their DNA and RNA viruses, sea urchins, insects, slime mold, yeast, and higher plants. It has also been found in the mitochondrial mRNA of mammals and insects. Most mRNA in cultured mammalian cells except histone mRNA contains poly A (16, 17). Since poly A is absent from bacterial mRNA (18) its presence is a distinguishing characteristic of eukaryotes. A compilation of reports on the occurrence of poly A is given in Table I.

Methods for the Isolation and Detection of Poly(+) mRNA

Perhaps one reason that the presence of poly A in mRNA went undetected for so long is that poly A(+)mRNA is lost in conventional procedures for RNA isolation in which polyribosomes or cytoplasmic extracts dissolved in a buffer containing

sodium dodecyl sulfate (SDS) are shaken with phenol at neutral pH (15, 19). Under these conditions the poly A(+)mRNA does not dissociate from protein, and is found at the phenol-water interface when the phases are separated by centrifugation. Moreover, some of the poly A may actually be sheared from the mRNA (19). Histone mRNA, which lacks poly A, is readily isolated by this procedure (19). Two special procedures for the deproteinization of poly A(+)mRNA are in widespread use. These include shaking with phenol at pH 9 (15) and shaking with a mixture of phenol and chloroform at neutral pH (19). Poly A(+)mRNA can also be recovered by conventional phenol extraction at neutral pH if the polyribosomes are first treated with a proteolytic enzyme (20, 19). This observation led Perry et al. (19) to infer that there is a protein tightly bound to poly A. The existence of this protein has now been directly demonstrated (21).

There are several ways in which poly A, or poly A(+)mRNA can be separated from other polyribonucleotides. The simplest, but least specific, are those in which poly A is bound to esterified cellulose (such as Millipore filters) or to cellulose itself (15, 22). The basis for the selective binding of poly A to cellulose or cellulose esters is not known. Other more specific methods make use of the ability of poly A to hybridize with oligo dT or poly U. In one of the most widely used and dependable techniques oligo dT bound to cellulose is prepared by organic synthesis. Poly A is hybridized to the oligo dT at high ionic strength (0.1–0.5 M Na^+ or K^+) at room temperature. The poly A-lacking RNA is then washed away and the poly A-containing RNA eluted with a buffer of low ionic strength (23). In other methods which utilize the same principle, poly U is covalently bound to agarose (24) or else immobilized on cellulose or glass fibers by means of cross linking with UV light (11, 25). In still another successful approach, poly A-containing RNA is hybridized with poly U in solution under conditions which permit the formation of a triple helix containing one strand of poly A and two strands of poly U. The poly A-lacking and poly A-containing RNA are then adsorbed to hydroxylapatite and eluted sequentially with 0.25 M and 0.5 M sodium phosphate buffer (17).

A useful technique for isolating and detecting poly A (but not RNA which contains it) makes use of the fact that poly A is resistant to hydrolysis by ribonucleases A and T_1, so that it is possible to digest away all poly A-lacking RNA while leaving poly A itself intact (10–12). The poly A can then be separated from the ribonuclease digestion products by means of affinity chromatography as described above, or by electrophoresis on polyacrylamide gels.

Properties of Poly A

It has been established beyond a doubt that poly A is located at the 3′-OH terminus of mammalian mRNA and HnRNA. This conclusion applies also to the mRNA of vaccinia, a DNA virus, and polio and AMV which are RNA viruses. It has been reached independently by several laboratories using a variety of techniques. The simplest is to show that alkaline hydrolysates of poly A isolated by RNase treatment contain adenosine, but not adenosine-3′,5′-phosphate. Poly A located at the 5′-terminus would be expected to yield an adenosine-3′,5′-phosphate, whereas internal poly A would yield only adenosine-3′-phosphate. This technique has been used by Kates (11) for vaccinia mRNA, Mendecki et al. (26) for Sarcoma 180 poly A, and McLaughlin et al. for yeast poly A (27). A quite different approach has been used by Molloy et al. (28) in the case of L-cell poly A. These authors treated isolated mRNA with a highly purified exonuclease which digests RNA sequentially from the 3′-terminus. They found that the RNA lost its ability to bind to Millipore filters when only a small fraction of the total nucleotides had been removed. Furthermore, they found that poly A isolated by RNase treatment could be completely hydrolyzed by the exonuclease, which could be true only if each fragment had a 3′-OH terminus and was located at the 3′-terminus of mRNA. Similar experiments were done by Sheldon et al. (29) on vaccinia mRNA and poly A derived from mRNA and HnRNA of uninfected cells, using the same exonuclease and also polynucleotide phosphorylase, another enzyme which can digest RNA sequentially from the 3′-terminus. A third method makes use of the fact that the 3′-terminal nucleoside of RNA can be radioactively labeled by reduction with [^{3}H]borohydride after periodate oxidation. The RNA is then hydrolyzed and the labeled trialcohol identified by chromatography. This method has been used to demonstrate the 3′-terminal location of poly A in globin mRNA by Burr and Lingrel (30), poliovirus mRNA by Yogo and Wimmer (31), AMV mRNA

TABLE I

Occurrence of Poly A in mRNA

Source of mRNA or poly A	Method of Detection*	Reference
Mammalian cells		
HeLa	Hm	11
HeLa	S	13
HeLa	Hf, G	14
Sarcoma 180	Am, Af, S	15
L cell	Hm	17
Other animal cells		
Sea urchin	Hm	43
Sea urchin	Hm, S	44
Insect (*Chronomus tentans*)	Hm	135
Insect (*Drosophila melanogaster*)	G	136
Insect (*Aedes albopictus*)	G	136
Mitochondria		
Mammalian (HeLa, CHO)	G, Hm	136
Mammalian (mouse ascites)	Hm, Am, Af, G	137
Insect (*Drosophila, Aedes*)	Hm, G	136
DNA virus-infected cells		
Vaccinia	Hm, S	11
Herpes simplex	G, Af	138
Adenovirus	G, Hm, Am	48
SV40	Hm	139
RNA tumor viruses (virions)		
AMV	Hm	140
AMV	Hm, Am	141
AMV	Af, B	32
Murine sarcoma	Hm, Am, B, G	141
Rous sarcoma	B	142
Rous sarcoma	Am, Af, G, B	143
Murine leukemia	S	143
Murine leukemia	Hm	140
Feline leukemia	Hm	140
Mason-Pfizer agent	Hm	140
Visna	Hm	144
Other RNA viruses (virions)		
Polio	Hf, G, B	36
Polio	G, B	31
Equine encephalitis	Hf, G, B	36
Sindbis, Columbia SK	S	50
Vesicular stomatitis virus		
(RNA complementary to virion RNA)	Hm, G	51
	Hm	52
	G, B	53
Other organisms		
Slime mold	Hm	90
Yeast	Hm, Hf, G	27
Yeast	G	145
Higher plant (*Phaseolus aureus*)	Hm, B, G	146

TABLE I—*Continued*

Source of mRNA or poly A	Method of Detection*	Reference
Specific kinds of mRNA		
Rabbit globin	G	12
Rabbit globin	Hm	147
Duck globin	G	148
Mouse immunoglobulin light chain	Hm	20, 149, 150
Chick ovalbumin, avidin	Am	151
Chick procollagen	Am	152
Chick keratin	Am	153
Rat tryptophan oxygenase	Am	154
Calf lens crystallin	Hm, R	155

* Hm, hybridization of mRNA to oligo dT or poly U; Hf, hybridization of RNase-resistant poly A fragment to oligo dT or poly U; Am, adsorption of mRNA to Millipore filter or cellulose; Af; adsorption of RNase-resistant poly A fragment to Millipore filter or cellulose; B, base composition analysis of RNase-resistant poly A fragment; G, gel electrophoresis of RNase-resistant poly A fragment; S, sucrose gradient sedimentation of RNase-resistant poly A fragment; R, oligo dT primer-dependent template activity with AMV reverse transcriptase.

by Stephenson et al. (32), and HeLa cell mRNA and HnRNA by Nakazoto et al. (33). The poly A of L-cell, HeLa cell, and AMV mRNA have been sequenced and shown to contain no bases other than adenine (34, 35, 32).

The size of the poly A of newly synthesized mammalian mRNA and HnRNA is known to be 150–200 nucleotides long based on end groups analysis, e.g., the number of nucleotides per terminal nucleoside (11, 26, 33).[2] However, the poly A of polio virus, equine encephalitis, and AMV virion RNAs are considerably shorter (40–90 nucleotides) (36, 31, 32). The reason for these variations in size is not known, but possibly they are related to the metabolic instability of poly A. It has been shown that the poly A shortens as mRNA ages in uninfected mammalian cells (26, 37, 38). It is not known, however, if this shortening proceeds to completion, or whether it stops at a finite size.

Relationship Between Nuclear and Cytoplasmic Poly A

The finding that poly A occurs in HnRNA as well as in mRNA led to the hypothesis that poly A(+) HnRNA is the precursor of mRNA, with the poly A segment of the HnRNA being conserved in the processing. There have been some experiments which tend to support this hypothesis. For example, Jelinek et al. (39) reported that HeLa cells given a short pulse with [^{3}H]adenosine initially have more radioactivity in the poly A of HnRNA than in the poly A of mRNA. However, with more prolonged labeling the incorporation of radioactivity into poly A of HnRNA leveled off, while incorporation into poly A of mRNA continued to increase. They concluded that these results are consistent with a precursor-product relationship between the poly A of HnRNA and the poly A of mRNA. In addition, they claimed that at least 40% of the poly A of HnRNA can be chased into the poly A of mRNA in the presence of 3′-deoxyadenosine, a drug which inhibits poly A synthesis.

In contrast to these results, Perry et al. (40), working with L cells, concluded that the labeling kinetics of nuclear poly A and cytoplasmic poly A are *not* consistent with a simple precursor-product relationship. In their experiments, incorporation of [^{3}H]adenosine into the poly A of mRNA or the total cytoplasmic RNA reached a maximum rate while incorporation into the poly A of HnRNA was still increasing linearly. From a detailed analysis of the labeling kinetics they concluded that most nuclear poly A, perhaps as much as 90%, turns over within the nucleus. They furthermore concluded that the rate of synthesis of poly A in the nucleus is not sufficient to account for its rate of appearance in the cytoplasm, but rather that the results are consistent with considerable cytoplasmic synthesis of poly A. If their nuclear fraction

[2] This is certainly the most dependable way to estimate size. Polyacrylamide gel electrophoresis is often used to size RNA molecules, but poly A migrates more slowly in gels than heteropolymeric RNA of the same chain length, and poly A standards of known size are difficult to obtain.

was contaminated with cytoplasmic poly A, the intranuclear turnover could have been overestimated. However, it is unlikely that all of the apparent intranuclear turnover could be explained on this basis. In other experiments LaTorre and Perry (41) found that when L-cell poly A synthesis is interrupted by 3'-deoxyadenosine, more than 50% of nuclear poly A turns over within the nucleus and does not reach the cytoplasm.

In attempting to explain the discrepancy between their results and those of Jelinek et al., Perry et al. pointed out that the labeling kinetics experiments of Jelinek et al. had been carried out with low concentrations of [^{3}H]adenosine in concentrated and presumably nongrowing cell suspensions. On the other hand, the experiments of Perry et al. were carried out with relatively high concentrations of low specific activity [^{3}H]adenosine which was replenished occasionally during the labeling so as to assure a constant specific activity of RNA precursor pools. Furthermore, the cells were in exponential growth at all times during the labeling. Thus, Perry et al. suggested that Jelinek et al. had observed a premature cessation of incorporation of radioactivity into the poly A of nuclear RNA due to the depletion of radioactivity in the precursor pool. In order to explain the continuing incorporation of label into cytoplasmic poly A under these circumstances, it is necessary to assume that cytoplasmic poly A is synthesized from a separate precursor pool which is more slowly depleted than the nuclear pool. The existence of such a slowly turning over cytoplasmic pool of ribonucleotides is strongly suggested by the work of Plagemann (42). The data obtained by Jelinek et al. and by LaTorre and Perry on the turnover of nuclear poly A in the presence of 3'-deoxyadenosine are quantitatively in fairly good agreement. However, there is a difference in interpretation; Jelinek et al. chose to emphasize that a considerable fraction of nuclear poly A can be chased into the cytoplasm, whereas LaTorre and Perry emphasized that a roughly equal fraction of nuclear poly A *cannot* be chased into the cytoplasm. On balance, it seems likely that a significant fraction of nuclear poly A does turn over within the nucleus. The finding rules out a simple precursor-product relationship between all poly A(+)HnRNA and mRNA.

A set of independent observations which support the idea that poly A is added to mRNA in the cytoplasm has been obtained by Slater et al. (43) and Wilt (44). These authors found that there is extensive net synthesis of poly A in sea urchin embryos immediately after fertilization. This synthesis apparently represents addition of poly A to maternal mRNA which was made before development and stored in the cytoplasm. It takes place in a subribosomal (40–60S) cytoplasmic particulate fraction before the engagement of the mRNA into polyribosomes (43), and can occur in the absence of transcription and even in enucleated merogons (44). It is not clear whether this synthesis constitutes completely *de novo* formation of poly A or elongation of previously existing poly A sequences. In spite of this ambiguity, cytoplasmic synthesis of poly A has been conclusively demonstrated in sea urchins. It seems reasonable to assume that addition of poly A to mRNA occurs in a similar manner in sea urchin and mammalian cells.

Biosynthesis of Poly A

Experiments using actinomycin D and labeling kinetics initially suggested that addition of poly A to HnRNA and mRNA takes place after they have been transcribed. Darnell et al. (45) found that actinomycin D inhibits the synthesis of total HnRNA to a much greater extent than the synthesis of poly A. Although actinomycin D does not bind to dA·dT base pairs and therefore, should not prevent the independent transcription of poly A from a dA·dT template, it would presumably prevent the transcription of poly A as the 3'-terminal segment of a larger molecule. Labeling kinetics studies by Mendecki et al. (26) and Perry et al. (40) have shown that the poly A segment is the earliest-labeled part of cytoplasmic mRNA. This could be true only if the poly A is the last part of the mRNA to be synthesized. Moreover, the lag between the appearance of label in poly A and the appearance of label in the remainder of the mRNA is too great to be accounted for as the difference in labeling time between the 3'- and 5'-termini (40).

Studies with actinomycin D and labeling kinetics, while showing that poly A is added after transcription, do not rule out the possibility that poly A itself is transcribed from DNA. Evidence that poly A is not transcribed from DNA comes from pyrimidine tract analysis of human and mouse DNA by Birnboim et al. (46), and Harbers and Spencer (47). These studies showed that there are no detectable large dA·dT sequences from which poly A could be transcribed. Some DNA-

RNA hybridization studies indicated that vaccinia virus DNA and HeLa cell DNA contain sequences capable of hybridizing with poly A (11, 48). However, the fidelity of the hybrids was not tested, and there is no evidence that these hybrids correspond to stretches of dA·dT base pairs sufficiently long to direct the synthesis of a 150–200 nucleotide poly A sequence.

The most convincing evidence for nontranscriptional synthesis of poly A comes from the studies with sea urchin embryos cited above which demonstrated cytoplasmic addition of poly A to previously synthesized maternal mRNA. Most probably, poly A is synthesized from ATP by a terminal addition enzyme such as the one first described by Edmonds and Abrams (49). Enzymes such as this are of very widespread occurrence, but the individual enzyme or enzymes responsible for the in vivo polyadenylation of mRNA and HnRNA have not been identified.

Function of Poly A

There are two general hypotheses about the role of poly A. One is that it plays a part in the processing of HnRNA into mRNA. The major evidence in favor of this hypothesis is that (supposedly) the poly A of HnRNA is conserved and transported to the cytoplasm as the poly A of mRNA and furthermore, that 3′-deoxyadenosine inhibits the entrance of mRNA into the cytoplasm while having relatively little effect on the synthesis of HnRNA (16, 45). On the other hand, there is much evidence which casts doubt on its validity. Firstly, histone mRNA lacks poly A but is nevertheless synthesized and transported to the cytoplasm. Secondly, as discussed above, it seems likely that much poly A turns over in the nucleus and that at least some poly A is added to mRNA in the cytoplasm. In view of these data, the results obtained with 3′-deoxyadenosine must be interpreted with caution. Presumably, cytoplasmic synthesis of poly A would be sensitive to the drug. Furthermore, since it is an analog of adenosine it could have profound effects on adenine nucleotide metabolism and therefore, on all ATP-requiring metabolic processes, in addition to inhibiting poly A synthesis.

The other hypothesis is that poly A functions cytoplasmically in the translation of mRNA. This idea was first suggested to several investigators by evidence that poly A is found in the mRNA of viruses which replicate in the cytoplasm, so that there is no need for their mRNA to be transported out of the nucleus. This is the case for vaccinia, a DNA virus, and polio, equine encephalitis, and sindbis, which are RNA viruses (11, 31, 36, 50). Indeed, Johnston and Bose have noted a correlation between the presence of poly A in high molecular weight virion RNA and the messenger function of that RNA (50). They have further suggested that the absence of poly A correlates with the absence of messenger function, so that the complementary strand and not the virion RNA itself serves as messenger. This appears to be true at least in the case of vesicular stomatitis virus (51–53). The fact that poly A is added to maternal mRNA in the cytoplasm of sea urchin embryos also suggests a cytoplasmic function for poly A.

However, it appears that the presence of poly A is not required in order for mRNA to be translated. For example, histone mRNA is known to lack poly A, yet it is undoubtedly capable of being translated. Moreover, bacteriophage mRNA which lacks poly A can be faithfully translated in a mammalian cell-free system (54, 55), and mRNA from which poly A has been removed enzymatically can be translated in a eukaryotic cell-free system virtually as well as the same mRNA before removal of its poly A (56, 57).

The above results eliminate the possibility that poly A is required for translation. However, they do not rule out that it may have a more subtle regulatory role in translation. Differences in the turnover of poly A(+)mRNA and histone mRNA are consistent with this possibility. Recent experiments which avoided the use of inhibitors have shown that poly A(+)mRNA is highly stable and turns over stochastically (with first order kinetics) (58–60). Histone mRNA, while only slightly less stable, behaves differently and possibly turns over with linear (zero order) kinetics (60). This difference in turnover may be correlated with the absence of poly A from histone mRNA. However, there could also be differences between histone mRNA and poly A(+)mRNA with respect to secondary structure, associated proteins, or nontranslated sequences other than poly A.

It has been suggested that poly A itself may be responsible for the high stability of poly A(+)mRNA (58). This possibility has not been ruled out, but would be most difficult to prove. It is known that poly A shortens as mRNA ages. If the presence of poly A confers stability, then one

would predict that mRNA is degraded and lost from the polyribosomes only after the poly A has been reduced to its minimum size (whether this be zero nucleotides, or greater than zero nucleotides). Since mRNA turns over stochastically, any involvement of poly A in conferring stability requires that the shortening of poly A must proceed in a random, nonordered fashion, i.e. the probability of mRNA decay is independent of poly A length. On the other hand, if poly A has no role in determining mRNA stability, then mRNA could well persist in the polyribosomes after the poly A has been reduced to its minimum size. Establishing the role of poly A in mRNA stability will require the means to detect mRNA which lacks poly A after a steady state label or long chase.

In summary, it seems unlikely that poly A functions primarily either in the processing of HnRNA into mRNA, or in enabling mRNA to be translated. However, it could have some role in translation. In spite of extensive research the function of poly A continues to be elusive and largely a matter for speculation.

STABILITY OF mRNA

It has been known for many years that some differentiated cells have mRNA which is quite stable. Perhaps the best-known example is the reticulocyte, in which the mRNA for globin continues to function for days after its synthesis has ceased (61). Other examples of stable mRNAs in differentiated cells are the mRNAs for calf lens crystallin (62) and cocoonase (63). It furthermore appears that egg and early embryonic mRNAs are quite stable. The ability of sea urchin embryos to progress through early development when transcription is inhibited by actinomycin D led Gross and Cousineau (64) to postulate that early development utilizes stable maternal mRNA which was synthesized before fertilization. The existence of maternal mRNA including histone mRNA in a cytoplasmic subribosomal particulate fraction of unfertilized sea urchin eggs has now been demonstrated more directly by DNA-RNA hybridization experiments (65, 66) and in vitro protein synthesis (67). Evidence of a different sort for the stability of mRNA in oocytes comes from the work of Gurdon et al. (68), who found that heterologous globin mRNA injected into frog oocytes continues to function for several days.

Until recently, however, it was generally believed that the half-life of mRNA in cultured cells was only about 2.5–4 h. This estimate was derived from the rate of decay of protein synthesis, or disaggregation of polyribosomes, in cells treated with a sufficiently high dose of actinomycin D to inhibit completely transcription (69, 70). The accuracy of this estimate was questioned by Cheevers and Sheinin (71), who found evidence that some mRNA in 3T3 cells has a half-life of at least 6 h by measuring the kinetics of incorporation of labeled precursors into polyribosomal mRNA of cells treated with a low dose of actinomycin D so as to suppress selectively the synthesis of rRNA.

With the discovery that most newly synthesized mRNA in cultured cells contains poly A it became possible to isolate mRNA, and therefore, directly to measure mRNA stability without relying on the use of inhibitors of transcription. Greenberg (58) measured the kinetics of approach to steady state labeling of mRNA in exponentially growing L cells and concluded that mRNA turns over with first order kinetics and has a half-life of 10 h in cells growing with a doubling time of 15 h. At the same time Singer and Penman (72) published results showing that the relatively rapid decay of protein synthesis in HeLa cells in the presence of actinomycin D is due to a failure of initiation of protein synthesis and not to the exhaustion of mRNA. Subsequently, they published the results of pulse-chase experiments showing that HeLa cell mRNA consists of two populations, one-third with a half-life of 7 h, and two-thirds with a half-life of 24 h (59). The high stability of mRNA in growing cultured cells has now been confirmed by others (73–76) and is generally accepted.

It is remarkable that poly A(+)mRNA, which in cultured cells must consist of a great diversity of mRNA species, seems to turn over rather homogeneously. While Singer and Penman, using pulse-chase experiments (59), found two classes in HeLa cells, a transformed human cell line of epithelial origin, Greenberg (58) and Perry and Kelley (60), using kinetics of approach to steady-state labeling found only one class in L cells, a transformed mouse cell line of fibroblastic origin. More detailed studies using pulse-chase experiments rather than kinetics of approach to steady-state labeling suggest that L cells also may have two mRNA populations which differ in stability (E. Bard, personal communication). However, Abelson et al. (77), in pulse-chase experiments on 3T3 and 3T6 cells, which are nontransformed mouse fibroblast cell lines, found only one class of poly A(+)mRNA with a half-life of 9 h. This was true for both

growing and resting (contact-inhibited) cultures. Of course, these results do not exclude the possibility that there are minor constituents of mRNA with other half-lives. Nevertheless, the fact that the majority of the mRNA falls into only one or two classes with respect to half-life argues strongly that differential stability of mRNA does not have a major role in the control of translation, at least in cell lines such as HeLa, L, 3T3, and 3T6.

As mentioned above, histone mRNA seems to turn over differently from poly A(+)mRNA. In early experiments with actinomycin D it was found that histone synthesis decays more rapidly than total protein synthesis (70, 78). However, in a recent experiment in which histone mRNA stability was measured by kinetics of approach to steady state labeling in parallel with a measurement of the stability of poly A(+)mRNA. Perry and Kelley (60) found that L-cell histone mRNA seems to turn over with zero order kinetics and has a fixed lifetime of 11 h. This compares with a mean lifetime of 15 h for the poly A(+)mRNA. In these experiments histone mRNA was identified on the basis of its size and the sensitivity of its synthesis to cytosine arabinoside, a specific inhibitor of DNA synthesis. Although the data were not sufficiently precise to exclude the possibility that the turnover of histone mRNA follows an aging function (with the probability of decay increasing as the RNA ages) it was quite clear that the histone mRNA has a mode of turnover different from that of the poly A(+)mRNA. This difference may exist because poly A(+)mRNA functions throughout the cell cycle (excepting mitosis), whereas the functioning of histone mRNA is probably restricted to the S period.

Significance of mRNA Stability

The lifetime of poly A(+)mRNA in proliferating cells is long enough so that a substantial fraction of it must persist from one cell division to the next. For example, in L cells the mean lifetime of mRNA is approximately one cell generation. Since the mRNA turns over with first order kinetics, this implies that about 40% of the mRNA survives more than one cell generation. Indeed, the postmitotic formation of polyribosomes which contain only mRNA synthesized before mitosis has been documented (79, 80).

The long lifetime of the mRNA implies that cells cannot regulate protein synthesis within the time span of one cell generation simply by terminating transcription of mRNA. Instead, regulation of protein synthesis must occur at the translational level by means of initiators or repressors of protein synthesis. There is good evidence for the existence of mRNA-specific initiation factors (81), but their importance remains unclear. Numerous instances in which isolated mRNA has been successfully translated in protein synthesizing systems derived from other cell types or species seem to argue strongly against a need for specific initiation factors. The evidence for translational control by means of repressors has been reviewed by Tomkins et al. (82). This evidence consists largely of various instances in which the rate of synthesis of a protein is *increased* by treating cells with inhibitors of transcription such as actinomycin D. The proposed explanation for this phenomenon, first described by McCauslan (83), which is known as "superinduction," is that translation is controlled by labile repressors which require transcription for their continuing synthesis. The hypothetical repressor molecule has yet to be identified, and could conceivably be either RNA or protein. The occurrence of superinduction cannot be considered as conclusive evidence for the existence of translational repressors. Alternative explanations of superinduction are possible (84). However, there can be no doubt that some form of translational control exists. The reversible inhibition of translation which occurs during mitosis is sufficient proof of this (79, 80).

GENES FROM WHICH mRNA IS TRANSCRIBED

As a result of the discovery that eukaryotic DNA consists of both unique and repeated DNA sequences (85), it became of interest to determine the degree of repetition of DNA sequences from which mRNA is transcribed. The usual method for making this type of measurement is to allow radioactively labeled mRNA to hybridize in solution with a large excess of denatured DNA. Under these conditions the hybridization of RNA to DNA is a second order reaction paralleling the renaturation of DNA, so that RNA transcribed from repeated DNA sequences hybridizes at the low values of D_0t (DNA concentration multiplied by time) at which repeated DNA sequences renature, and RNA transcribed from unique DNA sequences hybridizes at the high values of D_0t characteristic for the renaturation of unique DNA sequences. The amount of hybrid can be assayed in one of three ways: (*a*) it can be trapped on a

nitrocellulose filter which adsorbs hybridized RNA while allowing unreacted RNA to pass through; (*b*) it can be separated from unreacted RNA by chromatography on hydroxylapatite; (*c*) it can be detected as RNA resistant to ribonuclease. The first attempt to make such a measurement on mRNA was made by McCarthy et al. (86).

It is now apparent that most mRNA in eukaryotic cells is transcribed from DNA with a low degree of repetition. Greenberg and Perry (87) found that about 80% of hybridizing L-cell mRNA is transcribed from nonrepeated DNA, but about 20% may be transcribed from DNA sequences with a 10^4-fold redundancy. This is in contrast to HnRNA, which has a higher proportion of nucleotides (32%) transcribed from DNA sequences with a similar degree of repetition. mRNA is transcribed largely from nonrepeated DNA in organisms as diverse as mouse (87), sea urchin (88, 89), and slime mold (90). It seems to be generally agreed that repeated sequence DNA is represented to a greater extent in HnRNA than in mRNA in these same organisms (87, 89, 90). This unequal distribution of repeated sequence transcripts may be a universal attribute of eukaryotes, but its significance remains unclear.

Some disagreement exists as to whether repeated sequences are represented at all in mRNA. Goldberg et al. (88) have asserted that sea urchin mRNA contains virtually no repeated sequence transcripts. However, these results seem to be in slight disagreement with those of McColl and Aronson (89), who found a low but detectable level of repeated sequence transcripts in sea urchin mRNA. On the other hand, it has been acknowledged by Klein et al. (91) that HeLa cell mRNA does contain repeated sequence transcripts (about 6% of the total mRNA, or one-sixth of the hybridizing mRNA). It seems likely that repeated DNA sequences are represented to a greater extent in the mRNA of higher eukaryotes than in the mRNA of lower eukaryotes. A question of great importance is whether these repeated sequence transcripts are entire molecules, or segments of molecules composed in part of repeated sequence transcripts and in part of unique sequence transcripts.

Multiplicity of Genes Coding for Specific Proteins

The genes for a few specific proteins have been characterized with respect to the number of copies per genome. The histone genes of sea urchins were the first to be characterized in this way by Kedes and Birnstiel (92). Histone genes are present in 400–1,000 copies per genome (92, 93). These copies are identical, or nearly so, since they form hybrids which have a high thermal stability and therefore, little or no mispairing of bases. Moreover, histone genes are apparently conserved in evolution, since histone mRNA from sea urchins can hybridize with DNA from distantly related organisms (93, 94). This finding is in agreement with amino acid sequencing studies on histones. In addition, histone genes seem to be clustered, since high molecular weight DNA which hybridizes with histone mRNA bands in CsCl density gradients at a somewhat higher density than the bulk of sea urchin DNA (92). Also, Pardue et al. (95) have shown by means of *in situ* hybridization with sea urchin histone mRNA that histone genes are localized in a single band of *Drosophila* polytene chromosomes. Since histone mRNA is transcribed from repeated DNA sequences it is exceptional in this respect as in so many others, e.g., lack of poly A, distinctive mode of turnover, and rapid entrance into the cytoplasm (96).

Another gene which has been extensively analyzed by means of hybridization is the one for globin. It has been found that duck and mouse globin mRNAs are transcribed from DNA sequences present in one to five copies per genome (97–99). Furthermore, there is no difference between duck liver DNA and reticulocyte DNA with respect to the multiplicity of globin genes (99). Therefore, the extensive accumulation of globin mRNA in reticulocytes must be accounted for by rapid transcription and high stability rather than by selective gene amplification. Although globin mRNA is transcribed predominantly from nonrepeated DNA sequences, certain early reports which erroneously concluded that globin genes are present in a high multiplicity suggest that a small portion of globin mRNA may be transcribed from highly repeated DNA sequences (100, 101).

The gene for silk moth fibroin was also found to be present in only one copy per genome in silk glands by Suzuki et al. (102). The amount of DNA coding for fibroin was measured by hybridizing DNA with saturating amounts of labeled fibroin mRNA rather than by following the hybridization kinetics of the RNA in the presence of an excess of DNA. In this case also, gene amplification is ruled out as a mechanism for the extensive accumulation of a particular kind of mRNA.

Several attempts have been made to characterize immunoglobulin genes by means of hybridization kinetics at DNA excess (103–106). This type of measurement is of exceptional interest in the case of immunoglobulin genes since the strict germ line theory of antibody diversity predicts that the constant region of immunoglobulin chains should be coded for by repeated DNA sequences, whereas the variable region should be coded for by less highly repeated sequences. On the other hand, the somatic theory of antibody diversity predicts that immunoglobulin mRNA should be transcribed from DNA sequences with a uniform degree of repetition with the possible exception of untranslated regions of the mRNA. All of the published reports agree that at least some portion of immunoglobulin mRNA is transcribed from repeated DNA sequences. However, estimates of the size of this fraction vary from as low as 26% (104) to at least 50% (103). Also, estimates of the degree of repetition of these sequences range from 40 copies per genome (103) to 5,000 copies per genome (105). Workers in three laboratories concluded that the results are consistent with the germ line theory of antibody diversity (103, 105, 106), but in one laboratory that the results are consistent with the somatic theory (104). Unfortunately, there are uncertainties about the purity of the immunoglobulin mRNA preparations used by the various investigators and also about the extent to which mRNAs other than immunoglobulin mRNAs are transcribed from repeated sequences. Therefore, no strong conclusion can be reached on the basis of the foregoing results.

Nature of Repeated Sequence Transcripts in mRNA

The only genes known to occur in multiple copies are those coding for histones. The repeated sequence transcripts in total mRNA, which usually has a very small proportion of histone mRNA, differ from histone mRNA in that they hybridize with DNA sequences which have a higher multiplicity, perhaps as high as 10^4 copies per genome (87). Also, they differ from histone mRNA in that they form hybrids of lower thermal stability than the hybrids formed by unique sequence transcripts (87). This observation suggests that the repeated DNA sequences with which they hybridize are not identical. The high degree of repetition of the repeated sequence transcripts found in mRNA makes it unlikely that they are translated into proteins. Instead, it is probable that they, like poly A, represent untranslated segments of mRNA molecules.

There is now considerable evidence for the interspersion of unique sequence transcripts with repetitive sequence transcripts in mRNA and in HnRNA. Dina et al. (107) have found that unfragmented molecules of *Xenopus* mRNA hybridize to DNA more extensively than fragmented molecules at low values of D_0t. A similar observation has been made by Firtel and Lodish (90) in the case of slime mold mRNA and HnRNA, and by Holmes and Bonner (108) in the case of mouse HnRNA. This is the result expected if mRNA and HnRNA molecules consist of transcripts of repeated DNA sequences in tandem with transcripts of unique DNA sequences. When intact molecules containing repeated sequence transcripts are hybridized at low D_0t the entire molecule should complex with DNA to form a structure consisting of a duplex region associated with an unpaired region, but when fragmented molecules are hybridized at low D_0t only those fragments containing a repeated sequence transcript should associate with DNA. However, there is some disagreement about interspersion of repeated and unique sequence transcripts in mRNA; Klein et al. (91) were unable to find evidence for interspersion in HeLa cell mRNA.

If the repeated sequence transcripts in mRNA are untranslated segments, then one can predict that they should be found either at the 5′-terminus, or near the 3′-terminus and adjacent to poly A. This should be true since eukaryotic mRNA is believed to be monocistronic (109–111), so that the coding region of the mRNA cannot be interrupted by an untranslated sequence. Dina et al. (112) have obtained rather strong evidence for the existence of a repeated sequence transcript at the 5′-terminus of *Xenopus* mRNA. This was done by specifically labeling mRNA molecules at the 5′-terminus with [^{32}P]cyanoethyl-phosphate, then fragmenting the molecules and hybridizing them. It was found that the ^{32}P-labeled 5′-terminal fragments hybridized at considerably lower values of D_0t than the bulk of the mRNA fragments which were labeled with ^{3}H. The 5′-terminal fragment seems to be transcribed from DNA sequences present in about 2,000 copies per genome and has a size of about 50 nucleotides. This size estimate is based on the size of the RNase-resistant fragment of mRNA hybridized at low D_0t and is probably a minimum estimate. In the case of slime mold mRNA a portion of the

repeated sequence transcripts has been found to consist of a 25-nucleotide sequence of oligoadenylic acid (113). This sequence is located near the 3′-terminus, but it is distinguishable from the larger poly A sequence since it can be separated from the latter by means of digestion with RNase A (but not RNase T_1). The oligo A is probably transcribed as a part of slime mold HnRNA, whereas the poly A is added posttranscriptionally. Since some of the repeated sequence transcripts of slime mold HnRNA are lost during processing into mRNA it has been inferred that they are located near the 5′-terminus (90).

In summary, it seems likely that most mRNAs other than histone mRNAs are transcribed from genes which are present in one or a few copies per genome. However, it is fairly well established that mRNA contains transcripts of repeated DNA sequences. Except for histone mRNA these repeated sequence transcripts are probably untranslated segments of mRNA molecules which are located near the termini. Although there is still no experimental evidence, it is possible that different kinds of mRNA may have similar or identical untranslated sequences in addition to poly A. There are no data whatsoever concerning the possible function of repeated sequence transcripts in mRNA. It can only be speculated that they, like poly A, may have some role in the processing of HnRNA into mRNA or in the control of mRNA translation.

PROTEINS ASSOCIATED WITH mRNA

The occurrence of proteins associated with mRNA was first suggested by the work of Spirin and his colleagues. These workers found that in the early embryonic development of fish and sea urchins, rapidly labeled cytoplasmic RNA which is messenger-like in size and base composition exists in the form of ribonucleoprotein particles which sediment in sucrose gradients at about the same rate as ribosomal subunits. These particles are not ribosomal subunits, however, since after formaldehyde fixation they band in CsCl gradients at a lower density than ribosomal subunits, presumably because they contain a higher proportion of protein. It was suggested that these particles contain newly synthesized mRNA on the way from the nucleus to the polyribosomes, and they were designated as "informosomes." The literature on informosomes has been reviewed by Spirin (114).

Particles with similar properties have been found in the cytoplasm of L cells by Perry and Kelley (115) and HeLa cells by Spohr et al. (116). These authors were careful to minimize contamination of their cytoplasmic extracts by high specific activity RNP from broken nuclei. Furthermore, mRNA released from polyribosomes by treatment with EDTA, puromycin, temperature shock, or amino acid starvation is in all cases found in particles with properties similar to those of informosomes (115, 117–120). This is true also for mRNA released from polyribosomes during mitosis, and for the free viral mRNA of infected cells (121–123).

Although free ribonucleoprotein particles containing mRNA are of very widespread occurrence, their biological significance has been questioned by Baltimore and Huang (124). These authors showed that RNA exposed to cytoplasmic extracts tends to associate with protein and form particles with the sedimentation properties and buoyant density of informosomes or mRNP. However, these artificial complexes are not formed at salt concentrations of 0.15 M, whereas mRNP released from polyribosomes is stable at even higher salt concentrations (0.25–0.5 M) (118). Also, mRNP released from L-cell polyribosomes by EDTA is stable upon centrifugation through Cs_2SO_4 density gradients without prior fixation, although ribosomal subunits are unstable under these conditions (unpublished results of the author).

Thus far, either proving or disproving the biological significance of mRNP and informosomes has been a very intractable problem, since no function has been found for the protein moiety of the particles. Some experiments have suggested a role for the proteins, but they are not conclusive. For example, Weeks and Marcus (125) isolated a "messenger fraction" analogous to mRNP of animal cells from ungerminated wheat embryos. They found that this fraction promotes polyribosome formation and initiation of protein synthesis in a wheat embryo cell-free system. Treatment of this fraction with pronase or *N*-ethylmaleimide caused it to lose activity, and furthermore, RNA extracted from this fraction was inactive in promoting protein synthesis. Unfortunately, investigators working with animal cells have not been able to obtain similar results. Several laboratories have used mRNP containing globin mRNA to direct synthesis of globin in cell-free systems. This mRNP was obtained either by EDTA release from reticulocyte polyribosomes, or from the postribosomal supernate of reticulocytes (125–128). In

either case the mRNP was found to direct globin synthesis with about the same efficiency and fidelity as deproteinized globin mRNA. Nudel et al. have shown that EDTA-released globin mRNP does *not* contain initiation factors (126).

Analysis of the proteins of mRNP by means of SDS-polyacrylamide gel electrophoresis has given highly interesting results. The proteins of globin mRNP released by treatment with puromycin and high salt or EDTA have been characterized in this way and found to consist of two to three major bands which are distinct from ribosomal proteins in that they have a higher molecular weight (50,000–130,000 daltons) (21, 129, 130). The uniqueness of this pattern suggests that mRNP is a real entity rather than an artefact. It has furthermore been shown that some of these proteins are phosphorylated and that their dissociation from the RNA is resistant to high concentrations of salt (130).

The presence of high molecular weight proteins associated with mRNA now appears to be a general characteristic of eukaryotic cells. Blobel has shown that mRNP released from polyribosomes of reticulocytes, L cells, and hepatocytes by treatment with puromycin and high salt contains two proteins with molecular weights of 78,000 and 52,000 daltons (21). Bryan and Hayashi have obtained similar results with chick embryo cerebral mRNP (131). It seems likely that other proteins are present as well. Lindberg and Sundquist (132) have devised an ingenious method for the isolation of mRNP. Polyribosomes from KB cells are treated with EDTA and passed through an oligo dT cellulose column. Poly A-containing mRNP, like poly A(+)mRNA, binds to the oligo dT cellulose, whereas ribosomal subunits pass through. The mRNP fraction can be eluted with formamide and contains two nonribosomal proteins in addition to the two found by Blobel (21), and by Bryan and Hayashi (131). Pederson and Kumar (133) have obtained similar results with oligo dT-isolated HeLa cell mRNP. Interestingly, mRNP from adenovirus-infected cells contains one additional protein not found in uninfected cells (132).

A remarkable result of all the investigations is that cells which have a large diversity of mRNAs, such as L cells, HeLa cells, KB cells, hepatocytes, and brain cells, have a rather small number of major proteins (on the basis of size) associated with the mRNP—no more than five. This result suggests that the same proteins are bound to many different kinds of mRNA. It does not exclude the possibility that there exist a larger number of unique messenger proteins, but these would necessarily be much less abundant than proteins common to many mRNAs, and therefore, much more difficult to detect. One example of a protein common to many different kinds of mRNA has been clearly demonstrated. This is a protein which binds to poly A. It has been shown by Brawerman and Kwan (134) and Blobel (21) that when polyribosomes or mRNP are digested with RNase the poly A is released in the form of mRNP. The protein of this mRNP has been shown by Blobel (21) to consist of a single species with a molecular weight of 78,000 as estimated by SDS-polyacrylamide gel electrophoresis. This finding has great significance, for it is the first instance in which a protein has been shown to be associated with a particular region of an mRNA molecule. The specificity of this association is the strongest evidence obtained thus far for the biological reality of mRNP.

SUMMARY AND CONCLUSIONS

In this review evidence has been considered which permits several conclusions about eukaryotic mRNA. (*a*) mRNA sequences are found in very large HnRNA molecules. However, the processing of these sequences into mRNA has not been directly demonstrated. (*b*) Most mRNA except histone mRNA contains poly A which is located at the 3′-terminus. The poly A is added posttranscriptionally by a terminal addition enzyme. In sea urchin embryos poly A addition takes place in the cytoplasm. The cellular site of poly A addition in other eukaryotes is still not entirely clear; there is still considerable controversy about this matter. The poly A grows shorter as the mRNA ages, but it is not known whether this loss of poly A goes to completion. The function of poly A is not known. Since there is probably considerable intranuclear turnover of poly A, its presence is not sufficient for the processing of HnRNA into mRNA. Poly A could have a role in translation although its presence is not necessary for translation. (*c*) Poly A(+)mRNA turns over with first order kinetics and is quite stable, much of it persisting for more than one generation in growing cells. Histone mRNA is also quite stable, but probably turns over with zero order kinetics. (*d*) Most mRNA other than histone mRNA is transcribed from nonrepeated DNA sequences. However, a fraction

of it, perhaps as much as 20% in mammalian cells, is transcribed from highly repeated DNA sequences. These repeated sequence transcripts may represent untranslated segments of mRNA molecules located near the termini. (*e*) mRNA released from polyribosomes or isolated from postribosomal supernates is associated with proteins. The number of proteins associated with mRNP released by EDTA or puromycin and high salt is rather small (2–5). The function of these proteins is unknown, and whether the association has any biological significance is still open to question except in the case of one protein which is specifically associated with poly A.

In examining these conclusions it is apparent that mRNA has attributes which are completely unexplained, namely, the presence of poly A and transcripts of repeated DNA sequences, both of which are presumably untranslated. In addition, it seems to be associated with mRNA-specific proteins. Furthermore, it is apparent that mRNA is involved in processes which are not understood. These include the conversion of nuclear precursors to functional cytoplasmic mRNA and the regulation of protein synthesis at the translational level. It seems likely that the information for these processes resides in the untranslated sequences of mRNA and HnRNA. Although nothing is known about the mechanism of these processes it seems reasonable that the interaction of proteins with the untranslated sequences could be involved. The relatively small number of major proteins associated with mRNA in cells which have a large diversity of mRNAs implies that there are proteins common to may different kinds of mRNA. It follows that these proteins are associated with nucleotide sequences common to many different kinds of mRNA, namely poly A and transcripts of repeated DNA sequences. Another possibility is that proteins are associated with regions of similar secondary structure common to many different kinds of mRNA, although the primary structure of these regions could be quite different. The existence of a protein specifically associated with poly A has been demonstrated, and it seems likely that other examples of the interaction of specific proteins with specific regions of mRNA molecules will be found.

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Note Added in Proof: Recently, Diez and Brawerman (1) have demonstrated cytoplasmic elongation of poly A chains in actinomycin-treated mammalian cells. Other new results support the idea that poly A has a role in determining mRNA stability. It has been found by Huez et al. (2) that deadenylated globin mRNA is initially translated in a mammalian cell-free system or in *Xenopus* oocytes with about the same efficiency as normal globin mRNA; however, with prolonged translation, the extent of synthesis of globin directed by deadenylated mRNA relative to the extent of synthesis directed by normal mRNA becomes progressively smaller.

Finally, workers in several laboratories (3–6) have obtained evidence that both the variable and constant regions of immunoglobulin kappa chain mRNA are transcribed from nonrepeated DNA sequences. These results favor the somatic theory for the origin of antibody diversity and also strongly suggest that the repeated DNA sequence transcripts present in immunoglobulin mRNA correspond to untranslated segments located at the 5'-terminus.

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