# Biogenesis of mRNA: Genetic Regulation in Mammalian Cells

In mammalian cells, unlike bacteria, messenger RNA arises from modified nuclear RNA after transcription.

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The experimental description of the mechanics as well as the regulation of bacterial protein synthesis appears to have entered a final stage. Virtually every step involved in the assembly of amino acids into protein—chain initiation, elongation, termination—can be performed in the test tube with components purified from bacterial cells (*1*). The steps in bacterial messenger RNA (mRNA) biogenesis are almost as well understood. The functional RNA polymerase and several regulatory proteins have been isolated and shown to participate in the control of synthesis of specific mRNA, both under conditions where only RNA is produced and in coupled systems where all the steps occur between the initiation of mRNA production and completion of functional enzyme protein (*1*). These awesome biochemical achievements with bacterial preparations probably would not have been possible, certainly not at this time, if a decade of successful bacterial genetics had not provided evidence concerning the mode of regulation that exists—namely, control of transcription of mRNA by regulatory proteins.

Presented with the challenge of understanding protein synthesis in mammalian cells in detail comparable to the present understanding in bacteria, the cell biologist might, after a first appraisal, despair. The genetic methods for isolating and characterizing mutants which might affect the availability of mRNA do not exist for mammalian cells and, if developed, will certainly present greater practical difficulties in their characterization and experimental use compared to bacteria. Even in yeasts, perhaps the most easily manipulated genetic system among eukaryotic cells, regulatory mutants known to affect the availability of mRNA have not yet been reported.

The purpose of this article is to claim reason for optimism in the attempts to understand regulation of gene expression in cultured eukaryotic cells through biochemical studies in spite of the lack of genetic details. An apparent first order understanding of gene expression in cultured eukaryotic cells seems possible through biochemical studies with cultured cells without the necessity for regulatory mutants.

As will be discussed, such studies have already contributed to our knowledge about mRNA biogenesis. When experiments concerning mRNA production can be coupled with experiments concerning the changing rate of specific protein synthesis in cells which respond to a known extracellular influence (such as hormones) at least some elementary information about modes of regulation should result.

## The Biogenesis of mRNA in Mammalian Cells

It now appears that mRNA in mammalian cells (*2–4*), like the predominant types of RNA in these cells [that is, ribosomal RNA (rRNA) and transfer RNA (tRNA)], is derived by post-transcriptional modification of larger RNA precursor molecules (*5*). The mRNA precursors are part of a class of high-molecular-weight nuclear RNA molecules (between 5000 and 50,000 nucleotides long) whose base composition resembles that of DNA [(U substituted for T (*6*)] (*7–10*). Most of the labeled RNA that can be isolated from cells after brief exposures to radioactive RNA precursors is contained in this high-molecular-weight nuclear RNA, yet this material does not accumulate in large amounts, that is, it "turns over" rapidly. We have used a descriptive term for this class of RNA, heterogeneous nuclear RNA, abbreviated HnRNA, rather than call it mRNA precursor because, as will be described, only a small portion of it becomes mRNA.

The question was raised some time ago whether mRNA might be derived from HnRNA (*7, 11*) in a manner similar to that in which rRNA and tRNA are derived from precursor molecules (*5, 7, 12, 13*). Two properties of rRNA and precursor rRNA were crucial to the proof of their relation to each other. (i) The 18*S* and 28*S* rRNA constitutes most of the cellular RNA and both rRNA and the 45*S* precursor rRNA are individual molecular species, so that it was relatively easy to isolate these RNA molecules in pure form (*7, 10, 13, 14*). (ii) It was found that rRNA and its precursor, in addition to being similar in base composition (*7, 10, 14*), share chemical markers, for example, methyl groups (*12, 15*), which have been utilized to prove detailed sequence similarities (*2, 12, 14*).

Even if HnRNA were a precursor to mRNA, both classes of RNA would be expected to be composed of many chemically different molecules, increasing the difficulty of testing the potential precursor-product relationship. However, two recent findings concerning sequence similarities between HnRNA and mRNA have provided evidence that mRNA is derived from HnRNA.

First, it was shown that cells transformed by the small DNA tumor virus SV40 contain the virus DNA as part of the cell genome (*16*). In such transformed cells, HnRNA molecules considerably larger than virus-specific polysomal mRNA were found to contain regions complementary to virus DNA (*2*). Whereas the HnRNA molecules containing virus-specific sequences were heterogeneous in size and contained host cell sequences (*17*), the virus-specific mRNA was discrete in size and lacked host cell sequences (*17*). Evidence has also been obtained in cells lytically infected with DNA viruses that nuclear virus-specific sequences exist which are much larger than cytoplasmic sequences (*2*). Thus, it appears that a processing step occurs which

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cleaves the HnRNA to yield discrete mRNA fragments containing only a portion of an HnRNA molecule.

The second sequence that has been shown to be shared by HnRNA and mRNA was discovered many years ago (*18–21*), but was not recognized to be part of HnRNA and mRNA molecules until recently. This sequence is about 200 nucleotides long and is unusual because it contains only adenylic acid residues (see below); this polyadenylic acid region is termed poly(A). That such a special sequence is present in both HnRNA and mRNA (but not in other cellular RNA molecules—for example, rRNA or tRNA) fortifies the idea that mRNA is derived from HnRNA.

## Presence of Poly(A) in Both HnRNA and mRNA

Kates (*22*) refocused attention on poly(A) when he found that vaccinia mRNA contained at its 3′OH end a relatively uniform, ribonuclease resistant segment that consisted largely of adenylic acid residues. This finding recalled earlier reports (*18–21*) of ribonuclease resistant adenylate-rich fragments in thymus and liver cells. More recent work has established that both HnRNA and mRNA contain a fairly discrete poly(A) segment as part of the polynucleotide chain (that is, not dissociable by treatment of the RNA molecules with dimethylsulfoxide) (*23–26*). When cells are briefly exposed to labeled adenosine, the labeled poly(A) obtained from HnRNA and mRNA is the same size as determined by migration during gel electrophoresis (*27*) (Fig. 1).

## 3′OH Location of Poly(A)

Evidence from several types of experiments indicate that the poly(A) of both mRNA and HnRNA molecules exists only at the 3′OH end of these molecules. Poly(A) obtained after ribonuclease digestion from either mRNA or HnRNA contains 1 adenosine residue per 200 adenylic acid residues, indicating that poly(A) is 200 nucleotides long and is located at the 3′OH terminus (*28*). Digestion of mRNA and HnRNA with an exonuclease that requires a free 3′OH quickly removes the poly(A) from both types of molecules, giving further support to a 3′OH localization (*29, 30*). These findings agree with recent demonstrations that a considerable fraction of the 3′OH adenosine termini of poly(A) becomes labeled when whole HnRNA and mRNA molecules containing poly(A) are oxidized with periodate and are reduced with [$^3$H]borohydride (*31*).

Further, recent experiments with poly(A) derived by T1 or pancreatic ribonuclease (or both) give conclusive evidence for the location of the poly(A) at the 3′ terminus of both HnRNA and mRNA as well as information about the composition of the nucleotides adjacent to poly(A) from the two sources (*32*). T1 ribonuclease cleaves RNA on the 3′ side of guanylic acid (that is, 5′---GpXp---3′) (*6*) and pancreatic ribonuclease on the 3′ side of pyrimidines (5′---PypXp---3′). T1 ribonuclease derived poly(A) segments contained no guanylic acid; pancreatic ribonuclease derived poly(A) segments contained no pyrimidines, indicating that poly(A) consists entirely of adenylic acid and that poly(A) is present only at the 3′ terminus of mRNA and HnRNA. Poly(A) segments derived by T1 ribonuclease showed that the structure of the 3′ end of poly(A)-terminated molecules (both HnRNA and mRNA) was G($C_2$U) $A_{200}$. This finding is consistent with the idea that mRNA is derived from HnRNA. The pyrimidine nucleotides adjacent to poly(A) may be a defined sequence or a limited set of sequences in both HnRNA and mRNA molecules, a question that can be answered by sequence analysis of the 5′ end of T1 derived poly(A).

## How Is Poly(A) Synthesized?

Poly(A) was discovered during the study of an enzyme isolated from thymus nuclei that specifically incorporates adenylic acid into a polyribonucleotide primer without a DNA template (*18*). This is in contrast to DNA directed RNA polymerases (bacterial or mammalian), which require a DNA template and which can initiate RNA chains with a 5′ terminal nucleotide and propagate them toward the 3′OH terminus (*5*). Thus, if poly(A) is localized at the 3′OH terminus of HnRNA and mRNA molecules, it might be synthesized by a non-DNA dependent enzyme (such as the Edmonds-Abrams enzyme) (*18*). Three findings suggest that such a posttranscriptional addition of poly(A) is likely. (i) In cultured cells, actinomycin D, which prevents DNA-dependent RNA synthesis by more than 80 to 90 percent within 1 to 2 minutes after its addition to a cell culture, has almost no effect on poly(A) synthesis during the ensuing 1 to 2 minutes (Fig. 2) (*3, 4*). Thus, it seems that the normal movement (some 5 to 10,000 nucleotides in 1 to 2 minutes) (*5*) of the RNA polymerase along the DNA template is not required for continued poly(A) synthesis. (ii) The DNA of adenovirus type 2, from which large, nuclear, virus-specific RNA sequences are transcribed during virus replication, contains no region to which poly(A)

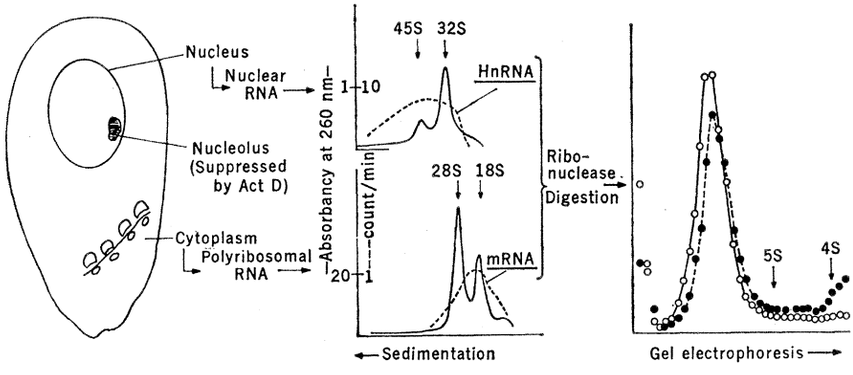


Fig. 1. Poly(A) from HnRNA and mRNA of HeLa cells. The left and center portions show the isolation by sucrose gradient sedimentation of labeled HnRNA from the nucleus and mRNA from the polyribosomes of HeLa cells. The majority species of RNA, 45*S* and 32*S* ribosomal precursor RNA in the nucleus, and 28*S* and 18*S* rRNA in the cytoplasm serve as absorbancy markers in the course of fractionation (*9, 12*). The right portion of the graph demonstrates that ribonuclease digests of HnRNA or mRNA labeled with adenosine for very brief times (20 minutes or less) contain poly(A) that migrates identically during gel electrophoresis [-●-, nuclear poly(A); -○-, cytoplasmic poly(A); see (*27*)].

will hybridize; that is, it contains no long stretches of poly dT (*33*). Nevertheless, virus-specific nuclear RNA, as well as the smaller virus-specific mRNA molecules in the polyribosomes, contain poly(A) of the same size as the cellular poly(A). (iii) Deoxypyrimidine nucleotide stretches can be isolated from DNA because they are resistant to acid hydrolysis (*34*). The DNA of CELO virus, an avian adenovirus (*35*), as well as HeLa cell DNA (*36*) do not contain pyrimidine-rich regions large enough to be transcribed into the 200 nucleotide segments of poly(A). It appears, therefore, that poly(A) is probably added to HnRNA molecules by a DNA-independent process after transcription.

Because of the rapid rate of RNA chain synthesis, it is difficult to distinguish experimentally the stepwise addition of individual nucleotides from the union of two polynucleotides. Nevertheless, because after very short label times poly(A) is found entirely associated with HnRNA, and not as a separate entity, it is suggested that poly(A) is synthesized by the stepwise addition of single adenylate residues to preexisting HnRNA molecules (*4*).

### Poly(A) Is Added in the Nucleus and Has a Nuclear Role

When HeLa cells are labeled with [$^3$H]adenosine for 1.5 minutes or less almost all (more than 95 percent) of the poly(A) is in the nucleus as part of HnRNA molecules (*4*). This result implies that the nucleus is by far the most active, if not the only, site of synthesis of the 200-nucleotide poly(A) segment. After 20 minutes of labeling there are equal amounts of labeled poly(A) in the nucleus and cytoplasm. In each succeeding 20-minute interval, the radioactivity in cytoplasmic poly(A) increases by an amount equal to the total labeled nuclear poly(A), while the nuclear poly(A) increased only about 25 percent. This leads to a fourfold greater amount of labeled cytoplasmic poly(A) compared to that in the nucleus by 120 to 160 minutes of labeling. The greater accumulation of labeled poly(A) in the cytoplasm was obtained both with growing cells and with cells in which ribosomal RNA synthesis had been stopped by a low dose of actinomycin D. In contrast, the total radioactivity in HnRNA remains much greater than that in mRNA for many hours, a circumstance that originally led to the conclusion that most of the HnRNA turned over (*10, 37*), (see Fig. 1). Therefore, during the processing of mRNA from HnRNA, the conservation of poly(A) in mRNA is far greater than the conservation of the HnRNA molecule. These results are consistent with the transfer of most of the poly(A) to the cytoplasm, but the possibility of some poly(A) turnover in the nucleus cannot be excluded.

The exploration of the physiological role of poly(A) in the nuclear biosynthesis and transport to the cytoplasm of mRNA has been facilitated by use of the drug cordycepin, which is 3′deoxyadenosine (3′dA). Penman, Rosbash, and Penman (*38*) found that in HeLa cells, 3′dA, which acts to terminate RNA chains prematurely (*39*) stopped the synthesis of some RNA molecules but not of others. For example, in cells treated with 3′dA, rRNA synthesis was quickly and completely halted, but incorporation of labeled precursor into HnRNA was not affected; mRNA, however, failed to appear in cytoplasmic polyribosomes. An explanation for these results and a suggestion of the importance of poly(A) in mRNA biogenesis came from finding that the synthesis of the 200-nucleotide poly(A) segment of HnRNA was stopped by the drug (*3, 4, 28*). In agreement with earlier results, labeled mRNA appearance was reduced in the presence of 3′dA by about 80 percent (Fig. 3A) and virtually none of the 200-nucleotide poly(A) segment became labeled in mRNA (*40*).

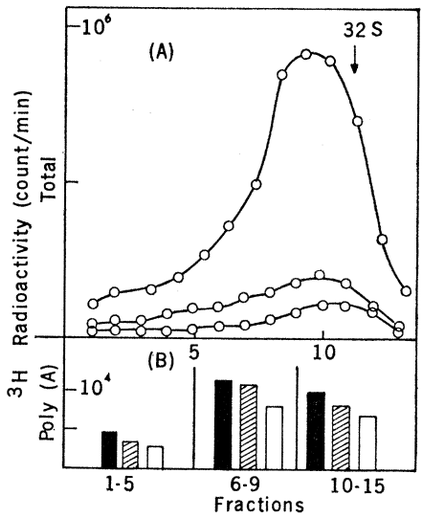


Fig. 2. Effect of actinomycin on total HnRNA synthesis compared to poly(A) synthesis. (A) Growing cells, and cells previously treated with a high dose of actinomycin D (7.5 μg/ml) for 1 or 2 minutes, were exposed to [$^3$H]adenosine for 1.5 minutes, and labeled nuclear RNA was separated on a sucrose gradient. The direction of sedimentation is from right to left. The effect on total incorporation (-○-) is shown in the top graph (top curve, no actinomycin; middle curve, 1-minute treatment with actinomycin; bottom curve, 2-minute treatment with actinomycin). (B) Molecules of various sizes (fractions 1 to 5, 6 to 9, and 10 to 13) were collected from each of the three RNA preparations. The poly(A) content of each size class from all three preparations was then determined. Solid bar, control; hatched bar, 1-minute treatment with actinomycin; open bar, 2-minute treatment with actinomycin. [The details of such experiments are described in (*3, 4*).]

An additional experiment indicated that 3′dA affected mRNA biogenesis at a step after transcription. If cells were briefly labeled with [$^3$H]uridine and then treated with actinomycin to stop further transcription, a measurable amount of previously synthesized RNA (presumably HnRNA) subsequently appeared in the polyribosomes. The cytoplasmic appearance of this "preformed mRNA" was blocked by the simultaneous addition of actinomycin and 3′dA (Fig. 3B) (*40*). Thus the posttranscriptional addition of poly(A) must be allowed to proceed or some step in the derivation of mRNA from HnRNA does not occur. These results suggest a nuclear role for poly(A) either in the proper cleavage of HnRNA or the transport of mRNA (or both) to the cytoplasm.

The fact that viruses which replicate in the cytoplasm also contain poly(A) does not necessarily conflict with the prediction of a nuclear role of poly(A). The mRNA molecules of such viruses may encounter a similar problem to that of cell mRNA in getting from the site of manufacture to the site of translation. For example, the replication of poliovirus RNA (which contains a 3′ terminal segment of poly(A) about 90 nucleotides long) (*41*) occurs on smooth membranes, whereas virus protein synthesis occurs on "rough" membranes, that is, those membranes bearing ribosomes (*42*).

While the precise role or roles of the poly(A) remains unknown, it is possible that it has both a nuclear and a cytoplasmic function. For example, recent experiments have shown that after arrival in the cytoplasm, the poly(A) segment gradually becomes shorter (*27, 28*). It has also been suggested that poly(A) may have specific pro-

teins bound to it while in the polyribosomes (*43*). Obviously further work with isolated components of protein synthesizing systems is needed to learn what, if any, cytoplasmic functions may be served by poly(A).

It might be asked whether the pathway of mRNA formation involving poly(A) synthesis is an abnormal aberration of cultured mammalian cells. This is certainly not the case. Edmonds and Abrams originally demonstrated the existence of adenylate-rich fragments in thymus nuclei (*18*), Hadjivasilou and Brawerman demonstrated its existence in rat liver (*19*), and Lim and Canellakis (*23*) reported such fragments in reticulocytes. Messenger RNA's responsible for the synthesis of hemoglobin (*44*), immunoglobulins (*45*), and ovalbumin (*46*) have been isolated from cells not grown in culture and shown to contain poly(A). These findings as well as the fact that 75 to 90 percent of the mRNA from polyribosomes of cultured cells contain poly(A) (*24, 25, 47, 48*) indicate that a major pathway of mRNA biogenesis in mammalian cells involves the addition of poly(A). Recently, invertebrates (*49*) and slime molds (*50*) have been shown to possess poly(A) both in nuclear and mRNA fractions; thus, all eukaryotic cells may employ a mechanism of mRNA biosynthesis that involves the addition of poly(A) to nuclear molecules.

One interesting exception to the finding that most all mRNA from eukaryotic cells may contain poly(A) is the group of mRNA's which direct histone synthesis (*47, 48*). These small mRNA molecules, which are formed only during the DNA synthesis phase of the cell cycle, exit more rapidly from the nucleus as compared to the majority of mRNA's and appear to lack poly(A) (*47, 51*). Whether histone mRNA is derived from a larger precursor molecule is not known. The lack of poly(A) in histone mRNA suggests that at least two mechanisms for the manufacture of mRNA exist, a nonpoly(A) and a poly(A) pathway, with the latter being much more common.

## Uncertainties about the HnRNA-mRNA Relationship

Many important points about the proposed HnRNA-mRNA conversion are uncertain and deserve mention here. Some time ago, kinetic analysis indicated that the majority of the nucleotides incorporated into HnRNA never reached the cytoplasm, and thus a large part of each HnRNA or all of some HnRNA molecules must turn over in the nucleus (*7, 8, 10, 37*). Every HnRNA molecule as isolated from cell nuclei does not contain a poly(A) sequence (*4, 48*), but it remains unknown which of the following explanations accounts for this finding. (i) Some or most HnRNA's are not precursors to mRNA and, therefore, "turn over" without ever containing poly(A); (ii) some HnRNA molecules are nascent—that is, not yet complete to the 3′OH end—but might eventually contain poly(A); and (iii) some HnRNA molecules are cleavage products from the 5′ portion of larger molecules and don't ever contain poly(A). If explanations (ii) or (iii), or both, are correct, every HnRNA might be transcribed for the purpose of yielding one mRNA. While it is possible to conclude that the 3′OH end of HnRNA molecules is the location at which poly(A) is added and from which mRNA is then derived, it is not certain that transcription actually ceases at the point where poly(A) is added. It is possible that an internal cleavage in the HnRNA exposes a poly(A) addition site. Some of these uncertainties will be discussed later in the section on possible models of regulation.

## Regulation of Gene Expression in Mammalian Cells

The preceding sections have summarized some of the details of mRNA manufacture in eukaryotic cells. From the number of steps involved between initiation of transcription and subsequent participation of mRNA in protein synthesis, it seems clear that many sites for the control of protein synthesis may exist. Unfortunately even almost complete knowledge of the steps in mRNA synthesis and transport will not guarantee understanding of the control of protein synthesis. Before going further into the discussion of regulation of gene expression, it is necessary to recognize two major levels at which control is possible: (i) the provision of mRNA to the protein synthesizing apparatus, and (ii) the extent of use of the mRNA molecules for protein synthesis.

Events within the cell which allow the accumulation of mRNA molecules will be called regulation: control of the production of mRNA during RNA polymerase action will be called *transcriptional regulation*; control of the number of mRNA molecules made available after the completion of transcription but before translation will be called *posttranscriptional regulation.* Changes in the output of protein directed by a given amount of mRNA will be called *translational modulation.*

An early and lucid description of these possible levels of control was provided by Scherrer and Marcaud (*52*) who used the term "cascade regulation" to embrace all these possibilities. They argued that, because mammalian cells and their genomes were so much more complex than bacteria, control at all levels should be expected.

Because of the inability to measure a specific mRNA, most attempts to gain information about fluctuating levels of mRNA in mammalian cells have been indirect. Various workers have studied enzyme activities that fluctuate after a cell is stimulated with a specific molecule, or have studied differentiating cells for the appearance of specific differentiated protein products. Studies of this sort have uncovered substantial evidence of translational modulation, but have not yet clarified the details of transcriptional or posttranscriptional regulation of mRNA formation.

One of the best cells for the study of protein synthesis regulation is a derivative of a liver hepatoma in which an increased synthesis of tyrosine aminotransferase (TAT) occurs after the cells are treated with corticosteroids (*53*). From work on this system, several conclusions can be made about control of enzyme synthesis which may be generally applicable to protein synthesis in mammalian cells.

1) Induction (increased rate of de novo enzyme synthesis) is accomplished by the accumulation of additional mRNA molecules that direct the increased synthesis of enzyme protein (*53*). Induction of ovalbumin synthesis by estrogen treatment and hemoglobin synthesis by erythropoietin also show evidence of increased mRNA molecules as the basis for increased specific protein synthesis (*46, 54, 55*).

2) The interruption of enzyme synthesis on cytoplasmic polyribosomes (deinduction) requires a positive action involving further synthetic events, or at least additional RNA synthesis and perhaps additional protein synthesis (*53*). Simply stopping the synthesis

of RNA does not result in a prompt cessation of protein synthesis as is the case in bacteria, undoubtedly because the half-life of mRNA in mammalian cells is longer than in bacteria (*11, 56*). These findings recall earlier experiments showing that the normal interruption of virus-specific synthesis of thymidine kinase in cells infected with vaccinia-virus required the independent synthesis of additional RNA and probably protein molecules (*57*).

3) Induction and deinduction of ongoing TAT synthesis are possible only during a portion (from midway in the G1 phase through the S phase) of the cell division cycle (*58*).

The complexities of the control of TAT synthesis, involving both a mechanism of mRNA accumulation and a mechanism for interruption of mRNA function, suggest caution in the interpretation of results in differentiating cells. It has been reported recently that erythroid precursor cells, which can be stimulated to enter hemoglobin production, contain no detectable hemoglobin mRNA before stimulation, while hemoglobin mRNA could be detected after stimulation (*54*). These results might indicate a control of transcription, but other means of control may also be involved, as suggested by analogy with TAT induction. For example, the unstimulated erythroid precursor cell might transcribe an HnRNA precursor to hemoglobin mRNA at only a brief period during the cell cycle. Such a precursor might escape detection since, without the stimulating agent, the precursor might be destroyed and not processed into hemoglobin mRNA. Thus mRNA would not accumulate, and regulation would have involved both a transcriptional and a posttranscriptional event.

## Models for mRNA Formation and Regulation in Mammalian Cells

The two areas of experimentation discussed so far—that is, the pathway of mRNA biogenesis and changing rate of synthesis of particular proteins in the cytoplasm have not yet been connected by experimental results. Nevertheless, since it is now possible to describe with some confidence the physical form of the transcription product from which mRNA arises and to measure some specific mRNA molecules (*46, 54, 59*), it is appropriate to reconsider the points at which regulation of formation of a specific mRNA might occur. Furthermore, in view of recent advances we can specify necessary, realizable experiments in order to choose between the possible modes of regulation. We should point out that the models we describe below are concerned with production of mRNA molecules and not with events involved in the modulation of protein synthesis.

In Fig. 4 we show two models in which mRNA production is regulated by transcription only and two models where mRNA production is regulated by both transcriptional and posttranscriptional events. In all of these models we assume that controlled initiation of transcription is necessary if proper chain synthesis is to proceed.

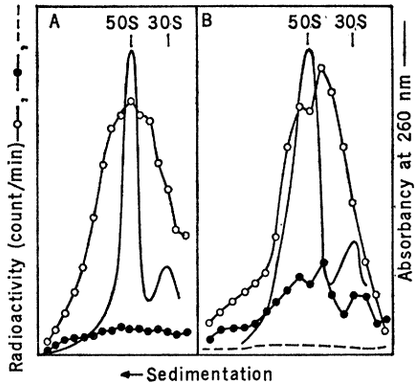


Fig. 3. Effect of 3′-deoxyadenosine on mRNA synthesis. (A) Cells in which rRNA formation was suppressed (Fig. 1) were labeled for 25 minutes with [$^3$H]uridine either after no further treatment (control, -●-) or after a 10-minute exposure to 3′-deoxyadenosine (-○-). Messenger RNA from polyribosomes (see Fig. 1) was assayed by sucrose gradient sedimentation as radioactive polyribosomal RNA released by ethylenediaminetetraacetate (EDTA) into material sedimenting from 20*S* to 60*S*. The profile of absorbancy at 260 nm comes from ribosomal subunits released from polyribosomes. (B) Growing HeLa cells were labeled for 5 minutes with [$^3$H]uridine, and one-third of the culture was removed onto frozen medium. Actinomycin D (7.5 μg/ml) was added to the remaining two-thirds, which was then equally divided; one portion received in addition to the actinomycin, 3′-deoxyadenosine (100 μg/ml). After 25 minutes at 370°C, the drug-treated samples were rapidly chilled. The polyribosomes from each sample were isolated and their content of labeled mRNA was assayed by EDTA release as in (A). ---, 5-minute labeling; -○-, 5-minute labeling followed by treatment with actinomycin for 25 minutes; -●-, 5-minute labeling plus treatment for 25 minutes with actinomycin plus 3′-deoxyadenosine [for details of experiments see (*3, 40*)].

Although it is theoretically possible that chain initiation is not controlled, this possibility seems a priori so unlikely that regulated initiation of transcription is included in all models. In those eukaryotic cells where transcription of HnRNA at particular chromosomal loci can be studied (the specific puffing patterns on insect chromosomes), transcription occurs only at certain times in the life cycle of the organism (*60*). Thus is seems reasonable to assume that regulation of the initiation of transcription plays a role in determining the portions of DNA to be transcribed.

Models in Fig. 4, A and B, depict two situations where transcriptional regulation of HnRNA synthesis is automatically followed by mRNA biogenesis involving poly(A) addition at the completed 3′ end of the HnRNA. Two cases are outlined. First, where only one mRNA per HnRNA exists, termination of RNA synthesis would be automatic when the RNA polymerase "reached the end" (designated t for termination). Biogenesis of mRNA would also follow automatically through poly(A) addition and cleavage (at site c). Each mRNA region or structural gene region is thus bounded by a cleavage and termination site.

The second possibility is that several mRNA regions per HnRNA might exist either scattered through the HnRNA or as a polycistronic region at the 3′ end (Fig. 4B). In such cases, both a proper termination signal would be needed at the 3′ end of any mRNA region and a regulated ability (a "read-through" signal) would be needed so that the RNA polymerase would only stop after the proper mRNA region had been synthesized. Again the 3′ terminal mRNA region would automatically be processed into a usable mRNA molecule. It is also conceivable that, if more than one mRNA existed in an HnRNA molecule, the processing of the 3′ terminal mRNA might expose a second mRNA which would then be processed.

In these models the regulation of the initiation of transcription would not necessarily be governed in a manner identical to that in bacteria. For example, it is known that genes which code for enzymes on the same biosynthetic pathway form one operon in bacteria, but are scattered throughout the genome in eukaryotes. This correlates with the finding of polycistronic bacterial mRNA's from entire operons,

while individual mRNA's are probably the rule in eukaryotic cells (*5, 61*). Also the induction of bacterial enzymes is possible throughout the cell cycle although this may not be true in eukaryotes. These and other potential differences in eukaryotic cells have provoked considerable speculation about the details of transcriptional regulation in eukaryotic cells (*62*).

Our present purpose, however, is not to concentrate on possible differences between prokaryotes and eukaryotes in the details of transcription, but to call attention to the fact that the point of regulation of mRNA production could be the same, that is, transcription. This is true even though the mechanical steps in the manufacture of mRNA in a mammalian cell are different from those in a bacterium.

The second class of models describing how cells generate mRNA involve posttranscriptional regulatory steps as well as regulated transcription. In these models (Fig. 4, C and D) the correct processing of any particular HnRNA molecules containing a potential mRNA would require a successful encounter with a posttranscriptional regulator. HnRNA molecules might contain only one possible mRNA (structural gene), in which case regulation could be accomplished by either destroying or not destroying a completed HnRNA (Fig. 4C). If an HnRNA contained more than one "structural gene" region, the regulation might involve an initial cleavage at the proper internal site (for example, at the site labeled "t") followed by poly(A) addition and cleavage at a second site "c."

In considering how posttranscriptional regulation of mRNA production might occur, one could suggest as possible regulatory events poly(A) addition, HnRNA cleavage, or transport of mRNA to the cytoplasm. Past that point, the fate of the mRNA falls into the category of translational modulation.

If posttranscriptional regulation does, in fact, occur, an important point needs specific emphasis. Posttranscriptional regulation necessarily implies transcriptional overproduction of potential mRNA. If potential mRNA's in excess of what eventually serve in the cytoplasm are not transcribed, then posttranscriptional regulation cannot occur. A corollary to this point is that even if excess transcription occurs but there is always a fixed probability of a potential mRNA getting to and functioning in the cytoplasm, then again posttranscriptional events might take place but posttranscriptional regulation in the sense we are using the term would not occur. In connection with this emphasis on the necessity for excess transcription, one proposed category of translation modulation requires special comment. In oogenesis it has been proposed that mRNA storage occurs in the egg cytoplasm, and only after fertilization is the mRNA utilized (*63*). It is true that such a series of events might seem to qualify as regulation of the availability of mRNA even though overproduction had not occurred but, in fact, if the egg becomes fertilized, the fate of the mRNA is to be used. Such a situation may simply be viewed as an exaggerated case of transcriptional modulation.

How would any of these models function in a situation where increased mRNA production occurred, for example, in TAT-mRNA induction? If the synthesis of TAT-mRNA were regulated entirely by transcription, the assumption would be that, during late G1 and the S phases, TAT-HnRNA would be normally formed at a low rate in uninduced cells, followed by the automatic processing of the HnRNA to yield the uninduced amount of TAT-mRNA and enzymes. Induction by steroid hormone would effect the proposed cytoplasmic stabilization of TAT-mRNA (*53*) and the promotion of an increased transcription rate. (This could involve either a small number of polymerases moving faster or more polymerase molecules synthesizing TAT-HnRNA.) Implicit in this scheme is the synthesis of TAT-HnRNA only during periods of the cell cycle when TAT-mRNA molecules can be accumulated to increased levels.

If, alternatively, the manufacture of additional TAT-mRNA molecules involved posttranscriptional regulation, then the major regulating event would be the preservation of more TAT-mRNA from the TAT-HnRNA during the time in the cell cycle when TAT-HnRNA production normally occurs. It is also possible that TAT-HnRNA might be synthesized at all times but

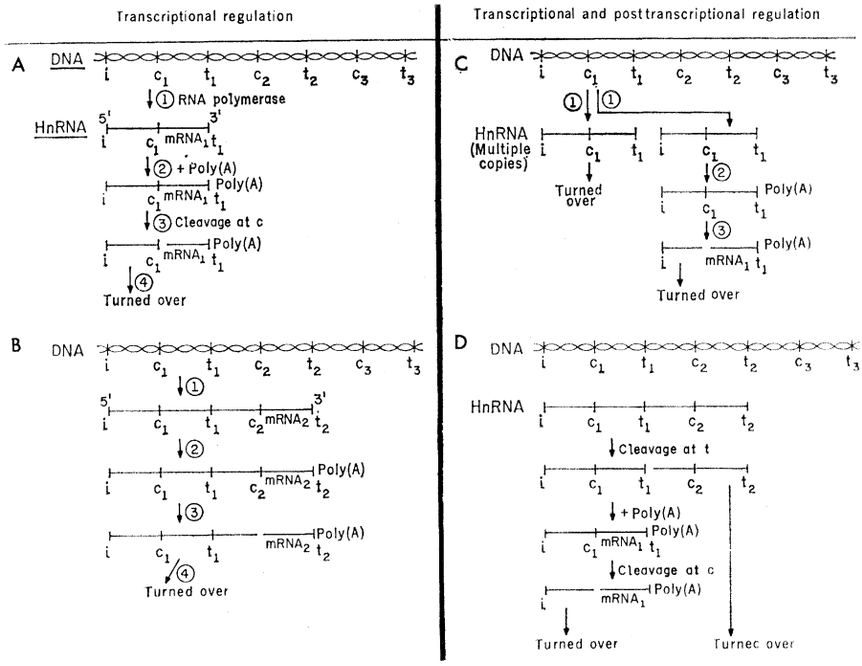


Fig. 4. Four models of regulation of mRNA formation in eukaryotic cells. The symbols are: i, initiation of transcription; c, cleavage points at 5′ end of mRNA; t, for termination at 3′ end of DNA-encoded region of mRNA; $c_1$, $t_1$, $c_2$, $t_2$, and so on indicate multiple sites on same molecule. The four steps in mRNA biosynthesis from HnRNA are: ① DNA dependent transcription by RNA polymerase; ② posttranscriptional addition of poly(A); ③ enzymatic cleavage at 5′ end of mRNA; ④ turnover of unused region(s) of HnRNA. (A) Transcriptional regulation at initiation site only. (B) Transcriptional regulation at initiation site and termination site ($t_1$ is passed by in favor of $t_2$). (C) Posttranscriptional regulation where one-half of the HnRNA molecules yield an mRNA and one-half are destroyed. (D) Posttranscriptional regulation where specific cleavage at $t_1$ reveals the 3′-terminus of mRNA, for processing and the $mRNA_2$ region is discarded.

might only be in a form where processing could occur during late G1 and S phase. Increased processing of TAT-HnRNA into TAT-mRNA during the G1-S phase would again be the regulatory device responsible for induction.

## Proposed Experiments and Conclusion

The models described raise experimental questions in two areas where possible answers may be at hand. The first has to do with the structure of the HnRNA. Do HnRNA molecules contain only one mRNA region and is the potential mRNA sequence always at the 3′ end? If so, then models involving correct RNA polymerase read through or correct internal cleavage (models in Fig. 4, B and D) become unlikely. More difficult but perhaps also answerable is whether, even though all mRNA sequences may be at the 3′ end of HnRNA, an HnRNA can contain several potential different mRNA's at the 3′ end. These questions are being or can be studied in two systems where specific mRNA and HnRNA can be recognized and isolated: (i) cells transformed by DNA viruses (*17*), and (ii) cells producing large amounts of two or more proteins the genes for which are linked [for example, beta and delta chains of human hemoglobin (*64*)].

The second area of experimentation which could help one choose among the models of Fig. 4 involves systems like TAT induction or stimulated erythropoiesis. Several experimental questions could be clearly phrased if the mRNA and the HnRNA for the protein in question could be accurately measured. (i) Is the RNA for an inducible mRNA synthesized during a phase in the cell cycle when induction is not possible (for example, hepatoma cells during the G2 or early G1 phase of the cell cycle)? (ii) When the number of mRNA molecules of a given type is being increased is there a larger amount of corresponding HnRNA?

Answers to these questions should go a long way toward ascertaining whether or not posttranscriptional regulation is just an often suggested possibility or a reality in eukaryotic cells. What seems clear at the moment is that the biochemical mechanisms of mRNA formation in eukaryotes differs radically from bacteria. It would surprise a great many people if the types of regulation didn't differ also. The challenge, however, is not to settle for this latter possibility as likely but to prove or disprove it.

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