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mRNA Stability in Mammalian Cells

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INTRODUCTION AND BACKGROUND

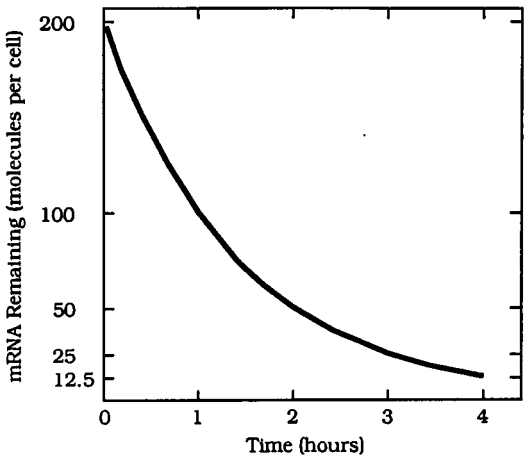
The primary goal of this review is to discuss how cytoplasmic mRNA half-lives are regulated and how mRNA decay rates influence gene expression. The topic is important, because mRNA stability influences gene expression in virtually all organisms, from bacteria to mammals. In mammalian cells, the abundance of a particular mRNA can fluctuate manyfold following a change in the mRNA half-life, without any change in transcription. The processes that regulate mRNA half-lives can, in turn, affect how a cell grows, differentiates, and responds to its environment. In the interests of clarity and brevity and because mRNA degradation in yeast cells is summarized elsewhere (264a), this review focuses primarily on mammalian cells. Other systems and organisms are discussed when relevant. Three major questions are addressed. Which sequences in mRNAs determine their half-lives? Which enzymes degrade mRNAs? Which (*trans*-acting) factors regulate mRNA stability, and how do they function?

How Are mRNA Decay Rates Determined?

Investigators who study mRNA stability will be familiar with the material in this section. It is included here for others as a brief review on how mRNA decay constants are determined and how the decay rate affects mRNA abundance.

The derivation of the mRNA decay constant (k_d) is based on the assumption that mRNA decay, like radioactive decay, is a stochastic process. Therefore, the change in mRNA concentration at any time point (dC/dt) is a first-order process, depending on the amount of mRNA (C) present at that time. The simplest way to derive the rate of change is to imagine an idealized situation in which transcription of a particular gene stops completely at time zero (Fig. 1). The subsequent reduction in mRNA abundance is then a direct indication of mRNA half-life, and $C/C_0 = e^{-k_d t}$, where C_0 is the mRNA concentration at time zero (Fig. 1). The unary minus indicates that the mRNA is being degraded. If the half-life of a mRNA is 1 h, as depicted in the figure, then $k_d = 0.693\ h^{-1}$ ($\ln 0.5 = -0.693$). If there are 200 molecules per cell at time zero, $dC/dt = -69.9$ and -34.6 molecules per h at 1 and 2 h, respectively.

There are three important practical and/or theoretical caveats about this deceptively straightforward analysis. (i) For situations of normal cell growth and in the absence of transcription inhibitors, it seems unlikely that gene transcription ceases very abruptly. (ii) In a population of cells, transcriptional repression is unlikely to occur with such high synchrony that mRNA depletion will begin simultaneously in all cells. (iii) It has never been satisfactorily shown that mRNA decay is a completely stochastic process. Many mRNAs are degraded by a multistep pathway in which the later steps are dependent on the earlier ones. For example, poly(A) sh ortening often precedes decay of the mRNA body (see the section on mRNA cleavage sites and mRNases, below). Therefore, k_d (measured by a molecular hybridization assay in which the amount of full-length [polyadenylated plus deadenylated] mRNA is quantitated at each time point) will depend on the poly(A) shortening rate and the age of the mRNA population at the time the analysis begins. It is difficult to assess the extent to which these sorts of variables affect mRNA half-life measurements in the laboratory, and they might be minimal, depending on the cell type and the mRNA of interest. However, there are circumstances in which such effects are probably not trivial and mRNA decay might not be stochastic. For example, the poly(A) tracts of globin mRNAs in young, newly formed reticulocytes that have just entered the circulation are longer than those in older reticulocytes (231, 232, 302). As a result, the measured k_ds for globin mRNAs might differ in the two reticulocyte populations. In summary, mRNA decay kinetics do not necessarily follow the idealized situation depicted in Fig. 1.



$$\frac{dC}{dt} \propto C$$

$$\frac{dC}{dt} = -k_d C$$

$$\frac{1}{C} dC = -k_d\, dt$$

$$\int \frac{1}{C} dC = \int -k_d\, dt$$

$$\ln C = -k_d t + A$$

$$C = e^{-k_d t + A}$$

$$C = (e^{-k_d t})(e^{A}) = (e^{-k_d t})(C_0)$$

$$\frac{C}{C_0} = e^{-k_d t}$$

$$\ln\left(\frac{C}{C_0}\right) = -k_d t$$

$$\ln 0.5 = -.693 = -k_d t_{1/2}$$

FIG. 1. Calculation of the mRNA decay constant, k_d. The graph is not semilogarithmic but, instead, has two linear axes, to emphasize that the rate of change of mRNA concentration (dC/dt) itself changes over time and is proportional to C ($dC/dt \propto C$). The calculations assume completely stochastic mRNA decay, as for radioactive decay.

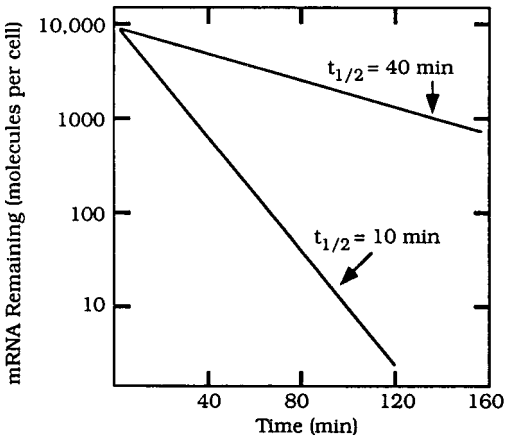


FIG. 2. Effect of a fourfold change in the mRNA decay constant on mRNA abundance. Note that, in contrast to Fig. 1, mRNA concentration (molecules per cell) is plotted on a logarithmic scale.

How Does k_d Affect mRNA Abundance and the Rate at Which mRNA Levels Change?

The stabilities of many short-lived ($t_{1/2}$ = 2 h or less) mRNAs change in response to environmental and other factors, but the new half-life might differ by only two- to fourfold from the original one (see the sections on *trans*-acting regulatory factors and on effects of hormones, growth factors, and ions on mRNA stability, below). Although a change of this magnitude seems modest, it can affect mRNA abundance by orders of magnitude over a short period. For example, the half-lives of histone mRNA during and at the end of S phase are approximately 40 and 10 min, respectively (9, 142, 145, 236). If a cell contains approximately 10^4 histone mRNA molecules during S phase and if histone gene transcription ceases abruptly at the end of S phase, an idealized graph of amount of histone mRNA per cell versus time illustrates the large change in abundance caused by the seemingly modest (fourfold) change in half-life (Fig. 2). Failure to destabilize the mRNA would cause it to persist through mitosis and into the next G_1 phase. If the mRNA were translated, excess histones would accumulate and damage or kill the cell (225). Therefore, two- to fourfold fluctuations in mRNA half-life can have significant effects on mRNA and protein abundance.

mRNA stability also affects the rate of change of mRNA abundance following an increase in transcription. (This topic has been reviewed at length [137–139], and a mathematical analysis is available in the pharmacological literature [123].) If genes X and Y are transcribed at the same rate but the half-lives of their mRNAs differ by 10-fold (where mRNA X is 10-fold more stable than mRNA Y), the ratio of X to Y at steady state will be 10:1 (Fig. 3). If the transcription rate of both genes suddenly increases 10-fold (arrow, Fig. 3), the steady-state level of each mRNA will eventually increase 10-fold and the ratio of X to Y will again be 10:1. However, the shorter-lived mRNA will reach half of its new steady state 10-fold faster than the longer-lived mRNA (139). This relationship holds for any system with zero-order input (in this case, transcription) and first-order output (decay). In summary, mRNA stability determines not only the rate of disappearance of a mRNA but also its rate of induction.

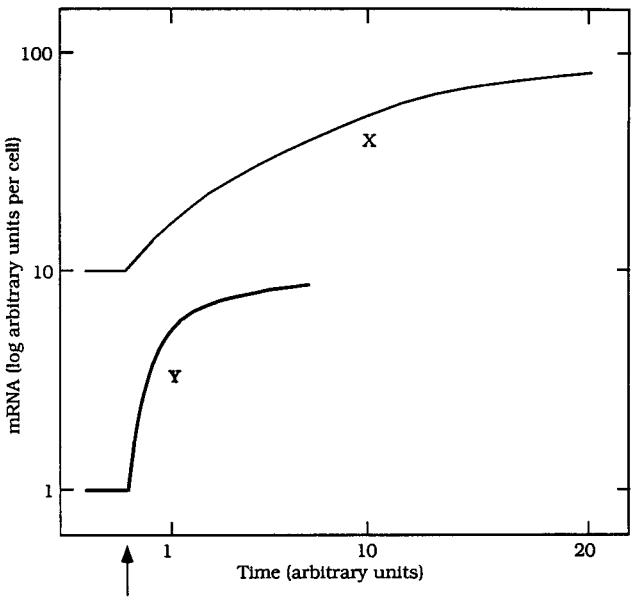


FIG. 3. Rate of change of two mRNAs (stable and unstable) following transcriptional up-regulation (137–139). mRNAs X and Y are transcribed at the same rate. mRNA X is more abundant, because it is 10-fold more stable. The arrow indicates the time at which transcription of both mRNAs increases 10-fold.

Is There a Correlation between mRNA and Protein Half-Lives?

It would make little sense to synthesize very stable proteins like globin from very unstable mRNAs. If it became necessary for whatever reason for the cell to repress globin gene transcription, the mRNA would disappear rapidly but the protein would persist and the biological effect of transcriptional repression would be nullified. It makes more sense to have unstable mRNAs encode unstable proteins. Unfortunately, relatively few cases are known in which both mRNA and protein half-lives have been carefully measured in the same cells under the same conditions. Some nonsecreted, unstable proteins are encoded by unstable mRNAs (139), but the relationship should be addressed further, because methods for measuring mRNA and protein stability are imperfect (see below) and are complicated by the facts that half-lives might change over time and that the chemical half-life of a mRNA might not reflect its functional half-life.

MEASURING mRNA HALF-LIFE

Some standard methods for half-life determination have been reviewed (270). Here, I summarize these methods and describe newer techniques in more detail. Most studies on mRNA stability have been performed with cultured cell lines, but I believe that it is also important to measure mRNA half-life in intact animals and will therefore discuss some whole-animal methods as well.

Transcription Inhibitors

Actinomycin D, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB), cordycepin, and α-amanitin are the most frequently used inhibitors (25, 68, 323, 332). Cells are simply cultured with inhibitor and harvested at different times thereafter. Although inhibitors provide an efficient and rapid way to block transcription, they have some limitations. α-Amanitin is a specific RNA polymerase II inhibitor (68) but does not enter

all cells. Actinomycin D and related inhibitors can, among other things, block translation and affect ATP pools (270), and half-lives measured with actinomycin D sometimes differ considerably from those obtained by other methods with less toxic compounds or transcriptional pulse-chase technology (242, 243, 280, 321, 328, 332, 333). A particularly relevant study compared the approach-to-steady-state method (see below) with actinomycin D and DRB to measure immunoglobulin heavy- and light-chain mRNA half-lives in mouse myeloma cells (142). The values ranged from 2.4 h in actinomycin to approximately 6 h in DRB.

Pulse-Labeling with Nucleosides and "Chasing" with Unlabeled Nucleosides

In the pulse-chase method, radioactive nucleosides are added to the cell culture medium for brief periods, during which they rapidly enter cells, are triphosphorylated, and are incorporated into RNA. The radioactive medium is then removed and replaced with fresh medium containing excess, unlabeled nucleosides to "chase" the intracellular radioactive nucleotide pool. The time-dependent decay of newly synthesized mRNA is then monitored by filter hybridization or other methods. Although the pulse-chase procedure is ideal because it avoids metabolic inhibitors, it is often difficult to label the mRNA of interest to a high specific activity, to deplete the intracellular nucleotide pool efficiently enough to block completely the synthesis of radiolabeled mRNA during the chase period, and to avoid reutilization of labeled nucleotides generated by RNA catabolism. As a result, the mRNA signal might be weak and continued mRNA synthesis complicates data analysis. In an effort to overcome these limitations, cells can be cultured with glucosamine, which traps uridine in the form of UDP-*N*-acetylhexosamine (200). The intracellular specific activity of radiolabeled UTP is thereby elevated, and the unlabeled-uridine chase is more efficient. While this refinement deals with the nucleotide pool problem, it does not overcome the low-sensitivity issue.

A variant of the pulse-chase method exploits the capacity of thiouridine-labeled RNA to bind to mercury (163). Cells are cultured for 1 h in medium containing thiouridine. Total-cell or cytoplasmic RNA is then isolated and fractionated on a mercury-agarose column. The bound (newly synthesized) RNA is eluted and hybridized to a probe for the mRNA of interest or analyzed by PCR, which provides a measure of the mRNA synthesized during the 1-h period (N). The total amount per cell of the mRNA (C), the amount in the bound plus the unbound fractions, is then determined by standard hybridization methods. From these measurements, a ratio of total mRNA per cell to amount synthesized during the labeling period is obtained. If the thiouridine was rapidly incorporated into RNA, N should be approximately equivalent to the amount degraded during the labeling period, and the mRNA half-life is calculated by applying the decay formula: $(C - N)/C = e^{-k_d t}$.

Approach to Steady State

The approach-to-steady-state method avoids some of the complications of the pulse-chase technique (132). Cells are labeled continuously with a radioactive nucleoside. The accumulation of radioactivity in the mRNA of interest is quantitated by molecular hybridization, and the time required for the mRNA to reach steady state (when its synthesis and decay rates are equal) is determined. The mRNA half-life is then calculated from standard stochastic decay equations. This method avoids the requirement for chasing the radioactive nucleoside but fails to overcome the specific activity problem encountered with low-abundance and very short-lived mRNAs.

Transcriptional Pulse-Chase or Short-Term Promoter Activation

Several techniques have been described to measure mRNA stability in mammalian and yeast (97) cells by using short-term activation of an inducible promoter. Prior to induction, the promoter is silent, or nearly so. Following induction, the promoter is activated briefly and then shuts down, and the mRNA half-life is determined simply by monitoring the subsequent rate of mRNA loss. In mammalian cells, the gene of interest is placed downstream of the serum-inducible c-*fos* promoter (166, 330). The gene is transfected into 3T3 fibroblasts, which are cultured for 24 to 36 h in low (0.5%) serum concentration, causing the cells to enter G_0 and repressing the c-*fos* promoter. The cells are then exposed to 10 to 15% serum, which induces the c-*fos* promoter for only 15 to 30 min, after which it shuts down (133, 184, 241). As a result, the mRNA of interest is synthesized for only a brief time, increases in abundance during that time, and then disappears at a rate dependent on its half-life. This system can also be used with permanent cell lines (151) and has two advantages over other methods. (i) mRNA synthesis is repressed without resorting to toxic chemicals but with a high degree of synchrony. (ii) Little or no reinitiation of c-*fos* promoter-driven genes is observed for at least 10 h, ensuring an efficient chase and offering the possibility of measuring the half-lives of relatively long-lived mRNAs (328). One potential disadvantage is that serum starvation/addition could affect mRNA half-lives.

For mRNAs like growth hormone or globin, whose half-lives are longer than one cell division time, the structural gene is linked to a *Drosophila* heat shock protein promoter (146). The construct is transfected into cells, and the promoter is transiently induced by a brief temperature shock. Cells are then returned to 37°C, and the mRNA level is quantitated for hours or days thereafter. Assuming that the promoter is efficiently silenced and is not induced during subsequent cell cycles, the mRNA half-life is calculated after accounting for dilution by cell division. One disadvantage is the potential effect of the heat step on mRNA stability.

mRNA Degradation In Vitro

mRNA decay rates have been measured in various ways in extracts from mammalian cells (reviewed in reference 295). The most common approach is to prepare crude cytosol, polysomes, or messenger ribonucleoprotein from nucleated cells (10–12, 19, 21, 182, 251, 297, 353, 366) or reticulocytes (126, 150, 384), incubate the extract under appropriate conditions, and monitor the decay of the endogenous (cell-derived) mRNA. Another approach is to incubate protein-free mRNA substrates with cell extracts or purified RNases, but there is some uncertainty whether reliable half-life measurements can be obtained consistently with protein-free substrates. In any event, no inhibitors are required to compare mRNA half-lives in vitro. Moreover, since each step in the mRNA degradation pathway occurs more slowly in vitro than in intact cells, mRNA decay intermediates that might be difficult to detect in cells (because of their extremely short life spans) can be readily detected in vitro. One disadvantage is the low or nonexistent translational capacity of some in vitro extracts, which precludes

investigating potential links between mRNA stability and the process of translation.

mRNA Decay in Animals

An appreciation of the significant role of mRNA stability in gene expression has come primarily from experiments with cultured cells. However, I believe that tissue culture models have unavoidable limitations and that animal studies will reveal new and important insights into how mRNA stability influences biological processes. The regulation of mRNA stability is likely to be an essential component in the pathways whereby tissues and organs respond to "stresses" like starvation, infection, inflammation, exposure to toxins, and tissue invasion by neoplastic cells. If so, it will be necessary to exploit reliable techniques for measuring mRNA stability in intact organisms.

One technique involves giving the animals, usually mice or rats, injections of an intravenous bolus of α-amanitin plus actinomycin D, which efficiently blocks transcription (180, 360). The tissue of interest, usually liver, is then harvested. This method will have to be validated for other tissues, because α-amanitin is not effective in all tissue culture cells and might not be effective in all tissues of the animal. Moreover, long-term toxicity might limit the effective measuring time. A second method has been used to compare the relative half-lives of two or more mRNAs. Each gene of interest is linked to a *H-2* histocompatibility gene promoter, which is expressed constitutively, and transgenic mouse strains, each expressing one transgenic mRNA, are constructed (235). The mRNAs are expressed in hepatocytes, and since each transgene should be transcribed at the same rate, any difference in steady-state mRNA level from one strain to another should reflect post-transcriptional processes.

Another approach exploits a tetracycline-repressible promoter and should be useful for both tissue culture cells and animals (128). Two constructs must be expressed within the same cell. The first encodes a chimeric transcription factor containing the repressor of the tetracycline resistance operon (from the *Escherichia coli* transposon Tn*10*) linked to the activating domain of VP16, a transcription factor from herpes simplex virus. The second includes core sequences from an immediate-early cytomegalovirus promoter plus seven tetracycline operators, downstream of which is placed the gene of interest. In tissue culture cells transfected with both constructs, synthesis of the mRNA of interest requires the chimeric transcription factor and is rapidly repressed by tetracycline, permitting the mRNA half-life to be measured under conditions in which a nontoxic inhibitor ensures efficient repression of only a single gene. The tetracycline-repressible system also functions in intact animals (118). Chloramphenicol acetyltransferase is produced in several tissues of transgenic animals expressing the transcription factor gene plus a chloramphenicol acetyltransferase gene driven by the tetracycline-cytomegalovirus promoter. If the animals are treated for 7 days with slow-release tetracycline pellets, chloramphenicol acetyltransferase activity decreases 20- to 60-fold compared with the activity in untreated animals. The lag time between implanting the pellets and observing a decline in protein or mRNA levels is not yet known. Since tetracycline rapidly represses tetracycline-responsive promoters in tissue culture cells (128) and has no apparent toxicity in animals at the required doses (118), this technique could become the method of choice, particularly as the production of transgenic mice becomes less expensive and more widespread.

SEQUENCE DETERMINANTS OF mRNA STABILITY

Introduction

Most investigators have exploited chimeric mRNAs to identify sequences affecting mRNA stability (reviewed in references 81, 270, 300, and 307). Genes with segments from stable and unstable mRNAs linked in such a way as to maintain the reading frame are transfected into cells, and the stability of the mRNA is assayed by any of the techniques described above. Although experiments of this sort have generated important information, they must be interpreted cautiously. Since many variables (primary and secondary structure, translation rate, intracellular location, etc.) influence mRNA stability, even minor changes in mRNA structure can affect stability to some extent, and some sequence changes might influence the half-life indirectly (369). For example, a truncated mRNA might be more stable than its wild-type counterpart, not because it has lost a nuclease cleavage site but because it is translated more efficiently. It is important to note that mRNAs can contain two or more well-separated stability determinants, each of which might specify a distinct decay pathway or a distinct response to some regulatory factor.

We have organized the discussion of determinants on the basis of their location (Fig. 4). In some cases, one signal specifying mRNA decay might encompass two or more of these regions. Such is the case for growth-associated protein (GAP-43) mRNA, which contains a stability determinant consisting of 115 nucleotides encoding the carboxy terminus of the protein plus 178 nucleotides from the 3′ untranslated region (3′-UT), the combination of which can form a large, thermodynamically stable, evolutionarily conserved duplex (254).

cis Determinants of mRNA Stability

Poly(A). It seems likely that poly(A) has multiple functions affecting nuclear processing of pre-mRNA, transport to the cytoplasm, translation, and cytoplasmic mRNA stability (reviewed in references 31, 273, 306, and 307). Two observations imply that poly(A) protects mRNAs from rapid degradation. (i) Deadenylation is the first step in the decay of many mRNAs (30, 46, 116, 201, 230, 275, 329, 330, 348, 354, 355, 378). If deadenylation is an obligate step, without which the mRNA body is not degraded, poly(A) must protect the mRNAs to some extent. (ii) A poly(A)-poly(A)-binding protein (PABP) complex at the mRNA 3′ terminus protects mRNAs from rapid destruction in vitro (30). Polyadenylated mRNA substrates are rapidly degraded when incubated in extracts depleted of PABP but are stabilized when the extracts are replenished with excess exogenous PABP. mRNAs lacking a 3′ poly(A) tract are unstable with or without added PABP (30, 298). These results do not imply that deadenylation automatically triggers mRNA degradation, because some deadenylated or oligoadenylated mRNAs are relatively stable in cells (62, 64, 121, 171, 183, 329). They do suggest a role for poly(A) in protecting mRNA from rapid or indiscriminate degradation. In yeast cells, the PABP-poly(A) complex seems to function as a signal for degradation catalyzed by a PABP-dependent nuclease (209, 309–312). The basis for the apparently discrepant results in mammalian extracts and yeast cells remains to be resolved. In any event, in mammalian cells the quantity of PABP is approximately threefold greater than necessary to bind up all of the poly(A) (125). Therefore, considering the high affinity of PABP for poly(A) (308), most or all of the poly(A) in a mammalian cell is probably complexed with PABP.

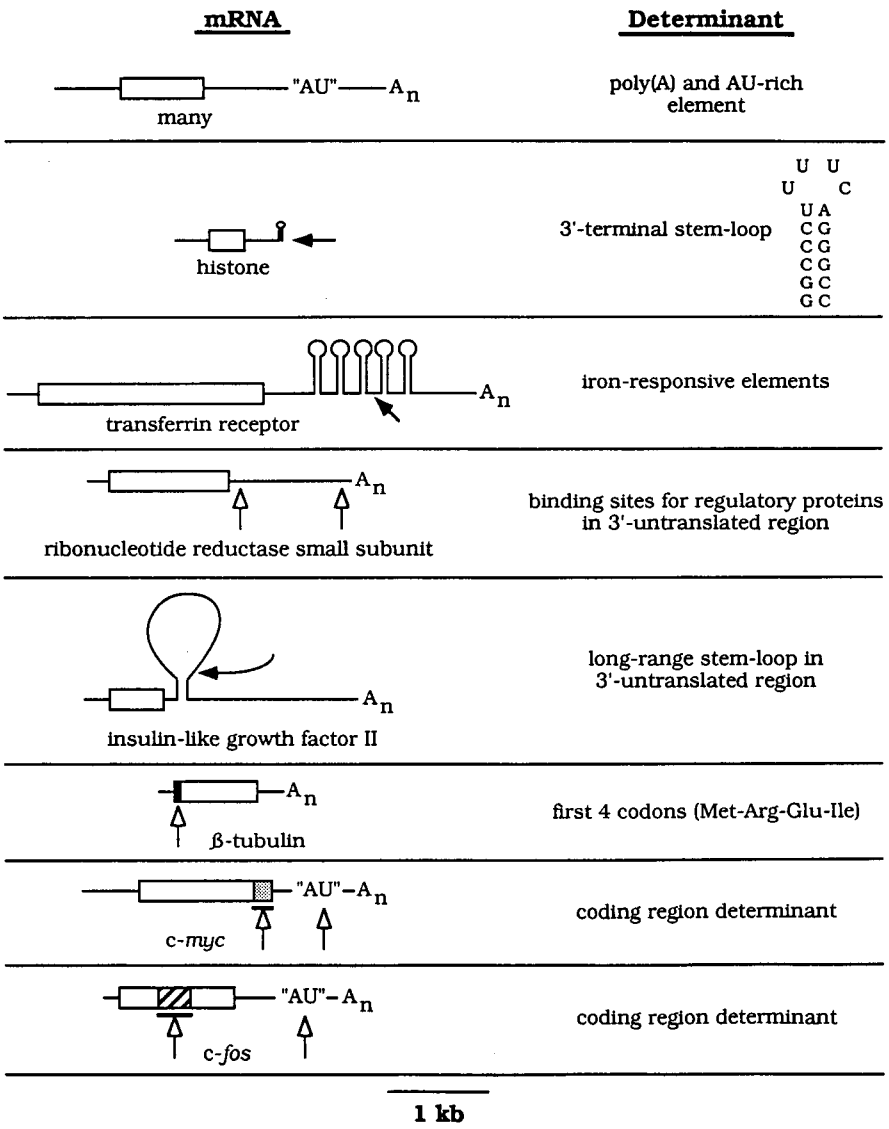


FIG. 4. Diagram of some mRNA stability determinants. Open arrows indicate the locations of the determinants. Solid arrows indicate determinants that are also cleavage sites in cells and, in some cases, in vitro. AU denotes an AU-rich element. Straight lines and boxes indicate untranslated and coding regions, respectively.

A related issue concerns how the translation-enhancing function of poly(A) is linked to its putative mRNA stability function. Poly(A) facilitates translation (reviewed in references 31, 273, 306, and 307; see also reference 268), and translation affects mRNA stability (see the section on mRNA stability and translation, below). Therefore, it will be important to determine the extent to which deadenylation influences mRNA stability by decreasing translation. Perhaps in vitro mRNA decay systems with the capacity to translate and degrade mRNAs will be useful for addressing this issue.

3′ untranslated region. The majority of papers dealing with mRNA stability determinants have identified mRNA decay signals in 3′-UTs, suggesting that the half-lives of most mRNAs are influenced by this region. Transfection experiments have revealed how the 3′-UT can function as an instability determinant independent of the remainder of the mRNA. A few well-characterized 3′-UT determinants, some of which are protein-binding sites, are discussed below.

(i) Histone mRNA 3′-terminal stem-loop. Cell cycle-regulated histone mRNAs lack poly(A), but their 3′-UTs affect the rates at which the RNA is processed in the nucleus, transported, translated, and degraded (reviewed in references 218 and 320). The mRNA is scarce (perhaps less than 200 molecules per cell) during G_1 phase but accumulates to approximately 10,000 to 40,000 molecules per cell during S phase. Histone genes are transcribed rapidly, and the mRNA half-life is 40 min throughout S phase. At the end of S phase, the transcription rate drops, the efficiency of pre-mRNA processing decreases, and the cytoplasmic mRNA half-life falls to 10 min, resulting in rapid disappearance of the mRNA from the cell (9, 24, 141, 145, 154, 212, 236, 238). A chimeric mRNA containing only the 3′-terminal 30 nucleotides of histone mRNA appended to globin mRNA is regulated posttranscriptionally as if it were wild-type histone mRNA (198, 213, 262, 341). Therefore, the 30 nucleotides contain the signal(s) essential for mRNA destabilization at the end of S phase. The critical structure seems to be a 3′-terminal 6-bp stem and 4-base loop, the so-called stem-loop motif present in all his-

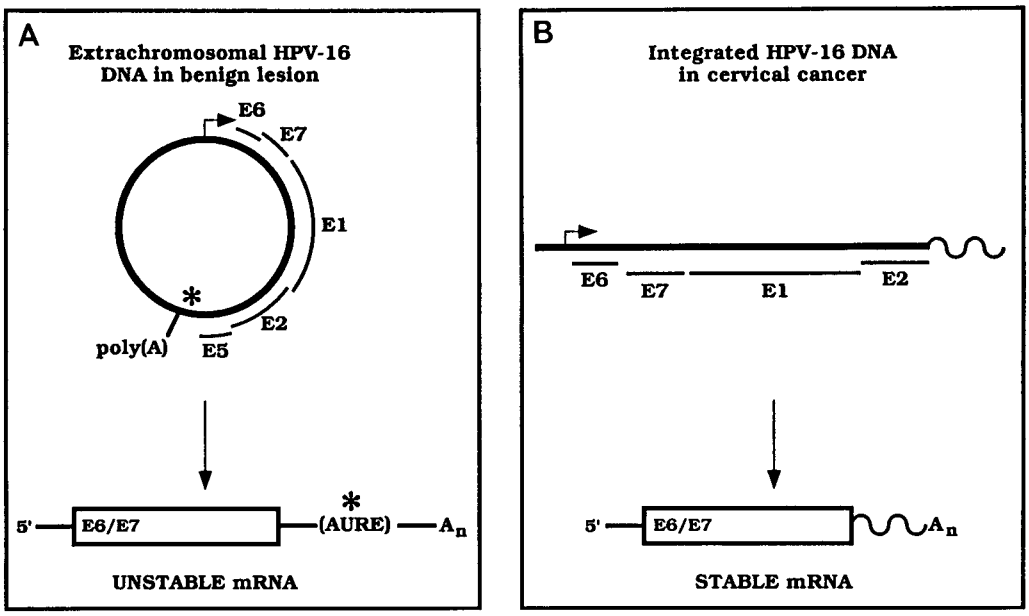


FIG. 5. Expression of human papillomavirus oncoproteins E6/E7 and mRNA stability (adapted from reference 162). (A) Unintegrated viral DNA in benign lesions generates unstable mRNA by virtue of the AURE in the mRNA 3′-UT. (B) DNA integration occurs in cervical cancer and creates a new transcription unit in which E6/E7-encoding mRNA contains a 3′-UT derived from cellular, not viral, sequences (wavy line). If the cellular sequences lack destabilizing signals or contain stabilizing signals, the resulting transcript is stabilized and more E6/E7 transforming proteins are synthesized.

tone mRNAs that are regulated as a function of the cell cycle (Fig. 4). Histone mRNAs that retain the stem-loop structure but are elongated and polyadenylated downstream fail to be regulated properly and are probably not destabilized at the end of S phase (198, 213, 262). Therefore, the stem-loop must be located at or very near the 3′ terminus to be effective as a regulatory signal. Histone mRNA is also improperly regulated if 500 or more nucleotides are inserted into its 3′-UT between the translation termination codon and the 3′ terminus, perhaps because the distance between the stem-loop and the last translating ribosome is critical (131). In summary, most or all of the signals required for histone mRNA processing and stability reside in the stem-loop and adjacent sequences. A stem-loop-binding protein and histone proteins themselves might be involved in the regulation process (see the section on *trans*-acting regulatory factors, below).

(ii) AU-rich elements (AUREs). Two observations link AU-rich elements with mRNA instability. (i) mRNAs whose 3′-UTs contain an AURE and/or an oligo(U) region tend to be unstable (54). (ii) If an AURE from the 3′-UT of an unstable mRNA, for example, one encoding granulocyte-macrophage colony-stimulating factor (GM-CSF), is placed within the 3′-UT of β-globin mRNA, the chimeric transcript decays with a half-life of less than 30 min (325). β-Globin mRNA lacking the AURE is stable for well over 2 h in the same transfected cells.

The exact definition of an AURE is unclear at this time (see below), and many AU-rich RNA sequences can function as mRNA-destabilizing signals. On the other hand, it is important to note that different AUREs affect mRNA half-lives to different extents. The c-*fos* mRNA AURE is a potent destabilizing element and an important determinant of the biological effect of c-*fos* and v-*fos* genes in cells (116, 166, 195, 226, 286, 290, 302, 359, 363), and it contains two subregions or domains (62). Domain I is approximately 40 to 50 nucleotides, is AU rich, and includes several AUUUA pentamers, while the adjacent domain II segment contains a U-rich region of approximately 20 nucleotides. If three AUUUA pentamers in domain I are changed to AUUAA or AUAUA, the deadenylation rate decreases only slightly compared with that for mRNA with three wild-type AUUUAs, but the mRNA body is stabilized at least fivefold. If the AUUUA pentamers are unchanged but the U-rich region is deleted, the deadenylation rate decreases and the mRNA is stabilized approximately twofold. These and related experiments suggest that AUUUA sequences facilitate degradation of the mRNA body, while the U-rich segment promotes deadenylation and enhances the destabilizing function of the AUUUAs (7, 64). These c-*fos* studies also clearly illustrate how the stability of a single mRNA can have significant effects on cell physiology. One of the major differences between the highly oncogenic v-*fos* gene and the much less oncogenic c-*fos* gene resides in the stability determinants encoded in their respective mRNA 3′-UTs. The c-*fos* gene is only weakly oncogenic in fibroblasts, but its oncogenicity increases approximately 20-fold if the AURE-containing region is deleted (195, 226, 290, 304).

A related observation appears to account for the correlation between cervical cancer and the integration of human papillomavirus type 16 DNA into the host chromosome. The viral DNA exists as extrachromosomal circles in benign lesions but is usually integrated into the chromosome in cervical carcinomas (Fig. 5) (reviewed in reference 162). Integration occurs in such a way as to disrupt the early DNA region, changing the structure of the mRNA encoding two oncogenes, E6 and E7. E6/E7 mRNA transcribed from unintegrated circles contains a virus-derived AURE and is short-lived. When the genome is integrated, the 3′-terminal region of E6/E7 mRNA is derived from cellular sequences located downstream from the integration site. Unless these sequences by chance include an AURE or some other destabilizing element, the resulting mRNA is likely to be stable, as is the case in one cell line expressing integrated DNA (162). The stabilized mRNA probably generates excess E6/E7 proteins, perhaps facilitating neoplastic transformation.

In summary, there is little doubt that AUREs influence mRNA half-life. However, AURE-mediated destabilization is probably a complicated process, and some important questions must be answered. (i) Is there a "core" or consensus AURE motif (defined as a minimal AURE causing the shortest possible mRNA half-life)? If so, why and how is the core sequence more effective than other AUREs? There appear to be at least three functional classes of AUREs, on the basis of the capacity of different 3′-UT segments to effect mRNA destabilization by accelerating poly(A) shortening and degradation of the mRNA body (64). A nonamer, UUAUUUA(U/A)(U/A) or UUAUU UAUU, reiterated several times and placed within the 3′-UT of a stable mRNA, is the most effective destabilization element of several related AU-rich sequences tested (189, 395). Interleukin-3 (IL-3) mRNA has eight AUUUA pentamers in its 3′-UT and is unstable in a mast cell line cultured in low-calcium medium. It remains unstable if two but not three of its AUUUAs are deleted (350). A mRNA with four closely spaced AUREs is unstable in a reticulocyte extract, but separation of the four AUREs stabilizes the mRNA (126; see also reference 64). (ii) Are all AUREs default or constitutive destabilization signals, or do some function as regulatory signals, affecting mRNA stability, translation, or both only under special circumstances? Some AURE-containing mRNAs that are unstable while translated become stabilized sixfold or more if they are not translated, implying that their AUREs function as destabilizing signals only when the mRNAs are ribosome associated (4, 364; see also reference 178). AUREs can also depress translation under some circumstances (185, 364), and hormones might influence mRNA metabolism via AUREs. For example, interferon mRNA and protein levels are similar when interferon genes with or without the AURE are expressed from a constitutively active thymidine kinase promoter (276). Therefore, the AURE affects neither the stability nor the translation of this mRNA. However, when cells are cultured with glucocorticoids, the level of the AURE-containing mRNA declines whereas that of the AURE-free mRNA is unchanged, suggesting that the AURE does not affect basal expression but does mediate the glucocorticoid response. When primary resting T cells are "activated" by being exposed to antibodies to two receptors, CD3 and CD28, several AURE-containing mRNAs, including GM-CSF, interferon, and IL-2, are stabilized but c-*myc* mRNA, which also contains an AURE, is not (206).

These observations imply that AUREs have several functions that depend on the mRNA, the cell type, and the cell growth conditions. For example, c-*myc* mRNA might remain unstable in activated T cells because it, like c-*fos* mRNA, contains more than one instability determinant and any stimulus blocking AURE-mediated destabilization is counteracted by the second determinant (32, 151, 317, 329, 330, 380, 381). We favor the following ideas to account for some of the observations summarized above. (i) The destabilizing activity of an AURE can be increased or decreased as a result of interactions in *cis* with other sequences (e.g., a U-rich region) and with AU-binding proteins (AUBPs) (see the section on *trans*-acting regulatory factors, below). (ii) AUREs can function as constitutive destabilizing elements and/or, under certain conditions, as regulatory elements. Some evidence supporting a regulatory role for AUREs was mentioned above. In fact, the first paper demonstrating AURE-mediated destabilization also suggested a regulatory function (325). The cultured T cells used in these experiments contain little or no GM-CSF mRNA unless they are incubated with either phytohemagglutinin or phorbol ester. If, following induction, the cells are treated with actinomycin D, the GM-CSF mRNA level decreases rapidly in phorbol ester-treated but not in phytohemagglutinin-treated cells (325). Although a complete understanding of this observation is not possible without additional experiments, one interpretation is that the AURE, which exists in the mRNA regardless of the inducing agent, affects the mRNA half-life in different ways by interacting with different regulatory factors whose expression, in turn, depends on the treatment regimen (phorbol ester versus phytohemagglutinin).

(iii) Iron-responsive element (IRE). The mRNAs encoding transferrin receptor and ferritin, both of which affect iron homeostasis, are regulated posttranscriptionally by processes dependent on the intracellular iron concentration. The transferrin receptor imports iron into cells, and ferritin is a major intracellular iron storage protein. Regulation of mRNA function is achieved through the iron-responsive element, a 23- to 27-bp stem with a mismatched C and a 6-nucleotide loop with C at its 5′ end (reviewed in references 136 and 176) (Fig. 4 and 6). The IRE functions by binding an iron-regulatory protein (IRP; formerly called the IRE-BP for IRE-binding protein [see the section on *trans*-acting regulatory factors, below]) and has different effects depending on its location within the mRNA. The 3′-UT of transferrin receptor mRNA contains five IREs, three of which serve to regulate the mRNA half-life. The 5′-UT of ferritin mRNA contains a single IRE that affects translation.

The levels of transferrin receptor mRNA and intracellular iron are inversely correlated. When intracellular iron is abundant, the IRE-IRP complex does not form and transferrin receptor mRNA is relatively unstable. If iron-loaded cells are treated with desferrioxamine, an iron chelator, iron levels decrease, the conformation of the IRP changes, the IRE-IRP complex then forms, and transferrin receptor mRNA is stabilized by 20- to 30-fold (57, 178, 242). As a result, transferrin receptor synthesis increases, as does the quantity of iron-transferrin complex imported into the cell. Under low-iron conditions, the IRP also binds to the ferritin IRE. In this case, however, ferritin mRNA translation is repressed. Thus, the reciprocal responses of transferrin receptor and ferritin mRNAs achieve iron homeostasis by exploiting IREs to regulate mRNA half-life and translation.

(iv) Long-range stem-loop of insulin-like growth factor II (IGF-II). Insulin-like growth factor II is expressed primarily in fetal cells but is also found in adult serum and probably plays an important role in cell proliferation and differentiation. A remarkable stem-loop structure in the 3′-UT of human, mouse, and rat IGF-II mRNAs is both an endonuclease cleavage site and an mRNA stability determinant (Fig. 4). The significance of the stem-loop was first recognized by observing that cells contain both full-length mRNA and two IGF-II mRNA decay products, a very stable 1.8-kb polyadenylated 3′ fragment, plus a larger but less stable 5′ fragment (also discussed in the section on mRNA cleavage sites and mRNases, below). These products are probably generated by endonucleolytic cleavage within the 3′-UT, approximately 1.7 kb upstream from the poly(A) addition site (227, 228, 251). Two segments are required for cleavage: one (~100 nucleotides) beginning 75 nucleotides 3′ of the translation termination site, the other (~300 nucleotides) beginning approximately 2 kb 3′ of the termination site (316). As determined by both computer analysis and RNase sensitivity assays, the two regions, although separated by almost 2 kb, form a stable duplex structure with several outcrops of additional stems and loops. Cleavage occurs in one of the loops. Changing the sequence of the major stem by inverting the nucleotide sequences reduces or blocks endonucleolytic cleavage, even though the duplex structure is maintained (316). Therefore, either the sequence of the stem-

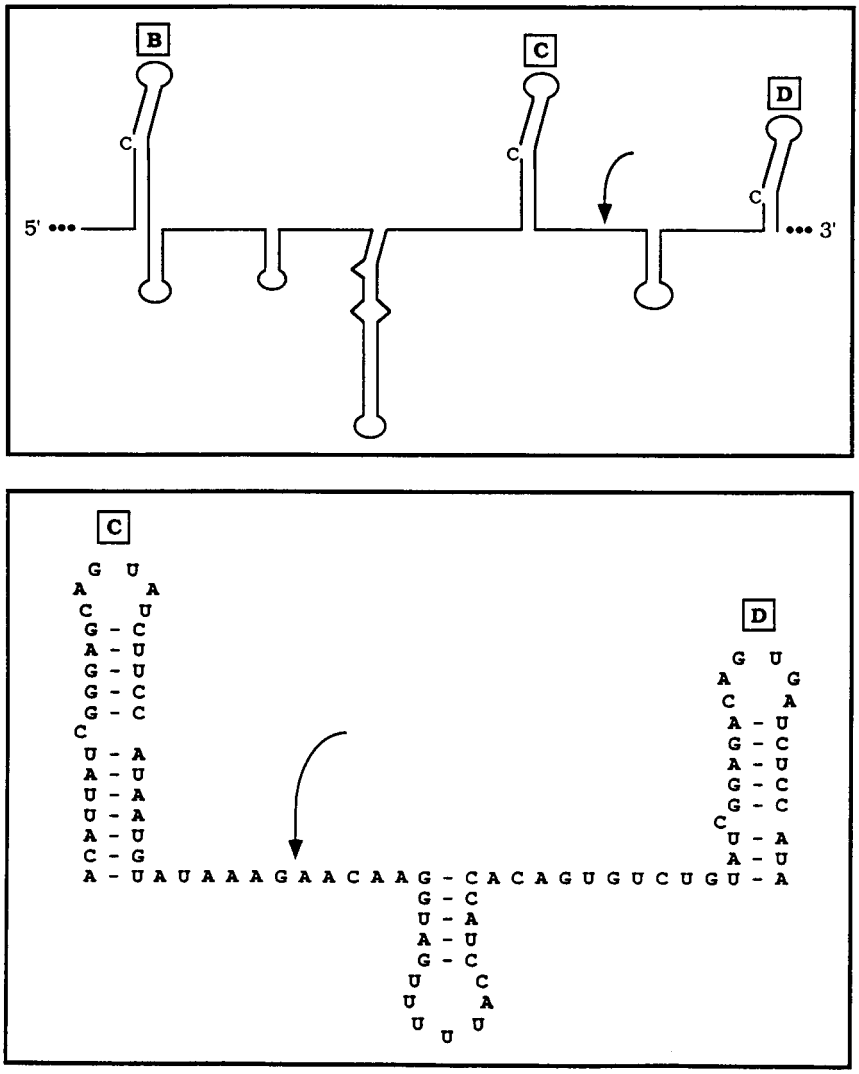


FIG. 6. The IRE of transferrin receptor mRNA and the site of endonucleolytic cleavage (33, 136). A portion of the mRNA 3′-UT is diagrammed at the top of the figure, showing three of the five IREs (B, C, and D). The sequence between elements C and D is shown below, and the arrow in both diagrams indicates where the mRNA is cleaved in cells.

loop is critical for function or the complementary strands form a duplex sufficiently different from the wild-type structure to be unrecognizable by the degradation factors. Two stem-loops can be placed far apart from each other in the 3′-UT, and each is cleaved. Therefore, each stem-loop is presumably recognized as an independent cleavage site.

In summary, several sequences in 3′-UTs can influence mRNA stability (see also the section on mRNA cleavage sites and mRNases, below). It is important to reemphasize, however, that 3′-UTs might also influence mRNA half-lives indirectly, for example, by affecting translation or mRNA localization (reviewed in reference 376). For example, the 3′-UT directs c-*myc* mRNA to cytoskeleton-bound polysomes (152), and localization to a particular compartment of the cell could affect the mRNA half-life (see below).

mRNA coding region. Three observations illustrate the importance of the coding region in determining the mRNA half-life. (i) Mutations in the coding region of mRNAs like c-*fos*, c-*myc*, and tubulin can result in significant changes in the mRNA half-life (see below). Some coding-region stability determinants are also protein-binding sites. (ii) The half-lives of c-*myc* and c-*fos* mRNAs lacking most of their 3′-UTs, including the AURE, are only 1 to 2 h, which is still relatively short compared with those of many other mRNAs (42, 116, 165, 166, 188, 286). Therefore, the truncated mRNAs contain an instability determinant, which, by default, must be in the 5′-UT and/or coding region. (iii) For most or all mRNAs thus far investigated, the introduction of a nonsense mutation in the 5′ portion of the coding region destabilizes the mRNA (see the section on mRNA stability and translation, below).

c-*fos* mRNA and protein levels increase transiently after serum-deprived cells are exposed to serum or growth factors (133, 184, 241). Therefore, c-*fos* is a good example of the class of rapidly inducible genes involved in the immediate-early response. c-*fos* mRNA contains at least three mRNA destabilization signals, a bipartite AURE in the 3′-UT (see above) plus two others in the coding region (317, 329, 330, 372) (Fig. 4). One of the coding-region determinants has been well characterized, contains 320 nucleotides, is located near the center of the mRNA, and encodes the basic and leucine zipper regions

critical to c-*fos* protein function. If the 320-nucleotide sequence is placed in frame within the globin mRNA coding region, the resulting 5′-globin-*fos*-globin-3′ chimeric transcript is at least fourfold less stable than globin mRNA (329, 330). If a frameshift mutation is introduced into the globin-*fos*-globin gene, such that the mRNA sequence is changed by only a single nucleotide but the peptide encoded by the c-*fos* region is entirely different, the frameshifted mRNA is just as unstable as the original globin-*fos*-globin mRNA (372). In this case, therefore, the structure of the mRNA itself specifies the instability phenotype, independent of the encoded protein. In contrast, the destabilization of β-tubulin mRNA by tubulin monomers (see below) and the stabilization of growth-associated protein (GAP-43) by nerve growth factor (254) depend on the peptides encoded by their respective coding-region determinants.

The c-*myc* mRNA coding-region determinant specifies the C-terminal 60 amino acids, including part of the helix-loop-helix and all of the leucine zipper motif, and influences the mRNA half-life when translation is inhibited (Fig. 4). Full-length c-*myc* mRNA is destabilized during the differentiation of myoblasts to myotubes and accumulates in cells exposed to cycloheximide, but mRNA lacking the coding-region determinant is neither destabilized nor cycloheximide responsive (380, 381). In intact cells, globin mRNA containing an in-frame insertion of the c-*myc* coding-region determinant is two- to threefold less stable than globin mRNA with an insert from the stable glyceraldehyde-3-phosphate dehydrogenase mRNA (GAPDH) (151). The globin-*myc*-globin mRNA is also at least 10-fold less stable than globin-GAPDH-globin mRNA under certain conditions in cell-free mRNA decay extracts (see the sections on mRNA cleavage sites and mRNases and on *trans*-acting regulatory factors, below).

β-Tubulin mRNA stability is inversely correlated with the intracellular concentration of tubulin monomers. When drugs such as colchicine are used to induce microtubule depolymerization or when tubulin monomers are microinjected into the cytoplasm, β-tubulin mRNA is specifically destabilized by a process dependent on a determinant contained in the first 12 to 13 coding nucleotides (55, 78–80, 279) (Fig. 4). All downstream coding nucleotides can be deleted and replaced by sequences encoding a reporter protein, and the resulting chimeric mRNA is still destabilized when monomer levels rise (386, 387). The 4-amino-acid determinant (Met-Arg-Glu-Ile) must be located at the start of the coding region, not in the middle, and the mRNA must be associated with polysomes and be translated past codon 41 (259, 386). The mRNA is not destabilized by microtubule dissociation if cells are exposed to high doses of cycloheximide (120). The 4-amino-acid destabilization signal thus seems to be recognized only in the context of a nascent tubulin peptide on a translating (elongating) ribosome. The signal cannot function if it is buried within the ribosome, explaining why translation must proceed past amino acid 41, which permits the amino-terminal tetrapeptide to emerge from the ribosome exit domain. The subsequent steps leading to mRNA degradation are unclear. β-Tubulin protein does not bind in vitro to the nascent tubulin peptide (356). Therefore, the monomers might trigger destabilization indirectly, perhaps by inducing an interaction between the nascent peptide and a ribosome-associated mRNase. It will also be important to learn why a tetrapeptide specifies the degradation of β- but not α-tubulin mRNA (17).

5′ untranslated region and mRNA cap and the effects of mRNA localization. It is not clear whether 5′-UTs are major determinants of mRNA stability, but they can affect mRNA half-life, sometimes dramatically. In theory, the half-life of every mRNA can be affected by how its 5′-UT influences its translational efficiency, and introducing a translation-inhibiting stem-loop in the 5′-UT can change the mRNA half-life manyfold (4) (see the section on *trans*-acting regulatory factors, below). The length of the 5′-UT appears to influence c-*myc* mRNA half-life in a translation-independent manner. c-*myc* mRNAs with different 5′-UTs are generated in some lymphoma and plasmacytoma cells by reciprocal translocations between immunoglobulin and c-*myc* genes (reviewed in references 84 and 164). c-*myc* and immunoglobulin sequences are cotranscribed from these genes, generating chimeric mRNAs with a 5′-UT from the immunoglobulin intervening sequence and a coding region and 3′-UT from c-*myc* exons 2 and 3. If the 5′-UT is sufficiently long, the chimeric mRNAs are three- to eightfold more stable than wild-type c-*myc* mRNA (41, 165, 278, 285, 289). Since the chimeric and wild-type mRNAs seem to be translated with comparable efficiency, the immunoglobulin intron sequences probably have a direct role in stabilization. Moreover, the mRNA half-life correlates with intron sequence length. If the 5′-UT contains only 40 intron-derived nucleotides, the mRNA is as unstable as wild-type c-*myc* mRNA, while mRNA with 600 intron-derived nucleotides is stabilized fivefold (41). Therefore, the larger mRNA is stabilized by the intron sequences rather than destabilized by lack of c-*myc* exon 1 sequences. The c-*myc* mRNA 5′-UT affects the mRNA half-life in vitro, perhaps by interacting with a binding protein (269), and the adenovirus tripartite leader stabilizes viral mRNAs approximately fivefold at late times during infection (234).

mRNAs without caps are at least fourfold less stable than capped mRNAs in oocytes and in cell-free mRNA decay reactions (119, 220, 271, 327). Therefore, decapping could, in theory, be a rate-limiting step in mammalian mRNA decay. There is direct evidence for such a pathway in yeast but not mammalian cells (264a), although mammalian cells do contain 5′-to-3′ exoribonucleases (see the section on mRNA cleavage sites and mRNases, below).

All mRNA segments can influence mRNA stability by affecting mRNA localization (see above), and the half-lives of immunoglobulin and histone mRNAs change if the mRNAs are redirected from one cytoplasmic compartment to another. Immunoglobulin heavy-chain mRNA is approximately fivefold more stable in fully differentiated plasma cells than in B cells, an effect requiring mRNA localization to membrane-bound polysomes (161, 219). If the signal sequence is deleted, the mRNA is translated on free rather than membrane-bound polysomes and is not stabilized during B-cell differentiation, implying that mRNA stabilization is actually facilitated by the accumulation of endoplasmic reticulum during B-cell differentiation (219). Histone mRNA, which is normally translated on free polysomes, is not cell cycle regulated when localized on membrane-bound polysomes (390, 391).

Why Are Stable mRNAs Stable?

There is little doubt that unstable mRNAs contain instability determinants, but it is unclear whether stable mRNAs contain discrete stabilizing determinants or are stable by default. Two observations indicate that at least some stable mRNAs do contain stability determinants that are recognized primarily or exclusively in erythroid cells. (i) mRNAs other than globin that are stable in undifferentiated erythroid precursor cells are destabilized by unknown mechanisms as the cells differentiate to erythrocytes (22, 23) (see the section on effects of hormones, growth factors, and ions on mRNA stability, below). This process seems to be necessary to generate a fully differentiated cell (erythrocyte) in which 90 to 95% of the cyoplasmic protein is

hemoglobin. (ii) Site-directed mutations were made within the 3′-UT of the human α-globin gene, which encodes a very stable mRNA, and wild-type and mutant genes were expressed in an erythroid and a fibroblast cell line (370). Mutations changing the C residues in three C-rich regions cause two- to threefold reductions in α-globin mRNA expression in the erythroid line but not in the fibroblasts. Mutations in other parts of the 3′-UT fail to down-regulate mRNA expression in either line, demonstrating the specificity of the C-rich elements. The mutations affect mRNA levels even when the AUG start codon is mutated, indicating that the C-rich regions are recognized whether or not the mRNA is translated into globin. These data suggest that C-rich elements account for the long half-life of α-globin mRNA in erythroid cells. Proteins capable of binding to the α-globin 3′-UT and to poly(C) have been detected in cell extracts, but their relationship to α-globin mRNA stability is unclear, because they are found in fibroblasts as well as erythroid cells (368).

mRNA CLEAVAGE SITES AND mRNases

Little is known about the enzymes that degrade mRNAs in mammalian cells. They might be constitutive RNases with little or no specificity for a particular RNA sequence, in which case differential mRNA stability might be determined by mRNA tertiary structure and mRNA-protein complexes. Alternatively, some or all mRNases might be programmed to degrade specific mRNAs or classes of mRNAs. No mammalian mRNase has yet been identified unequivocally, but recent progress in purifying RNases and identifying mRNA decay pathways suggests that significant progress is near at hand. In this section, I describe some putative mRNA cleavage products observed in cells and cell extracts plus some RNase activities that might be responsible for generating the products. Although some of these enzymes are likely to be mRNases, proof of their intracellular function is lacking and is a major challenge for future work. It might be significant that most or all of the candidate mRNases thus far identified are unaffected by the inhibitor of RNase A-type enzymes (reviewed in reference 192).

Exoribonucleases

Poly(A) shortening and histone mRNA degradation proceed in a 3′-to-5′ direction (31, 46, 116, 230, 275, 297, 299, 326, 329, 330, 359, 366, 378). The activity responsible for degrading histone mRNA has been investigated with cell extracts, because the early steps in the histone mRNA decay pathway are identical in vitro and in intact cells (297, 298). In crude extracts, polysome-associated histone mRNA is degraded by a magnesium-dependent exoribonuclease that is active from 0 to 250 mM salt and is insensitive to the RNase A inhibitor (297, 298). Several exoribonucleases with these properties have been solubilized by high-salt extraction of polysomes, and one has been purified to homogeneity (56). It is a 33-kDa, divalent cation (Mg^{2+})-dependent protein capable of degrading single-stranded RNA (histone and many others) and double-stranded RNA in the absence of ATP. It also accelerates the degradation of polysome-associated histone mRNA when added back to cell-free mRNA decay reactions, and it degrades protein-free poly(A) but not poly(A) in a complex with PABP. The reaction products are nucleoside 5′-monophosphates. It does not degrade DNA. Therefore, it has all of the properties of the activity that degrades polysomal histone mRNA in vitro. If it is an authentic mRNase, its in vitro substrate specificity suggests that it is not restricted to degrading histone mRNA but might degrade any mRNA that has lost its poly(A) tract. Other mammalian 3′-to-5′ exoribonucleases have been described, including some that degrade poly(A), but they are primarily nuclear and are therefore unlikely to effect cytoplasmic mRNA degradation (14, 15). A PABP-dependent nuclease appears to catalyze mRNA deadenylation in yeast cells (209, 312) and is reviewed elsewhere (264a), and several *Xenopus* oocyte poly(A) nucleases are being investigated to assess their role in deadenylating mRNAs whose 3′-UTs lack U-rich sequences (258).

One or more 5′-to-3′ exonucleases degrade mRNAs in yeast cells (264a), and a cytoplasmic 5′-to-3′ exonuclease activity of unknown function has been identified in extracts from cultured mammalian cells (87). A related activity, purified from the postpolysomal (S100) fraction of reticulocytes, is a processive 5′-to-3′ exoribonuclease consisting of three bands of 54, 58, and 62 kDa in a sodium dodecyl sulfate-gel (338, 339). With capped mRNA as a substrate, the activity first cleaves between N_2 and N_3 of the sequence 5′-$GpppGN_1N_2N_3N_4$ . . .-3′ and then proceeds 5′ to 3′, generating nucleoside 5′-monophosphates. It is inhibited by millimolar concentrations of ATP and by the cap-binding complex eukaryotic initiation factor 4F. It is not understood why ATP inhibits the enzyme, but eukaryotic initiation factor 4F presumably shields and protects the substrate. There is no compelling evidence that mammalian mRNAs are degraded by 5′-to-3′ exoribonucleases, but it is intriguing that such enzymes do exist in mammalian cells, because enzymes with similar properties degrade some yeast mRNAs (155, 172, 191, 239, 240, 344).

Endoribonucleases

Several mRNAs, including 9E3 and gro α (involved in regulating cell growth [347, 349]), transferrin receptor (33), monocyte-derived neutrophil-activating peptide (an inflammatory protein synthesized by monocytes [179]), apolipoprotein (20, 34, 82, 288), and IGF-II (227, 228, 251, 316), are degraded by endoribonucleases. It is difficult to detect the intracellular decay products of most mRNAs, presumably because they are so short-lived, and there is no obvious clue why the decay products from this group of mRNAs are stable enough to be detected. They might form duplex structures that are resistant to most single-strand-specific RNases or be protected by bound proteins.

The activities responsible for cleaving gro α, apolipoprotein, monocyte-derived neutrophil-activating protein, and transferrin receptor mRNAs have not been identified in cell extracts, but the nature of the decay intermediates is instructive. gro α, a 1.3-kb mRNA, is very unstable in growing cells (half-life of probably less than 30 min) but is stabilized at least eightfold by IL-1. If IL-1 is added to cells for several hours and then removed, a 0.9-kb, nonpolyadenylated decay product appears during the time when the mRNA is being degraded (347). This product is generated by removal of 300 to 400 nucleotides from the 3′-UT. Since the downstream (3′) degradation product has not been detected, the responsible enzyme could conceivably be an exoribonuclease that begins cleaving at the 3′ terminus and pauses within the −300- to −400-nucleotide region. However, no intermediate degradation products (less than 1,300 but greater than 900 nucleotides) have been detected, implying that the 0.9-kb RNA is generated endonucleolytically. Moreover, 9E3 mRNA, which is related to gro α, is degraded endonucleolytically, because two 9E3 decay products indicative of endonucleolytic cleavage are observed (349).

The decay products of transferrin receptor mRNA were

analyzed in cells transfected with a transferrin receptor gene encoding a mRNA that is constitutively unstable because of a deletion of the 5′-most C of the IRE loop (Fig. 6). S1 nuclease mapping and primer extension assays detected two degradation products consistent with cleavage at the site noted in Fig. 6 (33). The 3′ degradation product is polyadenylated, indicating that cleavage occurs without prior deadenylation. The sequence near the cleavage site is apparently essential, because changing the sequence GAACAAG to CCCCCCC blocks cleavage.

An endoribonuclease capable of degrading deproteinized IGF-II mRNA has been identified in the postpolysomal supernatant (S100) of a rat liver cell line (251). Cleavage occurs in a loop located within the long-range stem-loop formed in the mRNA 3′-UT (see the section on sequence determinants of mRNA stability, above) and near a G-rich region that could form either a G-G (*syn-anti*) duplex or a G quadriplex.

Some endonucleolytic decay products are generated by cleavage within the coding region. To assess how premature translation stop codons reduce mRNA levels, transgenic mice carrying any of four human β^0-thalassemic globin genes were generated (202–204). (Humans homozygous for thalassemia usually suffer from anemia, and those with β^0-thalassemia make no β-globin. The β^0-globin genes used in these experiments each had a nonsense mutation.) Erythroid cells from these mice, but not from those carrying wild-type globin genes, contain both full-length and 5′-truncated globin mRNAs. The truncated mRNAs are polyadenylated and apparently arise in the cytoplasm (202–204). Surprisingly, their 5′ termini are capped and are located within the coding region but not at the premature stop sites. The mechanism by which these intriguing intermediates arise is unclear.

Polysome-associated c-*myc* mRNA is cleaved endonucleolytically in cell extracts following the addition of excess competitor RNA corresponding to the coding-region determinant (32). Cleavage occurs near the middle of the determinant by an endoribonuclease activity that is tightly bound to polysomes, is magnesium dependent, and is unaffected by the RNase A inhibitor. The competitor RNA might function by removing a protective protein from the coding-region determinant, thereby exposing the determinant to the endonuclease (see the section on *trans*-acting regulatory factors, below).

Several soluble endoribonucleases degrade deproteinized substrates at rates corresponding to some degree to the known intracellular half-lives of the mRNA substrates. For example, IL-2 mRNA is at least 10-fold less stable than globin mRNA in cells (206, 301), and a soluble (postpolysomal or S130) ~68-kDa RNase in extracts from a human T-cell line degrades deproteinized IL-2 mRNA faster than globin mRNA (156). The enzyme is magnesium dependent and is unaffected by the RNase A inhibitor. It will be interesting to determine which sequences the enzyme recognizes and whether enzyme activity is regulated during T-cell activation. A soluble RNase activity from reticulocytes rapidly degrades an IL-1 mRNA substrate containing four or five AUUUA sequences but only if those sequences are sufficiently close to each other (126); a substrate with four or five closely spaced AUUUAs is degraded more rapidly than RNA in which the same four or five AUUUAs are separated. A similar activity capable of cleaving within the 3′-UT of interferon mRNA has been identified in crude reticulocyte lysates (129). The activity apparently requires translation, because the mRNA substrate is not degraded in lysates containing cycloheximide. An as yet uncharacterized activity in reticulocyte lysates degrades a large (7-kb) isoform of IGF-I mRNA faster than it degrades a 1-kb isoform (150). The two isoforms differ in their 3′-UTs.

Several endoribonucleases with apparent specificity for a subset of mRNAs have been identified in amphibian cells and are described here because they are likely to have counterparts in mammalian cells. The activity of one enzyme is induced in the liver of estrogen-treated *Xenopus* and degrades a deproteinized albumin mRNA substrate more rapidly than it degrades ferritin (266), which is significant because albumin mRNA is destabilized following estrogen treatment (see the section on effects of hormones, growth factors, and ions on mRNA stability, below). Cleavage occurs primarily between the U and G of the sequence 5′-AYUGA-3′, which is found in the mRNA 5′ region, generating a 194-nucleotide degradation product that is similar or identical to an RNA fragment observed in the liver of estrogen-treated animals (105, 267). The enzyme functions without magnesium but is more active with it and is associated with 80S ribosomes. Another endoribonuclease specifically cleaves maternal homeo-box mRNAs in *Drosophila* cells and *Xenopus* oocytes (49). Cleavage occurs in the 3′-UT at a consensus sequence, 5′-ANCUACCUA-3′. The 120-kDa enzyme has been partially purified and is active on deproteinized RNA substrates (50). If radiolabeled substrate and a crude oocyte lysate are mixed and incubated, decay occurs slowly but is accelerated by addition of excess unlabeled competitor RNA, implying that the lysate contains a factor that shields the substrate from the enzyme.

trans-ACTING REGULATORY FACTORS

Many mRNAs do not have fixed half-lives. Rather, their stabilities change in response to environmental factors, cell growth rates, etc. (see the section on effects of hormones, growth factors, and ions on mRNA stability, below). Therefore, it is important to document such changes, to identify the stimuli that induce them, and to characterize the steps leading to them. We believe that mammalian cells contain a limited number of RNases dedicated to destroying mRNAs. Moreover, although RNases with considerable specificity for a few mRNAs have been identified (see the section on mRNA cleavage sites and messenger RNases, above), we are unaware of any "restriction RNase" that functions like a restriction endodeoxyribonuclease with absolute specificity for a short nucleotide sequence. Therefore, some mRNAs might be inherently more susceptible than others to RNase attack, but we believe that the intracellular half-lives of many or most mRNAs are determined primarily by other factors, including their affinity for RNA-binding proteins. Here, we focus on a few *trans*-acting factors (other than RNases themselves) that seem to influence mRNA decay. Three general features should be noted. (i) Some factors, and perhaps many, probably have an authentic regulatory role and do not act simply as constitutive stabilizers or destabilizers. For example, if PABP were bound to all mRNAs with equivalent avidity and protected them from rapid destruction and if the rate-limiting step in the decay of these mRNAs were deadenylation, the half-lives of the mRNAs would be identical, which is not the case. Therefore, the binding of this and other proteins probably varies from mRNA to mRNA and is regulated in some way. (ii) Some binding proteins appear to stabilize the mRNAs to which they are bound, while others act as destabilizers. (iii) An mRNA can be stabilized or destabilized as a result of the combined actions of different regulatory factors.

The list of putative mRNA regulatory factors and mRNA-binding proteins is growing rapidly, and there are too many to discuss each in detail (Table 1). Recently, many investigators have made important correlative observations in which cells are treated with activators or growth factors, the stability of a

TABLE 1. Hormones, growth factors, ions, and other factors that affect mRNA stability[a]

mRNA[b]	Effector	Response[c]	Reference(s)
Fatty acid synthase	Glucose, RU486	S	58, 324
Growth hormone, fibronectin	Glucocorticoids	S	96, 260
Procollagen, IL-1β, IFN, 3-hydroxy-3-methylglutaryl-CoA reductase	Glucocorticoids	D	135, 194, 276, 331
Vitellogenin (amphibian liver)	Estrogen	S	48
Very low density apolipoprotein I, vitellogenin (avian liver), albumin, transferrin	Estrogen	D	83, 124, 319
Thyrotropin-releasing hormone receptor	Thyrotropin-releasing hormone	D	249
Cytochrome P-450SCC	Adrenocorticotropic hormone	S	38
Coumarin 7-hydroxylase	Pyrazole	S	5
IL-2, c-*fos*, TNF, CSF, IFN, T-cell receptor, CD4, CD8	T-cell activators	S	206, 261
Acetylcholine receptor	Acetylcholine agonists	D	193
GAP-43	Nerve growth factor	S	253, 254
IL-1, glucose transporter 1	Tumor necrosis factor	S	127, 343
Nerve growth factor, gro α, IL-8, CSF	IL-1	S	18, 205, 346
TNF, CSF, IL-2, IFN	CD3, CD28 (T lymphocytes)	S	206
IL-3	Cyclosporin A	D	247
Angiotensinogen	Angiotensin II	S	177
Transferrin receptor	Iron	D	136, 176
Catalase, superoxide dismutase, tyrosine hydroxylase	Oxygen	S	76, 77, 92, 93, 112
Erythropoietin	Oxygen	D	122
Tissue factor	Lipopolysaccharide, endotoxin	S	44, 91, 314
Acetylcholine receptor, TNF, IL-3, GM-CSF	Calcium, protein kinase C	S	117, 159, 201, 211, 382
Ribonucleotide reductase	TGF-β1, phorbol esters	S	11, 67
GM-CSF, MONAP, TGF-β1	Phorbol esters, calcium	S	39, 111, 159, 179, 325, 366, 367
Contractile proteins (muscle), estrogen receptor	Phorbol esters, calcium	D	71, 394, 305
Cyclin B	Partial hepatectomy (rat)	S	360
GM-CSF	Irradiation	S	6

[a] This table is far from complete. Some interesting interactions, including the effects of β-adrenergic agonists on β_2-adrenergic receptor mRNA (85, 281) and glucose on glucose transporter mRNA (115, 214), have been omitted, because the nature of the response is somewhat unclear. The table is not meant to imply that the "effector" has a direct effect on mRNA stability. In any or all of these examples, the effectors might function indirectly, as with iron changing the conformation of the immediate effector, the IRP.

[b] IFN, interferon; CoA, coenzyme A; TNF, tumor necrosis factor; MONAP, monocyte-derived neutrophil-activating peptide.

[c] S and D indicate that the mRNA is stabilized or destabilized, respectively, in response to the effector.

mRNA increases or decreases, and, concurrently, proteins capable of binding to some region of the mRNA appear or disappear. One interpretation of these data is that the binding protein influences the mRNA half-life. However, the link between the protein and mRNA stability has not been investigated further in many cases, and for this reason, many of these interesting and surely important factors and binding proteins are not described here. The factors to be discussed are arbitrarily divided into two subgroups, those that bind to the mRNAs they affect and those that are not known to bind. The binding proteins, in turn, are subdivided into classes that either stabilize or destabilize mRNA.

RNA-Binding Proteins That Protect mRNAs from Degradation

Poly(A)-binding protein. Although poly(A) surely has a role in mRNA stability, if for no other reason than that deadenylation is the first decay step for many mRNAs, there is some controversy as to how it functions. Since most mRNAs are more stable when polyadenylated than when deadenylated (reviewed in references 31 and 306), poly(A) probably has some role in protecting mRNAs from rapid destruction, a conclusion supported by the following experiments (30). The half-life of deproteinized β-globin mRNA is greater than 60 min in cell-free mRNA decay reaction mixtures containing polysomes (30, 298). However, when the polysomes are depleted of PABP by any of several methods and are then incubated, the mRNA is degraded rapidly (30). Adding purified PABP to PABP-depleted reaction mixtures restabilizes the mRNA. PABP depletion does not affect the stability of deadenylated mRNAs, and several other RNA-binding proteins do not restabilize polyadenylated mRNA in depleted extracts.

These data suggest that the endogenous PABP present in polysomes binds to the β-globin mRNA substrate, forming a poly(A)-PABP complex that protects the mRNA from rapid destruction. Failure to form the complex in PABP-depleted extracts leads to rapid destruction of the substrate, suggesting that PABP protects polyadenylated mRNA. A different conclusion has been drawn from experiments with yeast cells containing a PABP gene whose expression can be induced or repressed. When PABP synthesis is inhibited, the average poly(A) tract increases from 20–60 to 40–90 nucleotides (209, 309, 310). These and other experiments suggest that PABP actually promotes poly(A) shortening in yeast cells. The apparent discrepancy between the in vitro experiments with mammalian cell extracts and the yeast genetic experiments must be resolved. Perhaps the mammalian extracts are deficient in factors that affect poly(A) metabolism in cells. On the other hand, PABP deficiency has pleiotropic effects in yeast cells, and even the notion that poly(A) is a translational enhancer in yeasts has been challenged recently by experiments with mutant strains deficient in poly(A) polymerase (268, 283). Therefore, other factors besides PABP deficiency might account for poly(A) elongation following PABP repression in yeast cells.

Proteins that bind to AU-rich regions. A family of proteins, the AUBPs, has the capacity to bind with high affinity to RNAs

containing AU-rich and, in some cases, U-rich regions (reviewed in references 51 and 62; see the section on sequence determinants of mRNA stability, above). Some family members are located primarily in the cytoplasm, while others are nuclear and still others shuttle between both compartments (170). Some observations suggest that AUBPs influence mRNA stability by interacting with AUREs. (i) Many unstable mRNAs encoding transcription factors, lymphokines, or cytokines contain AUREs in their 3′-UTs (54, 325; see the section on sequence determinants of mRNA stability, above). (ii) On the basis of gel shift assays, the abundance of some AUBPs increases or decreases during the same period when mRNAs are stabilized in response to some stimulus. For example, in peripheral blood mononuclear cells "activated" by phorbol esters plus a calcium ionophore, many lymphokine mRNAs are stabilized and AUBP levels increase by as much as 15-fold (206, 215, 325, 343, 382). The stabilization of mRNAs encoding glucose transporter 1 and amyloid precursor protein in tumor necrosis factor alpha-treated preadipocytes and in phorbol ester-treated mononuclear cells, respectively, also correlates with AUBP induction (343, 389). (iii) The activity or abundance of other AUBPs correlates inversely with the mRNA half-life. For example, the level of an AUBP called AU-B is inversely proportional to the GM-CSF mRNA half-life in T cells activated by a phorbol ester plus an antibody to CD3 (39, 40), and a 35-kDa protein that binds to β-adrenergic receptor mRNA is up-regulated when the mRNA is destabilized by β-adrenergic agonists (281). (iv) mRNAs with AUREs form polysomes that sediment slightly faster than mRNAs of the same size but lacking the AURE (313), perhaps because a large complex of one or more AUBPs is bound to the AUREs. (v) Two observations suggest that AUBPs affect mRNA stability in vitro. First, a protein called AUF1 with high affinity for AUREs and for poly(U) was first identified and purified by virtue of its capacity to induce mRNA destabilization in vitro (45, 393). Second, when polysomes isolated from peripheral blood mononuclear cells are incubated in vitro, GM-CSF mRNA is degraded with a half-life of 90 min (287). However, if a competitor RNA with AUREs is added to the system, the mRNA is destabilized approximately fivefold. Perhaps an endogenous AUBP is bound to and protects the mRNA, while the competitor RNA dissociates the AUBP and exposes an RNase cleavage site.

In summary, some evidence indicates that AUBPs influence mRNA stability, but there are many unresolved issues. Do AURE-AUBP complexes exist within cells, and, if so, what determines the binding affinity? If AUBPs influence mRNA half-life, are they active at all times or is their capacity to influence stability regulated in some way (see below)? Do AUBPs have other functions besides affecting mRNA decay?

Several observations suggest that the functions of AUBPs are regulated, not constitutive. For example, interferon mRNA has an AURE in its 3′-UT, but mRNA abundance in transfected cells is unaffected when the element is deleted (276). Either a second destabilizing element is present in the truncated mRNA or the AURE does not function as a constitutive destabilizing signal. It does appear to have a regulatory function, because glucocorticoids down-regulate wild-type interferon mRNA but not a mRNA lacking the AURE (276). Similarly, IL-3 mRNA is stabilized in cells exposed to the calcium ionophore A23187 in a manner largely dependent on the AURE (350).

One of the major questions about AURE function concerns the potential links between AUREs and translation (see the section on sequence determinants of mRNA stability, above). The issue is not yet resolved, and there are some conflicting results. Several experiments indicate a repressive effect of AUREs on translation. For example, mRNAs containing several AUUUAs in their 3′-UTs are translated less efficiently in oocytes and cell extracts than are mRNAs lacking AUUUAs (130, 185). In intact cells, c-*fos* mRNA lacking its AURE generates 5- to 10-fold more nuclear c-*fos* protein than does its AURE-containing counterpart (364). The effects of AUREs on mRNA half-life are not as clear. Some results indicate that some AURE-containing chimeric mRNAs are stabilized severalfold if their translation is blocked by inserting a stem-loop or IRE into their 5′-UT (4, 364; see also reference 178). Other results, again with chimeric mRNAs, reveal no half-life change by inserting a translation-blocking 5′ stem-loop (328). These experiments were performed with different cells and different RNAs and exploited different methods for measuring mRNA half-life. Perhaps the apparent discrepancies reflect the different experimental protocols.

Taken together, the data described above confirm that AUREs and AUBPs influence mRNA metabolism, but a picture consistent with a single function has not emerged. If AUREs and AUBPs affect translation, a regulatory pathway, or mRNA localization, any effects they might have on mRNA stability could be indirect. Is AUBP binding affected by hormones and other factors, as suggested by the glucocorticoid data (276)? Why do some cells contain multiple AUBPs (147), many of which seem to bind in vitro with similar affinity to different mRNAs? Does the apparent lack of binding specificity in vitro reflect the situation inside cells, or do mRNA secondary structure, polysome structure, and other binding/interacting proteins influence whether an AUBP binds to a particular mRNA in cells? Do AUBPs have other functions besides RNA binding? Some dehydrogenases with dinucleotide-binding (Rossmann fold) regions bind RNA (reviewed in reference 148). GAPDH binds both tRNA (334) and AUREs (246), and a 32-kDa protein with enoylcoenzyme A hydratase activity binds AUREs (248). If some AUBPs have dual roles as enzymes and mRNA stability factors, what, if anything, controls their capacity to function in either role? Can AURE-containing RNAs affect other RNAs in *trans* by sequestering AUBPs? Herpesvirus saimiri produces small nuclear ribonucleoproteins that are abundant, bind AUREs tightly, and could conceivably affect the half-lives of host mRNAs by sequestering host AUBPs (244).

Iron regulatory protein. The IRP is perhaps the clearest example of an RNA-binding protein that protects a specific mRNA from degradation (see the section on sequence determinants of mRNA stability, above). It is at least a "triple-duty" protein, acting as an mRNA stabilizer by binding to the 3′-UT of transferrin receptor mRNA, as a translational repressor by binding to the 5′-UT of ferritin and other mRNAs, and as the enzyme aconitase (reviewed in references 136 and 176). The binding affinity of the IRP to the IRE is apparently controlled by a "sulfhydryl switch" (86, 149). When iron is abundant, an iron-sulfur cluster becomes saturated with iron, affecting the conformation of the protein and reducing its affinity for the IRE. When iron is scarce, the IRP becomes a high-affinity binder, attaching to one or more IREs of transferrin receptor mRNA and protecting it from endonucleolytic attack.

Ribonucleotide reductase mRNA-binding proteins. Three proteins influence the stability of ribonucleotide reductase subunit 1 and 2 (RR1 and RR2) mRNAs by interacting with 3′-UT sequences. Proteins of 57 and 45 kDa bind to RR1 and RR2 mRNAs, respectively. p57 recognizes an 8-nucleotide sequence, CAAACUUC (12, 65, 66). When cells are exposed to phorbol esters, the level or binding activity of each protein decreases, and RR1 and RR2 mRNAs are stabilized. These

results, coupled with observations with competitor RNA in cell-free mRNA decay assays, indicate that both proteins function as mRNA destabilizers (12, 65, 66). Parallel experiments with inhibitors in intact cells imply that the activity of each protein is influenced by phosphorylation via a protein kinase C pathway (67). A distinct 75-kDa protein binds to a different section of the RR2 mRNA 3′-UT (11), and two observations suggest that it functions as a stabilizer: (i) p75 binding activity increases in transforming growth factor β (TGF-β)-treated cells, and transforming growth factor β stabilizes RR2 mRNA; and (ii) the stabilization effect can be reproduced in cell extracts, and the data suggest p75-dependent protection of the mRNA from RNase attack. If so, it seems particularly interesting that, as measured by gel shift assays, the abundance of p45, which is a destabilizer under normal growth conditions, seems to be unaffected when RR2 mRNA is stabilized by transforming growth factor β (66). If p45 and p75 are bound to RR2 mRNA at the same time, p75 might function as a dominant stabilizer, overcoming some or all of the destabilizing activity of p45. It will be important to understand how these proteins affect the mRNA in cells and to confirm the apparent dominance of p75. Is RR2 mRNA susceptible to degradation by different pathways, one of which occurs more rapidly than the other? If so, does p75 block the rapid pathway? If not, does protein binding induce a change in mRNA tertiary structure, making the mRNA more or less susceptible to mRNase attack? Would the mRNA become hyperstable in TGF-β-treated cells lacking p45? Are the half-lives of other mRNAs influenced by two or more factors with affinities for different segments of the same mRNA? For example, multiprotein binding could account for the fact that phorbol esters and gamma interferon both stabilize intercellular adhesion molecule 1 mRNA, but the phorbol ester effect depends on the 3′-UT, while the gamma interferon effect requires sequences in the coding region (255).

c-*fos* coding region determinant-binding proteins. Two proteins bind to a purine-rich segment of the 320-nucleotide c-*fos* mRNA coding-region stability determinant (63). One, of 64 kDa, is associated primarily with polysomes. The other, of 53 kDa, is found on polysomes and in the cytosol or postpolysomal supernatant. The proteins bind primarily to a 56-nucleotide purine-rich segment at the 5′ end of the determinant, and binding is inhibited by poly(A) and poly(G). The protein-binding site is a necessary part of the determinant but is not sufficient, because deletion of either the 56-nucleotide segment or the remainder of the 320-nucleotide region results in mRNA stabilization (63). It will be important to know whether additional proteins are required to bind to the 3′ segment of the region.

c-*myc* coding region determinant-binding protein. In cell-free mRNA decay reaction mixtures containing polysomes, c-*myc* mRNA is normally unstable, with a half-life of 1 h or less, and is degraded 3′ to 5′ (46, 297). However, the addition of excess sense strand competitor RNA corresponding to the 180-nucleotide coding-region determinant accelerates degradation eightfold, apparently by activating an endonuclease that cleaves the mRNA within the determinant (32). A 70-kDa polysome-associated protein, which binds with considerable specificity to coding-region determinant RNA, has been identified by gel shift assays and purified (32, 282). Purified p70 binds at least fivefold more strongly to c-*myc* than to N-*myc* mRNA, even though both mRNAs are similar in structure, and it fails to bind to globin mRNA. These and other data suggest that the protein is normally bound to polysome-associated c-*myc* mRNA and serves to protect it from endonucleolytic attack. In vitro, the exogenous competitor RNA might titrate the protein away from polysome-associated c-*myc* mRNA, exposing the coding region to the endonuclease. The major question is whether such an mRNA-protein complex exists in cells and functions in this manner. In spite of the apparent specificity of the protein for the c-*myc* determinant, there seems to be a ~1,000-fold excess of p70 over c-*myc* mRNA in cultured erythroleukemia cells (282). Is the excess protein necessary to bind up all the c-*myc* mRNA molecules in the cell, or does the protein interact with other mRNAs or have other functions? If the protein is bound to c-*myc* mRNA in cells, do ribosomes somehow ignore the protein and translocate past it, so that translation continues unabated, or do ribosomes displace the protein? If ribosome displacement does occur and if ribosomes protect the mRNA from nucleases, why is a binding protein required at all? If p70 is involved in regulating mRNA stability, how is protein-mRNA affinity regulated? Does this protein, like the IRP, undergo a conformational change that affects its binding affinity, and, if so, what induces the change? Do other proteins bind to the c-*myc* mRNA coding-region determinant? Antibodies to thymidylate synthase protein immunoprecipitate both their own mRNA and c-*myc* mRNA from polysomes (74), and thymidylate synthase binds to the c-*myc* coding-region determinant in vitro (73).

Perhaps the most important question about this and other coding-region-binding proteins is whether they actually influence mRNA half-lives in cells. mRNA-protein complexes are less susceptible than protein-free RNA to RNases in vitro (88), and overproduction of DEAD-box proteins in *E. coli* protects ribosome-free regions of rapidly transcribed mRNAs from degradation, presumably because the proteins bind to the mRNAs and shield them from endoribonucleases (158). Therefore, protection by binding proteins can occur in extracts and in intact cells. However, convincing evidence for mRNA shielding in mammalian cells is lacking, except perhaps for transferrin receptor mRNA and the IRP. The c-*myc* coding-region-binding protein has not been purified in sufficient amounts to add back to protein-depleted extracts and thereby to determine whether it stabilizes polysome-bound, protein-depleted c-*myc* mRNA. Moreover, it is easy to imagine this and other coding-region-binding proteins functioning primarily as translational regulators, for example, by slowing ribosome translocation, as occurs when thymidylate synthase protein binds to its own mRNA (72, 75).

Perhaps the binding proteins are used to activate backup or emergency decay pathways in some of the mRNAs containing two or more stability determinants. Each determinant might serve a separate function, and one might be silent under all but a few conditions. For example, the 3′-UT determinant of c-*myc* mRNA might provide a constitutive destabilization signal, specifying an mRNA half-life of 30 to 60 min and a 3′-to-5′ decay pathway under most conditions. The coding-region determinant, silent under those conditions, might be activated during starvation, inflammation, or other special circumstances in which it becomes necessary to destroy the mRNA very quickly.

trans-Acting Regulatory Factors Not Known To Bind Specifically to RNA

β-Tubulin, histones, and heat shock proteins: autoregulation of mRNA stability. As discussed in the section on sequence determinants of mRNA stability (above), tubulins are synthesized as monomers that aggregate to form microtubules, and elevated tubulin monomer levels trigger tubulin mRNA destabilization. For tubulin autoregulation to occur, the mRNA must be polysome associated, a specific 4-amino-acid

sequence at the amino terminus is essential, and the mRNA must be translated for a sufficient distance, presumably to permit the amino-terminal end of the nascent peptide to emerge from the ribosome exit domain. The steps involved in this pathway are unknown, but it is tempting to speculate that tubulin monomers transiently activate a ribosome-associated RNase at the translation site, inducing specific degradation of tubulin mRNA.

Histone proteins also seem to autoregulate the half-lives of their mRNAs, but it has been difficult to assess histone autoregulation in intact mammalian cells because histones are toxic when microinjected. However, histone mRNA is destabilized in the presence of DNA synthesis inhibitors and at the end of S phase (reviewed in reference 218), both of which are consistent with the following model (summarized in references 274 and 342). (i) After DNA synthesis stops, histone mRNA translation continues, generating an excess of free histones having no newly synthesized substrate (DNA) with which to interact. (ii) Free histones accumulate in the cytoplasm. (iii) The free histones trigger histone mRNA destabilization. Consistent with this model, polysome-associated histone mRNA is specifically destabilized by histone proteins in vitro (274). In cell-free mRNA decay reaction mixtures containing polysomes and postpolysomal supernatant (S130), but no histones, the histone mRNA half-life is approximately 30 to 60 min at 20°C. When exogenous histones are added, decay is accelerated three- to sixfold by a process that is specific with respect to the substrate and the inducer, because histones do not destabilize most other mRNAs and because other basic proteins, including protamines, do not destabilize histone mRNA (220).

The histones accelerate the usual (3′-to-5′ exonucleolytic) histone mRNA decay pathway, but it seems unlikely that they act by binding to histone mRNA. Histones are highly basic and bind avidly to any nucleic acid, which is not compatible with their apparent specificity for histone mRNA. Perhaps histones act indirectly by affecting other proteins bound to the mRNA stem-loop region. Several such proteins have been identified (108, 229), and one ~50-kDa stem-loop-binding protein has been characterized in detail (263, 264, 377). It is detected by gel shift assays, is found primarily in ribosomal high-salt wash fractions, and binds with high specificity to an RNA probe with the histone stem-loop sequence. Three observations support a role for the stem-loop-binding protein in regulating histone mRNA, in large part by affecting its stability and perhaps also its translation. (i) Changing highly conserved stem-loop nucleotides depresses stem-loop-binding protein binding. For example, the loop sequence of histone H2A mRNA is 5′-UUUC-3′. Changing that sequence to 5′-AUAC-3′, without changing the stem, abolishes binding (263). (ii) Histone genes encoding a mRNA with the AUAC loop are expressed poorly or not at all in transfected CHO cells, whereas wild-type genes are expressed well (264, 377). (iii) The presence of a wild-type stem-loop facilitates histone mRNA translation in cells (352). In summary, the stem-loop-binding protein, the histone proteins, the 3′-to-5′ exoribonuclease, and perhaps other factors as well appear to interact in some way so as to autoregulate histone mRNA stability, thereby guarding against inappropriate and untimely histone protein production. Recent in vitro data suggest that La, the RNA-binding protein associated with nascent RNA polymerase III transcripts and with small nuclear ribonucleoproteins, is also involved in the process (221).

Two observations suggest that heat shock protein 70 (HSP70) mRNA is autoregulated. (i) The half-lives of HSP70 mRNA in mammalian cells grown at 37 and 43°C are 50 min and greater than 8 h, respectively (357; reviewed in reference 388), and sequences in the 3′-UT of *Drosophila* HSP70 mRNA are responsible for its short half-life at normal growth temperatures (277). If mammalian HSP70 mRNA contains a related instability determinant, temperature shock might stabilize the mRNA by neutralizing the determinant. (ii) The HSP70 mRNA half-life seems to correlate inversely with the amount of cytoplasmic HSP70 protein, at least in yeast and *Drosophila* cells (102, 277), and mild heat shock in *Drosophila* cells leads, for unknown reasons, to deadenylation of at least 40% of total cell HSP70 mRNA (100). Therefore, heat-induced mRNA stabilization might be particularly important to maintain the level of an mRNA whose translational efficiency has been compromised as a result of deadenylation.

Virion host shutoff protein of herpes simplex virus. Herpes simplex virus, a lytic virus with a wide host range, is a significant human pathogen (294, 375). It also provides a striking example of how viruses affect host cell macromolecular metabolism, in this case by affecting mRNA stability. Almost all host cell mRNAs, even those with half-lives of over 20 h in uninfected cells, are degraded within 3 h after the onset of viral infection (reviewed in references 113 and 294). Viral mRNAs also are destabilized, but neither rRNAs nor tRNAs are affected (182, 186, 257). Destabilization is useful for the virus, because it facilitates ready access of viral mRNAs to the translation machinery without competition from host mRNAs. By accelerating viral mRNA decay, transitions between immediate-early to early to late viral gene expression occur smoothly, requiring only efficient transcription of the appropriate viral genes. mRNA destabilization results from the action of the virion host shutoff (vhs) protein, which is encoded by the viral UL41 gene, is a virion protein, and is carried into the cell by the virus (114, 186, 187, 256, 257, 315, 336). UL41 mutants fail to destabilize host and viral mRNAs during the initial phases of infection. The protein is phosphorylated and exists in several forms, with a major 58-kDa form in virions (181, 293). In its active form, vhs protein might be a homodimer or multimer, since certain UL41 mutants behave as dominant negatives in mixed infections with wild-type virus (187). The protein is synthesized during the later stages of infection, at which time it probably binds to the major viral transcription factor, VP16 (335). The vhs-VP16 interaction might be required to sequester the vhs protein and thereby to block its mRNA-destabilizing function as it accumulates late in infection but prior to its incorporation into virions (337).

There are several major questions about how vhs protein functions. (i) What is its role in the viral life cycle and/or in the establishment of latency? It is not required for viral replication in tissue culture, although UL41 mutant virus yields are lower than those of wild-type virus (187, 292). It has a significant phenotype in animals, because when it is injected intraperitoneally into 12-day-old mice, the lethal dose of wild-type virus is at least 2 orders of magnitude lower than that of a UL41 mutant virus (26). (ii) Is the vhs protein an mRNase or an activator of a latent cellular mRNase? When extracts from cells infected with wild-type virus are incubated under appropriate conditions, both polysome-associated and exogenous (in vitro transcribed, protein-free) mRNAs are rapidly degraded (182, 340). Destabilization is not observed with extracts from cells infected with UL41 mutants. In vitro translation assays and cell transfection studies showing that the vhs protein is the only viral gene product required to induce destabilization are consistent with its being an mRNase (291, 392). If so, it will be important to understand its specificity for mRNA. Does it recognize mRNA-specific signals like the cap or poly(A) or their associated proteins? Perhaps it is analogous to the bacteriophage T4 immediate-early ORF61.9 protein, an RNase that cleaves in the ribosome-binding (Shine-Dalgarno) site of

some T4 mRNAs (303, 362). The function of the phage and herpesvirus proteins might be to degrade mRNAs from one stage of infection, permitting mRNAs from later stages to accumulate and be translated efficiently.

p27^{rex} of human T-cell leukemia virus. Infection of T cells by human T-cell leukemia virus induces the synthesis of IL-2 receptor alpha chain (IL-2Rα), which influences the efficiency with which cells respond to IL-2. At least part of the up-regulation process involves an approximately fivefold stabilization of host IL-2Rα mRNA by the viral gene product p27^{rex} (168, 169). The stabilization mechanism is unknown but is particularly interesting for at least two reasons. (i) p27^{rex} is a nucleolar protein, and its nucleolar localization signal is absolutely required for IL-2Rα mRNA stabilization (373). At this point, it is unclear how a nucleolar protein influences cytoplasmic mRNA half-life. (ii) The IL-2Rα mRNA coding region is necessary for, and is thus the presumed target for, stabilization (169).

Regulatory factors affected by translation inhibitors. Translation inhibitors prolong the half-lives of most short-lived mRNAs by unknown mechanisms (see the section on mRNA stability and translation, below). They might affect the activity or abundance of *trans*-acting regulatory factors, some of which have been detected in crude cell extracts. For example, c-*myc* mRNA is stabilized four- to sixfold in cells exposed to cycloheximide (95). To identify the responsible factor(s), extracts from untreated and cycloheximide-treated erythroleukemia cells were incubated in vitro and the half-lives of polysome-associated c-*myc* mRNAs were compared. The mRNA is degraded at equivalent rates with polysomes from treated and untreated cells but is destabilized three- to fourfold in reactions containing postpolysomal supernatant (S130) from untreated but not treated cells (47). Perhaps the untreated cells contain a constitutive c-*myc* mRNA destabilizer activity that is either destroyed or inactivated when translation is inhibited. A destabilizer factor with the capacity to bind to AUREs and to poly(U) has been purified from erythroleukemia cells, but it is unclear whether this factor is responsible for the cycloheximide effect (45). Another factor present in extracts from an epithelial cell line and associated with polysomes or with mRNA itself stabilizes urokinase-type plasminogen activator mRNA (10). These sorts of constitutive regulatory factors could play important roles in mRNA stability, considering the fact that so many mRNAs are stabilized when translation is inhibited. Therefore, it will be important to purify them, to catalog the mRNAs they affect, and to clarify how they work.

EFFECTS OF HORMONES, GROWTH FACTORS, AND IONS ON mRNA STABILITY

Although environmental factors of all sorts affect transcription, a diverse group of factors, some of which are listed in Table 1, also appear to influence mRNA stability. In most cases, their mechanisms of action are not well defined, and it is unclear whether they act directly or indirectly. For example, a differentiation factor might stabilize or destabilize a group of mRNAs by inducing cell development, in which case the modified half-life would reflect the stage of differentiation, not the direct effect of the factor. It is impractical to describe all of the factors in detail, but a subset is discussed to highlight the following general principles: (i) factors like phorbol esters affect the half-lives of many mRNAs, presumably through some common pathway; (ii) other factors like iron and glucocorticoids influence one or a few mRNAs; and (iii) factors like estrogen stabilize some mRNAs and, in the same cell type, destabilize others.

Phorbol Esters and Related Compounds: a Calcium Response?

Exposure of tissue culture cell lines to phorbol esters or lipopolysaccharide usually up-regulates by at least twofold many mRNAs, including tissue factor (a procoagulant) in monocytes and endothelial cells (44, 91, 314), CSFs in monocytes and lymphocytes (111, 159, 160, 325), and transforming growth factor β in monocytes (366, 367). As determined by using metabolic inhibitors or cell extracts, up-regulation results, at least in part, from mRNA stabilization. Considering the pleiotropic responses of cells to phorbol esters and protein kinase C activation, there is no reason to think that a single pathway or mechanism accounts for all instances of mRNA stabilization. Different cells might respond in different ways, even to the same phorbol ester. Nevertheless, it is tempting to speculate that stabilization results from the transient accumulation or redistribution of intracellular calcium. Calcium ionophores like A23187 specifically stabilize IL-3 mRNA in cultured mast cells, which have been exploited to investigate signalling events in allergic reactions (382). Soon after the cells are treated with A23187, IL-3 mRNA accumulates, and the apparent stabilization requires AUREs in the mRNA 3′-UT (350). If the ionophore is removed and the cells are washed, IL-3 mRNA levels decrease. A23187 also stabilizes GM-CSF mRNA at least 10-fold in a thymoma cell line (159).

Although many mRNAs are up-regulated and perhaps stabilized by phorbol esters, it is important to note several that are destabilized by phorbol esters, including those encoding contractile proteins in muscle cells (71, 394) and estrogen receptor in the breast cancer cell line MCF-7 (305). It is unclear why these mRNAs respond differently from most others, but the response to calcium and phorbol esters (stabilization, destabilization, or neither) is probably cell or mRNA specific or both. For example, an AURE is required to stabilize IL-3 mRNA in ionophore-treated mast cells (350) but not to stabilize GM-CSF mRNA in a phorbol ester-treated thymoma cell line (160).

Estrogen

Estrogen is one of the most interesting mRNA stability-regulating factors because, like the phorbol esters, it stabilizes some mRNAs and destabilizes others, sometimes in the same cell type. It destabilizes vitellogenin mRNA in avian liver after several days of treatment (83, 124) but stabilizes vitellogenin mRNA in amphibian liver (48). Since most of the amphibian vitellogenin mRNA coding region can be deleted without affecting the estrogen response, the estrogen response determinant is probably located in the 5′- and/or 3′-UT (250). Moreover, vitellogenin polysomes are the same size in estrogen-treated and untreated cells, indicating that stabilization by estrogen is not secondary to a change in translation rate (37). Estrogen destabilizes amphibian liver mRNAs encoding some secreted proteins, including albumin and transferrin (265, 319, 383), and it induces an RNase activity with apparent specificity for albumin mRNA (105, 266, 267) (see the section on mRNA cleavage sites and mRNases, above). The pathways involved in regulating vitellogenin and albumin mRNA stability in amphibian hepatocytes might be similar to those in mammalian cells, some of which contain a protein with homology to an estrogen-inducible *Xenopus* protein that binds to the vitellogenin mRNA 3′-UT (103, 104).

How does estrogen induce vitellogenin mRNA stabilization and albumin mRNA destabilization in the same organ? Does it induce an mRNase to degrade albumin mRNA (105) and, at the same time, activate a shielding protein to stabilize vitel-

logenin mRNA (48, 104)? Is albumin mRNA destabilized because it has an unusually short (~17-nucleotide) poly(A) tract (319)? Is there a connection between vitellogenin mRNA stabilization and the fact that, in contrast to albumin mRNA, it has a much longer poly(A) tract (36)? Presumably, the answers to these questions are forthcoming, as more RNases and RNA-binding proteins are purified and characterized. Regardless of the specifics, however, estrogen, like iron, provides a clear illustration of the central role of mRNA half-life regulation in maintaining homeostasis in eukaryotic cells.

Cytokines, Growth Factors, and Kinases

It seems logical that cells would exploit posttranscriptional regulatory mechanisms to effect a rapid response to growth factors and inflammatory mediators. The regulation of cytokine and growth factor mRNA stability appears to be very complex, in large part because one factor can stabilize an mRNA encoding a second factor, which then affects the stabilities of other factors, etc. (see below). These interactions are beginning to be understood, but more work is required before a clear picture of the various regulatory networks is available.

Several mRNAs, including c-*myc* and c-*myb* (175, 199, 358) and 9E3, which encodes a growth factor (349), are stabilized severalfold following serum addition to serum-starved cells. 9E3 mRNA is normally very unstable in chicken embryo fibroblasts but is stabilized ~10-fold in cells making v-*src*, the protein kinase oncogene encoded by Rous sarcoma virus (35, 349). This finding, coupled with the known effects of serum on protein kinases, suggests that 9E3 mRNA might be stabilized following the phosphorylation of a mRNA stability regulatory factor.

IL-1, an early response inflammatory cytokine, and glucocorticoids, which are potent anti-inflammatory agents, are particularly interesting regulators. IL-1 stabilizes mRNAs encoding growth factors and inflammation-related proteins (18, 346, 347). For example, vascular endothelial cells produce few or no growth factors, ILs, or adhesion molecules but can be induced to do so following exposure to IL-1. When cultured fibroblasts are treated with IL-1, gro α mRNA is specifically stabilized (347). When IL-1 is withdrawn and the cells are washed, the mRNA is rapidly deadenylated, after which mRNA degradation products appear (347, 348) (see the section on mRNA cleavage sites and mRNases, above). IL-1 might be a paradigm for how cytokines influence cells by affecting mRNA stability and for how one cytokine affects the stability of another cytokine mRNA. For example, tumor necrosis factor stabilizes IL-1 mRNA (127).

Glucocorticoids, like estrogen, destabilize some mRNAs and stabilize others. They destabilize IL-1β (194), interferon (276), and collagen (135) mRNAs, which is consistent with their role as anti-inflammatory agents, but they stabilize fibronectin mRNA in several human cell lines (96) and human growth hormone mRNA in pituitary cells (260). Glucocorticoids also destabilize 3-hydroxy-3-methylglutaryl coenzyme A reductase mRNA in rat liver (331).

Understanding how hormones, growth and differentiation factors, and ions influence mRNA half-life is a major challenge in the mRNA stability field. The problem is unlikely to be straightforward or easy to solve, for several reasons. (i) In view of the complex interactions and pathways of signal transduction and hormone action, it might be difficult to distinguish how one stimulus elicits a particular response (change in mRNA half-life). (ii) Since individual tissue culture cell lines cannot possibly recapitulate organs and tissues, it might be necessary to exploit whole animal systems in order to understand how IL/cytokine mRNA stability is regulated. We believe that these issues are important and that the whole-animal studies are worth the considerable effort they will surely require, because rapid responses to inflammation, starvation, trauma, etc., are likely to depend in large measure on post-transcriptional regulatory events.

Differentiation Factors

As noted above, little is known about the regulation of mRNA stability during cell differentiation in intact mammalian organisms. However, studies with mammalian cell lines and primary cells clearly demonstrate that mRNA half-lives change significantly during cell differentiation, and theoretical models of gene expression during erythroid cell development predict that changes in mRNA half-lives are essential for the production of fully hemoglobinized erythrocytes from immature bone marrow hematopoietic progenitors (22, 23). The following examples summarize some of the tissue culture studies: (i) Immunoglobulin heavy-chain mRNA is approximately fivefold more stable in plasma cells than in B cells (161, 219). (ii) Most mRNAs except those encoding globin are destabilized when immature erythroid cells are induced to differentiate with dimethyl sulfoxide and related chemicals (22, 23, 183, 365). (iii) The mRNAs for PABP and nonmuscle actin are destabilized at least twofold during the differentiation of myoblasts to myotubes (1, 143). If differentiation is slowed or blocked by exposing the myoblasts to phorbol esters, contractile proteins themselves and the mRNAs encoding them are both destabilized (71, 394). The effect is specific, because GAPDH mRNA is not destabilized. These observations suggest that the rate and/or extent of myoblast differentiation correlates with the destabilization of specific groups of mRNAs. Contractile proteins and their mRNAs might be destabilized to conserve energy and amino acids and to restrain the cells from progressing to a later differentiation stage until the previous stage has been completed. (iv) In fibroblasts and chondrocytes, the half-lives of several collagen mRNAs vary, depending on whether the cells are grown attached to a substratum or in suspension (101, 106). It is unclear whether the effect is a direct consequence of attachment or an indirect response related to the developmental stage of the cells.

mRNA STABILITY AND TRANSLATION

mRNA half-life and translation are linked in ways that are not completely understood (reviewed in references 28, 29, 81, 98, 218, 270, 272, and 307), but two general observations clearly demonstrate the link. (i) Most mRNAs are stabilized in cells exposed to translation inhibitors, but different mRNAs might be stabilized for different reasons and few mechanistic details are known (see below and the section on effects of hormones, growth factors, and ions on mRNA stability, above). In mammalian cells, cycloheximide prolongs the c-*myc* mRNA half-life by slowing deadenylation but does not affect degradation of the mRNA body, once deadenylation is complete (190). In yeast cells, cycloheximide slows or blocks decapping, an essential early degradation step for some mRNAs (27). (ii) mRNA half-lives are often influenced by changing mRNA structure in ways that affect translation. For example, changing the sequence in the 5′-UT can affect translation initiation and changing the coding region can influence polysome structure, the translation elongation rate, and/or the termination site. Many such changes, whose primary effects are thought to be on translation, also influence mRNA half-life (see below).

Evidence that the Stability of an mRNA Can Be Influenced by Its Association with Ribosomes

In *E. coli*, translation elongation inhibitors like chloramphenicol are particularly potent mRNA-stabilizing agents, probably because they cause ribosomes to cluster on the mRNA and ribosomes protect (shield) the mRNA from degradation (89, 90, 318). Consistent with this model, initiation inhibitors like kasugamycin, which block polysome formation, destabilize *E. coli* mRNAs (318). In contrast, most mammalian cell mRNAs are stabilized by inhibitors that block either initiation or elongation (see, e.g., references 8, 95, 110, and 345). The following experiment illustrates how translation of an mRNA coding region from start to finish can influence mRNA stability. A chimeric mRNA including the coding region for hepatitis B virus surface antigen plus a 3′-UT with the AURE from GM-CSF mRNA is normally unstable (half-life, 18 min) in transfected monkey cells but is stabilized (half-life, 7 h) by placement of a stem-loop structure within the 5′-UT (4). The stem-loop, of course, slows or stops translation initiation. If a picornavirus internal ribosome entry site is introduced downstream of the stem-loop, the mRNA is translated (because ribosomes bypass the stem-loop) and is also destabilized. Therefore, the stem-loop per se does not account for mRNA stabilization but acts by virtue of the translation block.

These and other observations demonstrate a link between translation and mRNA stability. However, no one understands fully the nature of the link, and not all mRNAs respond in the same way to translational down-regulation. For example, a chimeric mRNA with an IRE in the 5′-UT, the transferrin receptor coding region, and a c-*fos* AURE in the 3′-UT is unstable whether or not it is translated (178). Analogous results have been obtained with mRNAs containing a 5′ stem-loop, a β-globin coding region, and a c-*fos* or GM-CSF AURE in the 3′-UT (328). These and other studies implicate at least four distinct pathways or mechanisms for translation-dependent mRNA stabilization, depending on the mRNA and the circumstance. (i) Continued translation is required to maintain the level or activity of a *trans*-acting factor (discussed in the next subsection on "*trans*" effects). (ii) The act of translational elongation is important for some mRNAs. β-Tubulin mRNA is not destabilized by tubulin monomers if elongation is completely blocked by high doses of cycloheximide (120). (iii) For other mRNAs, ribosomes must enter and translate a specific segment of the coding region for degradation to occur at the normal rate. Full-length c-*myc* mRNA is stabilized at least fourfold by exposure of cells to elongation inhibitors (95), but an mRNA lacking the carboxy-terminal coding segment is not stabilized by cycloheximide (381). Perhaps this region contains a cleavage site for a ribosome-associated RNase, and ribosomes must enter the region to juxtapose the RNase and its substrate (32, 151). (iv) Polysome structure is probably an important determinant of mRNA stability, and any translation inhibitor, by slowing or blocking initiation or elongation, could affect the mRNA half-life by modifying the polysome conformation. Some experiments on histone mutants support the notion that polysome structure influences the mRNA half-life. Histone mRNA levels are regulated as a function of the cell cycle, and the mRNA is abundant only when DNA is being synthesized. A 3′-terminal stem-loop motif located 40 to 50 nucleotides downstream from the translation termination site is absolutely required for proper regulation (see the sections on sequence determinants of mRNA stability and on *trans*-acting regulatory factors, above). If a segment of 500 or more nucleotides is inserted between the translation stop site and the stem-loop, the mRNA is not regulated properly and is not destabilized after DNA synthesis is inhibited (131). Yet, the unregulated mRNA retains the usual histone mRNA coding region and 3′-terminal stem-loop and could theoretically generate histone protein. Perhaps the inserted sequence alters the conformation of the polysomes so drastically, by increasing the distance between the translation stop and the stem-loop, that the normal regulatory signals are unrecognizable. Similarly, when a termination site mutation causes histone or α-globin mRNA to be translated past the normal stop codon and into the 3′-UT, the mRNAs are destabilized or are not properly regulated (53, 369). Perhaps ribosomes translating within what should be an untranslated region disrupt interactions between the mRNA and polysome-associated mRNA-binding proteins (368). If the translation initiation rate for amphibian liver vitellogenin mRNA is reduced but not completely repressed by an initiation inhibitor, polysome size is significantly reduced, because there are fewer ribosomes per mRNA, and the mRNA is not stabilized by estrogen, implying that estrogen regulation depends on whether the mRNA is fully or partially loaded with ribosomes (37).

In summary, there are probably several ways in which mRNA translation influences mRNA half-life. At least some mRNases appear to be associated with ribosomes (295, 296). Therefore, it makes sense that mRNA degradation can occur as the mRNA is being translated. However, there are exceptions to this observation as well. Tyrosine aminotransferase mRNA is stabilized by translation elongation inhibitors like cycloheximide but not by initiation inhibitors like pactamycin (110).

Evidence that Translational Inhibitors Affect the Stability of Some mRNAs by a "*trans*" Effect

Most short-lived mRNAs (half-life, $<\sim$5 h) analyzed thus far are stabilized by translation inhibitors. Since these mRNAs share no obvious common sequence or structure other than the cap and poly(A), it seems reasonable to suppose that many are stabilized via a "*cis*" effect: i.e., they are not translated or are protected by ribosomes. Other mRNAs might be stabilized by virtue of a "*trans*" effect, whereby a labile factor normally required to degrade the mRNA is destroyed or inactivated after translation stops. An amphibian oocyte mRNA called Eg2 is deadenylated after fertilization but persists as free messenger ribonucleoprotein until the end of blastulation, when it is finally degraded. Cycloheximide stabilizes the mRNA as embryos reach the end of blastulation, but the mRNA does not reassociate with polysomes (43). In other words, stabilization occurs without a change in translational status, perhaps because the synthesis of a *trans*-acting factor is blocked. Cycloheximide also stabilizes MFA2 mRNA in yeast cells through a "*trans*" effect, because MFA2 mRNA containing a stem-loop in its 5′-UT is not translated in cycloheximide-treated cells but is stabilized (27). c-*myc* mRNA is more stable in cycloheximide-treated than untreated cells, and polysome-associated c-*myc* mRNA is also more stable in cell extracts prepared from cycloheximide-treated cells than in those from control cells (47; see the section on *trans*-acting regulatory factors, above). Presumably, a labile, cycloheximide-sensitive factor present in extracts from untreated cells facilitates c-*myc* mRNA degradation. Histone mRNA is stabilized in cells treated with translation inhibitors (reviewed in reference 218), perhaps because histone proteins, which normally trigger mRNA destabilization at the end of S phase, do not accumulate to a sufficient level when translation is blocked. Consistent with this model, polysome-associated histone mRNA is destabilized by addition of histone proteins to cell extracts (222, 274) (see the section on

trans-acting regulatory factors, above). Since little or no translation occurs in these extracts, histone-induced destabilization is independent of translational elongation.

In summary, it seems likely that translation can affect mRNA stability by different mechanisms, depending on the mRNA and the circumstances. To understand these mechanisms, it will be necessary to invent novel ways to visualize polysome structure and to identify and characterize *trans*-acting factors. Perhaps in vitro mRNA decay systems with large translational capacities will be useful.

Nonsense-Mediated mRNA Decay

In organisms ranging from bacteria to humans, mRNAs are destabilized by 10-fold or more when they contain premature translation termination sites. Maximal destabilization usually occurs when the termination site is closest to the initiation site. In *E. coli*, premature stop codons reduce mRNA abundance by two related mechanisms involving rho-dependent transcription termination downstream of the stop codon and formation of a ribosome-free region between the translation and transcription stop points (3, 252, 371, 385). The polar and mRNA destabilization effects of nonsense mutations in yeasts and mammals have been investigated for over 15 years but are less well understood (60, 167, 207). Two facts are well established for yeasts and mammals. mRNAs containing nonsense mutations are less abundant than their wild-type counterparts, and translation itself, or recognition of the altered reading frame, is important for down-regulation. However, there are three major questions. How is the premature stop codon recognized? Does instability occur in the nucleus, the cytoplasm, or both? What is the mRNA degradation pathway?

Nonsense-mediated mRNA destabilization in yeast cells is discussed elsewhere (264a), but it is important to note here that premature translation stop codons in yeast cells appear to change or shortcircuit the usual decay pathway. At least some yeast mRNAs are degraded by a three-step pathway: poly(A) shortening, decapping, and then 5′-to-3′ exonucleolytic decay (97, 98, 155, 272). If the mRNA contains a premature stop codon, decapping and 5′-to-3′ decay occur without deadenylation (240). The factors responsible for destabilization are being identified genetically and include a protein encoded by the *UPF1* gene (196, 197). In mutant strains lacking pUPF1, mRNAs with premature stop codons are degraded at approximately the same rate as wild-type mRNA. Although the mechanism of action of pUPF1 is unknown, it probably functions in the cytoplasm, where it is associated with polysomes (16, 272).

The situation is less clear for mammalian cells. The following observations imply that nonsense codon-mediated mRNA decay is linked to translation but occurs in the nucleus or at least is nucleus-associated. (i) The human triosephosphate isomerase (TPI) gene contains seven exons, and genes containing nonsense mutations in exon 1 through the first half of exon 6 generate approximately fivefold less steady-state mRNA than the wild-type gene does (94). However, the cytoplasmic half-life of TPI mRNAs with a premature translation stop is 33 h, which is approximately the same as that of the wild-type mRNA, as measured both by the actinomycin D technique (69) and by the c-*fos* promoter transcriptional pulse-chase technique (29). Premature stop codons also have little or no effect on the cytoplasmic half-life of dihydrofolate reductase mRNA in actinomycin D-treated cells (361; but see below). (ii) The quantity of prematurely terminated mRNA is increased three- to fourfold, i.e., almost to the level of wild-type mRNA, by expressing the mRNA plus a suppressor tRNA, which recognizes the nonsense triplet as a missense codon, or by placing a hairpin structure in the 5′-UT, which reduces translation initiation (28). These data indicate a role for translation in nonsense-mediated mRNA decay. (iii) The level of fully spliced, nonsense codon-containing TPI mRNA is reduced approximately fivefold in highly purified preparations of nuclei, compared with the level of wild-type mRNA (29). The half-life of the mRNA in nuclei is also reduced compared with that of the wild-type mRNA. These observations imply that the nonsense codon exerts its effect while the mRNA is within or associated with the nucleus. Consistent with this interpretation, premature stop codons do not decrease the abundance of nuclear TPI pre-mRNA (70). (iv) Exon skipping is observed in a small percentage of transcripts from transfected TPI genes containing a splice site mutation; in other words, the exon with the premature stop codon is excised and the resulting transcripts, which thus lack the premature stop, are more stable than normally spliced mRNAs that retain the premature stop (29).

Three models have been proposed to account for how premature stop codons are recognized in the nucleus (29, 361). (i) A translational translocation model suggests that translation begins as soon as the mRNA coding region emerges from the nucleus. The translating ribosomes then facilitate splicing of the remaining portion of the pre-mRNA and perhaps also enhance export. Premature stop codons slow or otherwise disrupt processing and export. As a result, some of the nuclear mRNA or pre-mRNA is degraded. This model implies that splicing occurs in a 5′-to-3′ direction, which is not the case for dihydrofolate reductase pre-mRNA (173). (ii) A similar model envisions that fully spliced mRNA is translated as it emerges, 5′ end first, out of the nuclear pore, and that the premature stop codon somehow interferes with this process and promotes rapid decay of the nucleus-associated mRNA. (iii) A nuclear scanning model proposes an undefined scanning process within the nucleus to recognize and degrade mRNAs with premature stops. If nuclei contain ribosomes and translation factors and are capable of translation, a mechanism for nuclear scanning would be relatively easy to imagine. Nuclei do contain some sort of as yet uncharacterized nonsense codon-recognizing capacity, because premature translation codons affect splicing and promote exon skipping, at least of some pre-mRNAs (99, 245).

Some results are not consistent with any of these models or with the data on intranuclear mRNA degradation. (i) β-Globin mRNA levels have been compared in hematopoietic tissues of transgenic mice expressing the wild-type β-globin gene and genes encoding mRNAs with premature stop codons. mRNAs with premature stops are less abundant than wild-type mRNA, as described for TPI (202). However, the globin mRNAs in transgenic mice appear to be degraded in the cytoplasm, not the nucleus (203, 204). (ii) mRNAs with nonsense mutations are degraded faster than their wild-type counterparts when injected into the cytoplasm of amphibian oocytes (374). (iii) The presence of a nonsense codon in an immunoglobulin κ light-chain gene results in inefficient nuclear splicing, accounting, at least in part, for reduced mRNA production (210). (iv) The half-lives of adenine phosphoribosyltransferase mRNAs with and without premature stop codons are 16 h as measured with actinomycin D in transfected tissue culture cells. However, if the transfected genes are placed under the control of a tetracycline-repressible promoter and transcription is blocked with tetracycline (see the section on measuring mRNA half-life, above), the half-lives of the wild-type and mutant mRNAs are 8 and 2 h, respectively (61).

To summarize, it is not clear whether every mRNA containing a premature stop codon is degraded in the same way in all cells or how nonsense codons are recognized while the mRNA

or pre-mRNA is within or associated with the nucleus. Perhaps the effects of nonsense mutations depend on the cell and the gene product. Cell specificity would explain why β-globin mRNAs with nonsense codons are unstable in erythroid cells (202–204, 217; see also reference 157) but are as abundant and apparently as stable as wild-type mRNAs in fibroblasts (216, 237).

Regardless of the mechanistic details, several observations reveal important roles for nonsense codon-mediated mRNA decay in maintaining cell viability. (i) In yeast cells lacking functional pUPF1, unspliced pre-mRNAs capable of producing potentially harmful peptides accumulate on cytoplasmic polysomes (144). (ii) In *C. elegans*, mutations in *smg* genes have similar effects to *UPF1* mutations in yeast cells, in that mRNAs with a premature stop codon are as abundant as wild-type mRNAs (284). Four nonsense alleles of a myosin H-chain gene, *unc-54*, are dominant in a *smg* mutant background but not a wild-type background, implying that the smg protein eliminates mRNAs encoding potentially harmful proteins. The products of the *UPF1*, *smg*, and related genes thus seem to make up a "surveillance" system that degrades mRNAs with premature stop codons and pre-mRNAs that escape to the cytoplasm. Failure to degrade these "mistakes" can result in the accumulation of toxic peptides.

SUMMARY AND PERSPECTIVES

mRNA stability plays a major role in gene expression in mammalian cells, affecting the rates at which mRNAs disappear following transcriptional repression and accumulate following transcriptional induction. An array of exogenous factors, from hormones to viruses to ions, influence mRNA half-lives in ways that are incompletely understood.

Significant progress in this field will probably depend on the identification and characterization of mRNases and relevant mRNA-binding proteins and on new insights into how translation, polysome structure, and *trans*-acting factors influence mRNA stability. While these studies might best be performed with tissue culture cells, I believe that, ultimately, a satisfactory understanding of the impact of mRNA stability on cell growth, differentiation, neoplasia, and the inflammatory response requires experiments with intact organisms. For example, there exists a significant literature on mRNA stability during the differentiation of oocytes and embryos of lower organisms, including slime molds (13, 59, 153), protozoans (208, 223, 224), flies (109, 233, 379), and amphibians (107, 140, 174), and changes in mRNA half-lives in mammalian cell lines cultured with various hormones, cytokines, and inducers of differentiation have been documented extensively. What is lacking is information on the enzymes and regulatory factors affecting mRNA stability in the organs of intact mammals.

There are many practical reasons for studying mRNA stability. If certain classes of mRNAs are regulated by common factors like AU-binding proteins, it seems logical to consider designing inhibitors to target such factors and block or enhance their activity. This approach might be particularly rewarding if mRNAs in specialized tissues like muscle, blood, or liver are regulated by tissue-restricted factors (see the section on sequence determinants of mRNA stability, above). We have not discussed plant mRNA stability in this review, although considerable progress has been made in recent years (reviewed in references 2, 52, 134, 322, and 351). Perhaps novel insights about how plants regulate mRNA stability will reveal useful ways for enhancing plant growth, fighting pests, and fixing nitrogen. In any event, the rate of new discoveries about mRNA stability in recent years encourages the hope that within a short time, some of the mRNases, RNA-binding proteins, and *trans*-acting factors involved in mRNA degradation will be identified and characterized.

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