INTRODUCTION
THE ROLE, MECHANISM
AND REGULATION OF
mRNA TURNOVER
1.1 GENE EXPRESSION AND mRNA TURNOVER

Eukaryotic gene expression involves various processes which are as follows:
Chromatin decondensation,
Transcription,
RNA splicing and processing,
Turnover of primary transcript,
Transport of mRNA from nucleus to cytoplasm,
mRNA degradation in cytoplasm,
Translation and
Posttranslational modification of proteins.

Expression of a eukaryotic gene can be regulated at any of these steps.

The quantity of a particular protein synthesized in a given time depends on the level of the mRNA coding for that protein and the efficiency of its translation. The level of mRNA is in turn, determined by the rate at which it is synthesized, spliced, processed and transported to cytoplasm and its rate of decay. The decay rate, thus, determines the time for which the mRNA stays undegraded in the cytoplasm and controls the cytoplasmic abundance of the mRNA and hence its availability for translation. Importantly, the decay rate also determines the time taken for any perturbations occurring in transcription rate to get manifested in the cytoplasmic steady state level of the mRNA.

It is known that the steady state levels of several mRNAs are determined to a significant extent by their transcription rates and that cell type specific mRNA expression patterns are brought about primarily by transcriptional regulation. But several examples are also known of mRNAs the levels and/or changes in levels of which, correlate more with their respective turnover rates rather than transcription rates. mRNA turnover is thus, an important mode of regulating gene expression.

1.2 SIGNIFICANCE OF MESSAGE STABILITY IN CELLULAR PHYSIOLOGY

Of considerable importance for the cell are the mRNAs of housekeeping genes. The housekeeping mRNAs code for enzymes involved in essential functions. These transcripts form 50% of the mRNA sequence complexity in the mammalian cell (Ryffel and McCarthy, 1975; Hastie and Bishop, 1976; Cabrera et al., 1984). Different housekeeping mRNAs of varying abundances (ranging from 5 to 700 transcripts per cell) were studied in mouse L cells and developing sea urchin embryos (Cabrera et al., 1984; Carneiro and Schibler, 1984) to understand how their abundance correlates with their transcription rate and turnover rate. It was found that in many of the cases, either an abundant mRNA is made from slowly transcribing gene or a scarce mRNA is made from a rapidly transcribed gene. But it was found that in a vast majority of the cases the abundance correlates very well with the half life of the mRNA. From these results, it was clear that the levels of house keeping mRNAs are determined primarily by their turnover rates, which means, mRNA turnover plays an important role in determining the expression of a significant number of important genes throughout the life time of the organism.
It is also known that mRNA turnover plays a major role in determining the expression of several very essential genes during important processes like embryogenesis, development and cellular differentiation (Kafatos, 1972; Davidson, 1986), inflammatory response, etc. The storage of maternal mRNAs in oocytes (Davidson, 1986) is a very well studied example. The oocyte maternal mRNAs are required to synthesize important proteins during early embryogenesis. During oogenesis, the abundance of these mRNAs is maintained by regulating both transcription and turnover. After maturation, the oocyte can remain in a semiquiescent state for a long time until it is activated to mature into an egg that can be fertilized. So, during this period, the maternal mRNAs are stored in a nuclease resistant, untranslated form and hence, exhibit very high stability. The mechanism of this storage is not very clear yet. Soon after fertilization, these mRNAs are activated and translated, and during the same time interval, the stability of many of them starts decreasing suddenly leading to their complete degradation within a short time period (Woodland et al., 1979; Brower et al., 1981; Paynton et al., 1988). Stabilization of α and β crystallin mRNAs (Yoshida and Katoh, 1972) and simultaneous degradation of several other mRNAs are very much responsible for the high accumulation and enrichment of the crystallin mRNAs during the differentiation of lens cells. An important postnatal step in the development of the parotid gland is the proliferation and differentiation of the cells that eventually produce parotid gland secretary protein (psp). Though the psp gene is transcriptionally active during the early postnatal life, the mRNA is very unstable (Shaw et al., 1986) as a result of which, not much of psp protein is made. But, as the secretory cells continue to differentiate, the mRNA becomes stable, and hence, abundant production of psp protein occurs. The high percentage (90 to 95%) of hemoglobin in erythrocytes is partly due to the selective degradation of the nonglobin mRNAs during red cell development (Aviv et al., 1976; Mishina et al., 1984 Krowczynska et al., 1985). During inflammatory response, the cell proliferation and differentiation are regulated by cytokines and lymphokines, and the levels of their mRNAs rise and fall as the tissue healing goes to completion. Many of these mRNAs were found to be very unstable, which enables the cell to rapidly adjust their levels according to its requirement (Peltz et al., 1991).

1.3 mRNA TURNOVER: THE PROCESS AND REGULATION

1.3.1 Intrinsic and Extrinsic stabilities of mRNAs and the levels of regulation of mRNA turnover

One working hypothesis (Peltz et al., 1991) is that turnover rates depend on intrinsic or passive factors, as well as, extrinsic or regulated factors. The former include constitutive mRNA degradatory enzymes and cofactors of the cell, interaction of the mRNA with which determine its intrinsic turnover rate. The latter involve additional cytoplasmic factors whose interaction with mRNA results in changes in its turnover rate from the intrinsic value. Some mRNAs may have fairly constant half lives under all cellular conditions and hence have only intrinsic (and hardly any extrinsic) turnover rate. Whereas others can have their turnover rates constantly subjected to modulation by multiple factors so that they have no intrinsic half life.
Regulation of message stability occurs at 2 levels. The first level of regulation is the differential intrinsic stabilities of different mRNAs according to the cellular requirement for those mRNAs. Messages coding for many of the regulatory polypeptides like transcription factors, cytokines, etc., which are usually expressed very transiently depending on the needs of the cell, are often highly unstable. The instability of the mRNA in these cases makes it possible for changes occurring in their transcription rate to get immediately reflected in the protein level, enabling the cell to make very rapid changes in their expression. Moreover, since many of such proteins are deleterious if expressed under circumstances when they are not required, it is important for the cell to down regulate their expression as rapidly as possible once their function is over. On the other hand, the requirement for a temporary uncoupling of transcription and translation makes the high stability of oocyte maternal mRNAs (see section 1.2) mandatory. Similarly, many mRNAs coding for house keeping proteins are also relatively stable because it permits transcriptional economy for these genes as their products are required by the cell at fairly constant levels (Hentze, 1991).

The second level of regulation involves modulation of the stability of a given mRNA under different conditions (in other words, it deals with extrinsic stabilities). There are numerous examples of this kind and they will be discussed in different sections of this chapter.

1.3.2 mRNA turnover process: A general outline

The mRNA turnover process can be considered to involve roughly the following steps:

(a) Appearance of the physiological signal that triggers the change in decay rate of an mRNA.
(b) Reception of the signal by the degradation machinery followed by change in its activity.
(c) The occurrence of the appropriate changes in the decay rate of the target mRNA, as a result of the above 2 processes.

The molecule(s) or molecular complex(s) that comprise the degradation machinery have to perform 3 important functions.

(a) Sensing the signal.
(b) Identification of the right target mRNA whose decay rate needs to be altered.
(c) The modulation of the decay rate of the target mRNA.

In the case of mRNAs which show very distinct intrinsic and extrinsic decay rates, the degradation machineries determining those decay rates may be very different; however, they may share certain common factors.

1.3.3 Physiological signals affecting mRNA stability

The physiological signals that lead to regulation of message stability are both intracellular and extracellular. Most of the extracellular signals probably affect mRNA turnover indirectly and not directly. Some of these signals are discussed below:
Heat shock

Heat shock gene expression is induced soon after cells are exposed to heat or other kinds of stress (Burdon, 1986). This occurs by regulation operating at several levels of gene expression including mRNA stability (Yost et al., 1990). The mRNA encoding the major heat shock protein, HSP70 is stabilized by at least 10 fold upon subjecting mammalian or Drosophila cells to heat shock or other kinds of stress (DiDomenico et al., 1982; Lindquist et al., 1982; Theodorakis and Morimoto, 1987). The intrinsic decay rate of this mRNA is restored once the stress is removed. Since HSP70 is a molecular chaperone (Beckmann et al., 1990; Gething and Sambrook, 1992), the regulation of its expression has important implications regarding the survival of the cells under stress. Drosophila HSP70 mRNA harbors certain stability determinants in its 3' region (Simcox et al., 1985). Many observations suggest that HSP70 influences stability of its own mRNA (DiDomenico et al., 1982; Theodorakis and Morimoto, 1987).

In yeast, heat shock leads to a transient drop in the levels of many of the mRNAs coding for ribosomal proteins by causing an increase in their turnover rates (Hernuer et al., 1988).

Hormones and growth factors

Hormones affect gene expression at both transcriptional and posttranscriptional levels. Several examples are known of mRNAs whose abundance is regulated by hormones at least partly by controlling their stability (Shapiro et al., 1987). The effects of estrogen, glucocorticoids, growth hormone, interleukin 1 and epidermal growth factor (EGF) on mRNA stability have been studied. In addition, some hormones and growth factors in serum seem to affect cell growth by regulating mRNA decay rates.

Estrogen dependent regulation of mRNA stability has been extensively studied. The abundance of the egg yolk precursor protein, vitellogenin, made in hepatocytes is regulated by estrogen (Shapiro and Brock, 1985). In Xenopus hepatocytes, vitellogenin mRNA is undetectable in the absence of estrogen. But it accumulates to \( \sim 5 \times 10^4 \) molecules/cell upon treating the cells with estrogen over several days. This upregulation is caused by both transcriptional activation and mRNA stabilization (Brock and Shapiro, 1983a and b). The vitellogenin mRNA has a half-life of 500 hours in presence of estrogen and upon withdrawal of the hormone, it gets degraded with half-life of 16 hours. Such exploitation of both transcriptional and posttranscriptional mechanism by the organism may be very essential to bring about abundant production of a protein in response to the hormone in the case of single or low copy number genes like vitellogenin. On the other hand, the rapid disappearance of the protein is ensured upon hormone withdrawal by destabilization of the mRNA. This mRNA stabilization process does not require protein synthesis (Brock and Shapiro, 1983a) and is suggested to be mediated by the cytoplasmic estrogen-binding protein. This protein has a 100 fold lower binding efficiency than the nuclear estrogen receptor, though its specificity for estrogen binding is similar to the latter, and it does not translocate to nucleus after binding estrogen. On this basis, it has been suggested that at low concentrations of estrogen, only the nuclear receptor might bind the hormone leading to transcriptional activation alone and higher concentrations of the hormone...
cause both transcriptional activation and mRNA stabilization probably because of the mediation of both the receptors in the process (Peltz et al., 1991). In this way vitellogenin expression can be controlled in response to a wide range of hormone concentrations.

An interesting aspect of estrogen effect is that it varies with the mRNA or cell type or species. In addition to vitellogenin mRNA stabilization, it also causes destabilization of many of the mRNAs coding for the liver secretory proteins including albumin, gamma-fibrinogen, transferrin etc. (Schöenberg et al., 1989; Pastori et al., 1991a) in Xenopus. Estrogen also induces vitellogenin II and apolipoprotein II mRNA expression in avian liver (Gordon et al., 1988). But this process differs in several ways from the vitellogenin mRNA induction seen in amphibian liver. During the 2nd day of hormonal treatment both of these mRNAs reach their new, higher steady-state levels, and turnover with a half-life of 13 hours in the presence of the hormone. Withdrawal of the hormone any time till the 3rd day does not alter their half-life values, indicating that mRNA stabilization does not contribute to estrogen-dependent accumulation of these mRNAs. However, if the hormone is withdrawn after 3 days, then these mRNAs decay rapidly with a half-life of 1.5 hours (Gordon et al., 1988). Thus, the half-lives of these mRNAs upon hormone withdrawal depend on the duration of prior hormone treatment. These results indicate that if estrogen treatment is continued for more than 3 days, it induces an mRNA destabilizing factor which remains inactive in the presence of estrogen but becomes activated following estrogen withdrawal.

EGF causes stabilization of α-actin, β-tubulin and EGF receptor mRNAs in human epidermoid carcinoma KB cells (Jinno et al., 1988). Treatment of H4-II-E rat hepatoma cells with insulin causes 57-fold drop specifically in albumin mRNA levels. Since the transcription rate decreases only about 5 fold, the process appears to occur mainly through a posttranscriptional mechanism (Straus and Takemoto, 1987). In the pituitary cell line, GH3, growth hormone mRNA gets stabilized upon treatment of the cells with thyroid hormone, T3 or glucocorticoids (Diamond and Goodman, 1985). Transfection experiments with chimeric constructs containing growth hormone structural gene under HSV-tk promoter also indicate that glucocorticoid-induced stabilization of growth hormone mRNA is accompanied by lengthening of its poly (A) tail (Paek and Axel, 1987). Similarly, the poly (A) tail of apoVLDLII mRNA also changes upon regulation of its stability by estrogen in hepatocytes of roosters (Cochrane and Deeley, 1988).

**Viral infection**

During viral infection, stability of both viral and host cell mRNA get regulated by the viral gene products. Half-lives of many adenovirus transcripts change during the course of infection. Some of the mRNAs generated by alternative splicing of the E1a and E1b gene primary transcripts are approximately 10 fold more stable late in infection, after DNA replication than early in infection (Wilson and Darnell, 1981). Some E1b gene products might also be in turn affecting the stability of late adenovirus mRNAs, because viruses with E1b gene mutations exhibit lesser cytoplasmic accumulation of late mRNA than their wild-type counterparts (Babiss and Ginsberg, 1984; Pilder et al., 1986). Adenovirus also affects cellular mRNAs. Although most host mRNAs are inefficiently transported in adenovirus infected cells, the stabilities of many of the mRNAs that
reach cytoplasm are modulated. For example, actin mRNA is 2-fold more stable in infected than in uninfected HeLa cells (Khalili and Weinmann, 1984). Herpes simplex virus, affects the stability of majority of cellular mRNAs. Within 1 to 2 hours after infection, host protein synthesis declines by 70 to 90% and most of the host cell mRNAs are rapidly degraded, a process called ‘virus-induced host shutoff’ (Fenwick and Clark, 1982). Even stable mRNAs (whose half-lives are close to cell generation time) get destabilized in this manner (Nishioka and Silverstein, 1978; Mayman and Nishioka, 1985; Strom and Frenkel, 1987). The mechanisms of this destabilization and host shutoff are complex and vary from cell to cell (Nishioka and Silverstein, 1978; Fenwick and Clark, 1982; Isom et al., 1983). Nevertheless, this process, with important exceptions, is very general affecting almost all host cell mRNAs (Oroskar and Read, 1989).

Several other viruses including influenza (Inglis, 1982), vaccinia (Rice and Roberts, 1983) and La Crosse (Raju and Kolakofsky, 1988) affect host cell mRNA stability. Influenza virus has evolved a unique mechanism for its replication linking host cell mRNA degradation with viral mRNA synthesis. The virion contains an endonuclease that cleaves host cell mRNAs 10 to 14 nucleotides downstream from their cap sites (Plotch et al., 1981). The resulting oligonucleotides, which include the mRNA cap, are then used by the RNA replicase as primers for viral mRNA synthesis. The large fragments of uncapped cell mRNA that are generated by the endonucleolytic cleavage are probably rapidly degraded.

Miscellaneous signals affecting message stability

Availability of nutrients in the environment is known to affect mRNA stability. In yeast, the meiosis specific, SPO11 and SPO13 mRNAs get stabilized when cells are shifted from rich media to sporulation media which lack glucose and nitrogen source (Surosky and Esposito, 1992). Similarly, decay rates of mRNAs encoding glycolytic enzymes alter when yeast cells are shifted from media containing glucose to media with lactose (Moore et al., 1991). Deprivation of H4 rat hepatoma cells for an essential amino acid (leucine, methionine, tryptophan or phenylalanine) under conditions in which the cells remain highly viable, leads to decrease in cytoplasmic albumin mRNA levels. The decrease is only partly accounted for by suppression of transcription and most of the down regulation appears to be through a posttranscriptional mechanism (Straus and Takemoto, 1988).

Ionic environment within the cell can also affect gene expression and mRNA levels. Increase in intracellular calcium and barium induces c-fos mRNA accumulation in an adrenal carcinoma cell line (Morgan and Curran, 1986). Dehydration leads to increase in poly (A) tail length of vasopressin mRNA in rodent hypothalamus (Zingg et al., 1988). The mechanism behind these processes are unclear, but likely to involve ion-induced changes in mRNA stability. Iron availability to cells is also known to regulate the stability of transferrin receptor mRNA and this has been discussed under ‘RNA binding proteins’ in section 1.4.2.

Human interferon mRNA is stabilized (leading to the elevation of interferon levels) when cells are exposed to poly (riC) (Nir et al., 1984). GM-CSF (granulocyte-macrophage colony stimulating factor) and G-CSF mRNAs are stabilized in macrophages and monocytes respectively,
cultured with LPS (Ernst et al., 1989; Thorens et al., 1987). When antibodies to CD28, a T-cell surface protein are added to T-cells exposed to CD3 antibodies, four cytokine mRNAs (IL2, tumor necrosis factor-α, GM-CSF, and interferon-gamma) are specifically stabilized (Lindsten et al., 1989). Calcium ionophore and phorbol ester treatments stabilize GM-CSF and IL3 mRNAs in PB-3c cells (Wodner-Filipowicz and Moroni, 1990) and phorbol ester stabilizes GM-CSF mRNA in human lymphoblastic cells (Shaw and Kamen, 1986).

Interferon is known to facilitate the degradation of viral mRNAs by activating RNase L and this has been discussed in section 1.4.3.

1.4 FACTORS AFFECTING mRNA TURNOVER

Half life of mRNAs is determined by complex interactions that depend on the primary and secondary structural features of the mRNA, its rate of translation, its location in cytoplasm, and types and amounts of cellular RNases, RNA binding proteins and regulatory factors that might be involved in the process of RNA turnover. The mechanism of mRNA turnover and its regulation are poorly understood. However, several factors affecting mRNA degradation are known. These can roughly be divided into cis- and trans- acting factors.

1.4.1 Cis-acting factors

Structural features present in almost all regions of the mRNA have been implicated in determination of half-life of the mRNA. Mutations in these cis-acting elements have been shown to alter the mRNA turnover. The nature of these elements vary in different mRNAs, and some mRNAs have more than one such cis-acting element located at different parts of the molecule. The cis-acting elements may include the secondary and tertiary structure of the mRNP, conformation of the mRNA in polysomes, nature of the bound factors etc., together with primary structure of the mRNA.

mRNA size

Earlier studies analyzing total mRNA populations indicated that larger the size of the mRNA, greater its sensitivity to nuclease and lesser the stability (Singer and Penman, 1973; Meyuhas and Perry, 1979; Santiago et al., 1986). However, later studies on specific transcripts could not confirm this (Ross and Pizarro, 1983; Harland and Misher, 1988; Shapiro et al., 1988). For example, β- and δ- globin mRNAs are similar in size and except for 3' region, are similar in sequence also. But they differ in their half lives significantly (Ross and Pizarro, 1983). Histone mRNA is small and unstable, while vitellogenin mRNAs are large and stable. Half-life data of yeast mRNAs also indicate the absence of any correlation between mRNA size and intrinsic stability (Peltz and Jacobson, 1993).
5'-cap structure

The 5' termini of all eukaryotic mRNAs possess a 7-methyl guanosine triphosphate linked to the first transcribed nucleotide by a 5'-5' triphosphate linkage. This structure is called cap and it is added immediately after start of transcription by the nuclear enzyme guanylyl transferase. mRNAs with 5'-cap have been shown to be more stable than those which lack the 5'-cap, both in cells and in vitro. (Furuichi et al., 1977; Peltz et al., 1987). The enzyme responsible for the rapid decay of the uncapped mRNAs has not been identified. But, it is likely to be a scavenger nuclease involved in the degradation of RNA fragments generated during RNA processing or turnover rather than an enzyme involved in the initial steps of mRNA decay; because, at least in vitro, this activity can be separated from the nuclease activity(s) that degrades capped mRNAs (Peltz et al., 1987). Moreover, in the mRNAs studied so far, the initial degradatory events have been found to occur near the 3'-ends (Ross et al., 1986; Brewer and Ross, 1988; Wilson and Treisman, 1988; Brown and Harland, 1990; Binder et al., 1989).

Poly (A) tail

Most of the mRNAs in eukaryotes possess the poly (A) tail at their 3' termini. The functions of the poly (A) tail are not fully clear, but it probably is involved in nuclear RNA processing and transport, mRNA stability and protein synthesis (Brawerman, 1981; Sachs, 1990). The observation that some mammalian viruses replicate exclusively in cytoplasm and synthesize polyadenylated mRNAs supports the notion that the poly (A) tail has a cytoplasmic function (Armstrong et al., 1972; Ehrenfeld and Summers, 1972; Weiss and Bratt, 1974). Moreover, it has also been shown that mRNAs lacking poly (A) tail can be transported to cytoplasm efficiently (Zeevi et al., 1982) and that splicing can occur without polyadenylation (Zeevi et al., 1981, 1982).

Two major observations suggest that poly (A) tail is one of the determinants of mRNA stability.

(1) In general, polyadenylated mRNAs turnover slowly compared to the deadenylated counterparts, indicating that probably the poly (A) tail protects the mRNA from nuclease attack. Studies on the half lives of polyadenylated and deadenylated mRNAs have been done following microinjection in amphibian oocytes, and they show that the histone mRNAs which normally lack poly (A) tail are stabilized when polyadenylated (Huez et al., 1978). Similar experiments with other mRNAs performed in cells or in vitro are also supportive of these results (Huez et al., 1975; Nudel et al., 1976; Drummond et al., 1985; Peltz et al., 1987; Harland and Misher, 1988).

(2) In the case of several mRNAs, degradation has been shown to be preceded by poly (A) tail removal in intact cells (Wilson et al., 1978; Colot and Rosbash, 1982; Restifo and Guild, 1986; Green and Dove, 1988; Wilson and Treisman, 1988; Swartout and Kinniburgh, 1989). Though this need not necessarily mean a cause-and-effect relationship between the two processes, the kinetics is highly suggestive in many cases. Some important examples which illustrate this point are as follows.
(a) When fibroblasts are starved for nutrients, and then cultured with serum, a transient induction of c-fos gene transcription occurs. The c-fos mRNA levels then increase and later fall as the transcription rate declines. During this period, when the mRNA is being degraded, its poly (A) tail also gets gradually shortened and ultimately removed completely. Moreover, deletions in the c-fos gene that lead to an increase in the mRNA half-life also cause increase in the half life of the poly (A) tail, indicating that poly (A) tail removal reflects mRNA decay (Wilson and Treisman, 1988).

(b) Elongation of human growth hormone mRNA poly (A) tract occurs during the same time period in which the mRNA is stabilized by glucocorticoids (Paek and Axel, 1987).

(c) The degradation of c-myc mRNA also appears to be preceded by poly (A) tail shortening in vivo (Swartout and Kinniburgh, 1989). When this was studied in an in vitro system (Brewer and Ross, 1989), three kinetic steps were identified, with poly (A) tail removal being the first step followed by the degradatory steps.

(d) Estrogen induced stabilization of apoVLDLII mRNA in avian liver is accompanied by lengthening of the poly (A) tail (Cochrane and Deeley, 1988).

Observations have also been made which indicate that poly (A) tail has multiple functions or is unrelated to stability and they are as follows.

(a) In slime mold, Dictyostelium discoideum, the turnover rates of several mRNAs fail to correlate with their poly (A) tail lengths (Palatnik et al., 1980; Shapiro et al., 1988).

(b) Some mRNAs remain relatively stable in spite of losing their poly (A) tails rapidly (Krowczynska et al., 1985; Baker et al., 1989).

(c) In Xenopus oocytes, polyadenylated and deadenylated interferon mRNAs have similar functional half lives (Sehgal et al., 1978).

So, it is important to note that not all deadenylated mRNAs are rapidly degraded.

**Sequences of the 3'-untranslated region**

Several mRNAs have sequences affecting their decay rates in their 3'-untranslated regions (UTRs). For example, β-globin mRNA is 6 times more stable than δ-globin mRNA in human bone marrow cells (Ross and Pizarro, 1983). But they are 92% homologous in their 5'-UTR and coding region sequences. Whereas, in the 3'-UTR sequence they differ by 50%. This indicates that the difference in their half lives is probably caused by differences in their 3'-UTR sequences. Several experiments involving transfection of cells with chimeric gene constructs followed by the study of stabilities of the resultant mutant transcripts as compared to the wild type mRNA, have been performed to analyze the mRNAs of proto-oncogenes (Treisman, 1985; Jones and Cole, 1987), histones (Pandey and Marzluff, 1987), transferrin receptor (Mullner and Kuhn, 1988), genes involved in stress (Simcox et al., 1985) and inflammatory responses (Shaw and Kamen, 1986), and TFIIIA genes (Harland and Misher, 1988) and they have strongly indicated the importance of the sequences of the 3'-UTRs in mRNA stability. For example, c-fos mRNA is very labile, and globin mRNAs are stable. However, a chimeric mRNA with 5' sequences of β- or δ-globin transcript and 3' sequences from c-fos 3'-UTR was unstable (Treisman, 1985; Kabnick and
Housman, 1988) indicating that the 3'-UTR of c-fos contains sequences that can impart instability to the mRNA and that they can function even when present in the context of heterologous sequences.

In yeast, the instability-conferring sequences present in several intrinsically unstable mRNAs have been determined by similar experiments involving chimeric mRNAs containing part of the sequences of a stable reporter mRNA (PGK1 or ACT1 mRNA) fused in frame to some of the sequences from the unstable mRNA under study. These experiments have revealed the presence of instability elements in the 3'-UTRs of STE2, STE3, HTB1 and MFA2 mRNAs (Heaton et al., 1992; Lowell et al., 1992; Muhlrad and Parker, 1992; Peltz and Jacobson, 1993). Since, experiments have also indicated that the 3'-UTR of MFA2 mRNA controls poly (A) shortening (Lowell et al., 1992; Muhlrad and Parker, 1992), it is possible that at least in this mRNA, the instability element causes destabilization through its effect on poly (A) shortening. Insertion of poly (G)\textsubscript{18} (but not other ribopolymers) into the 3'-UTR of PGK1 mRNA has been shown to cause 2 fold stabilization of the mRNA (Vreken et al., 1991). Since this insertion does not affect the translation of this mRNA, it is unlikely that this stabilization is a secondary effect of alteration in translatability. The significance of this observation is unclear.

The stability determinants of the 3'-UTR are varied in nature. The most well studied among them are the AU-rich segments. Several unstable mRNAs including those coding for proto-oncogene products and proteins involved in cell growth and inflammation contain these AU-rich sequences in their 3'-UTRs (Caput et al., 1986; Shaw and Kamen, 1986; Kabnick and Housman, 1988). The AU-rich sequence element of GM-CSF mRNA 3'-UTR, when placed in the 3'-UTR of the otherwise stable β-globin mRNA, destabilizes the latter significantly (Shaw and Kamen, 1986). Conversely, deletion of AU-rich segment from the 3'-UTR has been shown to markedly stabilize several unstable mRNAs (Jones and Cole, 1987; Reeves et al., 1987; Kabnick and Housman, 1988; Wilson and Treisman, 1988). These results indicate that the AU-rich region of the 3'-UTR contains destabilizer element which makes the mRNA (intrinsically) unstable. However, that the actual process may be more complex than this was indicated by the following observations:

(a) Deletion of AU-rich element from the 3'-UTR has little or no effect on the half life in some mRNAs like TFIIIA mRNA (Harland and Misher, 1988).

(b) Though most mRNAs bearing the AU-rich element in their 3'-UTRs are unstable (half life of 10 to 30 minutes), some are quite stable. E.g., the mRNA of proto-oncogene c-sis contains the AU-rich element in its 3'-UTR but has a half life of 3 hours (Press et al., 1988).

(c) Some mRNAs with AU-rich elements are unstable but not under all conditions. E.g., in T lymphoblasts, GM-CSF mRNA is unstable (half life ≤30 minutes) only when the cells are cultured in phytohemagglutinin; but exhibits a half life of >2 hours, when the cells are cultured in 12-O-tetradecanoyl phorbol-13-acetate (TPA; Shaw and Kamen, 1986).

These observations indicate that the AU-rich sequence elements of the 3'-UTR may be necessary but not sufficient to impart instability to mRNA. They probably need to bind some additional trans-acting factor(s) to exert their effect. For example, considering the case of GM-
CSF mRNA (Shaw and Kamen, 1986), its stability in cells cultured with TPA could be because in those cells such a trans-acting factor is inactive or absent.

Experiments with c-fos mRNA have indicated that the AU-rich sequence elements may have a role to play in deadenylation of mRNA also. The c-fos mRNA which is known to be very labile was shown to have an instability determinant in the coding region in addition to the AU-rich segment of its 3'-UTR. When the former element is deleted, both rapid degradation and poly (A) shortening of the mRNA depended on the presence of the AU-rich segment which contained three repeats of the pentanucleotide sequence AUUUA (Wilson and Treisman, 1988; Shyu et al., 1991). Interestingly, when three U to A mutations were introduced into the 3' -UTR in such a way that these three AUUUA repeats are disrupted without affecting the AU-richness, then the resultant mutant transcript exhibited higher stability than wild type c-fos mRNA but still underwent deadenylation as rapidly as the wild type transcript (Shyu et al., 1991). This indicated that deadenylation may be necessary but not sufficient for mRNA degradation and also that the dependence of poly (A) shortening on AU-rich element is not through the latter's effect of mRNA stability but rather direct.

The 3'-UTR of Xlhbox2B mRNA contains a repetitive sequence element which acts as the target for sequence-specific in vivo endonucleolytic cleavages in Xenopus oocytes. This sequence element has been shown to be sufficient to confer endonucleolytic cleavages to heterologous mRNA sequences attached to it (Brown and Harland, 1990).

In some cases, 3'-UTR bears sequence elements that are involved in the regulation of stability of the mRNA under some specific physiological conditions. In other words, they determine the extrinsic stability of the mRNAs. E.g., histone mRNA stability is regulated in cell cycle dependent manner. During the end of S phase, when DNA synthesis rate starts falling, histone mRNA gets destabilized. Inhibition of DNA synthesis by exogenously added inhibitors has also been shown to destabilize histone mRNA. Experiments involving transfection of cells with chimeric and mutated gene constructs, revealed that a histone specific, short, conserved stem-loop at the 3'-end of the mRNA is necessary for the DNA synthesis-dependent regulation of histone mRNA stability (Luscher et al., 1985; Levine et al., 1987). When this potential stem loop is placed at the 3'-end of globin mRNA, the resultant chimeric transcript exhibited DNA synthesis dependent regulation of its stability just like histone mRNA (Pandey and Marzluff, 1987). Mutant histone mRNAs with poly (A) tail or other additional sequences placed on the 3' side of the stem loop, however, do not get regulated as expected (Luscher et al., 1985; Levine et al., 1987). Experiments have also suggested that translation and polysome conformation are important for normal functioning of this stem loop structure. Because stabilities of mutant histone mRNAs with premature termination codon causing translation to stop at >300 nucleotides upstream to the stem loop or lacking normal termination codon (leading to translation into regions which are UTRs in wild type transcripts), are also not regulated like the wild type histone mRNA (Capasso et al., 1987; Graves et al., 1987).

Iron deprivation of cells is known to result in upregulation of transferrin receptor expression through the stabilization of the transferrin receptor mRNA. This is brought about by specific
elements called iron responsive elements (IREs) present in the 3'-UTR of that mRNA (Mullner and Kuhn, 1988; see under 'RNA binding proteins' in section 1.4.2).

ID elements are approximately 80 nucleotides long repetitive sequences present in 3'-UTR of several mRNAs, some of which are induced by serum stimulation of quiescent fibroblasts. If an ID element from an mRNA is placed in the 3'-UTR of another mRNA which is not serum inducible, the chimeric mRNA acquires the property of inducibility upon serum stimulation (Glaichenhaus and Cuzin, 1987). This effect appears to be brought about at a posttranscriptional level, perhaps by regulation of mRNA stability.

**Sequences of the coding region and translation**

Coding region sequences have been shown to be important in determining the stability of several mRNAs. In most of the cases, their influence on mRNA turnover is through their effects on translation.

In yeast, presence of nonsense mutations in mRNA is known to cause destabilization of the mRNA and this occurs in a position dependent manner; i.e., the mutations closer to the 5'-end of the mRNA have greatest effect and those which are close to the normal translational termination site have almost no effect (Losson and Lacroute, 1979; Peltz et al., 1993). That this destabilizing effect of nonsense mutations (termed nonsense mediated mRNA decay) is due to the premature translational termination caused by them is shown by the fact that the nonsense mutation containing mRNA is stabilized in a strain expressing the corresponding suppressor tRNA (Losson and Lacroute, 1979). Moreover, cycloheximide treatment stabilizes nonsense mutation containing PGK1 mRNAs which are otherwise very unstable compared to wild type PGK1 mRNA (Peltz et al., 1993). Extensive studies on the position dependence of nonsense mediated decay performed with yeast PGK1 mRNA (Peltz et al., 1993) have indicated the following.

(a) Specific sequences present on the 3' side of the nonsense mutation are required for nonsense mediated decay and they act probably by causing translational reinitiation.

(b) The inability of 3' nonsense mutations to trigger destabilization is due to the presence of specific stabilizing sequences which when translated cause inactivation of the nonsense mediated decay pathway.

Some of the genes coding for trans-acting factors involved in nonsense mediated decay have also been identified in yeast (Leeds et al., 1992). In the case of higher eukaryotes also it is known that nonsense mutations present in β-globin genes (in case of β+ thalassemia patients) cause a drastic reduction in β-globin mRNA half-life in bone marrow erythroid cells (Maquat et al., 1981). In the case of mouse triosephosphate isomerase mRNA and hamster dihydrofolate reductase mRNA, nonsense mutations have been shown to cause a significant drop in the mRNA steady state levels; however, this occurs not through mRNA destabilization but probably through changes in the rate of RNA splicing or transport of RNA across nuclear membrane (Cheng et al., 1990; Urlaub et al., 1989).
Studies on tubulin mRNA have clearly illustrated the requirement of translation for its degradation. Tubulin exists in cytoplasm in a dynamic flux between unpolymerized free tubulin heterodimers (made of one each of α- and β-tubulin polypeptides) and polymerized chains of tubulin or microtubules. The microtubules form major structural components of the cytoskeleton and mitotic spindle apparatus. The levels of tubulin mRNAs (and hence the rate of de novo tubulin synthesis) are inversely regulated as a function of free tubulin concentration in cytoplasm and several studies have suggested that this regulation occurs at the level of mRNA stability (Cleveland and Havercroft, 1983; Caron et al., 1985; Pittenger and Cleveland, 1985; Pachter et al., 1987). Extensive point mutagenesis performed by Yen et al (1988) indicate that only those mRNA sequences which when translated in-frame encode the N-terminal peptide, Met-Arg-Glu-Ile (MREI), and contain an open reading frame that extends for at least 42 codons (enabling the emergence of whole of that N-terminal tetrapeptide region from the ribosome), are subject to regulation by the free tubulin heterodimer concentration. α- and β-tubulins are the only 2 known proteins with this amino acid sequence motif in their N-termini. Further, protein synthesis inhibitors that induce dissociation of mRNA from polysomes (puromycin and pactamycin) cause decrease in tubulin mRNA degradation; whereas, inhibitors that trap the mRNA onto the polysomes (cycloheximide) have opposite effect (Pachter et al., 1987). These results indicate that the N-terminal tetrapeptide sequence, MREI, while emerging from the translating ribosomes somehow gives the signal for triggering the cotranslational degradation of the tubulin mRNAs.

Additional examples of translation dependent mRNA degradation are as follows. Human c-myc mRNA has some instability sequences in its coding region, which are functional only when they are present as a part of an efficiently translated open reading frame (Wisdom and Lee, 1991). The low stability conferred to yeast MA7α1 mRNA by a 42 nucleotide long sequence element present in its coding region is abolished if translation through that element is prevented by introducing a nonsense mutation on the 5' side of that element (Parker and Jacobson, 1990). This sequence element and the coding region instability element of HIS3 mRNA (another intrinsically unstable mRNA in yeast) were found to overlap with regions containing clusters of rare codons (Parker and Jacobson, 1990; Peltz and Jacobson, 1993). In the case of yeast PGK1 mRNA also, it has been shown that replacement of N-terminal 39% of the coding sequence with rare yeast codons, leads to a 3-fold drop in the mRNA abundance (Hoekema et al., 1987). Moreover, in yeast, studies have also shown that, in general, unstable mRNAs contain significantly higher percentage of rare codons than stable mRNAs (Herrick et al., 1990). Here, it is important to note that rare codons are known to slow down translational elongation and cause ribosome stalling.

In the case of mammalian systems, mRNA degrading activities have been shown to be associated with polysomes by in vitro experiments (Ross et al., 1987; Pastori et al., 1991b; Pastori and Schoenberg, 1993). Such polysome-associated nature of mRNA degrading activities also indicates that translation and mRNA degradation are probably related.

Apart from the above examples, drugs that block initiation and elongation of translation have been shown to cause increase in steady state levels of several mRNAs (Ernest, 1982; Dani et al., 1984; Stimac et al., 1984) often because of mRNA stabilization. This phenomenon is called...
superinduction of mRNA. However, here it is important to note that this need not always mean a direct requirement of translation for the degradation of that particular mRNA. Because, it could also be the result of secondary effects of the global translational block caused by these drugs. For example, superinduction of an mRNA can occur if the trans-acting factors (RNase(s) and/or their associated factors) necessary for the degradation of that mRNA, happen to be very unstable. This possibility can be ruled out if superinduction can be shown under conditions in which only the translation of the mRNA of interest is specifically inhibited. The c-myc mRNA superinduction has been attributed to the lability of a destabilizer activity (Brewer and Ross, 1989). However, in yeast, the cycloheximide induced stabilization (see above) of mutant PGK1 mRNAs containing premature nonsense codons occurs due to a direct requirement of translation for the degradation of these mRNAs (Peltz et al., 1993). At last, histone mRNA is a special case wherein, superinduction caused by cycloheximide (Baumbach et al., 1984; Stimac et al., 1984) is due to the prevention by cycloheximide of the accumulation of histone protein which causes destabilization of its own mRNA (Osley and Hereford, 1981; Peltz and Ross, 1987) through an autoregulatory circuit.

Studies have also shown that the instability-conferring AU-rich sequence elements of mRNA 3'-UTRs function in a translation dependent manner. For example, an altered β-globin mRNA bearing an AU-rich destabilizer element (taken from the 3'-UTR of GM-CSF mRNA) inserted in its 3'-UTR, decays rapidly and also associates with a 20S complex (whose nature is unknown) only if the mRNA is translated (though the translation for a short distance is sufficient for this) and ribosomes do not translocate through the AU-rich element itself (Savant-Bohnsale and Cleveland, 1992). Such an altered β-globin mRNA is also known to get superinduced by cycloheximide (Shaw and Kamen, 1986).

Mutations in the gene coding for tRNA nucleotidyl transferase (the enzyme that catalyzes the 3'-terminal CCA addition to the tRNA molecules during their maturation) have been shown to stabilize the unstable and moderately stable mRNAs in yeast (Aebi et al., 1990). At nonpermissive temperatures, the mutant, ts352, exhibits accumulation of the defective, short, immature tRNA molecules (Aebi et al., 1990), loss of protein synthesizing capacity and stabilization of many of the intrinsically unstable and moderately stable mRNAs (Peltz et al., 1992). The block in protein synthesis also accompanies a shift of the mRNAs to heavier polysomes which is also observed in cycloheximide-treated cells (Peltz et al., 1992) and is diagnostic of a reduction in the rate of translational elongation (Rose and Lodish, 1976; Schneider et al., 1984). The mRNA stabilization caused by the mutation was general (affecting all mRNAs examined, including those that were intrinsically unstable and moderately stable) and not limited to few specific mRNAs. Similar effects were also found in cycloheximide-treated cells. Thus, both cycloheximide treatment and ts352 mutation appear to influence mRNA turnover by affecting translation. But whether the effect of protein synthesis on mRNA turnover is direct or indirect is yet to be worked out.

Apart from MATα1 and HIS3 mRNAs discussed above, in yeast, the intrinsically unstable STE2, STE3 and CDC4 mRNAs also bear their instability determinants in their coding regions, as deduced by experiments involving chimeric mRNAs (Heaton et al., 1992; Herrick and Jacobson,
c-fos mRNA contains instability-conferring sequences both in the coding region as well as in its 3'-UTR. Even though β-globin mRNA is 40 times more stable than c-fos mRNA, a chimeric mRNA with c-fos coding region and UTR sequences from the β-globin mRNA (Shyu et al., 1989) is very unstable indicating that this coding region instability element can function even in heterologous context. However, the mechanism by which these coding region sequences confer instability to c-fos mRNA is not clear yet.

Sequences of the 5'-untranslated region

Unlike for 3'-UTR sequences, the involvement of 5'-UTR sequences in mRNA turnover has been shown only in a few cases. They are as follows.

As a result of reciprocal translocation, the c-myc gene is truncated in the neoplastic cells and hence, the c-myc mRNA produced in these cells lacks exon 1 (containing 5'-UTR) sequences but contains intron sequences linked to all of the coding and 3'-UTR sequences. This mRNA is 3 to 8 times more stable than the normal c-myc mRNA (Piechaczyk et al., 1985; Rabbits et al., 1985). The translational efficiency was found to be similar for the mutant and normal mRNAs (Butnick et al., 1985; Parkin et al., 1988), indicating that the stabilization is not due to any change in translational efficiency but due to either the loss of 5'-UTR sequences or the addition of intronic sequences. c-myc mRNA lacking exon 1 sequences is 3 times more stable than full length mRNA in vitro, indicating that the 5'-UTR has instability sequences (Pei and Calame, 1988). However, experiments with chimeric c-myc genes lacking exon 1 but containing different amounts of intron 1 sequences (Jones and cole, 1987; Bonnieu et al., 1988), have indicated that if ≥600 nucleotides of intron 1 are present in the chimeric mRNA, it exhibits 5 times higher stability than the unmodified mRNA, implying that the higher stability of the mutant mRNA in the neoplastic cells could also be due to the stabilization conferred to the message by intronic sequences. Experiments have also indicated that the exon 1 of c-myc gene contains sequence elements essential for estrogen dependent posttranscriptional regulation of c-myc mRNA. In the estrogen responsive cell line, MCF-7, the wild type c-myc mRNA levels increase upon estrogen treatment. Whereas, the c-myc mRNA lacking sequences of the exon 1 fails to respond to estrogen (Santos et al., 1988).

When a 40 nucleotide long sequence from 5'-UTR of c-fos mRNA is linked to coding and 3'-UTR sequences of β-globin mRNA, the resulting chimeric transcript is less stable than wild type β-globin mRNA, indicating that the 5'-UTR sequences of c-fos mRNA might contain a destabilizing element (Kabnick and Housman, 1988). In human microvascular endothelial cells, a truncated form of platelet derived growth factor-B mRNA which lacks some 5'-UTR sequences (but makes normal protein product) but not the full length mRNA is stabilized by cycloheximide treatment (Fen and Daniel, 1991). The 5'-UTR sequence which the truncated form lacks is known to have translation inhibiting effect on autologous or heterologous downstream sequences.
Posttranscriptional base modifications

The involvement of covalent posttranscriptional base modifications as one of the structural features of the cis-acting elements necessary for their stability determining function is an intriguing conceptual possibility. Because it is known that the nucleotides following the cap structure as well as many internal adenine residues can be methylated, and adenosine residues can be enzymatically converted to inosines. Kimelman and Kirschner (1989) have suggested that presence of such inosines may tag an mRNA for degradation.

1.4.2 RNases and other trans-acting factors

As mentioned already, very little is known about the trans-acting factors involved in mRNA turnover. Though several RNases from various eukaryotic organisms have been purified and studied in detail, information on their relationship with mRNA turnover is very limiting. Only in a few cases the RNase activities involved in specific mRNA degradation have been identified and partially characterized. The in vitro mRNA decay reactions have been very useful in the studies on trans-acting factors. mRNA binding proteins involved in stability regulation have also been studied in a few cases.

Role of poly (A) binding protein

The poly (A) binding protein (PABP) is a constitutive factor that can influence mRNA stability (Blobel, 1973; Adam et al., 1986; Sachs et al., 1986). It binds to poly (A) with very high specificity and may affect mRNA turnover through its effect on poly (A) metabolism (Bernstein and Ross, 1989). The poly (A)-PABP complex exists as an ordered structure with a periodicity of 25 to 27 A's (Baer and Kornberg, 1983). The amino terminal half of the protein contains 4 separate poly (A)-binding domains, any one of which can bind to poly (A). PABP can migrate off of one poly (A) tract and onto another (Bernstein and Ross, 1989).

Studies on the role of poly (A) tail in mRNA turnover have shown that, the presence of poly (A) tail correlates with higher mRNA stability and vice versa and the shortening and removal of poly (A) tail often precedes mRNA degradation in several cases (see under 'poly (A) tail' in section 1.4.1).

On the other hand, experiments involving in vitro mRNA decay reactions suggest that the poly (A)-PABP complex has a protective function in mRNA degradation (Bernstein et al., 1989). Normally stable mRNAs like β-globin, CAT and SV40 16S late, are degraded rapidly in these in vitro reactions if the extracts used in the reaction are depleted of PABP, by chromatography through poly (A) sepharose column or by the addition of excess of competitor poly (A). Addition of purified PABP to such PABP-depleted reactions, leads to restabilization of these mRNAs. However, histone mRNA (which normally lacks poly (A) tail) and deadenylated β-globin mRNA fail to get stabilized by such addition of purified PABP to PABP-depleted in vitro reaction, indicating that the stability conferred to the mRNAs by PABP specifically requires poly (A) tracts of the mRNAs. Moreover, addition of other RNA binding proteins does not restabilize the mRNAs.
in reactions with depleted extracts. Thus, the *in vitro* results indicate that poly (A) tail alone is necessary but not sufficient for protection of mRNA from rapid destruction. The poly (A)-PABP complex seems to be involved in protection rather than poly (A) alone. However, experiments performed in yeast, led to a different conclusion (Sachs and Davis, 1989). Here, steady state poly (A) tract lengths were studied in strains in which PABP expression can be experimentally manipulated, as compared to wild type strains. When the PABP expression was inhibited in these strains the average poly (A) tail length increased from a wild type value of 20-60 A's to 40-90 A's in the PABP deficient cells. Strains lacking a functional PABP gene are not viable, but seven independent suppressor strains are viable without PABP. In these suppressor strains also, average poly (A) length is higher than in wild type. One of the suppressor genes encodes a ribosomal protein. Thus, these genetic data suggest that PABP actually promotes rather than slows poly (A) shortening, in contrast to the *in vitro* results. It is possible that the cell free extracts used in *in vitro* mRNA decay reactions are deficient in essential components that affect poly (A) metabolism in the cytoplasm. However, since PABP deficiency has pleiotropic effects in yeast mutants (including excess production of one or other ribosomal subunit), PABP deficiency may not be directly responsible for poly (A) lengthening observed in PABP deficient strains. This discrepancy between *in vivo* and *in vitro* results is yet to be resolved. It has been suggested that affinity of PABP for poly (A) could vary from mRNA to mRNA and it is determined by *cis* - or *trans* -acting sequences or factors (Peltz et al., 1987; Bernstein et al., 1989).

Apart from PABP, most of the other known *trans*-acting factors are those that are specific to one or few related mRNAs and do not act on all mRNAs. Many of these *trans*-acting factors are not characterized fully.

**mRNA-specific RNase activities**

RNase activities involved in specific degradation of certain mRNAs have been partially characterized in a few cases. The properties of the 3' → 5' exonuclease activity involved in cell cycle dependent decay of histone mRNA have been studied in the crude *in vitro* mRNA decay systems (Ross et al., 1987) containing polysomes or ribosomal salt wash as the source of the nuclease. The endoribonuclease activity that is responsible for the occurrence of specific *in vivo* cleavages in a repetitive sequence element present in 3'-UTR of *Xlhbbox2B* mRNA of *Xenopus* oocytes (Brown and Harland, 1990; see under 'sequences of the 3'-UTR' in section 1.4.1) has been detected in and partially purified from the oocyte lysates (Brown et al., 1993). These lysates were also found to have another factor which is developmentally regulated and is inhibitory to this RNase activity. Both the RNase and inhibitor activities could be detected in Drosophila embryo lysates also (Brown et al., 1993). An estrogen induced endonuclease activity of *Xenopus* liver has been shown to specifically degrade albumin mRNA *in vitro* and characterized partially in liver polysomal extracts made from estrogen treated animals (Pastori et al., 1991b; Pastori and Schoenberg, 1993). None of these three activities is inhibited by human placental RNase inhibitor (RI), which is a 45 kDa protein that binds tightly and inhibits pancreatic type RNases (Blackburn and Moore, 1982; see section 4.1). An RI-resistant RNase activity has also been partially purified from human T lymphocyte cell line, Jurkat, and shown to selectively act on IL2 mRNA *in vitro* (Hua et al., 1993). An RNase activity that preferentially hydrolyzes
mRNAs containing AU-rich instability sequences (see under 'sequences of the 3'-UTR' in section 1.4.1) has been detected in mouse macrophage lysates and partially characterized (Beutler et al., 1988). An RNase activity that is selective for transforming growth factor (TGF) β1 mRNA has been detected in post-nuclear extracts of U937 promonocytes (Wager and Assoian, 1990) and shown to be blocked by prior treatment of the cells with TPA, which is known to induce the differentiation of these cells accompanied by the stabilization TGF β1 mRNA. In plasmacytoma P3X cell lysates, an RNase activity that preferentially degrades wild type c-myc mRNA relative to truncated c-myc mRNA (which is known to be more stable than wild type transcript in vivo also; see under 'sequences of the 5'-UTR' in section 1.4.1) lacking sequences of exon 1 has been detected and partially characterized (Pei and Calame, 1988). It will be interesting to see if such RNase activities detected in different systems in relation to the degradation of different mRNA species are related or have any commonalities. However, to address this question they need to be characterized more thoroughly.

The XRN1 gene of yeast codes for a 5' → 3' exonuclease that degrades RNAs with 5' mono- and triphosphates, well and poorly respectively. Capped RNAs are resistant to this enzyme (Stevens, 1978). Deletion of this gene slows down the decay of several mRNAs (Peltz and Jacobson, 1993). But, since, this gene (Larimer and Stevens, 1990) was found to be identical to other independently isolated genes, DST1, SEP1 and KEM1 thought to be involved in DNA strand exchange during recombination or nuclear fusion (Peltz and Jacobson, 1993) and its deletion also affects rRNA processing (Stevens et al., 1991), it is not clear if the mRNA stabilization observed is a direct effect of the deletion of this gene.

**RNA binding proteins**

RNA binding proteins involved in mRNA turnover have also been detected (often using gel mobility shift experiments) in some cases. The most important among these is the IRE binding protein (IRE-BP) which is a very well studied RNA binding protein involved in both the stabilization of transferrin receptor mRNA and inhibition of ferritin mRNA translation (Klausner et al., 1993). Binding of IRE-BP to the several IREs distributed in the 3'-UTR of transferrin receptor mRNA hinders the rapid destruction of the transferrin receptor mRNA mediated by an instability element(s) also present in the 3'-UTR, resulting in the stabilization of transferrin receptor mRNA (Casey et al., 1989; Harford and Klausner, 1990). The IRE-binding activity of IRE-BP is regulated in cells in response to iron availability. During iron starvation of cells, affinity of IRE-BP increases resulting in stabilization of transferrin receptor mRNA and inhibition of ferritin mRNA translation and in iron-replete cells, the opposite situation results due to presence of IRE-BP in an inactive form. Experiments have suggested that the activation and inactivation mechanism of IRE-BP involves reduction and oxidation of some of its sulphydryl groups whose presence in the reduced state is essential for the IRE binding activity (Hentze et al., 1989; Mullner et al., 1992; Yu et al., 1992).

At least, two protein factors have been shown to specifically interact with a 56-nucleotide purine-rich sequence located at the 5'-end of the 0.32 kb coding region instability determinant of
c-fos mRNA (Chen et al., 1992). Cytoplasmic factors that specifically bind to the 3' AU-rich sequence motifs (see under 'sequences of the 3'-UTR' in section 1.4.1) of unstable mRNAs have been studied in a few cases. Lymphocyte cytoplasmic extract has been shown to contain a factor that binds (AUUUA)$_4$ with high specificity forming a very stable complex (Malter, 1989). Bohjanen et al. (1991) have identified a factor in human T cell cytoplasmic extracts that is specific for the 3' AU-rich sequences of lymphokine mRNAs but not c-myc mRNA and appears only upon stimulation of T cells with αCD3 antibodies. The kinetics of induction of this factor parallels the lymphokine mRNA expression which is also known to be transiently induced upon αCD3 stimulation. Upon costimulation of T cells with phorbol myristate acetate (which is known to stabilize GM-CSF mRNA) and αCD3 antibodies, the binding of this factor to GM-CSF mRNA was reduced. These results indicate that this factor may be involved in specific positive regulation of lymphokine mRNA decay. These workers also find another binding activity that interacts equally to the 3' AU-rich sequences of both c-myc and lymphokine mRNAs and is present in unstimulated, αCD3 stimulated and αCD3 and phorbol myristate acetate costimulated T cell cytoplasmic extracts in equivalent amounts (Bohjanen et al., 1991). On the other hand, Brewer (1991) has purified two polypeptides both of which bind specifically (either together or independently) to 3' AU-rich sequences of c-myc and GM-CSF mRNAs from S130 extracts of human erythroleukemia (K562) cells. Though these polypeptides do not themselves possess RNase activity, they were found to be essential for specific degradation of polysome associated c-myc mRNA in in vitro mRNA decay reactions, indicating that the RNase activity provided by the polysome fraction was aided by these factors to preferentially degrade c-myc mRNA.

**RNA as a trans-acting factor**

RNA molecules may also be involved in the control of message stability as trans-acting factors in some cases. This has been suggested in the case of p53 mRNA (Khochbin and Lawrence, 1989). The p53 mRNA levels get down regulated through a posttranscriptional control mechanism probably acting at the level of mRNA stability, when murine erythroleukemia cells are induced to differentiate. This regulation was localized to the nuclear compartment of the cells and it was further found that a 1.3 kb long nuclear antisense RNA complementary to the first intron of p53 gene is involved in this process. This RNA accumulated to high levels when the cells were induced to differentiate. That this RNA is not a p53 pre-mRNA maturation product was evidenced by its antisense orientation and its enrichment in poly (A)$^+$ fraction. The small nonpolysomal B2 RNAs transcribed from repetitive elements in mouse genome contain short regions that are complementary to the AU-rich segments of some unstable mRNAs (Clemens, 1987), and hence could be involved in the decay of these mRNAs.

**1.4.3 Intracellular location**

Cellular compartmentation of mRNAs has been shown to affect their stability in a few cases which are discussed below. This phenomenon has not been studied in detail.

The antiviral state induced by interferon arises by various mechanisms, one of which involves the activation of an endoribonuclease called RNase L (Content, 1987). This process
also requires double stranded RNA apart from interferon. Interferon induces the synthesis of (2'-5')-A₅ synthetase. This enzyme becomes activated by binding to double-stranded RNA and then catalyzes 2'-5'-oligo (A) synthesis. This oligomer in turn, binds to and thereby activates RNase L. RNase L has been purified and shown to degrade many single-stranded substrates \textit{in vitro} (Silverman et al., 1988). However, in cells infected with certain viruses at low multiplicities, viral mRNA seems to be degraded selectively (Nilsen et al., 1982). Experiments have suggested that the specificity of RNase L might result from its localized activation at the sites of viral RNA replication (Baglioni et al., 1984; Nilsen et al., 1982). For example, during the early stages of reovirus RNA replication, partial duplexes are formed between the template RNA and the nascent RNA being synthesized. Such partial duplexes might activate the synthetase and eventually the RNase L at the site of replication so that viral mRNAs at that site are selectively degraded without affecting mRNAs located elsewhere in the cell.

Histone mRNA is normally translated on free ribosomes since histone is a cytoplasmic protein. But it can be directed to membrane-bound ribosomes by replacing the initial part of its coding region with sequences coding for signal peptide of a secretory protein. Such a chimeric histone mRNA does not get destabilized in response to inhibition of DNA synthesis, though it contains the 3’ sequences necessary for DNA synthesis dependent stability regulation (Pandey and Marzluff, 1987; Zambetti et al., 1987; see under ‘sequences of the 3’-UTR’ in section 1.4.1). Similarly, the half-life of immunoglobulin mRNA is approximately five fold longer in mature plasma cells than in immature B cells, and the percentage of mRNA located on membrane-bound polysomes is proportionally greater in the plasma cells (Mason et al., 1988).

Thus all these results together indicate that intracellular location of an mRNA can influence its stability.

1.5 CURRENT MODELS AND PERSPECTIVES

Thus, from the forgoing discussion, the regulation of RNA turnover appears to be very complex as the mechanisms greatly vary with the nature of the mRNA, cell type, organism, etc. The nature of the intra- and extracellular signals that affect mRNA turnover, the role of the mRNA functioning as the cis-acting element (which mediates the regulation of turnover), the primary and secondary structural features of the cis-acting elements etc., all differ in different cases. However, some general points do emerge from these data and they have formed the framework for some hypotheses that could explain at least some aspects of the mechanism of mRNA turnover process. As far as the cis-acting elements determining the intrinsic stability of mRNAs are considered, the AU-rich sequence elements (see under ‘sequences of the 3’-UTR’ in section 1.4.1) of 3’-UTR form an important class of destabilizer elements present in several unstable mRNAs most of which share the property of coding for protein products whose expression is required only transiently for the cell. Such a widespread occurrence of these elements in several unstable mRNAs has led to the suggestion that the instability caused by these elements could be mediated by RNase(s) and/or regulatory factors which specifically recognize and bind to these elements and the search for such \textit{trans}-acting factors (see under ‘mRNA-specific RNase activities’ and ‘RNA binding proteins’ in section 1.4.2). The protection
given by the 5'-cap structure and the poly (A) tail which has been demonstrated in several different mRNAs is suggestive of the presence of 5' → 3' and 3' → 5' exonuclease activities respectively in the cells.

The relationship between translation and mRNA turnover (see section 1.4.1) needs a special mention. In fact, in almost every example of coding region sequence element affecting mRNA turnover, there is some evidence or the other indicating the dependence of that sequence element on the process of translation for its function. As mentioned in section 1.4.1 (under ‘sequences of the coding region and translation’), nonsense mediated decay of yeast PGK1 mRNA is controlled by two cis-acting elements (apart from the nonsense codon itself), the upstream stabilizer sequence and the downstream sequence element which triggers nonsense mediated decay. Experimental evidences indicate that both of these cis-elements require translation for their function. The downstream sequence element contains three ATG codons of which, two are essential for its function (Peltz et al., 1993). This suggested that this sequence element probably triggers nonsense mediated decay by providing sites for translational reinitiation which was supported by the following observations (Peltz et al., 1993).

(a) The aminoacid biosynthesis inhibitor 3-aminotriazole which is known to reduce the capacity of cells to reinitiate translation, inhibits nonsense mediated decay (i.e., stabilizes nonsense mutant mRNAs specifically).

(b) Insertion of stem-loop structure which is known to inhibit both translational initiation and reinitiation, on the 5' side of the two critical AUGs prevents nonsense mediated decay.

However, this sequence element has another important feature which invokes an alternate explanation for its mode of functioning. The sequences flanking the two critical AUGs (but not the third AUG codon which is not essential for the function of this sequence element), contain a 9-nucleotides long stretch that is complementary to a part of 18S rRNA (Peltz and Jacobson, 1993). Moreover, a 14-nucleotides long stretch in the coding region instability element of MATα1 mRNA is also complementary to almost the same region of 18S rRNA (Peltz and Jacobson, 1993). This suggests that these sequence elements may be triggering decay by causing mRNA: rRNA base pairing. rRNA-mRNA interaction has been proposed to occur during mammalian internal initiation (Sonnenberg, 1991).

As mentioned in section 1.4.1 (under ‘sequences of the coding region and translation’), studies in yeast (both general study of large number of mRNAs and study of individual mRNAs) also suggest a strong correlation between the presence of high proportion of rare codons in mRNA and instability of mRNA.

Thus, an important point that emerges from all these studies is that, ribosome stalling can lead to triggering of mRNA decay. Because, both presence of rare codons and rRNA:mRNA base pairing are known to have the potential to slow down translation and lead to ribosome stalling. Ribosome stalling can be expected to trigger mRNA decay by one or a combination of both of the following mechanisms.
(a) Activation of a ribosome-associated nuclease as a result of the conformational changes in the ribosome caused by stalling.
(b) Exposure to attack by an active, soluble or ribosome-associated nuclease of a specific recognition site on the mRNA as a result of ribosome stalling.

In the case of nonsense mediated decay of PGK1 mRNA, results are suggestive of a two site model in which, first site potentiates cleavage process by causing ribosome stalling or translational reinitiation and second site forms the actual position of nuclease attack. Here, the nuclease itself or a factor that favors the nuclease attack either directly (by being an activator of the nuclease) or indirectly (by being a translation initiation factor required for the reinitiation event which may trigger the decay) is proposed to be ribosome-associated. Such a factor has been hypothesized to fall off from the ribosome when the upstream stabilizer sequence is translated by the ribosome.

In the case of tubulin mRNA decay, a direct interaction of free tubulin heterodimers with N-terminus of nascent tubulin peptide has been proposed to cause either activation of ribosome bound nuclease or stalling of the ribosome on the mRNA leading to formation of unprotected gaps on mRNA which may become targets for nuclease(s) (Gay et al., 1989).

These data and the increasing number of studies which show the polysome associated nature of nuclease activities capable of degrading mRNAs with high specificity (see under 'mRNA-specific RNase activities' in section 1.4.2) together greatly support the idea of ribosome associated nuclease(s) being involved in mRNA degradation. Thus, translation mediated degradation of mRNAs involving the triggering of nucleolytic attack upon ribosome stalling or translational reinitiation could be considered as a fairly generalized model for mRNA decay.

Superinduction of mRNA and stabilization of various mRNAs in ts352 mutant background (see under 'sequences of the coding region and translation' in section 1.4.1) are phenomena which also indicate the dependence of mRNA degradation upon translation. However, in these cases, it is not clear if the stabilization observed is the primary effect of translation since, the translation block is general.

The coding region instability determinants of the MATα1 and CDC4 mRNAs and the coding region of the unstable CLN3 mRNA (whose instability element has not been worked out yet) show a 10 nucleotide homology (Peltz and Jacobson, 1993). Since this homologous sequence occurs in different reading frames in these mRNAs, its role must be at the RNA level. The presence of such homologous sequences in the instability determinants raises the possibility of their action through similar mechanisms.

An interesting point that emerges from the studies on the role of cis-acting factors in mRNA turnover is the dependence of the different cis-acting elements on each other for their function. The influence of AU-rich sequence element and other 3'-UTR sequences on deadenylation observed in c-fos mRNA and yeast MFA2 mRNA respectively and the dependence of the AU-rich sequence element itself on the translation of upstream sequences for its destabilizing function in
β-globin-GM-CSF chimeric mRNA (see under ‘sequences of the coding region and translation’ in section 1.4.1) illustrate this point. The effect of the binding of IRE-BP to the IREs of transferrin receptor mRNA on the function of the destabilizing element present in the 3'-UTR of the same mRNA is another important example discussed in section 1.4.2 (under ‘RNA binding proteins’). These studies also indicate that the effects of cis-acting elements on mRNA stability could be often indirect.

The involvement of cis-acting elements in mRNA stability does not always mean a direct involvement of the primary sequence. Because, in the case of some sequence elements the secondary or tertiary structural features rather than the primary structure may be essential for interaction with trans-acting proteins, and this has been shown in many cases of RNA-protein interaction (Jaffrey et al., 1993 and references therein). Moreover, in vivo, the mRNA occurs as mRNP and hence, the RNA-protein interactions and intramolecular RNA-RNA interactions which determine the final tertiary structure of the mRNP, may play a major role in controlling the exposure of any cis-acting element to the trans-acting factors that may bind to it. The proposed role of polysome conformation in the functioning of the stem-loop structure of the histone mRNA 3'-UTR is worth mentioning in this context. In any case, a cis-acting element can act either both as recognition and cleavage site for a nuclease or only as a recognition site for a trans-acting factor which when bound, influences nucleolytic attack at some other specific site as in the case of transferrin receptor mRNA (see under ‘RNA binding proteins’ in section 1.4.2). It is also possible that the degradation machinery after binding to the recognition sequence causes cuts at several nonspecific sites in the vicinity. Finally, as in the case of tubulin, the protein sequence coded by the cis-acting element rather than the RNA sequence may be important as a recognition element.

1.6 OPEN QUESTIONS

Considering the number of different mRNAs present in a cell and the variety of signals that can affect mRNA stability under different conditions, mechanisms of target specificity in mRNA turnover are unknown in most cases. The cis-acting elements present in the mRNA may play an important role in the specificity of mRNA degradation, i.e., enable the identification of target mRNA by the degradation machinery. But these elements, though necessary, may not be sufficient for specifying the target. So, a complete understanding of the mechanism of specificity would require information on the trans-acting factors about which very little is known. Whether different target mRNAs are identified by different specificity factors but degraded by the same nuclease complex or the nature of nuclease activity also varies with different mRNAs or groups of mRNAs is not clear. In other words, we do not know what are the steps that are common and what are the steps that are different in the turnover mechanisms of the vast number of different mRNAs that a cell contains. However, at least some examples are known of coordinated regulation of related mRNAs as a group (Pastori et al., 1991a) wherein it is likely that not only the signal but also the mechanism is probably common for all the mRNAs of the group. Due to the limiting knowledge of trans-acting factors, the mechanistic details of degradation are also not understood very well yet. Similarly, the mechanism by which the degradation machinery senses the physiological signal is also not known in most of the cases. However, in the case of
transferrin receptor mRNA whose stability is controlled according to iron availability to cells, a model has been proposed (Klausner et al., 1993) to explain the mechanism by which iron regulates the RNA binding activity of the IRE-BP (see under 'RNA binding proteins' in section 1.4.2), which mediates the iron-dependent regulation of the stability of this mRNA.

Whether any common sequence elements (like the AU-rich sequences of 3'-UTR) exist among the 5'-UTR and coding region cis-acting elements of various mRNAs can be known only when more and more of them are characterized.

The relationship between translation and mRNA turnover also needs further study. The exact molecular mechanism by which ribosome stalling triggers nucleolytic attack the mRNA needs to worked out. In the case of nonsense mediated decay of the yeast PGK1 mRNA also, it is an open question whether the downstream sequence element triggers nonsensemediated decay by virtue of its ability to cause translational reinitiation or interact with 18S rRNA by base pairing. Understanding the mechanism of these processes would enable one to get information on the nature of conformational changes that ribosomes undergo during the steps of translation and the consequences of such conformational changes. Though lot of cis-acting elements are known which impart instability to mRNAs, hardly any stabilizer sequences are known so far. In this connection, the stabilizer sequence which when translated, inactivates nonsense mediated decay of PGK1 mRNA is of special importance.

The identification and purification of the RNases and other trans-acting factors involved in mRNA turnover can be made possible in future by a combination of genetic and biochemical approaches. Identification of in vivo cleavage sites of mRNA and the study of their primary and secondary structural features may also give important information on the nature of the nucleases involved; but such studies have been done only in a few cases (Ross et al., 1986; Cochrane and Deeley, 1989; Binder et al., 1989; Brown and Hartland, 1990). The characterization of cleavage sites of large number of mRNAs would help to understand the commonalities in their decay processes.

Investigations have so far mostly centered around unstable mRNAs and mRNAs whose stability gets extensively regulated (i.e., those which almost do not have any intrinsic stability) due to technical reasons and stable mRNAs like the house keeping mRNAs (which have almost only intrinsic stability) have not been studied much. Such studies may throw light on the mechanisms of constitutive or basal mRNA turnover processes. The lack of such studies could be one reason why the mechanisms of general mRNA turnover are not understood. No generalized hypothesis that can explain the mechanism of intrinsic or extrinsic mRNA turnover has been put forth yet.
### Table 1: Components of Eukaryotic mRNA Turnover Regulation

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Signal</th>
<th>Effect</th>
<th>Determinants</th>
<th>Special features</th>
<th>References</th>
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<tr>
<td>Apo VLDL II mRNA in rooster liver</td>
<td>Estrogen</td>
<td>Stabilization</td>
<td>Poly (A) tail</td>
<td></td>
<td>Cochrane and Deeley, 1988; Gordon et al., 1988.</td>
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<tr>
<td>iTE2, STE3, ITB1 and (\Delta F A 2) mRNAs of yeast</td>
<td>Intrinsic instability</td>
<td>3'-UTR sequences</td>
<td>In MFA2 mRNA, poly (A) shortening is mediated by 3'-UTR sequences</td>
<td>Heaton et al., 1992; Lowell et al., 1992; Muhlrad and Parker, 1992; Peltz and Jacobson, 1993.</td>
<td></td>
</tr>
<tr>
<td>mRNAs coding for photo-oncogene products and proteins involved in growth, differentiation, development and inflammation</td>
<td>Intrinsic instability</td>
<td>AU-rich sequences of 3'-UTR</td>
<td></td>
<td>Caput et al., 1986; Shaw and Kamen, 1986; Kabnick and Housman, 1988.</td>
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<tr>
<td>Xihbox 2B mRNA of Xenopus oocytes</td>
<td>Probably development related</td>
<td>Destabilization</td>
<td>Specific repetitive sequence element of 3'-UTR</td>
<td>Endonuclease activity specific to the repetitive sequence element (partially purified from Xenopus oocyte lysates) and a developmentally regulated inhibitor (also detected in the oocyte lysate) of the endonuclease activity</td>
<td>Brown and Harland, 1990; Brown et al., 1993.</td>
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<tr>
<td>Transferrin receptor mRNA</td>
<td>Iron deprivation</td>
<td>Stabilization</td>
<td>Iron responsive elements (IRE's) and an instability element present in 5'-UTR</td>
<td>Iron responsive element binding protein (IRE-BP)</td>
<td>Occupation of the IREs by the IRE-BP hinders the destabilizing function of 3'-UTR instability element. IRE binding activity of IRE-BP is increased during iron starvation as a result of reduction of essential sulfhydryl groups of IRE-BP and vice versa</td>
<td>Casey et al., 1989; Hentze et al., 1989; Harford and Klausner, 1990; Mullner et al., 1992; Yu et al., 1992; Klausner et al., 1993.</td>
</tr>
<tr>
<td>Nonsense mutant PGK1 mRNAs of yeast</td>
<td>Intrinsic instability (as compared to wild type mRNA)</td>
<td>Translational reinitiation sites present on the 3' side of nonsense mutation and stabiliser sequence in the 5' half of the mRNA</td>
<td>UPF gene products and proteins that interact with them</td>
<td>Sequences flanking the translational reinitiation sites contain a 9-nucleotide stretch complementary to 18S-rRNA sequence, suggestive of ability to cause mRNA : rRNA base pairing</td>
<td>Leeds et al., 1992; Peltz et al., 1993; Peltz and Jacobson, 1993.</td>
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<td>Tubulin mRNA</td>
<td>Depolymerisation of microtubules</td>
<td>Destabilization</td>
<td>Nascent N-terminal peptide MRE1 coded by the tubulin mRNA</td>
<td>Suggested to be free tubulin heterodimers</td>
<td>Free tubulin heterodimer is thought to recognise the N-terminal residues of the nascent peptide emerging from translating ribosome and lead to activation of ribosome associated nuclease or result in ribosome stalling which may eventually expose the nuclease recognition sites on the mRNA.</td>
<td>Pittenger and Cleaveland, 1985; Pachter et al., 1987; Yen et al., 1988; Gay et al., 1989.</td>
</tr>
<tr>
<td>c-myc mRNA</td>
<td>Intrinsic instability</td>
<td>Coding region instability</td>
<td>Coding region instability element</td>
<td>Destabiliser sequence is functional only if translation occurs through it</td>
<td>Wisdom and Lee, 1991.</td>
<td></td>
</tr>
<tr>
<td>MAT alpha 1, HIS3</td>
<td>Intrinsic instability</td>
<td>Coding region instability</td>
<td>Coding region instability element</td>
<td>In the case of HIS3 and MAT alpha 1 mRNAs, the destabiliser sequence is rich in rare codons and has been suggested to act by causing ribosome stalling</td>
<td>Parker and Jacobson 1990; Peltz and Jacobson, 1993.</td>
<td></td>
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<tr>
<td>STE2, STE3 and CDC4 mRNA of yeast</td>
<td>Intrinsic instability</td>
<td>Coding region instability</td>
<td>Coding region instability element</td>
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<td>c-fos mRNA and PDGF-B mRNA</td>
<td>Intrinsic instability</td>
<td>Destabilising sequences of 5'-UTR</td>
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<td>Kabnick and Housman, 1988; Fen and Daniel, 1991.</td>
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<tr>
<td>Beta globin mRNA, CAT mRNA and SV40 16S late mRNA</td>
<td>Intrinsic stability</td>
<td>Poly (A) tail</td>
<td>Poly (A) binding protein</td>
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<td>Bernstein et al., 1989.</td>
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<td>p53 mRNA</td>
<td>Differentiation inducing stimuli (like hexamethylene bisacetamide)</td>
<td>Destabilization</td>
<td>RNA fragment complementary to the first intron</td>
<td></td>
<td></td>
<td>Knochbin and Lawrence, 1989.</td>
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<tr>
<td>Albumin mRNA of <em>Xenopus</em> liver</td>
<td>Estrogen</td>
<td>Destabilization</td>
<td>Polysome-associated endonuclease induced by estrogen</td>
<td></td>
<td></td>
<td>Schoenberg et al., 1989; Pastori et al., 1991a and b; Pastori and Schoenberg, 1993</td>
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<tr>
<td>Vitellogenin mRNA of <em>Xenopus</em> liver</td>
<td>Estrogen</td>
<td>Stabilization</td>
<td>Proposed to be cytoplasmic estrogen binding protein</td>
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<td>Brock and Shapiro, 1983a and b.</td>
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<td>Gamma fibrinogen and transferrin mRNAs of</td>
<td>Estrogen</td>
<td>Destabilization</td>
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<td>Schoenberget al., 1989; Pastori et al.,</td>
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<td>Xenopus liver</td>
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<td>liver</td>
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<td>SP011 and SP013 mRNAs of yeast</td>
<td>Shift to sporulation media</td>
<td>Stabilization</td>
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<td>Surosky and Esposito, 1992.</td>
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<td>TGF beta 1 mRNA</td>
<td>Phorbol ester induced differentiation</td>
<td>Stabilization</td>
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<td>RNase specific to TGF beta 1 mRNA,</td>
<td>Phorbol ester treatment blocks RNase</td>
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<td>detected in PNS of cells</td>
<td>action Wager and Assolian, 1990.</td>
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<td>HSP70 mRNA</td>
<td>Heat shock</td>
<td>Stabilization</td>
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<td>Some observations suggest that HSP70</td>
<td>Theodorakis and Morimoto, 1987.</td>
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<td>influences stability of its own mRNA</td>
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1.7 OBJECTIVES OF THE PRESENT WORK

With the general objective to understand the process and regulation of mRNA turnover and the trans-acting factors involved in it, two approaches were taken in the present study.

(1) In one experimental strategy, efforts were focussed on the detection and characterization of the in vivo generated degradation intermediates of mRNA to gain insight into the functional properties of the machinery involved in mRNA degradation. The specific questions addressed were:

(a) Can we detect specific degradative cleavages of an mRNA in a particular physiological condition or cell type?
(b) If yes, what is their significance in terms of regulated mRNA degradation?
(c) Can we use such cleavages and the information on the cleavage sites to identify and characterize the nuclease system responsible for the degradation?

The studies on albumin mRNA cleavages in mouse liver described in chapter 3, were performed with the aim to answer mainly questions (a) and (b).

(2) Several different cellular RNases have been reported in the literature. For most of these RNases, the in vivo function is not known. It is possible that some of these RNases are involved in mRNA turnover in vivo. The cytosolic neutral RNase which is ubiquitously distributed in mammalian tissues, is believed to exist in vivo in free and latent form (complexed with a protein inhibitor) and its role in mRNA turnover is an open question. Studies on the modulation of expression and/or activity of such RNases under different physiological conditions would provide leads to understand not only their cellular role in specific or general mRNA turnover but also the putative mechanisms underlying the regulation of cellular RNase activity(s) about which, again, not much is known.

The studies performed on this enzyme-inhibitor system in an attempt to obtain information related to the regulation of such RNase activities are described in chapter 4 of this thesis.
"If we pull this off, we'll eat like kings."