DEGRADATION OF MESSENGER RNA IN MAMMALIAN CELLS

By

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ABSTRACT

Analyses of the response of hydrocortisone-induced tyrosine transaminase in cultured H-35 cells to inhibitors of translation (cycloheximide, puromycin) suggest: (1) that bound ribosomes stabilize messenger RNA \textit{in vivo}; (2) that messenger is degraded at a rate determined by the rate of translation. Since specific messenger RNAs of mammalian cells are degraded at quite different rates, there may be extensive heterogeneity either in the rate at which ribosomes traverse different messengers or in the number of ribosomes which translate specific messenger RNAs.

The messenger RNA (mRNA) of both procaryotic and eucaryotic cells is characterized by a relatively rapid rate of turnover \textit{in vivo}. Mechanisms involved in mRNA degradation in bacteria are rather well understood, owing largely to a series of elegant studies by Yanofsky and his colleagues on the translation of the tryptophan operon in \textit{E. coli} (Morse et al. 1969a,b; Mosteller et al. 1970) and by Levinthal and his colleagues, analyzing the lactose operon (Adesnik &
Briefly, Yanofsky and his collaborators have shown that the tryptophan mRNA is translated by a closely packed cluster of ribosomes and degraded by a 5'→3' exonuclease following immediately behind the last ribosome. Degradation of the mRNA is thus intimately coupled to translation. The tryptophan mRNA, like most bacterial mRNAs studied, has a functional and chemical life span of only a few minutes in the cell, perhaps because translation begins almost immediately upon initiation of mRNA synthesis and is completed quickly.

In mammalian cells the phenomenon of mRNA degradation is far more complicated and very poorly understood. Although they are degraded more rapidly than other classes of cellular RNA, the mRNAs of rat liver (considered in toto) have half-lives 2 to 3 orders of magnitude greater than those in bacterial cells (Wilson & Hoagland 1967; Wilson et al. 1967). Specific mRNAs of mammalian cells show far more heterogeneity with respect to rate of turnover than do bacterial mRNAs. Thus, half-lives of mRNAs coding for specific proteins in rat liver have been recorded as ranging from 60 minutes to 24 hours or greater (see below). These observations indicate that degradation of individual mRNAs is an important factor in the regulation of gene expression in higher forms. We report here some preliminary results from studies of mRNA degradation, together with some considerations that may provide a base for future probes into this problem.

METHODS

Culture of the rat hepatoma H-35 cells, the techniques used in their manipulations and in analyses of the inducible enzyme, tyrosine transaminase, were described in detail in a paper prepared for the 3rd Karolinska Symposium (Lee & Kenney 1971).

RESULTS

In order to estimate the intracellular stability of the mRNA coding for tyrosine transaminase in H-35 cells, we have assumed that treatment with actinomycin (to block further mRNA synthesis) does not alter the rate of degradation of existing mRNA. With this assumption, pulse-labelling measurements of the rate of transaminase synthesis, made at intervals after actinomycin treatment, should reflect the rate at which transaminase mRNA is degraded. Data from experiments of this kind (Fig. 1) show that the rate of enzyme synthesis drops in the first-order fashion expected of a random degradation process, and
Degradation of tyrosine transaminase mRNA. H-35 cells were pretreated with hormones or with L-leucine as indicated until a steady-state enzyme level was attained. At zero time actinomycin D (0.2 μg/ml) was added. At that time and at 2 hour intervals thereafter groups of cells were exposed to [14C] valine for 15 minutes, after which they were collected and the extent of isotope incorporation into the enzyme was determined immunochemically as described by Lee & Kenney (1971). Radioactivity data for hydrocortisone-treated cells have been multiplied by 0.1 to enable their inclusion into this plot.

Fig. 1.

Indicate that the transaminase mRNA is degraded with a half-life of about 2 hours. Treatment of the cells with either of the inducing hormones, hydrocortisone and insulin, or with excess L-leucine (which also increases the transaminase level) does not change the rate of transaminase mRNA degradation. The validity of this measurement, and of the assumption used in making it, is indicated by the fact that the kinetics of hormonal induction are almost exactly as expected if the transaminase mRNA is degraded at this rate (Lee et al. 1970).

Several years ago our laboratory reported that treatment of rats with cycloheximide or puromycin, inhibitors of protein synthesis, blocked the usually rapid degradation of tyrosine transaminase in rat liver (Kenney 1967). This was more recently confirmed in the cultured H-35 cells (Barker et al. 1971), and these experiments also yielded information relative to degradation of transaminase mRNA. Fig. 2 presents the results of an experiment in which the tyrosine transaminase of H-35 cells was first brought to a high, induced level by pretreatment with the transcriptional inducer, hydrocortisone. At zero time cycloheximide was added, which resulted in a rapid drop in the transaminase level for 1 hour, after which enzyme degradation slowed to come...
Fig. 2.

Effect of cycloheximide on hydrocortisone-induced cells. Cells were pretreated with hydrocortisone (10^{-6} M, 12 hours) until an induced steady-state was attained. At zero time cycloheximide (5 \mu g/ml) was added and enzyme activity was measured at the indicated times without further treatment (●). After 3 hours cycloheximide was removed from some of these (△) by washing three times with fresh medium. Adapted from Barker et al. (1971).

to a nearly complete stop after 4 hours. If degradation was not blocked the enzyme level would be expected to drop continuously at the known degradation rate (t^{1/2 \gg 2} hours).

When cycloheximide was removed by washing the cells after 3 hours, there was an almost immediate return to the original induced level, reflecting a burst of enzyme synthesis which in turn reflects transaminase mRNA which persisted for the 3 hour period during which translation was blocked. This interval represents nearly two half-lives of the transaminase mRNA (cf. Fig. 1); if mRNA degradation continued while translation was blocked the mRNA content of the cells would have been drastically reduced, but the data indicate that the original mRNA level was maintained. Thus when translation was blocked by cycloheximide, degradation of the transaminase mRNA also stopped.

Further support for the conclusion that mRNA degradation is coupled to
its translation can be derived from consideration of the rate of enzyme change after withdrawal of cycloheximide. Enzyme synthesis is unusually fast under these conditions, with synthesis of 240 enzyme units in 1 hour compared to the maximal rate of 90 units per hour during hydrocortisone induction. This translation appears to have as a consequence a rapid disappearance of messenger, for synthesis ceases completely 1 hour after cycloheximide removal. If the messenger retained its usual stability synthesis should persist for several hours (cf. Lee et al. 1970). Thus, the rate of translation appears to determine the rate of mRNA degradation.

A comparable experiment using puromycin instead of cycloheximide to block translation yields quite different results (Fig. 3). In preinduced cells treated with puromycin the transaminase level dropped slowly for 3 hours and then remained stable. If puromycin was withdrawn at the 3 hour point degradation of the enzyme was restored, but there was no enzyme synthesis comparable to that observed with cycloheximide as the inhibitor of translation. Thus in the

Fig. 3.
Effect of puromycin on hydrocortisone-induced cells. The experiment was carried out like that of Fig. 2 except that puromycin (200 µg/ml) was used in place of cycloheximide.
presence of puromycin the hydrocortisone-induced mRNA was not stabilized, as it was with cycloheximide.

The apparent discrepancy in results using the two inhibitors can be attributed to differences in the mechanisms by which they block translation. Cycloheximide is known to inhibit translocation; ribosome movement is stopped and the polysome structure is stabilized (Wettstein et al. 1964; Colombo et al. 1965; Stanners 1966). On the other hand puromycin results in release of growing polypeptide chains (as peptidyl puromycins) and the polysome structure is destroyed (Latham & Darnell 1965; Blobel & Potter 1967). Combining these observations with our functional measurements of capacity for transaminase synthesis, we make the following tentative conclusions: (1) bound ribosomes stabilize mRNA in vivo; (2) messenger is degraded at a rate determined by the rate of translation.

DISCUSSION

These preliminary observations suggest that, as in bacterial cells, the mRNAs of mammalian cells are degraded by a process associated with their translation. If this is so we are led to the conclusion that there must be considerable heterogeneity either in the rate at which ribosomes traverse different messengers or in the number of ribosomes which translate specific messenger RNAs, for we know that there is extensive variation in degradation rates for individual mRNAs of mammalian cells. To our knowledge, measurements of ribosome velocity and number of ribosomes which translate specific messengers have not been made for individual proteins in mammalian cells; we are currently attempting to do this in our laboratory.

We have been struck by the fact that turnover rates of tyrosine transaminase and its mRNA, in both rat liver and cultured hepatoma cells, are essentially identical. The significance of this relationship is far from clear, but it is not an isolated phenomenon. The turnover rates of many individual proteins of mammalian tissues have been analyzed, and some are known with a fair degree of accuracy. The known metabolic half-lives of five enzymes from rat liver and cultured hepatoma cells are listed in Table 1, together with measured or estimated values for the half-life of the mRNA coding for each enzyme. It must be emphasized that the latter values are not known with precision; no measurements of mRNAs as chemical entities have yet been made. Measurements (tyrosine transaminase, serine dehydrase, levulinate synthetase) have depended on analysis of enzyme synthesis or enzyme levels after actinomycin treatment, a procedure which involves assumptions that may not always be correct. For two others (tryptophan pyrrolase and glutamic-alanine transaminase) we have

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Table 1. Degradation rates of rat liver proteins and mRNAs.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Metabolic half-life (hours)</th>
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<tbody>
<tr>
<td></td>
<td>Protein</td>
</tr>
<tr>
<td>Tyrosine transaminase (TAT)</td>
<td>1.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TAT in cultured H-35 cells</td>
<td>2&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tryptophan pyrrolase</td>
<td>1-2&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glutamic alanine transaminase</td>
<td>30&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serine dehydrase</td>
<td>3-4&lt;sup&gt;h,i&lt;/sup&gt;</td>
</tr>
<tr>
<td>Levulinate synthetase</td>
<td>1&lt;sup&gt;k&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Superscript letters refer to data source as follows:

a, Kenney (1967). b, Kendrick & Kenney, unpublished data of in vivo experiments comparable to those of Fig. 1. c, inferred from induction kinetics, see text d, Lee et al. (1970). e, Fig. 1. f, Feigelson et al. (1959). g, Segal & Kim (1963). h, Pitot & Peraino (1964). i, Jost et al. (1968). j, Pitot et al. (1965). k, Tschudy et al. (1965).

Estimated mRNA stability from considerations of induction kinetics, based on our kinetic analysis of tyrosine transaminase induction (Lee et al. 1970). Briefly, this analysis shows that the difference between the rate of enzyme loss (after transcriptional induction ceases) and the known rate of enzyme degradation, is an indication of the stability of the induced mRNA. For example, tyrosine transaminase (t<sub>1/2</sub> ≈ 2 hours) is lost from H-35 cells (after cessation of hydrocortisone treatment) at a rate considerably slower than this (t<sub>1/2</sub> ≈ 5 hours), due to the existence in the cells of a hydrocortisone-induced intermediate which also decays with a half-life of about 2 hours (the mRNA). The rate at which tryptophan pyrrolase is lost from liver after hydrocortisone induction is nearly equivalent to the known rate of pyrrolase degradation; we infer that the induced mRNA is quite labile, with a half-life not greater than 2 hours. In contrast glutamic-alanine transaminase is lost from liver after steroid induction at a rate (t<sub>1/2</sub> = 84 hours) much slower than the rate of degradation (t<sub>1/2</sub> = 30 hours). Here we infer that the difference represents an induced intermediate (mRNA) of considerable stability, and calculate that its half-life must be of the order of 1 to 2 days.

This relationship, together with the apparent linkage between rates of enzyme synthesis and mRNA degradation discussed above, predicts that the translation rate for stable proteins in liver will be considerably slower than that for labile proteins. If so, the nature of the controls (feedback?) operating to govern these synthetic and degradative processes will provide an interesting target for future investigations.
REFERENCES


DISCUSSION

Kafatos: I am glad that your measurements also suggest no control at the chain elongation level. With respect to your belief that there is regulatory control exercised through change in ribosome packing, I will stick to my guess that this is not true in most systems. The value of arguing about this is that ribosome packing can be evaluated rather easily if the size of the protein in question is known and if the polysomes synthesizing it can be identified.

With respect to your model, I think it is important to make a statistical evaluation of the best fit lines, because I would argue that the data are not sufficient to exclude either alternative, even though they may fit one or the other better.

Kenney: The model is just one kind of evidence we have employed to make the argument about where the different hormones act.

Kafatos: What is the evidence you have for the mode of insulin action?

Kenney: One example is the pattern of inhibition by actinomycin. Induction by hydrocortisone is completely blocked by actinomycin. In the induction by insulin, if you treat with actinomycin, you see normal enzyme synthesis for a period of about two hours. Then it begins to slow, and it is not until five hours that it is blocked completely.
So, what one sees with actinomycin treatment in insulin induction is almost exactly what our model predicts, if insulin is acting on an existing message that is degraded with a half life of about two hours.

*Kafatos:* This type of argument does not distinguish between translational efficiency and recruitment. I would argue that you might be dealing with recruitment rather than efficiency control.

*Kenney:* If that were so, we would see the recruited message in the kinetic pattern of induction. If we remove the translational inducer, that is insulin after a steady-state is reached, there is no indication of any stable intermediate; *i.e.*, we immediately see first order enzyme degradation. So we argue that insulin has not recruited or caused the synthesis or anything that has any kind of stability. That is in contrast to the steroid induction, where we do see such an intermediate. So I think we would see recruitment in the kinetics, and we don't.

*Kafatos:* Since there are so many complications and so many levels of control, I suggest that the simplest way to settle the issue is by looking directly at ribosome packing. I would also like to make a comment about your parallelism between the half-lives of proteins and the corresponding messengers. It is quite interesting that you have such a parallelism in a number of cases, although I don't think it is a general phenomenon. In the system that I discussed, we have done parallel messenger and protein half life analyses. It is quite clear that there is no correlation in our system, and other investigators have also not found such a parallelism either.

*Paul:* I wonder how realistic it is to try to distinguish between chain elongation and ribosome packing. In at least one system there is good evidence that there is a reciprocal relationship between these. In the reticulocyte there is, of course, no transcription because there is no nucleus. There are two messengers which are both stable and two proteins which are both stable, *i.e.*, α- and β-globins. *Hunt et al.* (1968a) showed that α-globin is synthesized in small polysomes, whereas β- is formed mainly in pentasomes. This implies that in the α-polysomes there is less ribosome packing, which must be compensated for precisely by a more rapid rate of chain elongation.

*Kafatos:* I think that the difference between the polysome sizes is not as great as that. According to published data (*Lodish* 1971), it is approximately 40% rather than over 100%. With respect to a parallel change in chain elongation rate, which is what *Hunt et al.* (1968b) proposed, Lodish's recent evidence (*Lodish* 1971) suggests that in fact it is the number of messengers which compensates for the difference in ribosome packing rather than the rate of peptide elongation. I agree that haemoglobin is one case where we have a clear difference in translational efficiency; but since the difference is only 40%, I don't think it is important in overall gross schemes, which is what I have been discussing.

*Paul:* One point which one should not miss is that if Dr. Kenney's idea is right, that ribosome packing stabilizes messengers, then these observations provide an argument against it, because the stability of the two messengers seems to be the same.

*Mach:* I think that both Dr. Kafatos' and Dr. Kenney's talks show how difficult and dangerous it is to make predictions! I want to make a comment about the presentation of Dr. Kafatos in particular. If a protein is made, this is a proof that the message is there. If, however, a protein is not made, or less of it is made, this in no way allows you to tell that the message for the protein is no longer there. I think that it is an
important argument. This morning you first stated »I don’t think translational control plays an important role in ribosome packing and I am going to show you that.« Then, in your experiments on the double-labelling of proteins, your conclusions about messenger stability depended entirely on the assumption that there is no regulation at the level of translation! This is a somewhat circular argument. Indeed, if a protein ceases to be made, this could result either from control on the reading of its mRNA or from actual loss of that specific mRNA. The fact that there is no translational control in the reticulocyte is not so surprising, because it is a highly specialised cell. Although it would be foolish for a cell to have one initiation factor for each different mRNA, one could envisage a model of co-ordinate regulation of the level of translation with initiation factors functioning for groups of mRNAs. This would give the possibility of turning off or turning on many functions, for instance as the result of a single inducer. This would be much more economic for the cell. There is therefore a very likely possibility that when a protein is not made, the message is still there, but because of the shorter half life of certain initiation factors, this message might not be read as efficiently, or maybe not read at all.

Kafatos: First about the alleged circularity of my argument. Your comment is not justified, because I pointed out the possibility that changes in translational efficiency may account for the apparent differential stability of zymogous mRNA, and commented that this explanation is unlikely, in view of what we know about other systems (in which ribosome packing is rather constant) and the magnitude of the change in packing that would be required (40 × if the rate of chain elongation is constant). We have recently examined the size of zymogen polysomes with and without actinomycin, and it is the same; we have not yet measured chain elongation rates.

With respect to the rest of your comment, I would point out again that what I was trying to do was separate different aspects of what we usually call, vaguely, translational control, and specifically separate the recruitment process from the process of efficiency control. I would argue that if initiation factors are important, as they may well be, they will act as recruitment agents, as all-or-none controls. Except for the very brief transient period when the cell is in the process of acquiring a given type of initiation factor, I would predict that the translational efficiency in different phases of differentiation is essentially constant; any »translational control« that operates may in fact be what I like to call transcriptional-translational coupling: a mechanism whereby mRNA is moved from the inactive or masked to the recruited state.

Turkington: Martin et al. (1969) have presented evidence to support the idea that that inducibility in post-transcriptional control may change during different phases of the cell cycle. I wonder if you can measure messenger stability as a function of the cell cycle in synchronized cells?

Kenney: We have done some work with synchronized cells, but not that particular experiment. You realize that we differ entirely in our interpretation of the data cited by Martin et al. in support of their argument for post-transcriptional control. As far as we are concerned, post-transcriptional control is not involved in the steroid-mediated induction.

Turkington: I just wonder if the messenger might not have a different half life than the average half life that you determined in the cell population, depending on what part of the cell cycle it might be in. It might be that the messenger might be degraded at some particular point of the cycle.
**Kenney:** We have not made that measurement.

**Mach:** About the insulin effect on the induction of initiation, could you tell from your kinetic data whether this would be a protein specific effect of insulin, or whether, as Wool has suggested, it might be an overall effect on protein synthesis?

**Kenney:** The hydrocortisone effect that I have described is very specific. There is virtually no increase in total protein synthesis with the steroid hormone. With insulin there is a very definite increase, of perhaps 50% or more, in the rate of total protein synthesis. However, the synthesis of this transaminase is increased 5 or 10 fold by insulin, so it is a general effect that is better for some proteins than others, which we don't really understand.

**Baulieu:** Dr. Mach, did you say that initiation factor may have shorter half life than messenger RNA?

**Mach:** I said that if an initiation factor has a message, the half life of which is shorter than any of Kafatos' messengers, this would completely modify all of his data. And this is a sufficient condition to regulate the synthesis of a large number of proteins.

**Baulieu:** Dr. Kafatos, have you seen any shorter messenger RNA in your experiments? One hour or less? *

**Kafatos:** We have not seen them, but I don't think that is sufficient to exclude the possibility; if initiation factors are a very small fraction of the total protein synthesized, they would not have been observed. I am not clear whether Dr. Mach questions the variability of the non-specific messenger half lives or the contrast between stable and unstable mRNA classes. The evidence is much more definitive for the latter, because of the much greater disparity and because of the parallel with a lot of other highly differentiated cells. At least for haemoglobin and fibrin mRNA the evidence for stable mRNA is independent of actinomycin.

**O'Malley:** We have measured total intracellular levels of initiation factors in heterologous cross-over experiments under conditions where we can also quantify specific mRNA. There appears to be a hormone-induced change in the total cellular levels of initiation factors. Therefore, this is another potential regulatory site for hormone action. However, the rate-limiting event for induction of specific proteins still seems to be the intracellular accumulation of mRNA.

**Dixon:** I wonder whether you have done any Cuatrecasas type experiments with insulin bound to an agarose particle so that it cannot get into the cell? Is the effect still positive with such immobilized insulin?

**Kenney:** Yes and no. We have done experiments with immobilized insulin bound to Sepharose. It is fully effective. There is no difference at all between insulin bound to Sepharose and free insulin. However, we have gone a bit further and treated cells with the bound insulin and then centrifuged both the cells and the Sepharose particles away. Then we took that medium and put it on some other cells. By all available criteria, that medium contained all the insulin that was added with the Sepharose. So I am not sure that this is a particularly valid experiment.

**Ryan:** How about cyclic AMP in your enzyme system?

**Kenney:** Cyclic AMP will also induce synthesis of this enzyme and in terms of the parameters I have described, it acts much like insulin.
References: