Theodorakis, Nicholas G., and Antonio De Maio. Cx32 mRNA in rat liver: effects of inflammation on poly(A) tail distribution and mRNA degradation. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1249–R1257, 1999.—Previous studies showed that the expression of connexin 32 (Cx32), the polypeptide subunit component of the major hepatic gap junction, is reduced in liver by changes in mRNA stability during bacterial lipopolysaccharide (LPS)-induced inflammation. In this study, we examined the distribution of Cx32 mRNA poly(A) tail lengths during LPS-induced inflammation, because this is considered the first step in the degradation of many mRNAs. During LPS treatment, the first detectable change in Cx32 mRNA was a gradual shortening of its poly(A) tail, which reached a final size of ~20 nucleotides. However, the poly(A) tail did not disappear entirely before the bulk of Cx32 mRNA was degraded. Treatment with actinomycin D, which blocks the degradation of Cx32 mRNA after LPS administration, resulted in the appearance of a completely deadenylated mRNA, which otherwise could not be detected. On the contrary, treatment with cycloheximide resulted in a decrease in the stability of Cx32 mRNA without an apparent change of the poly(A) tail size. The effect of cycloheximide on Cx32 mRNA stability seems to be due indirectly to the induction of an inflammatory response by this drug. These results suggest that, similar for many mRNAs, shortening of the poly(A) tail is one of the first steps in the degradation of Cx32 mRNA during inflammation.

GAP JUNCTIONS ARE PROTEIN channels between adjacent cells through which ions and other small molecules (<1 kDa) can pass (4, 5). These channels are composed of two hemichannels opposed to each other on adjacent cells. These hemichannels are termed connexons, each is composed of a hexameric arrangement of six identical protein subunits, named connexins (Cx). In some cells, such as hepatocytes, gap junctions are found aggregated in large latticelike arrays containing hundreds of gap junctions (4). The expression of Cx genes is tissue specific (16). For example, Cx32 is the most abundant Cx expressed in normal liver (19). The liver also contains a second gap junction protein, Cx26. In rat liver, Cx26 is expressed at a lower level than Cx32; moreover, whereas Cx32 has a relatively homogeneous distribution throughout the liver, Cx26 is found predominantly in the periportal region (12).

Gap junctional intercellular communication is important for proper organ function. For example, in the heart, electrical conductance is propagated through gap junctions (18) and, in the liver, they are believed to play an important role in hepatic metabolism (13). Alterations in hepatic gap junction communication and expression have been observed after different pathological conditions. Thus expression of Cx32 is reduced in the liver during acute inflammatory states induced by the administration of bacterial lipopolysaccharide (LPS), a bacteria wall component (11). A similar decrease in Cx32 expression was observed after a regional hepatic ischemia-reperfusion injury (9), partial heptectomy (15, 23), and hepatic cholestasis (23). The reduction of Cx32 expression in the liver during LPS-induced inflammation is not caused by a reduction in Cx32 gene transcription, but rather is due to increased degradation of the Cx32 mRNA (11). To further understand the mechanism of Cx32 mRNA degradation after administration of LPS, the shortening of the Cx32 mRNA poly(A) tail was examined, because this is considered the first step in the degradation of many eukaryotic mRNAs.

METHODS

Animal preparation. Adult male Sprague-Dawley rats were fasted overnight and allowed water ad libitum before treatment with intravenous injection of LPS (1 mg/kg) or intraperitoneal injection of actinomycin D (2 mg/kg) or cycloheximide (6 mg/kg) as described previously (11). This dose of LPS does not result in mortality, but induces a rapid acute inflammatory response (24). At the indicated times, the rats were killed by pentobarbital injection and liver samples were removed.

Probes. A rat Cx32 cDNA was obtained from David Paul (Harvard University). This clone encodes a nearly full-length rat Cx32 cDNA cloned into the EcoRI site of pGEM-3, in an orientation such that transcription with T7 polymerase yielded an antisense RNA probe. To prepare a template for RNase protection, a Kpn I fragment was removed to construct a plasmid that contains the final 260 nucleotides of the rat Cx32 cDNA.

Transcription and RNA analysis. Rat liver nuclei were isolated, and transcription in isolated nuclei was performed as described (10). Total RNA was prepared by guanidine thiocyanate-acid phenol extraction (6) and analyzed by Northern blot as described (11). Cx32 mRNA levels were also analyzed by an RNA protection assay. RNA (5–10 µg) was incubated in 30 µl of hybridization buffer (80% formamide, 0.4 M NaCl, 40 mM PIPES, 20 mM EDTA) at 42°C for 16 h with a radiolabeled antisense RNA probe that corresponds to the final 260 nucleotides of the rat Cx32 gene (see Fig. 1). Ten volumes of RNase digestion buffer (10 mM Tris, pH 7.5, 0.3 M NaCl, 5 mM EDTA, 40 µg/ml RNase A) were added, and the mixture was incubated at 25°C for 60 min. The RNase digestion was terminated by the addition of 20 µl of 20% SDS.

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Cx32 mRNA poly(A) tail degradation during normal and inflammatory states. Previous studies showed a decrease in the stability of hepatic Cx32 mRNA during an acute inflammatory state induced by the administration of LPS (11). Because shortening of the poly(A) tail is the first step in the degradation of many eukaryotic mRNAs (3, 7), the distribution of Cx32 mRNA poly(A) tails in rat liver was analyzed during LPS-induced acute inflammation. Rats were intravenously injected with LPS, and liver samples were taken at 0, 2, 3, 4, or 5 h postinjection. Total RNA was isolated, and poly(A) tail distribution of Cx32 mRNA was analyzed by an oligonucleotide-directed RNase H cleavage assay (Fig. 2A). The poly(A) tail distribution was compared with the level of Cx32 mRNA determined by an RNase protection assay (Fig. 2B). In liver samples of untreated rats, Cx32 mRNA poly(A) tails displayed a heterogeneous distribution, with sizes of ~20–160 bases long (Fig. 2A, lane 1). One particular feature is the conspicuous periodicity of ~20–25 bases in the distribution of Cx32 mRNA poly(A) tail sizes that was calculated as described in METHODS and presented in Fig. 2C.

The average size of the poly(A) tail decreased between 2 and 3 h after LPS treatment but maintained the periodic distribution (Fig. 2A, lanes 2 and 3, and C). At this time little degradation of Cx32 mRNA was observed in the RNase protection assay (Fig. 2B, lanes 3 and 4). The Cx32 mRNA poly(A) tail lengths changed from 20–160 bases at time zero to 20–60 bases 5 h after LPS injection. This reflects a change in the average of poly(A) tail distribution from 78 bases (time zero) to 42 bases (5 h after LPS injection). Thus Cx32 mRNA poly(A) tail lengths became smaller concurrent with the disappearance of the message after LPS administration (Fig. 2, A–C). These data suggest that shortening of Cx32 mRNA poly(A) tail precedes degradation of the bulk of the message during inflammation. No completely deadenylated mRNA can be detected in liver samples of either untreated or LPS-treated rats. This can be seen by comparison of the size of the 3′-terminal fragment of Cx32 mRNA when it has been completely deadenylated by treatment with oligo(dT) plus RNase H (Fig. 2A, lane 6) with the size distribution of the fragments that still have poly(A) tails (Fig. 2A, lanes 1-5).

One possible explanation for the heterogeneous distribution of Cx32 mRNA poly(A) tail is that the different lengths represent mRNAs of different ages. Thus newly synthesized Cx32 mRNA may have a long poly(A) tail, which would get shorter as the message ages. Previous studies showed that after Cx32 mRNA levels decline after LPS treatment there is a reappearance of the message during recovery from LPS injection, presumably due to new RNA synthesis (11). Accordingly, the poly(A) tail distribution of Cx32 mRNA during recovery from LPS administration was examined (Fig. 3). The poly(A) tail length of Cx32 mRNA was 15–190 bases in samples of untreated animals; it decreased to 15–90 bases after 8 h of LPS injection and recovered to
15–195 bases within 12 h of LPS treatment (Fig. 3A, compare lane 8 with lanes 2–7). The appearance of Cx32 mRNA with a longer poly(A) tail corresponded with a rise in the level of Cx32 mRNA 12 h after LPS treatment (Fig. 3B, lane 8).

Effect of actinomycin D on Cx32 mRNA poly(A) tail distribution. Previous studies showed that the LPS-induced degradation of Cx32 mRNA could be prevented by the administration of the transcriptional inhibitor actinomycin D (11). We were interested in determining how treatment with this drug perturbed the degradation of Cx32 mRNA poly(A) tail. Therefore the poly(A) tail distribution of Cx32 mRNA was examined in liver samples obtained from rats treated with actinomycin D, LPS, or combinations of both (Fig. 4A). Administration of actinomycin D alone resulted in a shortening of the poly(A) tail of Cx32 mRNA (Fig. 4A, lane 2), although degradation of the message was not evident (Fig. 4B). The degree of poly(A) tail shortening after actinomycin D treatment was less than that observed after administration of LPS alone (Fig. 4A, lanes 3 and 4). When actinomycin D was given before LPS treatment, the degree of poly(A) shortening of Cx32 mRNA was reduced compared with LPS treatment alone (Fig. 4A, lanes 5 and 6). Treatment with actinomycin D resulted in the accumulation of a completely deadenylated Cx32 mRNA species, irrespective of whether it was given in combination with LPS or not (Fig. 4A, lanes 2, 5, and 6). To further investigate the effect of actinomycin D on Cx32 mRNA, the poly(A) tail distribution of Cx32 mRNA was analyzed at different times.
after treatment with actinomycin D. The presence of deadenylated Cx32 mRNA could be detected within 4 h of treatment with the transcriptional inhibitor, which continued to accumulate throughout 8 h of treatment (Fig. 5A). A gradual shortening of the poly(A) tail occurred concomitantly with the accumulation of the deadenylated mRNA, even though no appreciable Cx32 mRNA degradation was observed (Fig. 5B).

The results described above could be due to a direct effect of actinomycin D on the degradation process or by an indirect effect, such as preventing the synthesis or activation of an LPS-induced factor that causes the degradation of Cx32 mRNA. To distinguish between these possibilities, we performed an experiment in which rats were injected with LPS first, followed by an actinomycin D injection after 1 h. If an LPS-induced factor was required to cause the degradation of Cx32 mRNA, we reasoned that it should be synthesized within this time. However, if actinomycin D has a direct effect on the degradation of Cx32 mRNA, then it should block Cx32 degradation even if it was added after LPS. Deadenylated Cx32 mRNA was observed in samples from rats injected with actinomycin D, either with or without prior treatment with LPS (Fig. 6A). Nevertheless, LPS induced the degradation of Cx32 mRNA even if actinomycin D was added afterward (Fig. 6B). These results suggest that actinomycin D affects the activation of an LPS-induced factor necessary for degradation of Cx32 mRNA. Furthermore, actinomycin D treatment had a second effect: the accumulation of a deadenylated mRNA that was not previously detected in the absence of the drug.

Effect of cycloheximide on Cx32 mRNA poly(A) tail distribution. In contrast to actinomycin D, the translation inhibitor cycloheximide did not prevent the LPS-induced degradation of Cx32 mRNA; moreover, injection of cycloheximide alone was sufficient to cause the degradation of Cx32 mRNA (11). To further explore these effects, we examined the effect of the translational inhibitor cycloheximide on Cx32 mRNA poly(A) tails. Rats were injected with cycloheximide or saline and injected with LPS or saline 1 h later. After 6 h, livers were harvested for determination of Cx32 mRNA poly(A) tail lengths and message levels as described (Fig. 7). Cycloheximide pretreatment did not prevent the LPS-induced degradation of Cx32 mRNA (Fig. 7B). However, whereas LPS treatment caused Cx32 mRNA poly(A) tails to decrease in size, cycloheximide treat-
ment resulted in an average increase in the length of Cx32 mRNA poly(A) tails, irrespective of the addition of LPS (Fig. 7A). The Cx32 mRNA remaining after cycloheximide treatment had long poly(A) tails, indicating that probably only newly synthesized Cx32 mRNA remained.

We next examined Cx32 mRNA poly(A) tail lengths during a time course of cycloheximide treatment. Injection of cycloheximide caused a loss of Cx32 mRNA (Fig. 8B). Although the kinetics of Cx32 mRNA degradation are similar after either LPS or cycloheximide injection, they result in different distributions of poly(A) tails. Whereas LPS treatment results in shorter Cx32 mRNA poly(A) tails, cycloheximide treatment results in a longer average Cx32 mRNA poly(A) tail (Fig. 8A). This distribution appears to be the result of a preferential loss of Cx32 mRNA with short poly(A) tails.

The degradation of Cx32 mRNA induced by cycloheximide implied that cycloheximide treatment might be inducing an inflammatory response in the liver. To test this, we examined the transcription rates and mRNA levels for genes that encode mRNAs known to change after inflammation. Rats were injected with LPS or cycloheximide; liver samples were harvested at various times after injection for isolation of nuclei for run-off transcription analysis and total RNA for analysis by Northern blot. Cycloheximide treatment resulted in an elevation of the transcription of the genes encoding α2-macroglobulin and β-fibrinogen, which are acute phase response genes in rats (Fig. 9A). The kinetics and levels of transcription were similar to those observed after injection of LPS. We also examined transcription of the gene encoding phosphoenolpyruvate carboxykinase (PEPCK), which is known to be reduced after inflammation (24). Cycloheximide treatment attenuated PEPCK gene transcription with similar kinetics to that observed after LPS treatment (Fig. 9A). These results were confirmed by analyzing RNA levels by Northern blot (Fig. 9B). In general, RNA levels were commensurate with transcription rates; both α2-macroglobulin and β-fibrinogen mRNAs became ele-

Fig. 6. Cx32 mRNA poly(A) tails in livers of actinomycin D-treated rats after LPS treatment. Rats were injected or not with 1 mg/kg LPS; 1 h later they were injected intraperitoneally with 2 mg/kg actinomycin D. After 0, 3, or 6 h after actinomycin D injection, animals were killed, and their livers were removed for purification of RNA. Cx32 poly(A) tail length and mRNA levels were determined as described. A: Cx32 mRNA poly(A) tail distribution. Lanes 1 and 12: Cx32 mRNA 3′-end fragment from rat liver RNA that was deadenylated with oligo(dT) plus RNase H. Lanes 2-11: Cx32 mRNA 3′-end fragments from rat livers treated with LPS or actinomycin D. B: Cx32 mRNA levels. Cx32 RNA levels were determined by Northern blot as described. Lanes 1-10: Cx32 mRNA levels in rat livers treated with LPS or actinomycin D. Con, control.

Fig. 7. Cycloheximide (CHX) treatment accelerates the degradation of Cx32 mRNA species with short poly(A) tails. Rats were injected intraperitoneally with 6 mg/kg cycloheximide (+) or not (--), followed by injection with 1 mg/kg LPS (+) or saline (–) 1 h later. After 6 h, animals were killed, and their livers were removed for purification of RNA. A: Cx32 mRNA poly(A) tail distribution. Cx32 poly(A) tail length was determined as described. Lane 1: Cx32 mRNA 3′-end fragment from rat liver RNA that was deadenylated with oligo(dT) plus RNase H. Lanes 2-7: Cx32 mRNA 3′-end fragments from rat livers treated with LPS or CHX. B: Cx32 mRNA levels. Lane 1: fragments protected by hybridization of probe to tRNA. Lanes 2-6: fragments protected by hybridization of probe to RNA from rat livers treated with LPS and/or CHX.
vated after either cycloheximide or LPS treatment. Another gene, the expression of which parallels the acute phase response, metallothionein, was also elevated after administration of cycloheximide. PEPCK mRNA was reduced after LPS injection but not by cycloheximide injection, even though both treatments reduced the transcription rate of its gene. These results suggest that cycloheximide treatment results in an inflammatory or inflammatory-like response in the liver. The effects on Cx32 mRNA stability and poly(A) tail lengths could be due to two effects: a rapid shortening of poly(A) tails of messages that are already committed to degradation and a block in poly(A) shortening and degradation of messages that have long poly(A) tails.

DISCUSSION

In normal rat liver, the expression of Cx32 is characterized by a low rate of transcription and the presence of a very stable mRNA. During inflammation the level of Cx32 mRNA is altered by an increase in message degradation (1, 11). Consequently, changes in Cx32 mRNA stability play an important role in the expression of this gene within the liver. In the present study, the changes in Cx32 mRNA stability during LPS-induced inflammation were correlated by changes in the distribution of Cx32 mRNA poly(A) tail. The first detectable change in Cx32 mRNA is the shortening of the poly(A) tail, which occurs before the degradation of the bulk of Cx32 mRNA. Over time, the poly(A) tail becomes shorter as the message disappears. No completely deadenylated Cx32 mRNA was detected after any time point after the administration of LPS.

One of the most prominent features of the Cx32 mRNA poly(A) tail is a distinct periodicity of ~20–25 bases. This distribution is consistent with the distribution of bulk poly(A) tails of cytoplasmic mRNAs in cultured cells as described by Baer and Kornberg (2). In addition, a periodic distribution of poly(A) tail lengths has been observed in rabbit and mouse globin mRNA (14, 17). The periodic distribution of Cx32 poly(A) may be related to the physical properties of the poly(A) binding protein (PABP), which binds to poly(A) tails of 25–30 nucleotides (1). Our data suggest that the degradation of Cx32 mRNA proceeds by sequential loss of 20–25 nucleotide blocks of poly(A) tail from the 3' end. It is possible that the loss of these 20–25 nucleotide blocks of poly(A) tail is influenced by the binding of PABP. There are several possible explanations of how

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Fig. 8. Comparison of time course of LPS and cycloheximide treatments. Rats were injected intravenously with 1 mg/kg LPS or intraperitoneally with 6 mg/kg cycloheximide; at the indicated times livers were removed for purification of RNA. Cx32 mRNA levels and poly(A) tail lengths were determined as described. A: Cx32 poly(A) tail distribution. Lanes 1 and 9: Cx32 mRNA 3'-end fragment from rat liver RNA that was deadenylated with oligo(dT) plus RNase H. Lanes 2-8: Cx32 mRNA 3'-end fragments from rat livers treated with LPS for indicated times. Lanes 10-16: Cx32 mRNA 3'-end fragments from rat livers treated with cycloheximide for indicated times. B: Cx32 mRNA levels. Cx32 RNA levels were determined by Northern blot as described. Lanes 1-7: Cx32 mRNA 3'-end fragments from rat livers treated with LPS for indicated times. Lanes 8-14: Cx32 mRNA 3'-end fragments from rat livers treated with cycloheximide for indicated times.
this can occur. For example, there might be 3-exonuclease that degrades the poly(A) tail, which could be physically impeded by the binding of the PABP. Alternatively, a poly(A)-specific nuclease that requires the presence of the PABP could cleave the poly(A) tail in discrete units dictated by the binding of the PABP. Another observation is that the size distribution of Cx32 mRNA poly(A) tail seems to reflect the age of the message. Thus newer mRNA has a long poly(A) tail, whereas old messages have short poly(A) tails.

The effects of actinomycin D on Cx32 mRNA are complex. Previous studies have shown that administration of actinomycin D before LPS injection resulted in the stabilization of Cx32 mRNA (11). In the present study, we showed that actinomycin D does not stabilize the message if LPS is given first. One interpretation of this finding is that LPS affects the activation of a factor involved in the degradation of Cx32 mRNA. It is curious that administration of actinomycin D results in the appearance of a completely deadenylated mRNA species, regardless of whether it is given before, after, or in the absence of LPS treatment. Moreover, if actinomycin D is administered either in the absence of or before LPS treatment, a reduction in the length of the Cx32 mRNA poly(A) tail still occurs. However, this reduction is not as great as that which occurs if LPS is given before or in the absence of actinomycin D. We speculate that treatment with actinomycin D alone reveals the normal rate of Cx32 mRNA poly(A) shortening that occurs in the absence of stress. This rate appears to be ~25–50 residues/h, as estimated from the data in Figs. 4–6. If poly(A) tail shortening is the rate-limiting step in Cx32 mRNA degradation, then it would take ~25–50 h to degrade a message if the initial poly(A) tail size is ~200 nucleotides. This is in reasonably close agreement with data obtained from Kren and coworkers (15), who reported that the half-life of Cx32 mRNA in the presence of actinomycin D was 10.9 h in normal liver. Although we detected little or no degradation of Cx32 mRNA in the presence of actinomycin D in normal livers, we do note that a completely deadenylated species accumulates. The apparent discrepancy between the results of Kren and coworkers and ours could be due to the fact they analyzed poly(A)-selected mRNA, rather than total RNA. Therefore, the accumulated deadenylated mRNA that we observed would not have been detected in those studies. The appearance of deadenylated mRNA that occurs after actinomycin D treatment suggests that degradation of nonpolyadenylated mRNA might be inhibited under these conditions, although it is unclear how this could occur. One possibility is that actinomycin D treatment might result in an alteration in messenger ribonucleoprotein particle structure or transport that occurs when transcription is inhibited, as has been reported by Dreyfuss and coworkers (8, 20). It is interesting to note that there are other mRNAs, such as the c-fos mRNA, for which the degradation is altered by treatment with actinomycin D (22). The block in degradation of these mRNAs might also be due to an inhibition of the degradation of the deadenylated species.

Fig. 9. Effects of cycloheximide on transcription rate and mRNA levels in rat livers. Rats were injected with saline, cycloheximide (6 mg/kg), or LPS (1 mg/kg). Livers were isolated at the indicated times, and nuclei and total RNA were prepared. A: transcription in isolated nuclei. Nuclei isolated from rats treated with cycloheximide or LPS were incubated in presence of radiolabeled UTP. Radioactive runoff transcripts were purified and hybridized to filter-bound plasmids containing β-fibrinogen (β-fib), α2-Macroglobulin (α2 Mac), and phosphoenolpyruvate carboxykinase (PEPCK) sequences. Filters were washed and exposed to X-ray film. B: Northern blots. Total RNA isolated from rats injected with LPS or cycloheximide was separated on formaldehyde/agarose gels, blotted to Nylon membranes, and probed for labeled DNAs containing β-fibrinogen, α2-Macroglobulin, and PEPCK sequences. Filters were washed and exposed to X-ray film.
Curiously, the translation inhibitor cycloheximide has the opposite effect as actinomycin D. Cx32 mRNA levels decrease after administration of this drug. It is likely that cycloheximide is mimicking at least some aspects of the acute phase response. Indeed, we have observed that injection of cycloheximide causes an elevation in the transcription rate of β-fibrinogen and α2-macroglobulin genes, both of which are acute phase response genes in rodents and part of the inflammatory response (Fig. 9). Alternatively, cycloheximide may directly accelerate the degradation of Cx32 mRNA, as it does for β-tubulin (17a). We also note that after cycloheximide treatment Cx32 mRNA poly(A) tails have a longer average length, which is probably due to the loss of the species with the shorter tails. Perhaps a message needs some time before it is committed to the process of poly(A) shortening and degradation. In this scenario, these “older” mRNAs that have shorter poly(A) tails can be rapidly degraded after cycloheximide treatment. However, the “newer” mRNAs that have longer poly(A) tails have not yet been committed to the degradation pathway and are thus resistant to the effects of cycloheximide. It is perhaps not surprising that cycloheximide treatment might have multiple effects; for example, PEPCK mRNA is apparently stabilized by cycloheximide treatment (Fig. 9B).

A standard model for the degradation of eukaryotic mRNA has been derived from studies in yeast (3, 7). In this model, poly(A) tail shortening is the first step in the degradation of the message and is the step that apparently determines the half-life of the mRNA. After the poly(A) tail has been shortened to a minimal length, the 5’ cap structure of the mRNA is removed, and the mRNA is rapidly degraded by a 5’-exonuclease. The degradation of Cx32 mRNA has many features in common with this standard model, such as the shortening of the poly(A) tail being the first and rate-limiting step. However, we believe that the poly(A) tail of Cx32 mRNA is completely removed before the subsequent degradation steps. We propose that the degradation of Cx32 mRNA comprises the following steps, which are summarized in Fig. 10. The first step is a commitment of the mRNA to the degradation process. This step may be inhibited by cycloheximide. The second step is a shortening of the poly(A) tail, which is the rate-limiting step in degradation. During inflammation, the rate of poly(A) tail removal increases. After the poly(A) tail is reduced to short length, the removal of the final block of poly(A) may or may not occur at the same rate as poly(A) shortening. Although we have no evidence to suggest that the removal of the last block of poly(A) is kinetically different from poly(A) shortening, evidence from other laboratories suggests that it might not be the case. For example, purified yeast poly(A) nuclease cannot completely remove all of the poly(A) tail of a synthetic substrate (21). After all of the poly(A) tail has been removed, the deadenylated mRNA species is degraded very rapidly (Fig. 6). If the rate of degradation is very rapid compared with the rate of poly(A) removal, then the deadenylated mRNA species would not normally be detected. However, deadenylated mRNA is detected during actinomycin D treatment, suggesting that actinomycin D inhibits the degradation of the deadenylate mRNA. We suggest that after the poly(A) tail of Cx32 mRNA is removed, the subsequent degradation steps, which are rapid, are likely to be common to the degradation of many, if not most, mRNAs. We suggest that actinomycin D and cycloheximide inhibit different steps of the degradation pathway. Cycloheximide inhibits poly(A) tail shortening of mRNAs at an early step (but nevertheless accelerates poly(A) tail shortening of mRNA already committed to the degradation process), whereas actinomycin D inhibits the degradation of completely deadenylated mRNA.

In summary, our data suggest that shortening of the poly(A) tail of Cx32 mRNA is the first step in the degradation of this message during LPS-induced inflammatory states. It is also likely that shortening of the poly(A) tail is the rate-limiting step in the degradation of Cx32 mRNA. Finally, there is an apparent correlation between the size of the poly(A) tail and the age of the message.

Perspectives

Changes in gene expression are an important component in the cellular response to different disease conditions such as inflammation. Although transcriptional regulation has been the major focus of research in gene expression, it is becoming increasingly clear that post-transcriptional mechanisms also play an important role. Indeed, the concentration of any message is determined equally by its rate of synthesis (transcription rate) and its rate of degradation. Accordingly, mechanisms of mRNA degradation have gained attention recently. In some cases, shortening of the poly(A) tail has been observed to be the first step in mRNA decay.
whereas in others, an endonucleolytic cleavage occurs first. Previous studies showed that the expression of Cx32 is regulated by changes in mRNA stability. Data presented in this study suggest shortening of the poly(A) tail of Cx32 mRNA is the first step in degradation of the message. This information, in addition to other studies on the effect of inflammation at the transcriptional level suggest that this disease condition has multiple effects on cellular homeostasis. Attempts to control the inflammatory response require a profound knowledge of the mechanisms involved in gene expression.

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REFERENCES