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Evidence that the 5′ End of *lac* mRNA Starts to Decay as Soon as It Is Synthesized

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By monitoring the decay of the first 16% of the β-galactosidase message, we showed that the 5′ end started to decay before the 3′ end was completed and at a rate equivalent to that of the whole molecule. Thus, decay was neither from 3′ to 5′ nor from random internal fragmentation but rather proceeded in a net 5′ to 3′ direction.

The mechanism of mRNA decay is not known. The initial events in its breakdown could occur near the 5′ end, near the 3′ end, or at internal sites. The mRNA from the lactose operon contains three contiguous messages (*Z*, *Y*, and *A*), with 39 nucleotides before the *Z* start (9), 54 between *Z* and *Y*, and 69 between *Y* and *A* (3). From size analyses of decaying *lac* mRNA, it has been concluded that specific internal cleavages occur between the *Z* and *Y* messages and possibly *Y* and *A* messages (2, 8).

The fate of an individual message (mRNA for a specific polypeptide) is not as clear. Early experiments by Morikawa and Imamoto (10) and Morse et al. (11) showed that a proximal *trp* message can be lost before synthesis of a distal one, but this does not identify the mode or direction of decay of a single message. That the 5′ end can at least affect the decay rate has been suggested by changed rates of *trp* mRNA decay when the normal *trp* promoter is replaced by the phage λ p_L promoter (15). Also, nucleotide changes that affected a potential secondary structure before the start of the *lac* mRNA significantly change the decay rate of the first (*Z*) message for β-galactosidase (4). Recent studies have pointed to a possible importance of the 3′ end for decay, at least for some messages; e.g., message for phage λ *int* protein may be inactivated by attack at its 3′ end (5, 12), and it has been concluded that the mRNA for a membrane protein decays from 3′ to 5′ (14). In an earlier study (7), we observed a delay before *lacZ* mRNA decays at the final exponential rate even when rifampin inhibition is complete. This delay causes a shoulder in the curve, and one condition that would give such a shoulder would be a requirement that decay commences only after the message is completed (7).

Size analyses of decaying β-galactosidase message populations have shown that most of the message mass remains in the full-length molecule during decay (6, 8). This distribution is inconsistent with fragmentation by random endonucleolytic cleavages since the longest molecules would always be lost fastest and result in a progressive shift to smaller sizes with time (2, 8). Thus, the β-galactosidase message must decay directionally from one end to the other. However, these results could not distinguish between a 5′-to-3′ versus 3′-to-5′ direction.

Since the β-galactosidase message is so long (3,063 nucleotides [16]) and so strongly induced, it is possible to perform a simple experiment to determine whether the 3′ end has to be completed before decay commences. Transcription initiations are inhibited very quickly by rifampin in the permeable strain AS19 of *Escherichia coli* B (7, 13), and it is possible to follow the decay of a short time set of induced β-galactosidase messages.

E. coli B strain AS19 (13) was grown exponentially at 37°C in 400 ml of M9 medium (1) supplemented with glycerol (0.2%) and casein hydrolysate (0.1%), with a 40-min doubling time. When growth reached 3×10^8 cells per ml, bacteria were induced at time zero by adding isopropyl-β-D-thiogalactoside (IPTG) (to 0.5 mM). Cyclic AMP (to 0.5 mM) was added at −5 min to reduce catabolite repression, and [5-^{3}H]uridine (15 mCi and ~600 nmol) was added at −30 s. Rifampin was added (to 200 μg/ml) at +20 s to block transcription initiations. Samples (40 ml) were taken at the indicated times and brought to 0°C on crushed ice in the presence of chloramphenicol (to 100 μg/ml) and sodium azide (to 1 mM); this treatment should essentially block all metabolism within a few seconds (2). The RNA was partially purified at 0°C (2) and reacted for 18 h at 52°C to form a hybrid to 5 μg of denatured DNA carrying the first 479 base pairs (bp) for the start of the β-galactosidase mRNA (about 16% of the genome length). Hybridization procedures have been described (2). The DNA was a 789-bp *Hpa*I fragment from plasmid pMC3 (constructed by M. Calos) that also carries the 311 bp preceding the *lacZ* message region. The results for three cases could be predicted (Fig. 1). (i) The message population starts to decay at the final exponential rate as soon as the 5′ end is made (curve A); (ii) decay of the 5′ end does not commence until the molecule (3′ end) is completed (curve B) or (iii) until it is completed and degradation proceeds from the 3′ end to the 5′ end (curve C). The shorter the induction time, the more sharply defined are the expected differences in the results. The 20-s induction reported here was about as short a time as is practical to still be able to observe significant levels of the hybrid. However, with such a short induction, it is necessary to make two minor corrections that would be insignificant with much longer induction times. These are corrections for the short intervals during which rifampin inhibition becomes complete (curve b versus a) and before the final exponential rate is attained (curve A versus b). Both corrections would apply to any of the three mechanisms.

Curve a would be expected if rifampin shut off transcription initiations immediately and the 5′ end were vulnerable to degradation as soon as it was made. Curve b is curve a corrected for the incompleteness of rifampin inhibition. In a separate experiment, rifampin was added in separate flasks at −60, −20, −10, −5, 0, and +20 s with IPTG at zero time.

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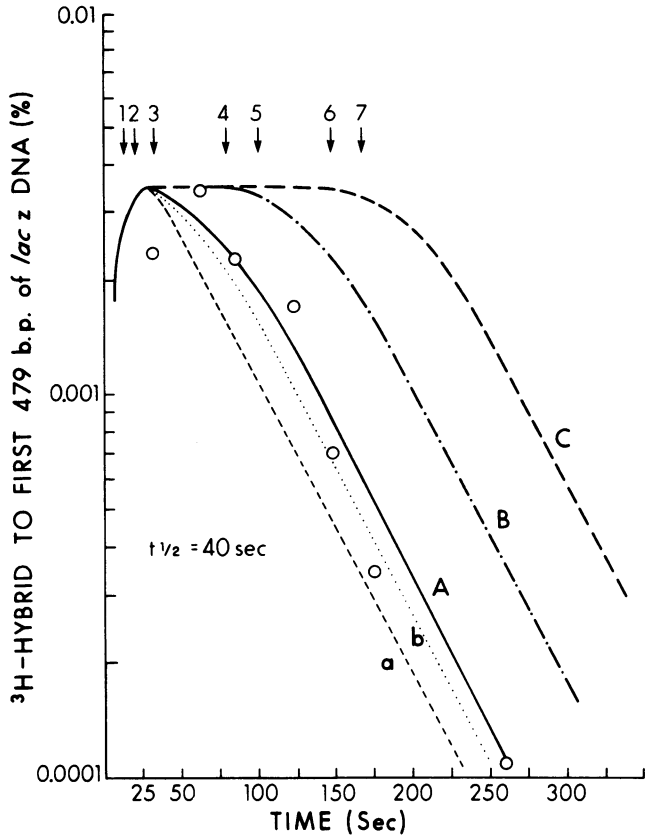

FIG. 1. Decay of the first 479 nucleotides of *lacZ* mRNA (for β-galactosidase). The points (○) show the percentages of [^{3}H]RNA that form hybrid after subtracting the nonspecific binding to a blank filter (~10 cpm). The numbers above the curve refer to the times of the following events in the culture: 1, the first RNA polymerase reaches the end of the 479-bp segment of *lacZ* DNA at 13 s (based on the observed induction lag of 80 s and transcription rate of 40 nucleotides per s [7]); 2, rifampin is added; 3, the last RNA polymerase completes the first 479 bp of *lacZ* at 33 s (20 + 13 s) to end synthesis of this RNA segment; 4, the first RNA polymerase reaches the end of *lacZ* (80 s); 5, the last RNA polymerase reaches the end of *lacZ* at 100 s; 6, the start of decay of the 3′ end of the first 479 nucleotides of *lacZ* mRNA if degradation were only 3′ to 5′ in direction and started from the end of the *lacZ* mRNA; 7, the maximal rate of decay of this proximal 479 nucleotides of *lac* mRNA would commence if decay were only 3′ to 5′ from the 3′ end of *lacZ* and there were no corrections (see below). The times for the preceding events were used to construct net decay curves by assuming the final decay rate ($t_{1/2}$ = 40 s) for each of four 5-s intervals of induction and summing the mRNA per milliliter at short time intervals, e.g., to construct curve a. Since the last RNA polymerase completes the 479 nucleotides at 33 s, decay could not commence at the final exponential rate before 33 s. However, there is a net loss of hybrid RNA before this time as soon as more than one-half of the RNA polymerases have completed the first 479 nucleotides. The corrections for the delay before rifampin inhibition is complete (curve b from curve a) and the delay before the final exponential rate is attained (curve A from curve b) are discussed in the text. Curve A would be expected if degradation of the 5′ end commenced as soon as it was synthesized, curve B if it commenced at the 5′ end only after the 3′ end was completed, and curve C if degradation was only from the 3′ end of the *lacZ* mRNA and in a 3′ to 5′ direction.

The yield of β-galactosidase was measured at 15 min (the amount of enzyme per ml was maximal before 15 min). The increments of β-galactosidase (0 to −5, −5 to −10, and −10 to −20 s) were used to correct curve a by the following. The total "leakage" of induction after rifampin addition (the yield when IPTG and rifampin were added simultaneously) was 43% of the total β-galactosidase observed when rifampin was added 20 s after IPTG. The next step was to determine how this total leakage was distributed with respect to time after rifampin addition; e.g., was the inhibition 90% or only 50% complete after 10 s of inhibitor? The amount of *lac* RNA induced during the first 5 s after rifampin addition is equal to the difference between the total β-galactosidase synthesized in a culture to which rifampin was added 5 s before IPTG and one in which they were added simultaneously. This difference was found to correspond to 19% of the total leakage observed or 14% above the level obtained without leakage. This additional amount of expected hybrid was added to the amount in curve a by adding the values from an identical curve shifted 5 s later and defining an area 14% of the area under curve a. The difference between a −5 and −10 s culture (12%) was treated similarly by shifting its contribution another 5 s. These corrections were continued until they were insignificant and generated the shoulder in curve a, or curve b.

Curve A is 5′ to 3′ degradation in which curve b is corrected for the delay before reaching the final exponential decline in *lacZ* message activity or mass. This very minor correction is over and above the correction for any delay in complete rifampin inhibition and accounts for the experimental observation that the *lacZ* message decays with kinetics that are consistent with two or more hits being necessary for degradation (7). These kinetics do not change the time when degradation commences or the final exponential rate, which is established by the slowest hit rate, but also gives a shoulder to the curve.

Curve B is the expected decay if the 5′ end does not decay until the *lacZ* message is completed. Curve C is the expected decay if it only occurs in a net 3′ to 5′ direction and initiates as soon as the *lacZ* message is completed. Curves B and C were corrected in the same way as was curve A.

The observed values for one experiment are shown (Fig. 1). They agree with the expected curve when the 5′ end is vulnerable as soon as it is synthesized. In all experiments, the hybrid yield started to decline at close to 60 s, which was before the molecule was completed. The 60-s lag is expected from the two small corrections, which become significant nonetheless, in such a short induction. However, the following conclusions would be the same even without these corrections.

There are a number of indirect lines of evidence that the degradation of most bacterial mRNAs start at the 5′ end (reviewed by Kennell, *in* W. S. Reznikoff and L. Gold, ed., *From Gene to Protein: Steps Dictating the Maximal Level of Gene Expression*, in press). However, we believe that this is the first rigorous evidence that the 5′ ends of a message population start to be degraded as soon as they are made. Combined with the size analyses that showed that the messages decay from one end to the other (6, 8), it follows that the initial attack is at the 5′ end and that degradation then proceeds by a net unidirectional 5′-to-3′ breakdown. Furthermore, since loss of the 5′ region was at a rate as fast as was the loss of the entire molecule (data not shown), it follows that no other additional decay process, i.e., one initiated by an attack internally or at the 3′ end, could play a significant part in the degradation. This directionality does

not rule out endonucleolytic or 3′-to-5′ exonucleolytic activities in the process, but such activities must be primarily limited to the decaying 5′ end of the molecules.

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