Stationary phase, amino acid limitation and recovery from stationary phase modulate the stability and translation of chloramphenicol acetyltransferase mRNA and total mRNA in *Escherichia coli*

Ann E. S. Kuzj, Poonam S. Medberry† and Janet L. Schottel

Author for correspondence: Janet L. Schottel. Tel: +1 612 624 6275. Fax: +1 612 625 5780.
e-mail: schot002@maroon.tc.umn.edu

Department of Biochemistry, University of Minnesota, St Paul, MN 55108, USA

The functional stability of the chloramphenicol acetyltransferase (*cat*) mRNA, as well as the functional stability of the total mRNA pool, change during the course of *Escherichia coli* culture growth. mRNA half-lives are long during lag phase, decrease during the exponential phase and increase again during the stationary phase of the bacterial growth cycle. The half-lives of *cat* mRNA and total mRNA also increase three- to fourfold during amino acid starvation when compared to exponential culture growth. Even though the stability of the *cat* message changes about fourfold during culture growth, the amount of *cat* mRNA per cell mass does not vary significantly between the culture growth phases, indicating that there are compensating changes in *cat* gene transcription. Translation of *cat* mRNA also changes during culture growth. In exponential phase, the rate of *cat* translation is about 14-fold higher than when the culture is in stationary phase. This is in contrast to the fourfold increase in stability of *cat* mRNA in the stationary-phase culture compared to the exponentially growing culture and indicates that active translation is not correlated with increased mRNA stability. When a stationary-phase culture was diluted into fresh medium, there was a five- to sevenfold increase in CAT synthesis and a threefold increase in total protein synthesis in the presence or absence of rifampicin. These results suggest that while mRNA becomes generally more stable and less translated in the stationary-phase culture, the mRNA is available for immediate translation when nutrients are provided to the culture even when transcription is inhibited.

Keywords: mRNA decay, *cat*, *Escherichia coli*, stationary phase, starvation, translation

INTRODUCTION

Escherichia coli produces the appropriate amounts of proteins needed to achieve the fastest growth rate possible in a given medium (Pedersen *et al.*, 1978a). Shifts in osmolarity, temperature and amount or type of carbon source or other essential nutrients may require drastic alterations in cellular protein composition (Neidhardt & Savageau, 1996). In particular, specific proteins are synthesized in response to starvation conditions (Groat *et al.*, 1986; Matin, 1991) and the ability to carry out protein synthesis during starvation is critical for maintaining viability (Reeve *et al.*, 1984). The amount of a particular protein in the cell is determined by the rate of transcription of the gene encoding the polypeptide, the rate of decay of the mRNA, the rate of translation of the mRNA encoding the polypeptide and the rate of degradation of the protein. Cellular protein concentrations could be changed in response to an environmental stimulus by changing the rate of any of these processes. Compared to the work on transcription,

†**Present address:** Berlex Biosciences, 15049 San Pablo Ave., PO Box 4099, Richmond, CA 94804, USA.

Abbreviations: CAT (*cat*), chloramphenicol acetyltransferase (gene); Cm, chloramphenicol; DEPC, diethylpyrocarbonate; Tc, tetracycline.

translation and protein degradation, relatively little has been done to study the involvement of mRNA decay in the cellular response to an environmental stimulus.

Within a cell, different mRNAs decay at different rates (Pedersen *et al*., 1978b). These differences in decay rates suggest that individual mRNA molecules vary in their susceptibility to degradation and that the rate of mRNA decay may be regulated. Several models have been proposed for the process of mRNA degradation that include 3′→5′ exonuclease activities and rate-determining mRNA cleavages followed by exonuclease digestion of the cleavage products (Belasco & Higgins, 1988; Petersen, 1992; Alifano *et al*., 1994). In addition, 3′ polyadenylation and 5′ phosphorylation of the transcript may also play important roles in regulating mRNA decay (Cohen, 1995; O'Hara *et al*., 1995; Xu & Cohen, 1995; Raynal *et al*., 1996). Several RNases critical to the process of mRNA degradation have been identified. Two of the most well-characterized endoribonucleases responsible for initiating decay of specific transcripts are RNase III and RNase E. RNase III cleaves substrate RNAs that are double-stranded and may contain a bulge or mismatched bases (Krinke & Wulff, 1990), whereas RNase E recognizes single-stranded sequences that are AU-rich (Cohen & McDowall, 1997). RNase E regulates the decay of a large number of transcripts in *E. coli* (Ehretsmann *et al*., 1992; Melefors *et al*., 1993) and is required for viability (Goldblum & Apirion, 1981; Arraiano *et al*., 1988). Polynucleotide phosphorylase and RNase II are exonucleases that remove nucleotides in procession from the 3′ end of transcripts (Deutscher, 1993a, b) and mutants lacking both of these RNases are no longer viable (Donovan & Kushner, 1986; Arraiano *et al*., 1988). Even though many RNases have been isolated from *E. coli* that are involved in processing rRNA, tRNA and mRNA, there may be additional RNases that are specifically involved in mRNA degradation (Deutscher, 1993a, b).

Regulation of mRNA degradation occurs in response to several changes in the culture environment such as starvation for required nutrients (Albertson *et al*., 1990; Albertson & Nystrom, 1994) and growth rate (Nilsson *et al*., 1984). In particular, *ompA* mRNA decay is growth-rate-regulated (Nilsson *et al*., 1984; Emory & Belasco, 1990; Georgellis *et al*., 1992) and the half-life of *ompA* mRNA also decreases rapidly when cells are shifted from nutrient-rich to nutrient-poor medium, indicating one way in which cells adjust rapidly to changing culture conditions (Georgellis *et al*., 1992). In contrast, the half-life of total mRNA has been shown to either not vary substantially with steady-state culture growth rate (Pato & von Meyenburg, 1970) or even increase as the growth rate decreases (Gray & Midgley, 1970). Studies with chloramphenicol acetyltransferase (*cat*) mRNA have indicated that the stability of this transcript changes in response to the composition of the culture medium and not to growth rate *per se* (Meyer & Schottel, 1991). Cultures shifted from aerobic to anaerobic conditions show an increase in *ompA* and *bla* mRNA stability and a three- to fourfold increase in the functional half-life of bulk mRNA (Georgellis *et al*., 1993). In addition, the half-lives of specific mRNAs as well as bulk mRNA have been shown to increase during starvation in *Vibrio* sp. S14 (Albertson *et al*., 1990) and in *E. coli* (Albertson & Nystrom, 1994). Obviously, growth rate, growth phase and medium composition impact the stabilities of specific transcripts as well as bulk mRNA. Since mRNA degradation plays an important role in the overall regulation of gene expression, this process may be critical in the regulation of culture growth and viability, particularly under conditions of stress and starvation.

Most studies of mRNA decay are carried out with cultures in exponential growth. Little work has been done to measure *E. coli* mRNA decay and translation in response to the variety of changes that occur as a culture progresses from the lag phase, through exponential phase and into stationary phase, or as growth slows when starved for a specific required nutrient. In this study, the median functional half-life of the total mRNA pool was measured at various times and conditions during the growth of *E. coli* cultures. The functional stability of *cat* mRNA from plasmid pACYC184 (Chang & Cohen, 1978) was also measured as a specific example of a well-characterized transcript that has the typical half-life of mRNAs in *E. coli* (Meyer & Schottel, 1991, 1992). The results presented here indicate that *cat* as well as total mRNA stabilities change during the course of culture growth and that increased transcript stability does not correspond to increased translation. In addition, there is an immediate increase in protein synthesis when a stationary-phase culture is diluted into fresh medium that does not require new transcription, suggesting the possibility of transcript stabilization and storage during stationary phase.

METHODS

Bacteria and growth conditions. *E. coli* strain MC1000 [*araD139* Δ(*ara leu*)7697 Δ*lac* χ74 *galU galK strA*] (Casadaban & Cohen, 1980) transformed with plasmid pACYC184 (Chang & Cohen, 1978) was used in these experiments. pACYC184 contains the *cat* gene and the tetracycline (Tc) resistance gene. The cultures were grown at 30 °C in Davis–Mingioli minimal salts medium (Davis & Mingioli, 1950) supplemented with 0·2% glucose, 4 μg thiamin ml^{-1}, 50 μg threonine ml^{-1}, 80 μg leucine ml^{-1} and 10 mM $NaHCO_3$ (Neidhardt *et al*., 1974). When used, Tc or chloramphenicol (Cm) was added to the medium at final concentrations of 12·5 or 25 μg ml^{-1}, respectively. Inclusion of Tc or Cm in the culture medium had no effect on CAT specific activity.

Functional half-life measurements. The functional half-life of *cat* mRNA was determined by quantifying the amount of labelled CAT protein produced during a 0·5 min pulse-labelling with [^{35}S]methionine at various times after the addition of rifampicin to the culture (Pedersen *et al*., 1978b; Albertson & Nystrom, 1994; Meyer & Schottel, 1991). Briefly, a sample of cells was removed from the culture at different times during the growth cycle and rifampicin was immediately added at 200 μg ml^{-1} final concentration to inhibit transcription initiation (Belasco & Brawerman, 1993). At various times after the addition of rifampicin, 5 ml of cells was removed and

labelled with 18 μCi [^{35}S]methionine ml^{-1} [1100 Ci mmol^{-1}; Amersham (1 Ci = 3·7 × 10^{10} Bq)] for 0·5 min. The labelling was stopped by the addition of 5·2 ml cold sonication buffer (50 mM Tris/HCl, pH 6·8, 10 mM $MgCl_2$) containing 300 μg spectinomycin ml^{-1} and 500 μg unlabelled methionine ml^{-1}. The cells were pelleted by centrifugation at 10000 ***g*** for 20 min, resuspended in 1·2 ml cold sonication buffer and pelleted at 12800 ***g*** for 5 min at 4 °C. The cells were then resuspended in 450 μl cold sonication buffer (pH 7·8) and lysed by sonication. The cellular extracts were cleared by centrifugation at 12800 ***g*** for 15 min at 4 °C and stored at −80 °C.

CAT was immunoprecipitated from each cleared extract according to Kessler (1976) using antiserum against purified CAT (Zaidenzaig & Shaw, 1976). An equal amount of extract protein from each sample was denatured and run on a 12·5% (w/v) SDS-discontinuous polyacrylamide gel (Laemmli, 1970). Fluorographs (Laskey & Mills, 1975) of duplicate gels were scanned by densitometry. *cat* mRNA functional half-life was calculated from the slope of the line generated by plotting ln(area) for each labelled CAT band as a function of time after rifampicin addition to the culture sample.

The concentration of protein in the cleared extracts was measured in duplicate by the method of Bradford (1976) using bovine serum albumin as standard. The effectiveness of transcription inhibition by rifampicin was verified during the different growth phases by quantifying the incorporation of [^{3}H]uridine (c.p.m.) into TCA-insoluble RNA with time after rifampicin addition (Gupta & Schlessinger, 1975).

Analysis of translational capacity. Translation of *cat* and total mRNA was measured by labelling a sample of cells for 0·5 min with 18 μCi [^{35}S]methionine ml^{-1} in the absence of rifampicin. Labelled cells and extracts were prepared as described for the functional half-life determinations. The total protein samples or the CAT immunoprecipitated samples were analysed by electrophoresis on 12·5% denaturing polyacrylamide gels (Laemmli, 1970) and fluorography (Laskey & Mills, 1975).

Determination of total mRNA functional half-life. To determine the functional half-life of total cellular mRNA, cell samples were taken at various times after the addition of rifampicin to the culture and pulse-labelled with [^{35}S]-methionine for 0·5 min. The count (c.p.m.) of TCA-precipitable protein (μg total protein)$^{-1}$ in each extract was calculated from the mean of at least two precipitation experiments (Pelham & Jackson, 1976). The functional half-life of the total mRNA population was determined from the slope of the line generated by plotting percentage c.p.m. incorporated (μg protein)$^{-1}$ (relative to time zero) versus time (min) after rifampicin addition.

***cat* mRNA measurements.** Total RNA was isolated from the cells in the presence of diethylpyrocarbonate (DEPC) as described previously (Meyer & Schottel, 1992). *cat* mRNA was quantified by dot blot hybridization of RNA samples to a *cat*-specific probe. The probe used for hybridization was a 977 bp *Sau*3AI fragment, isolated from a gel (Sambrook *et al.*, 1989), from the pACYC184 plasmid (Chang & Cohen, 1978) that contains the structural gene sequence for CAT. The *cat* probe was radiolabelled by the random primer method (Feinberg & Vogelstein, 1983) to a specific activity of greater than 1 × 10^{8} c.p.m. μg^{-1} and used at a concentration of approximately 0·5 ng ml^{-1}.

RNA samples were denatured in 6× SSC (1× SSC is 0·15 M sodium chloride, 0·015 M sodium citrate, pH 7·0), 7·4% (v/v) formaldehyde and 50% (v/v) formamide at 60 °C for 15 min and then stored on ice. The samples were spotted onto 2·5 × 2·5 cm^{2} sections of a Gene Screen Plus membrane (DuPont NEN) that had been soaked in distilled deionized water for 15 min, soaked in 20× SSC for 15 min and air-dried. Serial dilutions of an RNA sample were also spotted onto the filter to confirm the linear response of probe hybridization.

The filters were baked at 80 °C under vacuum for 2 h and prehybridized at 42 °C in 50% formamide, 2× Denhardt's solution (1× Denhardt's solution is 0·2 g l^{-1} each of Ficoll, polyvinylpyrrolidone and BSA), 0·5% SDS, 2× SSC, 0·1% sodium pyrophosphate, 10% (w/v) dextran sulfate and 0·2 mg sonicated calf thymus DNA ml^{-1} that had been denatured by boiling for 10 min. The probe was denatured, added directly to the prehybridization mixture and hybridized for at least 12 h at 42 °C. The filter was first washed in 2× SSC and 1% SDS once at 25 °C for 10 min, 2× SSC and 1% SDS twice at 65 °C for 30 min, 0·1× SSC and 1% SDS twice at 65 °C for 30 min and 0·1× SSC at 25 °C for 30 min. Radioactivity in individual squares was measured by liquid scintillation counting.

To quantify *cat* mRNA present per cell mass at different times during culture growth, an equal volume of MC1000 cells labelled with [^{3}H]uridine was added to each of the cell samples prior to RNA extraction. The count (c.p.m.) of *cat* gene probe hybridized to each RNA sample was corrected for the amount of [^{3}H]uridine-labelled MC1000 RNA in each sample.

RESULTS

The stability of *cat* and total mRNA during culture growth

The functional stability of *cat* mRNA was determined as a function of culture growth phase. During different stages of growth (labelled 1–5 in Fig. 1a), a sample of cells was removed for the half-life determination. The fluorographs in Fig. 1(c) indicate the amount of [^{35}S]-methionine-labelled CAT (μg total protein)$^{-1}$ in each extract that was synthesized during the 0·5 min labelling period at various times after the addition of rifampicin. The intensity of each band is proportional to the amount of *cat* mRNA in the cell that is being translated into full length polypeptide (Pedersen *et al.*, 1978b) and was used to calculate the *cat* mRNA functional half-life. The mRNA half-lives determined at each growth stage (1–5) are summarized in Table 1. The *cat* mRNA functional half-life was 0·7 ± 0·1 min during the mid-exponential growth phase and increased about fourfold when the culture was in either the lag or stationary phases of growth. These results indicate that when cells are in starvation conditions (such as stationary phase), the *cat* mRNA is functionally more stable than when the culture is growing exponentially.

In addition to the stability of *cat* mRNA, the half-life of total mRNA during culture growth was also determined. Portions of the [^{35}S]methionine-labelled cellular extracts prepared for the *cat* mRNA stability measurements were TCA-precipitated to quantify the amount of [^{35}S]-methionine incorporated into all soluble proteins during the 0·5 min labelling period. From the amount of label incorporated (μg total protein)$^{-1}$ in each extract, the

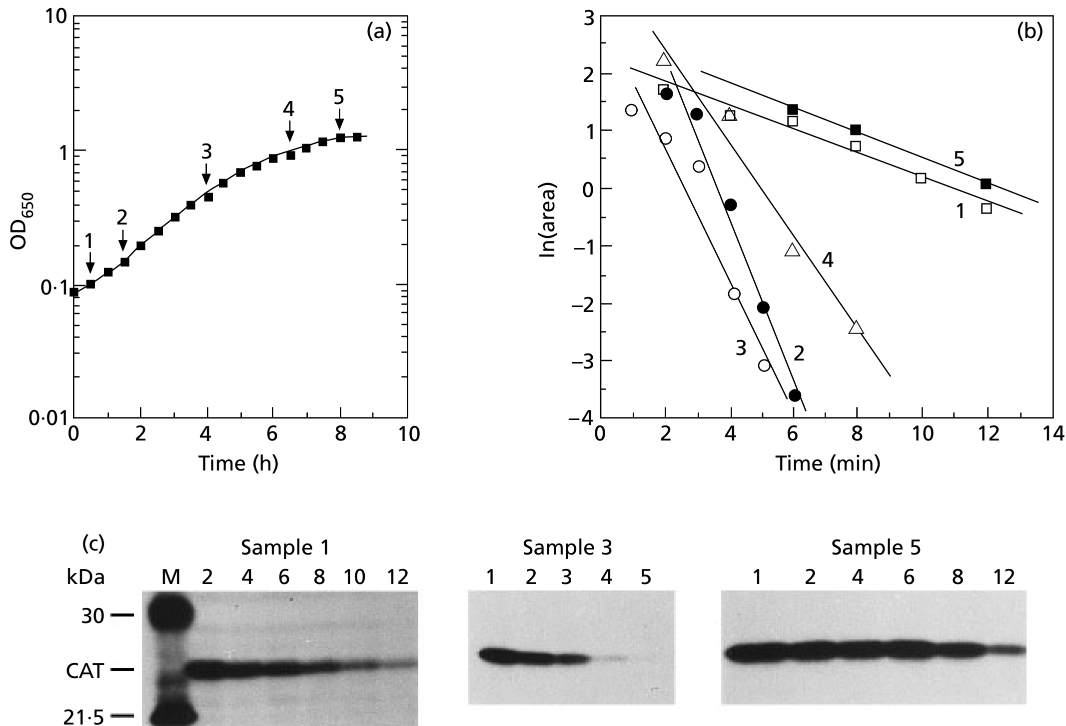

Fig. 1. *cat* mRNA functional decay determinations during growth of *E. coli* strain MC1000(pACYC184) in glucose minimal medium. (a) Culture growth was monitored by measuring OD_{650}. At the times labelled 1–5 a sample of the culture was removed and rifampicin was added to initiate a mRNA decay measurement. (b) The amount of [^{35}S]methionine-labelled CAT immunoprecipitated from each sample was quantified by densitometric scanning of fluorographs as shown in (c). The ln(area) of the CAT band was plotted as a function of time after rifampicin addition to the culture sample. Lines 1–5 correspond to samples 1–5 in (a); 1, lag phase (□); 2, early exponential phase (●); 3, mid-exponential phase (○); 4, late exponential phase (△); 5, stationary phase (■). (c) Three of the fluorographs used to calculate *cat* mRNA functional half-lives are shown. The number of each sample set (1, 3, 5) corresponds to the numbers in (a). Each lane of the fluorographs shows the amount of [^{35}S]methionine-labelled CAT that was immunoprecipitated from equal amounts of total extract protein. The number above each lane indicates the time (min) after rifampicin addition that cells were removed and labelled for 0·5 min with [^{35}S]methionine. The results are representative of two separate experiments. Lane M, radiolabelled Rainbow protein molecular mass markers (Amersham).

Table 1. *cat* and total mRNA functional half-lives during culture growth

Samples were taken from the culture at the growth phases indicated and correspond to the times labelled 1–5 in Fig. 1(a). The mRNA half-lives are the means of two determinations from two different cultures ± SD.

Sample	Culture growth phase	Functional half-lives (min)	
		cat mRNA	Total mRNA
1	Lag	2·9 ± 0·6	4·0 ± 1·4
2	Early exponential	0·7 ± 0·3	1·6 ± 0·06
3	Mid-exponential	0·7 ± 0·1	1·4 ± 0·05
4	Late exponential	0·8 ± 0·1	2·7 ± 0·6
5	Early stationary	2·8 ± 0·5	19·0 ± 0·8

median functional half-life of translated mRNAs at that particular stage of culture growth was calculated (Table 1). This number is not a mean of the functional half-lives of all cellular mRNA but is a median value which takes into account the cellular abundance of individual messages, their efficiency of translation and their functional half-life at the time of the determination. The total message pool was more labile in cells during exponential growth when compared to cells in the lag and stationary phases, similar to *cat* mRNA. The half-life measurements also indicate that the decay rate of the total mRNA pool is slower than that of *cat* mRNA during all growth phases.

cat mRNA and total mRNA functional stability during amino acid starvation

The functional stability of *cat* mRNA and total mRNA was measured during starvation of the culture for a required amino acid. MC1000(pACYC184), a leucine auxotroph (Casadaban & Cohen, 1980), was grown in leucine-limited medium (Fig. 2a). The culture grew exponentially to an OD_{650} of about 0·3 and then culture growth slowed due to leucine deprivation, analogous to a culture entering stationary phase. To recover growth,

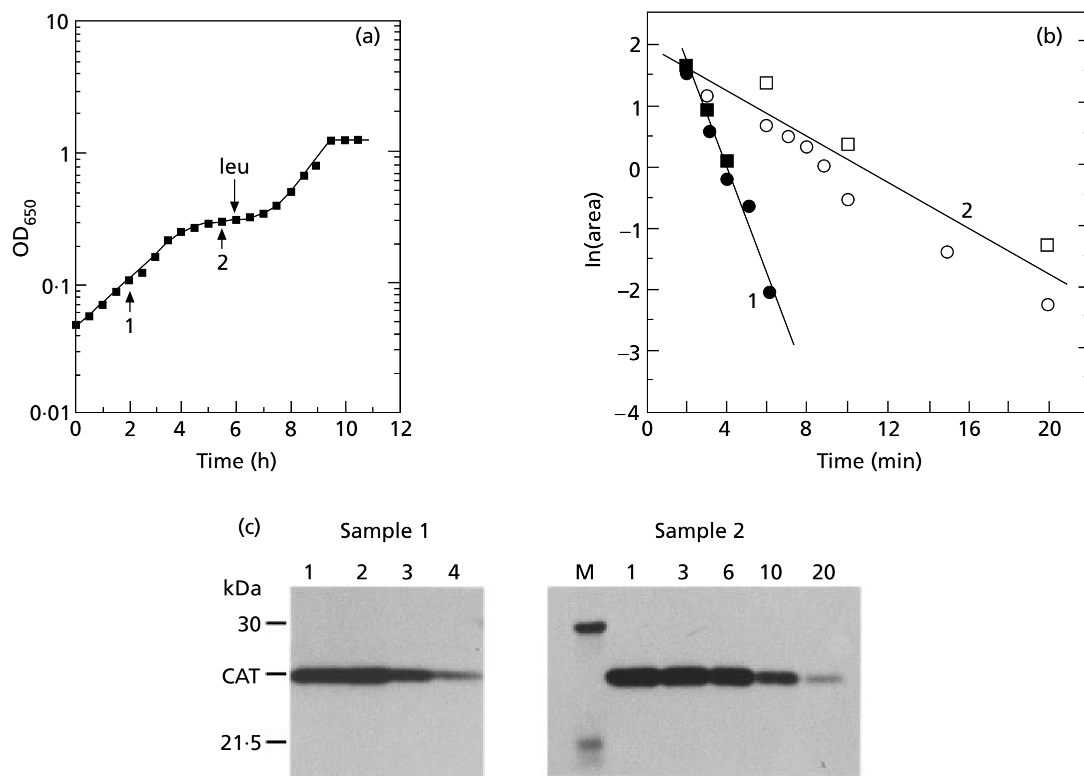

Fig. 2. mRNA decay measurements during growth in leucine-limited medium. (a) A culture of MC1000(pACYC184) was initiated in leucine-limited medium (2 µg leucine ml^{-1}). When growth stopped due to leucine deprivation, leucine was added back to the culture (at 80 µg ml^{-1}) at the time indicated by the arrow. Culture growth was monitored by OD_{650}. At the times labelled 1 and 2, a sample of the culture was removed to initiate a mRNA decay measurement. (b) The ln(area) of the CAT band, determined by densitometric scanning of fluorographs as shown in (c), was plotted as a function of time following the rifampicin addition to the culture sample. The results of two separate experiments are shown. The exponential-phase samples (1; ●, ■) and leucine-limitation-phase samples (2; ○, □) had mean half-lives of $0{\cdot}93\pm0{\cdot}04$ min and $3{\cdot}6\pm0{\cdot}8$ min, respectively. (c) To determine the *cat* mRNA functional half-lives, CAT was immunoprecipitated from equal amounts of cellular extract protein and electrophoresed as shown in the fluorographs. The number above each sample set (1, 2) corresponds to the numbers in (a). The number above each lane indicates the time (min) after rifampicin addition that cells were removed and labelled for 0·5 min with [^{35}S]methionine. Lane M, radiolabelled Rainbow protein molecular mass markers (Amersham).

80 µg leucine ml^{-1} was added back to the culture. After about an 80 min lag, the culture resumed exponential growth before entering stationary phase.

At the times indicated in Fig. 2(a) (1, 2), a sample of the culture was taken for *cat* mRNA and total mRNA functional half-life measurements. The amount of [^{35}S]-methionine-labelled CAT (µg extract protein)$^{-1}$ with time after rifampicin addition for each determination was used to calculate the *cat* mRNA functional half-lives. The *cat* mRNA functional half-life increased about fourfold during the leucine limitation phase when compared to the culture in exponential growth ($3{\cdot}6\pm0{\cdot}8$ min and $0{\cdot}93\pm0{\cdot}04$ min, respectively). These results are very similar to the increase in stability of *cat* mRNA observed between an exponentially growing culture and a stationary-phase culture in non-limiting medium (Table 1).

The functional half-life of total cellular mRNA was calculated from the amount of [^{35}S]methionine in TCA-precipitable protein (µg total protein)$^{-1}$ in each extract. Total mRNA became three times more stable during the leucine limitation phase ($9{\cdot}0\pm0{\cdot}4$ min half-life) when compared to its stability in an exponentially growing culture ($3{\cdot}2\pm0{\cdot}6$ min half-life). The trend toward mRNA stabilization during amino acid starvation is similar to the increased stability of total mRNA that occurs as the culture enters stationary phase (Table 1). The actual half-life values of total mRNA observed during leucine starvation were about twofold less than in stationary phase induced by carbon starvation which may reflect the different physiological states of the cells under these conditions or perhaps different mechanisms of stabilization.

Translation and abundance of *cat* mRNA during culture growth

The rate of *cat* mRNA translation at different times during the culture growth cycle was determined (Fig. 3,

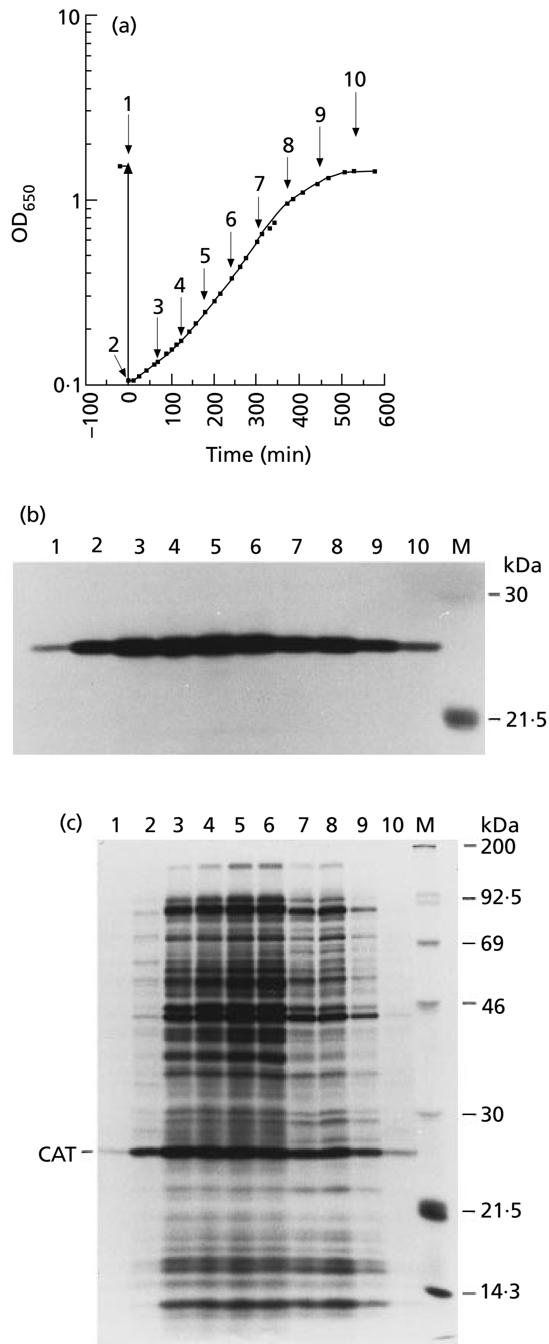

Fig. 3. Incorporation of [^{35}S]methionine into CAT and into all *E. coli* polypeptides at various times during culture growth. (a) Growth of the MC1000(pACYC184) culture was monitored at OD_{650}. At the times indicated (1–10), culture samples were withdrawn and labelled for 0·5 min with [^{35}S]methionine and cellular extracts were prepared. (b) A fluorograph of a 12·5% denaturing polyacrylamide gel showing CAT that was immunoprecipitated from equal amounts of extract protein from each of the samples (1–10) described in (a). (c) A fluorograph of the gel following electrophoresis of equal amounts of total cellular extract protein of samples 1–10. These [^{35}S]methionine-labelled samples correspond to those used for the immunoprecipitation of CAT shown in (b). Lanes M, radiolabelled Rainbow protein molecular mass markers (Amersham).

Table 2). The rate of CAT synthesis was slowest in the overnight stationary-phase culture, increased to a maximum in the early to mid-exponential phase and then decreased during the late exponential and stationary phases of growth. An approximately ninefold increase in the rate of CAT synthesis was observed within 2 min after dilution of the stationary-phase culture into fresh medium (samples 1 and 2). This burst in translation was not accompanied by a significant increase in *cat* mRNA levels (Table 2), suggesting increased translation of mRNA transcripts that were present in the stationary-phase culture prior to dilution.

The total amount of *cat* mRNA present per mass of cells varied less than twofold between the growth phases even though the decay rate of *cat* mRNA changed at least fourfold during the growth cycle (Table 1) and the CAT synthesis rate changed over a 24-fold range. These results suggest that transcriptional activity of the *cat* gene must change during culture growth, with low levels of transcription during the lag and stationary phases when the message is more stable and higher transcriptional activity during exponential growth when the message is more labile. The *cat* mRNA decay rate appears to be co-ordinated with transcriptional activity, thereby keeping the total amount of *cat* mRNA per cell mass fairly constant.

The burst in the rate of CAT synthesis observed upon dilution of a stationary-phase culture as well as the subsequent changes in translational rate that occur during the growth cycle may be dictated by the translational capacity of the cell, which is a function of amino acid pools, charged tRNAs, translational initiation and elongation factors and available ribosomes. The factors affecting the translational capacity of the cell should generally alter the synthesis rate of all cellular proteins. To determine whether the changes in the rate of CAT synthesis during culture growth are specific to *cat* mRNA or are a more general response of protein synthesis rate to growth phase, the rates of total protein synthesis (μg extract protein)$^{-1}$ during the growth cycle were determined (Table 2). The results indicate that the pattern of total protein synthesis is similar to the pattern of CAT synthesis (Fig. 3c) and suggests that the decrease in protein synthesis after the mid-exponential growth stage may be due to a decreasing ability of the cell to efficiently translate most mRNAs. However, when the rate of CAT synthesis relative to the rate of total protein synthesis was calculated (Table 2), a specific enhancement in *cat* mRNA translation during the lag phase of growth was indicated.

Effect of transcription inhibition on CAT synthesis

As described above, when a stationary-phase culture was diluted into fresh medium, the amount of *cat* mRNA per cell mass did not increase significantly in contrast to the ninefold increase in the rate of CAT synthesis (Table 2). To determine whether *cat* gene transcription is required for the burst of CAT synthesis

Table 2. Relative rates of CAT and total protein synthesis and amount of *cat* mRNA during culture growth

Sample*	Relative rate of CAT synthesis†	Relative rate of total protein synthesis‡	Relative rate of CAT synthesis§	Relative amount of *cat* mRNA‖	Relative translational efficiency¶
1	0·6	0·5	1·2	0·68	0·9
2	5·3	1·8	2·9	0·83	6·4
3	12·1	7·0	1·7	0·59	21
4	13·3	9·1	1·5	0·63	21
5	14·1	11·9	1·2	0·56	25
6	11·9	10·8	1·1	0·56	21
7	7·5	5·3	1·4	0·66	11
8	8·3	6·0	1·4	0·62	13
9	3·4	2·6	1·3	0·71	4·8
10	1·0	1·0	1·0	1·0	1·0

* Samples were taken from the culture at the times indicated in Fig. 3(a).

† The fluorograph in Fig. 3(b) was scanned by densitometry. The mean area of each CAT band from duplicate gels was calculated and expressed relative to sample 10.

‡ Triplicate samples of each cellular extract were TCA-precipitated. The count (c.p.m.) of [^{35}S]methionine incorporated into protein in 0·5 min (μg extract protein)$^{-1}$ was averaged and expressed relative to sample 10 [4·4 × 10^{4} c.p.m. min^{-1} (μg extract protein)$^{-1}$].

§ The rate of CAT synthesis is expressed relative to the rate of total protein synthesis.

‖ Samples identical to those described in Fig. 3 were also taken at the times indicated in Fig. 3(a) but poured over ice containing sodium azide and 1 ml MC1000 cells grown in the presence of [^{3}H]uridine and then quickly chilled to 4 °C. The amount of *cat* mRNA (OD_{650} unit)$^{-1}$ in each of the samples was determined by hybridization and corrected for yield of [^{3}H]uridine-labelled MC1000 RNA. The numbers are expressed relative to sample 10.

¶ The rate of CAT synthesis is expressed as a function of *cat* mRNA.

during the early lag phase, the rates of CAT and total protein synthesis (μg extract protein)$^{-1}$ and the amount of *cat* mRNA per cell mass were measured in cells treated with rifampicin (Fig. 4, Table 3). A stationary-phase culture was incubated for 1 min in the presence of rifampicin to inhibit transcription. This culture was then diluted 1:13 into fresh glucose minimal medium containing rifampicin. Samples were taken from the stationary-phase and diluted cultures (20 s after dilution) for translation rates and *cat* mRNA measurements. Analogous cultures not treated with rifampicin were also assayed for comparison.

The rate of CAT synthesis (μg extract protein)$^{-1}$ increased five to sevenfold after dilution of the stationary-phase culture in the presence and in the absence of rifampicin (Fig. 4b, Table 3). A threefold increase in the rate of total protein synthesis (μg extract protein)$^{-1}$ was also observed upon dilution of the stationary-phase cultures regardless of the presence of rifampicin (Fig. 4a, Table 3). The [^{35}S]methionine-labelled polypeptide patterns of the fractionated extracts obtained after dilution into medium without and with rifampicin (lanes 2 and 4, respectively, of Fig. 4a) appear virtually identical, as do the polypeptide patterns of the stationary-phase extracts (lanes 1 and 3 of Fig. 4a). Active synthesis of this group of polypeptides (including CAT) immediately after culture dilution did not appear to be affected by the presence of rifampicin and therefore is not dependent upon active transcription. In addition, the amount of *cat* mRNA per cell mass did not change significantly upon dilution of the stationary-phase cultures, in the presence or absence of rifampicin (Table 3). These results suggest that at least some transcripts, including *cat* mRNA, are stored in a relatively inactive state in stationary phase and become active for translation upon dilution of the culture into fresh medium.

DISCUSSION

The functional stability of *cat* mRNA changes during the *E. coli* growth cycle. Similar to *cat* mRNA, the functional half-life of the total mRNA pool is longer during the lag and stationary phases of growth compared to the exponential phase. These results emphasize the importance of carefully monitoring culture growth (Ingram *et al.*, 1983) to obtain reproducible half-life values and comparable mRNA decay measurements between different cultures in the same growth phase. The median functional half-life for total mRNA in an exponentially growing culture measured in this study is very similar to the 1–2 min half-life values reported for total mRNA (Pato & von Meyenburg, 1970; Pedersen *et*

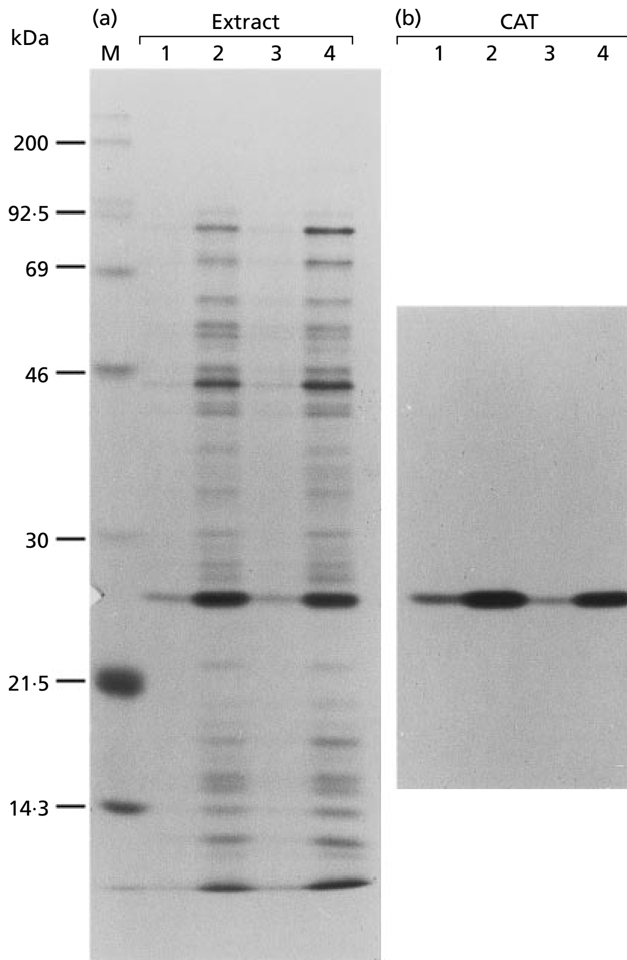

Fig. 4. The effect of rifampicin on protein synthesis before and after dilution of a stationary-phase culture into fresh medium. A culture of MC1000(pACYC184) was grown to early stationary phase and divided into two cultures. From one culture a sample was removed for pulse-labelling with [^{35}S]methionine for 0·5 min (lanes 1). This culture was then diluted 13-fold into fresh medium and within 20 s of dilution a sample was removed for pulse-labelling (lanes 2). To the second culture, 200 µg rifampicin ml^{-1} was added 1 min before a sample was removed for pulse-labelling with [^{35}S]methionine for 0·5 min (lanes 3). This culture was then diluted 13-fold into fresh medium containing 200 µg rifampicin ml^{-1} and a sample was removed for pulse-labelling (lanes 4). (a) Total extract protein was electrophoresed on a 12·5 % denaturing polyacrylamide gel and detected by fluorography. (b) A fluorograph of CAT protein immunoprecipitated from equal amounts of extract protein from each sample. Lane M, radiolabelled Rainbow protein molecular mass markers (Amersham).

al., 1978b; Albertson & Nystrom, 1994). The median functional stability values are weighted by the degree to which each message is expressed. A transcript which is abundant or translated efficiently would have a higher representation in the measurement than a transcript expressed to a lesser extent. Even though the pattern of change of total mRNA functional half-life with culture phase is very similar to that of *cat* mRNA, the half-life of the *cat* transcript is less than the total translated pool of mRNA at all stages of growth and indicates that the majority of the mRNAs being actively translated have somewhat longer functional half-lives than *cat* mRNA.

Changes in *cat* mRNA stability during culture growth are not explained by changes in growth rate. During the growth cycle, the *cat* mRNA functional half-life increases as the growth rate decreases. The longest half-life values were obtained from stationary-phase and lag-phase cultures while the shortest half-lives were obtained from an exponentially growing culture (Table 1). In contrast to these results, Nilsson *et al*. (1984) reported that the chemical half-life of the *E. coli cat* gene transcript is longer (2·3 min) when the bacteria are grown in L-broth (40 min generation time) than the 0·4 min half-life when grown in MOPS/acetate medium (200 min generation time), the opposite trend to that seen with *cat* mRNA decay during the growth cycle. Changing the growth rate by changing the culture medium has an obviously different effect on *cat* mRNA stability than changes in growth rate that occur during the growth cycle. Even though the results of Nilsson *et al*. (1984) might suggest that the *cat* mRNA decay rate is dependent on growth rate, additional experiments using steady-state chemostat cultures at different growth rates have indicated no effect of growth rate *per se* on *cat* mRNA half-life (Meyer & Schottel, 1991) and suggest that growth cycle parameters other than growth rate influence message decay.

Not all mRNAs become more stable during stationary phase when compared to the exponential phase of growth. The half-life of *E. coli ompA* mRNA decreased about fourfold when the culture entered stationary phase compared to its half-life during exponential phase (Nilsson *et al*., 1984; F. Kong & J. L. Schottel, unpublished). In addition, the half-life of the *Bacillus subtilis* succinate dehydrogenase message decreased from 2·6 to 0·4 min as the culture entered stationary phase (Melin *et al*., 1989). These results suggest the existence of different classes of transcripts that differ in their response to stationary-phase conditions. The *cat* mRNA is representative of those transcripts whose stability increases during stationary phase (Albertson *et al*., 1990; Albertson & Nystrom, 1994) in contrast to mRNAs, such as *ompA*, that have decreased stability as the culture enters stationary phase (Nilsson *et al*., 1984). A third class of mRNAs potentially exists which would not demonstrate a change in decay rate that is growth-phase-dependent. The decay rate of individual transcripts could therefore be regulated differentially in response to growth conditions.

Both *cat* mRNA and total mRNA also become functionally more stable during the premature non-growth phase induced by leucine deprivation, similar to the stabilization observed during a stationary phase that results from carbon starvation (Albertson *et al*., 1990; Albertson & Nystrom, 1994). These results are in agreement with previous reports which indicated that amino acid starvation delayed the apparent degradation of untranslated mRNA transcribed from polar muta-

Table 3. Effect of transcription on the rate of CAT and total protein synthesis before and after dilution of a stationary-phase culture

Sample*	Relative rate of CAT synthesis†	Relative rate of total protein synthesis‡	Relative amount of *cat* mRNA§
1	1·0	1·0	1·0
2	4·6	3·3	0·9
3	1·0	1·0	1·0
4	6·7	3·4	0·7

* Samples are as described in Fig. 4. A stationary-phase culture, before or after dilution into fresh medium (samples 1 and 2, respectively), was assayed without the addition of rifampicin. A stationary-phase culture, before or after dilution into fresh medium (samples 3 and 4, respectively), was assayed after rifampicin addition.

† The rate of CAT synthesis in 0·5 min (μg extract protein)$^{-1}$ was calculated from the area under a densitometric scan of the CAT band on the fluorograph shown in Fig. 4(b). The rates are the means of duplicate samples on one fluorograph. The rate of CAT synthesis after dilution of the culture (samples 2 and 4) is expressed relative to synthesis in the stationary-phase culture [samples 1 and 3 had 0·72 and 0·32 area units min^{-1} (μg extract protein)$^{-1}$, respectively].

‡ The rate of total protein synthesis was determined as described in Table 2 by quantifying the count (c.p.m.) of [^{35}S]methionine incorporated into protein in 0·5 min (μg extract protein)$^{-1}$. The results are the means of three determinations. The rate of total protein synthesis after culture dilution (samples 2 and 4) is expressed relative to synthesis in the stationary-phase cultures [samples 1 and 3 had 28 000 and 23 200 c.p.m. incorporated min^{-1} (μg extract protein)$^{-1}$, respectively].

§ The amount of *cat* mRNA per OD_{650} was corrected for yield of [^{3}H]uridine-labelled MC1000 RNA in each sample as described in Table 2. The results are the means of two determinations. The amount of *cat* mRNA in the diluted cultures (samples 2 and 4) is expressed relative to the stationary-phase cultures [samples 1 and 3 had 695 and 620 c.p.m. hybridized (OD_{650} unit)$^{-1}$, respectively].

tions of the *trp* operon (Morse & Guertin, 1971) and from the *lacA* gene (Kennell & Simmons, 1972). This, however, may not be the case for all transcripts since the *lacZ* mRNA does not appear to be stabilized during amino acid starvation (Kennell & Simmons, 1972). The biosynthetic rates of 16 proteins of the transcription/translation apparatus have been determined following amino acid limitation and revealed no one simple pattern of response (Reeh *et al.*, 1976). These results indicate that changes in mRNA stability do not respond in a uniform manner to amino acid starvation and that the stability of individual transcripts may be differentially regulated during amino acid starvation conditions, similar to the differential effect of stationary-phase growth conditions on changes in the decay rate of individual mRNAs.

Changes in *cat* mRNA stability during growth are not correlated with the translational efficiency of this message. During exponential growth, *cat* mRNA is actively translated even though the message is very labile. In a stationary-phase culture, *cat* mRNA appears to be present in a relatively non-translatable form that is also more protected from decay. Therefore, mRNA stability is not dictated simply by the frequency of translation (Petersen, 1993). In fact, inhibiting translation has been reported to reduce mRNA degradation in some cases (Schneider *et al.*, 1978; Lundberg *et al.*, 1988). In spite of a near 14-fold difference in the rate of *cat* mRNA translation when comparing exponential-phase to stationary-phase cultures, the total amount of *cat* mRNA per cell mass does not vary substantially. Translation of *cat* mRNA is therefore not simply dictated by mRNA stability or abundance but instead may be regulated in response to other parameters of culture growth such as the amino acid pool size and the availability of ribosomes and charged tRNAs. A twofold decrease in peptide chain elongation rate has been reported during starvation of an *E. coli* culture (Albertson & Nystrom, 1994). This trend in translational rates during culture growth is consistent with *in vitro* translation studies. Extracts obtained from late exponential-phase and stationary-phase *E. coli* cultures are lower in translational activity when compared to extracts made from early exponential-phase cultures (Minks *et al.*, 1978; Isaksson *et al.*, 1980). Therefore, the decrease in the rate of CAT and total protein synthesis after the exponential growth stage may be due to a decreasing ability of the cell to efficiently translate mRNAs.

Degradation and stabilization are two post-transcriptional processes that determine mRNA abundance during culture growth. In response to environmental stimuli, bacterial cells may produce or degrade factors that are involved in stabilization or degradation of a specific mRNA or class of transcripts. There are several possible mechanisms for the observed increase in mRNA stability which occurs during the stationary and lag phases of the growth cycle or during amino acid

starvation. Stabilization may involve the binding of a *trans*-acting molecule such as a protein or anti-sense RNA to the transcript. Data reported by Gorski *et al*. (1985) indicate that the stability of gene 32 monocistronic mRNA involves a *trans*-acting factor in addition to requiring specific sequences of gene 32 mRNA near the ribosome-binding site. mRNA stabilization could be the result of a decrease in ribonuclease activity during these times of slow growth (Morse & Guertin, 1971). Transcripts could also be protected from ribonucleases if they are loaded with ribosomes that are stalled and not actively translating the message (Schneider *et al*., 1978). In this case, the synthesis of ribosome modulation factor in stationary phase that is associated with 100S ribosome dimers (Yamagishi *et al*., 1993) may effectively sequester transcripts in a protective ribosome complex. Whatever the mechanism responsible for stabilization of mRNA during stationary phase, it must have an element of specificity since not all transcripts are similarly affected.

Stationary phase is very complex and can be caused by a variety of conditions, including starvation for required molecules such as carbon, nitrogen or phosphorus. Mechanisms have evolved that permit *E. coli* to survive when the conditions for growth become unfavourable. A number of proteins are synthesized by *E. coli* in response to starvation conditions whose continued activity is required for long-term cell survival (Matin, 1991; Hengge-Aronis, 1996). The genes that encode proteins required for survival under conditions of nutrient deprivation or heat shock are generally not expressed until those proteins are needed (Chuang *et al*., 1993). Stabilization of specific mRNAs during the stationary phase of growth may represent an additional mechanism for cell survival. Transcripts that are stabilized and 'stored' could become available for translation when the cells begin to recover from starvation, resulting in synthesis of a required protein even in the absence of active gene transcription. Examples of this type of translational regulation have been reported in eukaryotic cells. Some embryonic cells contain masked mRNAs in ribonucleoprotein particles that are not immediately translated but are available for protein synthesis in later stages of development (Jackson & Standart, 1990). These mRNAs appear to be stored but are available for immediate translation when production of those proteins becomes necessary. A similar type of mechanism may be used by *E. coli* to survive unfavourable growth conditions, such as those resulting in the stationary phase, and to respond to improved growth conditions experienced when starved cells are diluted into fresh culture medium.

It may be advantageous for cells to regulate expression of an antibiotic resistance gene like *cat* in this fashion. 'Storage' of the *cat* message during times of starvation could provide a means for immediate translation of the mRNA when more favourable growth conditions are encountered. During the lag phase, as the culture begins to prepare for exponential growth, active synthesis of CAT, which is not dependent on gene transcription, may be beneficial. *cat* mRNA is representative of a group of transcripts that are translated poorly in stationary phase but can be actively translated when growth conditions improve. Stabilization of particular mRNAs and regulation of their translation may be essential features of a cell's ability to quickly respond to its changing environment and thereby play an important role in cell survival during unfavourable growth conditions.

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