which apparently characterized latitudes beyond 45° in the late Eocene were altered during the first half of the Oligocene to conditions more like the present. Simultaneously, equatorial transport in the oceanic gyrals of the Pacific was restricted, giving colder surface waters around the western Pacific and thus less efficient poleward heat transport. Colder Oligocene surface waters in high latitudes led to less evaporation and therefore to decreased precipitation and colder air temperatures. The process of poleward heat transport by the oceans operates even less effectively today, and the consequences are seen in the still lower temperatures and precipitation rates.

Feedback mechanisms arising from the existence of warm oceans may have contributed to more efficient atmospheric transfer of heat energy away from the equatorial heat source as well, but the relative significance of these processes is difficult to quantify. Today, 20% of the total poleward heat transfer is effected by ocean currents; in the Eocene, certainly, a greater proportion would have been required. We emphasize the significance of the oceans to store and transport heat, particularly if given the proper configurations of land and sea, but this is with the recognition that other direct and indirect factors undoubtedly play a part in influencing climate[33].

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BIOLOGICAL SCIENCES

Stability of HeLa Cell mRNA in Actinomycin

SYNTHESIS of proteins from the mRNA in eukaryotic cells differs from prokaryotes in two important ways. In the mammalian cell, the translation of mRNA in the cytoplasm is remote from the transcription in the nucleus, whereas in bacteria these two processes occur almost simultaneously[1,2]. Bacterial protein synthesis ceases within several minutes of inhibition of RNA transcription with actinomycin[3]; hence, the mRNA is short-lived. In metazoan eukaryotic cells, a similar experiment indicates that protein synthesis is not immediately affected by inhibition of transcription. Thus the mRNA is much more stable—of the order of several hours[4]. In a few specialized systems, the lifetime of mRNA can be deduced by observing the decay of protein synthesis after RNA synthesis has ceased. The messenger for haemoglobin in the reticulocyte[5] and for silk fibroin in the silkworm[6] are examples of stable messenger molecules with a lifetime of several days. The measurement of messenger lifetime in cells with active RNA metabolism is more difficult. In the experimental approach mentioned above, new RNA synthesis was blocked with actinomycin and the subsequent decay of protein synthesis measured. Under these conditions, protein synthesis generally decreased with a 2.5 to 3 h half-life in a wide variety of systems[4,7–9]. Interpreting the decay of protein synthesis as due to a concomitant degradation of mRNA assumes that the availability of messenger molecules is limiting in protein synthesis. Here, we shall show that mRNA decay is not

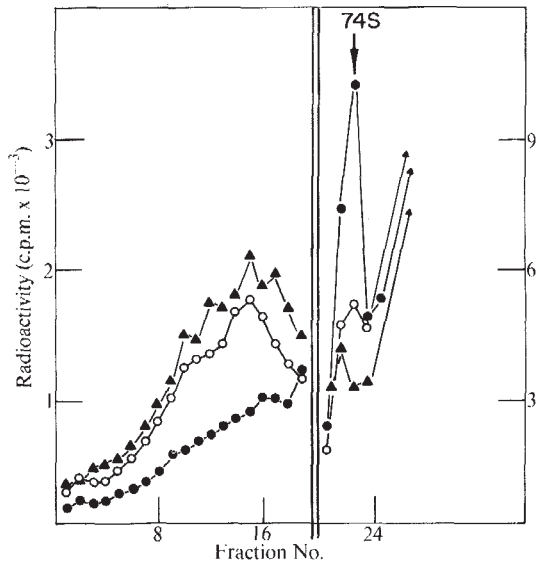

Fig. 1 The decay and rebuilding of polysomes in the presence of actinomycin. HeLa cells were grown in suspension culture as previously described[27]. 100 ml. culture was labelled for 15 h with 2 mCi of ^{14}C-uridine (Schwarz Biochemical, 35 mCi/mM), so that most of the label was in rRNA. Polysomes were allowed to decay in the presence of actinomycin (4 μg/ml., 3 h) and then 1 μg/ml. of cycloheximide was added to 1/3 the incubation mixture of HeLa cells (50 ml. at 4×10^5 cells/ml.) for 30 additional min. For control cells, the actinomycin was omitted. Cells were harvested by centrifugation at 2,000 r.p.m. for 2 min and resuspended in RSB buffer (0.01 M NaCl, 0.01 M Tris-HCl, *p*H 7.4, 0.0015 M $MgCl_2$). The cells were then broken with 'NP40' (Shell Oil Company, 0.5%). Nuclei were removed by centrifugation at 2,000 r.p.m. for 2 min and the cytoplasm layered on an RSB sucrose (15%–30% w/v) gradient (12 ml.) and centrifuged at 40,000 r.p.m. in a 'Spinco SW 40' rotor at 4° C for 1 h. Fractions were collected directly into vials and scintillation fluid (omnifluor, methoxy ethanol, toluene) added. ▲, Polysomes from normal cells; ●, polysomes from actinomycin treated cells; ○, subsequent cycloheximide treatment. Monomers (74S) are on the right-hand scale, polysomes on the left-hand scale.

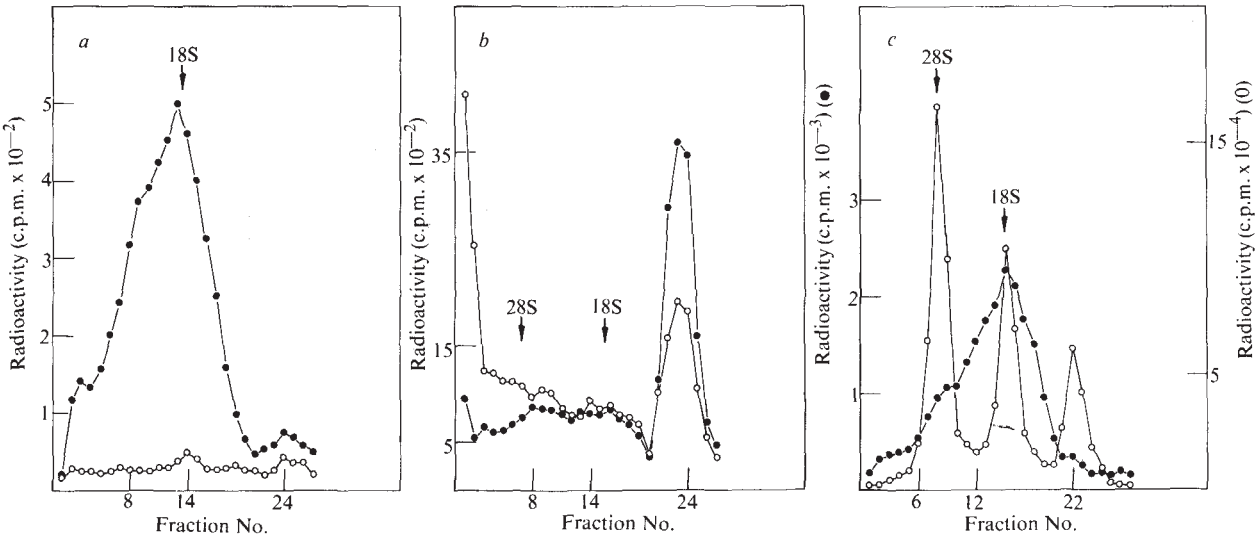

Fig. 2 Comparison of RNA hybridized to and washed through the filters under various conditions. *a* and *b* show the effect of cordycepin on the filter binding and non-binding material. 10 ml. of cell culture (2×10^6 cells/ml.) were exposed to a low level of actinomycin (0.04 μg/ml.) and ethidium bromide (1 μg/ml.). One half had cordycepin added (20 μg/ml.). The cells were then labelled with ^{3}H-uridine (New England Nuclear, 20 Ci/mM) for 90 min. *a* compares filter bound material in the presence and absence of cordycepin. *b* compares the washes from these filters. *c* represents the washed and bound material from cell cytoplasm labelled for 90 min (no drugs present) in 2 μCi/ml. of ^{3}H-uridine (4×10^5 cells/ml.). For all experiments, cells swollen in RSB buffer were lysed with NP 40 and the cytoplasm extracted with phenol after the method of Penman[26] except that the phenol and chloroform were mixed previous to the extraction. The pellet after precipitation with ethanol was resuspended in 2 ml. of SDS buffer and bound to a poly U filter. Poly U filters were made after the method of Kates[16]. The sample of total cytoplasm was passed through the poly U filter at about 1 ml. min^{-1}. The filters were washed with 4 ml. of SDS buffer and the material bound was eluted from the filter by passing 4 ml. of a formamide solution (50% formamide, 50% elution buffer, 0.01 M Tris, *p*H 7.4, 0.5% SDS) at 45° C through the filter. Carrier RNA was added to eluant (made 0.1 M NaCl) and wash. Both were precipitated with alcohol and then resuspended in 0.8 ml. SDS and layered on an SDS sucrose gradient (30%–15% w/v) and centrifuged at 25,000 r.p.m. in a 'Spinco SW27' rotor for 18 h. The gradients were collected, precipitated with 5% trichloroacetic acid and assayed to radioactivity. *a*: ○, cordycepin cytoplasm; ●, control (low actinomycin) cytoplasm. *b*: ○, cordycepin wash; ●, control (low actinomycin) wash. *c*, ●, eluant; ○, wash.

responsible for protein synthesis decay in actinomycin. Rather, mRNA appears quite stable after the administration of actinomycin. The subsequent decay of protein synthesis appears to be due to a failure in the initiation of translation.

The decrease in sedimentation pattern of polyribosomes after several hours treatment with actinomycin does not yield the expected result assuming a decay in the amount of mRNA. Disappearance of mRNA should reduce the total amount of polyribosomes but not shift the sedimentation distribution, yet polysomes from HeLa cells treated with actinomycin sediment slower than normal cells (Fig. 1). This decrease in size implies that mRNA becomes more lightly loaded with ribosomes, possibly because the rate of ribosomal initiation decreases relative to the rate of elongation. The experiment shown in Fig. 1 supports this possibility. Cycloheximide is added to both control and actinomycin treated cells at a concentration sufficient to decrease the rate of ribosome movement on message about 5-fold[10–12]. The slower initiation is apparently balanced by the diminished elongation rate and a significant recovery of normal sized polyribosomes occurs in the actinomycin treated cells. This resurrection of polyribosomes in cycloheximide suggests that much of the mRNA is still present in the actinomycin treated cells.

The recent discovery that nearly all mRNA contains a length of 3′ terminal polyadenylic acid now allows a quantitative measurement of the amount of mRNA in the cell[13–15]. By adapting a technique developed by Kates *et al.*[16], RNA containing this poly A sequence can be completely and selectively hybridized to polyuridylic acid immobilized on a glass fibre filter. The hybrid can then be melted off the filter and analysed in a sedimentation gradient. Fig. 2 shows the selectivity and efficiency of this hybridizing filter method. The first panel compares the sedimentation profiles of filter-retained and filter-transmitted RNA from the cytoplasm of HeLa cells labelled for 60 min in a low level of actinomycin (0.04 μg/ml.) sufficient to differentially suppress synthesis of rRNA[17–19]. The RNA hybridized and then eluted from the filter has a characteristic sedimentation pattern of mRNA[19]; it is heterogeneous and extending from 8S to approximately 30S with a maximum at 18S. The transmitted RNA shown in Fig. 2*b* consists of 4S RNA and a flat distribution of heterogeneous RNA extending to greater than 60S which has been shown to be unassociated with polyribosomes and is assumed to be unrelated to mRNA[19]. mRNA does not appear in the cytoplasm in the presence of cordycepin[20], an adenosine analogue; and indeed, panel A indicates that this drug inhibits the labelling of nearly all filter binding material but has little effect on transmitted RNA. Furthermore, the RNA bound to the filter is found associated with polyribosomes and is released by EDTA. It therefore appears to have the behaviour expected of mRNA. Fig. 2*c* indicates the powerful selectivity of the filters. Total cellular RNA was labelled without suppressing rRNA synthesis. The total labelled cellular RNA was bound to a filter. Although only a small percentage of this RNA is messenger, this filter technique selectively hybridizes mRNA uncontaminated by rRNA. Less than 0.2% of the input rRNA is retained on the filter.

The stability of mRNA in actinomycin was measured using poly U filters (Fig. 3). Actinomycin was added to cells after a 90 min labelling period, and measurements made at intervals several hours later. The bound RNA eluted from the filters and the transmitted RNA were analysed on sucrose gradients. The mRNA eluted from the filter is identical in its sedimentation distribution and purity in all samples. There was little decay of the mRNA eluted from the filter, even after 5 h in actinomycin. So mRNA in exponentially growing HeLa cells apparently has a half-life much longer than 6 h. It is possible, however, that actinomycin may be interfering with the normal messenger decay process, as in hormone-induced protein synthesis. We have measured the kinetics of labelling mRNA with the filter technique in the absence of any inhibitors. These experiments, to be described in another report, indicate a messenger lifetime in the order of 24 h in exponentially growing HeLa cells.

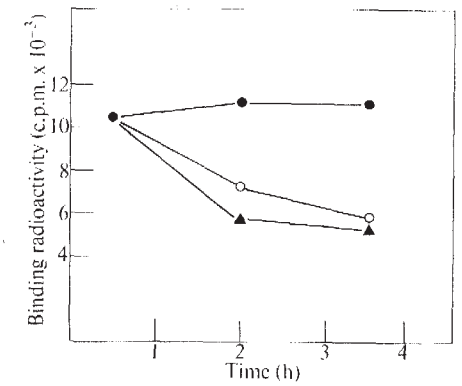

Fig. 3 Stability of messenger RNA in actinomycin and the effect of polyribosome dissociating agents. 70 ml. of cells (2×10^6 cells/ml.) was incubated for 1 h in ^{3}H-uridine (10 μCi/ml.) and then actinomycin (4 μg/ml.) was introduced into the culture. 20 min after the addition of actinomycin an aliquot of cells was removed and the remaining cells were divided into 3 separate mixtures: one received 200 μg/ml. puromycin (▲), one 10 mM NaF (○), and the last was a control (●). Aliquots were removed from each mixture at 2 h and 3.5 h after the addition of actinomycin. Cytoplasm was prepared as in Fig. 2. The samples were extracted with phenol. All samples were analysed with the poly U filters after the procedure as in Fig. 2 and run on gradients along with the wash, in order to correct for variations in recovery. Total counts bound to the filter are plotted.

Mechanisms do exist in the cell which can break down mRNA relatively rapidly. In the presence of puromycin (200 μg/ml.) or fluoride ion (10 mM), the messenger can be seen to decay significantly using the poly U filter technique (Fig. 3). Similar experiments done with total cytoplasmic RNA prelabelled in low actinomycin to suppress rRNA and not bound to poly U filters yielded the same result. Thus the loss of filter binding RNA does not result from the selective and exclusive loss of the poly A sequence from the mRNA. Because both puromycin[21] and fluoride[22] are polyribosome disaggregating agents, the data suggest that mRNA stability requires intact polyribosomes and that interruption of normal translation results in rapid messenger decay. Both drugs, however, can cause serious side reactions so that this conclusion must be tentative. Our experiments indicate that mRNA appears essentially stable in the presence of actinomycin. We show also that actinomycin has the additional effect of slowing the initiation of ribosomes onto the mRNA. Therefore, the rate of protein synthesis is not a measure of the availability of mRNA. The results using polyribosomal disruptors imply that messenger must be on polyribosomes in order to be stable. Although the demonstration of the existence of mRNA in the cytoplasm does not prove it functional, the fact that it can be used to build up polyribosomes for as long as 3 h after actinomycin treatment suggests that it probably remained functional for at least this long. A report in preparation indicates that after a 24 h chase, labelled mRNA is still associated with polyribosomes.

In some differentiated systems[23,24], some protein synthesis has been shown to be unaffected by actinomycin, possibly indicating a different initiation mechanism. Moreover, the apparent long lifetime of mRNA in actinomycin may explain a puzzling result. McAuslan showed in 1963 that actinomycin prevented the shutoff of early enzyme synthesis when administered 3 h after vaccinia virus infection[25]. Remarkably, synthesis of thymidine kinase continued linearly for 16 h. Should initiation of translation in vaccinia be unaffected by actinomycin, the long lifetime of the early vaccinia message could be understood in the light of our results. The apparent long life of mRNA in HeLa cells suggests that equating protein synthesis decay with messenger lifetime may often be incorrect. In particular, rapid changes in the rate of production of particular products may, in part, be controlled by mechanisms involving translational control.

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High Stability of Messenger RNA in Growing Cultured Cells

THE discovery that messenger RNA (mRNA) in mammalian cells contains sequences of polyadenylic acid (poly A) about 200 nucleotides long localized at the 3′-OH terminus of the mRNA molecule[1–5] has raised questions about the function of poly A, but has also provided a powerful new tool for the study of mRNA metabolism. I have measured the stability of mRNA in exponentially growing mouse L-cells without having to resort to the use of inhibitors of RNA synthesis, and found that poly A-containing mRNA has a half-life of 10 h, and thus turns over approximately once per cell generation (15 h). This high stability is in striking contrast to the 3–4 h half-life for mRNA in cultured cells obtained by following the decay of polyribosomes after treatment with a high dose of actinomycin D[6,7]. This, however, agrees with the finding of Cheevers and Sheinin[8] that there is a long-lived fraction of mRNA in cells treated with a low dose of actinomycin D. Singer and Penman[9] have simultaneously found that poly A-containing mRNA in HeLa cells turns over about once per cell generation.