On the translational error theory of aging
(error propagation/translational fidelity/clonal senescence)

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ABSTRACT Theoretical treatments of error feedback in translation have revealed that two different modes of behavior are possible, depending on the values of certain parameters. In mode I, the error frequency will rise steadily toward randomness, inevitably reaching whatever value is catastrophe for cell survival; the “error catastrophe” theory of aging implicitly assumes this mode of behavior. In mode II, the error frequency will converge to a stable value, which may or may not have toxic consequences. We have performed an experimental test of the behavior of the translation system in Escherichia coli cells: we altered the system’s intrinsic fidelity by means of the error-promoting drug streptomycin, and monitored the kinetics of change in error frequency by means of a specific assay of one kind of mistranslation (incorporation of cysteine into flagellin). We find that the system behaves according to mode II. Moreover, E. coli cells in which the error frequency has stabilized at a value as high as 50 times greater than normal continue to proliferate, albeit abnormally slowly, and their viability is not detectably reduced. Earlier results by Gorini and his associates point in the same direction. These observations diminish the plausibility of the error catastrophe theory of aging.

Orgel has pointed out that the fidelity of translation contains an element of positive feedback: errors in protein components of the translation apparatus itself (e.g., ribosome proteins or activating enzymes) would increase the frequency of subsequent translational errors (1). At first thought, this seems to imply that the fidelity of translation must deteriorate progressively, leading to an inevitable “error catastrophe.” The seeming inevitability of the error catastrophe has offered an attractive explanation of the equally inevitable death of normal mammalian cell lines or of organisms that undergo clonal senescence and, by implication, has offered an explanation of mortality itself (2–7).

However, this implication may be in error. In a significant amendment to the original theory, Orgel (8) noted that the error frequency might not increase indefinitely, but could approach a limiting value. This issue Orgel posed is as follows. Let C_n be the error frequency in “generation” n, assuming a simplified model in which successive generations of the protein synthetic apparatus are discrete and distinguishable. This error frequency can be partitioned between an intrinsic error frequency that occurs independently of prior errors, and a further contribution assumed to be linearly dependent on the preexisting error frequency in the synthetic apparatus. Let R be the intrinsic error frequency per generation, and let α be the proportionality constant between errors already present in the synthetic apparatus and new errors in freshly synthesized protein. Then:

\[ C_n = R + αC_{n-1}, \]

and

\[ C_{n-1} = R + αC_{n-2}, \text{ etc.} \]

Because \( C_1 = R \) by definition, \( C_n = R + Rα + Rα^2 + Rα^3 \).

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METHODOLOGY AND MATERIALS

E. coli strain C92 (HfrC, phoS− relA− spoT−) was employed in these studies. Conditions of growth, and the method for detecting cysteine incorporation into flagellin, were exactly as described previously (15). Briefly, the cells were double-labeled with [35S]sulfate and [3H]alanine under conditions where incorporation of sulfate into methionine is blocked altogether; flagella were highly purified and disassociated, and the flagellin monomer was resolved by sodium dodecyl sulfate/polyacrylamide gel electrophoresis; the 35S/[3H] ratio in the flagellin peak was recorded by liquid scintillation counting. The left-hand ordinate of Fig. 1 presents these 35S/[3H] ratios. In order to convert these ratios into absolute misreading frequencies, the pmol of cysteine incorporated per pmol flagellin made during the labeling period was determined, as described previously (15), in four experiments, two in uninhibited cells and two in

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cells growing in streptomycin at 2 μg/ml; these measurements showed that a \(^{35}S/^{3}H\) ratio of 1.0 corresponded on average to 0.95 ± 0.14 (SEM) \(10^{-14}\) pmol of cysteine per pmol of flagellin produced. The \(^{35}S/^{3}H\) values were accordingly scaled to this value on the right-hand ordinate of Fig. 1.

For the streptomycin studies, cells were grown in medium containing streptomycin at 2, 5, or 8 μg/ml. In the presence of these low concentrations of the drug, growth inhibition developed gradually, as other workers have observed (16), reaching maximal levels after a few generations; maximal growth inhibition by streptomycin at 2, 5, and 8 μg/ml was 15%, 20%, and 40%, respectively.

Total cell number was determined by cell count in a Petroff–Hauser chamber. Viable cell number was determined by plating on LB agar (21).

RESULTS AND DISCUSSION

Fig. 1 presents the results of two types of experiment. In the first, we simply added streptomycin and monitored the error frequency at intervals thereafter (filled symbols). It can be seen that the error frequency rose gradually, in agreement with Orgel’s original notion of positive feedback in error, as well as with observations on the gradual increase in nonsense suppression (17) and the production of thermolabile β-galactosidase (16) in similarly treated cells.

However, the error frequency did not continue to increase progressively, but rather reached a limiting value after a few generations of growth in streptomycin. In other experiments we found that the error frequency did not change between 8 and 16 generations of growth in streptomycin. This implies that a stable state of translational accuracy can be attained, even at an error frequency more than 20 times greater than normal, as in Fig. 1. In terms of Orgel’s 1970 model, this implies that \(\alpha\) is indeed less than one.

A more decisive test of this point is provided by the experiment illustrated in the open symbols of Fig. 1. In this experiment, we removed streptomycin after two generations of growth in its presence, thus reducing the intrinsic error frequency—Orgel’s \(R\)—back to its low, normal value. If \(\alpha\) were greater than one, then the first equation predicts that the error frequency should continue to rise. On the contrary, the error frequency dropped steadily after removal of streptomycin. This observation is inconsistent with any value of \(\alpha \geq 1\); instead, the data indicate a value of \(\alpha\) in the vicinity of 0.8.

In more general terms, our results show that a stable state of translational accuracy is maintained even at an error frequency much higher than normal. Moreover, a substantial increase in the error frequency, as seen after two generations in streptomycin, does not propagate itself into a further escalation of error toward catastrophe after removal of the drug. Both of these observations suggest that, whatever model one entertains, the parameters are such that translational accuracy converges to a stable value.

It follows that the immortality of vegetatively growing E. coli cells need not be ascribed to cellular selection, but may simply reflect the fact that the normal limiting error frequency is tolerable. In agreement with this view, we find that much higher limiting error frequencies are tolerable. Table 1 shows the error frequency and cell viability determined simultaneously in cultures grown in three concentrations of streptomycin for eight generations—long enough to reach a limiting error frequency as judged by Fig. 1. Even with the antibiotic at 8 μg/ml, cells that manifested an error frequency 53 times greater than normal were essentially all viable. Microscopic examination of these cells revealed no gross abnormalities. The cells did grow slower than normally, which is roughly consistent with the expected increase in the proportion of inactive enzymes produced through mistranslation. We conclude that a large increase in the error frequency is neither lethal in itself nor capable of triggering an escalating error catastrophe.

A considerable literature points to the occurrence of abnormal proteins in senescent cells, suggesting the possibility of
increased translational error (3–5, 18–20). The least we can say from our data is that a large increase in error frequency is not necessarily lethal for the type of cell we have studied. Therefore, the mere demonstration of increased translational error in senescent cells is clearly insufficient for arguing a causal relationship between translational error and cell death. What is required, our experiments emphasize, is an independent calibration of the error level that cells can or cannot tolerate. If other types of cells are as tolerant of error as E. coli appears to be, then evidence in support of the error catastrophe theory of aging will have to demonstrate very high error frequencies indeed in senescent cells.

Garvin et al. have measured the misreading of poly(U) in vitro by crude $S_{30}$ preparations derived at different times from cells growing in dihydrostreptomycin (table 2 of ref. 17). They detected a sizeable increase in poly(U) misreading, but this increase reached a constant value within a few generations of growth in the antibiotic. Our measurements of misreading in vivo (Fig. 1) show the same type of convergence. Both observations imply that the parameters of error feedback are such that stable states of translational accuracy are indeed attained, in agreement with theoretical considerations outlined by Goel and Ycas (11). It follows that error catastrophe is not an inevitable property of error feedback in protein synthesis.

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