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THE LOGNORMAL DISTRIBUTION FITS THE DECAY PROFILE OF EUKARYOTIC mRNA

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SUMMARY

A general program was written which simulates radioactive labeling of RNA in vivo. The program was used to determine the effect that different distributions of half-lives would have on the composite decay curve observed in a pulse-chase experiment. Four biologically relevant points emerge: 1) The published, experimentally determined composite decay curves for eukaryotic mRNA are not compatible with a normal, uniform, or exponential distribution of decay times. 2) The experimental curves are compatible with a lognormal distribution of decay times as well as the two-component discrete distribution previously hypothesized. 3) If the lognormal or some similar distribution were correct, about half the mRNA species would decay faster than what is presently called the "fast component of decay". This point is crucial to any argument about the fraction of poly(A) or other nuclear sequence that is transported to the cytoplasm. 4) If a particular mRNA species is found to decay at a constant rate for 3 half-lives, that is not only consistent with 1 half-life for all the mRNA, but also consistent with 20 different half-lives which are normally or uniformly distributed.

In addition to the decay of mRNA, the lognormal distribution is also compatible with data on the decay of poly(A)-containing nuclear RNA and total cellular protein.

INTRODUCTION

Eukaryotic cells contain thousands of species of mRNA and each species could conceivably be degraded with a different half-life. For human, mouse, and mosquito the shape of the decay curve of total mRNA has been interpreted in terms of two (or three) discrete decay components (1-5).

To explore whether other interpretations were possible, a computer simulation program was used to examine the way different continuous distributions affect the composite mRNA decay curve. The results show that the published, experimentally determined decay curves are not compatible with a normal, uni-

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form, or exponential distribution of decay times. However, the curves are compatible with a lognormal distribution of decay times.

The lognormal distribution is a skewed curve that peaks early and then declines slowly. Its name comes from the fact that the logarithm of the variate plotted against the frequency has the shape of a Gaussian curve. Just as a normal distribution can arise as the sum of many independent random events, the lognormal distribution can arise as the product of many independent random events (6).

The lognormal distribution appears often in biology and the social sciences. For example, the following parameters are lognormally distributed: 1) weight of human beings (7), 2) number of individuals in a species (8,9), 3) number of viral lesions in plants infected with tobacco mosaic or bushy stunt virus (10), 4) sizes of protein subunits and mRNA (11), 5) income in the U.S. (12), and 6) number of inhabitants per town (13).

MATERIALS AND METHODS

A general program (which will be described in detail at a later date) was written in Fortran IV to simulate the labeling of mRNA with radioactive isotopes. For the present application, the program solved a series of equations of the form:

$$\frac{dM}{dt} = -k_i M$$

where M = concentration of mRNA, t = time, and k_i = i^{th} rate constant for decay. The equation is solved for the first k_i for each increment of time. Then all the values are multiplied by a weighting factor which corresponds to the probability that a mRNA species will have that decay rate. Then the equation is solved for the next rate constant. All values are multiplied by the appropriate weighting factor and added to the previous values. So it goes until all the rate constants are exhausted.

RESULTS AND DISCUSSION

The computer program was used to analyze the effects of various distributions of decay times on the composite mRNA decay curve that would be observed during an ideal pulse-chase experiment. It was assumed that there is 1) no incorporation of radioactivity into RNA after the chase begins, and 2) a negligible reservoir of untransported mRNA in the nucleus. In an actual experiment, the initial part of the decay curve is obscured because both assumptions are not completely true in the beginning of the chase.

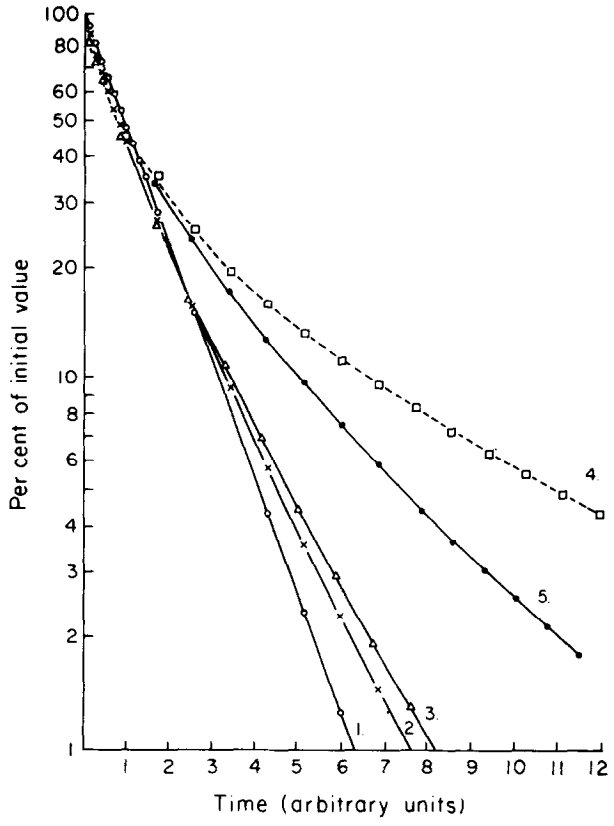

Fig. 1. Computer simulation of the composite chase curves that would result for different distributions around one median.
Curve #1 - All mRNA have half-lives of 1 unit of time.
Curve #2 - A "wide" normal distribution of half-lives with a median time of 1 unit and a standard deviation of 1/2 unit.
Curve #3 - A uniform distribution with a median half-life of 1 unit and a range of .01 unit -1.99 units.
Curve #4 - A lognormal distribution with a median half-life of 1 unit and a standard deviation of a factor of 4 (i.e. 64% of the values are between 1/4 and 4 units and 95% of the values are between 1/16 and 16 units).
Curve #5 - An exponential distribution with a median half-life of 1 unit. In other words, if C is the number of mRNA with a half-life of 1 unit of time, ½C mRNA have a half-life of 2 units of time, ¼C mRNA have a half-life of 3 units of time, etc.

Fig. 1 compares the composite curves that would result from five different distributions of decay times (see figure legend for details). Three points deserve comment: 1) For a normal distribution (curve #2), even a large standard deviation relative to the mean results in a curve whose shape is close to that of curve #1. 2) As the deviation of the lognormal distribution (curve #4) increases, the curvature becomes more marked. 3) Since

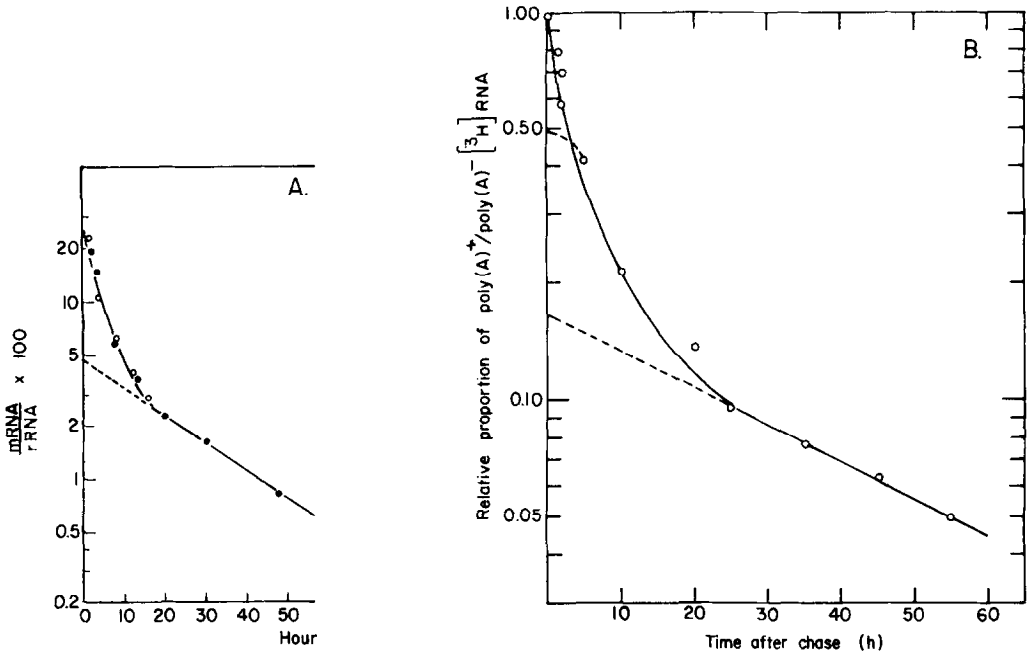

Fig. 2. A) Decay of mRNA from a cell line derived from Aedes albopictus (data from Spradling et al.;4). Cells were pulsed from 1 hr with (^{3}H)-uridine and then chased in the presence of 5 mM uridine. At various times the cpm in poly(A) containing mRNA relative to rRNA was determined.
B) Decay of mRNA in cultured erythroblastic mouse spleen cells (data from Bastos et al.; 5). Cells were pulsed for 2 hr with (^{3}H)-uridine and then chased in the presence of 20 mM uridine and 15 mM cytidine.

the exponential distribution (curve #5) is a function of only one parameter, its curvature is fixed. The slope in the regions of 100%-50% (of the maximal value) is about 3 times the slope in the region of 10%-5%.

Fig. 2 shows previously published, experimentally determined curves for a mosquito cell line (4) and cultured mouse erythroblastic spleen cells (5). Notice that the slope in the region of 100%-50% is 6-10 times the slope in the region of 10%-5%. Therefore, the computer simulations show that, of the common types of distributions tested, only the lognormal distribution is compatible with the data.

The published data on the half-lives of individual mRNA hint that decay times have a lognormal rather than a 2-component discrete distribution. Indirect measurements of mRNA half-life by the use of actinomycin D in rat liver

indicate that the half-life for: 1) levulinate mRNA is 1 hr (14), 2) tyrosine aminotransferase mRNA is 2 hr (15), 3) alanine aminotransferase mRNA is 13 hr (15), and 4) albumin mRNA is greater than 48 hr (16). In addition, direct measurement of the decay curve of mRNA in cultured mouse spleen cells was fit to two kinetic components with half-lives of 3 hr and 35 hr, yet globin, the only specific mRNA examined, decays with a half-life of 17 hr (5). In rat embryo cells transformed by adenovirus, the composite decay curve has an initial half-life of over 180; yet the two adenovirus-specific mRNA which were examined have half-lives of 35 and 100 min (17).

Implication of the Lognormal Distribution

If the lognormal or some similar distribution were correct, as Fig. 1 shows, about half the mRNA species would decay faster than what is presently called the "fast component of decay". Thus, half the mRNA in mosquito and mouse spleen (see Fig. 2) would decay faster than 2.5 hr and 4 hr, respectively. These estimates for median half-lives are upper values because the chases were not instantaneous and the reservoirs of mRNA in the nucleus were not negligible. A more accurate estimate for mosquito and mouse spleen mRNA would be 1.2 and 2 hr, respectively.

This abundance of rapidly decaying mRNA suggests that much, and maybe even all, the nuclear poly(A) could be transported to the cytoplasm. Previously, Perry et al. (18) had examined the kinetics of accumulation of poly(A) in the nucleus and cytoplasm of mouse L cells. The results indicated that, if mRNA has a half-life of 6 hr or longer, the great majority of the nuclear poly(A) is not transported to the cytoplasm. Then, Puckett et al. (19) showed that, if an appreciable fraction of the mRNA has a half-life of less than 2 hr, the accumulation curves were consistent with the transport of all the poly(A) to the cytoplasm. Therefore, if the observed composite decay curves of mRNA do reflect a lognormal distribution of decay times, the data is consistent with transport of much or all of the nuclear poly(A).

This agrees with recent experiments which indicate that poly(A) is conserved during the processing of late adenovirus-2 mRNA (20).

Generality of the Lognormal Distribution of Half-Lives

The lognormal distribution may describe the decay profile of a number of classes of macromolecules. In addition to the decay of mRNA, curve number 4 in Fig. 1 resembles the curves for the decay of proteins in rat fibroblasts (21) and the decay of poly(A) containing heterogeneous nuclear RNA in Drosophila and human cells (22,23).

One mRNA, One Half-Life?

Bastos et al. (5) have found that globin mRNA decays exponentially at a constant rate for at least three half-lives. This is consistent with all the globin mRN s turning over at the same rate, but it is also consistent with 20 different globin mRNAs with 20 different decay times which are normally or uniformly distributed (see Fig. 1). Therefore the "cause" of a 17 hr half-life for globin mRNA should be investigated if heterogeneity were ever to be found in globin ribonucleoprotein particles.

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