Equations describing the effects of the transient stability of subpopulations of nascent hnRNAs on the kinetics of turnover of hnRNA

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The equations that have been used previously to analyse the rate of decay of hnRNA implicitly assume that nascent hnRNAs are degraded stochastically. This assumption is inconsistent with electron-microscopic studies of transcription cited here, which show that nascent hnRNAs are not degraded during transcription, implying that hnRNA degradation occurs only after termination of transcription and release of the hnRNA from chromatin. Equations are derived describing the accumulation of radioactivity hnRNA during continuous labelling assuming that nascent hnRNAs are stable and that hnRNAs decay with first-order kinetics only after completion of transcription. The effects of the transient stability of nascent hnRNAs on the kinetics of hnRNA turnover can become important when the half-life of the hnRNA is shorter than the time to transcribe an hnRNA from the point of initiation to the point of termination. These equations should prove useful in studies of hnRNA turnover that require a precise description of the labelling kinetics of nascent and completed subpopulations of hnRNA.

INTRODUCTION

The rates of synthesis and decay of hnRNA have been measured in a variety of eukaryotic cells with the goal of understanding how rates of synthesis and decay interact to regulate the accumulation of hnRNA and mRNA. These analyses have always been based on equations that implicitly assume that hnRNA is a homogeneous population that decays with first-order kinetics [1–7]. However, the assumption that hnRNA is a homogeneous population all of which decays with first-order kinetics must be incorrect, because hnRNAs are stable during transcription. The stability of nascent hnRNAs is clearly demonstrated by electron-microscopic studies of transcription in a variety of organisms which show that nascent hnRNAs on active transcription units are organized into arrays of fibrils whose length increases linearly as the distance along the chromatin from the point of initiation of transcription increases [8–12]. This morphology of active transcription units implies that transcription is initiated at a specific point on the genome and that nascent hnRNAs are not degraded or released from chromatin until the RNA polymerase reaches a specific point of termination. It follows that nascent hnRNAs are stable, and therefore that it is imprecise to analyse hnRNA decay with equations that assume that both nascent and completed hnRNAs decay stochastically.

The present paper is concerned with the effects of a transiently stable subpopulation of nascent hnRNAs on the kinetics of hnRNA turnover. I have derived equations that describe for the first time the accumulation of radiolabelled hnRNA during continuous labelling assuming that nascent hnRNAs do not decay during transcription, and that only after transcription is completed do the hnRNAs decay with first-order kinetics. These equations imply that the effects of the transient stability of nascent hnRNAs increase as the

ratio of the half-life to the time to transcribe an hnRNA from end to end decreases. In the Results and discussion section I describe the effects of the transient stability of nascent hnRNAs on measurements of hnRNA turnover in several experimental systems.

DERIVATIONS

The equations previously used in analysing hnRNA turnover by the continuous-labelling method implicitly assume that radiolabelled nascent and completed hnRNAs decay with first-order kinetics according to the reaction:

where K_s is the rate of synthesis of hnRNA (c.p.m./min or mol/min), K_d is the first-order rate constant of decay and is related to the half-life ($T_{1/2}$) by $K_d = \ln 2/T_{1/2}$, and R_t is the total amount of radiolabelled hnRNA (c.p.m. or mol). Assuming a steady-state system, and that the nucleoside triphosphate pools equilibrate instantaneously and their specific radioactivity remains constant during the pulse, the change in radiolabelled hnRNA is described by the differential equation:

$$\frac{dR_t}{dt} = K_s - K_d R_t \quad (2)$$

which integrates to:

$$R_t = \frac{K_s}{K_d} (1 - e^{-K_d t}) \quad (3)$$

The following equations apply to a population of hnRNAs in which nascent hnRNAs are stable and the hnRNAs after completion of transcription decay with first-order kinetics. This reaction is given by the equation:

where K_{cd} is the first-order rate constant of hnRNAs after completion of transcription, R_n and R_c are the amount of radiolabelled nascent and completed hnRNAs respectively, and T is the length of time to transcribe an hnRNA from its point of initiation to its point of termination. For simplicity, I have assumed that all of the transcription units in the population of hnRNAs are exactly the same length, that there is no lag between the addition of the last nucleotide to the 3' end of the hnRNA and the start of stochastic decay, and that no hnRNA is transported to the cytoplasm as mRNA.

Different equations are required to describe the accumulation of radiolabelled nascent and completed hnRNAs during the labelling periods when $0 \leq t \leq T$ and $t \geq T$. When the labelling period is shorter than T , each completed hnRNA will contain radioactive nucleotides incorporated along a fraction, t/T , of its length. Therefore the rate of flow of radiolabelled hnRNA from the nascent subpopulation into the completed subpopulation will be given by $(t/T)K_s$. The rate of accumulation of radiolabelled nascent hnRNAs (R_n) will be described by the differential equation:

$$\frac{dR_n}{dt} = K_s - (t/T)K_s \quad 0 \leq t \leq T \quad (5)$$

and its integral:

$$R_n = \frac{2tTK_s - t^2K_s}{2T} \quad 0 \leq t \leq T \quad (6)$$

The rate of accumulation of radiolabelled completed hnRNAs (R_c) during the labelling period $0 \leq t \leq T$ will be described by the differential equation where K_{cd} is the first-order decay constant of completed hnRNAs:

$$\frac{dR_c}{dt} = \frac{tK_s}{T} - R_cK_{cd} \quad 0 \leq t \leq T \quad (7)$$

which integrates to become:

$$R_c = \frac{K_s}{TK_{cd}^2} (tK_{cd} - 1 + e^{-K_{cd}t}) \quad 0 \leq t \leq T \quad (8)$$

The total amount of radiolabelled hnRNA is the sum of eqns. (6) and (8):

$$R_t = \frac{2tTK_s - t^2K_s}{2T} + \frac{K_s(tK_{cd} - 1 + e^{-K_{cd}t})}{TK_{cd}^2} \quad 0 \leq t \leq T \quad (9)$$

When $t \geq T$, all nascent hnRNAs will be uniformly labelled from end to end, and the equation describing the accumulation of radiolabelled hnRNA can be broken into three parts: (1) eqn. (6) evaluated at $t = T$; (2) the amount of completed hnRNAs that accumulates during the interval $0 \leq t \leq T$ is given by eqn. (8) evaluated at $t = T$; during the interval $t \geq T$, these completed hnRNAs will decay by the first-order kinetics; (3) hnRNAs that are completed during the interval $t \geq T$ will accumulate with kinetics that are described by eqn. (3).

$$R_t = \frac{K_s}{2T} + \frac{K_s[TK_{cd} - 1 + e^{-K_{cd}T}][e^{-K_{cd}(t-T)}]}{TK_{cd}^2} + \frac{K_s[1 - e^{-K_{cd}(t-T)}]}{K_{cd}} \quad t \geq T \quad (10)$$

RESULTS AND DISCUSSION

Measurements of the half-life of hnRNA in eukaryotic cells have always been based on a simple model of hnRNA turnover that I believe is incorrect because it assumes that all molecules of hnRNA are equally likely to be degraded. I believe that the work cited in the Introduction demonstrates that nascent hnRNAs are stable during transcription and it is only after transcription of the primary transcript has been completed that the completed hnRNA begins to decay stochastically. I have derived equations here that describe the simplest model of accumulation of radiolabelled hnRNA during continuous labelling: one in which radioactive nucleotides are incorporated into nascent hnRNAs, nascent hnRNAs do not decay during transcription, and after termination of transcription the completed hnRNAs decay with first-order kinetics. It should be noted that several simplifying assumptions were made in deriving these equations, and so they do not apply perfectly to any experimental situations.

To illustrate how the transient stability of nascent hnRNAs affects the kinetics of accumulation of radiolabelled hnRNA during continuous labelling, I have graphed eqns. (3) and (9)+(10) in Fig. 1 using two different half-lives, 0.1 and 1.0 (arbitrary unit). The continuous line (—) shows that the accumulation of radiolabelled hnRNA described by eqn. (3) follows a curve of decreasing slope that asymptotically approaches a steady-state plateau amount of hnRNA. Eqns. (9)+(10) define curves (—) that have similar, but not identical, shape (see logarithmic plots in Fig. 2), and the amounts of hnRNA at steady state are higher owing to the temporary stability of nascent hnRNAs. I have also graphed the accumulation of radiolabelled nascent hnRNA that is described by eqn. (6). This also defines a curve of decreasing slope (—) that, in contrast with eqns. (3) and (9)+(10), reaches a steady-state plateau at a definite time, $t = T$, when all nascent hnRNAs have been completely labelled from end to end. The steady-state amount predicted by eqn. (10) is the sum of the steady-state amounts predicted by eqns. (3) and (6).

Graphing eqns. (3) and (9)+(10) in the form normally used in the analysis of hnRNA decay in continuous-labelling experiments, $\log(1 - R/R_\infty)$ versus t , demonstrates effects of the transient stability of nascent hnRNAs on turnover kinetics that are not obvious in linear plots. When eqn. (3) is graphed in this form as shown in Fig. 2, it always produces a straight line with slope $-T_{1/2}$ for three different half-lives. For comparison, eqns. (9)+(10) were graphed for the same three half-lives such that the half-lives are 0.1-, 0.3- and 1.0-fold of the time it takes the RNA polymerase to transcribe from the point of initiation to the point of termination, T . The logarithmic plots of eqns. (9)+(10) do not produce straight lines, but rather produce lines that curve downward and asymptotically approach a straight line with slope $-T_{1/2}$. This downward curvature reflects the fact that when t is close to 0 the completed hnRNA transcripts will have radiolabelled nucleotides incorporated only near their 3' ends, so the instantaneous rate of decay of radiolabelled completed hnRNA is lower than the rate of decay of total completed hnRNA. As t increases to and exceeds T , the completed hnRNA transcripts will contain radioactive nucleotides along an increasing fraction of their length, and the instantaneous

Fig. 1. Effects of the transient stability of nascent hnRNA on the kinetics of accumulation of radiolabelled hnRNA during continuous labelling

Eqns. (3) (—), (6) (----) and (9)+(10) (— · —) were graphed against t for $K_s = 1$ and $T = 1$. The units of time and radioactivity are arbitrary. Panel (a) $K_d = K_{cd} = 0.693$; panel (b) $K_d = K_{cd} = 6.93$.

Fig. 2. Effects of the transient stability of nascent hnRNAs on the kinetics of decay of hnRNA as measured by the continuous-labelling method

Eqns. (3) (—) and (9)+(10) (— · —) were evaluated for varying t for $K_s = 1$ and $T = 1$. R was calculated for $t = \infty$ by using eqns. (3) and (10). Panel (a) $K_d = K_{cd} = 6.93$; panel (b) $K_d = K_{cd} = 2.31$; panel (c) $K_d = K_{cd} = 0.693$. The units of time and radioactivity are arbitrary.

rate of decay of radiolabelled completed hnRNA asymptotically approaches the rate of decay of total completed hnRNA.

Fig. 2 also shows that using eqn. (3) to analyse the rate

of decay of a population of hnRNAs that contains a temporarily stable population of nascent hnRNAs will generally produce estimates of half-lives that are too long, and that the size of the errors increases as T_1/T decreases.

When $T_{1/2}/T = 1$ (Fig. 2c) there is practically no difference between the lines described by eqns. (3) and (9)+(10), and the errors resulting from using eqn. (3) to analyse decay kinetics will be slight (approx. 10%). When $T_{1/2}/T$ is less than 1 (0.1, Fig. 2a; 0.3, Fig. 2b), the lines described by eqns. (3) and (9)+(10) are quite different. The actual extent of the errors would depend on the individual experimental system and the method of weighting data points in the data reduction. A rough approximation of these errors can be obtained by comparing T with the time when the line defined by eqns. (9)+(10) intersects $0.5R_{\infty}$. For $T_{1/2}/T$ equal to 0.1 and 0.3, the apparent half-lives based on eqn. (3) exceeded the real half-lives by about 3.2- and 1.5-fold respectively. It should be noted that when $T_{1/2}/T$ is very small (e.g. 0.1) eqns. (9)+(10) are very insensitive to $T_{1/2}$, so using eqns. (9)+(10) to measure rates of decay would require extremely accurate measurements of hnRNA turnover.

Whether previous measurements of the rate of hnRNA decay are in error because it was assumed that nascent hnRNAs decay with the same kinetics as completed hnRNAs reduces to the question: what is that ratio $T_{1/2}/T$ in various organisms? T , the interval required to transcribe an hnRNA from end to end, is a function of the size of the primary transcript and the rate at which the RNA polymerase adds nucleotides to nascent hnRNAs. It has been estimated that the median transcription unit in HeLa cells is about 5 kilobases [13]. Assuming an RNA polymerase elongation rate of about 100 nucleotides/s for polio virus [14], it would take about 50 s to transcribe an hnRNA of this size completely. This is about 25–84-fold shorter than the half-life of hnRNA measured in mouse L-cells, rabbit erythroid cells and HeLa cells, 21–70 min [3, 6, 7]. Since Fig. 2 indicates that there is essentially no effect of the stability of nascent hnRNAs on the kinetics of hnRNA decay when the ratio $T_{1/2}/T$ is greater than 1, estimates of the half-life of hnRNA in mammals would not be in error even if the average transcription time were 25-fold longer than assumed above.

By comparison, the transcription times of hnRNA in sea-urchins appear to be sufficiently long to create problems with using eqn. (3). Busby & Bakken [11, 12] have estimated that it takes 35–70 min to transcribe an average-sized hnRNA in embryos of *Strongylocentrotus purpuratus* from their measurements of the size of transcription units (20 kilobases in blastulae, 26 kilobases in gastrulae) and the RNA polymerase elongation rate in blastulae (6–9 bases/s [5]). Estimates of the average half-life of total populations of hnRNA in sea-urchin embryos based on eqn. (3) have ranged from 7 to 23 min [1, 2, 5]. Since these estimates of the average half-life of sea-urchin hnRNA are at least 1.5–10-fold shorter than the average transcription time, the real half-lives of sea-urchin hnRNA may be even shorter, because eqn. (3) leads to estimates of half-life that are too long when $T_{1/2}/T$ is less than 1. I have not attempted to use eqns. (9)+(10) to estimate the half-life of sea-urchin hnRNA because these equations do not apply to populations of total hnRNA consisting of transcription units of different lengths. The purpose of this analysis is simply to establish

that populations of hnRNA do exist in which $T_{1/2}/T$ is small enough for the transient stability of nascent hnRNAs to affect measurements of rates of hnRNA decay.

The large difference in the nucleotide elongation rate of the mammalian and sea-urchin RNA polymerases, 7.5 compared with 100 bases/s [14, 15], would be expected to produce substantial differences in the relative proportions of nascent and completed hnRNAs. The fraction of hnRNA in nascent and total hnRNAs can be calculated by using eqns. (6) and (10) as described in the legend to Fig. 1. Assuming a uniform transcription unit size of 20 kilobases and an RNA half-life of 23 min, I calculate that nascent hnRNAs would comprise, respectively, 4.7% and 40% of the total mass of hnRNA at steady state in mammals and sea-urchins. This difference in the abundance of nascent and complete hnRNAs might affect hnRNA utilization in mammals and sea-urchins.

Finally, I should point out that the transient stability of nascent hnRNAs may explain why the kinetics of decay of labelled hnRNA in the presence of actinomycin D shows a long-lived component not seen in the absence of the drug [6, 16]. This slowly decaying component of hnRNA has been explained as a side effect of the drug. A simpler explanation is that transcription of prelabelled nascent hnRNAs continues very slowly in the presence of actinomycin D, and that after termination of transcription the completed hnRNAs decay by normal processes. Alternatively, it is possible that when actinomycin D prevents the completion of transcription of nascent hnRNAs the nascent hnRNAs decay abnormally.

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