Half-Life and Rate of Synthesis of Globin Messenger Ribonucleic Acid

DETERMINATION OF HALF-LIFE OF MESSENGER RIBONUCLEIC ACID AND ITS RELATIVE SYNTHETIC RATE IN ERYTHROID CELLS

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The specific radioactivity of mouse globin mRNA in blood reticulocytes was measured after injection of [^3H]uridine into anaemic mice up to 60h before collection of reticulocytes. From these data, the decay of the acid-soluble nucleotide pool in the marrow and the relative marrow-cell composition it is possible to build models that allow the cell life-times and half-life of mRNA in the erythroid cells of the marrow to be calculated. Best fit of models to these data favour a model with either one or two cell divisions from the onset of mRNA synthesis. The single-cell-division model has cell times of 20, 13 and 7h respectively for the basophilic erythroblast, polychromatophilic erythroblast and reticulocyte. The two-cell-division model has cell times of 12, 12, 12 and 7h for the basophilic erythroblast 1 and 2, polychromatophilic erythroblast and reticulocyte respectively. Both models have an mRNA half-life of 17h and a constant rate of mRNA synthesis until enucleation at the reticulocyte stage, when synthesis stops. A declining rate of mRNA synthesis can be accommodated in a two-cell-division model, when synthesis halves at each cell division and cell times are essentially the same as above, but mRNA half-life is either 9h in the basophilic and polychromatophilic erythroblasts and 17h in the later cells, or 10h in the basophilic erythroblasts and polychromatophilic erythroblasts and 14.5h in later cells. In all cases it is clear that mRNA synthesis occurs over a time-period of only 30-36h and that mRNA cannot be pre-synthesized in precursor erythroid cells.

Evans & Lingrel (1969), by measuring the specific radioactivity of globin mRNA after injection of [^3H]uridine into anaemic mice at various times before collection of reticulocytes, estimate that most of the mRNA synthesis occurs in basophilic erythroblasts. However, their work did not take into account the rate of destruction of mRNA in these cells. I thought that the data presented by Evans & Lingrel (1969), if extended, could be used to determine the half-life of globin mRNA and the relative rate of synthesis of mRNA in each cell compartment of the bone marrow, as well as estimates of the number of and duration of cell divisions used during erythropoiesis in the anaemic mouse.

Induction of anaemia

Swiss Cox-strain mice (25g) were injected daily with 0.1ml of 0.8% neutralized phenylhydrazine, and blood and bone marrow were collected 1 day after the last injection. In general seven injections were given and blood was collected on day 8, i.e. 7 days after the first injection.

Collection of reticulocytes, preparation of ribosomes and labelling reticulocytes

These procedures were exactly as described by Evans & Lingrel (1969). The same amount (50 μCi) of [^3H]uridine was injected into groups of ten mice at various time-intervals before collection of the blood 8 days after the first injection with phenylhydrazine.

Analysis of RNA

Ribosomes (approx. 8mg/ml in 5mM-Tris-HCl, pH 7.4) were made 0.5% in sodium dodecyl sulphate by addition of a 10% (w/v) stock solution, incubated for 5 min at 37°C and layered over precooled sucrose gradients.

Experimental

Materials

All chemicals were reagent grade. Sucrose (ribonuclease-free) and [^3H]uridine (8Ci/mmol) were from Schwarz/Mann (Orangeburg, N.Y., U.S.A.). Sodium dodecyl sulphate was recrystallized from ethanol.

Sucrose gradients were made by mixing 5% (w/v) and 22.6% (w/v) sucrose in 5mM-Tris-HCl in an Isco gradient former in the ratio of 16ml:21ml to obtain a convex 37ml gradient. However, under these conditions centrifugation at 27000 rev./min for neither 28h nor 36h at 2°C in the Spinco SW 27.1 rotor separated the mRNA sufficiently for accurate determination of its specific radioactivity. Consequently the mRNA region was made 0.1M in NaCl and the mRNA was precipitated with 2vol. of ethanol at -15°C. The mRNA was dissolved in 0.2ml of 0.1M-NaCl-0.005M-Tris-HCl, pH 7.4, and made 0.5% in sodium dodecyl sulphate as above and layered on 5ml linear 5-20% (w/v) sucrose gradients containing 0.1M-NaCl-0.005M-Tris-HCl, pH 7.4, and centrifuged at 50000 rev./min for 6.75h in the Spinco SW 50.1 rotor at 2°C.

Radioactivity and specific radioactivity determination

Although the centrifuge tubes from the SW 50.1 rotor were emptied by upward displacement through an Isco u.v. monitor, accurate readings of the absorption of each fraction were obtained by taking 0.2ml from each fraction for dilution with 0.8ml of 0.1M-sodium phosphate, pH 7.0, and reading the E_{260} in a spectrophotometer. The diluted fraction (1.0ml) was transferred to a scintillation counter and mixed with 9ml of a mixture containing 1 vol. of Beckman BBS-3 solubilizer and 5 vol. of toluene containing 0.5% 2,5-diphenyloxazole (PPO) and 0.05% 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene (dimethyl-POPOP). The mixture was counted for radioactivity in a Packard 3000 series liquid-scintillation spectrometer. All counts were corrected to d.p.m. This counting procedure was accurate when 0.1M salt was present in the sucrose solutions but gave low and variable readings of undiluted material from sucrose gradients in the absence of salt.

Specific radioactivity of mRNA was calculated by assuming an extinction coefficient of 25 at 260nm for a 1 mg/ml solution of RNA in a 1 cm cell.

Bone marrow

Bone marrow from the rear-leg bones of an anaemic mouse was collected and washed in 40% foetal calf serum-60% Hanks' balanced salt solution (Grand Island Biological Co., Grand Island, N.Y., U.S.A.) and taken up in 0.5ml of the washing medium. Cell counts were made in a haemocytometer and smears were made for differential cell counts. Radioactive-pool measurements were made on 0.4ml of the marrow sample after centrifugation and addition of 1.1ml of cold 5% trichloroacetic acid; 1ml of trichloroacetic acid supernatant was added to 9ml of the toluene-BBS-3 scintillator solution described above and counted for radioactivity in the Packard liquid-scintillation counter.

Haemoglobin content of erythrocytes and reticulocytes

Blood from anaemic and non-anaemic mice was collected by heart puncture and cell counts were made in a haemocytometer. Reticulocytes were stained with Methylene Blue and differential counts made on smears. Alternatively reticulocytes could be counted as the larger-diameter cells in the suspension.

Haemoglobin was determined by the cyanmet-haemoglobin method (Hycel Inc., Houston, Texas, U.S.A.).

Model of erythropoiesis

The two models of erythropoiesis, following a cell from the start of mRNA synthesis to its appearance in the blood, which are proposed for the anaemic mouse, are shown in Fig. 1. Both assume that mRNA synthesis starts at a point in the basophilic erythroblast at time T_3 or T_4 (depending on whether there is one or two cell divisions) before the cell reaches the bloodstream. Similarly, mRNA synthesis is assumed to stop when the nucleus is expelled from the polychromatophilic erythroblast at time T before the cell reaches the bloodstream. The times in each cell compartment are then T_1 , $T_2 - T_1$, $T_3 - T_2$ and $T_4 - T_3$ respectively and the cellular composition of the marrow, omitting the pronormoblast, is defined from the cell times as follows.

R , P , Ba_2 and Ba_1 are the relative concentrations of the marrow reticulocyte, polychromatophilic erythroblast and basophilic erythroblast 2 and 1 respectively.

In the two-cell-division model:

$$R + P + Ba_1 + Ba_2 = 1 \quad (1)$$

$$\frac{R}{P} = \frac{T_1}{T_2 - T_1} \quad (2)$$

$$\frac{R + P}{2Ba_2} = \frac{T_2}{T_3 - T_2} \quad (3)$$

and

$$\frac{Ba_2}{2Ba_1} = \frac{T_3 - T_2}{T_4 - T_3} \quad (4)$$

For the single-cell-division model:

$$\text{eqn. (1) becomes } R + P + Ba = 1 \quad (5)$$

$$\text{eqn. (2) remains the same and}$$

$$\text{eqn. (3) becomes}$$

$$\frac{R + P}{2Ba} = \frac{T_2}{T_3 - T_2} \quad (6)$$

It is assumed that the rate of mRNA synthesis is a pseudo-zero-order reaction which is not affected by the size of the pool of nucleotide triphosphates and

Fig. 1. Models for mRNA synthesis during erythropoiesis in an anaemic mouse

Abbreviations used are: Baso., basophilic erythroblast; Poly. polychromatophilic erythroblast; Retic., reticulocyte. T_1 – T_4 are times for a cell at the beginning of each stage to reach the circulating blood. Time t indicates the measurement of time for integration of cells during each stage. (a) Single-cell-division model; (b) two-cell-division model.

that on cell division, since the size of the cell diminishes by a factor of two (Borsook, 1965), the specific radioactivity of the radioactive pool of nucleotide triphosphates remains the same.

Radioactive mRNA synthesis may be calculated by assuming a common pool of radioactively labelled precursors (A) with a decay rate K_1 .

The synthesis of radioactive mRNA (B) is described thus:

where K_0 is the rate constant for the synthesis of mRNA. The decay of labelled mRNA is described by the scheme,

where K_2 is the decay rate of mRNA.

From these schemes (7, 8 and 9) the rate of production of labelled mRNA becomes

$$\frac{dB}{dt} = K_0[A] - K_2[B] \quad (10)$$

and

$$A = A_0 e^{-K_1 t} \quad (11)$$

where A_0 is a constant defined by initial conditions; hence

$$\frac{dB}{dt} + K_2[B] = K_0 A_0 e^{-K_1 t} \quad (12)$$

The general solution of this differential equation is

$$B = \alpha_1 e^{-K_2 t} + \alpha_2 e^{-K_1 t} \quad (13)$$

when

$$\alpha_2 = \frac{K_0 A_0}{K_2 - K_1} \quad (14)$$

hence,

$$B = \alpha_1 e^{-K_2 t} + \frac{K_0 A_0 e^{-K_1 t}}{K_2 - K_1} \quad (15)$$

Now, if $B = 0$ at $t = 0$,

$$\alpha_1 = \frac{-K_0 A_0}{K_2 - K_1} \quad (16)$$

and solution of the differential equation becomes

$$B = \frac{K_0 A_0}{K_1 - K_2} (e^{-K_2 t} - e^{-K_1 t}) \quad (17)$$

when $K_1 \neq K_2$.

It is now possible to apply eqn. (17) to the case where an injection of radioactive precursor to mRNA is given to an anaemic mouse at time T before collection of blood. Clearly when $T < T_1$ no label incorporated into mRNA will appear in the blood. When $T_2 > T > T_1$, only cells in the polychromatophilic-erythroblast stage mature and pass into the blood, and hence their labelled mRNA will be found in the blood. The total label is found by integrating over the interval T_1 to T as follows, when $t = 0$ at the end of the polychromatophilic-erythroblast stage (see Fig. 1).

$$R = \int_{t=0}^{t=T-T_1} \frac{B_t e^{-K_2(T-t)} dt}{T_2 - T_1} \quad (18)$$

The term $e^{-K_2(T-t)}$ represents the decay of mRNA after mRNA synthesis stops at the end of the polychromatophilic-erythroblast period since all cells are labelled for a total time T .

This integral becomes

$$R = \frac{K_0 A_0}{(T_2 - T_1)(K_1 - K_2)^2} [(K_1 - K_2)(T - T_1)e^{-K_2 T} + e^{-K_2 T - (K_1 - K_2)(T - T_1)} - e^{-K_2 T}] \quad (19)$$

The proportion of cells in the polychromatophilic-erythroblast compartment is P , hence the total labelled mRNA reaching the blood after injection at time T is

$$R_T = \frac{P}{(T_2 - T_1)} \cdot \frac{K_0 A_0}{(K_1 - K_2)^2} [(K_1 - K_2)(T - T_1)e^{-K_2 T} + e^{-K_2 T - K_1(T - T_1)} - e^{-K_1 T}] \quad (20)$$

When $T_3 > T > T_2$, i.e. when the labelling spreads into the basophilic-erythroblast period, the integral of label in the polychromatophilic-erythroblast period becomes

$$R_{poly.} = \frac{PK_0 A_0}{(T_2 - T_1)(K_1 - K_2)^2} [(K_1 - K_2)(T_2 - T_1)e^{-K_2 T_2} + e^{-K_2 T_1 - K_1(T_2 - T_1)} - e^{-K_2 T_2}]e^{-K_2(T - T_2)} \quad (21)$$

where $e^{-K_2(T - T_2)}$ represents further decay of the mRNA synthesized in this period.

Labelling of mRNA in the basophilic erythroblasts is defined now by the following integral.

$$R_{Baso.} = \frac{Ba}{T_3 - T_2} \int_{t=0}^{t=T-T_2} [Be^{-K_2(T-t)} + 2B_{T_2-T_1}e^{-K_1 t - K_2[T-(t-T_2-T_1)]}]dt \quad (22)$$

The second part of the equation is included to allow for each basophilic erythroblast becoming two polychromatophilic erythroblasts on cell division. This integral becomes:

$$R_{Baso.} = \frac{K_0 A_0 Ba}{(T_3 - T_2)(K_1 - K_2)^2} [(K_1 - K_2)(T - T_2)(e^{-K_2 T} + e^{-K_1 T_1 - K_1(T - T_1)} - e^{-K_2 T} - 2(e^{-K_2(T_2 - T_1)} - e^{-K_1(T_2 - T_1)})(e^{-K_1(T - T_2)} - e^{-K_2 T_1} - e^{-K_2[T-(T_2 - T_1)]})] \quad (23)$$

The sum of $R_{poly.}$ and $R_{Baso.}$ thus represents the total labelled mRNA reaching the blood when $T_3 > T > T_2$.

If the model were for a single-cell division this would be the final label. If we consider the two-cell-division model, Ba would be replaced by Ba_2 in eqn. (22) and the label in the basophilic-erythroblast 1 stage (when $T_4 > T > T_3$) is

$$R_{Baso.1} = \frac{Ba_1}{(T_4 - T_3)(K_1 - K_2)^2} [(T - T_3)(K_1 - K_2)e^{-K_2 T} + e^{K_2 T_3 - K_1(T - T_3)} - e^{K_2 T} - 2(e^{-K_2(T_3 - T_2)} - e^{-K_1(T_3 - T_2)})(e^{-K_1(T - T_3)} - e^{K_2 T_2} - e^{K_2[T-(T_3 - T_2)]}) - 4(e^{-K_2(T_2 - T_1)} - e^{-K_1(T_2 - T_1)})(e^{-K_1(T - T_2)} - e^{K_2 T_1} - e^{-K_1(T_3 - T_2)} - e^{K_2[T-(T_3 - T_1)]})] \quad (24)$$

This value is added to the sum of contributions by the polychromatophilic-erythroblast and basophilic-erythroblast-2 stages (i.e. eqn. 21 and 22 at $T = T_3$ multiplied by $e^{-K_2(T - T_3)}$).

These equations were used in a program written in APL for use on the IBM 360/65 computer in the time-sharing mode to provide values of R at any time relative to R at 24h. Factors of rates of synthesis were introduced into eqns. (23) and (24) to allow for changes in rate of mRNA synthesis in the basophilic-erythroblast cell times relative to the rate in the polychromatophilic-erythroblast cell time. The specific radioactivity of mRNA found was calculated to give $R = 1$ at 24h and the sum of squares of deviations from experimental and calculated points was minimized to obtain the curve fit.

Results

Degree of anaemia of mice at various times before collection of reticulocytes

The mice were given seven daily injections of phenylhydrazine and blood was usually collected on the eighth day. To determine if there was the same degree of erythropoiesis over the periods of injection, up to 60h before collection, bone-marrow and blood samples were taken from mice on days 5, 6, 7 and 8. No significant differences could be detected between differential bone-marrow counts taken on mice killed on these days, and the reticulocyte count showed a progressive increase (Table 1). It was considered that the marrow was equally active in erythropoiesis over the period of injection, which was 1 day longer than that chosen by Evans & Lingrel (1969). Some difficulty was found in obtaining good bone-marrow smears, however, and reproducibility of differential counts depended somewhat on the quality of the slides (Table 1c). The greatest variation was in the percentage of reticulocytes found.

The haemoglobin content of cells from anaemic and non-anaemic mice is given in Table 2 and from this values of 17pg/cell for normal and 26pg/cell for anaemic mice can be calculated. A value of 16pg/cell is usually quoted for non-anaemic mice. Cell diameters of the mouse reticulocytes are greater than those of the erythrocytes (9 against 6 μ m). The same difference is shown by anaemic and non-anaemic rabbits (Lehmann & Huntsman, 1961). It is then

Table 1. *Degree of anaemia in mice after various periods of injection with phenylhydrazine*

For details see the text. Each of the values in (a) and (b) was from a single mouse.

(a)	No. of injections	Blood taken after day	Percentage of reticulocytes	
	4	4	—	36.6
	5	5	38	48
	6	6	40.5	66
	7	7	75	78.5

(b) Differential bone-marrow counts

No. of injections	Marrow taken after day	Percentage of erythroid cells	Proportion of cells (% of erythroid cells)				No. of cells counted
			Pronormoblast	Basophilic erythroblast	Polychromato- philic erythroblast	Reticulocyte	
5	5	75.8	5.3	35.2	39.8	20.6	2041
6	6	72.1	2.9	36.8	41.1	19.3	2049
7	7	66.6	2.6	33.2	38.9	26.5	2808

(c) Mean values of differential bone-marrow counts from eight mice after seven injections taken after 7 days

Percentage of erythroid cells	Proportion of cells (% of erythroid cells, $\pm$ S.D.)				No. of cells
	Pronormoblast	Basophilic erythroblast	Polychromatophilic erythroblast	Reticulocyte	
76.2 $\pm$ 6.2	4.0 $\pm$ 1.4	33.9 $\pm$ 3.2	37.2 $\pm$ 4.1	24.0 $\pm$ 5.6	2756

Table 2. *Haemoglobin content of reticulocytes and erythrocytes of anaemic and non-anaemic mice*

Six mice in each state were used. The difference between the haemoglobin contents is significant (Student's $t = 3.22$, 10 degrees of freedom; $0.01 > P > 0.005$). Assuming that the reticulocytes in both animals are similar, then by solving the simultaneous equations: $0.725x + 0.275y = 23.4$; $0.035x + 0.943y = 17.6$, the reticulocyte (x) contains 26 pg and the erythrocyte (y) 17 pg of haemoglobin.

State of mice	$10^{-12} \times$ haemoglobin/ cell (g, $\pm$ S.D.)	Percentage of reticulocytes ($\pm$ S.D.)
Anaemic	23.4 $\pm$ 3.98	72.5 $\pm$ 4.5
Non-anaemic	17.6 $\pm$ 1.92	5.7 $\pm$ 2.7

likely that the anaemic mouse by-passes the polychromatophilic stage in erythropoiesis exactly as in the rabbit (Borsook, 1965).

Specific radioactivity of globin mRNA

Centrifugation of sodium dodecyl sulphate-treated ribosomes from [3 H]uridine-injected rabbits under conditions similar to those of Evans & Lingrel (1969) was not sufficient to separate the mRNA from rRNA and tRNA, and a further

centrifugation step was used on the isolated mRNA region. In all but two cases a good separation of mRNA from other RNA species was obtained and the specific radioactivity over the peak area was constant (Fig. 2). A plot of specific radioactivity of mRNA against time of injection is shown in Fig. 3 and is remarkably close to the values given by Evans & Lingrel (1969).

Measurement of the half-life of the acid-soluble pool of 3 H radioactivity in the bone marrow

To estimate the relative rate of synthesis of mRNA accurately it is necessary to know the half-life of the pool of 3 H-labelled nucleotides in the bone marrow. Blood and bone marrow from mice injected seven times with phenylhydrazine were taken 0.5, 2, 4, 8 and 16 h after injection with 50 μ Ci of [3 H]uridine and the acid-soluble radioactivity was determined. The results are shown in Table 3 and Fig. 4. More than half of the radioactivity in the acid-soluble fraction of the blood evaporated under vacuum, and was presumed to have been metabolized uridine. The least-squares fit to the data for bone marrow gives a half-life of 6 h for the radioactivity in the acid-soluble nucleotide pool.

In more recent experiments, analysis of acid-soluble radioactivity by adsorption on to acid-washed

activated charcoal (Sigma Chemical Co., St. Louis, Mo., U.S.A.) (Tsuboi & Price, 1959) showed that 70% of the acid-soluble radioactivity is absorbed in both blood and bone marrow after 1 or 2 h injection times, and that decay of the radioactivity in blood now is similar to that found for the marrow (6h).

Fit of the data to various models of erythropoiesis

Two general models of haemoglobin-mRNA synthesis in erythropoiesis are used (Fig. 1). The first uses a single cell division after onset of mRNA synthesis, the RNA synthesis being in the basophilic erythroblast and the polychromatophilic erythroblast. The second is a two-cell-division model which assumes mRNA synthesis during two classes of basophilic erythroblast (early and late) and the

polychromatophilic erythroblast. In both models it is assumed that mRNA synthesis continues until the nucleus is expelled from the polychromatophilic erythroblast, and that mRNA synthesis starts at the beginning of the first cell cycle, although the latter assumption is not critical except in calculating relative cell concentrations. Parameters that are altered are cell times, rates of mRNA synthesis and mRNA half-life.

Single-cell-division model

Table 4 shows various attempts to fit the data with mRNA half-lives in the range 10–20h, and variation of cell times, the criterion for fit being a minimal sum of squares of the difference between experimental and theoretical data.

Fig. 2. Sucrose-density-gradient centrifugation of partially purified radioactive mRNA from reticulocytes collected at various times after injection of anaemic mice with $[^3\text{H}]$ uridine

Sedimentation was for 6.75 h at 50000 rev./min in the Spinco SW 50.1 rotor at 2°C as described in the Experimental section. Direction of sedimentation is from left to right and fraction size is 0.2 ml. —, E_{254} ; ●, radioactivity; ○, specific radioactivity in arbitrary units. Injection of $[^3\text{H}]$ uridine was at (a) 12 h, (b) 20 h, (c) 36 h and (d) 44 h before collection of reticulocytes.

Fig. 5 shows that a good fit can be obtained by using half-lives of mRNA between 15 and 20 h with a half-life of 0–10 h for the pool.

The calculations were made by assuming that the rate of mRNA synthesis is the same in both the basophilic and polychromatophilic erythroblasts. If the mRNA-synthesis rate is doubled in the basophilic erythroblast, a good fit can be obtained by lowering the pool half-life to less than 1 min, i.e. a true pulse label, with an mRNA half-life of 15 h [Table 4(v)].

With a pool half-life of 6 h it is not possible to obtain a good fit even if the half-life of mRNA in the basophilic erythroblast was lowered to 12 h [Table 4(vi)]. The half-life of the pool does not affect the shape of the curve to any great extent and it has no effect at the point after which all the cells are labelled, i.e. past 40–42 h. If these data alone are used to calculate the half-life of mRNA, a least-squares fit to the log plot of values from 36 h to 60 h gives a half-life of 14.5 h.

Two-cell-division model

Similar least-squares values to those obtained in the single-cell-division model could be obtained with 17h half-life for mRNA (Table 5, Fig. 6); however, it was also possible to get reasonable fits with shorter half-lives provided that the rate of mRNA synthesis was decreased by a factor of 2 after each cell division [Table 5(iii)]. The best fit obtained in this way was by using an 11h half-life and cell times of 7, 15, 16 and 16h for the reticulocyte, polychromatophilic and two basophilic erythroblasts respectively.

Fig. 3. Specific radioactivity of mRNA after injection of anaemic mice at various times before collection of reticulocytes

For details see the text. ●, This work; Δ, points from work by Evans & Lingrel (1969).

Table 3. Radioactivity of blood and bone marrow after injection with [³H]uridine

Mice had seven injections of phenylhydrazine and were injected with uridine after 7 days. Five mice were used for each point. Least-squares slope of log (radioactivity) versus time in bone marrow is -0.110 , giving a half-life of 6.3h.

Time after injection (h)	Blood (c.p.m./ml $\pm$ s.d.)	Bone marrow (c.p.m./10 ⁷ cells $\pm$ s.d.)
0.5	178 000 $\pm$ 49 000	224 $\pm$ 87
2	133 000 $\pm$ 82 000	177 $\pm$ 51
4	138 000 $\pm$ 78 000	162 $\pm$ 32
8	128 000 $\pm$ 35 000	57 $\pm$ 19
16	109 000 $\pm$ 25 000	37 $\pm$ 14

Fig. 4. Plot of decay of ³H radioactivity in the acid-soluble pool from bone-marrow cells

For details see the text.

Table 4. Summary of best-fit data for a single-cell-division model

Half-life (h)		Cell time (h) and percentage composition			Sum of squares of differences between theory and experimental†
mRNA	Pool	Reticulocyte	Polychromatophilic erythroblast	Basophilic erythroblast	
(i) 20	6	7 (25.0%)	11 (39.3%)	20 (35.7%)	0.046
(ii) 17	10	7 (22.6%)	13 (41.9%)	22 (35.5%)	0.046
(iii) 17	6	7 (22.6%)	13 (41.9%)	22 (35.5%)	0.049
(iv) 14.5	6	7 (20.0%)	15 (42.9%)	26 (37.1%)	0.059
(v) *15	0.001	6 (19.7%)	13 (42.6%)	23 (37.7%)	0.046
(vi) †14.5	6	7 (22.6%)	13 (41.9%)	22 (35.5%)	0.081
Cell composition (from Table 1c)		25%	38.7%	35.3%	

* In this model the rate of mRNA synthesis in the basophilic erythroblast was twice that in the polychromatophilic erythroblast.

† In this model the rate of mRNA synthesis in the basophilic erythroblast was twice that in the polychromatophilic erythroblast and the mRNA half-life in these two cell compartments was 12h.

‡ A curve drawn through the experimental points to obtain a best fit by eye gave a sum-of-squares value of 0.026. The ratio of the sum-of-squares values is significant at the 5% level when this ratio is greater than 2.8 by using an F test with 11 degrees of freedom for each variance.

Fig. 5. Plot of computer-generated curves for mRNA specific radioactivity relative to the value at 24h for a single-cell-division model

Cell parameters for (a) are from Table 4 (i), for (b) from Table 4 (ii), for (c) from Table 4 (iii), for (d) from Table 4 (v). Note that in (d) the rate of mRNA synthesis halves at the polychromatophilic-erythroblast stage and the pool half-life is extremely short. —, Computer-generated plot; ●, experimental points.

Reasonable fits could also be obtained with the declining rate of mRNA synthesis with longer final half-lives for mRNA if the half-life in the polychromatophilic and basophilic erythroblasts was decreased [Table 5(iv) and (v)].

Discussion

Although the model has been written specifically in terms of basophilic erythroblast, polychromatophilic erythroblast and reticulocyte, it is in fact a general model. For instance, mRNA synthesis may cease during any portion of the polychromatophilic-erythroblast stage, and hence the mRNA-synthesizing stages might be classified as one or two stages of basophilic erythroblast and/or the pronormoblast. For this reason, and the relative inaccuracy of the measurements, the relative cell times obtained from bone-marrow differential counts (Table 1c and eqns. 2,

5 and 6) of 0.24:1:0.55:0.35 for the pronormoblast, basophilic erythroblast, polychromatophilic erythroblast and reticulocyte respectively were not used in the curve-fitting procedure. However, the relative cell composition from Table 1 is compared with the predicted values in Tables 4 and 5. It should be noted that the shape of the curve imposes severe restrictions on possible cell times since no mRNA is labelled at an injection time of approx. 7h before collection of reticulocytes. It was also found that the time of cell division in either model was extremely restrictive in terms of excellence of fit of the computer-generated curve.

Half-life of mRNA

The range of half-lives calculated from all of the experimental data is between 10 and 20h even when the rate of mRNA synthesis varies in each cell

Table 5. Summary of best-fit data for a two-cell-division model

Cell time (h) and percentage composition				Relative rate of mRNA synthesis (baso. 1: baso. 2: poly.)	Sum of squares of differences between theory and experimental ‡
Half-life (h)		Reticulocyte	Polychromatophilic erythroblast		
mRNA	Pool			Basophilic erythroblast	
		2	1		
(i)	17	7 (25.0%)	12 (42.9%)	12 (21.4%)	12 (10.7%)
(ii)	14.5	7 (20.3%)	14 (40.6%)	18 (26.1%)	18 (13.0%)
(iii)	11	7 (20.6%)	15 (44.1%)	16 (23.5%)	16 (11.8%)
(iv)	*17	7 (26.7%)	11 (41.9%)	11 (20.9%)	11 (10.5%)
(v)	†14.5	7 (26.4%)	12 (45.3%)	10 (18.9%)	10 (9.4%)
Cell composition (from Table 1c)		25%	38.7%	35.3%	

* In this model the half-life of mRNA was set at 17h in the reticulocyte and 9h in the earlier cells.

† In this model the half-life of mRNA was set at 14.5h in the reticulocyte and 10h in the earlier cells.

‡ The ratio of the sum-of-squares values is significant at the 5% level when this ratio is greater than 2.8 using an F test with 11 degrees of freedom for each variance.

compartment. If it is considered that mRNA synthesis occupies about 35h, the period beyond this, i.e. from times of injection longer than 40h, will be the measure of the mRNA half-life in the reticulocyte. This was found to be 14.5h. This value is very similar to the values obtained by using all of the data.

Previous studies where the half-life of globin mRNA was estimated were in the rabbit and embryonic chick. In the rabbit a value of 20h was found by decay of protein synthesis in reticulocytes (Schulman, 1968), and in bone marrow and reticulocytes a value of 2.5h was found by using decay of protein synthesis in the presence and absence of actinomycin D (Armentrout *et al.*, 1965). In chick embryos a value of 4-5h can be calculated from the published data of decay of protein synthesis in the presence of actinomycin D (Campbell *et al.*, 1971). However, the work of Singer & Penman (1972) has shown that actinomycin D has an effect on initiation of protein synthesis as well as on synthesis of RNA. By isolation of poly(A)-containing mRNA in HeLa and L cells, values of the half-life of the general mRNA in these cells are calculated to be between 10 and 15h (Singer & Penman, 1972; Greenberg, 1972). It is thus clear that globin mRNA has a similar half-life to general mRNA in other mammalian cells and that long life is not a special characteristic of mRNA in differentiated cells, but perhaps a common characteristic of mRNA in all mammalian cells.

Cell time and number of cell divisions in the erythroid series

Cell times for the embryonic cells in the chick have been determined accurately (Weintraub *et al.*, 1971) as well as for the cells in rat bone marrow (Lord, 1970). In the non-anaemic adult rat a cell time of 8.2h for pro- and early normoblasts and 15.7h for intermediate normoblasts is found, with an average of 12.8h for all dividing normoblasts. In human erythroblasts values of 11 and 26.4h have been calculated for pro- and basophilic erythroblasts (Boll, 1972). In this paper two models have been considered, involving either one cell division after initiation of mRNA synthesis and mRNA synthesis only in basophilic and polychromatophilic erythroblasts, or two cell divisions, dividing the basophilic-erythroblast fraction into an early and a late fraction. Since the mice are anaemic and by-pass the orthochromatic erythroblast stage, it is difficult to equate the values in the rat with the values obtained here. However, for the best fit in the one-cell-division category a cell composition of 22.6% reticulocytes, 41.9% polychromatophilic and 35.5% basophilic erythroblasts, and cell times of 7, 13 and 22h respectively was found [Table 4 (ii) and (iii)], whereas for the best fit in the two-cell-division model the

Fig. 6. Plot of computer-generated curves for mRNA specific radioactivity relative to the value at 24h for a two-cell-division model

Cell parameters for (a) are from Table 5 (i), for (b) from Table 5 (iii), for (c) from Table 5 (iv) and for (d) from Table 5 (v). Note that in all of the curves except (a) there is halving of the rate of mRNA synthesis at each cell division. —, Computer-generated plot; ●, experimental points.

cell composition would be 25.0, 42.9, 21.4 and 10.7% with cell times of 7, 12, 12 and 12h [Table 5 (i)]. It would appear that the 22h life for a basophilic erythroblast may be longer than expected, whereas the 12h life found for the two-cell-division model is closer to the expected values. In the best fit for the model involving a variable rate of mRNA synthesis [Table 5 (iii)] two 16h cell divisions are required for the basophilic erythroblasts, and the proportion of basophilic and polychromatophilic erythroblasts is significantly different from the experimental data (Table 1c). The ratio of the sum-of-squares value in the various tests is only significant at the 5% level when the ratio is greater than 2.8 by using an F test with 11 degrees of freedom for each variance.

RNA-synthesis rate during differentiation

It has long been known that the rate of RNA synthesis in early cells is much greater than in later cells. Values from normal human bone marrow give a ratio of 100:39:15 for immature, basophilic and polychromatophilic erythroblasts respectively (Schmid *et al.*, 1966). In the anaemic rabbit the ratio is 100:34 in basophilic and polychromatophilic erythroblasts (Borsook, 1965). In most of the best-fit models it has been assumed that the rate of synthesis of mRNA is the same in basophilic erythroblasts as in polychromatophilic erythroblasts, and it might be assumed that this applies to the high-molecular-weight mRNA precursor as well. It would therefore appear that the synthesis of rRNA must account for

the difference in these rates. If one assumes that rRNA is stable, then the linear decline of specific radioactivity of 18S and 28S rRNA from 36h to 12h shown by Evans & Lingrel (1969), and also confirmed by the present work, indicates that the rate of rRNA synthesis declines by half at each cell division.

The declining rate of rRNA synthesis in the basophilic and polychromatophilic erythroblasts agrees with the experimental data in rabbit erythroid cells (Hunt, 1972). These data indicate that the total erythroid-cell-maturation time from differentiation or onset of mRNA synthesis to elimination from the bone marrow must be approx. 36–40h. If this value is used then the cell times longer than 20–22h for the basophilic erythroblasts in the single-cell-division model [Table 4 (iv)] or the two-cell-division model [Table 5 (ii) and (iii)] would be eliminated.

Conclusions

Although it is not possible to define erythroid-cell differentiation perfectly from the present data, the best-fit curves limit the half-life of mRNA to close to 15h and the total time of synthesis of globin mRNA to approx. 30–36h. If there are two stages in the basophilic erythroblast compartment their cell times are 10–12h; if only one stage the cell time is 20–22h, and the cell time of the polychromatophilic erythroblast and reticulocyte in the bone marrow is 11–13 and 7h respectively. It is not possible to decide whether globin-mRNA synthesis is constant throughout the basophilic-erythroblast and polychromatophilic-erythroblast stages or if it is decreased by a factor of 2 after cell division. However, by relating the amount of mRNA synthesis to protein synthesis predictions of the overall rate of mRNA and protein synthesis in each cell type can be made in each case.

This is discussed in the following paper (Hunt, 1974).

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