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

RATE OF SYNTHESIS OF GLOBIN MESSENGER RIBONUCLEIC ACID CALCULATED FROM DATA OF CELL HAEMOGLOBIN CONTENT

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By the use of the favoured models defining mRNA synthesis and half-life from the preceding paper (Hunt, 1974) and the known content of globin in a reticulocyte it is possible to estimate the absolute rate of mRNA and globin synthesis and the mRNA and globin content in each type of erythroid cell. The best model requires an mRNA-synthetic rate of 3000 molecules per h/cell. This rate compares favourably with the estimated chain-extension rate of 43 nucleotides/s in *Escherichia coli* (Manor *et al.*, 1969) provided that the four α- and β-chain cistrons per cell are transcribed by polymerases spaced 50 nucleotide base pairs apart. Similar calculations can be made for erythropoiesis in the chick embryo, where cell times and relative globin content at each mitosis have been measured (Campbell *et al.*, 1971), but where no reliable estimates of mRNA half-life have been made. In this case it was estimated that a constant rate of mRNA synthesis at 10000 molecules per h/cell through six cell divisions is necessary if the mRNA half-life is 15h; after the sixth mitosis the mRNA synthesis would stop and its half-life would increase to approx. 20h. If an mRNA half-life of 4.5h is used, the synthesis rate through the six mitoses would be 21000 molecules per h/cell, ceasing at the sixth mitosis, when the half-life would need to increase to 25h. The chain-elongation rate for the four α- and β-globin cistrons per cell would be 1–2 times higher than in *E. coli* and would either require a greater rate, polymerases spaced between 25 and 50 nucleotide base pairs apart on the DNA, or limited gene replication. These possibilities are discussed in the light of the low values found for globin cistron multiplicity in ducks and mice.

In the preceding paper (Hunt, 1974) attempts were made to estimate the duration of synthesis of globin mRNA and its half-life during erythropoiesis in the anaemic mouse.

Two models were made which satisfied the experimental data, and a half-life of 15–17h was calculated with an overall synthesis period of approx. 30–36h. However, they differed in the number of cell divisions that would be necessary during this time and whether the rate of mRNA synthesis was constant during this period.

From the knowledge of the total globin content of circulating reticulocytes and the time for synthesis of a globin chain it is possible to calculate the rate of synthesis of mRNA required to reach this content by using the various models proposed. A similar calculation can be made for chick-embryo erythropoiesis studied by Campbell *et al.* (1971) where the number and time of each cell division has been determined, and the haemoglobin content measured at each mitosis. In this case it is possible to vary the rate of mRNA synthesis during each cell cycle and its half-life to obtain best fits to the experimental data.

In both cases computer calculations are made to speed the curve-fitting process.

The estimated rates of globin mRNA synthesis in the chick and mouse are approx. 10000 and 3000 molecules per h/cell, values which are lower than previous calculations made for rabbit globin-mRNA synthesis from simpler assumptions (Hunt, 1972). These values are similar to those calculated by Kafatos (1972) from data on increase of various proteins synthesized in insects and mammalian embryos.

These rates are discussed in relationship to the rate of mRNA synthesis in bacteria and maximal rates of synthesis per DNA molecule in a cell.

Methods

Mouse erythropoiesis

Cell times and mRNA half-lives are taken from Tables 5 and 6 of the preceding paper (Hunt, 1974). The synthesis time for a globin chain is taken as 30s (Knopf & Lamfrom, 1965), and it is assumed that

each globin mRNA is transcribed simultaneously by five ribosomes so that the rate of synthesis of protein in the cell is 600 globin chains per h/mRNA molecule. The globin concentration is 26 pg/cell (Hunt, 1974).

Chick-embryo erythropoiesis

The cell times for the six mitotic stages of erythropoiesis were originally taken as 10, 10, 17, 17, 28 and 78 h (Campbell *et al.*, 1971) although minor modifications were made later. Haemoglobin cell concentrations at each mitosis were published as 17.5, 22, 30, 47.5, 70 and 100% of the final haemoglobin.

Haemoglobin concentration in primitive chick erythrocytes is taken as 94 pg/cell, which is equivalent to 3.3×10^9 globin chains/cell (O'Connor, 1952).

Calculation of amount of mRNA and protein during cell growth

Let K_1 be the rate of mRNA synthesis in molecules/h and K_2 its rate of decay h^{-1}.

R is the concentration of mRNA in the cell and P is the concentration of protein in the cell, both in molecules.

T_1, T_2, etc. are the times of each cell division after onset of mRNA and specific protein synthesis.

If the concentration of precursors of mRNA synthesis is not rate-limiting at any time during mRNA synthesis then

$$\frac{dR}{dt} = K_1 - K_2R \tag{1}$$

Therefore on integration

$$\text{const.} + t = -\frac{1}{K_2}[\ln(K_1 - K_2R)] \tag{2}$$

or

$$K_1 - K_2R = \text{const.} \times e^{-K_2t} \tag{3}$$

Assuming that $R = R_0$ at $t = 0$:

$$K_1 - K_2R_0 = \text{const.} \tag{4}$$

and the general solution of the equation is:

$$R_0^t = \frac{K_1 - (K_1 - K_2R_0)e^{-K_2t}}{K_2} \tag{5}$$

This equation is the same as that derived by Kafatos (1972).

Hence, at a cell division at time T_1 the new initial concentration for the new cell is $R_{T_1}/2$ and t becomes $t - T_1$.

Protein synthesis

It is assumed that the time of synthesis of a globin chain on a ribosome is 30 s and that five ribosomes synthesize globin on one mRNA molecule.

$$\frac{dP}{dt} = 120 \times 5 \times \text{R molecules/h} \tag{6}$$

During the first cell division, i.e. from $t = 0$ to $t = T_1$, $R_0 = 0$.

Hence

$$\frac{dP}{dt} = 600\frac{K_1}{K_2}(1 - e^{-K_2t}) \tag{7}$$

On integration this becomes

$$P = \frac{600K_1}{K_2}\left(t + \frac{e^{-K_2t}}{K_2}\right) + c \tag{8}$$

Again assuming that at zero time no globin chains were present;

$$P_0^t = \frac{600K_1}{K_2^2}(K_2t - 1 + e^{-K_2t}) \tag{9}$$

The generalized equation for $T_2 > t > T_1$ is

$$\frac{dP}{dt} = \frac{600\left[K_1 - \left(K_1 - \frac{K_2R_{T_1}}{2}\right)e^{-K_2(t-T_1)}\right]}{K_2} \tag{10}$$

which integrates to

$$P = \frac{600K_1(t - T_1)}{K_2} + \frac{600\left(K_1 - \frac{K_2R_{T_1}}{2}\right)e^{-K_2(t-T_1)}}{K_2^2} + \text{const.} \tag{11}$$

since at $t - T_1 = 0$, $P = \frac{P_{T_1}}{2}$.

Therefore

$$\text{const.} = \frac{P_{T_1}}{2} - \frac{600\left(K_1 - \frac{K_2R_{T_1}}{2}\right)}{K_2^2} \tag{12}$$

and

$$P_0^{(t-T_1)} = \frac{600}{K_2^2}\left[K_1K_2(t - T_1) - (1 - e^{-K_2(t-T_1)})\left(K_1 - \frac{K_2R_{T_1}}{2}\right)\right] + \frac{P_{T_1}}{2} \tag{13}$$

These equations were put into a computer program which generated R and P at regular intervals, calculated P at the end of each mitotic period (T_1, T_2 etc.) and calculated average values for P and T during each mitosis.

Table 1. *Summary of accumulation of haemoglobin and mRNA in various models of erythropoiesis assuming various mRNA half-lives and using a single cell division as described in the preceding paper (Hunt, 1974)*

The average amount of globin in the blood reticulocyte is set as $9.2\times10^8\pm0.1\times10^8$ molecules/cell (see the text).

(*a*) mRNA half-life 20h: rate of synthesis 3000 (3350)* molecules/h

Cell type	Cell time (h)		Percentage of blood reticulocyte haemoglobin		Average mRNA molecules/cell	
Basophilic erythroblast	20	(20)	11.8	(13.4)	25200	(28100)
Polychromatophilic erythroblast	11	(11)	27.0	(30.7)	33500	(37400)
Marrow reticulocyte	7	(7)	48.8	(55.5)	36800	(47100)
Average in marrow			27.0	(30.7)	31400	(35000)
Blood reticulocyte	96	(48)	100	(100)	9400	(17700)
Ratio of marrow/blood			0.27	(0.307)	3.33	(1.98)

(*b*) mRNA half-life 17h: rate of synthesis 2930 (3250)* molecules/h

Cell type	Cell time (h)		Percentage of blood reticulocyte haemoglobin		Average mRNA molecules/cell	
Basophilic erythroblast	22	(22)	13.3	(14.7)	25400	(28100)
Polychromatophilic erythroblast	13	(13)	30.9	(34.1)	33400	(37000)
Marrow reticulocyte	7	(7)	55.1	(60.7)	35900	(39800)
Average in marrow			30.1	(33.2)	31100	(34500)
Blood reticulocyte	96	(48)	100	(100)	7800	(15100)
Ratio of marrow/blood			0.301	(0.332)	4.01	(2.29)

* The values in parentheses are obtained by using a shorter life (48h) for the blood reticulocyte.

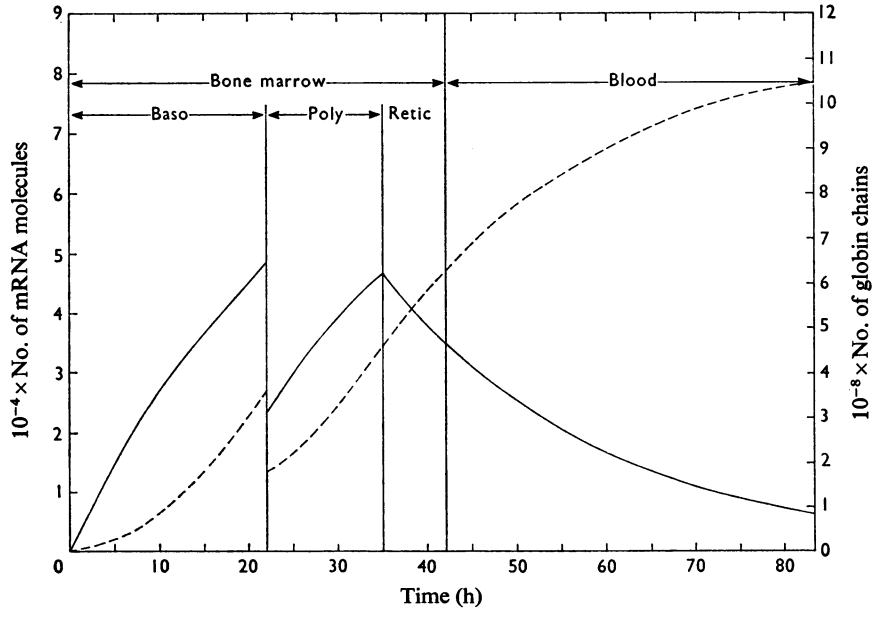

Fig. 1. *Computer-generated curves for accumulation of mRNA and globin chains in a single-cell-division model for anaemic mouse bone marrow*

Parameters for cell constants are given in Table 1 (*b*). Abbreviations used are: Baso, basophilic erythroblast; Poly, polychromatophilic erythroblast; Retic, reticulocyte. ——, mRNA; – – – –, globin chains.

Results

Mouse erythropoiesis

Table 1 and Fig. 1 summarize the data obtained from the single-cell-division models most favoured from the preceding paper (Hunt, 1974). The average globin and mRNA content of reticulocytes in the peripheral blood was calculated on the basis of an average life of the reticulocyte of either 2 or 4 days. Since the animals were made anaemic for 7 days before collection of blood, and reticulocytes first appear at day 3–4 (Hunt, 1974) the choice of these two extremes seems reasonable. The ratio of the

Table 2. *Summary of accumulation of haemoglobin and mRNA in various models of erythropoiesis assuming various mRNA half-lives and using a double cell division as described in the preceding paper (Hunt, 1974)*

The average amount of globin in the blood reticulocyte is set as $9.2\times10^8\pm0.1\times10^8$ molecules/cell.

(*a*) mRNA half-life 17h: rate of synthesis 3400 (3750)* molecules/h

Cell type	Cell time (h)		Percentage of blood reticulocyte haemoglobin		Average mRNA molecules/cell	
Basophilic erythroblast 1	12	(12)	5.3	(5.8)	18800	(20700)
Basophilic erythroblast 2	12	(12)	17.8	(19.8)	31300	(34500)
Polychromatophilic erythroblast	12	(12)	27.9	(31.0)	35100	(38700)
Marrow reticulocyte	7	(7)	52.1	(57.9)	38500	(42500)
Average in marrow			29.4	(32.6)	33400	(36800)
Blood reticulocyte	96	(48)	100	(100)	8300	(16100)
Ratio of marrow/blood			0.294	(0.326)	4.01	(2.29)

(*b*)

Cell type	Cell time (h)		mRNA Half-life (h)	mRNA Rate of synthesis (molecules/h)		Percentage of blood reticulocyte haemoglobin		Average mRNA molecules/cell	
Basophilic erythroblast 1	11	(11)	9	12000	(13000)	14.6	(15.9)	54700	(59300)
Basophilic erythroblast 2	11	(11)	9	6000	(6500)	38.6	(42.0)	56200	(60900)
Polychromatophilic erythroblast	11	(11)	9	3000	(3250)	42.0	(45.6)	34300	(37200)
Marrow reticulocyte	7	(17)	17	0	(0)	61.8	(67.1)	30600	(33100)
Average in marrow						43.7	(47.5)	40000	(43400)
Blood reticulocyte	96	(38)	17	0	(0)	100	(100)	6600	(12600)
Ratio of marrow/blood						0.437	(0.475)	6.05	(3.45)

(*c*)

Cell type	Cell time (h)		mRNA Half-life (h)	mRNA Rates of synthesis (molecules/h)		Percentage of blood reticulocyte haemoglobin		Average mRNA molecules/cell	
Basophilic erythroblast 1	10	(10)	10	12200	(13000)	12.8	(13.7)	53400	(56900)
Basophilic erythroblast 2	10	(10)	10	6100	(6500)	34.9	(37.3)	57400	(61100)
Polychromatophilic erythroblast	12	(12)	10	3050	(3250)	41.3	(44.2)	36800	(33200)
Marrow reticulocyte	7	(7)	14.5	0	(0)	63.8	(68.3)	32500	(34700)
Average in marrow						43.3	(46.4)	41100	(43800)
Blood reticulocyte	96	(48)	14.5	0	(0)	100	(100)	5900	(11400)
Ratio of marrow/blood						0.433	(0.464)	6.96	(3.83)

(*d*)

Cell type	Cell time (h)		mRNA Half-life (h)	mRNA Rate of synthesis (molecules/h)		Percentage of blood reticulocyte haemoglobin		Average mRNA molecules/cell	
Basophilic erythroblast 1	16	(16)	11	8500	(8800)	20.4	(21.0)	52600	(54400)
Basophilic erythroblast 2	16	(16)	11	4250	(4400)	53.5	(55.2)	52400	(54300)
Polychromatophilic erythroblast	15	(15)	11	2125	(2200)	56.0	(57.8)	30900	(32000)
Marrow reticulocyte	7	(7)	11	0	(0)	77.5	(79.9)	25000	(25900)
Average in marrow						55.6	(57.4)	37300	(38600)
Blood reticulocyte	96	(48)	11	0	(0)	100	(100)	3300	(6500)
Ratio of marrow/blood						0.556	(0.574)	11.35	(5.95)

* The values in parentheses are obtained by using a shorter life (48h) for the blood reticulocyte.

average amount of mRNA in the blood and in the marrow gives a ratio of globin-synthetic rates, and the average amount of mRNA in each cell compartment an estimate of the globin-synthetic rate in each cell compartment of the marrow.

In both cases the peak of synthetic activity is in the marrow reticulocytes, although the polychromatophilic erythroblasts are very close in amount. However, only about 50% of the globin is made in the marrow cells.

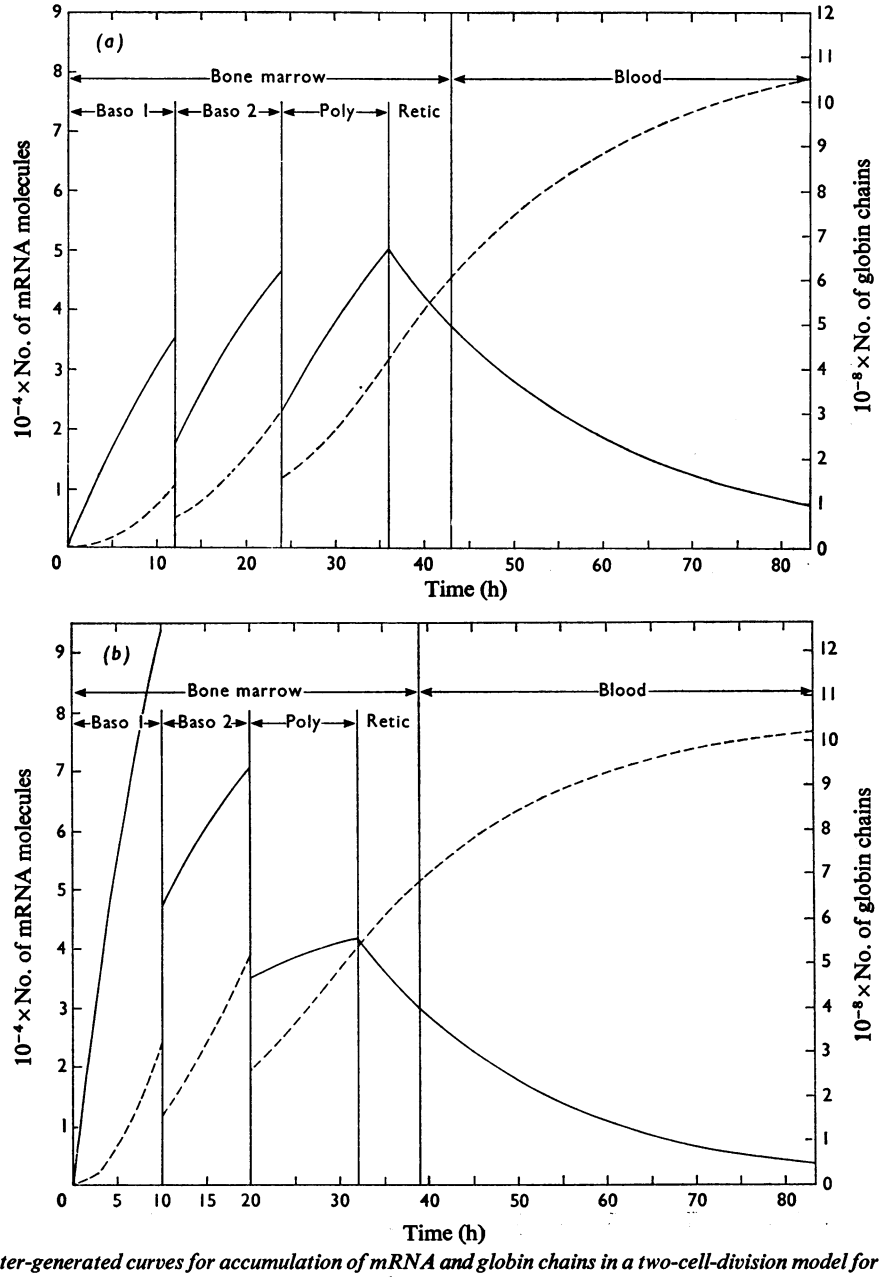

Fig. 2. *Computer-generated curves for accumulation of mRNA and globin chains in a two-cell-division model for anaemic mouse bone marrow*

Parameters for (*a*) are given in Table 2 (*a*), in which the rate of mRNA synthesis is constant and for (*b*) in Table 2 (*c*), where the rate of mRNA synthesis is halved at each cell division. Abbreviations are as for Fig. 1. ——, mRNA; – – – –, globin chains.

In Table 2 and (Fig. 2) the values for the two-cell-division model are shown for models assuming either a constant rate of mRNA synthesis (Table 2, *a*) or a declining rate of synthesis (Table 2, *b–d*). In the former case the overall results are not dissimilar to those of the single-cell-division models except that

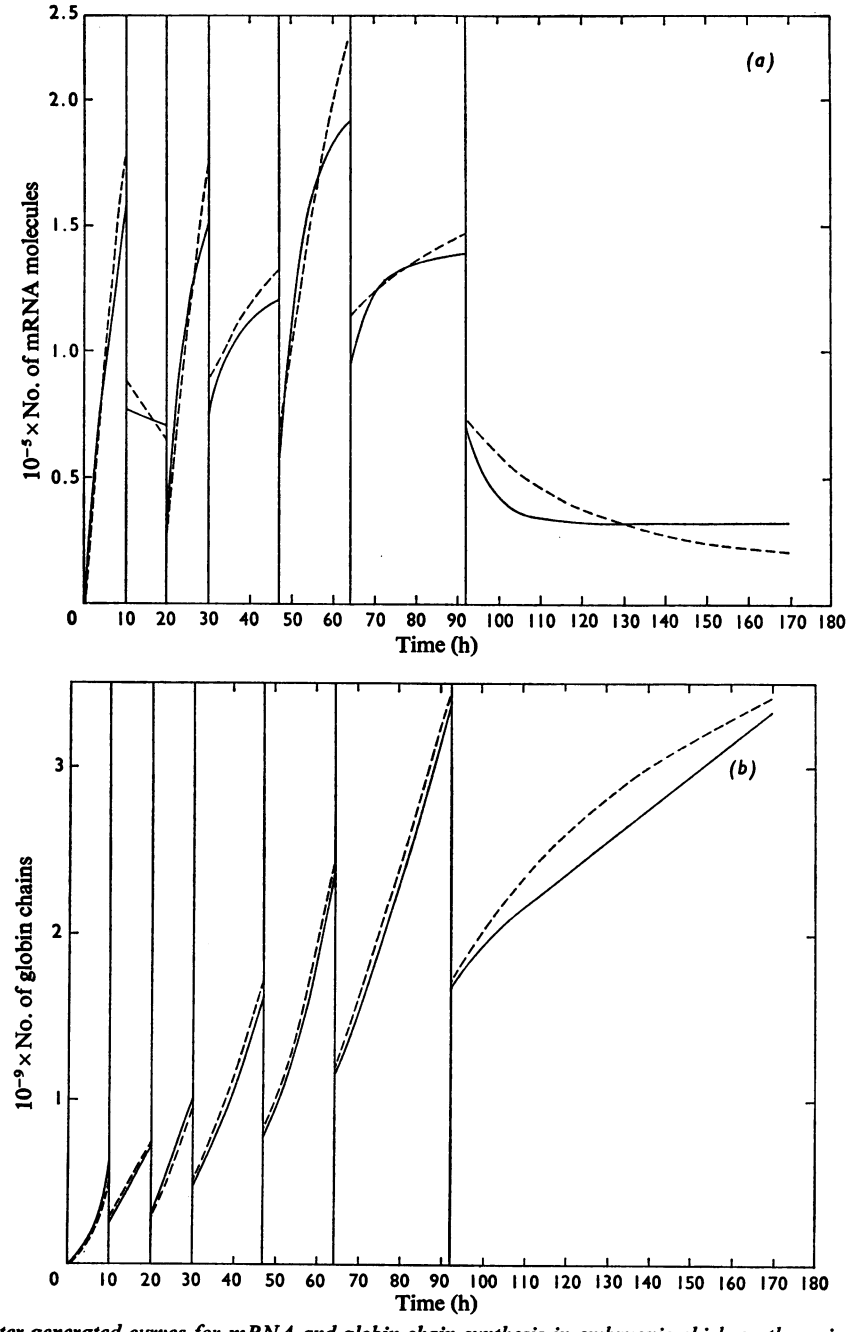

Fig. 3. *Computer-generated curves for mRNA and globin-chain synthesis in embryonic chick erythropoiesis with data from Campbell et al.* (1971)

(*a*) mRNA; (*b*) globin chains. ——, 4.5h half-life (Table 4*a*); – – – –, 20h half-life (Table 3*b*).

the basophilic-erythroblast-2 stage would be expected to contain 20–30% of the final haemoglobin concentration.

However, in the latter case, where the initial rate of globin-mRNA synthesis is high, some 60–70% of the globin is synthesized in the bone marrow, and the peak of globin-synthetic rate is now found in the basophilic-erythroblast-1 and -2 stage. The amount of globin in the basophilic-erythroblast-2 stage is 40–50% of the final amount. The rate of globin synthesis in the blood reticulocytes is less by a factor of 2 than that found in the other models.

Chick-embryo erythropoiesis

In this case it was necessary to match the contents of globin at each mitosis, taking into account the final globin content of 3.3×10^9 chains/cell. The correctness of fit was defined by the least-squares value from observed and expected mitotic haemoglobin values, and values lower than 5 were considered a good fit. In initial trials the rate of globin-mRNA synthesis was varied and the mRNA half-life set at 4.5 h or 20 h. To obtain the values quoted by Campbell *et al.* (1971) it was necessary to vary the rate of mRNA synthesis in an extraordinary way, and consequently the average globin-synthetic rate at each mitosis (Table 3 and Fig. 3). No change of rate of mRNA degradation or of mRNA synthesis in a unidirectional manner allowed a good fit to be obtained. However, on re-examination of the data presented by Campbell *et al.* (1971) it appeared that a certain amount of rounding-off of haemoglobin contents had been made, and recalculation of their data produced a lower value for the haemoglobin content at the first mitosis, with revisions in other values (Table 4).

Now a good fit can be obtained by assuming a constant rate of mRNA synthesis until the last mitosis, after which it falls to zero, but with an mRNA half-life which varies from 4.5 h to 26 h during the last stage (Fig. 4), slight adjustments were also made to the cell times; however, it is considered that these were not major changes. Even with these changes it has not been possible to fit the data to a constant rate of synthesis in each cell compartment and a half-life of 15 h increasing to 23 h at the last stage unless

Table 3. *Estimates of chick-embryo-mRNA and globin synthesis assuming a final globin content of* 3.3×10^9 *molecules/cell and using the amounts of globin present at each mitosis, from Campbell et al.* (1971)

(*a*) mRNA half-life 4.5 h.

No. of divisions after differentiation	Cell time (h)	Percentage of globin at mitosis*		mRNA-synthesis rate (molecules/h)	Average mRNA (molecules/cell)
1	10	17.5	(17.5)	30500	104600
2	10	22.1	(22)	10750	73600
3	10	30.1	(30)	27500	111200
4	17	47.5	(47.5)	19000	107200
5	17	70.1	(70)	31000	155000
6	28	100.1	(100)	21375	129600
7	78	100	(100)	5000	35300

Least squares is 0.04.†

(*b*) mRNA half-life 20 h.

No. of divisions after differentiation	Cell time (h)	Percentage of globin at mitosis*		mRNA-synthesis rate (molecules/h)	Average mRNA (molecules/cell)
1	10	16.9	(17.5)	21470	105000
2	10	22.0	(22)	6	75400
3	10	30.0	(30)	18050	114900
4	17	48.5	(47.5)	6460	113000
5	17	70.5	(70)	14820	158900
6	28	100.4	(100)	5700	132400
7	78	100	(100)	589	35800

Least squares is 1.86.†

* The values in parentheses are the observed values obtained from data presented by Campbell *et al.* (1971).

† The least-squares value is the sum of the squares of the differences between observed and calculated values of globin at each mitosis.

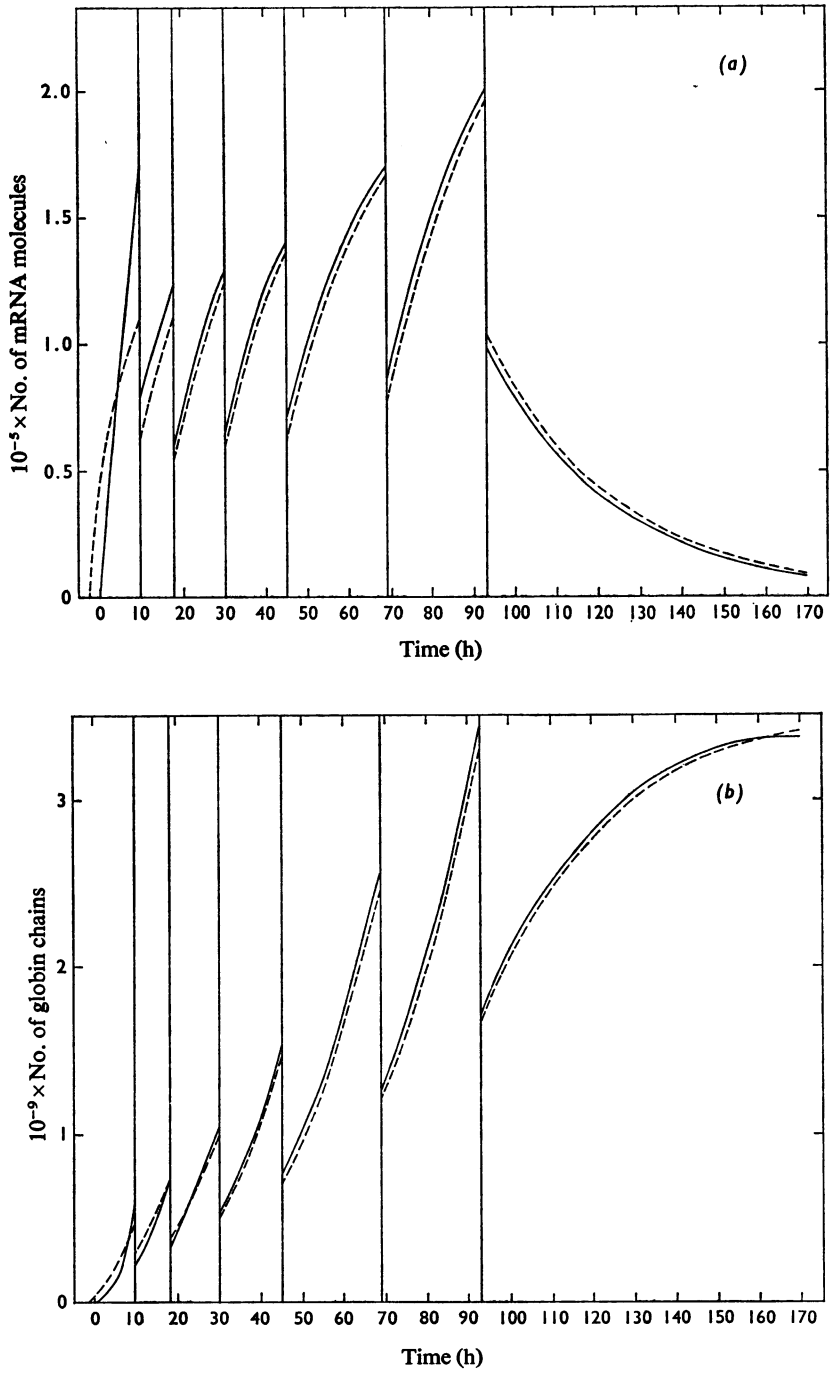

Fig. 4. *Computer-generated curves for mRNA and globin-chain synthesis in embryonic chick erythropoiesis with revised data from Tables* 4(*b*) *and* 4(*c*), *assuming a general half-life of* 15 *h until the fifth division*

(*a*) mRNA; (*b*) globin chains. —— In this model the rate of mRNA synthesis in the first cell is double that in subsequent cells. ---- In this model the rate of mRNA synthesis is constant but the cell time of the first cell is increased.

Table 4. *Estimates for chick-embryo mRNA half-life and synthetic rate using revised haemoglobin amounts at each mitosis and a relative constancy of mRNA synthesis and half-life*

Final haemoglobin approximately 3.3×10^9 chains/cell. For details see the text.

(*a*)

Cell division	Cell time (h)	Percentage of globin at mitosis*		mRNA half-life (h)	mRNA-synthesis rate (molecules/h)	Average mRNA (molecules/cell)
1	10.5	13.1	(14.5)	4.5	21000	72000
2	9.5	22.6	(22.3)	4.5	21000	94700
3	11	31.0	(31.6)	4.5	21000	101700
4	14.5	43.4	(43.8)	4.5	21000	107800
5	25.5	76.3	(76.5)	4.5	21000	118700
6	24	100.3	(100)	6	21000	145300
7	75	100	(100)	25	0	36200

Least squares is 2.85.†

(*b*)

Cell division	Cell time (h)	Percentage of globin at mitosis*		mRNA half-life (h)	mRNA-synthesis rate (molecules/h)	Average mRNA (molecules/cell)
1	9.7	14.3	(14.5)	15	20000	86800
2	8.3	21.9	(22.3)	15	10000	102300
3	12	31.4	(31.6)	15	10000	99900
4	15	43.6	(43.8)	15	10000	108700
5	27	75.6	(76.5)	15	10000	130000
6	27	100.7	(100)	20	10000	152100
7	77	100	(100)	21.5	0	36200

Least squares is 1.69.†

(*c*)

Cell division	Cell time (h)	Percentage globin at mitosis*		mRNA half-life (h)	mRNA-synthesis rate (molecules/h)	Average mRNA (molecules/cell)
1	14.5	14.9	(14.5)	15	10000	60700
2	9.5	21.4	(22.3)	15	10000	85400
3	13	32.4	(31.6)	15	10000	98200
4	15	44.2	(43.8)	15	10000	109000
5	24	75.8	(76.5)	15	10000	130000
6	24	100.8	(100)	20	10000	152100
7	77	100	(100)	21.5	0	36200

Least squares is 2.78.†

* The values in parentheses are the observed values as recalculated from data presented by Campbell *et al.* (1971).

† The least-squares value is the sum of the squares of the differences between observed and calculated values of globin at each mitosis.

the rate of synthesis of globin mRNA is doubled in the first mitosis. It is possible to obtain a constant synthesis rate but only by increasing the cell time of the first mitotic step (Fig. 4, Table 4).

Discussion

It is possible to take the predictions of the rates of protein synthesis and accumulation of protein from the various models of mouse erythropoiesis and compare them with published data.

Rate of protein synthesis

In anaemic rabbit bone marrow, Lingrel & Borsook (1963) found a globin-synthetic rate of 4.6×10^6–24×10^6 chains/h in the marrow and 3.7×10^6–12×10^6 chains/h in blood reticulocytes; the highest ratio between bone marrow and blood was 3.

In the anaemic mouse, Bordin *et al.* (1972) found 1.7×10^6 chains per h/cell in blood reticulocytes but only 0.5×10^6 chains per h/cell in spleen erythroblasts.

In all cases these were rates measured in cell suspensions *in vitro*. Since the rabbit and mouse have the same haemoglobin content in reticulocytes, similar bone-marrow erythroid cell composition and similar rates of onset of reticulocytosis on treatment with phenylhydrazine, it may be assumed

that their erythropoietic systems show similar kinetics.

In Tables 1 and 2 the average mRNA contents in bone marrow for the 15–20h half-life models are approx. 30000 molecules/cell, which would produce a globin-synthetic rate of 18×10^6 chains per h/cell, very close to the rate from Lingrel & Borsook's (1963) data. Similarly, the rate of globin synthesis in the blood reticulocytes would be one-third to one-quarter of that in bone marrow if a 96h reticulocyte life is assumed. In the models using a declining synthetic rate and a half-life less than 11h for mRNA (Table 3 *b–d*) the bone-marrow globin-synthetic rate is 27×10^6 chains per h/cell, but the rate in blood reticulocytes is between one-seventh and one thirteenth of the rate in bone marrow. Lingrel & Borsook (1963) found an average of 6×10^5 ribosomes/cell in marrow, which would be sufficient to handle 1.2×10^5 mRNA molecules, that is, four times the number required by the model. In the blood reticulocytes they found 6×10^4–12×10^4 ribosomes, just sufficient for the favoured 6×10^3–10×10^3 mRMA molecules/cell, assuming that all are in pentaribosome structures.

Haemoglobin content of erythroid cells

Most studies of haemoglobin content of erythroid cells have relied on staining and microspectrophotometric techniques with various criteria used for the stage of cell development. Thorell (1947), in his estimates of haemoglobin content of normal human and rat marrow, found 3–8% of the final erythrocyte haemoglobin concentration in basophilic erythroblasts (cell diameter 8.5–9.5μm), 16–40% of final haemoglobin in polychromatophilic erythroblasts (diameter 6.9–8.0μm) and close to 100% of final haemoglobin content in all orthochromatic erythroblasts. In the rabbit foetal liver (Grasso *et al.*, 1963) 6–15% of final haemoglobin was found in pronormoblasts and basophilic erythroblasts, 20–25% of final haemoglobin in polychromatophilic erythroblasts and 38% of final haemoglobin in orthochromatic erythroblasts. None of these systems is strictly comparable with the anaemic mouse; however, the indications would favour a low haemoglobin content for the polychromatophilic erythroblast and little or no haemoglobin accumulation in the pronormoblast. Again, these values, although imprecise, do not support the declining-rate-of-synthesis model.

Rate of protein synthesis in erythroid cells

Most of the data on protein-synthesis rates is calculated from grain counts in radioautography and can only deal with overall protein-synthetic rates. Borsook *et al.* (1968) claim that a fraction rich in pronormoblasts makes haemoglobin at 20% the rate of later cells. In experiments (J. A. Hunt, unpublished work) using fractionated bone-marrow erythroid cells from anaemic rabbit, my own interpretation is that the rate of protein synthesis does not differ greatly between basophilic or polychromatophilic erythroblasts and marrow reticulocytes, but that the rate in pronormoblasts is very low and probably nil. There is certainly no lowering of the rate of haemoglobin synthesis as suggested by the two-cell-division declining mRNA-synthetic rate model.

Rate of RNA synthesis

In the preceding arguments the rate of mRNA synthesis most favoured in the mouse erythroid cells is close to 3000 molecules per h/cell, and is constant throughout the period of mRNA synthesis. The maximum rate for a declining-rate-of-synthesis model with a short mRNA half-life is 12000 molecules per h/cell. Since there is at least a diploid chromosome set present in each cell, and separate genes for α- and β-chain production, these rates are equivalent to 750 and 3000 molecules/h per globin gene. These values compare favourably with rates proposed by Kafatos (1972) by examination of overall rate of specific protein synthesis in insects and chick and mammalian embryos.

When we examine proposed rates of synthesis of mRNA in the chick embryo several points become clear. The rate of mRNA synthesis necessary to attain a given protein concentration in a cell is dependent mostly on the half-life of the mRNA, provided that the rate of translation of the mRNA remains constant throughout the time of development of the cell. However, it is impossible to allow a declining mRNA-synthetic rate in terms of a decrease by half at each cell division under any of the half-life conditions. It is possible, from the 15h half-life model, that the rate of mRNA synthesis in the first cell in which mRNA synthesis starts is twice that of all later cells, but even this can be overcome by increasing the cell time of the first mitosis to 14.5h instead of 10h; however, it is more likely that there is an inaccuracy in measurement of the low amounts of haemoglobin in these cells. For chick erythropoiesis, the mRNA synthetic rate is between 10000 and 20000 molecules/h per cell, depending on the mRNA half-life, so that the mRNA synthesis would be between 2500 and 5000 molecules/h per haemoglobin gene.

The maximum rate of RNA-chain extension in *E. coli* has been calculated by Manor *et al.* (1969) as 43 nucleotides/s at 37°C, and in order to accommodate the overall rate of rRNA synthesis in *E. coli* some 90 molecules of polymerase are needed to transcribe the regions of DNA for 16S and 23S rRNA at one time. Since the rRNA cistrons in *E. coli* occupy approx. 5000 nucleotide base pairs, the spacing of the polymerases is then 55 base pairs. This is the spacing seen by Miller & Bakken (1972)

for transcription of presumptive *E. coli*, amphibian and HeLa-cell ribosomal cistron regions. In order to transform the lowest globin-mRNA-synthetic rate of 750 molecules/h proposed in the mouse to a chain-extension rate it is assumed that there is one RNA polymerase molecule per 55 base pairs and the extension rate is thus

$$\frac{750 \times 55}{3600} = 11.4$$

nucleotides/s, or 45.2 nucleotides/s for the maximum rate. With the same criteria for the chick-embryo erythropoiesis, the chain-extension rates are between 38.2 and 76.4 nucleotides/s. These values are comparable with the chain-extension rate in *E. coli*. In HeLa cells chain extension in the 45S rRNA precursor is 2.3min/chain or 90 nucleotides/s (Greenberg & Penman, 1966).

In earlier calculations based on a burst of mRNA synthesis (Hunt, 1972), I calculated that the rate of synthesis of globin mRNA should be ten times greater than in *E. coli* and 100 times that of rRNA in mammalian cells. The rates in mammalian cells were overall rates and assumed that all of the ribosomal cistrons were active. From data in HeLa cells it appears that the overall rate for rRNA with a 24h doubling time requires only 20 polymerase molecules/rRNA cistron (Miller & Bakken, 1972), whereas the active cistrons are shown to have 100–150 polymerase molecules transcribing them at one time, hence indicating that some rRNA cistrons are inactive. Presumably the same is the case in erythroid and liver cells, where the overall chain-extension rate was calculated as a maximum of 0.04 nucleotide per base pair/s (Hunt, 1972).

The conclusion that must be drawn is that in both mouse and chick-embryo erythroid cells the genes controlling the synthesis of globin mRNA are at least as active as in *E. coli* and HeLa-cell rRNA synthesis and therefore it must be predicted that if only four genes are available in each cell, and that the mRNA is made as at least a 50S heterogeneous nuclear RNA molecule, these four regions could be seen in electron micrographs similar to those seen in lampbrush chromosomes of oocytes (Miller *et al.*, 1970) densely packed with polymerase molecules in a region at least 5μm in length. If they are less densely packed with polymerase it would be necessary to predict either that the rate of chain elongation is increased or that there is an amplification of the basic genetic information as a part of differentiation, i.e. localized polyteny. However, the amplification need not be great, say less than tenfold, and could be extrachromosomal, which would make it hard to detect. Already most experimental data on the number of globin genes for each α- and β-chain produce a value of 1–5 per haploid genome (Bishop *et al.*, 1972; Harrison *et al.*, 1972), although some of these data are for DNA extracted from non-erythroid tissues.

If it was considered that mRNA for globin was stored in the precursor cell and released at a controlled rate during subsequent cell divisions, and that its half-life was infinite, then in the case of the chick-embryo cells a precursor cell would need to produce enough mRNA to provide an average content of approx. 10^5 molecules per cell in 2^6 cells. That is, it would have to contain 6.4×10^6 molecules of mRNA, which would need to be doubled every cell division of the precursor cell. If this doubling was in 10h the globin-mRNA synthetic rate would be 640000 molecules per h/cell, which is 64 times the rate required at present for continuous synthesis and certainly would require some kind of gene amplification in the precursor cell. However, the evidence of continued mRNA synthesis in mouse erythroid cells would indicate that this model must be unlikely.

Conclusions

A model of mRNA synthesis may now be put forward which has the following features. Initiation of globin-mRNA synthesis occurs as an act of terminal differentiation when, in erythroid cells, other mRNA synthesis declines in rate or ceases, since it is observed that the overall rate of protein synthesis declines during erythroid-cell maturation (Borsook, 1965). The increase in relative proportion of haemoglobin synthesis may be caused by a differential stability of globin and non-globin mRNA (Kafatos, 1972), but if non-globin-mRNA synthesis was maintained total protein synthesis per cell would have to increase. The globin mRNA is immediately used for synthesis of globin, and mRNA synthesis continues at a constant rate while ribosomal RNA synthesis may be declining, until the terminal division occurs when mRNA synthesis ceases. In the anaemic animal the termination of synthesis may occur on premature ejection of the nucleus from the cell. The half-life of globin mRNA is of the order of 15h, but this half-life may vary by being shorter in earlier cells. There is no evidence for extrachromosomal DNA amplification unless the amplified DNA replicates at each cell division to keep the rate of mRNA synthesis constant. The rate of mRNA synthesis is such that a gene under maximal transcription by one polymerase per 50 base pairs, that is, by about one initiation per second, and a chain-extension rate of about 50 nucleotides/s, would be capable of supplying the cell with enough α-and β-globin mRNA even if the α- and β-globin genes were presented only once in each haploid set, provided that they were open to transcription throughout most of the cell time.

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