

# Model for the regulation of mRNA translation applied to haemoglobin synthesis

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*A kinetic analysis of protein synthesis on ribosomes predicts that certain treatments which reduce the overall rate of polypeptide chain initiation will inhibit translation preferentially of mRNAs with lower rate constants for polypeptide chain initiation. The effects of several inhibitors on  $\alpha$  and  $\beta$ -globin synthesis by reticulocyte extracts agree with these predictions.*

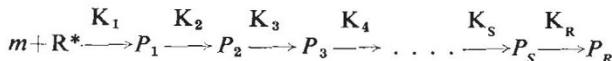
SEVERAL factors determine the rate of initiation of translation of an mRNA molecule. First, different mRNAs seem to differ in the rate of attachment to ribosomes and/or in other stages of initiation of protein synthesis. For example, in the reticulocyte each molecule of  $\alpha$ -globin mRNA initiates protein synthesis only 60% as frequently as does each  $\beta$ -globin mRNA<sup>1,2</sup>. Similarly, the frequencies of initiation of translation of the ten monogenic reovirus mRNAs differ at least tenfold<sup>3</sup> and the rates of initiation of translation of the three cistrons of bacteriophage RNA differ more than twentyfold<sup>4-8</sup>. In this latter case, it has been proposed that the primary nucleotide sequence about the initiation codon and/or the tertiary structure of the mRNA near this codon determine the rate of ribosome attachment<sup>5,7</sup>.

Second, under certain conditions there are alterations in the overall rate of polypeptide chain initiation in a cell. For instance, during mitosis the rate of chain initiation is only one third of that during other phases of the cell cycle, although the rate of polypeptide chain elongation is unaltered<sup>9</sup>. In reticulocytes, the absence of haemin results in formation of an inhibitor of binding of the initiator Met-tRNA<sub>f</sub> to 40S ribosomes<sup>10-12</sup>; initiation of synthesis of all reticulocyte proteins is restricted<sup>13-15</sup>.

In this communication I derive a simple kinetic rate equation for initiation and elongation of polypeptide chains in eukaryotic cells. A principal result of these calculations is that any reduction in the rates of polypeptide chain initiation steps at or before binding of mRNA will result in preferential inhibition of translation of mRNAs with lower initiation rate constants (the poorer mRNAs). I present several new results on translation of  $\alpha$  and  $\beta$ -globin mRNA in the rabbit reticulocyte which are predicted by, and consistent with, this conclusion. I will show, further, that changes in the levels or rate-limiting components required for initiation of synthesis of all proteins (for example, initiation factors, Met-tRNA<sub>f</sub>, 40S subunits, and so on) could explain many results previously ascribed to mRNA specific factors.

## The model

Polypeptide synthesis on ribosomes can be described by the following scheme:



The binding of Met-tRNA<sub>f</sub> to the 40S subunit is the first step in protein synthesis in eukaryotic cells<sup>16-19</sup>;  $R^*$  is the concentration of the Met-tRNA<sub>f</sub>-40S ribosome complex able to bind an mRNA;  $m$  is the concentration of the mRNA, and  $K_1$  is the rate constant for ribosome binding and formation of the 80S initiation complex  $P_1$ . Although there are several

factors and several steps involved in this process, the use of an overall rate constant  $K_1$ , possibly different for different mRNAs, is sufficient and does not affect the nature of the final rate equation. It is assumed that mRNA-specific factors are not involved in mRNA binding. The polypeptide contains  $S$  amino acids and the message  $S$  codons;  $P_i$  ( $i=1, 2, \dots, S$ ) represents a nascent chain of  $i$  amino acids.  $P_R$  is the released polypeptide chain.  $K_i$  ( $i=2, 3, \dots, S$ ) is the rate constant for addition of the  $i$ th amino acid, and  $K_R$  is the rate constant for release of the completed polypeptide chain. Ribosomes are known to cover many more codons on a message than those being decoded at any instant; the value  $L$  is the number of codons covered by one ribosome.

MacDonald and Gibbs<sup>20,21</sup> have derived rate equations for polypeptide synthesis in a very general context, and my derivation follows closely their analysis. It is necessary to make several simplifying assumptions in order to yield a relatively simple analytical expression which can readily be compared with experimental data. First, we must assume that rate constant for addition of any amino acid is the same, that is  $K_i$  ( $i=2, \dots, S$ ) =  $K_e$  the single elongation rate constant. Following ref. 20 I define  $n_i$  as the probability that an mRNA contains a ribosome with a nascent chain  $i$  amino acids long. I make the crucial assumption here that under all conditions ribosomes are uniformly distributed along an mRNA (see below), that is that  $n_i = n$  for all  $i=1, \dots, S$ . It is also assumed that  $K_R \leq K_e$  that is that release of the completed chain does not limit the overall rate of protein synthesis. Given these assumptions, the flux of ribosomes,  $q_e$  across any given codon in an mRNA is given by equation (10) of ref. 20.

$$q_e = K_e n (1 - nL) [1 - n(L - 1)]^{-1} \quad (1)$$

In the steady state this value represents the net amount of protein synthesised per mRNA, hence the total amount of protein produced per unit time,  $Q_e$ , is given by

$$Q_e = mq_e = mK_e n (1 - nL) [1 - n(L - 1)]^{-1} \quad (2)$$

The number of new polypeptide chains initiated per unit time,  $Q_i$  is given by the following equation

$$Q_i = mR^* K_1 (1 - nL) \quad (3)$$

where the term in brackets is the probability that the initiation codon is not covered by a ribosome and, hence, available for polypeptide chain initiation. This equation follows from the definitions of  $K_1$  and  $R^*$  (ref. 21).

Since, in the steady state, the number of new chains initiated is balanced by the number elongated or completed, we can set  $Q_i = Q_e$  and solve for  $n$ :

$$n = [L - 1 + K_e(K_1 R^*)^{-1}]^{-1} \quad (4)$$

and combining equations (3) and (4),  $Q = Q_i = Q_e$  is given by

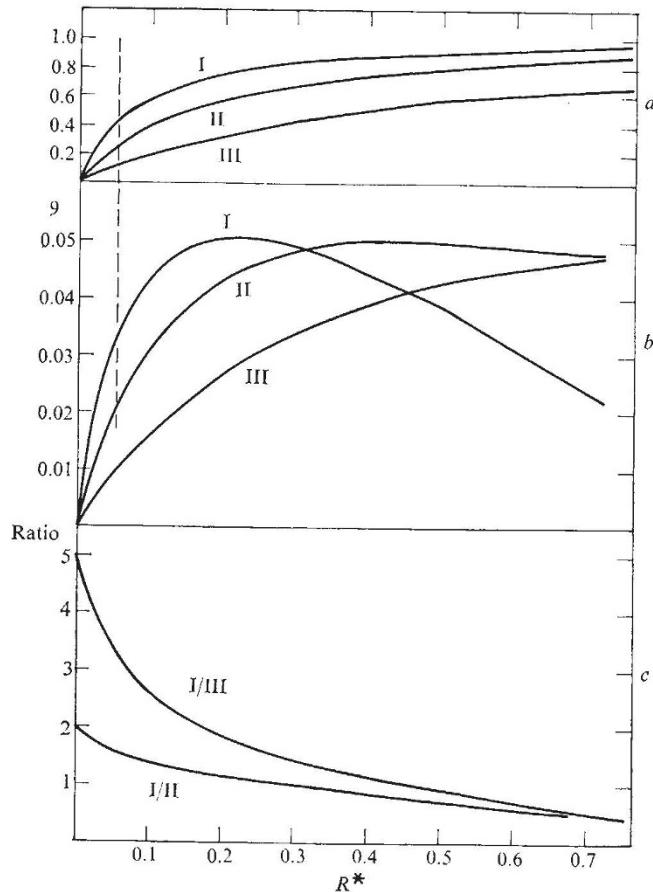
$$Q = mR^* K_1 \left[ 1 - \frac{L}{\frac{K_e}{K_1 R^*} + L - 1} \right] \quad (5)$$

The average number of ribosomes per mRNA is equal to  $nS$ ,

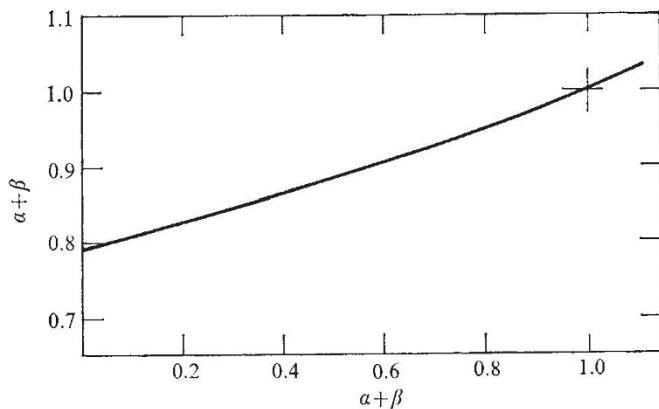
and the fraction of codons on a message covered by a ribosome is given by  $nL$ . In comparing the translation of two different types of mRNA in the same cell, I assume that  $K_e$  (and  $R^*$ ) are the same for all mRNAs. In the case of greatest interest here, the rabbit reticulocyte, we have shown directly that the rate of elongation of the  $\alpha$  and  $\beta$  polypeptides is the same<sup>2</sup>.  $K_1$  (and hence  $n$ ) will, in general, be different for different mRNAs.

$R^*$ , the concentration of Met-tRNA<sub>f</sub>-40S complex capable of binding mRNA, is a key element in this equation; it can be taken as a measure of the overall rate of polypeptide chain initiation in the cell. Reductions in the rate of formation of 40S subunits, or of binding of Met-tRNA<sub>f</sub> to them, will result in a lowered value of  $R^*$ ; likewise, addition of an inhibitor of mRNA binding, such as poly(I) on aurintricarboxylic acid, will also result in a reduction of  $R^*$ .

The crucial assumption in the derivation of equation (5) is that ribosomes are on the average distributed uniformly along an mRNA, that is  $n_i = n$  for all  $i$ . This is the case for the  $\alpha$  and  $\beta$ -globin mRNA in the normal rabbit reticulocyte<sup>22,23</sup>. In the specific experimental situations considered below— inhibition of polypeptide chain initiation in the reticulocyte—it can safely be assumed that in the steady state ribosomes will continue to be distributed uniformly on the average (but at a lower density). However, in cases where initiation is increased



**Fig. 1** Theoretical calculation of parameters of protein synthesis as a function of  $R^*$ . A value of 1 is assumed for  $K_e$  (rate constant for elongation) and 12 is taken for  $L$ , the number of codons covered by one ribosome. Rate constants for chain initiation,  $K_1$ , are taken as 1.0 (curve I); 0.48 (curve II) and 0.20 (curve III). *a*, The fraction of codons occupied by a ribosome,  $nL$ , plotted as a function of  $R^*$ , using equation (4). *b*, Amount of protein synthesised per mRNA,  $q = Qm^{-1}$  plotted as a function of  $R^*$ , using equation (5). *c*, Ratio of amount of protein synthesised by the different mRNAs, plotted as a function of  $R^*$ . As detailed in the text translation of  $\beta$  and  $\alpha$ -globin mRNAs can be approximated by curves I and II, respectively. The dashed line represents the conditions obtaining in the normal reticulocyte, where  $n^\beta$  (curve I) = 0.0340 and  $n^\alpha$  (curve II) = 0.0211.



**Fig. 2** Theoretical calculation of the effect on relative synthesis of  $\alpha$  and  $\beta$ -globin chains by inhibiting polypeptide chain initiation at stages at or before binding of mRNA. The values,  $K_{\alpha}^a/K_{\beta}^b = 0.48$  and  $m^a/m^b = 1.57$  were calculated, as detailed in the text, using only the result that, under normal conditions obtaining in the reticulocyte, each  $\alpha$  mRNA contains, on the average, three ribosomes while each  $\beta$  contains five. These values and equation (5) were used to calculate the ratio of  $\alpha$  to  $\beta$  chains produced as a function of total globin synthesis; for the latter a value of 1.0 corresponds to synthesis in the uninhibited reticulocyte.

from the normal, relative to chain elongation (high values of  $R^*$ ) the assumption of uniform distribution may no longer be valid. These cases have been considered theoretically by MacDonald *et al.*<sup>20,21</sup>.

In Fig. 1*b* the relative translation of three mRNAs is plotted as a function of  $R^*$  with values for  $K_1$  set at 1 (curve I), 0.48 (curve II) and 0.20 (curve III). The value of  $K_e$  is set arbitrarily at 1, and a value of 12 for  $L$  is used. Since a molecule of  $\beta$ -globin mRNA (147 codons) can apparently contain a maximum of 12 ribosomes<sup>24</sup> a value of 12 ( $= 144 \div 12$ ) appears reasonable; it is consistent with the size of mRNA protected from nuclease digestion by a ribosome<sup>8</sup>. (The relative positions of the three curves in this panel is unaffected if  $L=7$  or  $L=20$  is used.)

Note that, with increasing  $R^*$ , the rate of protein synthesis increases to a maximum, and then declines. This means that above a certain density of ribosomes on the mRNA ribosomes interfere significantly with movement of each other along the mRNA. (The optimum value of  $n$  can be calculated from equation (2) by setting  $dq/dn=0$ ; it is given by equation (7) of ref. 21.) It should be stressed that, because of the assumption  $n_i=n$  used in deriving equation (5); the curves in Fig. 1 may not be correct at high values of  $R^*$ .

The crucial point to emerge from Fig. 1 is that the relative translation of two mRNAs with different values of  $K_1$  changes monotonically with  $R^*$  (Fig. 1*c*). At very low values of  $R^*$  the amount of protein synthesised by a species of mRNA is given by

$$Q_{lim} = mK_1R^* \quad (6)$$

Increasing the value of  $R^*$  results in proportionally greater stimulation of translation of the mRNA with a lower value of  $K_1$ . Conversely, reducing  $R^*$  (inhibiting chain initiation nonspecifically) results in a proportionally greater inhibition of translation of the mRNA with lower  $K_1$ .

### Application to synthesis of $\alpha$ and $\beta$ globin

Cell-free extracts of rabbit reticulocytes synthesise globin for about 1 h at rates comparable to that of the intact cell. These extracts, as do the intact cells, synthesise equal amounts of  $\alpha$  and  $\beta$ -globin chains<sup>1,2</sup>. Each message for  $\alpha$  globin contains on the average five ribosomes ( $n^\beta = 5/147 = 0.0340$ ) while each  $\alpha$ -globin mRNA contains three ( $n^\alpha = 3/142 = 0.0211$ )<sup>1,26</sup>. Since the rate of elongation of the two chains is the same<sup>2</sup>, I conclude that under conditions obtaining in the cell each  $\alpha$  mRNA

**Table 1** Inhibition of synthesis of  $\alpha$  and  $\beta$  globins

Reaction	Time of labelling	% Total globin synthesis	Ratio $\alpha:\beta$ globins synthesised
Control (plus haemin)	0-10	100	1.02
Minus haemin	0-10	94	0.99
Plus double-stranded RNA	$10^{-3}$ $\mu\text{g ml}^{-1}$	92	0.98
Plus preincubated lysate	$3 \times 10^{-3}$ $\mu\text{g ml}^{-1}$	93	0.96
	0-10	18	0.83
Control (plus haemin)	10-30	100	1.02
Minus haemin	10-30	17	0.80
Plus double-stranded RNA	$10^{-3}$ $\mu\text{g ml}^{-1}$	27	0.86
	$3 \times 10^{-3}$ $\mu\text{g ml}^{-1}$	15	0.75
Control (plus haemin)	0-20	100	1.01
Plus ATA $2 \times 10^{-5}$ M	0-20	45	0.91
$4 \times 10^{-5}$ M	0-20	21	0.82
Plus poly(dI) $6.6 \mu\text{g ml}^{-1}$	0-20	63	0.92
$13.2 \mu\text{g ml}^{-1}$	0-20	17	0.75
Plus poly(dT) $6.6 \mu\text{g ml}^{-1}$	0-20	61	0.89
$11.4 \mu\text{g ml}^{-1}$	0-20	38	0.80
Plus pactamycin $5 \times 10^{-8}$ M	0-20	75	1.11
$1 \times 10^{-7}$ M	0-20	54	1.13
	$2 \times 10^{-7}$ M	17	1.29

Reticulocyte cell-free protein synthesis reactions (0.06 ml) have been described in detail<sup>2,3</sup>. They contained  $2 \times 10^{-6}$  M nonradioactive tyrosine, and  $^{14}\text{C}$ -tyrosine ( $5 \mu\text{Ci ml}^{-1}$ , 480 mCi mmol $^{-1}$ ; New England Nuclear Corp.) was added during the indicated labelling period. Unless otherwise noted, reactions also contained 20  $\mu\text{g ml}^{-1}$  haemin. Aliquots of the reaction were taken to measure total incorporation of radioactive tyrosine into protein. After incubation, about 600,000 c.p.m.  $^{3}\text{H}$ -tyrosine-labelled rabbit haemoglobin was added. Globin was isolated and digested with trypsin; the tyrosine-containing peptides were resolved by paper electrophoresis and detected by autoradiography (ref. 2). The measure of  $\alpha$ -chain synthesis was the average of the ratios of  $^{14}\text{C}$  to  $^{3}\text{H}$  radioactivity in peptides  $\alpha\text{T4}$  and  $\alpha\text{T6}$ ;  $\beta$ -chain synthesis was the ratio of  $^{14}\text{C}$  to  $^{3}\text{H}$  radioactivity in peptide  $\beta\text{T4}$ . These three relatively N-terminal peptides were utilised so that peptide labelling represents predominantly (over 98% in the control reaction) polypeptide chains initiated during the course of the cell-free reaction.

initiates protein synthesis only 60% as frequently as does each  $\beta$  mRNA and that there must be 1.7 ( $=1 \div 0.6$ ) times as much  $\alpha$  mRNA as  $\beta$  mRNA<sup>1,2</sup>. Further studies showed that any putative  $\alpha$ -mRNA-specific or  $\beta$ -mRNA-specific initiation factors or specific ribosomes do not limit the rate of  $\alpha$  or  $\beta$ -globin synthesis (unpublished results of G. Temple and myself).

Since at the value of  $R^*$  which obtains in the riboculocyte  $n^{\beta} \div n^{\alpha} = 1.6$ , I calculate from equation (4) that  $K_1^{\alpha} \div K_1^{\beta} = 0.48$ . Since  $Q^{\alpha} = Q^{\beta}$ , from equation (3) I calculate that  $m^{\alpha} \div m^{\beta} = 1.57$ , in agreement with a previous estimate (ref. 1). The probability that an mRNA will have no ribosomes translating it is given by  $(1-n)^s$ . For  $\alpha$  mRNA in the reticulocyte lysate ( $n^{\alpha} = 0.0211$ ,  $s=142$ ), this value is 0.049 while for  $\beta$  mRNA ( $n^{\beta} = 0.0340$ ,  $s=147$ ) it is 0.0062. Recent evidence showed that there is indeed 10 times more  $\alpha$  mRNA unattached to ribosomes than  $\beta$  mRNA<sup>27,28</sup>.

The relative translation of  $\alpha$  and  $\beta$  mRNAs, as a function of  $R^*$ , are thus given by curves II and I, respectively, of Fig. 1b. In Fig. 2 are plotted the same data but in a form which eliminates  $R^*$  — the ratio  $\alpha:\beta$  as a function of the total rate of polypeptide chain initiation (the sum of  $\alpha$  and  $\beta$  chains synthesised). Figures 1b and 2 make one crucial prediction: any treatment which (nonspecifically) inhibits the rate of polypeptide chain initiation at stages at or before mRNA binding will preferentially inhibit translation of  $\alpha$ -globin mRNA.

Table 1 shows the results of experiments involving six different treatments, all of which do not affect polypeptide chain elongation or termination, and all of which block initiation at steps at or before binding of mRNA. All preferentially inhibit translation of  $\alpha$  mRNA. The data in this table follows very closely the theoretical curve of Fig. 2.

When a reticulocyte lysate is incubated in the absence of haemin, protein synthesis is normal for about 8 min (at 30° C). Synthesis continues at a decreasing rate, and ceases by 15–30 min; concomitantly there is buildup of an inhibitor which blocks binding of Met-tRNA<sub>f</sub> to the 40S subunit<sup>10–15</sup>. Table 1 shows that there is equal synthesis of  $\alpha$  and  $\beta$  globin during the first 10 min in reactions with or without haemin; and also from 10 to 30 min in the presence of haemin. By contrast, in a lysate incubated in the absence of haemin there is a marked reduction in the ratio  $\alpha:\beta$  synthesised from 10 to 30 min, during the time inhibition is manifest. In a second experiment,

a lysate was incubated for 1 h in the absence of haemin, to build up the initiation inhibitor, and then a small amount was added to a fresh lysate. Again, the amount of  $\alpha$  chain made in the second reaction was only 70% that of  $\beta$  (Table 1).

Globin synthesis in the presence of  $10^{-2}$   $\mu\text{g ml}^{-1}$  double-stranded RNA (in the presence of haemin) is similar to that of a reaction in the absence of haemin<sup>13,29,30</sup>. There is also produced in this case an inhibitor which blocks binding of Met-tRNA<sub>f</sub> to the 40S subunit<sup>31</sup>. Table 1 shows that double-stranded RNA has no effect on the amount of  $\alpha$  or  $\beta$  chains made during the first 10 min of incubation. By contrast, during the next 20 min, when inhibition of initiation is manifest, there is preferential inhibition of synthesis of  $\alpha$  globin.

Aurintricarboxylic acid, at low concentrations, is a specific inhibitor of chain initiation<sup>32,33</sup>. It blocks the binding of mRNA to the Met-tRNA<sub>f</sub>-40S complex (ref. 34 and unpublished data). In kinetic terms ATA can be viewed as lowering the concentration ( $R^*$ ) of Met-tRNA<sub>f</sub>-40S complexes which can bind mRNA. Table 1 also shows that ATA preferentially inhibits initiation of synthesis of  $\alpha$  chains.

A large number of synthetic polyribonucleotides and polydeoxyribonucleotides are also specific inhibitors of chain initiation and also block binding of mRNA to the Met-tRNA<sub>f</sub>-40S complex (ref. 34 and unpublished data). Like ATA, these can be viewed as reducing the concentration of  $R^*$ . We have shown that all 11 polynucleotides tested preferentially block  $\alpha$ -chain synthesis<sup>35</sup>. Table 1 shows recent results for two of those, poly(dT) and poly(dI).

It is interesting that the initiation inhibitor pactamycin which acts at a stage after binding of mRNA to the Met-tRNA<sub>f</sub>-40S complex<sup>36,37</sup> does not preferentially inhibit  $\alpha$ -chain synthesis. This result is not in disagreement with the theory, which only pertains to steps at or before mRNA binding. There results give confidence that my theory is correct at least as pertains to synthesis in rabbit reticulocyte lysates.

### Application to other systems

That one can alter in a predictable manner the relative translation of different mRNAs by nonspecific changes in polypeptide chain initiation components has implications for other systems. In cases where the rate of cellular protein synthesis is either increasing rapidly with time—such as after fertilisation of the quiescent sea urchin embryo (reviewed in

ref. 38)—or decreasing—such as after infection of cultured cells by poliovirus<sup>39</sup>—one might reasonably expect that different mRNAs would be affected to different extents.

Although the above calculations assume that mRNA-specific factors do not regulate polypeptide chain initiation, they could easily accommodate them; in this case  $K_1$  for any mRNA would be a function of the concentration of such factors. Whether mRNA specific factors do, in fact, exist is at present controversial. In this context it is useful to point out the three eukaryotic systems available which can translate exogenous mRNA at very high efficiency (greater than 10 molecules of protein produced per mRNA added). The rabbit reticulocyte lysate<sup>40,41</sup>, the mixed cell-free system of Schrier *et al.*<sup>42</sup> and the *Xenopus* oocyte (into which the mRNA is microinjected)<sup>43</sup>. In each of these systems a wide variety of exogenous mRNAs have been translated, and in no case is there any evidence for mRNA specificity. Palmiter has shown for instance, that a molecule of ovalbumin mRNA is translated in a crude reticulocyte lysate with nearly the same rate of chain initiation and elongation as obtains in the intact hen oviduct<sup>44</sup>.

In contrast, RNA-specific factors have been observed in cell-free systems, such as preincubated extracts from mouse ascites cells<sup>44-50</sup>, in which there is synthesis of, at best, one molecule of protein per molecule of mRNA added<sup>49</sup>. It is clear that in such extracts some component or components required for polypeptide chain initiation (and elongation) are limiting (ref. 51 and reviewed in ref. 52). The present theory shows that addition to such extracts of a component required for initiation for all mRNAs can stimulate preferentially translation of certain mRNAs. It is at present difficult to prove or disprove the notion that mRNA-specific factors, are, in fact, nonspecific components which are required for translation of all mRNAs, particularly since the  $K_1$  constant of a mRNA could be changed considerably by reagents used in its isolation, such as organic solvents and detergents. Addition of unfractionated rabbit globin mRNA to an extract of ascites cells generally results in synthesis of about twice as much  $\beta$  globin as  $\alpha$  globin. Nudel *et al.*<sup>44</sup> reported a factor preparation from reticulocyte ribosomes which will preferentially stimulate translation of  $\alpha$  mRNA in the ascites extract. At least in the intact reticulocyte  $\alpha$  mRNA has a lower  $K_1$  than  $\beta$  mRNA; the present theory predicts that addition of whatever initiation factor limits synthesis in the ascites extracts would preferentially stimulate  $\alpha$ -globin synthesis. Whether this factor, and the others<sup>45-49</sup> supposedly specific for translation of  $\beta$ -globin mRNA or myosin mRNA are real entities, artefacts of cell-free synthesis, or, as postulated here, nonspecific components required for all chain initiation, remains for future work.

Finally, the theory developed here is applicable to transcription of DNA by RNA polymerase; under conditions of limitation of some factor required for transcription initiation, the theory predicts a selective inhibition of transcription of those operons with a lower rate constant for polymerase attachment and RNA synthesis initiation.

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