A model for regulation of mammalian ribosomal DNA transcription

Co-ordination of initiation and termination

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Based on recent experimental data about transcription initiation and termination, a model for regulation of mammalian ribosomal DNA transcription is developed using a simple kinetic scheme. In this model, the existence of the transition pathway from the terminator to the promoter increases the rate of ribosomal RNA precursor synthesis. In addition to this 'non-transcribed spacer' traverse of RNA polymerase I, the co-ordination of initiation and termination allows a rapid on/off switch transition from the minimum to the maximum rate of ribosomal RNA precursor synthesis. Furthermore, taking account of the participation of two factors in the termination event, we propose a plausible molecular mechanism for the co-ordination of initiation and termination. This co-ordination is emphasized by repetition of the terminator unit.

INTRODUCTION

In mammalian cells, ribosomal RNA genes (rDNA) are copied about 200–500 times in tandem arrays of repeated units (Long & Dawid, 1980; Hadjiolov, 1985). Each unit consists of a 13 kbp region that is transcribed into a large precursor rRNA molecule, and the surrounding 'non-transcribed spacer' (NTS) region (Fig. 1). The rDNA transcription is well regulated in response to growth rate or amino acid starvation (Grummt *et al.*, 1976). When protein synthesis is inhibited by treatment with puromycin or cycloheximide, the resultant inhibition of rRNA synthesis occurs rapidly (Mishima *et al.*, 1979; Muramatsu *et al.*, 1979). Beginning in the early 1980s, many studies using cloned rDNA focused on the molecular mechanism of transcription initiation by RNA polymerase I (pol I) (Mishima *et al.*, 1981; Miller & Sollner-Webb, 1981; Grummt, 1981). From these studies, a general model for the rDNA promoter was suggested in which the promoter contains two segmental regions, i.e. 'proximal promoter domain' and 'upstream promoter domain' (Grummt, 1982; Yamamoto *et al.*, 1984; Miller *et al.*, 1985; Sollner-Webb & Tower, 1986). Furthermore, using phosphocellulose chromatography of cell extracts, Mishima *et al.* (1982) have revealed three activities (designated A, C and D) which appear to be necessary for the accurate transcription of mammalian rDNA.

Recently, it has been demonstrated that the NTS is at least partly transcribed in *Drosophila* (Murtif & Rae, 1985), *Xenopus* (Moss, 1983) and mouse (Grummt *et al.*, 1986b). In *Drosophila* (Tautz & Dover, 1986) and *Xenopus* (Labhart & Reeder, 1986), pol I is considered to read rDNA through the NTS, probably terminating transcription close to the promoter for the next gene at the 3' end of the spacer region. In mouse, termination is thought to occur near the end of 28 S rRNA at tandemly repeated termination sites (Grummt *et al.*, 1985), and NTS transcription is terminated at the terminator region,

T_0 , upstream of the initiation site (Grummt *et al.*, 1986b). In addition, evidence that a terminator-binding factor(s) stimulates transcription initiation by binding to the upstream T_0 is given by Grummt *et al.* (1986b). Henderson & Sollner-Webb (1986) have suggested that polymerases on tandem rDNA traverse the entire spacer to the next promoter terminator, where they are made available and positioned to favour reinitiation. However, little is known about the role of the NTS traverse of pol I or the upstream terminator affecting rRNA synthesis in the regulation of transcription.

Taking account of these new findings, we propose a model for the regulation of mammalian rDNA transcription. This model can explain the rapid on/off switch of rRNA synthesis, as well as the role of the NTS traverse of pol I and the significance of the tandemly arranged termination sites and the upstream terminator (T_0).

THEORY

For simplification we consider only three states of pol I in the rDNA transcription system, i.e. a state on the

Fig. 1. Diagram of tandemly arranged mouse rDNA

The closed box represents the transcribed region containing 18 S, 5.8 S and 28 S rRNA coding region. The thin line represents non-transcribed spacer. The open box shows a terminator unit and the hatched box a promoter region.

Abbreviations used: rDNA, ribosomal RNA genes; NTS, non-transcribed spacer; pol I, RNA polymerase I.

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promoter (P), a state on the terminator (T) and a state in the free pool (F). Pol I in the free pool binds to the promoter to construct the transcription initiation complex with several factors at a rate of v_i . The enzyme on the promoter transcribes the rDNA template to the rDNA terminator at a rate of v_s . Then some fraction of pol I on the terminator is released from the rDNA template at a rate of v_T and the other fraction continues to traverse NTS to the promoter of the next rDNA unit at a rate of v_R . Thus the ratio $v_T/(v_T + v_R)$ represents the fraction of pol I dissociated from the terminator. Taking account of the tandem arrangement of rDNA units and the equivalence of each unit (Fig. 1), we may write the following kinetic scheme:

where a transition from P to T produces a ribosomal RNA precursor (pre-rRNA).

According to this kinetic scheme, we may write the equilibrium equations as follows:

$$v_i[F] + v_R[T] = v_s[P] \quad (2)$$

$$v_s[P] = (v_R + v_T)[T] \quad (3)$$

$$v_T[T] = v_i[F] \quad (4)$$

where [F], [P] and [T] are the amounts of pol I in the states of F, P and T respectively.

Using the equilibrium equation (4), and setting

$$[F] + [P] + [T] = [\text{pol}]_{\text{total}} \quad (5)$$

where $[\text{pol}]_{\text{total}}$ is the total amount of pol I, we obtain [P] as follows:

$$[P] = [\text{pol}]_{\text{total}} - (v_i + v_T)[T]/v_i \quad (5')$$

We eliminate [T] in eqn. (5') using eqn. (3) to obtain [P] as follows:

$$[P] = \frac{[\text{pol}]_{\text{total}} v_i (v_R + v_T)}{v_T v_s + (v_s + v_R + v_T) v_i} \quad (6)$$

With this equation, the rate of pre-rRNA synthesis, V , is as follows:

$$V = \frac{d[T]}{dt} = v_s[P] = \frac{[\text{pol}]_{\text{total}} v_i (v_R + v_T) v_s}{v_T v_s + (v_s + v_R + v_T) v_i} \quad (7)$$

Theoretical calculations were performed on the basis of eqn. (7).

RESULTS AND DISCUSSION

The effect of the NTS traverse of pol I on the rate of pre-rRNA synthesis

For fixed values of v_s and v_T , the theoretical curves of V as a function of v_i are drawn in Fig. 2, corresponding to the various values of NTS traverse rate, v_R . The graphs show a hyperbolic response curves as predicted by eqn. (7). The maximum values of synthesis rate increase according to the augmentation of the NTS traverse rate (v_R) (Fig. 2). Even for many other values of v_T , the maximum values of V increase in response to the increase in v_R (results not shown).

Fig. 2. The effect of NTS traverse of pol I on pre-rRNA synthesis rate (V)

The theoretical curves of V (eqn. 7) as a function of v_i are drawn for the various values of v_R . The values of the constants v_s , v_T and $[\text{pol}]_{\text{total}}$ are 1000, 1 and 100 respectively. The value of ordinate multiplied by 9.2×10^2 is the actual calculated value.

Fig. 3. The effect of NTS traverse of pol I and co-ordination of initiation and termination on pre-rRNA synthesis rate (V)

The theoretical curves of V (eqn. 11) as a function of $[f_{T2}]$ are shown for various values of m . The values of the constants v_s , K and $[\text{pol}]_{\text{total}}$ are 1000, 1 and 100 respectively. The values of the constants k_m and k_i ($1 \leq i \leq m-1$) are 1 and 0 respectively. The value of ordinate multiplied by 9.2×10^2 is the actual calculated value.

The effect of the NTS traverse of pol I and co-ordination of initiation and termination on the rate of pre-rRNA synthesis

In addition to rDNA template and pol I, at least four factors are known to be involved in the initiation of mouse rDNA transcription. Three of these are factors $A(f_A)$, $C(f_C)$ and $D(f_D)$ (Mishima *et al.*, 1982). The other factor (f_{T2}) that binds to the terminator unit (designated T_0) upstream of the promoter also increases transcription initiation activity (Grummt *et al.*, 1986b). Using the assumption that:

$$v_i = k[f_A][f_C][f_D][f_{T2}] = K[f_{T2}] \quad (K = k[f_A][f_C][f_D]) \quad (8)$$

in eqn. (7), we obtain:

$$V = \frac{[\text{pol}]_{\text{total}} v_s (v_R + v_T) K[f_{T_2}]}{v_T v_s + (v_s + v_R + v_T) K[f_{T_2}]} \quad (9)$$

As will be discussed later, it is reasonable to consider that the initiation stimulating factor (f_{T_2}) also interacts with the terminator at the 3' end of the rDNA repeat and stimulates pol I to traverse NTS beyond the terminator, so that the pol I reinitiates transcription at the promoter of the next rDNA unit without entering the free pool. Therefore, the dissociation rate of pol I, v_T , is considered to decrease in accordance with the increase in the amount of initiation stimulating factor, f_{T_2} , i.e.,

$$v_T = \sum_{i=1}^m k_i [f_{T_2}]^{-i} \quad (10)$$

As will be shown later, m corresponds to the number of termination units. k_i represents the degree of contribution of each term, $[f_{T_2}]^{-i}$, to v_T . Thus we may replace v_T in eqn. (9) with this formula to obtain an arranged form of the equation as follows:

$$\begin{aligned} V &= \frac{[\text{pol}]_{\text{total}} v_s \left\{ v_R K[f_{T_2}] + \sum_{i=1}^m k_i K[f_{T_2}]^{(1-i)} \right\}}{v_s \sum_{i=1}^m k_i [f_{T_2}]^{-i} + (v_s + v_R) K[f_{T_2}] + \sum_{i=1}^m k_i K[f_{T_2}]^{(1-i)}} \\ &= \frac{[\text{pol}]_{\text{total}} v_s \left\{ v_R K[f_{T_2}] + \sum_{i=1}^m k_i K[f_{T_2}]^{(1-i)} \right\} [f_{T_2}]^m}{v_s \sum_{i=1}^m k_i [f_{T_2}]^{-i} + (v_s + v_R) K[f_{T_2}] + \sum_{i=1}^m k_i K[f_{T_2}]^{(1-i)} [f_{T_2}]^m} \\ &= \frac{[\text{pol}]_{\text{total}} v_s \left\{ v_R K[f_{T_2}]^{(1+m)} + \sum_{i=1}^m k_i K[f_{T_2}]^{(1+m-i)} \right\}}{v_s \sum_{i=1}^m k_i [f_{T_2}]^{(m-i)} + (v_s + v_R) K[f_{T_2}]^{(1+m)} + \sum_{i=1}^m k_i K[f_{T_2}]^{(1+m-i)}} \quad (11) \end{aligned}$$

For the various values of m , the theoretical curves of V as a function of $[f_{T_2}]$ are obtained (Fig. 3). The slope of the sigmoidal curve gets steeper the larger the value of m . It is considered that the value of m represents the strength of co-ordination of initiation and termination. These graphs demonstrate how the co-ordination of initiation and termination contributes to the drastic phase transition from no pre-rRNA synthesis to maximum synthesis. The theoretical curve ($v_R = 100$ and $m = 8$) coincides well with the experimental data which show a rapid increase in rRNA synthesis in response to the addition of amino acid (Grummt *et al.*, 1976). Many studies have demonstrated that a low level of pre-rRNA synthesis (not zero level) is maintained even when the cells are exposed to amino acid starvation (Grummt *et al.*, 1976) or cycloheximide treatment (Mishima *et al.*, 1979). This is thought to reflect that transcription initiation factors are maintained to some extent regardless of cell conditions.

A plausible molecular mechanism for the co-ordination of initiation and termination

Although nothing is known about the molecular mechanism for the co-ordination of initiation and termination, we will describe a plausible mechanism that takes account of the following experimental results. (i)

Termination of transcription occurs at a repeated 18 bp sequence motif downstream of the 3' end of the 28S rRNA coding region in mouse and is mediated by a nuclear factor(s) binding to the motif (Grummt *et al.*, 1986a). (ii) Competition experiments have shown that pol I can read beyond the termination site without the nuclear factor(s) (Grummt *et al.*, 1986a). (iii) The 18 bp sequence (T_0) necessary for termination of transcription also exists 171 bp upstream of the transcription initiation site and mediates the termination of NTS transcription. It has been suggested that pol I at T_0 can traverse into the promoter without entering the free pool (Grummt *et al.*, 1986b; Henderson & Sollner-Webb, 1986). (iv) The terminator-binding factor(s) also stimulates transcription initiation by binding to the T_0 region (Grummt *et al.*, 1986b).

We designate one of the terminator-binding factors that stimulates transcription termination to release pol I and transcript from the template, f_{T_1} , and another factor that stimulates transcription initiation at the promoter, f_{T_2} . We assume that in the presence of f_{T_1} , f_{T_2} suppresses pol I release so permitting the pol I to traverse NTS beyond the terminator, regardless of NTS transcription. In the absence of these two factors, pol I continues to transcribe NTS beyond the terminator (Fig. 4a). In the presence of excess f_{T_1} , pol I reaching the terminator stops transcription and is released from the template (Fig. 4b). As shown in Fig. 4(c), in the presence of a small amount of f_{T_2} and excess f_{T_1} , pol I can pass the terminator units bound by both f_{T_1} and f_{T_2} until it confronts a terminator unit without f_{T_2} and is dissociated from the template. In the presence of excess f_{T_1} and f_{T_2} , pol I can enter the NTS beyond the repeated terminator units, and the NTS-traversing pol I can then reinitiate transcription at the promoter of the next rDNA unit (Fig. 4d). In this case, pol I in the free pool also can be stimulated by f_{T_2} to initiate transcription.

On the basis of this assumption, in the presence of excess f_{T_1} , free f_{T_2} is thought to be related to terminator-binding ones in the following kinetic scheme:

where t is a terminator unit; tf_{T_1} , a complex of a terminator unit and f_{T_1} ; f_{T_2} , f_{T_2} in the free pool; $tf_{T_1} f_{T_2}$, a complex of a terminator unit, f_{T_1} and f_{T_2} ; h_1 , association constant; h_2 , dissociation constant.

From this kinetic scheme, we can derive the probability that m times repeated terminator units are all occupied by f_{T_2} in equilibrium. By equating the probability and the ratio of pol I to go through the terminator to pol I reaching the terminator, we obtain the termination rate (v_T) as follows:

$$\begin{aligned} v_T &= v_R \left[\frac{1}{2^m} \left\{ \left([t] + \frac{h_2}{h_1} \right) [f_{T_2}]^{-1} + 1 + \left[\left([t] + \frac{h_2}{h_1} \right) [f_{T_2}]^{-2} \right. \right. \right. \\ &\quad \left. \left. \left. - 2 \left([t] - \frac{h_2}{h_1} \right) [f_{T_2}]^{-1} + 1 \right]^{\frac{1}{2}} - 1 \right\} \right] \quad (13) \end{aligned}$$

where $[t]$, $[f_{T_2}]$ and m represent the amount of the terminator units, the total amount of f_{T_2} and the number of the repeated terminator units respectively (see Appendix). In mouse, the terminator units are repeated eight times (i.e. $m = 8$). Eqn. (13) has essentially the same

Fig. 4. Schematic diagram of a plausible molecular mechanism of co-ordination of initiation and termination

The symbols used on rDNA are the same as those in Fig. 1. Pol I, the wavy line, the open circle and the closed circle represent pol I, transcript, termination factors f_{T1} and f_{T2} , respectively. (a) Without both termination factors, pol I continues to transcribe NTS beyond the terminator. (b) In the presence of excess f_{T1} , pol I stops transcription at the first f_{T1} -bound terminator unit and is released with the transcript from the template. (c) In the presence of f_{T2} and excess f_{T1} , pol I can traverse beyond terminator units occupied by both termination factors, f_{T1} and f_{T2} releasing transcript. When the pol I confronts a terminator unit without f_{T2} , the pol I is dissociated from the template. (d) In the presence of excess f_{T1} and f_{T2} , pol I traverses NTS beyond the terminator up to the promoter of the next rDNA unit. The termination factor f_{T2} can also stimulate transcription initiation at the promoter.

form as eqn. (10) relating to $[f_{T2}]$. When the rate of initiation complex formation (v_i) depends on the amount of f_{T2} ($[f_{T2}]$) in the form of $v_i = K[f_{T2}]$, a sigmoidal response curve like that shown in Fig. 3 can be obtained.

In this model for rDNA transcription regulation, the existence of the transition pathway from the terminator to the promoter at the rate of v_R was shown to increase the level of pre-rRNA synthesis rate, most likely by preventing pol I from entering the free pool. The effect of co-ordination of initiation and termination in the presence of NTS traverse of pol I contributes to the sudden on/off switch transition of transcription level within a small level of initiation factor range, which may be advantageous to living cells by allowing a quick response to change in circumstances.

The values of the transition rates in real cell systems are not known, except for the transcription elongation rate of pol I, which has been estimated to be approx. 30 nucleotides/s (Grummt, 1978). The relative values used here for the theoretical calculations might not necessarily reflect the real values. However, a wide variety of sets of transition rate values was attempted for the evaluation of

pre-rRNA synthesis rate to obtain the same characteristic features of the theoretical curves as described above.

In this model, we did not take direct account of the effect of spacer promoters or enhancers, although the presence of a spacer promoter has been shown in mouse by Grummt *et al.* (1986b). However, augmentation of the value of v_i can easily reflect the effect of these elements. Further experiments [e.g. identification and characterization of the termination factors (f_{T1} and f_{T2})] will also be necessary to prove the proposed mechanism of co-ordination of initiation and termination.

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APPENDIX

The termination rate, v_T (eqn. 13), is derived as follows. From the kinetic scheme (12), we get the following equilibrium equation:

$$h_1[tf_{T1}][f_{T2}]_{free} = h_2[tf_{T1}f_{T2}] \quad (14)$$

$[tf_{T1}]$, $[f_{T2}]_{free}$ and $[tf_{T1}f_{T2}]$ represent the amounts of tf_{T1} , free f_{T2} and $tf_{T1}f_{T2}$ respectively. Setting:

$$[f_{T2}]_{free} + [tf_{T1}f_{T2}] = [f_{T2}] \quad (15)$$

$$[tf_{T1}] + [tf_{T1}f_{T2}] = [t] \quad (16)$$

the amount of two factor-bound terminator unit, $[tf_{T1}f_{T2}]$, is represented as follows:

$$[tf_{T1}f_{T2}] = \frac{1}{2} \left\{ [f_{T2}] + [t] + \frac{h_2}{h_1} - \left[[f_{T2}]^2 - 2 \left([t] - \frac{h_2}{h_1} \right) [f_{T2}] + \left([t] + \frac{h_2}{h_1} \right)^2 \right]^{\frac{1}{2}} \right\} \quad (17)$$

The probability that a terminator unit is occupied by f_{T2} is represented as:

$$[tf_{T1}f_{T2}]/([tf_{T1}] + [tf_{T1}f_{T2}]) = \frac{1}{2[t]} \left\{ [f_{T2}] + [t] + \frac{h_2}{h_1} - \left[[f_{T2}]^2 - 2 \left([t] - \frac{h_2}{h_1} \right) [f_{T2}] + \left([t] + \frac{h_2}{h_1} \right)^2 \right]^{\frac{1}{2}} \right\} \quad (18)$$

and the probability for pol I to go through the m times repeated terminator units is represented by

$$\{[tf_{T1}f_{T2}]/([tf_{T1}] + [tf_{T1}f_{T2}])\}^m = \left\{ \frac{1}{2[t]} \left\{ [f_{T2}] + [t] + \frac{h_2}{h_1} - \left[[f_{T2}]^2 - 2 \left([t] - \frac{h_2}{h_1} \right) [f_{T2}] + \left([t] + \frac{h_2}{h_1} \right)^2 \right]^{\frac{1}{2}} \right\} \right\}^m \quad (19)$$

On the other hand, the ratio (r_R) of pol I to traverse NTS beyond the repeated terminator units to pol I reaching the terminator is represented from the kinetic scheme (1) by:

$$r_R = v_R/(v_R + v_T) \quad (20)$$

Therefore, by equating the formulae (19) and (20), we obtain the termination rate (v_T) as follows:

$$v_T = v_R \left[\frac{1}{2^m} \left\{ \left([t] + \frac{h_2}{h_1} \right) [f_{T2}]^{-1} + 1 + \left[\left([t] + \frac{h_2}{h_1} \right)^2 [f_{T2}]^{-2} - 2 \left([t] - \frac{h_2}{h_1} \right) [f_{T2}]^{-1} + 1 \right]^{\frac{1}{2}} \right\}^m - 1 \right] \quad (13)$$