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Role of metal ions in the assembly and decay of the transcription initiation complex on tRNA gene in yeast extracts

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Studies on formation of the transcription initiation complex on the $tRNA^{Tyr}$ gene in yeast extracts with the use of Sarkosyl-challenge assay proved that a single magnesium ion is indispensable for the complex assembly in the absence of nucleotides. Other cations do not substitute for Mg^{2+}. The optimal KCl concentration for transcription of the gene by the assembled initiation complex was found to be twice as high as that for the assembly process and close to that observed previously for the TFIIIC (or tau) factor dissociation from the promoter. The preformed complex remained stable for several hours at room temperature and its decay was not influenced by ionic strength. The data seem to support the notion that the TFIIIC factor is used only for assembly of the initiation complex and is not necessarily involved in the subsequent steps of transcription.

RNA polymerase III; tRNA gene; Transcription complex; K^+ and Mg^{2+} ions; Sarkosyl

1. INTRODUCTION

Assembly of the transcription initiation complex on eukaryotic tRNA gene proceeds in several steps [1–3]. First, the TFIIIC (tau) factor binds to an internal promoter of the gene, then the next factor, TFIIIB, binds to the tau–DNA complex and interacts with an upstream promoter element. RNA polymerase III recognizes this triple complex and initiates transcription.

The role of metal ions in this complicated process is not clear. RNA polymerase is known to bind magnesium at the catalytic center and this ion is involved in chelation of nucleotides (NTPs) during RNA synthesis [4,5]. The yeast tau factor does not require Mg^{2+} but does require a rather high concentration of K^+ for promoter binding [6]. On the other hand, the transcription process is strictly Mg^{2+}-dependent and requires a concentration of K^+ lower than that necessary for optimal binding of tau [7]. This apparent difference in salt requirements between the first step and the overall process of transcription led us to investigate the influence of Mg^{2+} and K^+ ions on formation of the transcriptionally active complex.

Here we show that magnesium is absolutely required for assembly of the initiation complex, not only for catalysis, and that KCl concentrations optimal for transcription of the gene by the preformed complex and for tau dissociation are similar.

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2. EXPERIMENTAL

Saccharomyces cerevisiae (pep4-3) cells were extracted as described earlier [8]. The plasmid containing 12 copies of SUP4-o $tRNA^{Tyr}$ gene cloned in the *Bam*HI site of pBR322 was a generous gift from Dr. A. Sentenac (CEN, Saclay). The specific transcription was performed under previously described conditions [9] using the Sarkosyl-challenge method [10]. After preincubation of the template with the extract for various times and at different salt concentrations, Sarkosyl was added to a final concentration of 0.01% together with NTPs and salts to 70 mM KCl and 5 mM $MgCl_2$ (unless otherwise noted). The detergent at 0.01% prevents formation of a complex without affecting RNA synthesis (vide infra). The results are expressed as percentage of the amount of pre-$tRNA^{Tyr}$ formed in a control reaction. All chemicals were from Sigma, Bio-Rad or Amersham ([α-^{32}P]UTP).

3. RESULTS AND DISCUSSION

We noticed [11] that Sarkosyl, a known detergent inhibitor of initiation in the polymerase II [10] and III [12] systems, fully blocked transcription of a tRNA gene in yeast extract when it was present at 0.01% concentration during preincubation of template with the extract. The detergent did not affect transcription when added after formation of the complex. Thus, Sarkosyl inhibits assembly of the complex, and the number of pre-tRNA molecules synthesized after addition of Sarkosyl and NTPs can be used as a measure of the amount of the active complex formed. Since kinetic parameters of Sarkosyl inhibition determined for polymerase II in crude extract [13] and with purified transcription factors [10] are very similar, the use of the Sarkosyl method in cell extracts in studies on assembly of the initiation complex seemed fully justified.

Using the Sarkosyl-challenge method, we determined the half-time for formation (Fig. 1) and stability (data

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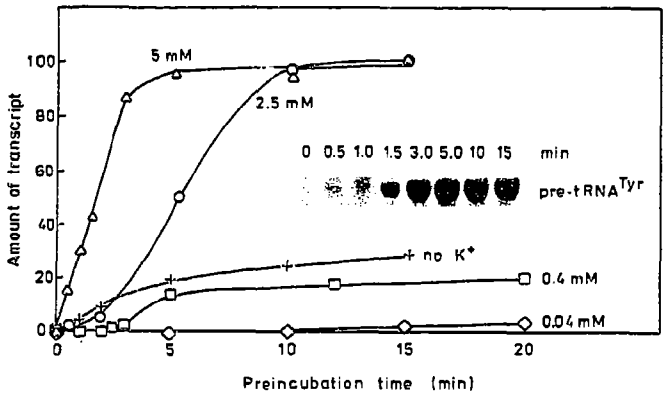

Fig. 1. Kinetics of formation of the transcription initiation complex at various $MgCl_2$ concentrations. [KCl] was 70 mM except for the curve marked 'no K^+' (5 mM $MgCl_2$). The autoradiogram of 6% PAGE of RNA polymerized by the complex assembled in standard salt conditions is shown as inset. The peak areas of the scanned autoradiogram correspond to the curve marked '5 mM'. The data for each curve are normalized to the 15 min time point at optimal salt concentrations. A single extract preparation was used for all experiments.

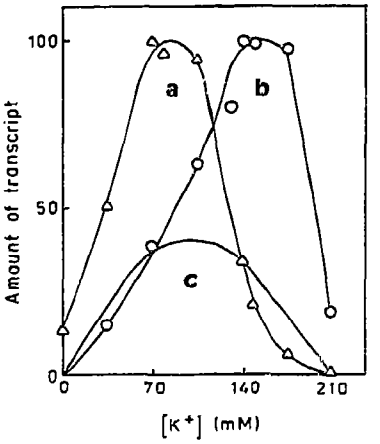

Fig. 3. Ionic strength optima for assembly of the initiation complex and for pre-tRNA^{Tyr} polymerization. (Curve a) The complex was preformed at different [KCl] and during polymerization the salt concentration was 140 mM. (Curve b) The complex was preformed at 70 mM KCl and for polymerization the salt concentration was varied. (Curve c) The same salt conditions were applied during complex assembly and RNA polymerization. In each case, Sarkosyl was added after preincubation.

not shown) of the initiation complex. These amount to 2.5 min and 7 h, respectively, in optimal salt conditions. The time data are very close to those obtained recently by footprinting with partially purified factors [2], which confirms again applicability of the Sarkosyl method for kinetic studies.

As can be seen from Fig. 1, the time of formation and the amount of the complex formed depend on K^+ and Mg^{2+} concentration. The decay time is salt independent in the range of 35 mM $\leqslant$ [KCl] $\leqslant$ 140 mM and 2.5 mM $\leqslant$ [$MgCl_2$] $\leqslant$ 10 mM (not shown). Magnesium but not potassium ions are indispensable for complex formation. At low [Mg^{2+}] the kinetic curves show a lag phase,

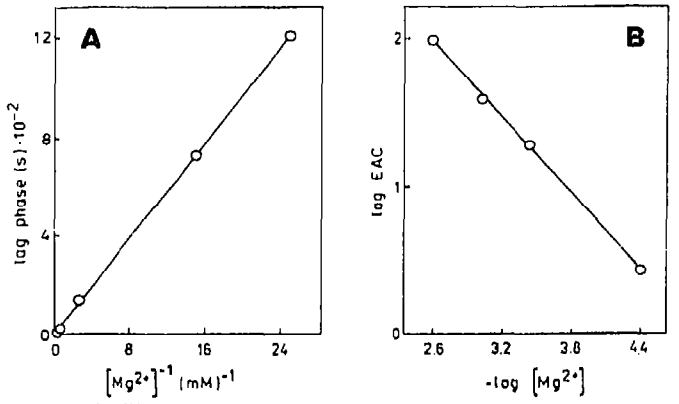

Fig. 2. (A) Plot of lag-times in assembly of the initiation complex observed at different [Mg^{2+}] (Fig. 1) as a function of its reciprocal. The length of the lag-phase was taken as the time at which the transcription signal was twice as high as the background level. (B) Double logarithmic plot of the equilibrium amount of the complex (EAC) determined in the Sarkosyl assay vs. [Mg^{2+}]. Average data from three independent experiments are plotted.

the magnitude of which depends approximately linearly on the reciprocal of Mg^{2+} concentration (Fig. 2A). This is characteristic for magnesium activation in enzyme kinetic systems in which a single Mg^{2+} ion binds to one of the reaction components and makes the interaction between them possible [14].

The plot of logarithm of the equilibrium amount of the initiation complex (EAC) vs negative logarithm of [Mg^{2+}] gave a straight line with the slope of -0.9 ± 0.1 (Fig. 2B). According to the macromolecular binding theory [15,16], $-(\log K_b/\log [Mg^{2+}]) \sim n$, where K_b is the ligand binding constant and n is the number of ions released (when the slope of log K_b vs $-\log [Mg^{2+}]$ is positive) or bound (when the slope is negative). The plot shown in Fig. 2B clearly indicates that a single Mg^{2+} ion must be bound to protein component(s) of the system for formation of the initiation complex. At present it is impossible to conclude whether binding of TFIIIB or polymerase is directly Mg^{2+} dependent.

The demonstrated magnesium requirements are different from those observed for the HeLa 5 S gene transcription system, where Mg^{2+} together with ATP was necessary for formation of the rate limiting initiation complex [17]. In our case ATP is not required in the assembly.

Another feature of the system which becomes manifest from Fig. 1 is that K^+ ions are not indispensable for assembly; to some extent they can be substituted by Mg^{2+}. The other divalent cations we checked in the assembly assay and in transcription by the preformed complex, were Mn^{2+}, Zn^{2+} and Ca^{2+}. Only the presence of Mn^{2+} resulted in a very weak transcription signal. Monovalent cations, K^+, Na^+ and Li^+ were equivalent in supporting assembly, while Li^+ was three times less

efficient in polymerization of RNA than were K^+ and Na^+ (not shown).

Using Sarkosyl-challenge assay we also established optima of ionic strength separately for the complex assembly and for the pre-tRNA synthesis by the preformed complex. Fig. 3 shows that the former process (curve a) requires two-fold lower KCl concentration than the latter (curve b). It was shown previously [6] that at 185 mM KCl almost half of the tau–promoter complexes was stripped. The pre-tRNA synthesis by the preformed initiation complex is optimal between 140 and 180 mM KCl. The fact that the polymerization is most effective at a similar, high ionic strength as the decomposition of the tau–promoter complex supports the recent model [18] according to which the tau factor is involved only in the assembly of the initiation complex.

In a complementary experiment, we determined the stability of the initiation complex formed at the KCl concentration (0.3 M) known to displace completely the tau factor from the complex reconstituted from partially purified components [18]. We preformed the complex at standard ionic strength, then [KCl] was increased to 0.3 M and, after 10 min, the initial salt concentration was restored by dilution of the reaction mixture with simultaneous addition of Sarkosyl and NTPs. The amount of the transcript formed was identical with that obtained in the control reaction in which the 'salt jump' step was omitted (not shown). Thus, as soon as the initiation complex is assembled, the salt induced dissociation of tau does not indeed influence transcription.

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REFERENCES

[1] Gabrielsen, O.S., Marzouki, N., Ruet, A., Sentenac, A. and Fromageot, P. (1989) J. Biol. Chem. 264, 7505–7511.
[2] Kassavetis, G.A., Riggs, D.L., Negri, R., Nguyen, L.H. and Geiduschek, E.P. (1989) Mol. Cell. Biol. 9, 2551–2566.
[3] Palmer, J.M. and Folk, W.R. (1990) Trends Biochem. Sci. 15, 300–304.
[4] Beal, R.B., Pillai, R.P., Chuknyisky, P.P., Levy, A., Tarien, E. and Eichhorn, G.L. (1990) Biochemistry 29, 5994–6002.
[5] Szafrański, P., Smagowicz, W.J. and Wierzchowski, K.L. (1985) Acta Biochim. Polon. 32, 329–349.
[6] Camier, S., Gabrielsen, O., Baker, R. and Sentenac, A. (1985) EMBO J. 4, 491–500.
[7] Ruet, A., Camier, S., Smagowicz, W.J., Sentenac, A. and Fromageot, P. (1984) EMBO J. 3, 343–350.
[8] Koski, R.A., Allison, D.S., Worthington, M. and Hall, B.D. (1982) Nucleic Acids Res. 10, 8127–8143.
[9] Smagowicz, W.J., Ruet, A., Camier, S., Sentenac, A. and Fromageot, P. (1983) Nature 304, 747–749.
[10] Hawley, D.K. and Roeder, R.G. (1985) J. Biol. Chem. 260, 8163–8172.
[11] Smagowicz, W.J., Szafrański, P. and Wierzchowski, K.L. (1989) 19th FEBS Meeting, Rome, Abstr. p.TH 25.
[12] Klekamp, M.S. and Weil, P.A. (1986) Arch. Biochem. Biophys. 246, 783–800.
[13] Hawley, D.K. and Roeder, R.G. (1987) J. Biol. Chem. 262, 3452–3461.
[14] Segal, I.H. (1975) Enzyme Kinetics, Wiley-Interscience, New York.
[15] Record Jr., M.T., deHaseth, P.L. and Lohman, T.M. (1977) Biochemistry 16, 4791–4796.
[16] Schellman, J.A. (1975) Biopolymers 14, 999–1018.
[17] Bieker, J.J., Martin, P.L. and Roeder, R.G. (1985) Cell 40, 119–127.
[18] Kassavetis, G.A., Brawn, B.R., Nguyen, L.H. and Geiduschek, E.P. (1990) Cell 60, 235–245.