Relative Affinities of Nucleotide Substrates for the Yeast tRNA Gene Transcription Complex

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Apparent Michaelis constants for nucleotides in transcription of yeast tRNA gene by homologous RNA polymerase III with auxiliary protein factors, were found to be remarkably higher in initiation than in elongation of RNA chain. This supports presumptions regarding topological similarities between catalytic centers of bacterial and eukaryotic RNA polymerases.

The catalytic center of *Escherichia coli* RNA polymerase includes two distinct sites for binding of initiating (5′-terminal) and elongating nucleotides. Binding of the initiating NTP is by an order of magnitude weaker than binding of the consecutive substrates [1, 2]. It was not clear whether the same was valid also for eukaryotic transcription. The available K_m data, approximating affinities of NTP's to purified yeast polymerase II, did not exhibit analogous differentiation [3], but the authors have measured only unspecific transcription. The RNA polymerase III system seems to be particularly interesting for such a comparison, because in this case transcription factors remain bound to intragenic promoters and may interfere with substrate binding during polymerization.

We have analyzed the kinetics of pre-tRNA synthesis by a complete and homologous yeast polymerase III transcription complex. Its assemblage was achieved by incubating (for 10 min at 25 °C) partially purified cell extract [4] from a *Saccharomyces cerevisiae* 20B-12 (trp 1, pep 4–3) strain with recombinant plasmid DNA containing 12 copies of the yeast SUP4-o $tRNA^{Tyr}$ gene. The protein-DNA complex was further separated from 80% of the extract proteins by filtration through a Bio-Gel A-0.5 m minicolumn (18 µg protein and 0.6 µg DNA/2 ml bed). The transcription complex was stable for at least 8 h at room temperature and was essentially free of nucleolytic processing, RNA degradation and phosphatase activities.

The level of pre-$tRNA^{Tyr}$ synthesis was monitored as a function of concentration of one nucleotide ($3K_m \geq [NTP] \geq 0.3K_m$, Fig. 1) maintaining the other three at a saturated, constant level

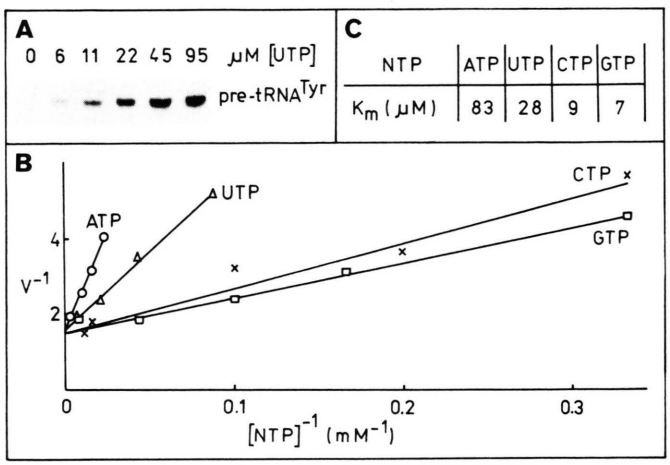

Fig. 1. Kinetics of yeast SUP4-o $tRNA^{Tyr}$ gene transcription *in vitro*. Incubations were performed for 30 min at 25 °C in 20 mM Hepes-KOH (pH 7.5), 5 mM $MgCl_2$, 70 mM KCl, 0.1 mM EDTA, 0.1 mM DTT and 10% (v/v) glycerol with NTP's as indicated in the text including [α-^{32}P]UTP or GTP (0.5 Ci/mmol) and with the transcription complex (0.07 µg DNA complexed with extract proteins/50 µl assay mixture). The 110 nucleotides long pre-$tRNA^{Tyr}$ was resolved by Urea-PAGE (6% acrylamide). For autoradiography (A) the gels were exposed to pre-flashed Hyperfilm-β_{max} (Amersham) at −70 °C. RNA bands were then quantified by laser densitometry. For easy interpretation, the data (average of duplicate assays) are presented as double reciprocal plots of transcription initial velocities (arbitrary units) versus NTP concentrations (B). Apparent Michaelis constants (C) were estimated using nonparametric formalism of Eisenthal and Cornish-Bowden (for relevant computer program see ref. [13]).

Abbreviations: K_m, apparent Michaelis constant; NTP, ribonucleotide 5′-triphosphate; $AMPPCH_2P$, adenosine 5′-(βγ-methylene) triphosphate; s^4UTP, 4-thiouridine 5′-triphosphate.

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([NTP] $\geq 7K_m$: 0.6 mM ATP and 0.2 mM of the other NTP's). The same approach has been applied routinely to determine kinetic constants for substrates and inhibitors of *E. coli* polymerase. The observed rate of yeast $tRNA^{Tyr}$ gene transcription was a hyperbolic function of nucleotide input, thus reminiscent of steady-state, one-substrate kinetics. K_m constants calculated from this model (Fig. 1) suggest that affinity of the 5′-terminal nucleotide, ATP (5′-end of the pre-$tRNA^{Tyr}$: pppApApApUpApC...), to the catalytic center of polymerase III is much lower than affinities of UTP, CTP or GTP which are bound exclusively in the elongation phase of transcription (the comparison is justified by almost equal content of A, T, C and G residues within the tRNA gene). Thus in this respect the yeast system is analogous to the *E. coli* system. Higher affinity of nucleotide substrates in the RNA elongation step may result from opening or activation of the elongation, i+1 site [5] by the presence of nascent RNA chain at the i site. In particular, the opening might be due to conformational change of the i+1 site and the exposure of ribose 3′-OH group of growing RNA for interaction with the subsequently incoming nucleotide.

Among other similarities to the *E. coli* enzyme [6–8], we found that in the yeast polymerase III system: (1) ATP may be replaced by $AMPPCH_2P$ without loss of transcriptional efficiency, (2) s^4UTP does not substitute for UTP, and (3) 2′-dNTP's inhibit transcription very poorly (not shown).

Our data supplement earlier findings that bacterial and eukaryotic nuclear RNA polymerases share common structural features such as: (1) $\beta\beta'\alpha_2$-like subunit design comprising the region directly involved in polymerization, (2) similar antigenicity of the catalytically active largest subunits, and (3) amino acid sequence homology (rev. in refs. [9–11]). Also the size and location of DNA unwinding around the transcription start site are similar for the *E. coli* enzyme and yeast polymerase III [12]. It thus seems reasonable to assume that, as soon as the initiation complex is formed and the enzyme correctly positioned on the template, the catalytic properties of polymerases in the initiation and elongation steps of RNA synthesis are similar.

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[1] D. D. Anthony, C. W. Wu, and D. A. Goldthwait, Biochemistry **8,** 246 (1969).
[2] T. D. Yager and P. H. von Hippel, in: *Escherichia coli* and *Salmonella typhimurium:* Cellular and Molecular Biology (F. C. Neidhardt, ed.), pp. 1241–1275, American Society for Microbiology, Washington, D.C. 1987.
[3] M. Sawadogo, B. Lescure, A. Sentenac, and P. Fromageot, Biochemistry **20,** 3542 (1981).
[4] R. A. Koski, D. S. Allison, M. Worthington, and B. D. Hall, Nucleic Acids Res. **10,** 8127 (1982).
[5] W. J. Smagowicz and K. H. Scheit, Nucleic Acids Res. **4,** 3863 (1977).
[6] P. Szafrański, W. J. Smagowicz, and K. L. Wierzchowski, Acta Biochim. Polon. **32,** 329 (1985).
[7] H. R. Rackwitz and K. H. Scheit, Eur. J. Biochem. **72,** 191 (1977).
[8] J. E. Sylvester and D. Dennis, Biochem. Biophys. Res. Commun. **75,** 667 (1977).
[9] D. Armaleo, J. Theor. Biol. **127,** 301 (1987).
[10] A. Lazcano, J. Fastag, P. Gariglio, C. Ramirez, and J. Oro, J. Mol. Evol. **27,** 365 (1988).
[11] N. Iwabe, K. Kuma, H. Kishino, M. Hasegawa, and T. Miyata, J. Mol. Evol. **32,** 70 (1991).
[12] G. A. Kassavetis, B. R. Braun, L. H. Nguyen, and E. P. Geiduschek, Cell **60,** 235 (1990).
[13] Z. W. Kamiński and E. F. Domino, Computer Meth. Progr. Biomed. **24,** 41 (1987).

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