COOPERATIVE EFFECTS OF RNA POLYMERASE FROM HIGHER PLANT CELLS AND *ESCHERICHIA COLI*: A COMPARISON

Klaus GROSSMANN and Hanns Ulrich SEITZ
Institute of Biology I, University of Tübingen, Auf der Morgenstelle 1, D-7400 Tübingen, FRG

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1. Introduction

RNA polymerase from *Escherichia coli* is an allosterically regulated enzyme whose activity and specificity is controlled by low molecular weight mediators, primarily by ppGpp. RNA synthesis, in *Escherichia coli* is tightly coupled to protein synthesis by two other effectors of RNA polymerase, fmet $tRNA_f^{met}$ and EF-TuTs [1]. The intracellular level of ppGpp is closely linked to both protein synthesis and the metabolic state of the cell [2]. These effectors may ensure that the production of ribosomes and mRNA is directly related to translation under different levels of nutrient and energy supply.

In eukaryotic cells there is little evidence for selective regulators of transcription, analogous to ppGpp. In [3] we described a coarse regulation of RNA synthesis in higher plant cells by nucleotides and divalent cations. In these cells RNA synthesis can be controlled by the interaction of such low molecular weight mediators. Eukaryotic cells, as opposed to prokaryotes, contain multiple forms of DNA-dependent RNA polymerase with specific roles and properties. But these enzymes have in common with the prokaryotic enzyme that they are all structurally heteromultimers. Although the subunit structures of eukaryotic RNA polymerases are more complex, in a number of respects they strongly resemble the bacterial enzyme. In spite of all structural differences, it would seem unlikely that a regulatory mechanism of such a basic process as the transcription of ribosomal RNA has been altered during evolution. In an attempt to answer this question we compared the properties of RNA polymerase I of higher plants with those of *Escherichia coli*. Here, the enzyme kinetics and the regulatory role of NTP and divalent cations, as low molecular weight regulators of RNA synthesis, are presented.

Abbreviations: DTT, 1,4-dithiothreitol; NTP, nucleoside triphosphate; NTPase, nucleoside triphosphatase

2. Materials and methods

Freely suspended callus cells of parsley (*Petroselinum crispum*) were cultured as in [4]. RNA polymerase I was purified from parsley and soybean hypocotyls according to [3]. RNA polymerase from *Escherichia coli* MRE 600 was purchased from Boehringer (Mannheim). RNA polymerase activity was assayed as in [3]. For special treatments see the figure legends.

3. Results and discussion

RNA polymerase I isolated from callus cells of parsley and from soybean hypocotyls seems to be an allosterically regulated enzyme which utilizes the complex of NTP and divalent cations as substrate [3]. NTP if present in concentrations exceeding the molar concentrations of divalent cations, acted as allosteric inhibitors and led to a sigmoid curve, if initial velocity was plotted against substrate concentration. Free divalent cations, on the other hand, had a promotive effect on RNA polymerase I. In this case, a Michaelis-Menten relationship was obtained. In order to analyse the mechanism of regulation by NTP not complexed by divalent cations the data were plotted according to Hill, a method widely used to provide an index of cooperativity and to have a diagnostic tool in the determination of the minimum number of ligand binding sites [5].

In fig.1, kinetic data of RNA polymerase I from parsley and soybean are plotted according to Hill. The concentration of unlabeled NTP (ATP, CTP, GTP in equal amounts) and divalent cations was increased, maintaining a 1:1 ratio. In addition, each reaction mixture contained 1 mM NTP (ATP, CTP, GTP in equal amounts) in excess of this ratio. $n = 0.67$ was obtained for RNA polymerase I from parsley, as well as for the soybean enzyme, at <30% substrate saturation; at >30%, $n = 4.7$. Such a curve with two different slopes may indicate a mixed type of cooperativity. $n = 0.67$ might signify a negative cooperativity

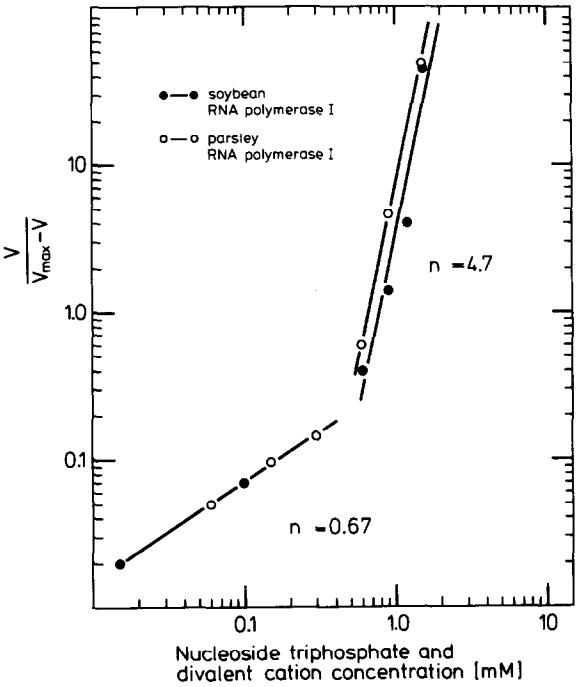

Fig.1. Effect of increasing substrate concentration on RNA polymerase I from parsley and from soybean. Hill plots. Parsley RNA polymerase activity was assayed in 0.5 ml reaction mixtures containing 50 mM Tris · HCl (pH 8.0), 20 mM $(NH_4)_2SO_4$, 25% glycerol (v/v), 20 mM DTT, 100 μg/ml heat-denatured salmon sperm DNA and 0.1 mM [5′-^{3}H]uridine 5′-triphosphate (6 μCi). Increasing amounts of nucleoside triphosphates (ATP, CTP, GTP in equal amounts) and divalent cations ($MgCl_2$ and $MnCl_2$ in a 6:1 ratio) were added to the reaction mixtures maintaining a NTP/cation ratio of 1:1. NTP, 1 mM (ATP, CTP, GTP in equal amounts) was added to each reaction mixture in excess of the 1:1 NTP/cation ratio. The reaction was started by adding 9 μg enzyme protein (20 μl). The assay conditions for soybean RNA polymerase were the same as for parsley with the exceptions that 50 mM $(NH_4)_2SO_4$ was present and the divalent cations were represented only by $MgCl_2$.

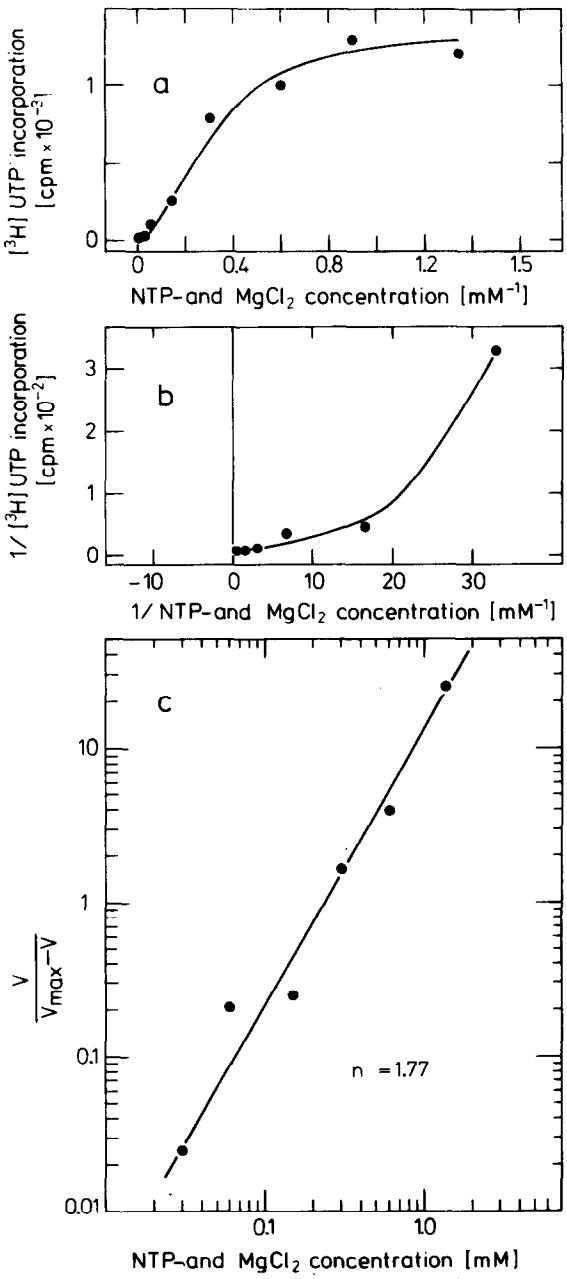

Fig.2. Effect of increasing substrate concentration on RNA polymerase from *E. coli*. The activity was assayed in 0.5 ml reaction mixtures containing 40 mM Tris · HCl (pH 7.9), 75 mM KCl, 25% glycerol (v/v), 100 μg/ml heat-denatured salmon sperm DNA, 0.1 mM DTT and 0.05 mM [5′-^{3}H]uridine 5′-triphosphate (4 μCi). Nucleoside triphosphates (ATP, CTP, GTP in equal amounts) and $MgCl_2$ were added to the reaction mixtures in increasing amounts, while maintaining a 1:1 NTP/cation ratio. NTP, 1 mM (ATP, CTP, GTP in equal amounts) was added to each reaction mixture in excess of the 1:1 NTP/cation ratio. The reaction was started by adding 0.5 unit enzyme (10 μl) and the mixture was incubated at 32°C for 30 min. (a) Velocity versus substrate concentration plot; (b) Lineweaver-Burk plot of the data in fig.2a; (c) Hill-plot of the data in fig.2a.

while $n = 4.7$ indicates a positive cooperativity, with a minimum number of 5 ligand binding sites.

Similar experiments were conducted on *E. coli* polymerase. Fig.2a, in which initial velocity is plotted against substrate concentration, shows a sigmoid curve. In the corresponding Lineweaver-Burk plot (fig.2b), a non-linear relationship was observed which is characteristic for allosteric behaviour and positive cooperativity. From the Hill plot, $n = 1.77$ was calculated, indicating a positive cooperativity, with a minimum number of two ligand binding sites on *E. coli* RNA polymerase. It should be mentioned that the enzyme obeyed regular Michaelis-Menten kinetics if divalent cations were added in excess of a 1:1 ratio of NTP and divalent cations (not shown).

Our results are in good agreement with findings obtained by other techniques which demonstrated that the RNA polymerase from *E. coli* has two nucleotide binding sites: one involved in the initiation of RNA chains and the other in their elongation. It is not yet clear whether one or both of the α-subunits bear the binding sites of the nucleotides [6,7].

These kinetic data provide good evidence that NTP in concentrations exceeding the 1:1 NTP/divalent cation ratio, are allosteric inhibitors of *E. coli* polymerase, leading to a positive cooperativity with at least two substrate binding sites.

Comparison of the kinetic data of RNA polymerase from higher plant cells and *E. coli* supports the view that NTP and divalent cations may act as low molecular weight regulators in transcription in prokaryotes as well as in eukaryotes. It would be reasonable to assume that the eukaryotic RNA polymerase, with a higher complexity of subunit structure, also shows a higher degree of cooperativity of the substrate binding sites, reflecting a more complex regulation of eukaryotic RNA synthesis.

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