TRANSCRIPTION SPECIFICITY OF *XENOPUS LAEVIS* RNA POLYMERASE A

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1. Introduction

Since the demonstration that type A RNA polymerase from *Xenopus laevis* ovaries selectively transcribes ribosomal cistrons in vitro [1], the probable significance and roles of the major classes of eukaryotic RNA polymerases have received considerable clarification [2,3]. In particular, form AIII enzyme may be equated with form C which occurs both in nuclei and in soluble form in the cytoplasm after cell disruption [2]. This enzyme is probably involved in the transcription of 5 S and tRNA genes in vivo [3]. Furthermore, an RNA polymerase with properties similar to form C has been isolated from *Xenopus* oocytes by a technique similar to our own [4,5]. It was therefore important to clarify further the properties of the enzyme which selectively transcribes ribosomal genes in vitro, and in particular to determine whether type A or C was responsible for the observed specificity.

2. Materials and methods

RNA polymerases were purified from *Xenopus laevis* ovaries to the stage of DEAE-cellulose chromatography as described previously [5]. Mainband DNA and DNA enriched in ribosomal cistrons by a single caesium chloride centrifugation ('rDNA') were prepared from *Xenopus* erythrocytes as outlined elsewhere [1]. DEAE-Sephadex (A-25) chromatography was performed as described in the appropriate figure legend. RNA polymerase activity was assayed as described previously [5].

3. Results and discussion

RNA polymerase activity eluted from DEAE-cellulose by 0.15 M ammonium sulphate [5] was diluted to 0.05 M ammonium sulphate and adsorbed onto DEAE-Sephadex as described in the legend to fig. 1. RNA polymerases were then eluted by a gradient of 0.05–0.6 M ammonium sulphate. Two discrete

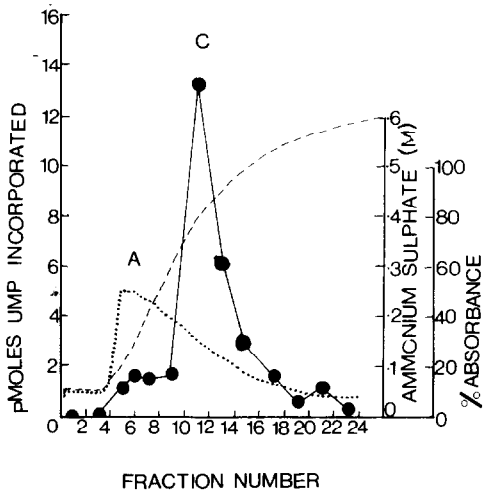

Fig. 1. DEAE-Sephadex chromatography of *Xenopus* RNA polymerases. 1670 units of RNA polymerase (0.15 M ammonium sulphate eluate [5]) were diluted to 0.05 M ammonium sulphate and adsorbed onto a 1.6 × 10 cm column of DEAE-Sephadex previously equilibrated with 50 mM Tris–HCl pH 8.0, 25% (v/v) glycerol, 0.1 mM EDTA, 0.1 mM dithiothreitol (TGED) and 50 mM ammonium sulphate. The column was washed with 50 ml of the same buffer and eluted with a linear gradient of 0.05–0.6 M ammonium sulphate/TGED in a total volume of 50 ml. Flow rate was 36 ml/hr and 2.0 ml fractions were collected; all operations were at 4°C. 100 μl aliquots of each fraction were assayed for RNA polymerase activity [5] with [^{3}H]UTP at 160 μCi/μmole. (. . . . .) % Absorbance at 260 nm; (– – – –) Ammonium sulphate concentration; (●——●) RNA polymerase activity.

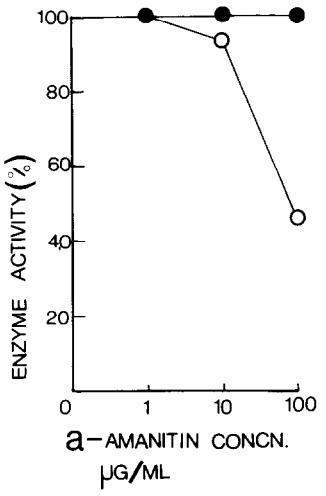

Fig. 2. Effects of α-amanitin. RNA polymerase activity was assayed as described elesewhere [5], in the presence of the α-amanitin concentrations indicated. 100 μl aliquots of the peak fractions shown in fig. 1. were used as enzyme sources. (●——●) RNA polymerase peak 'A'; (○—○) RNA polymerase peak 'C'.

peaks of activity were revealed when the fractions were assayed; the first and smallest eluted at about 0.1 M ammonium sulphate, the second and largest at about 0.35 M ammonium sulphate.

Aliquots from the two peak fractions were tested for sensitivity to the drug α-amanitin as shown in fig. 2. Form A enzyme is known to be totally resistant to α-amanitin, whereas form B is inhibited by very low concentrations (< 0.1 μg/ml) and form C by high concentrations (> 10 μg/ml) of the compound [2,6,7]. Fig. 2 clearly demonstrates that the small peak of RNA polymerase activity is form A, whereas the large peak has form C characteristics and is fundamentally sensitive to α-amanitin.

Since the enzyme which demonstrated specificity in the transcription of ribosomal genes [1] was clearly a mixture of A and C enzymes, it remained to be resolved as to which was actually synthesising the ribosomal RNA. Measurements of U:G incorporation ratios by forms A and C on both mainband and 'rDNA' templates are documented in table 1. Ribosomal RNA precursors are known to have a high proportion of G+C [8]. Whereas form A enzyme synthesised a G rich product on 'rDNA' form C enzyme did not transcribe this DNA at all at the enzyme: template ratio employed. Conversely, both enzymes synthesised U rich RNA species on mainband DNA.

Table 1
U:G Incorporation ratios

Transcription complex	Incorporation: UTP	GTP	Ratio UTP:GTP
	pmoles	pmoles	
A enzyme, 'rDNA'	0.15	0.45	0.33
C enzyme, 'rDNA'	0	0	–
A enzyme, mainband DNA	1.23	0.43	2.80
C enzyme, mainband DNA	7.00	2.00	3.50

100 μl aliquots of RNA polymerases A and C were assayed in the presence of either 0.75 μg 'rDNA' or 7.5 μg mainband DNA, and with nucleotides containing either [^{3}H]UTP or [^{3}H]GTP (Both at 700 μCi/μmole).

Form C RNA polymerase demonstrates different chromatographic properties on the two ion exchange resins DEAE-cellulose and DEAE-Sephadex [2], a feature which has caused confusion in enzyme nomenclature. Form C virtually cochromatographs with form A on DEAE-cellulose [2,7] but elutes at much higher ionic strength from DEAE-Sephadex and can be separated from form A by the use of this ion exchanger [2]. Thus the 'Form A' enzyme previously reported from *Xenopus* ovaries did contain the A type of enzyme [1,5] but was predominantly form C as revealed in figs. 1 and 2. The latter RNA polymerase is relatively more abundant in *Xenopus* ovaries than in most other tissues so far examined, a finding confirmed elsewhere [4]. The low ionic strength extraction procedure developed for RNA polymerase purification from *Xenopus* ovaries [5] clearly solubilised all three major forms of the enzyme.

The data of table 1 suggest that in the mixture of forms A and C, form A was probably responsible for the transcription of the ribosomal cistrons. Form C, like form B [1], did not transcribe these genes. This was not an artefact of the different 'rDNA' and mainband DNA concentrations, since both enzymes are active with low concentrations of mainband DNA [1].

The observation goes some way towards explaining certain specificity characteristics observed earlier: in particular, the relatively low proportion of form A compared with C could explain the high enzyme: 'rDNA' ratio needed for specificity and for the increase in rifamycin AF/0-13 resistance [1]. The inactivity of form C on 'rDNA' at the high enzyme: DNA ratio could also explain the observed variation in U:G incorporation ratios [1].

In conclusion, the data presented above confirmed the suspicion that the 0.15 M ammonium sulphate RNA polymerase fraction was not homogeneous, but contained a mixture of forms A and C. Form A was indicated to be the species involved in the transcription of ribosomal genes.

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