Volume 227, number 2, 122–126 FEB 05493 January 1988

# Variable RNA polymerase populations in the life cycle of *Trypanosoma brucei*

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Received 23 November 1987; revised version received 30 November 1987

DNA-dependent RNA polymerase from blood forms and culture forms of the parasitic protozoan *Trypanosoma brucei* was resolved into multiple peaks of activity by DEAE-Sephadex chromatography. The enzyme from the two forms was found to be different by several criteria, suggesting that it is subject to developmental control.

RNA polymerase; Enzyme activity; Developmental regulation; *(Trypanosoma)*

## 1. INTRODUCTION

When passing through their insect and mammalian hosts, kinetoplastid protozoan parasites such as *Trypanosoma brucei*, the species responsible for sleeping sickness in man, undergo a series of stage-specific morphological and physiological changes [1–3]. At least some of these changes appear to be caused by altered patterns of RNA accumulation [4–7]. The switching of hosts being abrupt, it should be to the parasite's advantage to evolve its transcriptional apparatus to sense and respond to changed conditions rapidly, such as a difference in temperature, in the new host. New patterns of transcription have been shown to be associated with modifications of DNA-dependent RNA polymerase in, for example, encysting acanthamoebae [8] and sporulating bacteria [9]. To determine whether the developmental transformation of *T. brucei brucei*, a subspecies infecting animals, is associated with a change in the properties of its RNA polymerase, I compared partially purified enzyme from in vitro cultured insect-stage trypanosomes [10] (culture forms) with that from parasites grown in the bloodstream of rats (blood forms). The enzyme was different in the two forms, indicating that it is subject to developmental control.

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## 2. MATERIALS AND METHODS

### 2.1. *Parasites*

Blood and culture form stocks of *T. brucei*-427 [11] were kindly provided by Dr Fred Opperdoes (Brussels). Blood forms were grown in Wistar rats and purified by DEAE-cellulose chromatography according to Lanham [12]. Culture forms were grown at 27°C in SDM79 medium [13] supplemented with heat-inactivated 10% foetal calf serum and 100 $\mu$g/ml gentamycin, and harvested in late exponential phase by centrifugation at 1800 × *g* for 20 min at 4°C. Parasites were stored at −80°C, for a maximum of 3 weeks, until use.

### 2.2. *Enzyme extraction and chromatography*

Enzyme extraction and chromatography were performed at 2–4°C. Crude extracts were prepared essentially as described by Kitchin et al. [14]. DEAE-Sephadex A-25 was from Pharmacia. Column beds were 24 × 1.5 cm, and were equilibrated with TGED [50 mM Tris-HCl (pH 7.9), 25% (v/v) glycerol, 100 $\mu$M EDTA, 100 $\mu$M dithiothreitol]. Flow rate for all operations given below was 30 ml/h. Crude lysate protein, in a constant volume of 30 ml TGED, was applied to the column, washed with 60 ml TGED, and the enzyme activities were eluted with a 50 ml, 0–500 mM linear gradient of ammonium sulphate, followed by 15 ml of 500 mM ammonium sulphate, in TGED, 60 × 1 ml fractions were usually collected. A 0.05 ml aliquot was taken from each fraction for enzyme assay, 0.1 ml aliquots from suitably spaced fractions were taken for conductivity measurements, and the rest stored for a maximum of 2 weeks at −80°C with no detectable loss of activity.



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00145793/88/$3.50 

2.3. *Enzyme assay*

The assay mixture contained, in a volume of 0.1 ml, 0.5 mM each of ATP, GTP and CTP, and 0.005 mM UTP (Sigma); 20 $\mu$g heat-denatured calf thymus DNA (Sigma type I); 1 mM dithiothreitol (Sigma); 2 mM $MnCl_2$ (analytical grade); 50 mM Tris-HCl (pH 7.9); 2 $\mu$Ci [5-$^3$H]UTP (10 Ci/mmol, Amersham); and 0.05 ml of the enzyme fraction. Reaction mixtures were pre-incubated in ice for 10 min and shifted to 37°C for 12 min. Reactions were stopped by depositing 0.085 ml of the assay mixture onto DE-81 filter discs (Whatman). Filters were dried quickly in a stream of air and, at room temperature, washed extensively with 5% $Na_2HPO_4$, followed by one wash in water and three washes in absolute alcohol. Filters were air-dried and counted for tritium under 5 ml of OCS scintillator solution (Amersham).

## 3. RESULTS AND DISCUSSION

Soluble extracts from the two stages of the parasite were applied to columns of 'DEAE-Sephadex A-25' and eluted with ammonium sulphate (fig.1). The polymerase activity from the blood form extract eluted in a large peak at about 75 mM of the salt and, when increased amounts of protein were applied to the column (fig.1c), a much smaller peak of activity eluted at about 25 mM salt. These results confirm the recent original observations of Earnshaw et al. [15] on the multiplicity of the blood form enzyme. There has, however, been no report so far on the nature of the RNA polymerase in trypanosome culture forms. As shown in fig.1, extracts from culture forms also resolved into two peaks of activity, at about 25 and 75 mM salt, but in this case the 25 mM peak was readily seen even when smaller amounts of protein were analyzed, and both peaks were of comparable size; in the experiment shown in fig.1c, the activity optima, expressed as cpm [$^3$H]UMP incorporated per assay, were about 7000 and 30000 for the blood form peaks and about 13000 each for the culture form peaks. [Until a nomenclature for

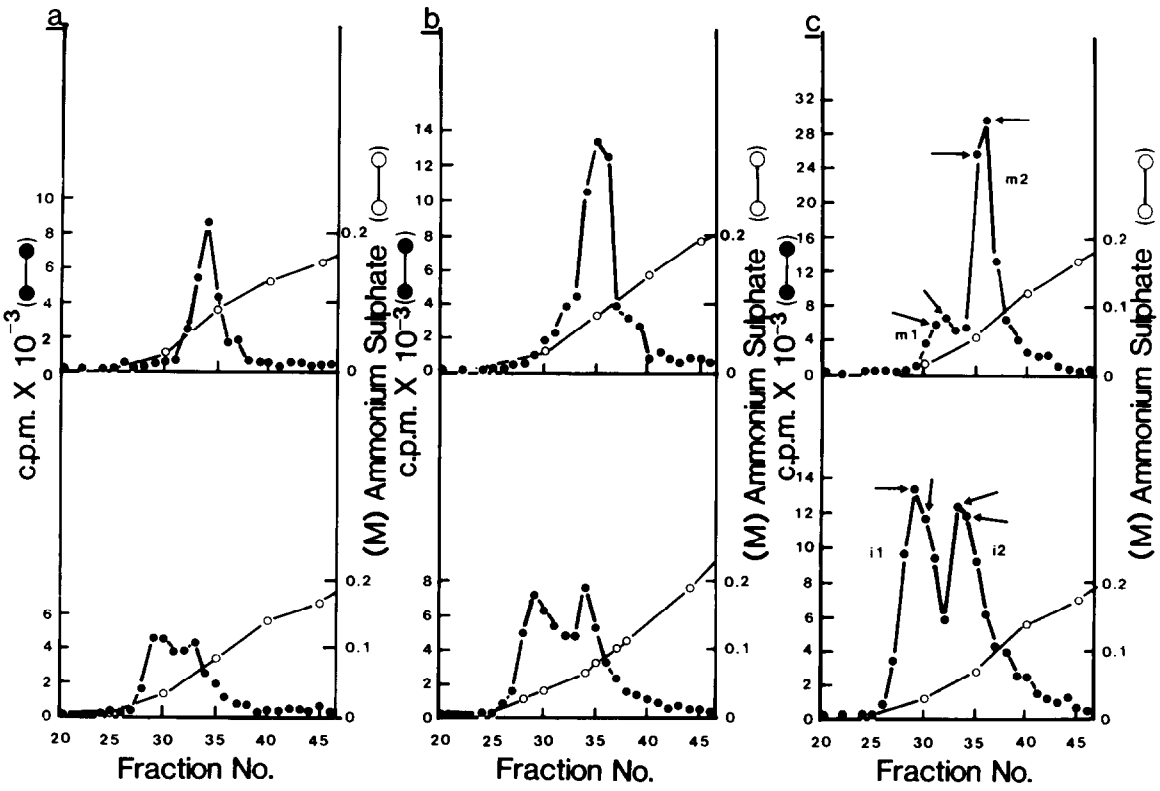


Fig.1. Resolution of blood form and culture form RNA polymerase activity by DEAE-Sephadex chromatography. Top row of graphs: blood form extracts; bottom row: culture form extracts. (a) 30 mg; (b) 60 mg; (c) 120 mg crude lysate protein applied to column. Enzyme activity is given as tritium in the UMP incorporated into RNA in the assay (section 2). Only the region of the chromatogram showing activity is plotted. Arrows and lettering by the peaks in c are explained in the text and in fig.2.

trypanosome RNA polymerase is agreed upon, the '25 mM' and '75 mM' peaks from the blood form will be referred to as pol-m1 and pol-m2 (for mammalian) respectively, and the corresponding culture form peaks as pol-i1 and pol-i2 (for insect) (fig.1c).] All four activities retained their elution patterns when re-chromatographed under identical conditions (fig.2). The difference, reproduced in several experiments, between the pol-m1/pol-m2 and pol-i1/pol-i2 activity ratios in carefully controlled chromatographic and assay conditions (fig.1), together with other data given below, indicate that the RNA polymerase population in *T. brucei* is under some form of developmental control.

The four enzyme activities were tested for their response to $\alpha$-amanitin, the divalent cations $Mg^{2+}$ and $Mn^{2+}$, $(NH_4)_2SO_4$, and different temperatures (fig.3). The results are compared in table 1.

There were obvious similarities between pol-m1 and pol-i1, and between pol-m2 and pol-i2, notably in elution patterns from the column, response to $\alpha$-amanitin, temperature optima, and number and position of $Mn^{2+}$ optima.

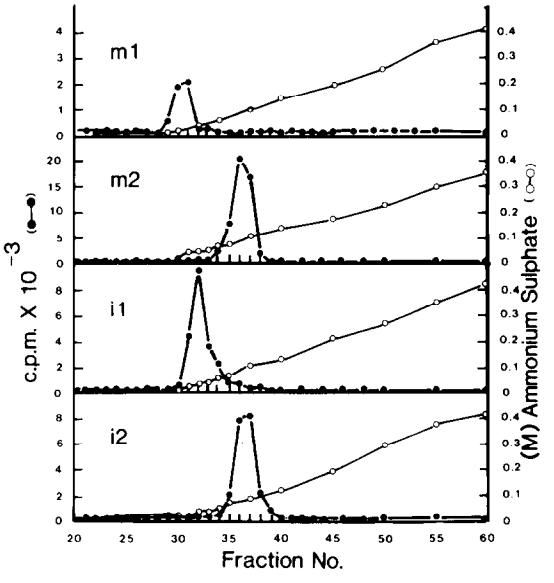


Fig.2. Re-chromatography of peak enzyme fractions from experiment shown in fig.1c. Fractions (denoted by arrows in fig.1c) were pooled and desalted at 4°C by passing through Sephadex G-25 dispo-columns (10 ml; Pharmacia) equilibrated with TGED, and chromatographed separately and assayed under conditions identical to those given in section 2.

Nonetheless, they also displayed significant differences. Thus, pol-m1 had a higher $Mg^{2+}$ optimum than pol-i1, and its activity was much less stimulated by intermediate temperatures. In the re-chromatography experiments (fig.2) pol-m1 was seen to be unstable, and only a small proportion of the activity was recovered. This instability is also apparent in fig.3d and table 1, where pol-m1 decays more than pol-i1 at higher temperatures. The most striking difference between pol-m2 and pol-i2 was that pol-m2 had an almost 4-fold higher preference for $Mn^{2+}$ over $Mg^{2+}$ than had pol-i2. To account for these observations, one may speculate that two fairly distinct polymerases, here called pol-1 and pol-2, are each reversibly modified to produce its related blood and culture form versions, as follows:

pol-m1 ⟷ pol-1 ⟷ pol-i1;

pol-m2 ⟷ pol-2 ⟷ pol-i2.

Kooter and Borst [16] and Laird et al. [17], in transcriptional studies with whole nuclei taken from blood forms, found that the synthesis of variant surface glycoprotein RNA and rRNA was highly resistant to $\alpha$-amanitin. The present evidence (fig.3a) would suggest that pol-m1 and pol-i1 are the activities responsible for this transcription. The transcription of the 'mini-exon' repeat sequence [18] and the synthesis of 5 S RNA were found by these authors to be sensitive to $\alpha$-amanitin at about the same concentrations as those here shown to inactivate pol-m2 and pol-i2. The synthesis of tubulin and phosphoglycerate kinase RNA was shown to be sensitive to even lower concentrations of the toxin (50% inhibition at 5 $\mu$g/ml), implying that the parasite has yet other polymerases, undetected here. (Note also that $\alpha$-amanitin had a slight stimulatory effect on pol-m1 and pol-i1 and, at lower concentrations, even on pol-m2 and pol-i2. The drug rifampicin has a similar effect on mutant *Escherichia coli* RNA polymerase [19].)

The two $Mn^{2+}$ optima for pol-m1 and pol-i1 must mean that, if the peaks constitute single enzymes, they each have more than one active conformation which this cation can induce. Alternatively, pol-m1 and pol-i1 may each represent multiple enzymes, having quite different $Mn^{2+}$ optima.

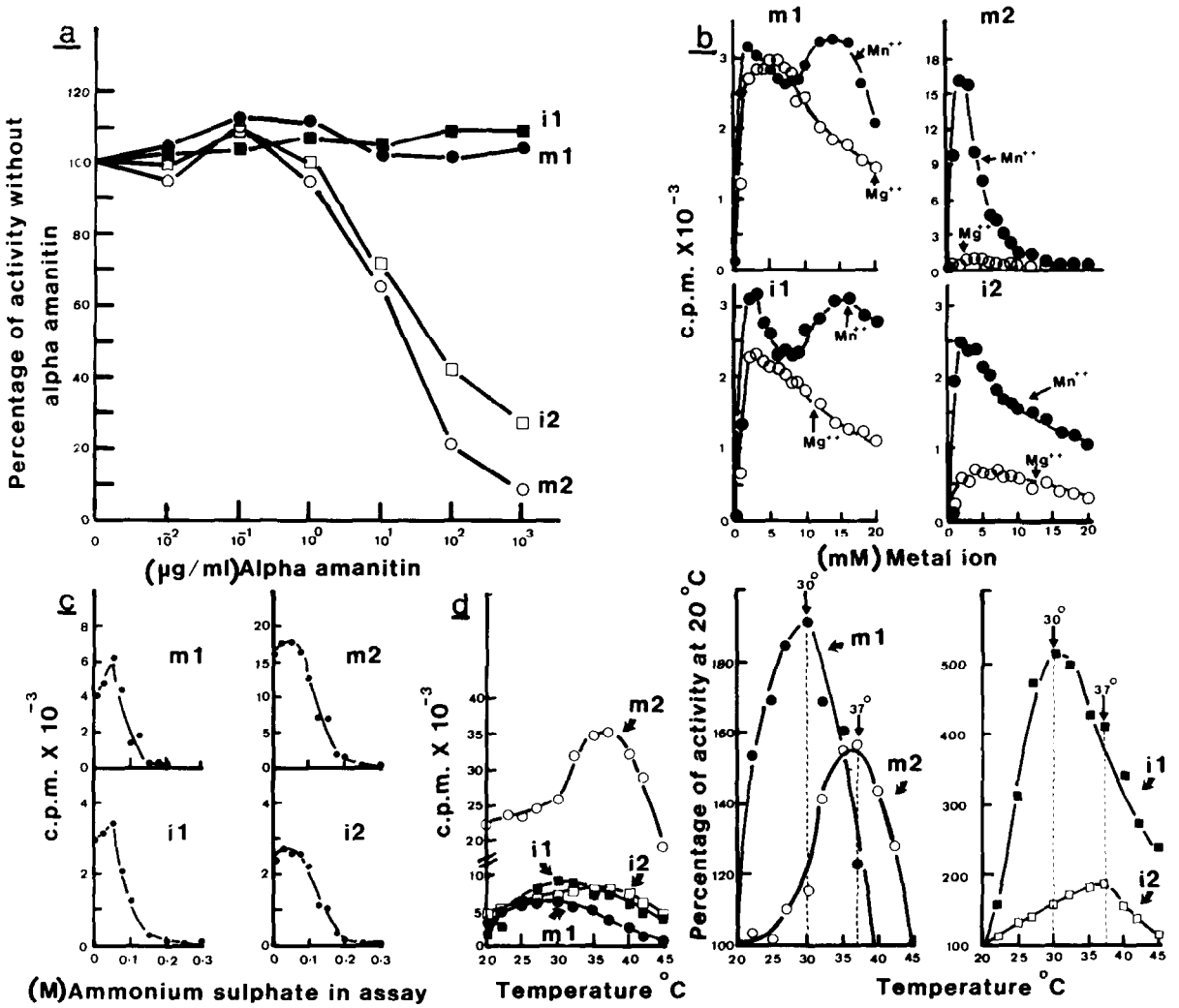


Fig.3. Response of the enzyme peaks to (a) $\alpha$-amanitin; (b) $Mg^{2+}$ and $Mn^{2+}$; (c) $(NH_4)_2SO_4$; (d) temperature. The two-peak enzyme fractions from experiments similar to those shown in fig.1b,c were desalted as described in the legend to fig.2, before use. All assays, except where indicated, were as described in section 2. Enzyme activity is given either as cpm tritium incorporated in the assay, or as a percentage of such incorporation under a particular assay condition. The pattern of results shown was obtained in at least two separate experiments. Each point on the graph is the mean of three values (within an error of 5% of the mean) obtained in one such experiment. (a) $\alpha$-Amanitin, kindly provided by Dr T. Wieland, was dissolved in water. The assay components, given in section 2, together with appropriate amounts of $\alpha$-amanitin, were preincubated with the enzyme in ice for 10 min. The corrected mean full activities without $\alpha$-amanitin, in cpm $^3H$ incorporated, were about 7000 for pol-m1, 21000 for pol-m2, 5300 for pol-i1 and 4000 for pol-i2. (b) The assay components given in section 2, minus $Mn^{2+}$, were first mixed with the enzyme and pre-incubated with the appropriate concentration of the divalent cation for 10 min in ice (d, left). Assay conditions were as those described in section 2 except that following pre-incubation of the reaction mixtures in ice they were transferred to the given temperatures for 5 min; (d, middle and right), replots relative to activity at 20°C, taken from data in graph on the left.

The temperature optima of 30°C (pol-m1 and pol-i1) and 37°C (pol-m2 and pol-i2) approximate the ambient temperatures of the culture/insect form (26–29°C) and the blood form (37–39°C). We may speculate that a reserve of RNA polymerases with selected temperature optima is a device for ensuring rapid and efficient transcriptional adjustment in the parasite's life cycle; for example, it may allow a rapid alteration in the pattern of promoter choice when the parasite is

Table 1

Comparison of blood and culture form RNA polymerases (based on data in fig.3)

| | Pol-m1 | Pol-i1 | Pol-m2 | Pol-i2 |
|---|---|---|---|---|
| $(NH_4)_2SO_4$ elution point from Sephadex (mM) | 25 | 25 | 75 | 75 |
| % resistance to $\alpha$-amanitin at | | | | |
| $10^{-1}$ $\mu$g/ml | >100 | >100 | >100 | >100 |
| $10^{0}$ $\mu$g/ml | >100 | >100 | 95 | 100 |
| $10^{1}$ $\mu$g/ml | >100 | >100 | 70 | 70 |
| $10^{2}$ $\mu$g/ml | >100 | >100 | 20 | 40 |
| $10^{3}$ $\mu$g/ml | >100 | >100 | 10 | 25 |
| $Mg^{2+}$ optimum (mM) | 6 | 2 | 4 | 4 |
| $Mn^{2+}$ optimum (mM) (1st) | 2 | 2 | 2 | 2 |
| (2nd) | 10–15 | 10–15 | – | – |
| Activity at $Mn^{2+}$ optimum/activity at $Mg^{2+}$ optimum | 1.1 | 1.4 | 14.5 | 3.6 |
| $(NH_4)_2SO_4$ optimum (M) | 0.05 | 0.05 | 0.05 | 0.05 |
| Temperature optimum (°C) | 30 | 30 | 37 | 37 |
| Activity at 30°C/activity at 20°C | 1.9 | 5.1 | 1.2 | 1.5 |
| Activity at 37°C/activity at 20°C | 1.3 | 3.8 | 1.5 | 1.8 |
| Activity at 45°C/activity at 20°C | 0.9 | 2.3 | 0.8 | 1.1 |

abruptly deposited from one temperature to another.

The possibility that the different RNA polymerases seen in blood and culture forms are due to some contaminating microorganism has been ruled out by microscopy. Furthermore, the pedigree of the parasites has been well established in other laboratories [11], and confirmed here by a Southern analysis of variously restricted DNA from the two forms, using a *T. brucei* mini-exon probe (pCL102) [20] (kindly provided by Dr Jan Kooter) and *Chlamydomonas reinhardtii* $\alpha$- and $\beta$-tubulin probes [21] (kindly provided by Drs Carolyn Silflow and Joel Rosenbaum) (not shown).

The molecular basis and full significance of this developmental adjustment of RNA polymerase remain to be explained. Several speculations may nonetheless be made. For example, there is much more mitochondrial (kinetoplast) activity in the insect form of trypanosomes than in the blood form, and the prominent pol-i1 peak may represent a polymerase involved in the transcription of insect form mitochondrial genes, whereas the residual, highly labile pol-m1 may be the enzyme found in blood form mitochondria. The isolation of DNA coding for the polymerase subunits and the use of in vitro blood form to insect form transformation protocols, along the lines described by Overath et al. [22], should prove most valuable in understanding this phenomenon further.

*Acknowledgements:* I am grateful to Dr Staffan Normark for his interest in this work, to both him and Dr Glenn Björk for discussion, and to Miss Ingegerd Söderström for help with the rats. This work is being supported by the Swedish Medical Research Council (MFR).

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