CONTROL OF RNA TRANSCRIPTION IN NUCLEI AND NUCLEOLI OF *PHYSARUM POLYCEPHALUM*

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1. Introduction

RNA synthesis in nuclei isolated from synchronised cultures of *Physarum polycephalum* shows an apparent biphasic pattern over the mitotic cycle which implies that there may be control over the transcriptional activity of both polymerases A and B [1,2]. Using a modified method of isolating both nuclei and nucleoli, we have recently demonstrated that the size-distribution of RNA synthesised in vitro using the endogenous polymerase activities is very similar to that synthesised in vivo. In nucleoli it appears that rRNA (ribosomal RNA) precursors are correctly re-initiated, synthesised and processed [3]. We thought it worthwhile, therefore, to re-investigate the specific activity of the endogenous polymerases present in both nuclei and nucleoli during the mitotic cycle using α-amanitin to inhibit the nucleoplasmic polymerase B and the rifamycin derivative AF/013 to inhibit reinitiation [4]. The results show that whereas polymerase B activity shows a maximum in mid-S phase, decreasing to a constant value for the rest of interphase, the nucleolar polymerase A activity remains constant throughout interphase even though the number of genes (template) and the number of polymerase molecules doubles during the mitotic cycle. Two possible control mechanisms are discussed.

2. Materials and methods

Liquid cultures of *Physarum* (strain M_3CVIII) were grown at 26°C as in [5]. Mitotically synchronous cultures 5 cm diam. were grown on filter paper [6]. Observations on mitosis were made as in [7]. Nuclei were prepared by a modification of the method in [8] and the nucleoli isolated by sonication of the nuclei as in [3]. The standard assay system for RNA synthesis contained in final vol. 0.3 ml: 50 mM Tris–HCl, pH 7.5; 1 mM $MnCl_2$; 0.1 M KCl; 0.5 mM dithiothreitol; 0.15 mM ATP, CTP, GTP; 0.005 mM UTP; 2 μCi [^{3}H]UTP (Radiochemical Centre, Amersham). The sample of nuclei or nucleoli (5×10^6) was added at the beginning of the reaction to tubes preincubated 30°C for 3 min. The reaction was terminated by the addition of 5% trichloroacetic acid containing 1% sodium pyrophosphate at 0°C. The acid-soluble material was filtered under suction onto Whatman GF/C filters, washed with a further 50 ml trichloroacetic acid solution, dried and counted. The number of nuclei or nucleoli per assay tube was determined by staining with Giemsa's stain and counting in a haemocytometer. At least 6 independent determinations were performed for each experiment. The rifamycin derivative AF/013 was added in dimethyl sulphoxide to final concn 60 $\mu g \cdot ml^{-1}$ when required; α-amanitin (Boehringer) in Tris–HCl, pH 7.5 was used at concn 16.5 $\mu g \cdot ml^{-1}$. Polymerase B was inhibited to 50% by 0.5 $\mu g/ml^{-1}$ α-amanitin. No further decrease in polymerase activity was observed up to 200 μg/ml α-amanitin, indicating the absence of polymerase C activity, a result consistent with [15].

3. Results

The endogenous RNA polymerase activity of nuclei was studied over the mitotic cycle, in the presence

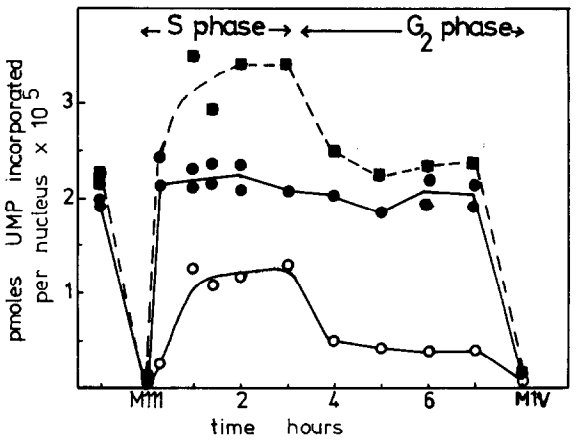

Fig.1. RNA transcription in isolated nuclei over the mitotic cycle. (▪- -▪) In the absence of α-amanitin. (•—•) In the presence of α-amanitin. (○—○) Difference curve.

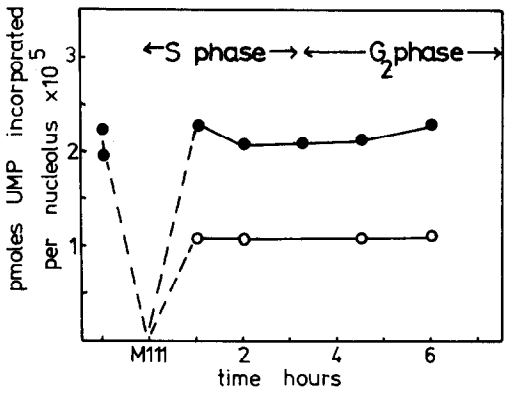

Fig.2. RNA transcription in isolated nucleoli over the mitotic cycle. (•—•) nucleoli alone. (○—○) nucleoli in the presence of 60 $\mu g \cdot ml^{-1}$ rifamycin AF/013.

and absence of α-amanitin, by harvesting 1 surface culture 1 h before mitosis III and at 30 min or 1 h intervals thereafter through the next mitotic cycle. All cultures used to obtain one set of data were checked to ensure that they went through mitosis within 10 min of each other. All cultures up to the next mitosis were >98% synchronous provided that they were no larger than 5 cm diam. The result of such an experiment is shown in fig.1. All RNA synthesis is completely shut off at metaphase in agreement with observations in vivo [9]. The endogenous RNA polymerases begin actively synthesising RNA as soon as daughter nuclei are visible by phase-contrast microscopy and reach the interphase maximum rate after 10 min. This is observed either in the presence or absence of α-amanitin, indicating that the complete reconstruction of the nucleolus into one integrated organelle is not required before rRNA synthesis can proceed. In early-S phase the nucleolus still exists as separate sub-particles each containing DNA, protein and RNA [10].

In the absence of α-amanitin RNA synthesis shows a biphasic uptake of precursor with a maximum in S phase which falls to a lower plateau in mid-G_2 phase before decreasing to zero at metaphase. This pattern was also observed in the absence of KCl and is similar to that in [12]. RNA synthesis does not begin to decrease significantly until prophase, 30 min before mitosis, and then drops quite rapidly to zero at metaphase. In contrast, the RNA synthesis observed in the presence of α-amanitin was maintained at a constant level from 20 min after mitosis until prophase. No biphasic pattern of RNA synthesis was observed. A similar pattern was observed in the absence of KCl.

The RNA synthetic activity of polymerase A in isolated nucleoli was investigated over the mitotic cycle. The pattern is similar to that obtained in the presence of α-amanitin in whole nuclei (fig.2). The RNA synthesis remains constant throughout interphase even though there has been a doubling in the number of rRNA genes during the cell cycle [11,13]. The activity decreased by ~50% in the presence of the rifamycin derivative AF/013 and remained constant throughout interphase showing that the rate of initiation by polymerase A is also unchanged.

4. Discussion

The combined RNA polymerase activities in nuclei isolated from synchronised cultures of *Physarum* rise sharply from zero at metaphase to a pronounced maximum in mid-S phase before decreasing to a constant level throughout the rest of interphase and finally to zero just before the next mitosis. The use of α-amanitin, a drug which specifically inhibits polymerase B, shows that the maximum in S phase is due entirely to polymerase B activity. This decreases by over 50% in G_2 despite a uniform doubling in the

number of mRNA genes by the end of S phase [11–14] and a uniform 2-fold increase in the amount of enzyme by the end of G_2 [15]. Thus the synthesis of mRNA appears to be under strict control through the mitotic cycle with the maximum rate of synthesis occurring in mid-S phase. mRNA transcription might be coupled with replication in *Physarum* [16] but this would not account for the continued activity of polymerase B in G_2.

By contrast polymerase A activity, responsible for the synthesis of rRNA, rises to a level 20 min after metaphase which remains constant throughout interphase only decreasing and falling to zero during prophase. This constant level of activity is maintained against a background doubling in the number of rRNA genes by the end of G_2 phase and a doubling in the activity and presumably amount of enzyme during the mitotic cycle [11,12,15]. Therefore, the production of rRNA in *Physarum* is not proportional to the total activity of polymerase present in the nucleus neither to the amount of template, and must therefore be subject to some form of strict transcriptional control.

The patterns of RNA synthesis observed here in nuclei are qualitatively similar to those in [1,2] although we do not observe such a large decrease in activity in mid-interphase. This difference might be attributed to the different assay conditions employed here and also to the method of isolation of the nuclei and nucleoli (W. D. Grant, personal communication). The ionic conditions used here (0.1 M KCl) probably approach closely those prevailing in the nucleus in vivo because the RNA synthesised in vitro has a molecular weight distribution similar to rRNA precursors observed in vivo and the RNA transcripts appear to be processed in a similar manner [3]. This suggests that correct re-initiation, elongation and termination of RNA chains is taking place.

The activity of polymerase A in isolated nucleoli is similar in all respects to that found in nuclei in the presence of α-amanitin (fig.2). The activity rises sharply from zero during reconstitution reaching its maximum level before the nucleolus has properly reformed, and remains constant throughout interphase before decreasing to zero at the next mitosis. The specific activity is reduced by a constant amount (~50%) at all times during interphase in the presence of the rifamycin derivative AF/013 which is thought to inhibit initiation [4]. This suggests that the rate of re-initiation of new RNA chains and the combined rates of elongation and termination remain constant throughout interphase even though the amount of template (rDNA) has doubled and the total activity of polymerase has doubled.

Two mechanisms of control may be considered to account for the above observations in nucleoli. In the first case we assume that the endogenous polymerase activity is either enzyme limiting or template limiting. In either situation the specific activity would be expected to at least double during the cell cycle and the fact that it does not imply that re-initiation, elongation and termination are inhibited to a degree which just offsets the increase in amounts of enzyme or template. In either case, inhibition may be caused by factors acting on the enzyme or on the template.

In the second case we assume that either all newly synthesised template or all newly synthesised enzyme (or both) is rendered transcriptionally inactive. This condition is maintained throughout interphase until metaphase when even the parent activity is completely inhibited. After nuclear division all the template and polymerase become activated and available for transcription during the next mitotic cycle.

These data are not inconsistent with the observation that the rate of dilution of rRNA increased 5–6-fold over the mitotic cycle of *Physarum* [14] but merely suggest that some post-trancriptional control is operative. The processing, transport and degradation of mature rRNA must be controlled in such a way that 5–6-fold molecules enter a stable pool of rRNA in G_2 phase compared with S phase even through the rate of production of rRNA precursors remains constant throughout.

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References

[1] Mittermayer, C., Braun, R. and Rusch, H. P. (1966) Biochim. Biophys. Acta 114, 536–546.

[2] Grant, W. D. (1972) Eur. J. Biochem. 2, 94–98.
[3] Davies, K. E. and Walker, I. O. (1977) J. Cell. Sci. 26, 267–279.
[4] Meilhac, M., Tysper, Z. and Chambon, P. (1972) Eur. J. Biochem. 28, 291–300.
[5] Daniel, J. W. and Baldwin, H. H. (1964) in: Methods in Cell Physiology, (Prescott, D. M. eds) Vol. 1, pp. 9–54, Academic Press, New York, London.
[6] Mohberg, J. and Rusch, H. P. (1969) J. Bact. 97, 1411–1418.
[7] Guttes, E., Guttes, S. and Rusch, H. P. (1961) Develop. Biol. 3, 588–614.
[8] Mohberg, J. andRusch, H. P. (1971) Exp. Cell Res. 66, 306–316.
[9] Kessler, D. (1967) Exp. Cell Res. 45, 676–680.
[10] Lord, A., Nicole, L. and LaFontaine, J. G. (1977) J. Cell. Sci. 23, 25–42.
[11] Zellweger, A., Ryser, V. and Braun, R. (1972) J. Mol. Biol. 64, 681–691.
[12] Newlon, C. S., Sonenshein, G. E. and Holt, C. E. (1973) Biochemistry 12, 2338–2345.
[13] Braun, R. and Evans, T. E. (1969) Biochim. Biophys. Acta 182, 511–512.
[14] Hall, L. and Turnock, G. (1976) Eur. J. Biochem. 62, 471–477.
[15] Hildebrandt, A. and Sauer, H. W. (1976) Biochim. Biophys. Acta 425, 316–321.
[16] Fouquet, H., Böhme, R., Wick, R., Sauer, H. W. and Scheller, K. (1975) J. Cell Sci. 18, 27–39.