PN 6149

DNA-dependent RNA synthesis in isolated nuclei from *Saccharomyces carlsbergensis*

A DNA-dependent synthesis of RNA has been proved to occur in the nuclei of a number of mammalian and plant tissues (*e.g.* refs. 1–3). Moreover, the presence of enzymes catalyzing RNA synthesis under the direction of DNA has been established in various microorganisms (*e.g.* refs. 4–6) and substantial purification of one of these enzymes has been reported[7].

As a result of our studies on protein synthesis in protoplasts of *Saccharomyces carlsbergensis*[8,9] we became interested in the nuclear synthesis of RNA. Therefore, we have studied the RNA synthesizing activity of nuclei isolated from the same protoplasts according to the method of ROZIJN and TONINO[10]. In this paper, preliminary results are presented, indicating the occurrence of DNA-dependent RNA-synthesis in our preparations of isolated nuclei.

The nuclei were isolated from *S. carlsbergensis* cells in a medium containing 0.02 M potassium phosphate (pH 6.5), 0.0005 M $MgCl_2$ and 8 % polyvinylpyrrolidone ("PVP medium").

The following procedure was used: *S. carlsbergensis* cells were converted to protoplasts as described previously[11]. The protoplasts were lysed in PVP medium containing 0.02 % Triton X-100. The lysate was homogenized in a Potter-Elvehjem type homogenizer and diluted immediately with an equal volume of a 0.6 M solution of sucrose in PVP medium. The resulting homogenate was centrifuged at $3000 \times g$ for 7 min to sediment the nuclei. The crude nuclear sediment thus obtained was purified either by washing twice with 0.6 M sucrose–PVP medium (preparation A), or by high-speed centrifugation on a layered gradient of 1.2–2.0 M sucrose in PVP medium (preparation B). Preparation A was still contaminated with small amounts of membraneous material, whereas the contamination in preparation B was negligible. Full details of the isolation procedure will be published elsewhere[10].

As it was easier to obtain preparation A in substantial amounts than preparation B, we used preparations A for the routine determination of DNA-dependent RNA synthesis as well as for studying the sucrose gradient ultracentrifuge patterns of newly synthesized RNA. However, from the data in Table I it can be seen that pure preparations of nuclei — although less active — also show RNA-synthesizing ability. Routinely, the complete incubation medium in which RNA synthesis was studied contained 40 mg polyvinylpyrrolidone, 200 mg sucrose, 50 mg glucose, 20 μmoles $MgCl_2$, 10 μmoles phosphate buffer (pH 6.8), 2 μmoles UTP, 2 μmoles CTP, 2 μmoles GTP, 0.8 μmole [^{14}C]ATP ($3 \cdot 10^5$ counts/min) and 3–5 mg nuclear protein per ml.

After 30 or 60 min of incubation at 30°, a solution was added which contained, for each ml of medium, 6 mg of carrier RNA, 0.1 ml of 10 % sodium laurylsulphate and 0.05 ml of 1 M NaCl. This was followed by phenol extraction of the RNA according to KIRBY[12]. After 15 min centrifugation at $3000 \times g$, the water layer was separated from the phenol layer. The nucleic acid was precipitated from the water layer by the addition of cold 10 % trichloroacetic acid. As it proved to be rather difficult to wash out all [^{14}C]ATP, 10 mg [^{12}C]ATP was added to the water layer

before the precipitation of RNA with trichloroacetic acid. Subsequently, the RNA precipitate was washed as described before[13], dissolved in formic acid, plated, and counted in a Philips electronic counter type P.W. 4032 after evaporation of the formic acid. Data of illustrative experiments with preparations A and B respectively are presented in Table I.

TABLE I

SYNTHESIS OF RNA FROM *S. carlsbergensis*

RNA synthesis at 30° by isolated nuclei from *S. carlsbergensis*, determined as the incorporation of ^{14}C label from [^{14}C]ATP. Complete system as described in the text; the time of incubation was 60 min.

Preparation of nuclei	*Remarks*	*Radioactivity of RNA precipitate (counts/min/mg)*
A	Complete system	94
A	Nuclei preincubated 10 min with 10 μg DNAase/ml	32
A	Nuclei preincubated 10 min with 10 μg actinomycin D/ml	45
A	RNA-extract treated with 10 μg RNAase*/ml	16
A	No UTP	46
A	No CTP	66
A	No GTP	55
B	Complete system	18

* EC 2.7.7.16.

From the results shown in Table I, it can be concluded that isolated nuclei from *S. carlsbergensis* show a DNAase (EC 3.1.4.5) and actinomycin D-sensitive RNA synthesis. For an optimal activity of the system all four ribonucleoside triphosphates are needed.

As we were interested in knowing something about the size of the RNA being synthesized in the nuclear preparations, we studied ultracentrifuge patterns of the labeled RNA preparations in a 20–5 % sucrose gradient. Again, the RNA was extracted with phenol from an incubation medium to which RNA was added as described above, precipitated at pH 5 from the water layer by the addition of a double volume of cold ethanol, redissolved in a small volume of versene–NaCl–Tris buffer solution of concentrations, 0.001, 0.02 and 0.005 M, respectively, and carefully titrated to pH 7. Subsequently, a 0.2 ml sample was layered on a 5 ml 20–5 % sucrose gradient and spun for 5 h at 37 000 revs./min in a SW 39 Spinco rotor. After puncture of the tube fractions were collected and their radioactivity was determined as described above, after the addition of another 6 mg carrier RNA per sample. Fig. 1 shows the gradient patterns of ^{14}C-labeled RNA as obtained after 30 and 60 min of incubation, respectively.

The gradient patterns shown in Fig. 1 strongly suggest that with increasing time of incubation a larger amount of RNA label moves to the heavier fractions of the

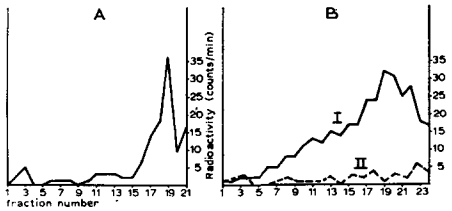

Fig. 1. Sucrose gradient patterns of ^{14}C-labeled RNA extracted after (A) 30 and (B) 60 min from an incubation medium as described in the text. Next to $3 \cdot 10^5$ counts/min [^{14}C]ATP per ml, the medium used for experiment B contained $3 \cdot 10^5$ counts/min/ml [^{14}C]UTP. B I: Pattern of nuclear RNA; B II: Pattern of the same RNA, after a 10 min treatment with 10 μgr/ml RNAase. Ultraviolet-light absorption patterns are left out as they have to be attributed mainly to the carrier RNA.

gradient. As it is known[14,15] that after 5 h of 20–5 % sucrose gradient centrifugation the RNA with a sedimentation coefficient of 23 S and a mol. weight of $1.2 \cdot 10^6$ moves to about fraction 13, this would mean that isolated nuclei of *S. carlsbergensis* are still able to synthesize or at least to finish the synthesis of macromolecular RNA.

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1 S. B. WEISS, *Proc. Natl. Acad. Sci. U.S.*, 47 (1960) 1020.
2 V. G. ALLFREY AND A. E. MIRSKY, *Proc. Natl. Acad. Sci. U.S.*, 48 (1962) 1590.
3 R. C. HUANG, N. MAKESHWARI AND J. BONNER, *Biochem. Biophys. Res. Commun.*, 3 (1960) 689.
4 J. HURWITZ A. BRESLER AND R. DIRINGER, *Biochem. Biophys. Res. Commun.*, 3 (1960) 15.
5 A. STEVENS, *J. Biol. Chem.*, 236 (1961) PC 43.
6 S. B. WEISS AND T. NAKOMOTO, *J. Biol. Chem.*, 236 (1961) PC 18.
7 J. HURWITZ, A. EVANS, C. BOBINET AND A. SKALKA, *28th Cold Spring Harbor Symp. Quant. Biol., June* 1963.
8 V. V. KONINGSBERGER, in R. J. C. HARRIS, *Protein Biosynthesis*, Academic Press, London and New York, 1961, p. 207.
9 S. R. DE KLOET, L. B. BARNETT, H. P. J. BLOEMERS AND V. V. KONINGSBERGER, *Proc. Koninkl. Ned. Akad. Wetenschap.*, Ser. B, 66 (1963) 332.
10 TH. H. ROZIJN AND G. J. M. TONINO, in preparation.
11 G. J. M. TONINO AND E. P. STEYN PARVÉ, *Biochim. Biophys. Acta*, 67 (1963) 453.
12 K. S. KIRBY, *Biochem. J.*, 64 (1956) 405.
13 L. B. BARNETT, G. FRENS AND V. V. KONINGSBERGER, *Proc. Koninkl. Ned. Akad. Wetenschap.*, Ser. B, 59 (1962) 418.
14 C. G. KURLAND, *J. Mol. Biol.*, 2 (1960) 83.
15 H. P. J. BLOEMERS AND V. V. KONINGSBERGER, unpublished.

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