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ABSENCE OF COMPLETE REPRESSION OF A SPECIFIC PART OF THE DNA DURING *IN VITRO* TRANSCRIPTION OF YEAST DEOXYRIBONUCLEOPROTEIN

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SUMMARY

A deoxyribonucleoprotein isolated from *Saccharomyces cerevisiae* was used as a template for the *in vitro* synthesis of RNA with *Escherichia coli* RNA polymerase. No significant differences between the base composition of this RNA and RNA synthesized with DNA as template could be detected. The RNA's synthesized with DNA or deoxyribonucleoprotein both hybridize with DNA to the same extent. On extrapolation to an infinite RNA concentration it appears that 42–45 % of the DNA can be saturated. Self-annealing experiments show that at least 60 % of both kinds of RNA were self-complementary, indicating a high degree of symmetric transcription. No differences between the two kinds of RNA were observed when they were used in competition hybridization to compete for a natural RNA fraction, or for RNA transcribed from DNA.

From the resemblance in base composition and hybridization properties between RNA synthesized with DNA or deoxyribonucleoprotein as template it is concluded that permanent repression of a large part of the DNA in yeast deoxyribonucleoprotein is absent.

INTRODUCTION

There is increasing evidence[1–3] that in chromatin or deoxyribonucleoprotein isolated from various cell types of multicellular eukaryotes only a small part of the DNA is available for transcription *in vitro*. Several authors[2–5] have presented data indicating that RNA synthesized with deoxyribonucleoprotein as template closely resembles RNA that is present in the cell nucleus. From this observation it can be concluded that the specific restriction of the template activity of deoxyribonucleoprotein that exists *in vivo* is preserved during isolation. This restriction which, is characteristic for each tissue, is thought to be caused by histones acting as non-specific inhibitors with non histone proteins[4,6] or chromosomal RNA[5,7] having more specific regulatory functions.

It is not clear, however, whether such a situation also exists in unicellular eukaryotes. Previously, we have described the isolation of deoxyribonucleoprotein

Abbreviation: 1 × SSC, 0.15 NaCl–0.015 M trisodium citrate, pH 7.0.

from yeast[8,9]. The histones in this complex are definitely less basic than histones from higher organisms, and resemble similar proteins in *Neurospora crassa*[10] and *Parameciu*m[11]. The chromosomal proteins in yeast stabilize the DNA against thermal denaturation. The template activity of yeast deoxyribonucleoprotein is 50–60 % as compared with deproteinized DNA, when RNA polymerase from *Escherichia coli* is used at high enzyme/template ratios[8].

In a non-differentiating organism such as yeast, there seems to be no need to keep a large part of the DNA permanently in an inactive state. In order to determine if the quantitative suppression of RNA synthesis might be correlated with a qualitative restriction of transcription, we have characterized the RNA synthesized *in vitro* by means of base analysis and DNA–RNA hybridization. The results did not demonstrate any significant difference between RNA synthesized on yeast deoxyribonucleoprotein and yeast DNA, respectively. These results might imply that yeast histones do not function as permanent suppressors of part of the genome.

METHODS

Isolation of yeast deoxyribonucleoprotein

The deoxyribonucleoprotein was isolated essentially as described previously[8]. Yeast cultures in the stationary growth phase (commercial bakers yeast, *Saccharomyces cerevisiae*) were used. 5–20 g yeast was suspended in 0.05 M phosphate buffer pH 6.5 containing 1 mM $MgSO_4$ (phosphate–$MgSO_4$ buffer) and shaken for 5 min with glass beads (diameter 0.25–0.30 mm). The resulting desintegrate was centrifuged for 40 min at 8000 $\times g$ and the supernatant was discarded. The sediment consisted of two layers, a firmly packed bottom layer containing mainly cell walls and a loosely packed upper layer containing nearly all of the DNA. The upper layer was suspended in phosphate–$MgSO_4$ buffer and centrifuged for 20 min at 8000 $\times g$. The sediment was washed twice with phosphate–$MgSO_4$ buffer (20 min 8000 $\times g$), resuspended in 0.05 M Tris–HCl, pH 8.0, containing 5 mM $MgSO_4$ (Tris–$MgSO_4$ buffer), and then centrifuged for 20 min at 20 000 $\times g$. The resulting sediment was homogenized at a concentration of 0.5 mg DNA per ml in Tris–$MgSO_4$ buffer by means of a Potter–Elvehjem type tissue homogenizer with a very close-fitting pestle. 5 ml of the suspension was layered on a discontinous sucrose gradient, consisting of 10 ml 2 M and 10 ml 1.5 M sucrose, both in Tris–$MgSO_4$ buffer. Centrifugation in a Spinco SW25 rotor for 2 h at 22 500 rev/min yielded the DNA containing material as a pellet (purified chromatin).

This pellet was washed twice with 0.15 M NaCl–5 mM EDTA (pH 6.5) (20 min 20 000 $\times g$) and resuspended in 0.2 mM EDTA (pH 8.0) to a concentration of about 0.5 mg DNA per ml. After stirring for 1 h the suspension was homogenized during 2 min in a Bühler homogenizer and centrifuged for 30 min at 50 000 $\times g$. The supernatant, containing about 70 % of the DNA as soluble deoxyribonucleoprotein, was dialyzed overnight against 0.2 mM EDTA (pH 8.0). In some cases the deoxyribonucleoprotein was pelleted by centrifugation for 22 h at 105 000 $\times g$. All operations were performed at 0–4°. The deoxyribonucleoprotein was stored in 0.2 mM EDTA (pH 8.0) in liquid N_2.

Preparation of DNA

Yeast DNA was isolated as described by VAN DER VLIET *et al.*[8]. For the preparation of [^{3}H]DNA yeast cells were grown at 27° in a medium containing 1 % yeast extract and 5 % glucose[12], to which was added 400 μC per l of [2-^{3}H]adenine (spec. act. 1 C/mmole, Philips Duphar N.V., Petten, North Holland). The specific activity of the [^{3}H]DNA was 1250 disint./min per μg.

Single-stranded DNA and double-stranded DNA (RF) of bacteriophage ΦX174 were prepared according to JANSZ *et al.*[13]. 85 % of the RF DNA was in the closed circular duplex form (Component I).

Isolation of RNA

For the isolation of *E. coli* RNA, cells suspended in 0.01 M Tris–HCl (pH 7.4) 0.1 mM $MgSO_4$, to which 10 μg/ml polyvinyl sulphate was added, were disrupted by sonication. The homogenate was made 0.5 % with respect to sodium dodecyl sulfate and an equal volume of phenol containing 0.1 % oxychinoline was then added. After extraction and centrifugation the nucleic acids were precipitated with 2 vol. of 96 % ethanol–2 % potassium acetate. The precipitate was treated with deoxyribonuclease, followed by pronase as described below, after which the phenolextraction and precipitation with ethanol were repeated. The RNA was dissolved in 4×SSC (1×SSC = 0.15 M NaCl–0.015 M trisodium citrate, pH 7.0) and stored at −20°. Nuclear RNA from *Saccharomyces carlsbergensis*, labeled with [^{3}H]methylmethionine, was prepared by the procedure of SILLEVIS SMITT *et al.*[14].

In vitro synthesis and purification of RNA

RNA polymerase (EC 2.7.7.6) was isolated from *E. coli* Q13, lacking ribonuclease I and RNA phosphorylase[15], according to the method of CHAMBERLIN AND BERG[16] up to fraction 4. The specific activity was about 2000 units/mg protein. *In vitro* RNA synthesis with DNA or deoxyribonucleoprotein as template was carried out in 0.04 M Tris–HCl buffer, pH 7.9, containing per ml 4 μmoles $MgCl_2$, 1 μmole $MnCl_2$, 12 μmoles β-mercaptoethanol, 0.4 μmole each of unlabeled CTP, UTP and GTP and 0.4 μmole unlabeled ATP or [8-^{14}C]ATP (spec. act. 1–10 μC/μmole). 40–80 μg DNA or deoxyribonucleoprotein and 400 units RNA polymerase were added per ml, and the reaction mixture was incubated for 20 min at 37°. The reaction was terminated by treating the solution at 100° for 3 min, followed by rapid cooling. 20 μg/ml of deoxyribonuclease (ribonuclease free, Worthington Biochem. Corp., N.J., U.S.A.) was then added, and the mixture was incubated for another 30 min.

For the purification of the RNA the solution was brought to a concentration of 1×SSC, and heated for 3 min at 100°. Sodium dodecyl sulfate was then added to make a final concentration of 1 %. After 5 min at 37° an equal volume of phenol, containing 0.1 % oxychinoline and saturated with SSC, was added. The mixture was shaken for 10 min at 60°, cooled and centrifuged. The phenol phase was extracted once more with a solution of 1 % sodium dodecylsulfate in 1×SSC. The RNA in the combined waterlayers was separated from low molecular weight contamination by gelfiltration on Sephadex G-75 in 0.1×SSC. The peak fractions eluting after the void volume were collected, concentrated to the desired volume and stored at −20°.

The specific activity of the RNA preparations, calculated from the specific activity of [^{14}C]ATP and the base composition of the RNA, was in good agreement

with the measured values when these were corrected for RNA in the enzyme preparation and in the yeast deoxyribonucleoprotein. Characterization of the RNA by centrifugation through a 5–20 % linear sucrose gradient is 0.1 M NaCl–0.01 M sodium acetate, with ribosomal yeast RNA as marker shows it to sediment heterogeneously, with a peak at 8–9 S for RNA, synthesized on yeast DNA, and a peak at 7–8 S for RNA synthesized on yeast deoxyribonucleoprotein.

Base analysis of in vitro synthesized RNA

RNA was synthesized as described above, using as substrate a mixture of the four ribonucleoside triphosphates, each of which was ^{14}C labeled. The specific activity (about 10 μC/μmole) of the ribonucleoside triphosphates was determined after chromatography on Whatman paper No. 1, with isobutyric acid–0.5 M ammonia 10 : 6, v/v) as solvent. The nucleoside triphosphate spots, detected under ultraviolet light, were eluted with 0.01 M HCl (GTP with 0.1 M HCl) after which the concentration was determined spectrophotometrically and the radioactivity was measured.

The reaction mixtures were incubated at 37° for 10 min, 0.5 mg bovine serum albumin was then added, followed by an equal volume of cold 0.5 M $HClO_4$ to terminate the reaction. The precipitated RNA was washed 3 times with cold 0.5 M $HClO_4$ and hydrolyzed in 0.3 M KOH at 37° during the night. After neutralization with $HClO_4$ a mixture of the four 2′(3′)-nucleosidemonophosphates was added as marker, and the pH adjusted to 4–5. The hydrolysate was subjected to electrophoresis on Whatman 3 MM in 0.02 M citric acid–sodium citrate buffer (pH 3.5) during 60 min at 40 V/cm. The nucleotide spots were detected under ultraviolet light, cut out, eluted with 0.01 M HCl and their radioactivity measured.

DNA–RNA hybridization

RNA was hybridized to DNA by the membrane filter technique as described by GILLESPIE AND SPIEGELMAN[17]. Before use, the filters (MF30, Sartorius Membranfilter Gesellschaft, Göttingen), were heated for 10 min at 80° in 4×SSC. The filters were then loaded with heat-denatured DNA using 6×SSC at 4° to prevent renaturation, washed with 20 ml 4×SSC, dried at 4°, and baked for 2 h at 80° *in vacuo*. The retention of DNA on the filter was tested with [^3H]DNA. Annealing was carried out at 60° for 20 h in closed vials containing 0.5 ml 4×SSC–0.2 % sodium dodecyl sulfate[18]. Each vial contained two DNA-loaded filters and one without DNA. A hybridization temperature of 60–65° was found to be optimal, with a minimum of aspecific hybridization. Before the hybridization RNA preparations were heated in 0.1×SSC for 10 min at 80° and rapidly cooled to destroy secondary structure. After annealing the filters were washed on both sides with 100 ml 2×SSC and treated for 1 h at 20° with 5 ml of 2×SSC containing per ml 10 μg pancreatic ribonuclease and 5 units T_1 ribonuclease. The filters were then washed again with 100 ml 2×SSC on both sides, dried and dissolved in 2 ml ethyleneglycol dimethyl ether. After addition of 10 ml of a scintillation mixture, consisting of (toluene–PPO–POPOP)–Triton X-100–water (23 : 6 : 1, v/v/v), [^3H]DNA and [^{14}C]RNA were simultaneously counted in a Nuclear Chicago Mark I liquid scintillation counter. The radioactivity of the blank filters, always less than 0.1 % of the RNA input, was subtracted.

Assay for self-complementarity of the RNA

After self-annealing of the RNA in 4×SSC at 63° the ribonuclease resistance was measured according to BILLETER *et al.*[19]. Samples of RNA (2–4 μg) were incubated at 25° in 2×SSC, containing 4 μg pancreatic ribonuclease and 2 units T_1 ribonuclease per ml. After different periods of time the acid-precipitable radioactivity was measured. Part of the RNA was rapidly degraded, followed by a linear, slow breakdown of the remaining RNA, which was ascribed to degradation of double-stranded RNA[31]. By extrapolating this latter part of the curve to zero time the percentage of ribonuclease-resistant RNA can be calculated, defined as the amount of acid-precipitable RNA after ribonuclease treatment divided by the initial amount of acid-precipitable RNA, ×100 %. The values were corrected for the ribonuclease resistance of RNA that was heated at 80° in 0.01×SSC and rapidly cooled before incubation with ribonuclease.

RESULTS

The base composition of in vitro synthesized RNA

Analysis of the base composition of RNA, synthesized *in vitro* with yeast DNA or yeast deoxyribonucleoprotein as template (Table I), does not reveal significant differences between the two products. The base composition is independent of the ratio of template to enzyme. Even when an excess of template is used, the ratio of synthesized RNA to DNA input being only 0.07, the same base composition is found. There is only a small difference between the G+C content of the RNA and that of the template, indicating the absence of a shift to lower A+U/G+C ratios as observed with rat liver chromatin-primed RNA[20,21].

TABLE I

BASE COMPOSITION OF *in vitro* SYNTHESIZED RNA

Incubation was for 20 min. The ratio of RNA synthesized to DNA varied from 0.85 to 2.90. The values are the average of four separate incubations, each of which was analyzed in triplo.

Template	*RNA*				
	C	*A*	*G*	*U*	*A+U/G+C*
Yeast DNA	17.1±0.52	31.3±0.74	17.7±0.73	33.7±0.77	1.87±0.06
Yeast deoxyribonucleoprotein	16.7±0.47	32.3±0.30	18.6±0.75	32.2±0.75	1.83±0.05
ΦX174 DNA (viral strand)	22.9±0.46	31.4±0.66	20.7±0.98	24.8±0.88	1.29±0.04
ΦX174 DNA (RF)	20.7±0.40	23.6±0.47	25.5±0.36	30.3±0.30	1.17±0.02
	DNA (U = T)				*A+T/G+C*
Yeast (ref. 29)	17.3	31.5	18.5	32.7	1.80
ΦX174 (viral strand) (ref. 30)	19	25	23	33	1.38

In a control experiment, RNA synthesized on single-stranded DNA from coliphage ΦX174 was analyzed. The base composition of the RNA (Table I) appears to be complementary to that of the template, the A+U/G+C ratio being only slight-

ly lower than the theoretical value, in good agreement with the results of COHEN *et al.*[22]. When RNA synthesized with ΦX174-RF DNA as template was analyzed, the product was shown to be non-complementary, with a preferential transcription of the *minus* strand. This indicates that asymmetric transcription occurs in our system if the proper template is used.

Hybridization between DNA and complementary RNA

The RNA was further characterized by DNA–RNA hybridization. To measure the proportion of sequences in DNA which are complementary to the sequences of RNA synthesized on DNA or deoxyribonucleoprotein as template, the method of BISHOP *et al.*[23] was used. Immobilized DNA was annealed with increasing concentrations of cRNA and the RNA/DNA ratio (r) on the filter was assayed. The hybridization values, obtained with relatively moderate RNA to DNA excess, were extrapolated to infinite RNA concentrations. When $1/r$, multiplied by the RNA concentration, is plotted against the RNA concentration, a linear relationship is observed (Fig. 1). The reciprocal slope gives the apparent hybridization at saturating RNA concentrations (r_s). We find for DNA-primed RNA $r_s = 0.45 \pm 0.016$, and for deoxyribonucleoprotein-primed RNA $r_s = 0.42 \pm 0.026$. As the difference between these two values is not signficant it can be concluded that both RNA's saturate DNA up to the same level.

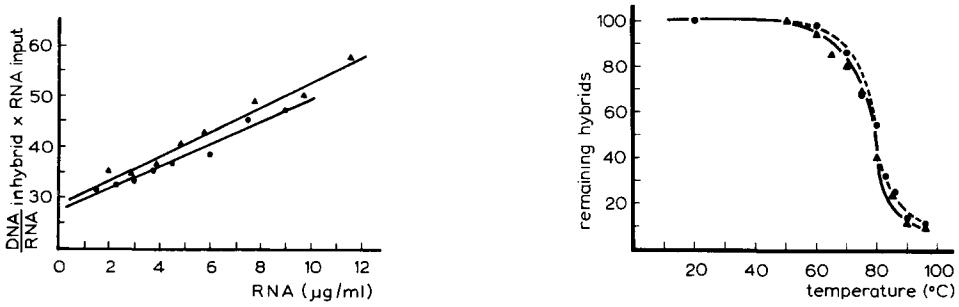


Fig. 1. Analysis of the hybridization between yeast DNA and complementary RNA, synthesized with DNA (●) or deoxyribonucleoprotein (▲) as template. The ordinate shows the ratio of RNA concentration to hybridization. The specific activity of both [^{14}C]RNA's was 53 000 disint./min per μg. Each point is the average value of 3 filters, containing 1.1 μg DNA (corrected for the average of 2 blank filters).

Fig. 2. Thermal stability of the hybrids in 2×SSC. MF30 filters, containing 2 μg of yeast DNA each, were hybridized with RNA synthesized with DNA as template (●, spec. act. 32 000 disint./min per μg, 12 μg/ml) or deoxyribonucleoprotein as template (▲, spec. act. 53 000 disint./min per μg, 10 μg/ml), respectively. After annealing the filters were washed with 2×SSC ,and sets of 2 DNA filters and 1 blank filter were heated for 10 min in 20 ml 2×SSC at the indicated temperatures. The filters were then treated with ribonuclease, washed, dried and counted as described in METHODS.

BISHOP *et al.*[23] concluded from hybridization experiments using RNA isolated from enzymatic hybrids with a known complementarity that the value of r_s in general underestimates the complementarity by a factor 2.0–2.5. Applying the same correction in our system results in a complementarity of at least 80 % between *in vitro* synthesized RNA and yeast DNA. This would mean that a high degree of symmetric transcription takes place, leading to the synthesis of self-complementary RNA.

Self-complementarity in the RNA was assayed by measuring the formation of double-stranded RNA resistant to ribonuclease after self-annealing under various conditions (Table II). Up to 60 % of the complementary RNA became resistant to ribonuclease after annealing for long times. This observation holds for both DNA- and deoxyribonucleoprotein-primed RNA.

TABLE II

RIBONUCLEASE RESISTANCE OF *in vitro* SYNTHESIZED RNA AFTER SELF-ANNEALING UNDER VARIOUS CONDITIONS

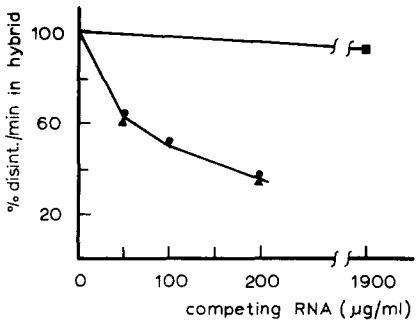
For details of the analysis see METHODS

Template for RNA synthesis	*Concn. of RNA (μg/ml)*	*Annealing time (h)*	*Percentage of ribonuclease resistant RNA*
Yeast DNA	12.4	21	28.7
		197	46.9
	24.7	21	36.6
		197	60.4
Yeast deoxyribonucleoprotein	10.7	21	26.1
		197	55.9

The degree of correct base pairing in the hybrids was tested by determining thermal stability in the following way. After annealing, the hybrid-containing membranes were washed and heated for 10 min at various temperatures in 2×SSC. After treatment with ribonuclease, the amount of hybrid remaining on the filters was measured. It can be seen (Fig. 2) that both hybrids melt over the same temperature range, with a T_m of 78.5°. This is 11° below the T_m of native yeast DNA, which amounts to 89.5° in 2×SSC. The lower T_m of the hybrids is of the same order of magnitude as has been found with cRNA of T_4[24] and mRNA from sea urchin eggs[25]. Based on the relation of LAIRD *et al.*[26], that 1 % uncorrect base pairing reduces the T_m 0.7°, the hybrid should contain 16 % mispaired bases. However, since enzymatic hybrids, in which a high degree of correct base pairing is to be expected, also melt at lower temperatures than native DNA, it is not sure that mismatching is the only explanation for the reduction in thermal stability of the hybrids. Another explanation could be a lower thermal stability, of mixed ribose–deoxyribose homoduplexes, compared to the corresponding ones containing only deoxyribose. The low molecular weight of cRNA might only explain a small part of the reduced thermal stability of the hybrids, as the average RNA chain length amounts to 300 nucleotides[32].

Competition hybridization

A high level of DNA saturation by complementary RNA, synthesized on deoxyribonucleoprotein or DNA, suggests a large homology between both kinds of RNA products. To test the degree of homology, labeled cRNA, synthesized on yeast DNA, was hybridized with DNA in the presence of increasing amounts of unlabeled *in vitro* synthesized RNA primed by deoxyribonucleoprotein or DNA (Fig. 3). Both RNA products compete for the DNA sites to the same extent. The experimental values are in agreement with the theoretical expectations for competition with



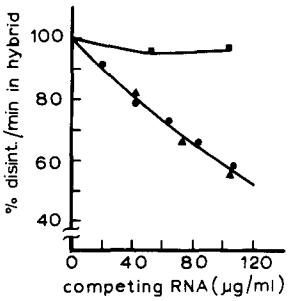


Fig. 3. Competition hybridization. ^{14}C-labeled complementary RNA, primed by DNA, (100 μg/ml) was hybridized with 0.8 μg DNA in the presence of increasing concentrations of unlabeled *E. coli* RNA (■), RNA synthesized with deoxyribonucleoprotein (▲) or DNA (●) as template. 100 % = 11 200 disint./min. Corrections were made for the increase in hybridization caused by the higher RNA input in the case of added competitor.

Fig. 4. Comparison of a natural yeast RNA with *in vitro* synthesized RNA by means of competition hybridization. Nuclear RNA from *S. Carlsbergensis* (3.5 μg), labeled with [*Me*-^3H]methionine, (spec. act. 26 117 disint./min per μg) was annealed with 20 μg DNA in the presence of increasing amounts of unlabeled, *in vitro* synthesized RNA, primed by deoxyribonucleoprotein (▲), yeast DNA (●) or ΦX174 DNA (■). 100 % = 1147 disint./min.

homologous RNA. No competition occurs with *E. coli* RNA. The results suggest a high degree of sequence homology between the transcription products of the DNA and deoxyribonucleoprotein.

To compare natural yeast RNA and cRNA, a nuclear RNA fraction from *S. carlsbergensis* was used. The fraction was labeled with [*Me*-^3H]methionine and contained more than 90 % ribosomal RNA and precursor rRNA, according to polyacrylamide electrophoresis. At saturating concentrations this RNA hybridized with 2.4 % of DNA from *S. cerevisiae*, reaching a plateau at a ratio RNA/DNA = 0.15. When 3.5 μg of this natural RNA was hybridized with 20 μg DNA in the presence of increasing concentrations of *in vitro* synthesized RNA (Fig. 4), yeast DNA- and deoxyribonucleoprotein-primed products both competed with the same efficiency. RNA transcribed from a heterologous source (ΦX174 phage DNA) did not compete. 33 % competition was obtained when a 22-fold excess of competitor yeast RNA was added (77 μg/ml), indicating that 2.3 % of the *in vitro* product shows sequence homology with the nuclear RNA fraction. Comparing this value with the hybridization plateau of 2.4 % indicates that the rRNA genes are transcribed *in vitro* to about the same extent as the other parts of the genome.

DISCUSSION

Deoxyribonucleoprotein prepared from the yeast *S. cerevisiae* acts as a template for DNA-dependent RNA synthesis in the presence of *E. coli* RNA polymerase. Compared to purified yeast DNA, the deoxyribonucleoprotein exhibits a lower template activity (50–60 %)[8]. This decrease in template activity may be due to an inaccessibility of a specific part of the deoxyribonucleoprotein to RNA polymerase or to a decrease in the efficiency of transcription of all of the DNA.

To discriminate between these two possibilities we have characterized the RNA synthesized *in vitro* on yeast DNA or yeast deoxyribonucleoprotein as template by means of base analysis and DNA–RNA hybridization. Both kinds of RNA appear to have the same base composition and hybridize with yeast DNA to the same extent, reaching a saturation value of at least 40 % upon extrapolation. The absence of difference in hybridization behaviour between both kinds of RNA, together with the high saturation values, suggest that the decreased template activity of yeast deoxyribonucleoprotein is not due to a complete repression of a specific part of the DNA. This notion is affirmed by the results of hybridization competition (Fig. 3). RNA transcribed from deoxyribonucleoprotein competes on an equal basis with RNA transcribed from pure yeast DNA for cRNA as well as for a nuclear RNA fraction. This indicates a high degree of sequence homology between the two transcription products. It also suggests that all of the DNA sequences are equally well transcribed *in vitro*, regardless of whether DNA or deoxyribonucleoprotein is used as the template. From the base composition of the *in vitro* synthesized RNA and from the occurence of self-annealing in our hybridization experiments, it can be concluded that the transcription of yeast DNA and deoxyribonucleoprotein by *E. coli* RNA polymerase is symmetric to a large extent. The symmetric transcription observed with templates from yeast may be inherent to the heterologous system as in our system double-stranded (RF) DNA from coliphage ΦX174 is transcribed asymmetrically. The specific restriction of transcription with deoxyribonucleoprotein from higher eukaryotes is observed with homologous RNA polymerase and with RNA polymerase from *E. coli* as well[2]. It is therefore unlikely that the lack of strand-selective transcription in our system will invalidate our conclusion that there is no complete repression of a specific part of the DNA in yeast deoxyribonucleoprotein.

In a previous publication[8] we reported that the chromosomal proteins in yeast deoxyribonucleoprotein affect the thermal denaturation and sedimentation properties of DNA in much the same way as do mammalian histones. The present results indicate that the chromosomal proteins in yeast deoxyribonucleoprotein depress the template activity of the DNA, not by blocking the transcription of a specific part of the DNA, but by lowering the efficiency of transcription of all of the DNA. It is now generally accepted that the organ-specific restriction of transcription in chromatin from plants and animals plays a decisive role in the determination of cell differentiation. The absence of complete repression of a specific part of the DNA in yeast deoxyribonucleoprotein may be related to the fact that yeast is a eukaryotic protist, which does not differentiate. In this connection it is worthwhile to mention that yeast histones are considerably less basic than histones from higher eukaryotes and that a very-lysine-rich type of histone is lacking in yeast[9]. The very-lysine-rich histone fraction (F_1) is believed to be involved in the condensation of chromatin fibers into more compact structures (metaphase chromosomes, heterochromatin). Such condensed chromatin masses are generally correlated with genetic inactivity. According to GEORGIEV *et al.*[27,28] removal of F_1-histone from mammalian deoxyribonucleoprotein leads to a complete loss of the specific restriction of transcription, although the template activity of the remaining partial deoxyribonucleoprotein is still lower than that of protein-free DNA. The template properties of yeast deoxyribonucleoprotein thus resemble those of mammalian deoxyribonucleoprotein from which the F_1-histone is removed.

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REFERENCES

1 J. PAUL AND R. S. GILMOUR, *J. Mol. Biol.*, 16 (1966) 242.
2 K. D. SMITH, R. B. CHURCH AND B. J. MC CARTHY, *Biochemistry*, 8 (1969) 4271.
3 C. H. TAN AND M. MIYAGI, *J. Mol. Biol.*, 50 (1970) 641.
4 J. PAUL AND R. S. GILMOUR, *J. Mol. Biol.*, 34 (1968) 305.
5 I. BEKHOR, G. M. KUNG AND J. BONNER, *J. Mol. Biol.*, 39 (1969) 351.
6 R. S. GILMOUR AND J. PAUL, *FEBS Lett.*, 9 (1970) 242.
7 R. S. HUANG AND P. E. HUANG, *J. Mol. Biol.*, 39 (1969) 365.
8 P. CH. VAN DER VLIET, G. J. M. TONINO AND TH. H. ROZIJN, *Biochim. Biophys. Acta*, 195 (1969) 473.
9 G. J. M. TONINO AND TH. H. ROZIJN, *Biochim. Biophys. Acta*, 124 (1966) 427.
10 R. S. DWIVEDI, K. DUTTA AND D. P. BLOCH, *J. Cell Biol.*, 43 (1969) 51.
11 R. E. ISAACKS, B. G. SANTOS AND W. J. VAN WAGTENDONK, *Fed. Proc.*, 29 (1970) nr. 3658.
12 H. FUKUHARA, *Proc. Natl. Acad. Sci. U.S.*, 58 (1967) 1065.
13 H. S. JANSZ, P. H. POUWELS AND J. SCHIPHORST, *Biochim. Biophys. Acta*, 123 (1966) 626.
14 W. W. SILLEVIS SMITT, G. NANNI, TH. H. ROZIJN AND G. J. M. TONINO, *Exp. Cell Res.*, 59 (1970) 440.
15 I. HARUNA AND S. SPIEGELMAN, *Proc. Natl. Acad. Sci. U.S.*, 54 (1965) 579.
16 M. CHAMBERLIN AND P. BERG, *Proc. Natl. Acad. Sci. U.S.*, 48 (1962) 81.
17 D. GILLESPIE AND S. SPIEGELMAN, *J. Mol. Biol.*, 12 (1965) 829.
18 H. O. HALVORSON AND W. S. VINCENT, *Exp. Cell Res.*, 57 (1969) 240.
19 M. A. BILLETER, R. C. WARNER AND C. WEISSMANN, *J. Mol. Biol.*, 17 (1966) 145.
20 K. MARUSHIGE AND J. BONNER, *J. Mol. Biol.*, 15 (1966) 160.
21 P. CHAMBON, H. KARON, M. RAMUZ AND P. MANDEL, *Biochim. Biophys. Acta*, 157 (1968) 520.
22 J. A. COHEN, I. V. D. SLUYS AND S. O. WARNAAR in V. V. KONINGSBERGER AND L. BOSCH, *Regulation of Nucleic Acid and Protein Biosynthesis*, B.B.A.Library, Vol. 10, Elsevier, Amsterdam, 1967, p. 98.
23 J. O. BISHOP, F. O. FOBERSTON, J. A. BURNS AND M. MELLI, *Biochem. J.*, 115 (1969) 361.
24 J. O. BISHOP, *Biochim. Biophys. Acta*, 174 (1969) 636.
25 M. EIGNER AND H. DENIS, *Biochim. Biophys. Acta*, 204 (1970) 617.
26 C. D. LAIRD, B. L. MCCONAUGHTHY AND B. J. MCCARTHY, *Nature*, 224 (1969) 149.
27 G. P. GEORGIEV, L. N. ANANIEVA AND Y. V. KOZLOZ, *J. Mol. Biol.*, 22 (1966) 365.
28 Y. V. KOZLOZ AND G. P. GEORGIEV, *Nature*, 228 (1970) 245.
29 E. CHARGAFF AND H. S. SHAPIRO, *Proc. Natl. Acad. Sci. U.S.*, 59 (1968) 161.
30 R. L. SINSHEIMER, *J. Mol. Biol.*, 1 (1959) 43.
31 H. F. TABAK AND P. BORST, *Biochim. Biophys. Acta*, 217 (1970) 356.
32 P. CH. VAN DER VLIET, Thesis, Utrecht 1971, p. 52.