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Phosphorylation of RNA Polymerases: Specific Association of Protein Kinase NII with RNA Polymerase I [and Discussion]

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Phil. Trans. R. Soc. Lond. B 1983 **302**, 135-142
doi: 10.1098/rstb.1983.0046

Phil. Trans. R. Soc. Lond. B **302**, 135–142 (1983) [135]
Printed in Great Britain

Phosphorylation of RNA polymerases: specific association of protein kinase NII with RNA polymerase I

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The activities of the three DNA-dependent RNA polymerases from a rapidly growing rat tumour, Morris hepatoma 3924A, and from rat liver were examined. The activity of RNA polymerase I was higher in the tumour than in the liver. The enhanced capacity for RNA synthesis was a result of a higher concentration of polymerase I in the tumour as well as of an activation of this enzyme *in vivo*. The possibility that the high specific activity of the hepatoma polymerase I resulted from phosphorylation was investigated. Two major cyclic-AMP-independent nuclear casein kinases (NI and NII) were identified; the activity of protein kinase NII in the tumour was ten times that in liver. Protein kinase NII was capable of activating and phosphorylating RNA polymerase I *in vitro*. This kinase could also stimulate RNA polymerase II activity, although to a lesser extent than RNA polymerase I. RNA polymerase III was not affected by protein kinase NII. Protein kinase NII was tightly associated with polymerase I and was found even in purified preparations of the polymerase. Antibodies against both RNA polymerase I and protein kinase NII were present in sera of patients with certain rheumatic autoimmune diseases. These results imply that RNA polymerase I and protein kinase NII are in close association *in vivo* as well as *in vitro* and that polymerase phosphorylation may regulate the rate of ribosomal RNA synthesis in the cell.

Introduction

The first step in the process by which cells convert their genetic information to protein is the transcription of DNA into RNA. The formation of RNA is a complex event that involves initiation of RNA synthesis at the appropriate site on the template, elongation of the RNA molecule and accurate termination of the nascent RNA. These steps are accomplished by the DNA-dependent RNA polymerases (EC 2.7.7.6) acting in concert with a variety of regulatory proteins. In eukaryotes there are three structurally distinct RNA polymerases responsible for the synthesis of different classes of RNA. Specifically, ribosomal, messenger and transfer RNAs are synthesized by RNA polymerases I, II and III, respectively. Although the structure and function of the polymerases have been known for almost a decade, little information is available on how the cell regulates the production of the specific RNAs. It is becoming increasingly clear that the synthesis of mRNAs and tRNAs is controlled, not at the level of the polymerases themselves, but rather by unique 'transcription factors' (for a review see Rose *et al.* 1983*b*). Although less information is available about the production of rRNA it is apparent that the activity of RNA polymerase I *in vitro* correlates well with the rate of rRNA synthesis *in vivo* (reviewed in Jacob & Rose 1978, 1980). Thus the enzyme itself may be one of the controlling elements in the synthesis of this important class of RNA. It was with this thought in mind that we (S.T.J. and K.M.R.) set out, several years ago, to determine whether RNA polymerase I was the rate-limiting factor in rRNA synthesis and, if so, what cellular constituents regulate the activity of this enzyme.

Results and discussion

Relative RNA polymerase activity in liver and hepatoma 3924A

One of the hallmarks of growing cells, and neoplastic cells in particular, is their rapid rate of rRNA synthesis relative to non-dividing cells. If RNA polymerase I were a critical factor in rRNA production, it would be expected that the activity of this enzyme would be higher in the dividing cell than in the resting cell. This possibility was investigated in detail by comparing

Table 1. Distribution of RNA polymerase activity in nuclei and cytoplasm of hepatoma 3924A and rat liver

(After homogenization in hypertonic sucrose, nuclei of liver and hepatoma 3924A were obtained by centrifugation (Rose *et al.* 1976). 'Cytosol' was prepared after dilution of the nuclear supernatant and removal of cellular organelle by high-speed centrifugation. Extracts were prepared and subjected to column chromatography on DEAE-Sephadex (Rose *et al.* 1976; Lin *et al.* 1976). One unit equalled 1 nmol UMP incorporated into RNA in 20 min at 30 °C. Data for liver were adapted from Lin *et al.* (1976).)

		RNA polymerase activity		
		I	II	III
tissue	cellular fraction	units mg^{-1} DNA equivalent (%)	units mg^{-1} DNA equivalent (%)	units mg^{-1} DNA equivalent (%)
hepatoma	nuclei	3.71 (92)	0.68 (66)	0.07 (37)
	cytosol	0.32 (8)	0.34 (34)	0.13 (63)
liver	nuclei	0.21 (84)	0.73 (64)	0.16 (58)
	cytosol	0.04 (16)	0.41 (36)	0.11 (42)

the RNA polymerase I activity from a rat hepatoma with that from normal adult rat liver. Indeed, initial studies demonstrated that the total activity of RNA polymerase I partly purified from nuclei of the rapidly growing Morris hepatoma, 3924A, was four to eight times greater (per milligram of DNA), than that obtained from rat liver (Rose *et al.* 1976).

Eukaryotic RNA polymerases exist in two states, one that is tightly complexed with the template and, presumably, actively engaged in RNA synthesis ('bound' or 'engaged' form), and one that is either 'free' or loosely bound to the template. In some cells there is an excess of 'free' enzyme. After appropriate stimulation this polymerase population becomes engaged in RNA synthesis (see Jacob & Rose 1978). In this context, then, it was important to determine whether the elevated activity of hepatoma nuclear RNA polymerase I was due to an increase in total RNA synthesizing capacity or was merely a reflection of a redistributed enzyme population. For this purpose RNA polymerases of liver and hepatoma 3924A were extracted from the postnuclear fraction as well as from the nuclei and separated by chromatography on DEAE-Sephadex. After chromatography, RNA polymerases I, II and III were pooled and analysed under optimal conditions. These data are presented in table 1. The most striking difference between the tissues was the total activity of RNA polymerase I. Based on tissue mass, 33 times more activity was obtained from the tumour than from the liver. Even when expressed per unit of DNA (a hepatoma 3924A cell has 73 chromosomes whereas a liver cell has 42), the total activity of RNA polymerase I in the tumour was still more than 15 times greater than that in the liver. Not only was there more RNA polymerase I in the tumour, but a greater proportion (92 %) of this enzyme was in the nucleus in tumour cells than in liver cells (43 %). Hence, although there is a smaller percentage of 'free' polymerase I in the hepatoma, redistribution of the enzyme does not account for the increased nuclear activity in the tumour. In

contrast to RNA polymerase I, there was little difference between the tissues with regard to total activity or cellular distribution of RNA polymerases II and III.

To determine whether the increased polymerase I activity in the hepatoma was due to a higher enzyme concentration or to enhanced polymerase activity, three approaches were taken. First, RNA polymerase I was purified to essential homogeneity from both hepatoma and liver nuclei. Not only was more enzyme (as mass of pure protein) recovered from the tumour than from liver, but the former's specific activity was greater (Rose *et al.* 1981 *b*). Second, the number of RNA polymerase I molecules in the liver and hepatoma was estimated by determining the number of nascent RNA chains under conditions of RNA synthesis that produce no more than one RNA chain per polymerase molecule. These measurements also suggested that there were more enzyme molecules in the tumour and that each enzyme was more active (Duceman & Jacob 1980). Finally, the amount of RNA polymerase I in the two tissues was estimated by immunological methods. These data indicated that the fourfold to sixfold higher concentration of enzyme in the tumour was not sufficient to account for the differences in total activity between the tissues (Rose & Jacob, in preparation).

Relative protein kinase activity in nuclei of liver and hepatoma 3924*A*

The observations described in the previous section suggested that the polymerase I from the hepatoma was activated *in vivo*. We next explored the mechanism by which this polymerase might be activated. There have been many reports that gene expression may be regulated by protein phosphorylation and that cyclic-AMP-independent protein kinases (casein kinases) appear to be responsible for the phosphorylation of non-histone chromatin proteins (see Krebs & Beavo 1979). Since, by definition, RNA polymerase I is a non-histone nuclear protein, it was of interest to investigate the characteristics of the casein kinases in nuclei of liver and hepatoma 3924A and delineate any putative role that these kinases may play in modifying polymerase I. Initial studies employing isolated nuclei from the two tissues suggested that there was more total casein kinase activity in the tumour than in the liver. In order to characterize these kinases, nuclear extracts were prepared and fractionated by chromatography on DEAE-Sephadex. Two major peaks of casein kinase activity were resolved by this procedure. One of these activities was not retained on the column and the other eluted from the column at approximately 0.1 M $(NH_4)_2SO_4$. In keeping with the nomenclature of Desjardins *et al.* (1972), these kinases were referred to as nuclear protein kinases NI and NII, respectively. Measurements of the relative activity in liver and hepatoma 3924A demonstrated that, on a tissue mass basis, activities of protein kinases NI and NII were three to four times and thirty to forty times higher, respectively, in the hepatoma than in the liver (Rose *et al.* 1983 *a*). Even when expressed on a DNA basis (table 2), the NII kinase activity was twelve times greater in the tumour than in the liver. This twelvefold increase in the hepatoma enzyme relative to liver persisted through several purification steps (Rose *et al.* 1981 *a*).

Since the activities of protein kinase NII and RNA polymerase I were higher to a similar extent in the hepatoma, it seemed possible that the NII kinase may play a role in polymerase I modification. For this hypothesis to be viable it was important to determine whether protein kinase NII was associated with the nucleolus, the site of rRNA synthesis and the subcellular location of RNA polymerase I. Liver nuclei and nucleoli were therefore prepared and analysed for GTP-dependent protein kinase activity. (Protein kinase NII is the sole nuclear casein kinase with the ability to use GTP as phosphoryl donor (see Rose *et al.* 1981 *a*).) As shown in table 3,

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on a DNA basis, there was almost ten times more GTP-dependent protein kinase activity associated with nucleolar DNA than with the nuclear DNA. This observation suggested that the relative concentration of this enzyme was substantially greater in nucleoli than in total nuclei. In fact, if the data in table 3 are computed on a nuclear basis (assuming 10 pg DNA per nucleus and that nucleolar DNA comprises 5 % of the nuclear DNA), it is evident that almost half of the nuclear NII kinase is located in the nucleolus.

TABLE 2. CASEIN KINASES FROM NUCLEI OF RAT LIVER AND HEPATOMA 3924A

(Nuclear extracts were prepared from liver and hepatoma 3924A and subjected to chromatography on DEAE-Sephadex as described by Rose *et al.* (1976) and in the legend to table 1. Protein kinase activity was measured by using [γ^{32}P]ATP and casein as described (Rose *et al.* 1981*a*). Results are the average of triplicate determinations from two separate experiments.)

	protein kinase activity (pmol phosphate (15 min)$^{-1}$ mg^{-1} DNA equivalent)	
source	NI	NII
hepatoma	700	530
liver	488	40

TABLE 3. GTP-DEPENDENT PROTEIN KINASE ACTIVITY IN NUCLEAR AND NUCLEOLAR CHROMATIN PREPARATIONS

(Rat liver was homogenized in 2.4 M sucrose containing 15 mM $MgCl_2$ and 0.25 mM spermine and nuclei prepared by differential centrifugation. Nuclei were washed once by gentle resuspension in buffer containing 10 mM Tris-Cl (pH 8) and 25 % glycerol and recovered by centrifugation. Nucleoli were prepared by sonication of isolated nuclei (resuspended in 0.34 M sucrose) followed by centrifugation through 0.88 M sucrose. Protein kinase NII was assayed essentially as described previously (Rose *et al.* 1981*a*) by using [γ^{32}P]GTP (56 μM), casein (2 mg ml^{-1}) and $MgCl_2$ (5 mM). Reactions, performed in triplicate with several concentrations of nuclei or nucleoli, were incubated for 5 min (nuclei) or 10 min (nucleoli) at 30 °C. Results, expressed per milligram DNA per minute, are the means ± s.e.m. Data were also computed per nucleus assuming 10 pg DNA per liver nucleus and that nucleolar DNA represented 5 % of the total nuclear DNA.)

fraction	kinase activity (pmol phosphate min^{-1} mg^{-1} organelle DNA)	kinase activity (10^{-7} pmol phosphate min^{-1} per nucleus)
nuclei	34 ± 4	3.4
nucleoli	300 ± 26	1.5

Since hepatoma 3924A nuclei contain significantly more protein kinase NII than liver nuclei do, it was of interest to determine whether the activity of this kinase was also enhanced in nucleoli isolated from the tumour. Unfortunately, nuclei from hepatoma 3924A are very heterogeneous in size and nucleoli isolated from this tumour were consistently contaminated with small unbroken nuclei. However, despite the presence of extranucleolar material, the 'nucleolar-enriched' fraction from the tumour (about 30 % nucleoli as estimated by light microscopy), had two to three times more GTP-dependent protein kinase activity per milligram of DNA than the pure nucleolar preparation from the liver (not shown).

Activation of RNA polymerases

If protein kinase NII were responsible for modifying the activity of RNA polymerase *in vivo* it might be expected that this kinase could also affect RNA synthesis *in vitro*. Typically, when protein kinase NII was added to polymerase I transcription systems, depending on the amount

of kinase and the time of incubation, a twofold to eightfold stimulation of RNA polymerase I activity was observed (Duceman *et al.* 1981). The elevation in RNA synthesis was not obtained when Mn^{2+} replaced Mg^{2+} (the divalent cation preferred by protein kinase NII) nor when 5′-adenylylimidodiphosphate and 5′-guanylylimidodiphosphate replaced ATP and GTP in the assay (Rose *et al.* 1983*a*). The requirement for a hydrolysable bond between the β and γ

TABLE 4. EFFECT OF PROTEIN KINASE ON RNA SYNTHESIS

(RNA polymerases I (Rose *et al.* 1981*b*), II (Stetler & Rose 1982) and III (Stetler & Rose, in prep.) were purified to essential homogeneity and RNA synthesis measured essentially as described (Duceman *et al.* 1981), in the absence and presence of optimal amounts of protein kinase NII. It should be noted that stimulation by protein kinase NII was dependent on the time of incubation as well as on the relative concentrations of polymerase and kinase. In general, activation of polymerase II reached a plateau at 30 min whereas that of polymerase I was still increasing at 90 min.)

RNA polymerase	activity (pmol UMP (30 min)$^{-1}$)		
	−kinase	+kinase	+kinase/−kinase
I	1.7 ± 0.7	6.4 ± 1.1	3.8
II	5.0 ± 0.2	12.4 ± 0.4	2.5
III	10.9 ± 0.1	11.3 ± 0.6	1.0

nucleotide phosphate indicated that activation of polymerase was mediated by protein phosphorylation. Because both the polymerase I and the kinase were highly purified, the enhanced RNA synthesis most probably involved phosphorylation of the polymerase itself rather than of other intermediary proteins. Further, because addition of protein kinase NI had no effect on RNA synthesis, phosphorylation of RNA polymerase I was presumably specific for the NII casein kinase (Duceman *et al.* 1981; Rose *et al.* 1983*a*).

We also investigated whether protein kinase NII altered the activity of the other RNA polymerases. For this purpose, RNA polymerases II (Stetler & Rose 1982) and III (Stetler & Rose, in prep.) were purified to essential homogeneity and RNA synthesis measured in the absence and presence of the protein kinase. As indicated in table 4, protein kinase NII enhanced RNA synthesis catalysed by polymerases I and II. Although optimal concentrations of kinase resulted in a threefold to fourfold stimulation of polymerase I activity, similar levels rarely yielded more than a doubling of polymerase II-catalysed RNA synthesis. Further, the activation of polymerase I was more prevalent after prolonged incubation (Duceman *et al.* 1981), whereas the stimulation of polymerase II reached a plateau after 30 min (Stetler & Rose 1982). Although the extent of stimulation of polymerase II by protein kinase NII was not as great as that of polymerase I, the activation did require hydrolysable phosphate at the terminal nucleotide phosphate (Stetler & Rose 1982). In contrast to RNA polymerases I and II, the activity of polymerase III was not affected by addition of protein kinase NII.

Phosphorylation of RNA polymerase polypeptides

That protein kinase NII actually phosphorylated RNA polymerase I was confirmed by incubating the two enzymes in the presence of [γ^{32}P]ATP and $MgCl_2$ and separating RNA polymerase I from the exogenous kinase by sucrose gradient centrifugation in the presence of 0.3 M KCl. Acid-precipitable ^{32}P was associated with the reisolated polymerase; analysis after acid hydrolysis demonstrated the presence of radioactive phosphate attached to serine and threonine (Duceman *et al.* 1981).

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Since hepatoma RNA polymerase I consists of several (eight) polypeptides, it was of interest to determine which of the enzyme-associated polypeptides were modified by protein kinase NII. Polyacrylamide gel electrophoresis under denaturing conditions indicated that polymerase I polypeptides of M_r 120000, 65000 and 25000 were phosphorylated *in vitro* by NII protein kinase (Rose *et al.* 1981*c*). Protein kinase NII itself consists of two polypeptides, M_r 42000 and 25000. *In vitro* this kinase autophosphorylates the smaller subunit (Rose *et al.* 1981*a*). It has been observed that protein kinase NII is associated with the essentially pure hepatoma RNA polymerase I and that this kinase accounts for two polypeptides (M_r 42000 and 25000) of the polymerase. Thus, owing to the presence of associated kinase activity, polymerase I can 'autophosphorylate'. The same polypeptides that are modified by excess exogenous protein kinase NII are also phosphorylated (to a lesser extent) in the absence of the NII kinase. In contrast to its affinity for RNA polymerase I, protein kinase NII is easily dissociated from RNA polymerase II (Stetler & Rose 1983), and no autophosphorylation of this polymerase can be detected.

TABLE 5. ANTI-RNA POLYMERASE I ANTIBODIES IN HUMAN SERA

(Sera were obtained from patients with SLE and MCTD as well as from others (15 normal individuals and 15 with cancer). Antibodies reacting with native RNA polymerase I were detected by solid-phase radioimmunoassay (Stetler *et al.* 1982). Individual polymerase I polypeptides were isolated by polyacrylamide gel electrophoresis, and antibodies reacting with each subunit were quantitated by radioimmunoassay (Stetler *et al.* 1982). Values are the percentages of sera tested that were capable of forming immune complexes with native enzyme or with a given polypeptide.)

	antigen						
	native RNA	individual polymerase I polypeptide/kDa					
diagnosis	polymerase I	190	120	65	42	25	18–21
SLE	100 (n = 18)	0	70	100	0	60	0 (n = 10)
MCTD	100 (n = 4)	0	0	67	100	50	0 (n = 4)
others	0 (n = 30)	0	0	0	0	0	0 (n = 3)

Production of anti-RNA polymerase I antibodies by humans with autoimmune diseases

The ability of protein kinase NII to phosphorylate RNA polymerase I and the close association of these two enzyme activities *in vitro* raised the possibility that there may also be a relation between these proteins *in vivo*. If a biological system capable of responding to RNA polymerase I were available and such a system also responded to protein kinase NII, a correlation between these two enzymes could be established. Since human rheumatic autoimmune diseases are characterized by the production of antibodies against nuclear and, in many instances, nucleolar antigens, it was of interest to ascertain whether sera from individuals with these diseases contained antibodies to RNA polymerase I or protein kinase NII, or both. Therefore sera from individuals with two types of rheumatic autoimmune diseases, systemic lupus erythematosus (SLE) and mixed connective tissue disease (MCTD), were examined for anti-RNA polymerase I antibodies. As indicated in table 5, all patients with SLE (n = 18) and MCTD (n = 4) had anti-RNA polymerase I antibodies. Neither normal (n = 15) individuals nor patients with cancer (n = 15) had antibodies against RNA polymerase I (although some of the latter individuals had circulating antibodies against defined tumour antigens). Hence the production of RNA polymerase I antibodies was correlated with the human autoimmune diseases (SLE and MCTD) that commonly have high titres of antinuclear antibodies. It should

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be noted that although 70 % of individuals with rheumatoid arthritis also had detectable anti-RNA polymerase I antibodies, the average 'titre' of these antibodies was only 14 % of that observed in individuals with MCTD (Stetler *et al.* 1982).

The presence of antibodies against protein kinase NII in sera of individuals with SLE and MCTD was next determined. Because the NII kinase subunits account for two of the polymerase polypeptides (M_r 42000 and 25000), the presence of antibodies against the kinase was first investigated by analysis of immune complexes with individual polymerase I polypeptides. As indicated in table 5, all SLE patients had antibodies against the polymerase I polypeptide of M_r 65000, in combination with antibodies against polypeptides of M_r 120000 or 25000, or both. Of interest relative to kinase polypeptides was the observation that 60 % of the SLE patients had antibodies against the 25000 Da polypeptide of the polymerase in addition to antibodies against the 65000 Da polypeptide. These data indicated that, in SLE, the production of anti-NII kinase antibodies was strongly correlated with the production of antipolymerase I antibodies.

We next examined the sera from individuals with MCTD. All (n = 4) of the MCTD patients had antibodies against one of the kinase-associated polymerase I polypeptides, that of M_r 42000. Since 75 % of these patients also had antibodies against the 65000 Da polypeptide, the production of antibodies reacting with the 65000 Da polypeptide was strongly correlated with the production of antibodies against the 42000 Da polypeptide. Interestingly, two of the individuals with MCTD also had antibodies against the 25000 Da polypeptide. That antibodies interacting with the 42000 and 25000 Da polypeptides of the polymerase I were actually anti-kinase antibodies was confirmed by demonstrating the ability of these sera to interact with the purified kinase itself (Stetler, Rose & Jacob, in prep.). Hence it is clear that a large proportion of individuals with the rheumatic autoimmune diseases SLE and MCTD can produce antibodies against both RNA polymerase I and protein kinase NII. It should be noted that none of the individuals with SLE or MCTD so far tested have had antibodies against the polymerase polypeptides of M_r 190000 or less than M_r 21000. Thus antibodies appear to be produced against only the polymerase I subunits that are associated with kinase activity (M_r 42000 and 25000) or that are phosphoryl acceptors (M_r 120000 and 65000) for this kinase. It should also be mentioned that individuals with SLE or MCTD are not significantly different from control individuals with respect to antibodies reacting with RNA polymerase II. Hence there is a strong and specific correlation between anti-kinase NII and anti-polymerase I antibody production in the two human rheumatic autoimmune diseases. These observations, coupled with the ability of protein kinase NII to phosphorylate and activate RNA polymerase I *in vitro*, suggest that there is a biological association between these two enzymes and imply that phosphorylation may play an important role in the regulation of ribosomal gene transcription.

The authors acknowledge Dr Cheston Berlin and Dr Marlin Wenger for providing samples of human sera. This work was supported in part by grants no. GM26740 (K.M.R.) and no. CA25078 and CA31894 (S.T.J.) from the U.S.P.H.S. K.M.R. is the recipient of a Research Career Development Award from the National Cancer Institute.

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References

Desjardins, P. R., Lue, P. F., Liew, C. C. & Gornall, A. G. 1972 Purification and properties of rat liver nuclear protein kinases. *Can. J. Biochem.* **50**, 1249–1259.

Duceman, B. W. & Jacob, S. T. 1980 Trancriptionally active RNA polymerases from Morris hepatomas and rat liver. Elucidation of the mechanism for the preferential increase in the tumour RNA polymerase I. *Biochem. J.* **190**, 781–789.

Duceman, B. W., Rose, K. M. & Jacob, S. T. 1981 Activation of purified hepatoma RNA polymerase I by homologous protein kinase NII. *J. biol. Chem.* **256**, 10755–10758.

Jacob, S. T. & Rose, K. M. 1978 RNA polymerases and poly(A) polymerase from neoplastic tissues and cells. *Methods Cancer Res.* **14**, 191–241.

Jacob, S. T. & Rose, K. M. 1980 Basic enzymology of transcription in prokaryotes and eukaryotes. In *Cell biology: a comprehensive treatise* (ed. L. Goldstein & D. M. Prescott), vol. 3, pp. 113–152. New York: Academic Press.

Krebs, E. G. & Beavo, J. A. 1979 Phosphorylation–dephosphorylation of enzymes. *A. Rev. Biochem.* **48**, 923–959.

Lin, Y. C., Rose, K. M. & Jacob, S. T. 1976 Evidence for the nuclear origin of RNA polymerases identified in the cytosol: release of enzymes from the nuclei isolated in isotonic sucrose. *Biochem. biophys. Res. Commun.* **72**, 114–120.

Rose, K. M., Bell, L. E., Siefken, D. A. & Jacob, S. T. 1981*a* A heparin-sensitive nuclear protein kinase. Purification, properties and increased activity in rat hepatoma relative to liver. *J. biol. Chem.* **256**, 7468–7477.

Rose, K. M., Duceman, B. W. & Jacob, S. T. 1981*b* RNA polymerases in neoplasia. In *Isozymes: current topics in biological and medical research* (ed. M. C. Ratazzi, J. G. Scandalios & G. S. Whitt), vol. 5, pp. 115–141. New York: Alan Liss.

Rose, K. M., Duceman, B. W., Stetler, D. A. & Jacob, S. T. 1983*a* RNA polymerase I in hepatoma 3924A: mechanism of enhanced activity relative to liver. *Adv. Enzyme Regula.* **21**. (In the press.)

Rose, K. M., Ruch, P. A., Morris, H. P. & Jacob, S. T. 1976 RNA polymerases from a rat hepatoma: partial purification and comparison of properties with corresponding liver enzymes. *Biochim. biophys. Acta* **72**, 60–72.

Rose, K. M., Stetler, D. A. & Jacob, S. T. 1981*c* Protein kinase activity of RNA polymerase I purified from a rat hepatoma: probable function of M_r 42000 and 24600 polypeptides. *Proc. natn. Acad. Sci. U.S.A.* **78**, 2833–2837.

Rose, K. M., Stetler, D. A. & Jacob, S. T. 1983*b* RNA polymerases from higher eukaryotes. In *Enzymes of nucleic acid synthesis and modification* (ed. S. T. Jacob), vol. 2. Florida: CRC Press. (In the press.)

Stetler, D. A. & Rose, K. M. 1982 Phosphorylation of deoxyribonucleic acid dependent RNA polymerase II by nuclear protein kinase NII: mechanism of enhanced ribonucleic acid synthesis. *Biochemistry, Wash.* **21**, 3721–3728.

Stetler, D. A. & Rose, K. M. 1983 Protein kinase NII: interaction with RNA polymerase II and contribution to immunological cross-reactivity of RNA polymerases I and II. *Biochim. biophys. Acta* **739**, 105–113.

Stetler, D. A., Rose, K. M., Wenger, M. E., Berlin, C. M. & Jacob, S. T. 1982 Antibodies to distinct polypeptides of RNA polymerase I in sera from patients with rheumatic autiommune disease. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7499–7503.

Discussion

L. A. Pinna (*The University, Padua, Italy*). Does Dr Rose have any idea whether NII maintains its structure once incorporated into the polymerase complex? In other words could one ever get active oligomeric NII out of the complex?

Kathleen M. Rose. We have noticed that when polymerase I is subjected to recentrifugation on sucrose gradients, some of the polymerase is denatured. Polymerase I subunits (detected by immunological methods) can be observed in fractions sedimenting at less than 16*S* (where the remaining intact polymerase is recovered). If we analyse for kinase across these gradients, we can detect activity at 7*S* as well as associated with polymerase at 16*S*. This observation suggests that intact kinase can be released from the polymerase complex.

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