Protein kinase NII and the regulation of rDNA transcription in mammalian cells

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ABSTRACT

Transcription of ribosomal RNA genes is generally accepted to correlate with cell growth. Using primary cultures of adult bovine aortic endothelial (ABAE) cells, we have shown that transcription of rDNA in confluent cells falls to 5% of the transcription level in growing cells. Protein kinase NII appears to be a limiting factor to promote rDNA transcription in isolated nuclei of confluent cells. Protein kinase NII was detected by immunocytochemistry in the cytoplasm, nuclei and nucleoli of growing cells while it was no longer present in nucleoli of confluent cells. The kinase activity, in isolated nuclei, was estimated by endogenous phosphorylation of a specific substrate, nucleolin. A 10% residual activity was present in confluent cell nuclei compared to growing cell nuclei. Concomitantly, the transcription 'in vitro' of rDNA in the corresponding nuclei was also highly reduced (by 85%). Addition of exogenous protein kinase NII to confluent cell nuclei induced a strong increase in the phosphorylation of specific proteins including nucleolin. In parallel, the transcription of rDNA was increased by a factor of 5, to nearly the level observed in nuclei prepared from growing cells. These data suggest that, in confluent cells, factors necessary for rDNA transcription machinery are present but inactive in the nucleolus and that the phosphorylation of one or several of these factors (nucleolin, topoisomerase I,...) by protein kinase NII is a key event in the regulation of rDNA transcription.

INTRODUCTION

The major steps of ribosome biogenesis occur in the nucleolus of eukaryotic cells where ribosomal genes are actively transcribed (1). Nascent pre-ribosomal RNA molecules (pre-rRNA) rapidly associate with ribosomal proteins and with specific proteins transitorily bound to preribosomes and are stepwise processed to mature forms. Numerous experiments using whole animals, intact cells, isolated nuclei, and nucleoli have shown that rRNA synthesis is regulated in response to a variety of treatments that alter, in particular, the rate of protein synthesis or cell growth. Thus, RNA synthesis is stimulated when cells are induced to proliferate (2), while conversely, it decreased upon entry into stationary phase (3).

One of the major process for rapid regulation of cellular functions is phosphorylation and dephosphorylation of proteins, catalyzed by protein kinases and phosphatases. In recent years, some nucleolar phosphoproteins potentially involved in various nucleolar activities have been described. Kuehn and co-workers (4) Daniels et al. (5) reported that, in *Physarum polycephalum*, the phosphorylation of nucleolar proteins is stimulated by polyamines and that a 70 kDa protein acts after phosphorylation as an activator of rRNA synthesis. Evidences have accumulated indicating that nucleolin, a major nucleolar phosphoprotein of mammalian cells, plays a key role in the modulation of rRNA synthesis and preribosome assembly

(6). Working on the effect of androgens on the activity of RNA polymerase I and on the phosphorylation of nucleolar proteins in rat prostate, Suzuki et al. (7) have shown that administration of androgens to castrated rats resulted in an increase in phosphorylation of nucleolin as well as in RNA polymerase I activity. Much of the phosphorylation of nuclear proteins is catalyzed by cyclic nucleotide-independent protein kinases. Two protein kinases, designated casein kinase NI and casein kinase NII, have been characterized in a number of cell types (8). Among the nuclear substrates of these enzymes that have been so far characterized (topoisomerases I and II, RNA polymerase II, high mobility group protein 14), we have shown that nucleolin is a preferential substrate for nucleolar NKII ($K_m = 1.7 \times 10^{-4}$ M) (9). These two enzymes are also detected in the cytoplasm of various cell lines (10).

In this paper, using primary culture of adult bovine aortic endothelial (ABAE) cells we show that protein kinase II is localized in the cytoplasm, the nucleus and the nucleolus of growing cells, while in confluent cells the same kinase is no longer detected in the nucleolus. Concomitantly, the synthesis of pre-rRNA is low in confluent cells. Addition of exogenous kinase NII to nuclei isolated from confluent cells enhanced the transcription of rDNA and promoted the phosphorylation of specific proteins, including nucleolin.

MATERIAL AND METHODS

Culture and ^{32}P labelling of adult bovine aortic endothelial cells (ABAE cells).

Primary cultures of ABAE cells were established from adult aortic arches as previously described (11). The cells were routinely grown at 37°C with 10% CO_2 in 10 cm plastic Petri dishes (Nunc) in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% heat inactivated calf serum (Seromed). Purified basic Fibroblast Growth Factor (bFGF) (1ng/ml of culture medium) was added every two days. Petri dishes were seeded at 1 to 2×10^5 cells. Quiescent confluent endothelial cells were obtained after seven days of culture.

Previous to pulse labelling, cells were incubated for 4 h at 37°C in 5 ml DMEM medium (0.1 mM phosphate). Cells were then labelled for one hour with 80 $\mu\text{Ci/ml}$ orthophosphate ^{32}P (Amersham). To measure the ^{32}P turnover of phosphoproteins of ABAE cells reaching confluence, subconfluent ABAE cells were incubated for 15 h at 37°C in 5ml low phosphate DMEM medium containing 80 $\mu\text{Ci/ml}$ orthophosphate. After labelling, cell medium was replaced with normal DMEM medium, and at various times, cells were harvested and nuclear proteins were isolated as described below.

Transcription by isolated nuclei and analysis of RNA :

Nuclei from confluent and exponential ABAE cells were isolated according to Schibler et al. (12). The transcription assays were carried out with 5×10^5 nuclei in a medium (0.2 ml final) containing 30 mM Tris-HCl at pH 8, 150 mM KCl, 5 mM MgCl_2 , 0.2 mM EDTA, 2.5 mM DTT and 0.5 mM of ATP, GTP and UTP, 0.05 mM CTP, 100 μCi of $\alpha\text{-}^{32}\text{P}$ CTP (Amersham, 400 Ci/mmol). The reaction proceeded at 30°C for 30min. RNA was extracted as described by Greenberg and Ziff (13). Labelled RNA was used to probe nitrocellulose filters with 1 and 4 μg of alkali-denatured rDNA fragments encoding 18S and 28S rRNA (2), U_3 cDNA (14), nucleolin and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) cDNA. Hybridization and washing conditions were carried out as described by Piechazyck et al., (15). Autoradiograms of the nitrocellulose filters were quantitated by densitometric scanning (16).

In vitro phosphorylation of nuclear proteins :

Phosphorylation of proteins in nuclei isolated from confluent and growing cells was carried out in the same medium as described for transcription assays except for ATP concentration (0.05 mM). 10 μ Ci of γ -³²P ATP (Amersham, 3000 Ci/mmol) were added. If not specified otherwise, 5 μ g/ml of leupeptine were added and the incubation was for 30 min at 30°C with or without 1.2 μ Units of purified kinase NII (9). The reaction was stopped by the addition of 1.5 volumes of buffer A : 20 mM Tris-HCl at pH 7.9, 0.15% Sarkosyl, 15 mM EDTA. An equal volume of buffer A saturated phenol was added. Phenolic phases that contains the phosphoproteins, was extracted twice with buffer A, acidified with 5% trichloroacetic acid and precipitated by 5 volumes of acetone. Proteins were washed with cold acetone, resuspended in electrophoresis sample buffer and heated for 1 min at 100°C. Samples were analysed by SDS-polyacrylamide gel electrophoresis (12% acrylamide, 1.3% bis-acrylamide) as described by Laemmli (17). Gels were fixed, and stained with Commassie blue. After destaining, phosphorylated proteins were detected by autoradiography of the gel with Trimax 3M. Protein kinase NII activity was determined as described by Caizergues-Ferrer et al. (9).

In vitro nucleolin desphosphorylation and phosphorylation :

Purified nucleolin (1 μ g) extracted from exponentially growing cells was dephosphorylated by 10 units of calf intestinal alkaline phosphatase (Boehringer Mannheim) 50 mM Tris-HCl, pH 8, 1 mM MgCl₂, 0.1 mM ZnSO₄ at 55°C for 90min. Unphosphorylated nucleolin (1 μ g) was phosphorylated by protein kinase NII (9) at 30°C for 15 min in 10 mM Tris-HCl, pH 7.9, 10 mM β -mercaptoethanol, 5 mM MgCl₂, 1 μ M ATP and 5 μ Ci γ -³²P-ATP at 3000 Ci/mmol (Amersham). Radioactivity incorporated was determined according to Amalric et al.(18). ATP content of the cells was measured by bioluminescence according to Teissie J. (19). Phosphoprotein phosphatase assay was carried out as described by Olson et al. (20) with slight modifications. In vitro labelled nuclei were incubated in 100 μ l of 0.33 M bis Tris pH 7.2 for 30 min at 37°C. Phosphoproteins were extracted and analysed as described above.

RESULTS*Transcriptional activity in confluent and growing ABAE cells :*

Primary cultures of ABAE cells were prepared from adult bovine aortas. Cells grow exponentially up to confluence in the presence of basic fibroblast growth factor. Confluent cells present structural and functional characteristics of differentiated endothelial cells. They can be maintained for several days and after dilution and replating growth resumes up to confluence. In confluent cells transcription is highly reduced compared to growing cells (80% and 95% respectively for total RNA and rRNA Fig.1A). Furthermore, the synthesis of specific RNAs is dependent or independent of cell growth. Using run-on experiments as an assay for transcribed genes, the transcription of GAPDH and nucleolin, two house-keeping genes, and of the U₃ RNA gene appeared equally efficient in nuclei purified from confluent and growing cells, while the transcription of rDNA in confluent cells is only 10% of that in growing cells (Fig1B). Thus in ABAE cells the production of pre-rRNA is highly regulated according to cell growth. Among the factors that could be involved in this process, we have followed the behavior of protein kinase NII, which has been shown to phosphorylate topoisomerase I and nucleolin, two proteins implicated in rDNA transcription.

Fig. 1: Transcription in growing and confluent ABAE cells.

A : Growing (E) and confluent (C) cells (10^5) were labelled in vivo for 1h with ^{32}P orthophosphate. RNA was extracted and the total amount of incorporated radioactivity into RNA was determined (1). An aliquot was hybrid-selected with rDNA (2).

B : Nuclei were prepared from exponentially growing (E) and confluent (C) cells. In vitro transcription assays were carried out with 5×10^5 nuclei as described in Material and Methods. ^{32}P labelled RNAs were hybridized with rDNA (1 μg and 4 μg), nucleolin (Nu) and GAPDH cDNAs (1 μg), U3 cDNA (3 μg) and PBR322 DNA (5 μg) as controls. Nu to PBR322 : 24h exposure ; rDNA : 2h exposure.

Protein kinase NII in confluent and growing ABAE cells.

Immunolocalization of protein kinase NII was carried out by immunofluorescence studies using a rabbit anti-mouse protein kinase NII antiserum. The antiserum did not display any cross reacting specificity for other components and recognized the nuclear enzyme (NII) as well as the cytoplasmic enzyme (CKII) (10). In exponentially growing ABAE cells, protein kinase NII was detected both in the nuclei and in the cytoplasm (Fig.2a). A high accumulation is observed in the nucleolus (Fig.2e). In confluent cells, the level and the distribution of the kinase II are drastically modified. CKII and NKII are barely detected in the cytoplasm and in the nucleus. The nucleolus appears unlabelled (Fig.2c,f). To correlate these variations of amounts of protein kinase NII with the endogeneous nuclear protein kinase NII activity, isolated nuclei from confluent and growing cells were incubated with $\gamma\text{-}^{32}\text{P}\text{-ATP}$. Nuclear protein kinase NII activity was estimated by in vitro phosphorylation of an endogeneous specific nucleolar substrate, nucleolin (9). We have previously shown that nucleolin is the major nuclear substrate of protein kinase NII, and that its phosphorylation level detected after PAGE fractionation is a reliable index of kinase NII activity (9). As shown in Fig.3 protein phosphorylation readily occurs in nuclei from growing and confluent cells lanes : 1 (–) and 2 (–). The phosphoprotein patterns were qualitatively similar, with nucleolin as major component. However the specific activity of nucleolin is lower in nuclei of confluent cells than in nuclei of growing cells (Table I). These two results taken together suggested that the protein kinase NII is present in limiting amount in nuclei of confluent cells.

In a similar experiment, exogeneous protein kinase NII purified as previously described

Fig. 2: Immunofluorescence microscopy of confluent and growing ABAE cells using rabbit anti-mouse casein kinase NII anti serum. a,b,e : growing cells ; c,d,f confluent cells ; a,c,f : immunofluorescence ; b,d : corresponding phase contrast (a-d, $\times 200$; e,f, $\times 600$). Immunodetection was performed according to Pfaff and Anderer (10) using a 1/50 serum dilution. White arrows = nucleoli.

Fig. 3: In vitro phosphorylation of nuclear proteins. Nuclei were isolated from growing (1) and confluent cells (2). One half (–) was incubated directly in the presence of α - ^{32}P ATP under conditions suitable for protein kinase NII assay (see Materials and Methods). The other half (+) was incubated in the presence of 1.2 μ units exogenous protein kinase NII. One unit is defined as the amount of enzyme that catalyses the incorporation of one mole of inorganic phosphate ^{32}P into dephosphorylated casein at 30°C. Nu : Nucleolin.

(9) from nuclei of exponentially growing cells was added to nuclei isolated from confluent or growing cells. A dramatic increase in the phosphorylation of nucleolin and a set of other proteins was observed in confluent cells nuclei (Fig.3: lane 2 +). The specific activity of nucleolin increased respectively by a factor 25 and 4 in confluent and exponentially growing cells (Table I). To get an insight into the in vitro efficiency of nucleolin labeling by the added exogeneous protein kinase NII, the following experiment was carried out. ^{32}P labeled nucleolin was purified from exponentially growing cells, grown in the presence of ^{32}P orthophosphate. One half was dephosphorylated in vitro by alkaline phosphatase. Control and in vitro dephosphorylated nucleolin were then used as substrates for in vitro

Table I. 'In vivo' and 'in vitro' nucleolin phosphorylation in confluent and exponentially growing ABAE cells.

		Confluent cells	Growing cells
		Specific activity (a.u.)	Specific activity (a.u.)
in vitro	Control	5	46
	+NKII	125	184
in vivo		0.6	29

Specific activity of nucleolin was obtained by densitometric scanning of immunoblot and by counting the ^{32}P incorporated in the corresponding nitrocellulose filter band. Values are in arbitrary units (a.u.).

Table II. In vitro phosphorylation of nucleolin by the protein kinase NII.

	Nucleolin Specific Activity (c.p.m./ μ g of nucleolin)	
Protein kinase NII (1.2 μ Units)	–	+
Native (Exp)	10,000	60,000
Alkaline phosphatase treated (Exp)	2,000	550,000

Exponentially growing (Exp) cells were labelled 15 hours with 32 P orthophosphate. Nucleolin was purified (9) and an aliquot was treated by alkaline phosphatase. Native and dephosphorylated nucleolin (1 μ g) were then phosphorylated in vitro in absence (–) or in presence of added protein kinase NII (+) as described in 'Material and Methods'.

phosphorylation by exogeneous protein kinase NII. As shown in Table II, dephosphorylated nucleolin is 10 fold better substrate than the native phosphorylated nucleolin extracted from exponentially growing cells.

Dramatic variations in the level of nucleolin phosphorylation were observed in vivo between growing and confluent cells, while the amount of the protein remained almost constant (Fig.4, lanes 1–4). As shown in Fig.4, in growing and confluent cells the incorporation of 32 P orthophosphate into most of nuclear proteins is similar after 15h labeling compared (lanes 5 and 6) except for nucleolin in which 32 P orthophosphate

Fig. 4: Amount of nucleolin and in vivo phosphorylation of nuclear proteins in ABAE cell according to proliferation state.

Electrophoretic pattern (12% SDS-PAGE) of nuclear proteins corresponding to 5×10^5 nuclei. Exponentially growing cells : 1,3,5,7—Confluent cells : 2,4,6,8. Coomassie blue staining : 1,2 Immunodetection of nucleolin by a polyclonal anti-nucleolin serum (3,4). Immunoblotting was performed as described by Bugler et al., (6). Autoradiograms (5–8) : cells were labelled in vivo with 32 P orthophosphate. 15 hours (5,6) or 1 hour (7,8). Exposure time = 15 hours without intensifying screen at -80°C . $\text{Mr} \times 10^{-3}$ = molecular weights were determined by running proteins markers in parallel 68 : serum albumin ; 43 : ovalbumin ; 30 : carbonic anhydrase ; 20 : soybean trypsin inhibitor ; 14 : lactalbumin ; Nu = Nucleolin (95 kDa).

Table III. ATP content, nuclear protein kinase NII and phosphatase activities in confluent and exponentially growing ABAE cells.

	Confluent cells	Growing cells
ATP (pmole/cell)	0.85	1.2
Nuclear protein kinase NII activity	10	100
Nuclear phosphatase activity	93	100

Determination of the ATP pool size and the enzymatic activities were carried out as described in 'Material and Methods'. Kinase and phosphatase activities were expressed as percentage of control (growing cells). The data is the average of three separate experiments.

incorporation is not detected in confluent cells. The specific activity of phosphorylated nucleolin is at least 50 times lower than in growing cells (Table I). These variations did not result from significant differences in the ATP pool size between the two growth states (Table III), but could result from a block in the turn-over of nucleolin phosphate groups. To investigate this hypothesis, subconfluent cells were grown in the presence of ^{32}P orthophosphate during 15 hours (Fig.5A, lane 1). After withdrawal of ^{32}P orthophosphate, the behavior of labeled nucleolin was followed in the cells that reached confluence after 24 h. As shown in figure 5, the chase induces variations in the pattern of nuclear protein phosphorylation (lane 1 to 5). In confluent cells (Fig.5A, lane 5) some proteins remained phosphorylated (48 kDa, 38 kDa) while others were rapidly dephosphorylated and among

Fig. 5: In vivo, phosphate turn over of nuclear proteins

A : Autoradiograms of a 12% SDS-PAGE of nuclear proteins labelled in vivo with ^{32}P orthophosphate. 1. subconfluent cells labelled for 15 hours. 2 to 5 : chase experiment 2, 3, 4, 5 correspond respectively to 6, 12, 24, 36 hours of chase after removal of ^{32}P orthophosphate. 5 : cells were fully confluent. Nu = Nucleolin ; H₂A = Histone H₂A.

B : Behavior of nucleolin specific activity (●) and of a 48 kDa nuclear protein (○) during the pulse-chase experiment.

a.u. arbitrary units (see legend of Table I).

them nucleolin. The turn over of nucleolin phosphate groups is completely achieved when cells reached confluence (Fig.5B).

Thus, in confluent cells, not only nucleolin is unphosphorylated but de novo phosphorylation of the protein does not occur after a pulse-labelling of 1 hour with ^{32}P orthophosphate (Figure 4 ; lane 8 compared with lane 7). Furthermore, phosphatase activity related to nucleolin is the same in confluent and growing cells (Table II). These results demonstrate that the residual protein kinase NII detected in vitro in confluent cell nuclei is in limiting amount to properly phosphorylate nucleolin in vivo.

Protein kinase NII and rDNA transcription in isolated nuclei.

The putative effect of protein kinase NII on rDNA transcription was investigated through run on experiments by addition of protein kinase purified from growing cells to nuclei isolated from confluent and growing cells. We found that the amount of pre-rRNA synthesized in vitro is ten times lower in nuclei of confluent cells than in nuclei of growing cells (Fig. 1A). Addition of purified protein kinase in the assay has no significant effect on RNA synthesized by nuclei of growing cells, (pre-rRNA as well as total RNA) but induces an increase in the synthesis of pre-rRNA and total RNA in nuclei isolated from confluent cells by a factor of 5 and of 1.75 respectively. A similar increase was observed whatever the time of incubation between 5 min and 30 min, suggesting that the protein kinase NII acts essentially on rDNA transcription and may be slightly on RNA stability (Table IV). The protein kinase NII stimulates RNA polymerase I activity in a dose dependent manner (Fig.6). The other RNA polymerase activities appear to be less affected by the exogenous protein kinase.

DISCUSSION

In the present study, we have shown that, in vitro, protein kinase NII plays a key role in the regulation of rDNA transcription in ABAE cells. Primary culture of ABAE cells is a particular suitable system to study correlations between cell growth and gene expression. The proliferation of sparse cells is bFGF dependent ; in the absence of the growth factor cells cease to proliferate (21). The sole addition of bFGF induces cell growth and induction of the transcription of a set of genes (2). Upon reaching confluence, these cells adopt morphological and differentiated properties of endothelial cells (22). In the presence of bFGF, these cells maintain viability for long periods of time.

Several reports have related ribosome biogenesis to cell proliferation (23), itself modulated

Table IV. Stimulation of rDNA transcription by protein kinase NII

Incubation time	5 min	30 min
	cpm hybridized	cpm hybridized
Protein kinase NII (μUnits)		
0	1100	1000
1.2	4300	5100
Stimulation factor	4	5

About 5×10^5 nuclei isolated from confluent ABAE cells were incubated 5 min or 30 min at 30°C in presence of $200 \mu\text{g/ml}$ α -amanitin. Newly synthesized labelled RNA was used to probe nitrocellulose filters with $4 \mu\text{g}$ alkali denatured rDNA fragments encoding 18S and 28S rRNA (2). Hybridized cpm were quantitated by filter counting. Values are the average of three different experiments.

Fig. 6: Effect of exogenous protein kinase NII on the transcriptional activity of isolated nuclei from growing and confluent cells. RNA was isolated and incorporated radioactivity in newly synthesized RNA was counted. Counts in pre-rRNA was determined by hybrid selection as described in legend of Fig.1. ○, △, total RNA ; ●, ▼, rRNA— ○, ● confluent cells ; △, ▲, growing cells.

by medium conditions (24,25), hormones (23), and growth factors (2). The increase in rRNA accumulation that occurs in cells stimulated to proliferate could result from an increase in rRNA transcription (26,27), or a decrease in rRNA degradation (27). Our results indicate that, in ABAE cells both mechanisms are used. In vivo, the residual accumulation of rRNA in confluent cells is 5% of the accumulation in growing cells, while, in vitro, the residual transcription of rDNA is 10% in run-on assays. Thus, in ABAE cells, regulation of rDNA gene expression is directly related to cell growth.

In vitro, factors that are necessary for accurate transcription from the rRNA promoter have been characterized (3,28,29). In in vitro assays, the transcriptional activity of cell free extracts reflects the in vivo transcription efficiency of the corresponding cells. In particular, regulation could occur directly by activation of the RNA polymerase I or through specific factors (3). On the other hand, we have established that nucleolin, a specific nucleolar protein, could play a role in the expression of rDNA, first by decondensation of chromatin (30) and secondly by interaction with the nascent transcripts (31). In run off assays, transcription from a rDNA promoter is blocked by nucleolin in the presence of a protease inhibitor, leupeptin. Leupeptin by itself has no effect on the in vitro transcription assay. In absence of leupeptin, nucleolin is processed in defined peptides and transcription occurs (32). This maturation process of nucleolin is clearly dependant on the phosphorylation level of the protein (33,34).

In this paper, we present evidence that nucleolin is unphosphorylated in confluent cells. In vivo, the protein did not incorporate ^{32}P orthophosphate. Protein that was previously labeled in growing cells rapidly lost phosphate groups as soon as the cells reached confluence. In vitro, dephosphorylated nucleolin is phosphorylated by the protein kinase NII 10 times more efficiently than the native protein extracted from growing cells. Another preferential substrate of the kinase NII is topoisomerase I. This enzyme is activated by phosphorylation and is necessary in vivo for efficient transcription of rDNA (35). Thus, protein kinase NII modulates the activity of one enzyme (topoisomerase I) and of nucleolin, two factors involved in the transcription of rDNA.

Another conclusion obtained from our studies is that the localization of the protein kinase NII in the nucleolus clearly parallels cell growth and transcription of rDNA. In confluent cells, the protein kinase is no longer detected in the nucleolus. According to a recent report

the cytoplasmic and the nuclear enzymes exhibited no differences with respect to structure and function (10). Furthermore, cDNAs clones encoding alpha and beta subunits of *Drosophila melanogaster* kinase II have been characterized and used to show that genes encoding these two subunits are unique (36). However, in confluent cells, the enzyme is present in the cytoplasm and the nucleus but is no longer accumulated into the nucleolus. This could result from a defect in a post translational modification of the enzyme, necessary for its nucleolar targeting or from the absence of a nuclear carrier (protein or an other molecule). Alternatively, an equilibrium in protein kinase concentration could exist in growing cells between the cytoplasm and the nucleus that would become unbalanced in confluent cells.

In conclusion, the results presented in this paper demonstrate that the protein kinase NII plays a key role in the regulation of rDNA transcription. The sole addition of the exogenous enzyme induces in vitro, pre-rRNA synthesis.

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REFERENCES

1. Sommerville, J. (1986) *Trends Biochem. Sci.*, **11**, 438–442.
2. Bouche, G., Gas, N., Prats, H., Baldin, V., Tauber, J.P., Teissie, J. and Amalric, F. (1987) *Proc. Natl. Acad. Sci.*, **84**, 6770–6774.
3. Sollner-Webb, B. and Tower, J. (1986) *Ann. Rev. Biochem.*, **55**, 801–830.
4. Kuehn, G.D., Affolter, H.U., Atmar, V.J., Seebeck, T., Gubler, U. and Braun, R. (1979) *Proc. Natl. Acad. Sci.*, **76**, 2541–2545.
5. Daniels, G.R., Atmar, V.J. and Kuehn, G.D. (1981) *Biochemistry* **20**, 2525–2532.
6. Bugler, B., Caizergues-Ferrer, M., Bouche, G., Bourbon, H.M. and Amalric, F. (1982) *Eur. J. Biochem.*, **128**, 475–480.
7. Suzuki, N., Matsui, H. and Hosoya, T. (1985) *J. Biol. Chem.*, **260**, 8050–8055.
8. Edelman, A., Blumenthal, D.K. and Krebs, E.G. (1987) *Ann. Rev. Biochem.*, **57**, 567–613.
9. Caizergues-Ferrer, M., Belenguer, P., Lapeyre, B., Amalric, F., Wallace, M.O. and Olson, M.O.J. (1987) *Biochemistry* **26**, 7876–7883.
10. Pfaff, M. and Anderer, F.A. (1988) *Biochim. Biophys. Acta*, **969**, 100–109.
11. Darbon, J.M., Tournier, J.F., Tauber, J.P. and Bayard, F. (1986) *J. Biol. Chem.*, **261**, 8002–8008.
12. Schibler, V., Hagenbuchle, O., Wellauer, P.K. and Pittet, A.C. (1983) *Cell*, **33**, 501–508.
13. Greenberg, M. and Ziff, E.B. (1984) *Nature*, **311**, 433–442.
14. Mazan, S. and Bachellerie, J.P. (1988) *J. Biol. Chem.* **263**, 19461–19467.
15. Piechaczyk, M., Blanchard, J.M., Marty, L., Dani, C., Panabieres, F., El Sabouty, S., Fort, Ph. and Jeanteur, Ph. (1984) *Nucleic Acid Res.* **12**, 6951–6963.
16. Blanchard, J.M., Piechaczyk, M., Dani, C., Chambard, J.C., Franchi, A., Pouyssegur, J. and Jeanteur, Ph., (1985) *Nature*, **317**, 443–445.
17. Laemmli, J.K. (1970) *Nature*, **227**, 680–685.
18. Amalric, F., Nicoloso, M. and Zalta, J.P. (1972) *FEBS Letters*, **22**, 62–72.
19. Teissie, J. (1986) *Biochemistry*, **70**, 368–393.
20. Olson, M.O.J. and Guetzow, K. (1976) *Biochem. Biophys. Res. Commun.*, **70**, 717–722.
21. Gospodarowicz, D., Moran, J., Braun, D.J. and Birdwel, C. (1976) *Proc. Natl. Acad. Sci.* **73**, 4120–4124.
22. Vlodasky, I., Jonhson, L.K. and Gospodarowicz, D. (1979) *Proc. Natl. Acad. Sci.* **70**, 1316–1320.

23. Cavanaugh, A.H. and Thompson, E.A. (1983) *J. Biol. Chem.* 258, 9768–9773.
24. Grummt, I., Smith, V.A. and Grummt, F. (1976a) *Cell* 7, 439–445.
25. Grummt, I. and Grummt, F. (1976b) *Cell* 7, 447–453.
26. Edwards, D.R., Parfett, C.L.J. and Denhardt, D.T. (1985) *Mol. Cell. Biol.* 5, 3280–3288.
27. Baserga, R. (1984) *Exp. Cell. Res.* 151, 1–5.
28. Mishima, Y., Financsek, I., Kominami, R., and Muramatsu, M., (1982) *Nucleic Acids Res.* 10, 6659–6670.
29. Grummt, I., Kuhn, A., Bartsh, I. and Rosennbauer, H. (1986) *Cell*, 47, 901–911.
30. Erard, M.S., Belenguer, P., Caizergues-Ferrer, M., Pantaloni, A. and Amalric, F. (1988) *Eur. J. Biochem.*, 175, 525–530.
31. Bourbon H., Bugler B. and Mathieu C. (1988) *J. of Cell. Biochem.* 12D, 37.
32. Bouche, G., Caizergues-Ferrer, M., Bugler, B. and Amalric, F. (1984) *Nucleic Acid Res.* 12, 3025–3035.
33. Bourbon, H., Bugler, B., Caizergues-Ferrer M. and Amalric F. (1983) *FEBS Letters* 155, 218–222.
34. Schneider, H.R., Mieskes, G. and Issinger, O.G. (1989) *Eur. J. Biochem*, 180, 449–455.
35. Durban, E., Goodenough, M., Mills, J. and Busch, H. (1985) *EMBO J.* 4, 2921–2926.
36. Saxena, A., Padmanabha, R. and Glover, C.V.C. (1987) *Mol. Cell. Biol.* 7, 3409–3417.

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