Journal of Cellular Biochemistry 29:115–126 (1985)

Posttranslational Phosphorylation of Specific Chromosomal Proteins and Transcription of hnRNA Genes in Isolated Nuclei: Retention of *In Vivo* Sensitivity to 5,6-Dichloro-1-β-D-Ribofuranosylbenzimidazole (DRB)

Mikael Holst and Endre Egyházi

Department of Histology, Karolinska Institutet, S-104 01 Stockholm, Sweden

The rapidly turning over phosphorylation of specific nuclear nonhistone proteins, especially 42-, 33-, and 30-kDa polypeptides, and its relation to the transcriptional activity of hnRNA genes was investigated in isolated nuclei from salivary gland cells of *Chironomus tentans*. Incubation conditions promoting the phosphorylation of nonhistone proteins as well as the transcriptional activity of RNA polymerase II were established. The pattern of ^{32}P incorporation into the nonhistone proteins found in isolated nuclei resembled that obtained in experiments with intact cells, and the endogenous RNA polymerase II retained its ability to reinitiate the transcription under *in vitro* assay conditions. In addition, the *in vivo* sensitivity of the phosphorylation of 42-, 33-, and 30-kDa polypeptides, like the sensitivity of the initiation of hnRNA transcription to 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB), were preserved in the nuclear preparation. The experimental data taken together provide further support for the idea that the activation of hnRNA genes is causally related to the phosphorylation of specific nonhistone proteins.

Key words: isolated nuclei, polytene chromosomes, nonhistone proteins, in vitro transcription, protein phosphorylation, initiation inhibition, 5,6-dichloro-1-β-D-ribofurano-sylbenzimidazole

A major interest in cell biology for many years has been the clarification of mechanisms and factors involved in the control of eukaryotic genes. The findings that chromatin proteins, especially those engaged in the transcriptional machinery, are phosphoproteins led to the suggestion that postsynthetic protein phosphorylation possesses a regulatory potential in the control of gene activity [1,2]. While a large body of indirect evidence suggests that postsynthetic phosphorylation/dephosphory-

Received April 24, 1985; accepted July 2, 1985.

lation may be an essential element in gene regulation, it is still an open question how gene activation can be controlled by protein kinases/phosphatases. Obviously, there are a variety of levels, including chromatin structure, recognition of promoter sites, initiation of transcription, etc, that may serve as targets for modification by addition of phosphate groups to specific nuclear proteins. The most suggestive support in favour of a causal relationship between phosphorylation and gene control comes from *in vitro* experiments demonstrating a stimulation of RNA polymerase I [3,4] as well as of polymerase II [4,5] activities by purified protein kinase NII, but correlative findings have also been obtained under *in vivo* assay conditions [6]. However, the establishment and characterization of the link between phosphorylation and gene regulation still remain to be done.

In previous reports we have described a set of nuclear phosphoproteins carrying rapidly turning over phosphate groups in *Chironomus tentans* salivary gland cells [7]. We further found that transcriptionally active gene loci, especially the tissue-specific Balbiani rings, were enriched in rapidly phosphorylated 42-, 33-, 30-, and 25-kDa polypeptides [6]. Thus the phosphorylation of these proteins was correlated with the transcriptional activity of hnRNA genes. Consequently, when hnRNA genes were inactivated at the level of chain initiation by the nucleoside analogue 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB), the transcription block coincided in time with the inhibition of the phosphorylation of 42-, 33-, and 30-kDa polypeptides [6]. Of special interest is the finding that the 42-kDa polypeptide is capable of cross-reacting immunologically with antibodies raised against a transcription stimulatory factor derived from Ehrlich ascites tumor cells [6]. An important question that arose from these findings was whether the inactivation of RNA polymerase at the level of transcription initiation by DRB is a consequence of the impaired phosphorylation of specific transcription factor(s). To address this question we have developed an *in vitro* transcription system of isolated salivary gland nuclei with the capacity to reinitiate hnRNA transcription simultaneously with a reactivation of the nuclear phosphorylation and dephosphorylation machinery by the endogenous protein kinases and phosphatases.

In the present communication we describe incubation conditions that enable the phosphorylation of the 42-, 33-, and 25-kDa polypeptides in a manner resembling that established in intact cells, as well as conditions that allow a reinitiation of hnRNA transcription. Furthermore, it will be shown that the sensitivity of protein phosphorylation and of hnRNA transcription against DRB is preserved in nuclei that were isolated from cells and transferred into the *in vitro* transcription medium.

MATERIALS AND METHODS

Labelling of Salivary Gland Cells

Salivary glands were explanted from fourth instar larvae of the dipteran *Chironomus tentans* [8] into a modified Cannon medium [9] (HEPES-Cannon, a modified Cannon medium without phosphate and with 14 mM HEPES + NaOH, pH 7.2) and incubated for 25 min. The glands were then transferred into 100 μl of a fresh medium containing 20 mCi per ml of [^{32}P]orthophosphate (carrier-free, NEN products, Boston, MA) and incubated for 10 min.

Isolation of Cell Nuclei

Isolation of nuclei from salivary glands was carried out as described by Sass [10] with minor modifications. Nuclear isolation medium contained 8 mM NaCl, 90

mM KCl, 0.5 mM $MgCl_2$, 0.5 mM $CaCl_2$, 15 mM Sörensen phosphate buffer, (pH 6.0), and 0.2% Nonidet P40 [7]. In addition, 0.2 mM PMSF and human placental ribonuclease inhibitor (5 U/ml; Amersham International plc, England) were added. The nuclear pellets were stored at −70°C in isolation medium. No loss of phosphorylation or transcriptional activity could be detected after storage for no longer than 3 days.

Labelling of Proteins in Isolated Nuclei, Extraction of Proteins, and Electrophoresis

Nuclei isolated from 15 salivary glands were suspended and washed in 50 μl of nuclear incubation medium (NIM) consisting of 10 mM HEPES (pH 7.8), 80 mM KCl, 25 mM $(NH_4)_2SO_4$, 10 mM $MgCl_2$, 10 mM mercaptoethanol, 0.2 mM DDT, and 0.5 mM each of GTP, CTP, and UTP. After centrifugation for 30 sec in an Eppendorf centrifuge, the supernatant was removed. The rinsing of the nuclear pellet was repeated once. (To make the conditions for phosphorylation assays as similar as possible to those used for transcription assays, unlabelled nucleoside triphosphates were included in the NIM.) The nuclear pellet was incubated in 2 μl of NIM containing 100 μM [γ-^{32}P]ATP (20–40 Ci/mmol) (NEN Research Products, Boston, MA) for 5–10 min at 18°C. The nuclear pellet was washed with cold fresh NIM and the proteins were extracted as described before [6]. Electrophoresis was run in a 12% sodium dodecylsulphate (SDS)-polyacrylamide slab gel. Protein separations were visualized by silver staining after fixation in 50% methanol plus 10% acetic acid for 30 min. The gels were then dried and autoradiographed on Kodak X-Omat AR film at −70°C. Dried gels and autoradiograms were scanned in a Shimadzu Dual-Wavelength Chromato-Scanner Model CS-930.

Labelling of RNA in Isolated Nuclei, Extraction of RNA, and Electrophoresis

Nuclei isolated from glands were suspended in 50 μl NIM, the supernatant was removed, and the nuclear pellet was incubated with 2 μl NIM supplemented with nuclease inhibitor (100 U/ml) and with 50–100 μM [α-^{32}P]ATP (600–700 Ci/mmol) (NEN Research Products, Boston, MA) for 45 min at 18°C. When DRB was used its concentration was 65 μM. The incorporation of label was stopped by addition of 100 μl of cold 70% ethanol. The ethanol wash was repeated twice. RNA was extracted with 100 μl 20 mM Tris-HCl buffer (pH 7.4), 0.5% SDS, and 0.1% proteinase K for 20 min at 20°C. For removal of free radioactivity, the RNA extract was subjected to chromatography on a Sephadex G-25 column equilibrated with the extraction medium. The void volume of the eluate was then mixed with 2.5 volumes of ethanol and the RNA was precipitated at −20°C overnight. The RNA precipitate was solubilized and subjected to electrophoresis in a 1.5% agarose gel slab.

RESULTS

The Phosphorylation of Nuclear Proteins in Isolated Nuclei in Comparison With That Found in Intact Cell Nuclei

A common feature of the nuclear phosphoproteins we are dealing with is the rapidity of the metabolism of posttranslationally added phosphate groups [7]. Thus the present experimental approach is essentially limited to the characterization of rapidly turning over phosphorylation. Figure 1 shows the electrophoretic pattern of

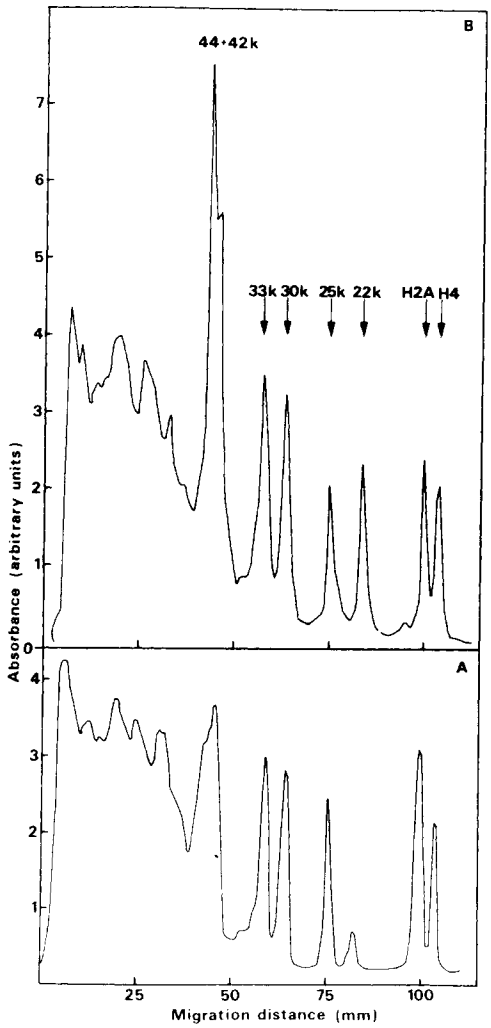

Fig. 1. PAGE analyses of rapidly phosphorylated nuclear proteins after labelling of intact cells (A) and isolated nuclei (B). Fifteen glands were labelled with $^{32}P_i$ in 100 μl HEPES-Cannon followed by preparation of the nuclei (A). For *in vitro* experiment, the nuclei were isolated from 15 glands and incubated with 100 μM of [γ-^{32}P] ATP in the NIM (B). The nuclear proteins were then solubilized in SDS sample buffer and prepared for electrophoresis as described previously [6]. For other data, see Materials and Methods.

^{32}P incorporation into nuclear proteins after labelling intact cells for 10 min *in vivo* (Fig. 1A) or after labelling isolated nuclei *in vitro* (Fig. 1B) with $^{32}P_i$ and [γ-^{32}P]ATP, respectively. In view of the finding that the incorporation of label into all proteins of interest reaches a steady state level after 10 min of exposure of isolated nuclei to [γ-^{32}P]ATP, we used 10-min incubation times throughout. As seen in Figure 1, the phosphorylation profile of nuclear proteins from *in vitro* labelled nuclei (Fig. 1B) shows a close resemblance to that derived from nuclei after incubation of intact cells (Fig. 1A). In accordance with previous observations, of the four core histones, it is

only H2A and H4 that carry rapidly metabolized phosphate groups [7,11]. The relative distributions of prominent ^{32}P-labelled nuclear proteins extracted from *in vivo* and *in vitro* incorporating nuclei are summarized in Table I. The relative incorporation of ^{32}P into 42-, 33-, and 30-kDa polypeptides is reasonably similar in the two nuclear samples. There are, however, differences in relative phosphorylation of histone H2A, which was reduced from 7.2% to 2.4%, and in the phosphorylation of the nonhistone 22-kDa polypeptide, which was approximately doubled after *in vitro* labelling of the nuclei.

The DRB Sensitivity of the Phosphorylation of 42-, 33-, and 30-kDa Polypeptides Is Resumed During In Vitro Assay Conditions

In previous communications we have reported that the phosphorylation of nonhistone 42-, 33-, and 30-kDa polypeptides and the histones H2A and H4 was inhibited and stimulated, respectively, by DRB in intact salivary gland cells [6,11]. Figure 2 shows the results obtained after incubation of isolated nuclei with (lanes B,D) and without (lanes A,C) DRB using an experimental design similar to that described for the experiment with intact cells. The phosphorylation of 42-, 33-, and 30-kDa proteins (lanes A,B) was reduced by 90%, 67%, and 86%, respectively, whereas the incorporation of ^{32}P into H2A and H4 slightly increased under the same conditions (Table II). The incorporation of label into the 25-, and 22-kDa polypeptides was also diminished after the DRB regimen. The effect of DRB on phosphorylation of the 22-kDa protein was difficult to assess in intact cells owing to a relatively poor incorporation of ^{32}P into this protein [6]. As seen in Figure 2, the 42-, 33-, 30-, 25-, and 22-kDa phosphoproteins could be visualized as distinct bands by silver staining, and the protein patterns of control (lane C) and DRB treated nuclei (lane D) coincide to a large extent. Thus the nucleoside analogue did not affect the level of protein phosphorylation by alteration of the protein content of the isolated nuclei under *in vitro* assay conditions.

We investigated the ability of nuclear protein kinases inhibited in living cells by DRB to recover their activities under *in vitro* assay conditions. To block phosphorylation, salivary glands were preincubated with DRB for 10 min before the explantation of nuclei into NIM. The sister glands, which were kept in DRB-free medium, were used as control. The isolated nuclei were then incubated with [γ-^{32}P]ATP in NIM for 10 min and the extracted proteins were separated by electrophoresis. The densitometer

TABLE I. Distribution of ^{32}P-Labelled Histone and Nonhistone Proteins in Living Cells and Isolated Nuclei*

Proteins	Incubation of intact cells (%)	Incubation of isolated nuclei (%)
H2A	7.2	2.4
H4	3.3	2.4
42 + 44 kDa	15.8	20.8
33 kDA	6.4	7.1
30 kDa	5.0	5.2
25 kDa	3.9	2.8
22 kDa	1.4	3.1

*Mean values for three experiments are expressed as a percentage of the total incorporation of label into nuclear proteins.

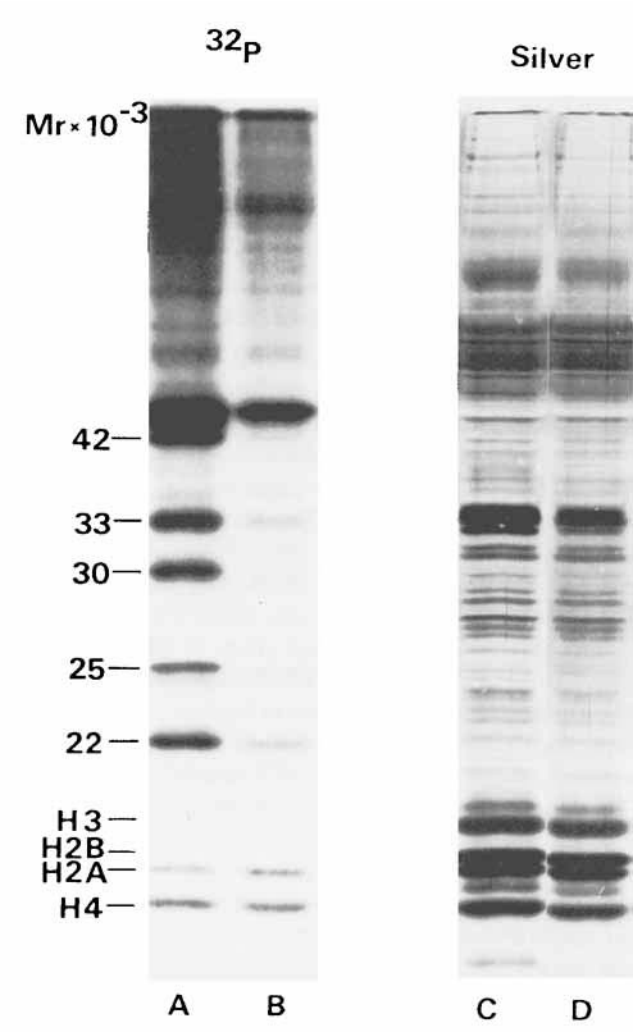

Fig. 2. PAGE analyses of rapidly phosphorylated nuclear proteins after labelling of isolated nuclei in the presence (B) and absence (A) of DRB. Fifteen glands were preincubated for 5 min in NIM containing DRB. They were then transferred to another volume of the same medium supplemented with [γ-^{32}P] ATP, and they were incubated for 10 min. Control nuclei were labelled in the absence of DRB in an otherwise parallel procedure. For more details, see Materials and Methods. Lanes A and B represent ^{32}P labelling. Lanes C and D represent silver staining of proteins from control and DRB-treated nuclei, respectively.

tracing of proteins derived from DRB-preincubated cells displays a close similarity to that of proteins obtained from nuclei processed in the absence of DRB throughout (Fig. 3). Thus the activity of protein kinases involved in the DRB-sensitive phosphorylation of histones H2A and H4 as well as of several nonhistone proteins is entirely reversible when nuclei prepared from DRB-treated glands are transferred to a DRB-free NIM.

TABLE II. The Effect of DRB on the Phosphorylation of Histone and Nonhistone Proteins*

Proteins	DRB (% of control)
H2A	140
H4	120
42 kDa	10
33 kDa	33
30 kDa	14
25 kDa	19

*The effect of DRB is expressed as a percentage of control. Each figure represents the average of three experiments.

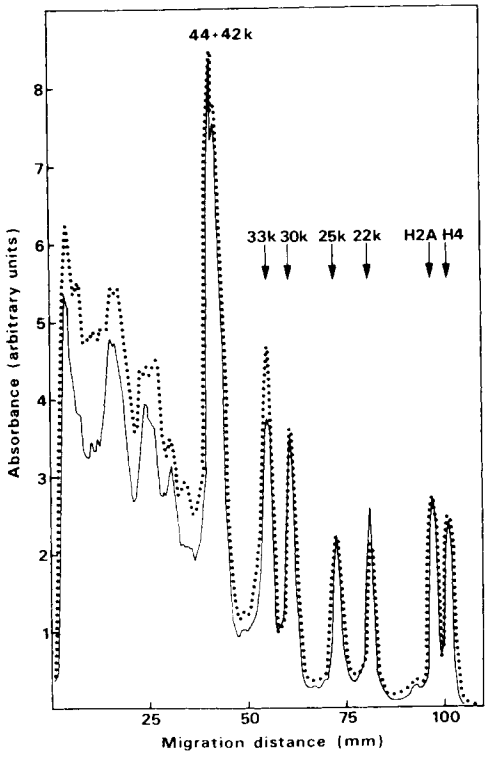

Fig. 3. The recovery of protein phosphorylation in nuclei isolated from glands treated with DRB. Fifteen glands were preincubated for 5 min in HEPES-Cannon containing DRB. Next, the nuclei were isolated and subsequently incubated with [γ-^{32}P] ATP in the absence of DRB. The control nuclei were derived from untreated glands and were labelled in an otherwise parallel procedure. (—), DRB-pretreated nuclei; (•••), untreated nuclei.

The Transcription of hnRNA Genes Appears To Be Reinitiated in Isolated Nuclei

The usefulness of a given *in vitro* transcription assay system for studies of gene regulation is highly dependent on its ability to transcribe relevant genes in a manner reflecting the *in vivo* situation with preservation of *in vivo* site(s) of regulation. It is a well-known fact that purified RNA polymerase II has to be supplemented with a crude cell extract or with exogenous purified transcription factors in assays aimed at a promotor-specific transcription of specific gene probes of naked DNA templates [12–14]. Transcription studies in isolated nuclei prepared under appropriate conditions of pH, ionic strength, temperature of the isolation medium, and the gentleness of cell disruption during isolation of the nuclei may have the advantage of preserving endogenous chromatin structure with its associated transcription factors and the ability to reinitiate the transcription. A technical problem associated with the evaluation of initiation competence of isolated nuclei is the difficulty to differentiate the incorporation of labelled RNA precursor into elongating but *in vivo* initiated nascent transcripts from that of *in vitro* initiated RNA chains. An approach to circumvent this problem is to use γ-thio analogues of ATP or GTP in the transcription system leading to the incorporation of the γ-thio triphosphate to initiating RNA chains only. The latter can then be selected by mercury-agarose affinity chromatography [15].

In our studies we have taken advantage of DRB in probing *in vitro* initiated transcription. By treatment of salivary glands with DRB for 30 min before isolation of nuclei, we were able to clear active hnRNA genes of growing nascent RNA chains. The rationale for this experimental design is the interference of DRB with the initiation process, but not with the elongation, [16,17] and the fact that the inhibition is reversible [18]. This means that incorporation of labelled RNA precursor into *in vitro* synthesized hnRNA molecules is expected to occur only if the transcribing isolated nuclei regain their capability to reinitiate the transcription. The results presented in Figure 4 show that the transcription of hnRNA retains its DRB-sensitivity under *in vitro* conditions and that a reinitiation of hnRNA takes place if nuclei from DRB-treated cells are incubated in NIM lacking DRB. The pattern of normal *in vitro* synthesized nuclear RNA (Fig. 4, lane A) displays label heterogeneously distributed in the 4–75 S range including a faint distinct band probably representing 75 S RNA of Balbiani ring origin [16]. The incubation condition used for isolated nuclei appeared favourable only for the transcription of hnRNA genes. Thus bands of preribosomal RNA known from *in vivo* studies [9] are lacking in the pattern, and furthermore, the treatment of nuclei with α-amanitin efficiently abolishes the incorporation of label into RNA (Fig. 4, lane D). In rare cases we observed the presence of preribosomal 38 S, 30 S, and 23 S RNA in our electrophoretic separation; however, we do not know the reason for this variability in the expression of rDNA genes. The incorporation of label into hnRNA was found to be linear for at least 80 min of incubation (data not shown). The transcription of hnRNA genes in isolated nuclei derived from glands pretreated with DRB is reinitiated if DRB is omitted in the course of *in vitro* incubation (lane B), whereas no significant incorporation of label can be detected in nuclei incubated in the continued presence of DRB (Fig. 4, lane C). Hence the labelling pattern shown in Figure 4, lane B, in all likelihood, reflects the size distribution of the *in vitro* initiated RNA chains. This means that the transcription time of the fastest moving molecules—that is, the position of the front of the spot,

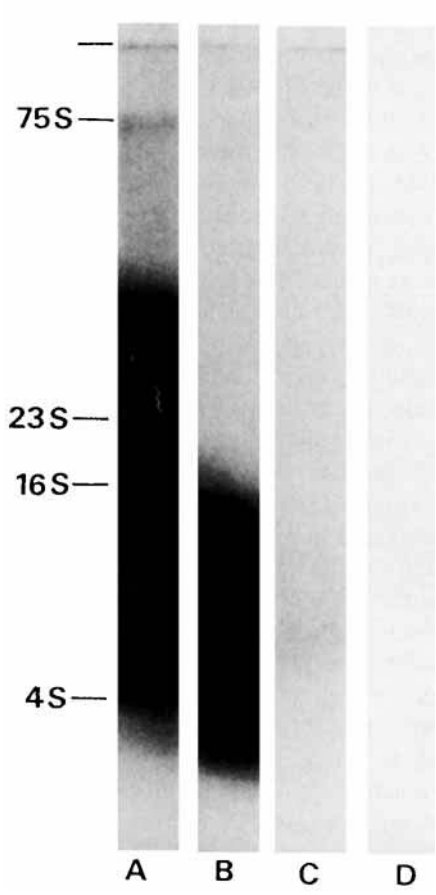

Fig. 4. Electrophoretic analyses of labelled RNA synthesized in isolated nuclei. Ten glands were labelled for 45 min with 50 μM of [α-^{32}P]ATP in 2 μl of NIM. Lanes B and C represent RNA from nuclei isolated from glands pretreated 65 μM DRB for 30 min before isolation of nuclei. The nuclei from DRB-treated cells were then labelled *in vitro* in the absence (lane B) and continued presence (lane C) of DRB. Lane D represents RNA from isolated nuclei labelled in the presence of α-amanitin (5 μg/ml). The labelled RNA from each sample was released by proteinase K/SDS treatment, and electrophoresis was carried out in 1.5% agarose gel slabs. *Escherichia coli* RNA was used as marker. The position of 75S RNA was determined in parallel analysis of Balbiani ring RNA. For other data, see Materials and Methods. Lane A) RNA from untreated nuclei.

which roughly comigrates with the 16 S RNA marker— is 45 min, indicating an elongation rate of less than 1 nucleotide per sec in isolated nuclei at 18°C. This is, in accordance with observations by others [19,20], more than one order of magnitude lower than that registered under corresponding *in vivo* assay conditions[16,19].

DISCUSSION

The main objective of the experimental work in this study was to prepare nuclear material and to compose a nuclear incubation mixture with capacities to maintain the function of both the nuclear phosphorylation/dephosphorylation machin-

ery by endogenous protein kinases/phosphatases and of polymerase II including the transcription initiation step. The outcome of the experiments shows that *Chironomous* salivary gland nuclei retain their ability to posttranslationally phosphorylate specific nonhistone proteins and to reinitiate hnRNA transcription after explantation into a well-defined synthetic medium. It is, of course, understood that a number of obvious limitations are associated with a system of isolated nuclei, implying that the efficiency of phosphorylation and RNA synthesis reactions measured under *in vitro* conditions can hardly reach their *in vivo* levels. Nevertheless, a transcription system of isolated nuclei, despite its limitations, may offer a potentially useful complement to pure *in vitro* systems (gene clones of naked DNA template plus total cell extract or purified factors) and to the entirely *in vivo* approaches. The *in vitro* systems of explanted nuclei appear to have selective advantages in studies aimed at elucidating the role of postranslational protein modifications in regulation of gene expression. An important feature of the present model system of salivary gland cell nuclei is the observed consensus between results from *in vivo* and *in vitro* experiments in responsiveness of phosphorylation of specific proteins and of hnRNA synthesis to administration of DRB. The phosphorylation of 42-, 33-, and 30-kDa polypeptides and the initiation of hnRNA chains in isolated nuclei and in nuclei of intact salivary glands [6] were inhibited to a similar extent at equivalent DRB concentrations. Another finding of relevance in this context is the resemblance in the kinetic appearance of ^{32}P incorporation into nonhistone proteins [7], although we have not yet established the site specificity of the *in vitro* added phosphate group(s).

A crucial question in the evolution of the *in vitro* transcription system is how accurately it reflects the physiological transcription initiation in corresponding intact cells. This in turn depends to a large extent upon the degree of the structural and chemical conservation of functional chromatin units and on the preservation of more or less loosely associated enzymes and transcription factors. A light-microscopic inspection of our isolated nuclei suggested that the structurally and functionally well-defined highly decondensed active transcription units, the Balbiani rings, as well as the highly condensed inactive bands maintained their *in vivo* state [7]. It seems unlikely that some loosely bound transcription factors, indispensable for accurate initiation, could have completely leached out from the nuclei and were subsequently lost in the course of nuclear preparation; however, we cannot exclude such a possibility. Thus our major argument in favour of the fidelity of transcription initiation in isolated salivary gland nuclei is based on the response of the transcriptional machinery to the action of DRB [16–18]. The results in Figure 4 shows that the reinitiation of hnRNA transcription occurs as soon as there is no DRB in NIM. The dose-response relationship as well as the sequence of events during inhibition of hnRNA labelling by DRB measured in isolated nuclei under *in vitro* assay conditions showed a reasonably good agreement with that established for living cells. The capacity of DRB to interfere with the transcription of hnRNA genes under *in vitro* conditions has also been documented by other investigators both in isolated nuclei of mammalian origin [21] and in a transcription assay with cloned gene probes together with whole cell extract [22]. A selective effect on transcription initiation with truncated DNA template (adenovirus major late promotor), reflecting the transcriptional events occurring *in vivo*, was demonstrated by Zandomeni et al [23]. Furthermore, it has been suggested that DRB affects the activity of one of the transcriptional factors involved in accurate and specific transcription. Thus DRB would appear to act only in *in vitro* assays in

which faithful initiation is taking place [23]. Another significant contribution to the evaluation of the function of DRB from *in vitro* transcription assays is that DRB possesses no measurable effect on enhancing premature transcript termination [23], which was concluded on the basis of *in vivo* data obtained from transcription studies in mammalian cells (for review, see ref [24]).

A question of general importance arising in view of accumulated DRB results is whether the cellular target for the inhibitory action of DRB represents a physiological site of gene control. Even though no direct evidence in support of such a possibility is at present available, there are observations compatible with the interpretation that DRB affects physiological site(s) of the regulation of hnRNA genes. (1) Adenosine, a natural constituent of living cells, exerts a similar inhibitory effect as does DRB on RNA synthesis if cells are incubated at elevated external concentrations. Nonetheless, the cells are capable of lowering their intracellular (toxic) level of adenosine after a period of inhibitory activity and thereby to reverse transcription inhibition, despite a constant excess concentration of extracellular adenosine [25]. (2) The activity of protein kinase NII, which promotes the phosphorylation of RNA polymerase II, is DRB-sensitive [26], although it is not yet known if it is the DRB-sensitive phosphorylation that confers competence to RNA polymerase II for initiation of transcription [3]. DRB-sensitive protein kinase activity is involved in the phosphorylation of a nonhistone 42-kDa protein that immunologically cross-reacts with an established transcription stimulatory factor [27]. Our *in vitro* system of isolated nuclei will hopefully enable us to identify and to test functionally the protein kinase NII-dependent and DRB-susceptible phosphoprotein(s) in phosphorylated and unphosphorylated states in transcribing isolated nuclei.

ACKNOWLEDGMENTS

We wish to thank Amina Ossoinak and Gunilla Carlsson for skillful technical assistance and Hannele Jansson for proficient secretarial work. We are grateful to Chana Szpiro for cultivation of the larvae.

This investigation was supported by the Swedish Cancer Society, Sven & Ebba Christina Hagbergs Stiftelse, and Karolinska Institutet (Reservations anslaget).

REFERENCES

1. Kleinsmith LJ: In Busch H (ed): "The Cell Nucleus." New York: Academic Press, Vol 6, 1978, p 221.
2. Stein GS, Plumb M, Stein JL, Phillips JR, Shepard EA: In Hnilica LS (ed): "Chromosomal Nonhistone Proteins." Boca Raton, Florida: CRC Press, Inc., Vol I, 1983, p 127.
3. Duceman BW, Rose KM, Jacob ST: J Biol Chem 256:10755, 1981.
4. Stetler DA, Rose KM: Biochemistry 21:3721, 1982.
5. Dahmus ME: Biochemistry 15:1821, 1976.
6. Egyházi E, Pigon A, Ossoinak A, Holst M, Tayip U: J Cell Biol 98:954, 1984.
7. Egyházi E, Pigon A, Holst M, Rydlander L: Chromosoma 88:24, 1983.
8. Beermann W: Chromosoma 5:139, 1952.
9. Ringborg U, Rydlander L: J Cell Biol 51:355, 1971.
10. Sass H: Chromosoma 78:33, 1980.
11. Egyházi E, Pigon A: Eur J Cell Biol 31:354, 1983.
12. Lewis MK, Burgess RR: In Boyer PD (ed): "The Enzymes." New York: Academic Press, Vol 15B, 1982, p 109.

13. Weil PA, Luse DS, Segall J, Roeder RG: Cell 18:469, 1979.
14. Manley JL, Fire A, Cano A, Sharp PA, Gefter ML: Proc Natl Acad Sci USA 77:3855, 1980.
15. Smith MM, Reeve AE, Huang RC: Cell 15:615, 1978.
16. Egyházi E: Proc Natl Acad Sci USA 72:947, 1975.
17. Egyházi E, Pigon A, Rydlander L: Eur J Biochem 122:445, 1982.
18. Egyházi E: Nature 262:319, 1976.
19. Cox RF: Cell 7:455, 1976.
20. Brooks TL, Green MH: Nucleic Acids Res 4:4261, 1977.
21. Winicov I, Button JD: Eur J Biochem 124:239, 1982.
22. Zandomeni R, Mittleman D, Bunick D, Ackerman S, Weinmann R: Proc Natl Acad Sci USA 79:3167, 1982.
23. Zandomeni R, Bunick D, Ackerman S, Mittleman B, Weinmann R: J Mol Biol 167:561, 1983.
24. Sehgal PB, Tamm I: Antibiot Chemother 27:93, 1980.
25. Holst M, Egyházi E: Eur J Biochem 147:631, 1985.
26. Zandomeni R, Weinmann R: J Biol Chem 259:14804, 1984.
27. Natori S: Mol Cell Biochem 46:173, 1982.