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DNA-dependent protein kinase specifically represses promoter-directed transcription initiation by RNA polymerase I

(ribosomal genes/*Xenopus*)

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ABSTRACT **DNA-dependent protein kinase (DNA-PK) is a nuclear enzyme that phosphorylates several transcription factors, but its cellular function has not been elucidated. Here I show that DNA-PK strongly inhibits promoter-directed transcription initiation by *Xenopus* RNA polymerase I *in vitro*. The repression is due to protein phosphorylation, since it is relieved by 6-dimethylaminopurine, an inhibitor of protein kinases. DNA-PK inhibits transcription from both linear and circular templates, but the repression is more efficient on linear templates. DNA-PK has no effect on promoter-directed transcription by RNA polymerases II and III. Partial fractionation of the *in vitro* transcription system shows that a protein fraction containing transcription factor Rib1, the *Xenopus* equivalent of human SL1, mediates the repression of transcription by DNA-PK. The present data suggest a role for DNA-PK in down-regulating ribosomal gene transcription.**

DNA-dependent protein kinase (DNA-PK) is a nuclear serine/threonine protein kinase consisting of a 350-kDa catalytic component (p350) and the human autoantigen Ku (1–7). Ku was originally found to bind to ends of double-stranded DNA (8), and it was reported that binding to double-stranded DNA ends was required for the activation of DNA-PK (2, 6). More recently, Ku was also found to bind to a variety of DNA structures bearing single- to double-strand transitions (9), and these DNA structures were also able to activate the kinase (10), suggesting that DNA-PK might have a role during transcription and DNA replication. DNA-PK has been found to phosphorylate several transcription factors as well as the C-terminal domain of RNA polymerase II (pol II) (reviewed in refs. 3 and 4). Transcription factor Sp1, for example, is phosphorylated by the DNA-bound enzyme only when bound to a GC box on the same molecule (6, 11). In all these cases, however, it has been difficult to demonstrate an effect of phosphorylation on the functional activity of the substrate. Thus, DNA-PK remains an enzyme with unknown function. In addition to identifying the *in vivo* and *in vitro* substrates of DNA-PK, it is therefore important to identify cellular processes that are regulated by this enzyme.

Here I report that purified human DNA-PK inhibits *Xenopus* ribosomal gene transcription *in vitro*, and I present evidence that transcription factor Rib1 (12), the *Xenopus* equivalent of human SL1 (13), plays a central role in the down-regulation of ribosomal gene transcription by DNA-PK.

MATERIALS AND METHODS

DNA-PK. For all the experiments shown, a preparation of purified HeLa DNA-PK (one Sp1 phosphorylation unit per μl; refs. 6 and 11) was used that was provided by S. P. Jackson (Wellcome/CRC Institute, Cambridge, U.K.). It was stored in a buffer containing 25 mM Hepes (pH 7.5), 0.2 mM EDTA, 10% (vol/vol) glycerol, 0.5 mM dithiothreitol, and 0.3 M KCl. Dilutions were made in the same buffer. Additional purified HeLa DNA-PK was provided by W. Dynan and S. Jesch (University of Colorado, Boulder). Both DNA-PK preparations were similarly active in inhibiting ribosomal gene transcription.

S-100 Extract and Fractionation. S-100 extracts from the *Xenopus laevis* tissue culture cell line Xl-K2 were prepared as described (14, 15). The basic pol I transcription factors including pol I were eluted from DEAE-Sepharose CL-6B (Pharmacia) with CB350 (column buffer containing 350 mM KCl; ref. 15). Termination was reconstituted by recombining the DEAE 0.35 fraction with the flow-through fraction (15). For further fractionation, the dialyzed DEAE 0.35 fraction was applied to heparin Sepharose CL-6B (Pharmacia) in CB100. Bound proteins were step-eluted with CB containing 0.2, 0.4, and 0.8 M KCl. Peak fractions were pooled and dialyzed against CB100. The 0.4 and 0.8 M fractions (H-0.4 and H-0.8) contained 8–9% of total S-100 proteins.

***In Vitro* Transcription Reactions.** Twenty-microliter reaction mixtures typically consisted of 10-μl protein fractions in CB100 and 10 μl of 2× transcription buffer (15, 16). The ribosomal minigene P-T3wt and the nuclease S1 probe used to detect initiation from the ribosomal gene promoter have been described (16–18). The plasmid containing the herpes simplex virus thymidine kinase gene (HSV-TK; refs. 19 and 20) was linearized at a unique *Kpn* I site. To detect correct initiation from the HSV-TK promoter, a 131-bp 5′-end-labeled *Eco*RI/*Bgl* II fragment was used. Correctly initiated transcripts protect a fragment of 56 nt from nuclease S1 digestion.

The single-round transcription reactions shown in Fig. 3*B* were performed as follows (final concentrations of components in the reaction mixtures are given in parentheses): 50 ng of linear P-T3wt was incubated with 10 μl of recombined H-0.4 and H-0.8 and 10 μl of 2× transcription buffer lacking phosphocreatine and nucleoside triphosphates. After 20 min at room temperature, 5′-adenylylimidodiphosphate (AMP-PNP) or ATP, GTP (250 μM each), and phosphocreatine (5 mM) were added to start transcription and incubation was continued for another 20 min. Complete elongation was started by adding 2.5 μl of a mixture containing UTP (250 μM), CTP (12.5 μM), [α-^{32}P]CTP (10 μCi; 650 Ci/mmol; 1 Ci = 37 GBq), and heparin (100 μg/ml). One microliter containing 0.25 μl of DNA-PK and 6-dimethylaminopurine (DMAP; 2.5 mM) was added at the points indicated in the diagram in Fig. 3*A*. dATP (250 μM) was always added along with DNA-PK.

For the experiment shown in Fig. 4*B*, 50 ng of linear P-T3wt was incubated with 5 μl of H-0.4, 5 μl of H-0.8, or 5 μl of each fraction, and 10 μl of 2× transcription buffer lacking phosphocreatine and nucleoside triphosphates but containing 200 μM dATP. Sets of three reaction mixtures also received 1 μl

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Abbreviations: AMP-PNP, 5′-adenylylimidodiphosphate; DNA-PK, DNA-dependent protein kinase; pol I, II, and III, RNA polymerases I, II, and III; DMAP, 6-dimethylaminopurine; HSV-TK, herpes simplex virus thymidine kinase.

containing 0.25 μl of DNA-PK (reactions 1–3), 0.25 μl of DNA-PK plus DMAP (2.5 mM) (reactions 4–6), or DNA-PK buffer (reactions 7–9). After 20 min at room temperature, DMAP (2.5 mM) was added to reaction mixtures 1–3 and 7–9, followed by 5 μl of H-0.4 to reaction mixtures 3, 6, and 9, and 5 μl of H-0.8 to reaction mixtures 2, 5, and 8. At this point, all reaction mixtures had exactly the same composition except for the presence or absence of DNA-PK. Transcription was started by the addition of 3 μl of a mixture containing AMP-PNP, GTP, UTP (250 μM each), CTP (12.5 μM), phosphocreatine (5 mM), and [α-^{32}P]CTP (10 μCi). Five minutes after the start of the reaction, heparin was added to 100 μg/ml and transcription was allowed to proceed for a total of 30 min. Appropriately exposed autoradiographs were scanned on an Apple OneScanner and analyzed using OFOTO 1.1 and NIH IMAGE 1.44 software.

RESULTS

DNA-PK Inhibits Ribosomal Gene Transcription *in Vitro*. To investigate whether DNA-PK would affect *Xenopus* ribosomal gene transcription, purified human DNA-PK was added to *in vitro* transcription reaction mixtures. Transcription of a linear ribosomal minigene (16) gives rise to a 410-nt transcript from the *Xenopus* ribosomal gene promoter to the pol I termination site T3 and a small amount of a 652-nt runoff transcript to the *Ssp* I site (Fig. 1*A*, lane 1). Addition of DNA-PK to the reaction mixture severely repressed transcription (lane 2). The repression could be relieved by the protein kinase inhibitor DMAP (16, 21) (lane 4), indicating that it was due to protein phosphorylation. Additional experiments confirmed that DNA-PK was inhibited by DMAP in protein phosphorylation assays (data not shown). I had previously

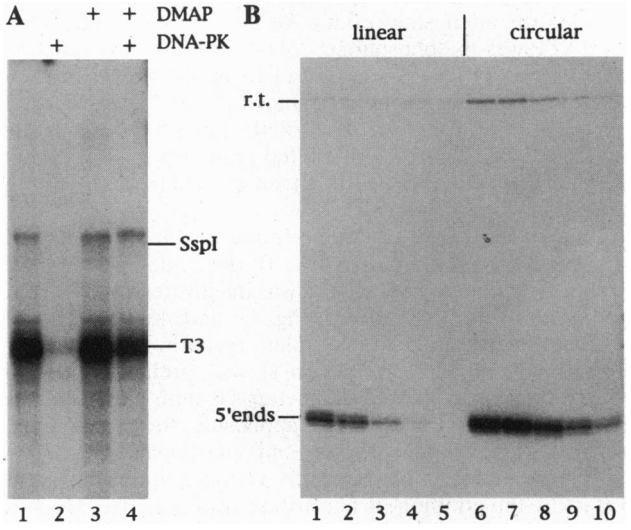

FIG. 1. DNA-PK inhibits ribosomal gene transcription *in vitro*. (*A*) The linear ribosomal minigene P-T3wt was transcribed in recombined DEAE-Sepharose fractions. RNA was labeled during transcription for 2 hr. T3, RNA terminated at the T3 terminator of the minigene; SspI, runoff transcript to the end of the template linearized with *Ssp* I. DMAP (2 mM) and/or DNA-PK (0.5 μl) were added as indicated above the lanes. At the template concentration used (5 ng/μl) the effect of DMAP in the control reactions without DNA-PK was very low (lanes 1 and 3). (*B*) Linear (lanes 1–5) and circular (lanes 6–10) minigenes were transcribed in recombined DEAE-Sepharose fractions. Unlabeled RNA was analyzed with a nuclease S1 protection assay using a probe specific for RNA 5′ ends at the pol I promoter. One microliter containing 0, 0.04, 0.1, 0.2, and 0.5 μl of DNA-PK was added to the reaction mixtures shown in lanes 1–5 and 6–10. r.t., Full-length nuclease S1 probe, indicative of readthrough RNA. Because there is a terminator upstream from the promoter (14), the readthrough is low even on circular templates.

reported that DMAP stimulates *in vitro* transcription due to the inhibition of an endogenous, unidentified protein kinase (16). The experiment shown in Fig. 1*A* was done under conditions where this endogenous DMAP effect was minimal (lanes 1 and 3), thus demonstrating that the DMAP-induced stimulation in lane 4 was due to inhibition of the added DNA-PK. As I discuss below, the endogenous DMAP effect and the present repression by DNA-PK appear not to be related.

Because DNA-PK has been shown to preferentially interact with DNA ends through its Ku component (5, 6), the effect of DNA-PK on transcription from linear and circular templates was compared. To be able to better measure total promoter-initiated transcription, the RNA was analyzed by a nuclease S1 protection assay (Fig. 1*B*, lanes 1–10). It is evident that transcription from linear templates was very sensitive toward increasing amounts of DNA-PK (lanes 1–5), while transcription from circular templates was more resistant (lanes 6–10). Densitometric analysis of the nuclease S1 signals of the RNA 5′ ends confirmed that to achieve the same level of transcriptional repression on a circular template as on a linear template, >2 times more DNA-PK was required (data not shown). The result is consistent with the idea that DNA-PK preferentially phosphorylates and inactivates components of the transcription complex via interaction with the ends of the transcribed template (6). It is also an important finding, however, that DNA ends are not essential for this repression (see *Discussion*).

The Repression by DNA-PK Is Specific for pol I. Fig. 2 shows that DNA-PK has no effect on promoter-directed transcription by both pol II and pol III. In the experiment shown in Fig. 2*A*, the pol I minigene was cotranscribed with a *Xenopus* somatic type 5S gene (22) in the crude S-100 extract. Both templates were used after linearization at the unique *Ssp* I site in the vector. In this system, DMAP leads to a stimulation of pol I transcription in the control reactions without DNA-PK (lane 2). As mentioned above, this stimulation is presumed to be due to the inhibition of an endogenous, unidentified protein kinase that represses transcription by pol I (ref. 16; see *Discussion*). Again, DNA-PK inhibited transcription by pol I (lane 3), and DMAP relieved this inhibition (lane 4). The cotranscribed pol III gene, which yielded the expected product of 120 nt, also showed some stimulation by DMAP (compare lanes 1 and 3 to lanes 2 and 4) but was unaffected by DNA-PK (lanes 3 and 4).

Fig. 2*B* shows a similar experiment in which the pol I minigene was cotranscribed with the HSV-TK gene. Both plasmids were used as linears. The promoter of this pol II gene had been well characterized in *Xenopus* cells, indicating that it is recognized by the *Xenopus* pol II transcription machinery (20). Unlabeled RNA from such cotranscription reactions was split into two aliquots and analyzed by a nuclease S1 protection assay using end-labeled DNA probes specific for the ribosomal and the HSV-TK promoter, respectively. Transcription by pol I (lanes 1–4) showed the same changes upon addition of DNA-PK and DMAP as described above for Fig. 2*A*, but initiation at the pol II promoter was not affected by DNA-PK (Fig. 2*B*, lane 8). Initiation at the HSV-TK promoter was by pol II, since it was sensitive to α-amanitin (50 μg/ml) (lane 10). The same amount of α-amanitin did not affect the ribosomal promoter (lane 5).

DNA-PK Affects a Step Before Initiation. As a first step toward identifying the process during transcription and the component of the transcription machinery that are inhibited by DNA-PK, the S-100 extract was fractionated over DEAE and heparin Sepharose columns to yield two fractions, H-0.4 and H-0.8. In agreement with a previous study (12), H-0.4 was found to contain RNA polymerase activity (data not shown). Western blot analysis showed that H-0.4 also contained the bulk of transcription factor xUBF, but xUBF was also detected

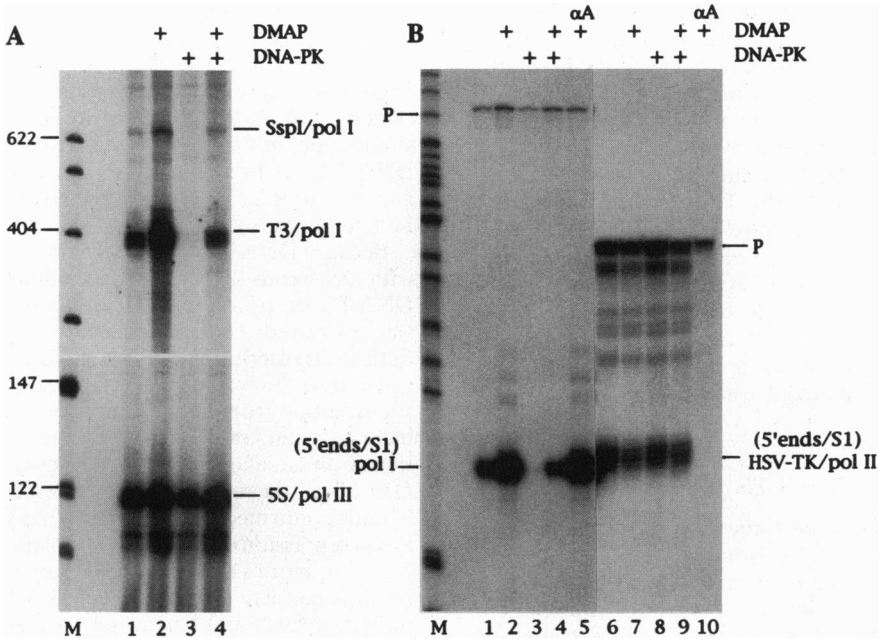

FIG. 2. DNA-PK does not inhibit transcription by pol II and III. (*A*) Cotranscription of linear P-T3wt and linear pX1s11 (5S gene) in the S-100 extract. RNA was labeled during transcription for 2 hr. DNA-PK (0.5 μl) and/or DMAP (2 mM) were added to the reaction mixtures as indicated above the lanes. Two areas of the same autoradiograph are shown. 5S/pol III, 120-nt 5S RNA. Lane M, end-labeled *Hpa* II-digested pBR322. The length (in nt) of some of the marker DNA fragments is indicated on the left. (*B*) Cotranscription of linear P-T3wt and linear HSV-TK in the S-100 extract. Unlabeled RNA was analyzed by nuclease S1 protection assay using probes specific for RNA 5′ ends at the pol I promoter (lanes 1–5) or specific for RNA 5′ ends at the pol II promoter (lanes 6–10). DNA-PK and DMAP were added as in *A*. The reaction mixtures analyzed in lanes 5 and 10 contained 50 μg of α-amanitin per ml. P, undigested nuclease S1 probe.

in H-0.8 (data not shown). The factor eluting from heparin Sepharose with high salt (H-0.8) was named Rib1 in *Xenopus* (12) and corresponds to factor SL1 in humans (13). In *Xenopus*, Rib1 and xUBF are the only factors that are known to be required in addition to pol I for initiation from the ribosomal gene promoter (12, 23). Since both H-0.4 and H-0.8 were required to reconstitute efficient transcription by pol I (data not shown), I conclude that pol I and Rib1 have been efficiently separated on the heparin Sepharose column.

Using this fractionated system, single-round transcription reactions were carried out to determine whether DNA-PK inhibits a step before or after initiation of transcription (Fig. 3). After assembly of initiation complexes in H-0.4 and H-0.8, transcription was started by adding the two initiating nucleotides AMP-PNP (a β,γ-nonhydrolyzable ATP analogue) and GTP. Based on the sequence of the 5′ end of the transcript (24), the majority of the elongation complexes should stall after synthesis of a 9-nt transcript. Elongation was allowed to proceed by adding CTP, UTP, and [α-^{32}P]CTP. Simultaneous addition of heparin prevented further initiation events. As expected, a control reaction in which AMP-PNP and GTP were omitted did not produce a detectable level of RNA (lane 7), and addition of heparin 5 min after CTP and UTP gave a 5 to 10 times stronger signal (lane 8) than the control reaction in which heparin was added along with CTP and UTP (lane 1), reflecting continuing initiation. Addition of DMAP (lane 2) slightly increased the signal as compared to the control reaction, suggesting that there was a low level of repression of transcription by endogenous protein kinases. If DNA-PK was present during the entire reaction, transcription was completely inhibited (lane 3), but the repression was relieved by DMAP (lane 4). If DNA-PK was active only during the assembly of the preinitiation complexes but was inhibited with DMAP at the time of initiation, the strong repression was still seen (lane 5). However, if DNA-PK was added only just after initiation and allowed to act on the stalled elongation complexes for the same total time, no repression was seen (lane 6). These results indicate that DNA-PK interferes with the process of initiation complex assembly.

In the reactions shown in Fig. 3*B* (lanes 1–8), AMP-PNP provided the adenosine residue for incorporation into RNA, and dATP was the phosphoryl donor for the kinase reaction. Even though DNA-PK was found to be active under these conditions (25), the reactions of lanes 1, 5, and 6 were repeated with ATP substituting for AMP-PNP (lanes 9–11). It can be seen that again DNA-PK inhibited transcription only when present before initiation (lane 10) but not when present during elongation (lane 11).

Evidence for a Role of Transcription Factor Rib1 in Mediating the Repression by DNA-PK. To determine which of the two heparin Sepharose fractions was inactivated by DNA-PK, the experiment diagrammed in Fig. 4*A* and shown in Fig. 4*B* was performed. Either the complete reaction mixture, H-0.4 alone, or H-0.8 alone (fraction-1) was preincubated with DNA-PK and template DNA. After 20 min, DNA-PK was inhibited by DMAP and, where applicable, the reaction mixture was supplemented with the missing heparin Sepharose fraction (fraction-2). At this point, transcription was allowed to start by the addition of nucleoside triphosphates. Heparin was added 5 min later to prevent further initiation and reinitiation.

Control reactions without DNA-PK showed that preincubation of single heparin Sepharose fractions led to weaker transcription (Fig. 4*B*, lanes 8 and 9) than preincubation of the complete system (lane 7). Additional controls indicated that the weaker signals in lanes 8 and 9 were caused by the fact that these reactions had less time to assemble functional initiation complexes after the addition of the second heparin Sepharose fraction (data not shown). Pretreatment of the complete reaction mixture with DNA-PK led to a strong inhibition of transcription (lane 1). A similar level of inhibition was also observed when H-0.8 was pretreated alone with DNA-PK (lane 3). Preincubation of H-0.4 with DNA-PK, on the other hand, had no significant effect on transcription (compare lanes 2, 5, and 8). Reactions in which DNA-PK was inhibited by

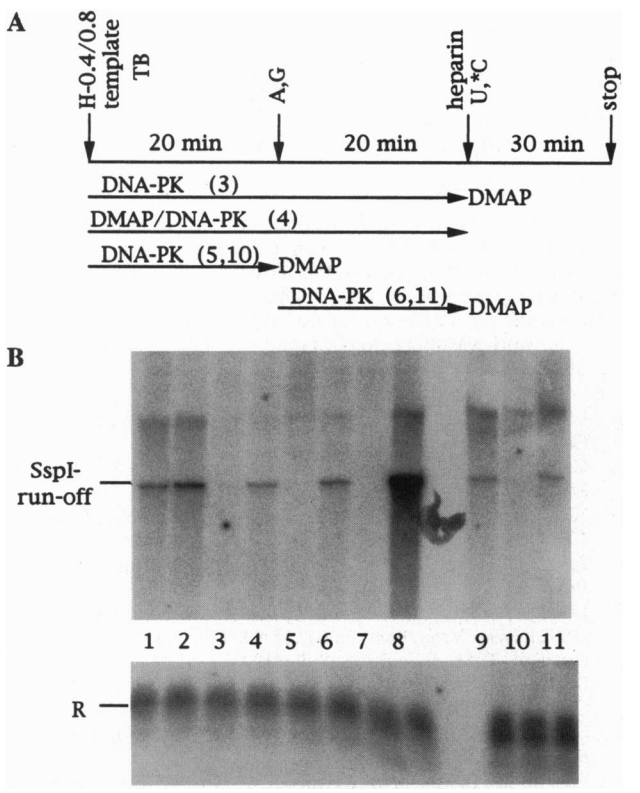

FIG. 3. DNA-PK affects a step before initiation. (*A*) Diagram of experiment to identify the step during transcription that is affected by DNA-PK. Horizontal arrows indicate additions of DNA-PK and DMAP. The reaction mixtures to which arrows apply are given in parentheses and correspond to the lanes in *B*. TB, transcription buffer; A, AMP-PNP in reactions 1–8 and ATP in reactions 9–11; *C, [α-^{32}P]CTP; G, GTP; U, UTP. (*B*) Result of transcription reactions outlined in the diagram in *A*. Control reaction mixtures not indicated in *A* were as follows: lanes 1 and 9, no addition; lane 2, DMAP; lane 7, no AMP-PNP and GTP; lane 8, heparin addition 5 min after UTP and [α-^{32}P]CTP. R, sample processing and recovery control (endogenous RNA of ≈80 nt present in H-0.4 that becomes end-labeled during the reaction). For the *Ssp* I runoff and the R bands, two areas of the same autoradiograph are shown.

DMAP from the beginning (lanes 4–6) gave a similar result as the control reactions without DNA-PK (lanes 7–9), except that the transcription signals were somewhat weaker throughout, most likely because of some residual DNA-PK activity. The bands in Fig. 4*B* were quantitated by densitometry and are shown in the graph in Fig. 4*C* expressed as percentage remaining transcription compared to the corresponding control reactions without DNA-PK (lanes 7–9). These results suggest that the inhibition of pol I transcription by DNA-PK is mediated by the transcription factors present in H-0.8: Rib1 and xUBF. However, Rib1 appears to play a central role in this inhibition, since H-0.4, which contains the bulk of xUBF, could not be inactivated by pretreatment with DNA-PK (lane 2), and since untreated H-0.4 could not rescue DNA-PK-treated H-0.8 (lane 3).

DISCUSSION

In the present paper, I report that purified DNA-PK strongly suppresses transcription by pol I *in vitro*. The inhibition is due to phosphorylation of a protein in the reaction, since the kinase inhibitor DMAP relieves the repression. The finding that repression by DNA-PK is also seen with circular templates indicates that DNA ends, which are most effective in activating DNA-PK, are not essential for this repression. Even though the DNA structure on the circular template that activates

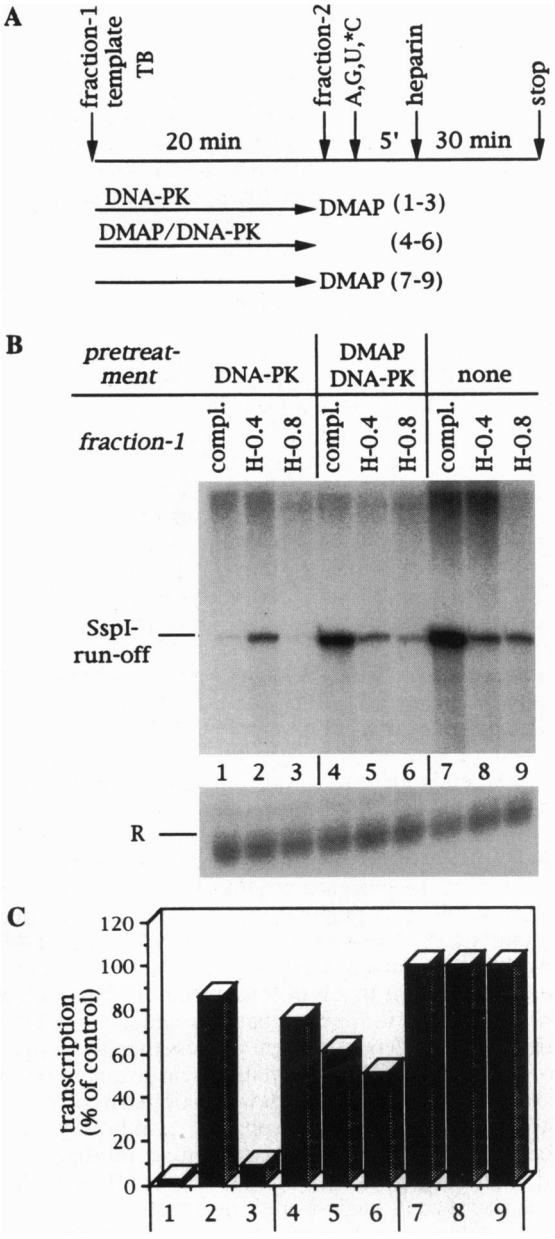

FIG. 4. The H-0.8 fraction contains the component inactivated by DNA-PK. (*A*) Diagram of the experiment to identify the fraction inactivated by DNA-PK. Horizontal arrows indicate the type of pretreatment of fraction-1 in the reactions given in parentheses (corresponding to lanes in *B*). See text for details. Abbreviations are as in Fig. 3*A*. (*B*) Result of transcription reactions outlined in the diagram in *A*. The type of pretreatment of fraction-1 is indicated above the lanes. compl., H-0.4 plus H-0.8 (complete); R, sample processing and recovery control as in Fig. 3*B*. (*C*) Densitometric analysis of transcription signals of the experiment shown in *B*. The signals are expressed as percentage remaining transcription compared to the corresponding control reactions without DNA-PK—i.e., the signals of lanes 1–3 and 4–6 are expressed as percentage of the signals in lanes 7–9, respectively.

DNA-PK is not known, it is intriguing that a recent study found that a dumbbell structure efficiently activated DNA-PK but to a slightly lower maximal level than a comparable linear DNA (10). DNA structures similar to dumbbell structures might at least transiently be present during promoter melting. It is thus possible that this same repression takes place during ribosomal gene transcription under normal *in vivo* conditions, where double-stranded DNA ends are not present.

The finding that cotranscription of a 5S gene and of the HSV-TK gene (having a classical pol II promoter with a

TATA box) in the same cell extract is not affected at all by DNA-PK shows that DNA-PK does not nonspecifically inhibit the transcription process but strongly suggests that DNA-PK acts on a factor specific for promoter-directed transcription by pol I. Furthermore, since the inhibition is also seen if the transcription factors are pretreated with DNA-PK, followed by inhibition of the enzyme and a single round of transcription, DNA-PK appears to inhibit a step during the assembly of the initiation complex on the ribosomal promoter. McStay *et al.* (12) showed that in *Xenopus* both Rib1 and xUBF are required to form a stable initiation complex. Rib1 has been defined only as a factor eluting from a heparin Sepharose column at ≈0.6 M KCl, and its polypeptide composition has not been determined yet. However, Rib1 appears to correspond to the human factor SL1 and the mouse factor TIF-IB, which have been shown to consist of the TATA-binding protein (TBP) and three pol I-specific TBP-associated factors (TAF_Is) (26, 27). The present finding that pretreatment of the Rib1-containing fraction with DNA-PK in the presence of DNA leads to the same inhibition of transcription as pretreatment of the complete reaction mixture strongly suggests that the inhibition is mediated by Rib1. Even though xUBF is clearly present in the Rib1 fraction (H-0.8) and may play a role in the inhibition process, xUBF by itself or in conjunction with pol I apparently cannot mediate the inhibition. The data are consistent with a mechanism, however, in which DNA-PK phosphorylates and inactivates the preinitiation complex of Rib1 and xUBF on the promoter.

I have recently identified two steps during ribosomal gene transcription *in vitro* that are sensitive to protein phosphorylation by endogenous protein kinases present in the S-100 extract (16). One of them manifested itself by a stimulation of transcription in the presence of DMAP (see also Fig. 2, lanes 1 and 2). While it is possible that the endogenous protein kinase inhibited by DMAP is a *Xenopus* DNA-PK, the DMAP-induced stimulation was seen only late in the reaction and depended on a factor that is different from the basic initiation factors. Therefore, it appears that this endogenous DMAP-sensitive kinase affects a different process than the exogenous DNA-PK. A second phosphorylation-sensitive step occurred before or during initiation (16). It was inhibited by okadaic acid and stimulated by protein phosphatase 1, indicating that the dephosphorylation of serine or threonine residues was involved. Like the repression by DNA-PK, the effect of okadaic acid was also seen in single-round transcription reactions. The available data are thus consistent with the notion that DNA-PK and protein phosphatase 1 antagonistically affect the same step during transcription initiation.

This work reports regulation of ribosomal gene transcription by DNA-PK *in vitro*. This is an important step in the search for a function of this nuclear kinase, even though the *in vivo* significance of the present findings remains to be shown. The observation that DNA-PK can repress pol I transcription on circular templates raises the possibility that DNA-PK might be involved in down-regulation of ribosomal gene transcription under normal cellular conditions, where DNA ends are not present. It is well known that pol I transcription is down-regulated when the cells shift from logarithmic growth to stationary phase. A growth-regulated factor has been identified and found to be closely associated with pol I (reviewed in refs. 23 and 28). However, there is evidence for additional mechanisms to regulate ribosomal gene transcription by both positive and negative factors (29–31). If DNA-PK is involved in growth regulation of the ribosomal genes, DNA-PK should be found in the nucleolus and be more active in resting cells. Consistent with such a model is a study which reported that Ku was found in the nucleolus of quiescent cells during early G_1 (32), and in a different study increased DNA-PK activity was found to accompany HL-60 cell differentiation (33). To test the *in vivo* significance of the present results, it will be important to examine in detail the subcellular location and activity of DNA-PK, to correlate it with ribosomal gene activity, and to find ways to selectively inhibit DNA-PK in the cell.

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