Vol. 96, No. 3, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 16, 1980 Pages 1216-1224

IS PROTEIN KINASE A SUBUNIT OF RNA POLYMERASE II, WHICH IS RESPONSIBLE FOR THE SPECIFICITY OF TRANSCRIPTION ?

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Received July 25,1980

SUMMARY: In vitro chromatin protein phosphorylation is enhanced in chromatin isolated from gibberellin-treated maize seedlings, as compared with chromatin from control plants. Studies of the influence of gibberellin on the template activity of maize chromatin, using homologous RNA polymerase II, showed that polymerase contains a factor dissociating in 0.35 M $(NH_4)_2SO_4$, which is decisive of the enzyme specificity. It was found that at this ionic strength protein kinase was removed from RNA polymerase. A role for protein kinase as a RNA polymerase II subunit, responsible for the specificity of transcriptase, is postulated.

INTRODUCTION

Chromatin isolated from gibberellin-(GA_3)-treated maize seedlings has shown a higher chromatin-bound RNA polymerase II activity than chromatin from control plants (1). Since in eukaryotic cells almost all mRNA contains a covalently linked poly/A/ segment (2,3), mRNA can easily be separated on an oligo-dT-cellulose column. Using this technique we have shown that poly/A/--rich RNA synthesized by chromatin from GA_3-treated plants stimulates protein synthesis in vitro about 14 times more efficiently than RNA synthesized by control chromatin (4). This observation supports the previous findings from our laboratory stating that GA_3 stimulates mRNA synthesis in vivo (5).

Moreover, there is evidence that activated chromatin shows enhanced phosphorylation of chromatin proteins (6).

0006-291X/80/191216-09$01.00/0

The aim of the present study was to investigate the effect of GA_3 treatment of maize seedlings on the template activity of chromatin, demonstrated by addition of homologous maize RNA polymerase. The effect of GA_3 on the chromatin-associated protein kinase activity was also studied.

MATERIALS AND METHODS

Maize (Zea mays var. 72K) seeds were grown in the dark at 26°C. Five days old seedlings were sprayed with GA_3 (10^{-5}M) or with water (control); after 24-h cultivation, the apical parts of shoots were harvested and used for chromatin preparation. Chromatin was isolated at pH 6.0, as described previously (1).

Assay of RNA synthesis. Standard mixture (0.1 ml) contained: 4 µmol Tris-HCl (pH 8.0), 5 µg bovine serum albumin, 40 nmol each of ATP, GTP, CTP, and 1 µCi of [^{3}H]-UTP (52 Ci/mmol, Amersham), 1 µmol 2-mercaptoethanol, 20% glycerol, 0.8 µmol $MgCl_2$, 0.15 µmol $MnCl_2$, 20 µmol KCl, and chromatin equivalent to 2-10 µg DNA. In the preparation of RNA polymerase, instead of chromatin 10 µg of denaturated calf thymus DNA (Sigma) or native homologous maize DNA were used.

After incubation at 30°C for 15 min, the reaction was terminated by addition of 5% ice-cold trichloroacetic acid (TCA) containing 10 mM sodium pyrophosphate. The precipitate was collected on a glass fiber filter (Whatman GF/A), washed, dried and counted for radioactivity.

DNA from maize seedlings was prepared according to Marmur (7) or Britten et al. (8).

The chromatin DNA content was determined as described by Giles and Myers (9), and protein was assayed according to Lowry et al. (10) or to Heil and Zillig (11).

Maize RNA was prepared after Grierson and Covey (12).

Assay for protein kinase activity. Standard mixture (0.1 ml) contained: 5 umol Tris-HCl pH 7.5, 1 µmol $MgCl_2$, 1 µmol 2-mercaptoethanol, [γ-^{32}P]-ATP (2-3 Ci/mmol) and chromatin (5-20 µg DNA). [γ-^{32}P]-ATP was prepared according to Böhm et al. (13). After 20 min. of incubation at 30°C, the reaction was terminated by addition of 3 ml of 5% ice-cold TCA containing 10 mM sodium pyrophosphate, 10 mM adenosine and 1% orthophosphoric acid The precipitate was collected on a glass fiber filter (Whatman GF/A). Filters were washed with 10 ml of the mixture for termination of the reaction, then with 40 ml of 5% TCA, and with 10 ml of 96% ethanol containing 20% of ethyl ether. Filters were dried and counted for radioactivity.

Sodium dodecyl sulphate gel electrophoresis. In vitro phosphorylated chromatin proteins were dissociated in a solution containing 2.5% SDS, 5% 2-mercaptoethanol, 0.03 M Tris-HCl pH 6.8, and 25% sucrose, at a concentration of about 20 µg protein per 1 µl, at 100°C for 5-10 min. Electrophoresis of these proteins was performed in 17% acrylamide gel containing 0.1% SDS and 0.37% Tris-HCl (pH 8.8), as described by Laemmli (14). Fluorography was carried out according to Bonner and Laskey (15).

Preparation of DNA-dependent RNA polymerase was performed by the method of Teissere et al., (16), as modified by Muszyńska (17). Five days old maize seedlings were ground in two volumes(v/w) of a solution containing 100 mM Tris-HCl (pH 7.85), 0.01 M $MgCl_2$, 0.01 M 2-mercaptoethanol and 10 mM glycerol.
The homogenate was centrifuged at 33 000 g for 20 min. The supernatant was brought to 50% saturation with ammonium sulphate, and after 30 min. the precipitate was collected by centrifugation at 12 000 g for 20 min. The precipitate was dissolved in buffer H (50 mM Tris-HCl pH 7.8, 5 mM $MgCl_2$, 0.1% TritonX100, 0.1 mM EDTA, 25 mM 2-mercaptoethanol, 25% glycerol), and dialyzed against the same buffer. This fraction was called preparation C (ionic strength equivalent to 35 mM $(NH_4)_2SO_4$). A part of this preparation was dialyzed to ionic strength equivalent to 350 mM $(NH_4)_2SO_4$ (controlled with a standard conductivity meter); it was called preparation A. Preparations C and A were treated by affinity chromatography on a column of heparin bound to Sepharose 4B (1.5 x 12 cm), prepared according to Teissere et al. (16). The column was washed with buffer H, till disapperance of absorption at 280 nm. In case of preparation A, the wash fraction (preparation W) was collected.

The enzymic activities were eluted with linear gradient of 0-1 M KCl in buffer H. Two RNA polymerase preparations were obtained: 1. from preparation C - enzyme preparation S (low ionic strength), and 2. from preparation A - enzyme preparation U (high ionic strength).

RESULTS AND DISCUSSION

Phosphorylation of chromatin proteins after GA_3 treatment of maize seedlings

The rate of ^{32}P incorporation into the nuclear phosphoprotein fractions varies at different physiological stages of the organism (6). There is also some evidence that hormonal treatment of animals increases the activity of kinases (17). Table I presents

TABLE I Effect of gibberellin (GA_3) on the chromatin-bound protein kinase activity in maize seedlings

Chromatin	Phosphorylation of chromatin proteins	
	cpm/µg DNA	increase in actibity %
From control maize seedlings	7 398	100
From GA_3-treated maize seedlings	19 279	260

Fig. 1. Densitography of fluorographs after polyacrylamide gel electrophoresis of chromatin phosphorylated proteins.
/-----/ chromatin from control maize seedlings
/———/ chromatin from gibberellin-treated maize seedlings

the influence of GA_3 on the chromatin-bound protein kinase activity. It was found that chromatin isolated from GA_3-treated seedlings contains an over two times higher protein kinase activity than chromatin from control plants. Fig. 1 shows that the increase in the phosphorylation rate concerns mainly non-histone chromosomal proteins (particularly within the higher molecular weight region). This is consistent with the observations of Murray and Key (19), and of Mazuś et al. (20).

Isolation of RNA polymerase

Patterns of the elution of the RNA polymerase activities (S and U) from a heparin-Sepharose 4B column were closely similar. Fig. 2 presents a typical pattern obtained for RNA polymerase (preparation S, low ionic strength).
This activity was characterized by a sharp peak. Table II shows some properties of enzyme preparation S. It was found that the total activity of the preparation is due to RNA polymerase II, and that no activity of RNA-dependent RNA polymerase is present.

Template activity of chromatin

Various preparations of maize RNA polymerase II were used to study the template activity of chromatin isolated from control

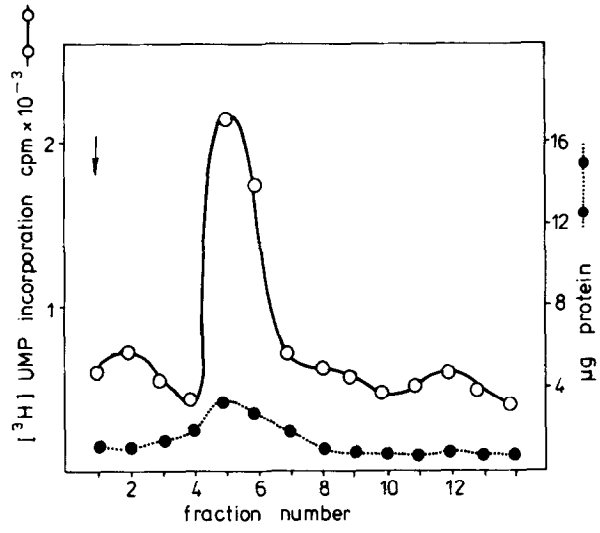
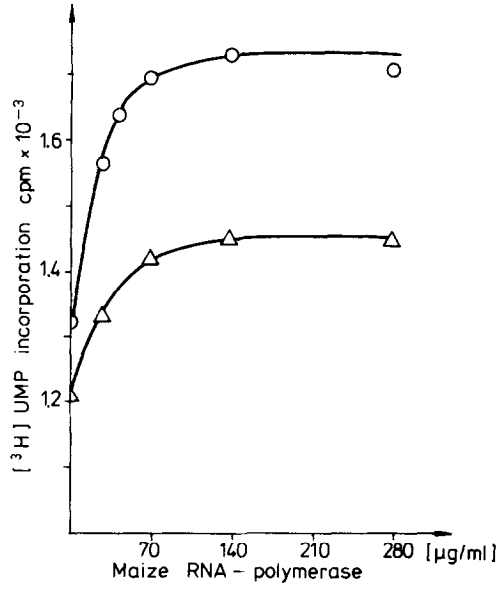
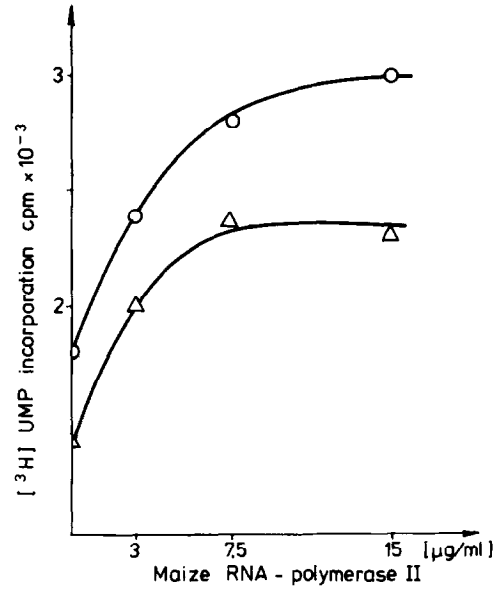


Fig. 2. Fractionation of maize RNA polymerase II on a heparin-Sepharose 4B column. Arrow indicates start of linear gradient of 0-1 M KCl.



(3)



(4)

Fig. 3. Template availability of chromatin isolated from control /-△-△-/ and from gibberellin-treated maize seedlings /-o-o-/, assayed using homologous RNA polymerase II preparation C, crude enzyme .

Fig. 4. Template availability of chromatin isolated from control /-△-△-/ and gibberellin-treated maize seedlings /-o-o-/, assayed using homologous RNA polymerase II (preparation S, low ionic strength).

TABLE II Properties of purified RNA polymerase II (preparation S, low ionic strength) isolated from control maize seedlings

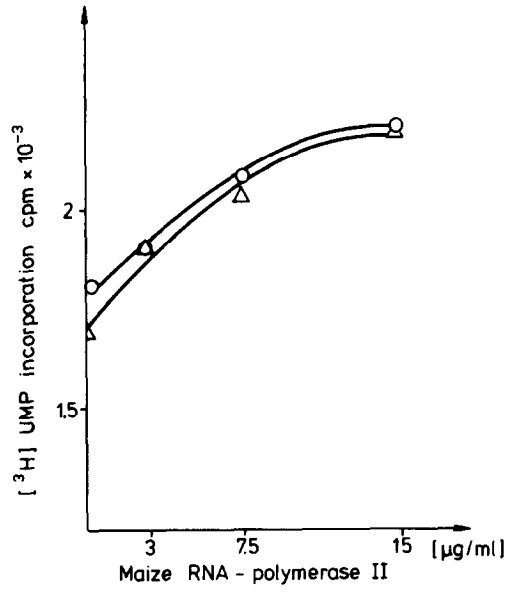
Additions in RNA polymerase assay	Incorporation of [^{3}H]-UTP cpm/µg protein
1. 10 µg of maize DNA	1230
2. 10 µg of maize DNA + Actinomycin D(20 µg)	ND[x]
3. 10 µg of maize DNA + α-amanitin (0.5 µg)	ND
4. Without DNA, + 10 µg of maize RNA[xx]	ND

[x] not detectable

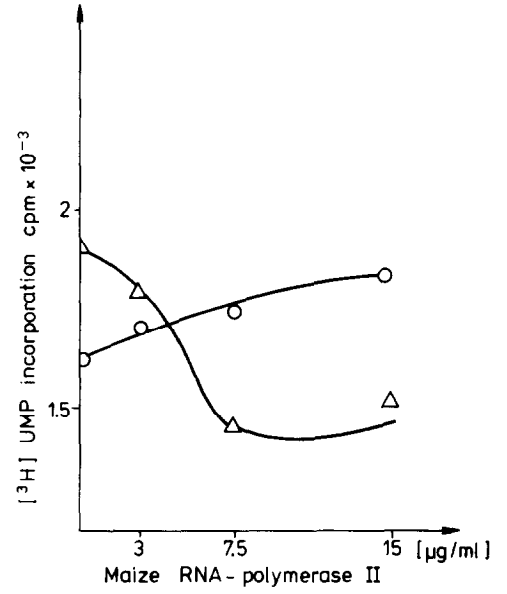
[xx] RNA was digested with DNAse free from RNAse

and GA_3-treated maize seedlings. Fig. 3 presents the data concerning the saturation of both chromatins with crude RNA polymerase II (preparation C). Chromatin isolated from the GA_3-treated plants was found to have more polymerase-binding sites than control chromatin. Data obtained for purified enzyme preparation S (low ionic strength) (Fig. 4) showed that the binding sites exhibit affinity only to RNA polymerase II (cf. Table II).

After dissociation of the enzyme preparation at high ionic strength prior to affinity chromatography on heparin-Sepharose 4B (preparation U), the specificity of polymerase II to the activated binding sites of chromatin disappeared (Fig. 5). When the wash from the column (fraction W) was added to the assay containing "unspecific" polymerase (preparation U), the GA_3 activation of chromatin reappeared (final part of the saturation plot in Fig. 6). Thus, preparation W contains some unidentified factor res-



(5)



(6)

Fig. 5. Template availability of chromatin from control /-△-△-/ and gibberellin-treated maize seedlings /-o-o-/, assayed using homologous RNA polymerase II (preparation U, high ionic strength).

Fig. 6. Saturation of chromatin with RNA polymerase II (preparation U, high ionic strength) in the presence of "wash" fracion (W) (34 µg of protein).
-△-△- chromatin from control maize seedlings
-o-o- chromatin from gibberellin-treated maize seedlings

ponsible for the specificity of RNA polymerase II for the activated binding sites of chromatin. Fig. 6 indicates that the ratio of this factor in preparation W to RNA polymerase II is very important. At low concentrations of polymerase, this factor even inhibited the transcription of GA_3-chromatin as compared with control chromatin. When the concentration of RNA polymerase II was increased, the specificity of the enzyme became appeared.

Teissere et al. (21) have studied the effect of auxin on gene expression in lentil roots, and reported similar dissociation of some factor from RNA polymerase I. Revie and Dahmus (22) have obtained a heat-stable factor which stimulated the polymerase II activity from lamb thymus; this factor dissociated from polyme-

rase II at higher ionic strengths (above 40 mM). Mazuś et al. (20) have reported that during purification of RNA polymerase II from wheat germ, some protein kinase activity was co-purified with RNA polymerase. Only dissociation at high ionic strength and sucrose-gradient centrifugation separate these activities.

On the basis of the present results and of the data from the literature, we put forward the hypothesis that the factor decisive of the RNA polymerase II specificity is a protein kinase playing the role of the transcriptase subunit, similar to the sigma subunit in E. coli polymerase.

Therefore, we checked also the protein kinase activity in our polymerase preparations. It was found that preparation S (low ionic strength) showed protein kinase activity, which decreased over 2 times in preparation U (high ionic strength) (unpublished data). This observation confirms our hypothesis. Purification of this protein kinase and examination of its function may be an interesting topic for further investigation.

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