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Transcription-Inhibiting Factor in Pea Seedling Chromatin

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Chromatin isolated from 5-day-old pea seedlings exhibits no template activity for chromatin-bound RNA polymerases. Pea chromatin completely inhibited transcription of the E. coli polymerase with calf thymus DNA or pea DNA as a template. More than 80% of the inhibiting activity was localized in the non-histone protein fraction of chromatin. It was found that the inhibiting factor is an enzyme which decomposes UTP and ATP to nucleoside monophosphates.

Non-histone proteins of chromatin exhibit several enzymatic activities (Rickwood et al., 1973; Kisch & Kleinsmith, 1974; Hirasawa et al., 1977) which seem of importance in regulation of transcription (Kostraba & Wang, 1972). Evidence has been presented that non-histone proteins are involved in the control of the in vitro RNA synthesis in plants (Yoshida & Sasaki, 1977; Hirasawa et al., 1978).

We have shown previously that gibberellic acid stimulated preferentially the synthesis of maize seedling mRNA (Wasilewska & Kleczkowski, 1976) and the transcription activity of chromatin (Jankowski & Kleczkowski, 1978). In the present work it was found that chromatin from pea seedlings used as a template in the transcription assay with homologous as well as with E. coli RNA polymerase, was fully inactive.

Materials and Methods

Dwarf pea seeds var. de Grace and maize seeds var. K-73 were sterilized in 0.15% sodium hypochlorite, soaked for 15 h in tap water, sown in trays covered with moist cotton wool, and cultivated in darkness at 21°C for five days. The apical parts of seedlings were harvested and used for chromatin preparation. The embryos

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of pea seeds were collected after 15 h of germination. Preparation of chromatin was based on the method of Lin et al. (1974) with a slight modification (Jankowski & Kleczkowski, 1978).

**Dissociation and fractionation of chromatin.** Chromatin was suspended in 10 mM-Tris/HCl buffer, pH 8.0, containing 10 mM-MgCl₂, placed on a magnetic stirrer, and solid NaCl was added to 3.0 M final concentration. Stirring was continued for 2 h. The dissociated chromatin was centrifuged at 200 000 g for 24 h. The sedimented DNA was removed, and the supernatant was treated with a saturated ammonium sulphate solution. The protein precipitated at 0.7 saturation, was sedimented at 15 000 g for 20 min, and the pellet was dissolved in 10 mM-Tris/HCl, containing 0.4 M-NaCl. Ammonium sulphate was removed by desalting on a Sephadex G-25 column. The desalted sample was loaded on a Bio-Rex 70 column equilibrated with 0.4 M-NaCl in 10 mM-Tris/HCl, pH 8.0. Protein was eluted with a stepwise gradient of 0.4, 1.0 and 3.0 M-NaCl (30 ml each). The collected fractions, monitored at 280 nm, were dialysed against water, lyophilized and used in the transcription assay.

Protein content was estimated by the method of Lowry et al. (1951), and DNA content by the diphenylamine method (Giles & Myers, 1965).

**Transcription assay.** E. coli RNA polymerase (Sigma Chem. Corp., Los Angeles Calif., U.S.A.) activity was assayed with calf thymus DNA as template under standard conditions (Lin et al., 1974). Pea chromatin or chromatin from maize seedlings, as well as the chromatin non-histone proteins were added to the assay mixture in the amount indicated in the Figures.

**Decomposition of nucleoside triphosphates by chromatin preparations.** [³H]UTP, 1 µCi or [³H]ATP, 1 µCi (both from the Radiochemical Centre, Amersham, England; spec. act. 52 and 19 Ci/mmol, respectively) was incubated at 37°C (Jankowski & Kleczkowski, 1978) for the desired time with chromatin (2 - 10 µg of DNA) or the non-histone protein fraction (0.5 - 5.0 µg of protein). The final volume of the incubation mixture was 0.1 ml. The reaction was terminated by addition of 4 vol. of cold (kept in solid CO₂) ethanol, and the sample was centrifuged.

**Thin-layer chromatography.** The supernatant obtained was spotted on a thin-layer cellulose chromatoplate (Merek 5718). The plate was developed in isobutyric acid/conc. ammonia/water (66:1:33, by vol.), and the radioactivity was located using a Berthold II TLC Chromatoscanner.

**RESULTS AND DISCUSSION**

Contrary to maize seedling chromatin (Jankowski & Kleczkowski, 1978), the chromatin isolated from dwarf pea seedlings did not show any detectable activity of chromatin-bound RNA polymerases. This observation suggested that pea chromatin contained a factor inhibiting RNA synthesis in vitro. This was confirmed by addition of pea chromatin to the E. coli RNA polymerase and calf thymus DNA transcription system. The E. coli RNA polymerase was active with maize chromatin, but not with pea chromatin (Fig. 1). Figure 2 shows that pea chromatin in the
Fig. 1. Effect of various templates on the activity of *E. coli* RNA polymerase. O, Calf thymus DNA, 5 μg; □, maize chromatin, 5 μg of DNA; △, pea chromatin from 5-day-old seedlings, 5 μg of DNA; ●, calf thymus DNA, 5 μg, supplemented with 2 μg of pea chromatin DNA, or 1 μg of pea chromatin non-histone protein.

Fig. 2. Effect of concentration and origin of DNA on the activity of *E. coli* RNA polymerase. O, Calf thymus DNA; ▲, purified pea DNA; △, pea chromatin DNA; ●, pea chromatin DNA supplemented with 5 μg of calf thymus DNA.
Fig. 3. Dependence of \textit{E. coli} RNA polymerase activity on the origin of chromatin. The polymerase was incubated with 5 \( \mu \)g of calf thymus DNA (○), and at the time indicated by arrows the following chromatin preparations were added: △, pea chromatin from 5-day-old seedlings, 2 \( \mu \)g of DNA; ▲, pea chromatin from embryos germinated for 15 h, 2 \( \mu \)g of DNA. The activity was unchanged on addition of 2 \( \mu \)g of maize chromatin DNA.

Amount equivalent to 0.5 \( \mu \)g of DNA inhibited transcription in about 65\%, and in the amount of 2.0 \( \mu \)g of DNA it caused 100\% inhibition of the \textit{E. coli} RNA polymerase activity, with calf thymus DNA as template. The inhibiting factor cannot be localized in DNA, since purified DNA from pea chromatin was active as template with \textit{E. coli} RNA polymerase (Fig. 2). When pea chromatin isolated

Fig. 4. Fractionation of dissociated pea chromatin on a Bio-Rex 70 column and distribution of the transcription inhibiting factor. For experimental details see Materials and Methods. ——, \( A_{260 \text{ nm}} \); ○, inhibition; NHP, non-histone protein; His, histones.
from 5-day-old seedlings was added during incubation of the assay mixture to the
*E. coli* RNA polymerase - calf thymus DNA system, the transcription was abruptly
inhibited (Fig. 3). In contrast, addition of maize chromatin under the same
conditions did not affect transcription. Chromatin isolated from pea embryos germinated
for 15 h showed a somewhat lower inhibitory effect as compared with chromatin
from 5-day-old seedlings (Fig. 3).

Since purified DNA from pea chromatin did not inhibit transcription (Fig. 2),
we fractionated pea chromatin into histones and the non-histone protein fraction,
and found that more than 80% of the total inhibitory activity was in the non-histone
protein fraction (Fig. 4). This may indicate that the minor inhibitory activity present
in the histone fraction was due to contamination with non-histone proteins. Heating
for 3 min at 70°C of pea chromatin, as well as of the non-histone protein fraction,
caused a complete loss of the inhibitory activity. Thermal lability of the inhibitor,
the results of chromatography on Sephadex G-25 and Bio-Rex, and non-diffusibility
during the chromatin - protein fractionation procedure, suggested that it could be
a protein. The sharp decline in the inhibitory activity upon heating (between 50 and
70°C) suggested that the chromatin inhibitor of transcription could be a protein
with an enzymatic activity.

We have found that alkaline phosphatase (EC 3.1.3.1) activity in pea chromatin
was 3 - 4 times higher as compared with that of maize seedling chromatin. Pyrophosphate
phosphohydrolase (EC 3:6.1.1) activity was also detected in pea chromatin (results not shown). The nucleoside triphosphate degrading activity in pea and maize chromatin, as well as in the non-histone protein fraction of pea chromatin
was tested. The results presented in Fig. 5 indicate that pea chromatin — but not
maize chromatin — contained a considerable nucleoside triphosphate degrading
activity, localized in the non-histone chromatin protein. Within 1 min incubation
of UTP with pea chromatin, under conditions applied in the transcription assay,
100% of the radioactivity of the labelled nucleoside triphosphate was found in UMP.
The same was observed with ATP (Fig. 5A). If instead of the equivalent of 5 μg of
chromatin DNA, the equivalent of 0.5 μg was used, after 1 min of incubation
more than 50% of the radioactivity was found in UMP (Fig. 5B). Maize chromatin incubated under those conditions did not show any detectable degradation of nucleo-
side triphosphates (Fig. 5B). When etiolated pea seedlings were replaced by light-
grown plants, the transcription was inhibited to the same extent. The same was
observed when dwarf pea was replaced by a normal, tall pea variety (results not
presented).

Since no nucleoside diphosphates were found among the degradation products
(Fig. 5), and pyrophosphate phosphorylase was detected in pea chromatin, it
may be concluded that the enzyme responsible for UTP and ATP degradation,
found in pea seedling chromatin, is a nucleoside pyrophosphohydrolase, similar
to ATP pyrophosphohydrolase (EC 3.6.1.8). Hirasawa *et al.* (1978), who used
pea cotyledon chromatin, reported that only orthophosphate was produced during
the degradation of UTP to UMP, and suggested involvement in this reaction of the
nucleotide phosphohydrolysing activity.
According to our knowledge, there is no single enzyme degrading in one step the nucleoside triphosphates to monophosphates, with orthophosphate as a reaction product. The presence of pyrophosphate phosphohydrolase in our chromatin preparation and the lack of nucleoside diphosphate as a degradation product in Hirasawa's et al. (1978) as well as in our preparations, support our suggestion that the enzyme(s) degrading UTP and ATP to UMP and AMP belong to the nucleoside pyrophosphohydrolases and not to nucleotide phosphohydrolases as suggested by Hirasawa et al. (1978). Our preparation from dwarf pea seedlings showed about ten times higher degradation of UTP to UMP as compared with the chromatin isolated by Hirasawa et al. (1978) from pea cotyledons. The pea cotyledon enzyme did not affect ATP while our preparation from pea seedlings degraded ATP and UTP to the same extent.

Our results have demonstrated that the chromatin isolated from various plant species shows differences in its template activity (maize and pea chromatin in Fig. 5). The rapid degradation of ATP and UTP as transcription substrates rendered impossible transcription in vitro with the dwarf pea chromatin. The physiological significance of the observed phenomenon as a possible mechanism of metabolic regulation on the transcription level in vivo seems hardly probable since ATP and UTP are important constituents of the cellular metabolic pool, involved not only in transcription but in other processes as well.
REFERENCES


CZYNNIK HAMUJĄCY TRANSKRYPCJĘ W CHROMATYNIE Z SIEWEK GROCHU

Streszczenie

Chromatyna izolowana z 5-dniowych siewek grochu nie wykazuje aktywności matrycowej dla polimerazy RNA związanej z chromatyną.

Chromatyna z grochu całkowicie hamuje transkrypcję RNA polimerazy *E. coli* z DNA z granicy ciecznej lub z grochu jako matrycy. Ponad 80% aktywności inhibitorowej było zlokalizowane we frakcji białek niehistonowych. Stwierdzono, że czynnik hamujący jest enzymem, który degraduje UTP i ATP do monofosforanów nukleozydów.

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