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Some biochemical properties of wheat chromatin

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Chromatin was isolated and purified from winter wheat shoots. Mass ratios of histone, nonhistone protein, and RNA to DNA in the chromatin were 1.17, 0.43, and 0.06, respectively. Tm values of the chromatin were 51.5° and 77.5°C in 0.1×SSC (pH 7.0). Optimum temperature of chromatin-directed RNA synthesis was 25° to 30°C. This chromatin considerably synthesized RNA at low temperature, 0° to 5°C. Optimum pH was about 8.0. Optimum concentrations of $(NH_4)_2SO_4$, $MgCl_2$, and $MnCl_2$ were 0.2 to 0.3 M, 16 mM, and 1 mM, respectively, both at low (4°C) and high (30°C) temperatures. The activity was almost completely inhibited with 100 μg/m*l* actinomycin D and partially with 0.1 to 100 μg/m*l* α-amanitin. About fifty percent of the total activity was α-amanitin-insensitive in 3-day-germinated shoots. The activity decreased during the periods of germination and cold treatment.

To study the regulatory mechanism of *in vivo* and *in vitro* syntheses of RNA and protein is interesting in a viewpoint of environmental control of growth and development of plants. Qualitative and quantitative alterations of RNA in nuclei are very important for induction of metabolic changes in responce to environmental changes. Winter wheat is an interesting material for study of germination at low temperature and low temperature-induced flowering. DNA-dependent RNA polymerases have been purified from wheat (POLYA and JARGENDORF, 1971 a; POLYA and JARGENDORF, 1971 b; POLYA, 1973; JENDRISK and BECKER, 1973). At the present time, regulation mechanism in RNA synthesis in wheat chromatin have not been defined. In the previous papers, certain changes of RNA and protein in winter wheat during germination and cold treatment were described (ISHIKAWA *et al.*, 1957a; ISHIKAWA *et al*, 1975b; ISHIKAWA and TATEYAMA, 1977; FUKUSHI *et al.*, 1977; SASAKI and TAZAWA, 1973; YOSHIDA and SASAKI, 1977). Our study has a purpose to elucidate the mechanism of low temperature effect on RNA synthesis in nuclei of winter wheat during germination and cold treatment.

In this paper, chemical composition and RNA-synthesizing activity of

Abbreviations: BSA, bovine serum albumin; PVS, polyvinyl sulfate; HEPES, N-2-hydroxyethylpiperazine-N-2-ethanesulphonic acid; SSC, standard saline citrate (0.15 M NaCl-0.015 M trisodium citrate, pH 7.0).

chromatin of winter wheat shoots are described.

Materials and Methods

Plant sources: *Triticum aestivum* L. cv. Mukakomugi (a type of winter wheat) was used throughout this study. After surface-sterilization with 0.1% Uspulun for 1 hr, seeds were rinsed in running tap water for 1 hr. The seeds were germinated in Petri-dishes at 24°C for 1 to 4 days in the dark. In cold treatment, seeds were kept in a cold room at 4°C in the dark for 15 to 60 days after 6 hr germination at 24°C. In this study, shoots were used after washing with sterile deionized water.

Isolation and purification of chromatin: For the determinations of chemical composition, UV-absorption spectrum and melting temperature, chromatin was isolated and purified from shoots by the method of BONNER *et al.*, (1968) and for the assay of chromatin-directed RNA-synthesizing activity by the method of LIN *et al.*, (1974).

Chemical composition: DNA was determined by the method of KECK (1956) and RNA by measurement of absorbance at 260 nm. Histone and nonhistone protein were determined by the method of LOWRY *et al.* (1951) using calf thymus histone and BSA as the standards, respectively.

UV-absorption spectrum: The spectra of DNA from wheat embryos and chromatin were determined in 0.1×SSC (pH 7.0) by means of Hitachi double-beam spectrophotometer Type 124 with integrating sphere in a cell of 10 mm of light path.

Half melting temperature (Tm): The Tm was estimated in 0.1×SSC with a Hitachi Perkin-Elmer spectrophotometer Type 139 equipped with a temperature-controlled cell.

Chromatin-directed RNA synthesis: The standard reaction mixture (0.25 m*l*) contained 10 μmoles Tris-HCl (pH 8.0), 2.5 μmoles $MgCl_2$, 0.25 μmoles $MnCl_2$, 25 μmoles $(NH_4)_2SO_4$, 2.5 μmoles β-mercaptoethanol, 0.1 μmoles each of ATP, GTP and CTP, 0.001 μmoles ^{3}H-UTP (1.5 μCi), 20 μg/m*l* PVS and an aliquot of chromatin (about 100 μg DNA). PVS was used as an inhibitor of RNase. Incubation was usually carried out at 30°C for 20 min. Radioactivity was determined in a toluene based scintillation solution. RNA-synthesizing activity was expressed as radioactivity incorporated into 5% TCA-insoluble materials per 100 μg DNA of chromatin in 20 min.

Chemicals: ATP, CTP, GTP and *E. coli* RNA polymerase were purchased from Boehringer Mannheim and DNase (RNase-free) from Worthington Biochem. Corp.. Actinomycin D and α-amanitin were obtained from Makor Chemicals, Ltd. and Boehringer Ingelheim, respectively. Tritium-

labeled UTP (27.6 Ci/mmol) was purchased from New England Nuclear Corp..

Results

Chemical composition, UV-absorption spectrum and Tm value: The mass ratios of histone, nonhistone protein and RNA to DNA of chromatin of 3-day-old shoots were 1.17, 0.43 and 0.06. This chromatin showed the typical UV-absorption spectrum of purified chromatin (BONNER *et al*, 1968) (Fig. 1).

This chromatin showed biphasic melting profile in 0.1×SSC, Tm values of which were 51.5° and 77.5°C (Fig. 2). Tm value of purified wheat DNA was 51.5°C in 0.1×SSC (unpublished). This chromatin had RNA polymerase activity. RNA polymerase II accounted for 27% of the total activity, as estimated by subtracting the value with 1 μg/ml α-amanition from that without the inhibitor. In chromatin obtained by the method of LIN *et al.* (1974), RNA polymerase II represented 41–50% of the total activity. Thus, this chromatin was used as the enzyme and template sources.

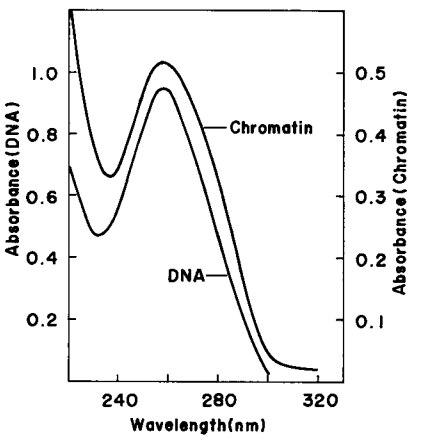

Fig. 1. UV-absorption spectra of purified chromatin and DNA. The spectra were measured in 0.1×SSC.

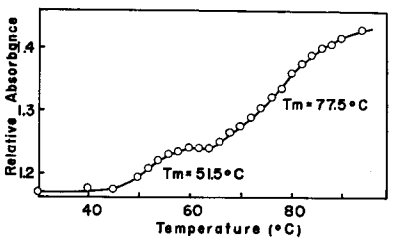

Fig. 2. Melting profile of purified chromatin. Optical density at 260 nm was plotted as a function of the temperature.

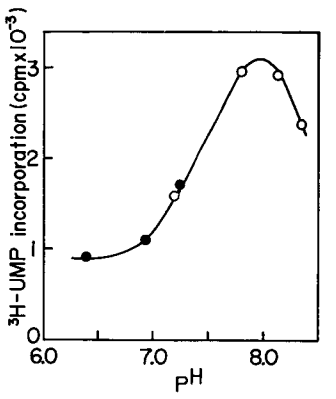

Fig. 3. Optimum pH for RNA synthesis. RNA synthesis was assayed in 0.01M Tris-HCl (○) or HEPES-NaOH (●) buffer solution.

Optimum conditions for RNA synthesis: Optimum pH was about 8.0 (Fig. 3). Optimum temperature of RNA polymerase in wheat chromatin was 24° to 30°C, and that of *E. coli* RNA polymerase was 37°C (Fig. 4). Wheat chromatin synthesized RNA considerably well even at 0° to 5°C, but *E. coli* RNA polymerase did not at all. RNA synthesis in the chromatin proceeded linearly for first 10 min at 24°C and began to decrease after 25 min. On the other hand, RNA synthesis at 3°C proceeded convexly for 3 hr (Fig. 5). The activity in wheat chromatin at low temperature was one-third

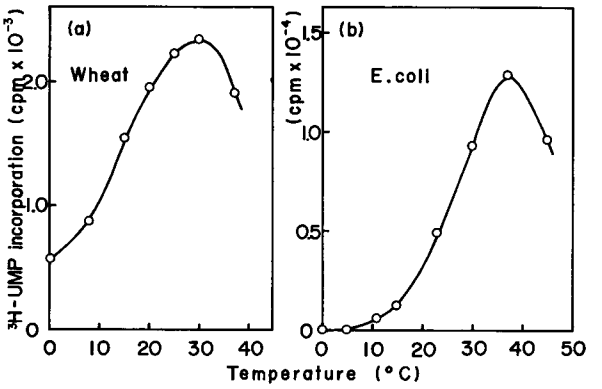

Fig. 4. Optimum temperature for RNA synthesis.
(a) Wheat chromatin-bound RNA polymerase activity. Reaction time was 20 min.
(b) *E. coli* RNA polymerase activity. Reaction time was 10 min. Chromatin, PVS and $(NH_4)_2SO_4$ were ommited from the standard reaction mixture, and 4μ moles/m*l* $MgCl_2$, 5.5 μg wheat DNA and *E. coli* RNA polymerase (1.25 units) were used.

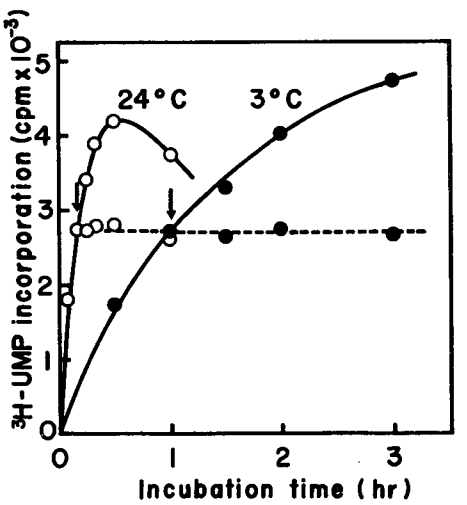

Fig. 5. Time courses for RNA synthesis at 3°C and 24°C. Reaction mixture was incubated at 3°C (●) or 24°C (○). The arrow shows the time of 1000-fold dilution of ^{3}H-UTP with unlabeled UTP.

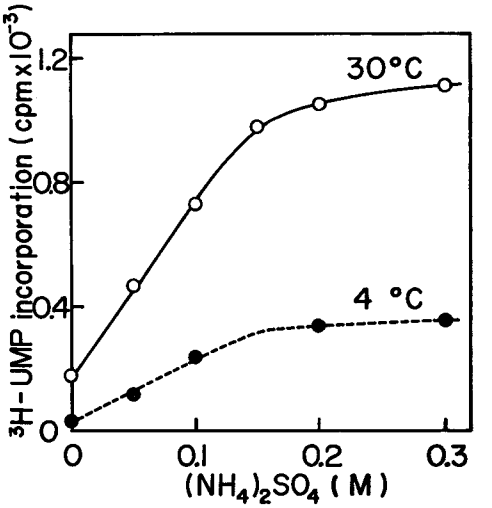

Fig. 6. Effects of various concentrations of $(NH_4)_2SO_4$ on RNA synthesis. The reaction was carried out at 4°C (●) or 30°C (○) for 10 min.

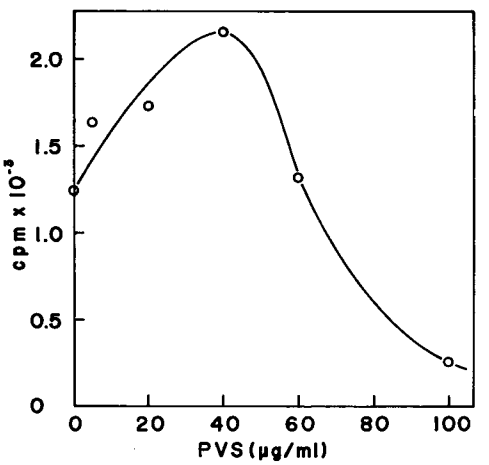

Fig. 7. Effect of PVS on RNA synthesis. Reaction time was 20 min at 30°C.

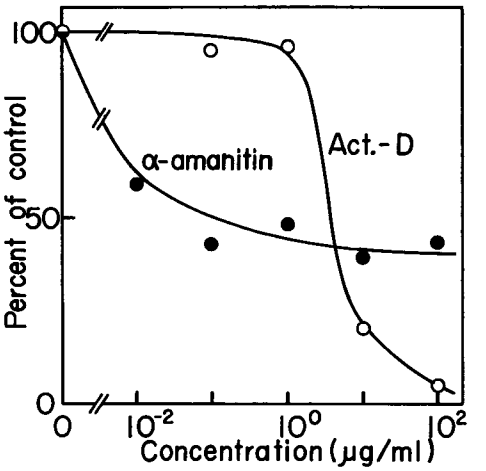

Fig. 8. Effects of actinomycin D and α-amanitin on RNA synthesis. The reaction was carried out in the presence of actinomycin D (○) or α-amanitin (●) for 20 min at 30°C.

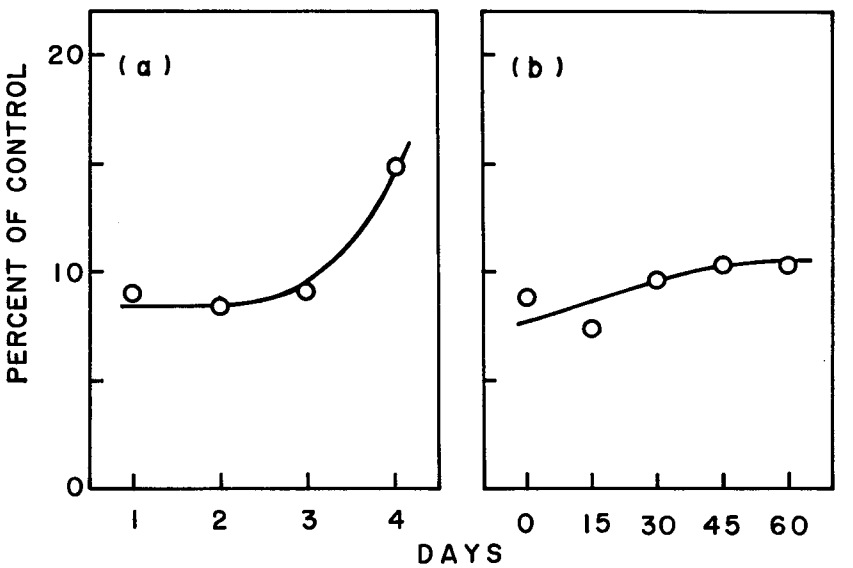

Fig. 9. Changes in template activity of wheat shoot chromatin during germination and cold-treatment. Whole template activity of chromatin (100 μg DNA) for RNA synthesis was assayed in the presence of excess *E. coli* RNA polymerase for 20 min at 30°C. The activity was expressed as % of control. Control: The activity of purified DNA (100 μg). (a): The activity of chromatin from 1-, 2-, 3-, or 4-day-germinated shoots. (b): The activity of chromatin from 0-, 15-, 30-, 45-, or 60-day-cold-treated (vernalized) shoots.

of maximum activity at 30°C. By addition of 1,000-fold unlabeled UTP at the indicated time, further incorporation of ^{3}H-UTP was stopped, and the degradation of ^{3}H-labeled product was not detected (Fig. 5). The chase experiments showed that the activity of endogenous RNase at 24°C and 3°C in the presence of PVS was negligible, because the labeled RNA product was not degraded for up to 60 min or 2 hr, respectively.

Optimum concentrations of $(NH_4)_2SO_4$, $MgCl_2$ and $MnCl_2$ for RNA synthesis were 0.2 to 0.3 M, 16 mM and 5 mM, respectively, both at 30° and 4°C (Fig. 6).

Effects of inhibitors on RNA synthesis: RNA synthesis in chromatin was stimulated with 5 to 40 μg/m*l* PVS, and was inhibited with higher than 40 μg/m*l* (Fig. 7). The RNA synthesis was inhibited with actimomycin D: 50% at 3 μg/m*l*, 90% at 37 μg/m*l*, and completely at 100 μg/m*l* (Fig. 8). The synthesis was partially inhibited with α-amanitin: 47 to 50% at 0.1 to 100 μg/m*l* (Fig. 8).

Changes in RNA-synthesizing activity during germination and cold treatment: Total (without α-amaitin), α-amanitin-sensitive and α-amanitin-insensitive (with α-amanitin) RNA-synthesizing activities in chromatin were measured at different periods of germination and cold treatment (Table 1). The activities at 30°C decreased from 2 to 4 days of germination and from 15 to 60 days of cold treatment: α-amanitin-insensitive activity decreased more strongly than α-amanitin-sensitive activity. The activity with excess

TABLE 1 RNA synthesis and the inhibition rate with α-amanitin during the periods of germination and cold treatment

Chromotin	Activity		Inhibition
	$-\alpha$-amanitin	$+\alpha$-amanitin	
days	cpm ^{3}H-UMP/100 μg DNA/15 min		%
(germination)			
2	7,808	5,114	34.5
3	4,714	2,635	44.1
4	2,558	1,443	43.6
(cold treatment)			
15	3,304	2,460	27.4
60	1,546	849	45.1

Chromatin was isolated from 2-, 3-, and 4-day-germinated shoots and 15- and 60-day cold-treated shoots. Chromatin-bound RNA polymerase activity was measured at 30°C for 15 min in the presence or absence of 1 μg/m*l* α-amanitin. For details, see Materials and Methods in the text.

RNA polymerase increased during the processes (Fig. 9). Growth of shoots cold-treated for 15 or 60 days corresponded to that of shoots germinated for 1 to 2 days or 3 to 4 days, respectively, although the RNA-synthesizing activity of the cold-treated shoots was lower than that of the germinated shoots.

Discussion

Chemical composition and UV-absorption spectrum suggest that chromatin used here was highly purified (Fig. 1).

Two distinct Tm values, 51.5° and 77.5°C, were shown in the chro matin (Fig. 2). The value of DNA purified from wheat embryos was 51.5°C (unpublished data). These results suggest that the value of 51.5°C in chromatin may be due to the presence of unmasked or nicked DNA which is active form for RNA synthesis, and the value of 77.5°C due to the presence of DNA-histone complex.

The chromatin had the activity to synthesize RNA in the presence and the absence of α-amanitin (Fig. 8). These results indicate that the chromatin was associated with native RNA polymerases I and II. The RNA-synthesizing activity was monophasically inhibited with α-amanitin : inhibition % was constant in a range of concentration from 0.1 to 100 μg/m*l*. This fact suggests that RNA polymerase III which is inhibited by high concentration of α-amanitin is labile and is lost during the preparation (ROEDER and RUTTER, 1970 ; WEINMANN *et al*, 1974). Therefore, chromatin-directed RNA synthesis in our study is due to RNA polymerases I and II : the activity of the former is 50 to 60% and that of the latter is 40 to 50% in 3-day-germinated shoots (Fig. 8).

Chromatin-directed RNA synthesis proceeded not only at high temperature, but also at low temperature (Figs. 4, 5). Presence of DNA-dependent RNA polymerase being active at low temperature, 0 to 5°C, is uncertain in higher plants. The existance of RNA polymerase activity at low temperature will give us a key to understand the mechanism of growth at low temperature and of induction of flowering by cold treatment in winter wheat. The activity of chromatin at 4°C as well as at 30°C was inhibited by heating. The result suggests that RNA-synthesizing reaction at low temperature is heat-labile. It is important to clarify the possibility that the activity at low temperature is depend on the presence of low-temperature-specific RNA polymerase or low-temperature-specific modification of chromatin structure. This low-temperature specific RNA polymerase or low-temperature-specific structure of chromatin may have a role in growth at low temperature or

vernalization of winter wheat.

The decline of the RNA-synthesizing activity during germination and cold treatment (Table 1) suggests that template activity and/or the amount of chromatin-bound RNA polymerase decreased during the both processes. The possibility of lowering the template activity for RNA synthesis may be excluded, because the template activity of chromatin tended to increase gradually during germination and cold-treatment (Fig. 9).

Studies on a characterization of RNA synthesized in chromatin during germination and cold treatment of winter wheat are in progress.

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