コムギ発芽胚とアリウロン層から分離したクロマチンのRNA合成能

誌名	The Japanese journal of genetics
ISSN	0021504X
巻/号	471
掲載ページ	p. 65-68
発行年月	1972年2月

JAPAN. J. GENETICS Vol. 47, No. 1: 65-68 (1972)

SHORT COMMUNICATION

TEMPLATE ACTIVITY FOR RNA SYNTHESIS IN WHEAT CHROMATIN FROM GERMINATING EMBRYOS AND ALEURONE LAYERS

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Received November 9, 1971

It has been assumed that cell and tissue differentiations are correlated principally with restriction of genetic transcription. One of the direct ways to investigate this problem is to examine properties of chromatin as a genetic material isolated from different cells. Aleurone cells in wheat and barley seeds are highly differentiated and have ceased to divide, but RNA synthesis occurs especially in response to gibberellic acid (Chandra and Varner 1965). The present paper deals with the template activity of chromatin prepared from germinating wheat embryos and aleurone layers, and from embryoless half-seeds treated with gibberellic acid (GA_3) by DNA-dependent RNA polymerase obtained from *Escherichia coli*.

MATERIALS AND METHODS

Two-day-old seedlings of *Triticum aestivum* (Cultivar. Shirasagi) were used for isolation of germinating embryo and aleurone layer chromatin. Half-seeds used for GA_3 response were obtained from transverse cutting of dry seeds. Following pre-incubation for 24 hr at 25°C, the half-seeds were transferred to flasks containing 1 mM sodium acetate buffer pH 4.8 and 10^{-5} M GA_3 or without the hormone. DNA was isolated according to Marmur (1961) from 2-day-old germinating embryos.

Chromatin preparation followed essentially the method of Shih and Bonner (1969). Germinating embryos or incubated half-seeds were homogenized in 0.34 M sucrose, 3 mM $CaCl_2$, 1 mM $MgCl_2$, 0.01 M Tris-HCl buffer pH 8.0 in a pre-chilled mortar and filtered through gauze and nylon mesh (37 μ). The filtrate was centrifuged at 1,700 g for 20 min, and the pellet was washed with the same solution several times. In the case of aleurone layer chromatin preparation from half-seeds, the washings were repeated several times more. The pellet was next washed with saline-EDTA (0.075 M NaCl, 0.024 M EDTA, pH 8.0) three times. The resulting pellet was suspended in 0.01 M Tris buffer pH 8.0 by using a Teflon homogenizer. It was then layered on 1.7 M sucrose in 0.01 M Tris pH 8.0, and centrifuged at 60,000 g for 90 min. The pellet was again suspended in 0.01 M Tris by using a Teflon homogenizer and dialysed against this buffer overnight. After stirring for 60 min, it was centrifuged at 10,000 g for 30 min. The supernatant contains the extracted chromatin, although aleurone layer chromatin might have some

impurity which is assumed from the pattern of ultraviolet absorption spectrum.

Template activity of chromatin or DNA to support RNA synthesis was assayed by the method of Chamberlin and Berg (1962) with *Escherichia coli* RNA polymerase purified up to the step of Fraction IV. The standard reaction mixture (0.25 ml) contained 10 μmoles of Tris buffer pH 7.9, 0.25 μmoles of $MnCl_2$, 1.0 μmole of $MgCl_2$, 100 μmoles each of CTP, GTP, UTP, or ATP. 50 μmoles of ^{14}C-ATP or ^{14}C-UTP (specific activity, 8 μCi/μM) were used.

RESULTS AND CONSIDERATION

It was found that the isolated chromatin from germinating wheat embryos and aleurone layers can prime RNA synthesis in the presence of exogenous *E. coli* RNA polymerase. As shown in Table 1, the abilities of embryo chromatin and aleurone layer chromatin to support RNA synthesis are different. The template activity of embryo chromatin is about a half of that of wheat seedling DNA, and further the activity of aleurone layer chromatin is about a half of that of embryo chromatin. One of the pos-

Table 1. Incorporation of ^{14}C-UMP into RNA synthesized on wheat chromatin isolated from germinating embryos and aleurone layers

Exp.	Template	μg DNA equivalent	$\mu\mu$moles UMP incorporated
1	Embryo chromatin	2.4	281
	Aleurone layer chromatin	2.4	133
2	DNA from seedlings	3.4	801
	Embryo chromatin	3.4	406
	Aleurone layer chromatin	3.4	232

Assay conditions described in text, incubation for 10 min at 37°C.
Each incubation was performed in triplicate.

Table 2. Incorporation of ^{14}C-UMP into RNA synthesized on chromatin from GA_3-treated and -untreated half-seeds

Exp.	Template	μg DNA equivalent	$\mu\mu$moles UMP incorporated	
			10 min	20 min
1. (Half-seeds incubated for 8 hr)	Control	9	13.7	18.0
	GA_3-treated	9	19.8	22.9
	(% increase)		(+48)	(+27)
2. (Half-seeds incubated for 16 hr)	DNA	10	152.0	198.9
	Control	10	78.7	97.9
	GA_3-treated	10	58.6	73.3
	(% increase)		(−26)	(−27)

40 g of embryo-less half-seeds were incubated with or without GA_3 at 25°C for 8 hr or 16 hr, and the chromatin was prepared. Assay conditions were the same as Table 1.

sible reasons for such a low template activity of aleurone layer chromatin, if regardless of the state of histone involved, might be the presence of some inhibitory enzymes such as ribonuclease whose activity was particulary high at pH 7.5.

Effect of GA_3 on the template activity of chromatin is shown in Table 2. In the chromatin from half-seeds incubated with or without 10^{-5} M GA_3 for 8 hours, GA_3 increases the template activity transcribed by *E. coli* RNA polymerase 48% over the control chromatin at 10-min assay incubation, but 27% over at 20-min incubation. However, 16 hour incubation showed that the template activity of the chromatin from GA_3-treated half-seeds decreased about 26% as compared with the control chromatin. The reason for decrease in template activity affected by GA_3 at 16 hour incubation is not obvious at the present time, but Chrispeels and Varner (1967) observed GA_3-stimulative production of ribonuclease in isolated barley aleurone layers. Precise investigation is necessary to resolve the problem in the present system. Effect of various amounts of added RNA polymerase on the template activity of chromatin was investigated. As shown in Table 3, the difference in ability to support RNA synthesis between chromatin

Table 3. Incorporation of ^{14}C-UMP into RNA synthesized on aleurone layer chromatin for various amounts of RNA polymerase added

Amounts of RNA polymerase (μg protein)	$\mu\mu$moles UMP incorporated		
	Control	GA_3-treated	% increase
10	20.6	32.0	55.3
20	31.0	38.0	22.6
40	70.4	89.7	27.4
60	139.8	184.8	32.2
80	205.6	218.9	6.5
100	230.0	240.8	4.7

Chromatin isolated from wheat half-seeds incubated for 8 hr at 25°C. Each assay contains chromatin corresponding to 4 μg of DNA, incubation for 20 min at 37°C.

from GA_3-treated and untreated half-seeds is maintained over a wide range of RNA polymerase concentrations. Addition of 60 μg of the polymerase assay to the aleurone chromatin and assay incubation for 10 min are considered to be suitable conditions for comparison of template activity in the present material.

McComb et al. (1970) reported that GA_3 increases the level of RNA polymerase associated with chromatin from dwarf pea plants. While, Matthysse and Phillips (1969) indicated that GA_3 does not interact directly with chromatin but requires a protein mediator. We tried an experiment about the effect of GA_3 on isolated chromatin. No increase of template activity was observed when GA_3 was added into the RNA synthesizing system consisting of *E. coli* polymerase and aleurone layer chromatin which obtained from the half-seeds incubated without GA_3. Further studies are in progress to ascertain the presence of a mediator responsible directly with GA_3.

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