Short Communication

Relationship between Poly(Adenosine 5'-diphosphate-ribose) Synthesis and Transcriptional Activity in Wheat Embryo Chromatin

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ABSTRACT
Chromatin-bound poly(adenosine 5'-diphosphate [ADP]-ribose) synthetase activity was highest in ungerminated wheat (Triticum aestivum L., cv. Mukakomugi) embryos, and it decreased after germination within 14 hours. In contrast, transcriptional activity was lowest in ungerminated wheat embryos, and it increased during germination for 24 hours or more. Histones, H1, H2A/H2B, basic nonhistone chromosomal proteins, and acidic nonhistone chromosomal proteins (molecular weight more than 10 kilodaltons) were ADP-ribosylated in wheat germ chromatin. Specific nonhistone chromosomal protein (molecular weight of 37 kilodaltons) in seedling chromatin was not found to be ADP-ribosylated.

There are many reports concerning poly(ADP-ribose) synthetase in mammalian cells (5, 6, 17) but only a few concerning that in plant cells (14, 22). In addition to a postulated role in the cell cycle, regulation of DNA repair and replication, gene expression, differentiation, proliferation, and transformation in animal cells (1, 3, 5–7, 11, 13, 15–17, 20); poly(ADP-ribose) may be involved in chromatin condensation (2, 12). Association of poly(ADP-ribose) glycohydrolase with chromatin has been reported in animal cells (5, 9) and plant (10) cells. Wheat germ nuclei contain the enzymes that participate in synthesis and degradation of poly(ADP-ribose) (22). A dynamic balance between poly(ADP-ribose) synthetase activity and poly(ADP-ribose) glycohydrolase activity may have an important role in regulation of nuclear function in many important physiological processes. We reported previously (19) that chromatin isolated from ungerminated wheat embryos appeared to have a number of aggregates with a mean diameter of 2,200 Å. The chromatin from ungerminated embryos may be present in a highly condensed state. Also, the chromatin from quiescent embryos appeared to have repressed transcriptional activity (23, 24). Therefore, we presumed that high poly(ADP-ribose) synthetase activity is closely connected with the repression of gene repression in nuclei of quiescent cells in higher plants. An attempt was made to understand the possible role of poly(ADP-ribose) in regulation of transcription during germination of wheat seeds.

MATERIALS AND METHODS
Plant Materials. Wheat germ was provided by Nippon Seifun Corp. Seeds of wheat (Triticum aestivum L., cv. Mukakomugi) were supplied by the Kitami Branch of the Hokkaido Experiment Station. Seeds were sterilized, washed, and then germinated at 24°C in the dark (19). After desired periods, the radicle-omitted embryos were separated by hand from the endosperm.

Chemicals. SVPDE, DNase I, and RNase T1 were purchased from Worthington Biochemical Corp. [Adenine-2,8-3H]NAD (3.39 Ci/mmol), [adenylate-82P]NAD (1.56 Ci/mmol), and [5,6-3H]UDP (83.7 Ci/mmol) were obtained from New England Nuclear Corp.

Isolation of Chromatin. Chromatin was isolated and purified by the method described in a previous paper (19). In some cases, chromatin from wheat germ was used as the material for ungerminated embryos.

Assay of Poly(ADP-ribose) Synthetase in Chromatin. Standard reaction medium consisted of 100 mm Tris-HCl (pH 8.0), 10 mm MgCl2, 1 mm DTT, 1 μCi [3H]NAD (5.5 × 105 cpm/180.5 pmol), and chromatin equivalent to 20 μg DNA in a final volume of 120 μl. The reaction was run for either 10 min or 20 min at 28°C. After the addition of 5 ml of 5% (w/v) TCA, the acid-insoluble materials were collected on filter paper (Whatman, GF/C) and were washed with 5% (w/v) TCA followed by 95% (v/v) ethanol. After drying, the radioactivity was determined in toluene-based scintillation medium using a Beckman LS 100 liquid scintillation system. In one experiment, [adenylate-82P]NAD was used.

Assay of Transcriptional Activity in Chromatin. The standard reaction medium consisted of 40 mm Tris-HCl (pH 8.0), 10 mm MgCl2, 110 mm (NH4)2SO4, 0.3 mm each of ATP, CTP, and GTP, 1 μCi [3H]UTP (6.0 × 105 cpm/1.75 mmol), and chromatin equivalent to 20 μg DNA in a final volume of 250 μl. The reaction was run for 20 min at 28°C and then stopped by the addition of 5 ml of 5% (w/v) TCA containing 10 mm P Pi. The acid-insoluble materials were collected on filter paper (Whatman, GF/C), washed with 5% (w/v) TCA containing 10 mm P Pi followed by 95% (v/v) ethanol, and dried; radioactivity was determined (18, 23).

Electrophoresis and Autoradiography. Chromatin was incubated with [32P]NAD (8.3 μCi, 5.1 nmol/ml) for 10 min at 27°C. Radiolabeled chromatin was collected by centrifugation and washed once with 10% (w/v), once with 5% (w/v) TCA, and twice with acetone. SDS-polyacrylamide gel electrophoresis of chromatin proteins was performed as described previously (18). The destained gel was photographed and exposed to Kodak X-Omat RP film, XRP-5, for 143 h at −80°C.

RESULTS AND DISCUSSION
Chromatin prepared from wheat embryos accepted the radioactive adenylate moiety of NAD. The reaction was inhibited with

1 Supported in part by grants from the Ministry of Education, Japan.

2 Abbreviations: SVPDE, snake venom phosphodiesterase; NHPs, non-histone chromosomal proteins.

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ADP-ribose, nicotinamide, thymine, thymidine, ATP, and CAMP and by pretreatment of chromatin with SVPDE (data not shown). Most of the radioactive adenylate moiety (over 70%) bound with chromatin was released by treatment with SVPDE, pronase, 0.3 N NaOH, and 0.4 N H2SO4 (Table I). These results indicate that the ADP-ribose moiety of NAD is bound mainly with histone by poly(ADP-ribose) synthetase. Also, over 40% of the radioactive adenylate moiety bound with chromatin was released by treatment with DNase I (Table I). This result suggests that the major ADP-ribosylated proteins are localized in the region of the chromatin that is susceptible to DNase I digestion.

The reaction took place linearly for 30 min. Therefore, the activity for 20 min was compared at the different germination stages (Fig. 1). The ADP-ribosylating activity was highest in ungerminated embryo chromatins, and it greatly decreased after germination for 14 h or more. In contrast, transcriptional activity was extremely low for the first 24 h germination; then it increased from 24 h to 72 h. Thus, transcriptional activity seems to have been repressed in chromatins that have high poly(ADP-ribose) synthetase activity. A lag of about 14 h occurred between the onset of decreasing poly(ADP-ribose) synthetase activity and the onset of increasing transcriptional activity. Events taking place during these intervals may be of critical importance in the regulation process in nuclei during germination. Since poly(ADP-ribose) glycohydrolase is present in wheat germ nuclei (22), a dynamic balance between poly(ADP-ribose) synthetase and poly(ADP-ribose) glycohydrolase may be important for the functional regulation of nuclei, as has been postulated for animal cells (6, 21).

When chromatin was pretreated with SVPDE, transcriptional activity increased. Absorbancy at 260 nm of 0.6 M KCl extract, including ADP-ribose moiety, was higher in germ chromatin than it was in seedling chromatin (data not shown). These observations seem to indicate that chromatin is endogenously ADP-ribosylated at different levels in ungerminated and germinated wheat embryos: high (low) poly(ADP-ribose) synthetase activity correlates with low (high) transcriptional activity.

A possible role of poly(ADP-ribose) in the G2 stage of continuously dividing cells has been shown in animal tissues (7). The low capacity in proliferating tissues versus the high activity in resting tissues has been reported (1, 6, 13, 17). The first cell division occurred after 14 to 18 h of germination at 25°C in wheat seeds of this study. Inasmuch as wheat embryos contain differentiated cells that start to proliferate upon germination, experiments using cells separated from meristematic and permanent tissues

### Table 1. Enzymic and Chemical Analysis of [32P] Adenylate-Labeled Material in Chromatin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Radioactivity %</th>
<th>Solubilized</th>
</tr>
</thead>
<tbody>
<tr>
<td>SVPDE, 200 µg/ml</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Pronase, 200 µg/ml</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>RNase T1, 200 µg/ml</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>DNase I, 100 µg/ml</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>DNase I, 200 µg/ml</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>NaOH, 0.3 N</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>H2SO4, 0.4 N</td>
<td>76</td>
<td></td>
</tr>
</tbody>
</table>

FIG. 1. Changes in ADP-ribosylating and transcriptional activities of chromatins during the germination of wheat seeds. Two parallel assays were performed two or more times (SE less than 10%).

FIG. 2. SDS-polyacrylamide gel electrophoresis and autoradiography of ADP-ribosylated proteins of chromatins from wheat germ and 3-d-germinated seedlings. Chromatins were incubated with [32P]NAD at 27°C for 10 min. S, SDS-gel electrophoreograms of whole proteins of chromatins; R, autoradiograms of whole proteins of chromatins; G, chromatins from wheat germ used as a material for ungerminated embryos; 3, chromatins from 3-d-germinated seedlings; o, specific NHP in seedling chromatin (mol wt, 37 kd); D.F., dye front. The experiment was performed twice.

must be performed to detect the true function of poly(ADP-ribose) in DNA replication and RNA synthesis during germination.

Germ chromatin with low transcriptional activity contains a number of aggregates with a mean diameter of 2,200 Å (19). Poly(ADP-ribose) is known to be involved in chromatin condensation (1, 12). From the results, we can infer that chromatin from ungerminated quiescent embryos is present in a highly condensed state because of ADP-ribosylation. However, there are more functions associated with chromatin that could be influenced by ADP-
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ribosylation of key enzymes or special proteins. ADP-ribosylation of RNA polymerases may be involved in the shut-off of transcription of certain genes in ungerminated embryos, as postulated in bacteriophage-infected Escherichia coli (4, 8). We have not determined whether wheat embryo RNA polymerases were ADP-ribosylated in chromatin.

To clarify which components of chromatin were ADP-ribosylated, radioactive ADP-ribose moiety in histone, NHPs, and nucleic acid fractions were determined. The radioactivity in whole proteins was higher in germ chromatin than it was in seedling chromatin, and about 80% of the activity was found in the 0.4 N H2SO4-soluble fraction. As shown in Figure 2, histone H1, H2A/H2B, basic NHPs corresponding to high mobility group proteins, and also NHPs having high mol wt (more than 10 kd) were markedly ADP-ribosylated. The radioactivity in each of the fractions was higher in germ chromatin than it was in seedling chromatin. Qualitative differences in ADP-ribosylated NHPs between germ and seedling chromatin were obscure. Differences in NHP species to be ADP-ribosylated may have an important role in the regulation of gene expression during germination.

LITERATURE CITED

1. Adamietz P R Bredehorst, M Oldekoop, H Hilz 1974 Nuclear poly(ADPR) and mono(ADPR) residues in tissues with different growth rates. FEBS Lett 43: 318-322

Page 543, column 1, line 8 from bottom should read: ...of gene expression in nuclei...
Page 543, column 1, line 1 from bottom should read: ...of the Hokkaido Agricultural Experiment...
Page 544, Figure 1 should be replaced with the following:

Fig. 1. Changes in ADP-ribosylating and transcriptional activities of chromatins during the germination of wheat seeds. Two parallel assays were performed two or more times (SE less than 10%).