Chromatin Structure in Interphase of the Shoot Apex during Germination of Barley Seeds[†]

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Inducing mutations at specific loci and altering the mutation spectrum have been long desired for crop improvement. The accessibility of the gene to the mutagen probably depends on the degree of coiling of chromosomes and chromosome regions. It has been suggested, thus, that mutagens mutate active genes with a higher frequency than inactive genes. Indeed, pulse treatment of alkylating compounds during different stages of the interphase in barley and rice seeds resulted in differential induction of mutations^{1)~3)}.

In the previous paper we found that in the first leaf of 5~6 h soaked barley seed the chromatins were dispersive fibrously and synthesized RNA along them⁴⁾. Hence, we have attempted to clarify chromosomal DNA replication and chromatin structure in the shoot apical meristem of the germinating barley seed.

1. Material and Methods

After dehusking over the embryo region to facilitate rapid and uniform germination, seeds of two-rowed barley (*Hordeum vulgare* var *distichon* cultivar Fuji 2-jyo) were allowed to germinate on moist filter paper in petri dishes. Every two hours after the initiation of seed soaking the embryos were excised from germinating seeds. To determine DNA synthesis period in the shoot apex during seed germination, autoradiographic studies were carried out with ³H-thymidine having a specific activity of 15.6 Ci/mM and a concentration of 2 μ Ci/ml.

The embryos were treated for two hours with ³H-thymidine, fixed in alcohol-acetic acid (3:1), and hydrolyzed in 1N HCl at 60°C for 10 min. After staining with Feulgen reagent, the shoot apex including fourth leaf primordium was separated and squashed in 45% acetic acid solution for autoradiographic evaluation. Labelling and mitotic indices were determined from these autoradiograms. Only the cells which had 3 or more silver grains on the nucleus were counted as actually labelled. Further studies were performed on the nuclei in which diffuse chromatin fibers were visible.

2. Results

In Fig. 1 the results obtained in this experiment are presented. The frequency of nuclei in which chromatin was dispersive varied depending upon the soaking time. It appeared even at 2 h soaking, decreased at 4~6 h soaking and increased again at 8~10 h soaking. Then, the frequency of nuclei with diffuse chromatin was decreased at 12 h soaking, increased to a noteworthy value at 14 h soaking, decreased suddenly at 16 h soaking, and rapidly increased again at 18 h soaking.

No interphase cells incorporating ³H-thymidine were observed before 12 h soaking. The labelled cells began to appear in 14 h soaked seeds. From the labelling curve it is evident that S phase consisted of two

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Fig. 1 Frequencies of nuclei with diffuse chromatin and of labelled nuclei in the shoot apex during germination of barley seed.

periods, namely there exist both early and late replicators. Autoradiograms showed that replication commenced in the telomere. The width between the 50% levels on the ascending side of the first peak and on the descending side of the second peak was measured. It indicated the period of chromosomal DNA replication (S phase) to be 7 h. Only a few dividing cells were appeared in the shoot apical meristem of 20 h soaked seed, and mitotic index increased gradually up to about 8% at 26 h soaking.

3. Discussion

Since ^3H -thymidine labelling started earlier than the initiation of division, cells in the shoot apex of dormant barley seeds stay in G_1 stage. The same data have been obtained in the shoot apex of the embryo in rice seed^{2),5)}. Using DNA cytophotometry, Avanzi, et al.⁶⁾ demonstrated the exclusive occurrence of nuclei with 2C (G_1) DNA content in the shoot apices of embryos of dry seeds of *Triticum durum*. It seems to be conclusive that the embryonic shoot apical meristems of the cereals, such as *Hordeum vulgare*, *Oryza sativa* and *T. durum*, are composed, at maturity, of G_1 cells.

Furthermore, Fig. 1 shows that S con-

sisted of two periods and each peak of labelling indices was found at 15 h and 19 h, respectively, after soaking seeds. This labelling index curve was similar to the prophase labelling index curve which was obtained with root tip cells of barley⁷⁾. Analysis of the autoradiograms has revealed that the euchromatic region has a tendency to complete earlier the replication than the heterochromatic region. This is in agreement with the results obtained with root tip cells of barley^{7),8)}.

Frequencies of the nuclei in which diffuse chromatin was visible increased at 8~10 h, 14 h and 18 h soaking. Two of these peaks, observed at 14 h and 18 h soaking, seem to be correspondent with the two peaks, 15 h and 19 h soaking, of labelling index curve. The semiconservative replication of eukaryotic DNA has been supported by enough proofs and accepted as a generality. However, a major question remains on the accommodation of semiconservative replication within complex chromosomes containing relatively enormous amounts of DNA⁹⁾. The obtained results have led to a general attitude that a conformational change of the chromatin has to occur to allow the replication of chromosomal DNA.

Few events have been identified that uniquely characterize the G_1 phase. It has been found by Kuroiwa and Tanaka¹⁰⁾ and Tatara and Yamaguchi⁴⁾ that the chromatin dispersed and synthesized RNA during G_1 period. As shown in Fig. 1, nuclei with diffuse chromatin increased at the middle part of G_1 , 4~6 h soaking.

Using root tip cells in germinating seeds of *Vicia faba*, Evans¹¹⁾ found that the G_1 stage was not a phase of constant radio-sensitivity in producing chromosome damage. Now, evidences are obtained that these nuclei with diffuse chromatin also perform DNA synthesis, probably a repair replication¹²⁾.

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