Characterization of the late replication banding patterns in barley chromosomes

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Replicating DNA regions on barley chromosomes were investigated by a replication banding technique. Of all chromosomal regions, the late replicating regions, in which DNA replicated in the last third of S phase, were detected as exceptionally distinct bands. Each late replicating region had its own timing and duration to replicate, which were not influenced by any translocations. This suggested that the late replication was highly associated with the structure of its related chromosomal region. By using the series of translocated chromosome lines, the late replication banding patterns of seven barley chromosomes were identified. The late replication banding pattern was similar to the C-banding pattern. The relationships of the late replicating regions with heterochromatin and C-banding regions are also discussed.

INTRODUCTION

Replication patterns of eukaryotic chromosomes have long been studied using replication banding techniques with DNA base analogues such as 5-bromodeoxyuridine (BrdU) (Gratzner, 1982). Mammalian chromosomes are clearly divided into early and late replicating regions: the former regions correspond to R-bands while the latter regions correspond to G-bands (Holmquist et al., 1982). In plants, attempts to investigate the replication patterns have recently been made and several patterns have been demonstrated in Allium (Cortes and Escalza, 1986; Taniguchi and Tanaka, 1991), Triticaceae (Kakeda and Yamagata, 1992), and Glycine (Yanagisawa et al., 1993). Even in these plant species, however, the arrangement of replicating regions within a chromosome, the replicating process, and the relationship of replication with chromosome structure have not been elucidated.

In mammals, the replication patterns of chromosomes with rearrangement such as translocation have been also well investigated. The X-autosomal translocation is known to change the timing of replication in each chromosomal region accompanied with the change of transcriptional activity (Camargo and Cervenka, 1984; Keitges and Palmer, 1986). Also in plants, changes in transcription have been reported to be caused by chromosome translocation, such as the transcription of nucleolar organizing regions (NOR) in translocation lines of wheat (Rieger et al., 1979). However, the replication patterns of translocated chromosomes in plants have not been investigated.

Kakeda and Yamagata (1992) reported that in barley the replication timing might be regulated over a rather wide chromosomal area, i.e., from the distal region in the early S phase to the proximal region in the late S phase. Aghamohammadi and Savage (1990) reported that a short BrdU pulse treatment made it possible to detect the replicating sites at a particular time of S phase. In this study, we used short BrdU-pulse treatment (5 min) and repeated the observation at short intervals (1 h) to identify the precise regions on barley chromosomes that replicate in the latest of S phase as the late replication banding pattern and to analyze the characters of the regions. The effect of chromosomal rearrangement on the late replication pattern was also investigated using translocated chromosomes.

MATERIALS AND METHODS

Plant materials. The six-rowed barley, Hordeum vulgare L., cv. Chikurin-Ibaraki No.1 (CI-1) and eight reciprocal translocation lines (TS3, 12, 13, 21, 30, 33, 41, and 51) derived from CI-1 (Makino, 1988) were used in this study. TS3 (6S-7S), TS12 (2L-6S), TS13 (4L-6S), TS30 (1L-6L), and TS33 (4S-6S), which have different sizes of chromosome 6H segments, and TS51 (2L-5S), which has the translocation on chromosome 5H, were used to examine the influence of translocation on the late replication. These six lines were also available to identify the late replication banding patterns of chromosomes 1H, 2HS, 4H, and 7HL, as well as 5H and 6H. TS21 (1L-2S) and TS41 (5L-7L) were used to determine the late replication banding patterns of chromosomes 2HL and 7HS, respectively.

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Replication banding. The root tips of two-day-old seedlings were immersed in 1 µg/ml BrdU solution for 5 min, followed by washing with distilled water for 15 min. Seedlings were then incubated in darkness on wet filter paper. The root tips of CI-1 were collected every hour up to 10 h after the end of BrdU treatment, and the roots of the translocated lines were collected 2 h after the end of BrdU treatment. All these procedures were carried out at 25°C. Collected root tips were pretreated in 0°C water for 16 h, and fixed in ethanol-acetic acid (3:1). Chromosome sample preparation was performed according to the standard method of enzyme maceration and air drying (Kakeda et al., 1991).

The immunological method of Kakeda and Yamagata (1992) was adopted to detect the BrdU combined with replicated DNA on the chromosomes. Chromosome samples were treated with 0.1N HCl for 10 min. After a brief rinse with cold water (4°C) and a wash with 50mM Tris-HCl, pH7.6, the samples were treated with the blocking reagent of normal rabbit serum (DAKO, diluted 1:5 with Tris-HCl, pH7.6) for 15 min at room temperature. Then the samples were treated first with mouse monoclonal anti-BrdU antibody (DAKO, diluted 1:200 with Tris-HCl) for 20 min, next with biotin-labeled rabbit anti-mouse immunogloblin (DAKO, diluted 1:200 with Tris-HCl) for 30 min, and finally with peroxidase-conjugated streptavidin (DAKO, 1:80 with Tris-HCl) for 30 min. The treated samples were washed in each step with Tris-HCl (pH 7.6) for 5 min, then with Tris-HCl containing 0.05% Tween-20 for 30 sec, and with Tris-HCl for 10 min. For staining, 0.05% DAB (diaminobenzidine tetrahydrochloride) in 0.01% H2O2 solution was dropped on the slides. The slides were incubated at room temperature for 15 min. After a brief rinse with cold Tris-HCl, the slides were dipped in 1% Giemsa (Merck) solution for counterstaining.

C-banding. The C-banding technique of Kakeda et al. (1991) was adopted to investigate the relationships between the late replicating regions and C-bands.

RESULTS

As the result of short pulse labeling at each hour of S phase, several late replication patterns which were different in the dimension and distribution of the replication signals were observed in the late S phase; the cells with the signals on the pericentromeric regions of most of the chromosomes and on the telomeric regions of several chromosomes (Fig. 1a), and the cells with the signals on the proximal regions of all chromosomes and the telomeric regions of most of the chromosomes (Fig. 1b). This indicated that the late replication continuously proceeds by small fragments within the proximal regions. The appearance of the late replication patterns in the time after the BrdU treatment showed that these patterns were specific to the last
third of S phase (Fig. 2).

Of all chromosomal regions, the late (the last third of S) replicating regions were detected as exceptionally distinct bands. The two satellite chromosomes, 5H and 6H, were selected to explain the late replication banding pattern. In both chromosomes 5H and 6H, late replication bands were observed in the region close to centromere, in the telomeric region of short arms, and at the ends of secondary constriction (Fig. 3). In chromosome 6H, a band was observed also in the telomeric region of its long arm. These bands showed marked differences in the frequency and timing of appearance in the late S. This indicated that the regions recognized as the late replication bands replicated in fixed order, and that each band had its own timing to replicate.

Fig. 4 shows the late replication bands of the translocated line TS13. As in CI-1, the late replication bands were clearly seen in all translocated lines.

TS3, TS12, TS13, and TS33 are reciprocal translocation lines concerning chromosome 6HS, differing in translocation breakpoint and partner chromosome. The 6HS seg-

Fig. 3. Late replication bands of chromosomes 5H and 6H. Idiogram of the late replication banding patterns of chromosomes 5H and 6H is presented on the right side. The number of dots on the right side of the idiogram show the appearance frequency of each band (one dot shows 10%).

Fig. 4. Metaphase plate of the translocated line TS13 with the 4H-6H translocation (arrowheads), which was stained by the replication banding method. Arrows indicate the breakpoints. Bar shows 10 μm.
ments in these translocated lines showed the same banding patterns as those of the corresponding part of the original chromosome 6HS in CI-1 (Fig. 5a). The 5HS segment of translocated chromosome in TS51 also showed the same banding pattern as that of the corresponding part of the original chromosome 5HS (Fig. 5b). These facts suggest that the late replication banding patterns of translocated segments are invariable, irrespective of breakpoint position and partner chromosome. Furthermore, the late replication banding pattern of the whole 6HS in TS30 having a translocation in the opposite arm was completely consistent with that of the original 6HS in CI-1 (Fig. 5a). Such coincidence of the patterns was also recognized between other whole arms having a translocation in the opposite arm and the respective original arms. This indicated that the late replication banding pattern of a chromosome arm is not disturbed by the translocation in the opposite arm. This also enabled us to determine the late replication banding patterns of six barley chromosomes, 1H, 2H, 4H, 5H, 6H, and 7H, without using another banding technique to identify each chromosome. The late replication banding pattern of the chromosome 3H was also determined by residual chromosomes in the original cell of CI-1. The late replication banding patterns of all barley chromosomes were obtained as shown in Fig. 6a.

The late replication banding pattern was similar to the C-banding pattern, although there were several replication bands that did not correspond to C-bands, and vice versa (Fig. 6b, c).

**DISCUSSION**

Short pulse labeling by BrdU at each hour of S phase enabled us to scrutinize the detailed procedure of the late replicating process of barley chromosomes. The late replicating regions on chromosomes proved to be detected as chromosomal bands, which differed in appearance frequency and timing. This shows that the replication of chromosomes proceeds within each band and that the replication timing of each band is genetically regulated. The unit size of replicon has been estimated to be approximately 50 kb long (Francis et al., 1985). Several dozens of them compose a 1-5 Mb replicon cluster replicating at the same time (Hand, 1978). Considering this size and the characteris-
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Fig. 6. a) Idiogram of the late replication banding patterns of barley chromosomes. The bands with the appearance frequency of 80%, 20–79%, and 19% are represented by black bars, striped bars, and open squares, respectively. b) Late replication-banded chromosomes of CI-1. All chromosomes were taken from a single cell. c) C-banding pattern of barley chromosomes.

It can therefore be said that the replication timing for each segment of autosomal chromosomes is determined not by its chromosomal position but by its structural character. As for X-autosome translocation, however, there are many instances that the early replicating regions of translocated autosomal segment replicated in the late S phase due to the "spreading effect" of translocated X chromosome (Camargo and Cervenka, 1984; Keitges and Palmer, 1986). This suggests that each chromosomal region has a feasibility of changing replication timing according to the function of the chromosome in which it is localized.

This is the first report showing the replication banding pattern of translocated chromosomes in plants. Our findings indicated that the replication banding pattern, as far as late replication is concerned, is regulated not by the relative position but by the structural condition of each region.
Our findings are in accord with those on autosome-autosome translocation in mammals. Moreover, the constancy of the late replication banding pattern, irrespective of translocation, enabled us to identify the late replication banding patterns of normal barley chromosomes using translocated chromosomes.

The similarity of the late replication banding pattern to the C-banding pattern (Fig. 6) would support the findings that heterochromatin (Lima-de-Faria and Jaworska, 1968) and C-banding regions (Bostock et al., 1972; Sperling and Rao, 1974) generally replicate in late S phase. This indicates that the C-band component is also predominant in the late replication bands. However, several late replication bands that did not correspond to C-bands were observed in this study. Heterochromatin regions are not uniform in structure (Cortes and Escalza, 1986). C-bands are usually regarded as the sites of highly repetitive DNAs, but not all C-bands contain highly repetitive DNAs (Arrighi et al., 1974; Wheeler et al., 1978). The minor incompatibility of the late replication banding pattern with C-banding patterns of normal barley chromosomes using translocated chromosomes.

REFERENCES


