GENE EXPRESSION OF rDNA IN TRANSLOCATION LINES OF BARLEY (*HORDEUM VULGARE* L.)

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ABSTRACT

Two structural mutant forms of *H. vulgare* with reconstructed karyotypes - T505 and T506 have been studied with respect to the position and the activity of rDNA genes. On the basis of the AgNO₃ staining and the number and size of silver stained nucleoli in somatic interphase cells and fluorescence in situ hybridization (FISH) we established a partial suppression of the expression of NOR7(5H) rDNA genes. When the NORs originally belonging to chromosomes 6(6H) and 7(5H) are combined in one chromosome, NOR6(6H) is dominant over NOR7(5H). Furthermore the dominance of the NOR6(6H) over NOR7(5H) is stronger when it is transposed. As shown by in situ hybridization the reason for the reduced nucleolus forming activity of NOR7(5H) is not due to eventual loss or deletion of rDNA.

Introduction

Eucaryotic rRNA genes are located in the nucleolus organizing regions (NORs), which are cytologically visible as secondary constrictions and are morphological sites around which the nucleoli develop at the end of mitosis. In fact NOR is often larger than the secondary constriction because each NOR include both transcriptionally active rRNA genes, which give rise to the secondary constriction on a metaphase chromosome, and silent, transcriptionally inactive rRNA genes (26, 7).

Activity of NORs can be modified by interspecific hybridization (differential amphiplasty, 19, 20) or, intra-specifically by chromosomal rearrangement (29). Among different types of chromosome rearrangement, the translocations are the most widely used chromosome structural changes (14, 15, 27). Reciprocal translocations between satellite chromosomes with breakpoints adjacent to nucleolar organizers result in modification of the normal formation and morphology of nucleoli and offer a useful tool for studies of alteration of gene activity in the NORs.

The 18s-5,8s-26s rRNA multigene families of barley (*Horedum vulgare* L.) are located in the NORs of the two satellite chromosome pairs 6 and 7 (respectively 6H and 5H according to the new classification since 1996, Seventh International Barley Genetics Symposium, Saskatoon, Canada) (5, 30, 33, 34, 37). Due to chromosome structural changes the nucleolus organizing regions are translocated in only one chromosome pair in chromosome structural mutants T505 and T506 (NOR6(6H) and NOR7(5H) are combined in one chromosome) (Fig. 1).

The activity of translocated NORs in the chromosome complement of T506 and T505 has previously been studied by Nicoloff *et al.* (22, 23). Initially they reported that only two of the four nucleolus organizing regions in the translocated chromosomes of line T505 are active in nucleolus formation while the other two are suppressed and remaining inactive throughout the cell cycle; hence the maximum number of primary nucleoli per nucleus is two. The authors proposed that the inactive NOR is the transposed ones.
Two years later (24, 29) it has been found that combination of all four NORs into a single barley chromosome pair (either in opposite arms of chromosome 6(6H) /T506/ or chromosome 7(5H) /T505/) (Fig. 1) result in a partial repression of the transposed rRNA cistrons. They obtain that the maximum number of primary nucleoli per telophase nucleus is two nucleoli of standard size (about 10 µm in diameter) and two micronucleoli (diameter about 1 µm). As they suggested the two micronucleoli were the product of the translocated NORs, which become translocated from chromosome 6(6H) to 7(5H) (e.g. T505) or conversely from chromosome 7(5H) to 6(6H) (e.g. T506).

Because of this contradiction we pose the aim to investigate the expression and organization patterns of translocated NORs in barley structural mutants T505 and T506 compared to the standard karyotype.

Materials and Methods

Plant material

In the present study the following three lines or varieties of barley were used:
- one possessing the standard karyotype – variety Elgina (2n=14):
- two homozygous translocation lines – T505 and T506 (Fig. 1).

The standard chromosome complement of barley contains two satellite chromosome pairs: one (pair 6(6H) with a longer satellite, the other (pair 7(5H) with a shorter one (25).

The translocation lines derived by gamma irradiation of standard variety Elgina (2n=14) (22, 23), combined all four NORs in opposite arms of a single satellite chromosome pair – 6(6H) /T506/ and 7(5H) /T505/ (22, 23, 24). Each of the two homologous satellite chromosomes in these karyotypes contains two secondary constrictions and two satellites.

Cytological techniques

For the identification of the active ribosomal sites (NORs and nucleoli) silver staining was performed as described by Lacadena (16).

Seeds were germinated for several days at 22-25°C. Root tips (about 1.5cm) were immersed for 3-4h in a solution of 0.025% colchicine saturated with α-bromonaphthalene to maximize metaphase number before fixation in freshly prepared absolute ethanol:glacial acetic acid (3:1 v/v) solution. Prior to making the preparations by squashing in 45% acetic acid the root tips were stained in acetocarmine (0.8-0.9% solution).

In-situ fluorescence hybridization /FISH/

The wheat clone pTa71 (14) which contains a 9kb EcoRI fragment including 18s-5.8s-26s rRNA genes and the intergenic spacer sequence was used. It was labeled by nick translation using biotin 14-dATP (Gibco BRL) at 16°C for 1.5h. The main hybridization...
Fig. 2. Satellite chromosomes and interphase cells of standard karyotype of *H. vulgare* var. *Elgina* after silver staining. The metaphase chromosomes (*left*) with silver bands and interphase cells with maximum four nucleoli (*right*) per nucleus.

zation procedure was similar to that of Rayburn and Gill (28). The prepared slides were treated with RNase (25 µg/ml) in 2x SSC for 60 min at 37°C. After washing with 2x SSC, the slides were air dried for 30 min. The hybridization mixture contained 50% deionized formamide, 2x SSC, 10% dextran sulfate and approximately 80ng DNA probe. The blocking DNA was 15-20 times the amount of the probe DNA. The hybridization mixture (30-40 µl per slide) was denatured for 10 min at 80°C and kept on ice for 10 min. The slides were incubated for 10 min at 78°C and then overnight at 37°C in a humid chamber. After hybridization, the slides were washed in 2x SSC twice (5 min each) at RT before detection of the hybridization sites by avidin-conjugated fluorochrome (avidin D, Vector Laboratory). The fluorochrome signal was amplified with biotinylated anti-avidin D (Vector Laboratory) followed by another layer of avidin-conjugated fluorochromes (32). After amplification the slides were washed three times in BN detection buffer at 37°C, drained and mounted in Vectashield Mounting Medium with PI (Vector Laboratory). The slides were kept at 4°C for 1-2 days to stabilize the fluorochrome before their examination with a Zeiss epifluorescence microscope.

**Results and Discussion**

**Differential Ag-staining**

Nucleolus organizing regions (NORs) of the barley standard karyotype are located in chromosome pairs 6(6H) and 7(5H). Transcription of rDNA, located in the NORs of the four barley satellite chromosomes, results in the formation of a maximum number of 4 primary nucleoli (10 µm in diameter) at late telophase of mitosis. According to Heitz (38) the “primary” number of 4 nucleoli per nucleus corresponds to the number of nucleoli expected from the presence of two satellite chromosome pairs with 4 NORs. Close to the interphase, cells show a gradual decreasing in the number of the nucleoli due to cell fusion (1).

After silver staining the NORs of the barley standard karyotype var. *Elgina* (2n=14) are visualized as four dark-stained bands on metaphase chromosomes (Fig. 2). The Ag-band in metaphase chromosome 6(6H) is larger than with the band of chromosome 7(5H) (Fig. 2) which confirmed that the NOR6(6H) is functionally stronger in the organizing nucleoli than the NOR7(5H)
Fig. 3. Interphase cells with different number and size of nucleoli in structural mutant lines T505.

The frequency of interphase nuclei with different numbers of nucleoli after silver staining. Because of nuclear fusion during the cell cycle, the maximum number of nucleoli regularly observed is of greater importance than average.

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Metaphase cells</th>
<th>Interphase cells with different number of nucleoli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>total number cells</td>
<td>4 macro nucleoli</td>
</tr>
<tr>
<td>Elgina</td>
<td>-</td>
<td>178</td>
</tr>
<tr>
<td>T505</td>
<td>72</td>
<td>4</td>
</tr>
<tr>
<td>T506</td>
<td>38</td>
<td>4</td>
</tr>
</tbody>
</table>

(35, 36). In the same karyotype (also after silver staining) were observed interphase cells with maximum number of four primary nucleoli, which corresponds to the number of Ag-bands and the number of the NORs. Two of primary nucleoli are little bit bigger in comparison with the others two (Fig. 2). The observed differences in the size of the nucleoli correspond to the size of Ag-bands in metaphase chromosomes. It is obvious that the bigger nucleoli and Ag-bands are characteristic to NOR6(6H) and the smaller ones – to NOR7(5H).

Nucleoli are phenotypic manifestation of the transcribed rRNA genes. Because of this the size of the nucleoli is the main criteria for the synthetic activity of the NORs (18). Due to chromosome structural changes of barley (1, 2, 22, 23, 24) the synthetic activity of rRNA cistrons in NORs may be changed and respectively, the normal formation and morphology of nucleoli may be modified.

In reconstructed mutant karyotypes of T505 and T506 are observed four primary nucleoli but they possess differences in the size towards standard karyotype. The size of two of them is 10 µm in diameter just as in standard karyotype (macronucleoli) but the others two are 1 µm in diameter and called micronucleoli (Fig. 3).

The presence of the micronucleoli is an indicative for the suppression of rDNA transcription activity. Based on this the both barley translocation lines T505 and T506 were found to show repressed nucleolus formation. Due to the fusion (22, 23) there is a polymorphism with respect to the number and the size of the nucleoli (Table). This polymorphism is not peculiar to the control line.

In T505 the most of the interphase cells are with two macronucleoli - 91.24% and
with one macro- plus one micronucleoli – 7.96% (Table). Significantly smaller is the percent of the cells with two macro- plus one micronucleoli and one macro- plus two micronucleoli – 0.54% and 0.23%.

Results obtained after silver staining for the mutant line T506 (Table) present that the percent of the interphase cells with maximum number of four primary nucleoli (2 micro- plus 2 macro) is several times bigger than T505 (where it is 0.02%) – 0.07%. In this line the percent of the others types of scored cells is considerably higher also – interphase cells with two macro- plus one micronucleoli is 1.04% and inter-phase cells with one macro- plus two micronucleoli is 0.8%.

According to Nicoloff et al. (24) and Reager et al. (29) micronucleoli in any case are products of the transposed ribosomal genes (NOR6(6H) or NOR7(5H)) (intra-chromosomal position effect) and are marker for the impaired rDNA transcription. However, results obtaining by us after silver staining shows, that in translocation lines with combined NOR6(6H) and NOR7(5H) on one chromosome always the transcription activity of NOR7(5H) is suppressed, and thereby formed micronucleoli.

In structural mutant form T505 presents that after transposition of NOR6(6H) in the long arm of chromosome 7(5H) (chromosome 75%) the NOR6(6H) expressed bigger Ag-band in comparison with NOR7(5H) (Fig. 4). These results entirely correspond to those established by Linde-Laursen (17) (according who NOR6(6H) represents a more extended secondary constriction and Ag-band than NOR7(5H)).

Shubert and Kunzel (31) established (in T505 and standard karyotype Elgina) that in 64% of evaluated chromosomes the AgNO$_3$ staining intensity of the both NORs is similar and only in 35% of chromosomes the NOR6(6H) is more effective than NOR7(5H) (in agreement with Appels et al (5)). The only reliable indication of dominance of NOR6(6H) over NOR7(5H) is the observation that nucleoli of standard size are exclusively associated with NOR6(6H) during the prophase I in the meiosis. This is in confirmation with our conclusion that the micronucleoli are not product of the transposed NOR6(6H) but they are products of the NOR7(5H).

Consequently the functional activity of NOR7(5H) in the both lines is always partial repressed in the presence of NOR6(6H), without matter if it is in its original site or in the transposed one, i.e. NOR6(6H) irrespective of its place always display more higher degree of expression. What is more the dominance of the NOR6(6H) is stronger over NOR7(5H) when it is transposed (T505). In its original locality it has less suppression effect on rRNA genes transcription of the transposed NOR7(5H) (T506).
In the mutant line T506 where the NOR7(5H) of chromosome 7(5H) is transposed on the long arm of chromosome 6(6H) after Ag-staining the number of the primary nucleoli (2 macro + 2 micro) is significantly greater than in line T505 (Table). Base on data obtained for both translocation lines (Table) we suppose that inactivation of the ribosomal genes in NOR7(5H) in the translocation line T505 is stronger than in the translocation line T506.

The mechanism underlying this phenomenon is presently unknown. Probably the structural organization of chromosomes and localization of heterochromatin in the satellite chromosomes affect on the suppression of NOR6(6H) over the NOR7(5H) genes. Georgiev et al. (11) after Giemsa N-banding established differences in the size of heterochromatin bands in the short arms of chromosomes 6(6H) and 7(5H) in H. vulgare (Fig. 1). The heterochromatin band in chromosome 6(6H) is more extended than in chromosome 7(5H). Probably the difference in the size of heterochromatin bands and the relative disposition have to some extent an influence on the degree of suppression of NOR6(6H) over NOR7(5H).

**Fluorescent in situ hybridization (FISH)**

Performing in situ hybridization with pTa71 in the standard karyotype of H. vulgare var. Freya were observed four hybridization bands corresponding to the four NORs of 6(6H) and 7(5H) chromosomes. In the interphase cells of the standard karyotype were observed four spots conforming to the maximum number of the nucleoli observed after Ag-staining (Fig. 5).

In the mutant lines T505 and T506 were obtained four hybridization bands in the metaphase chromosomes also but in contrast to the standard karyotype the bands are localized in the opposite arms of the one single chromosome pair - 6^3H and 5^6H (T505 and T506) (Fig. 6). The intensity of the hybridizing signals shows that the size of the NORs in the translocated site is the same as in their original site. The lack of hybridization signal in the original NORs sites demonstrate that the transposition is complete, i.e. neither significant loss nor NORs division has been occurred by the translocation of the NOR6(6H) and NOR7(5H) in their new sites (as it was observed by Gecheff et al. (8) and Georgiev et al. (12).

The four spots in the interphase cells possess equal sizes and equal intensities of the hybridization signals. We suppose the observed micronucleoli after Ag-staining in the translocation lines T505 and T506 are result of a partial suppression of the activity of the ribosomal genes after transposition.

Data obtained of Ag-staining and in situ hybridization of the translocation lines T505 and T506 showed that the transposition of NORs and their combining in only one chromosome pair results in suppression of rRNA genes of NOR7(5H) which lead to luck or presence of one or two micronucleoli. Anastassova-Kristeva et al. (4) in translocation lines T6-7a and T6-7d and Gecheff et al. (9) in mutant forms T13 and T17 have previously been obtained similar results.

This phenomenon, called by Nicolloff et al. (22, 24) intrachromosome nucleolar domi-
Fig. 6. In situ hybridization of metaphase chromosomes of T506 (upper row) and T505 (down row). The hybridization bands are localized on the opposite arms of one satellite chromosome pair.

In conclusion after combination by translocation of all four barley NORs into a single SAT chromosome pair the transcription activity of NOR7(5H) becomes more or less suppressed by the NOR6(6H) without matter if NOR7(5H) is transposed in chromosome 6(6H) or is localized in its original site in chromosome 7(5H). The only significant of the localization of the NORs is the fact the dominance of the transposed NOR6(6H) over NOR7(5H) (in T505) is stronger in distance of the case when it is in its original position (T506).

REFERENCES