Theor. Appl. Genet. 55, 247-251 (1979)

'Nucleolar Dominance' as Observed in Barley Translocation Lines with Specifically Reconstructed SAT Chromosomes

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Summary. Diploid homo- and heterokaryotypes of barley translocation lines with only one satellite chromosome pair containing two nucleolus organizer regions (NORs) in opposite arms were found to show repressed nucleolus formation by the transposed NOR as evident from the formation of only micronucleoli. The same was true for autotetraploid homokaryotypes and for translocation lines with all NORs tandemly arranged into the same chromosome arm. When NORs were transposed to chromosomes without NOR in the standard karyotype, the normal pattern of nucleolus formation remained unaffected. The modified mode of nucleolus formation after the combination of all NORs in one chromosome pair is interpreted to be due to intrachromosomal nucleolar dominance analogous to interchromosomal nucleolar dominance observed in certain interspecific hybrids.

Key words: Nucleolar dominance – Translocation lines – Nucleolus – Nucleolus association – Barley

Introduction

It has previously been reported (Nicoloff et al. 1977; Anastassova-Kristeva et al. 1977, 1979) that appropriate structural changes of barley chromosomes may result in modifications of the normal pattern of nucleolus formation. The standard karyotype of barley consists of 7 pairs of chromosomes, two pairs of which are nucleolar SAT chromosomes (chromosome pairs 6 and 7). Transcription of rDNA located in the nucleolus organizers (NORs) of the four barley satellite chromosomes results in the formation of a maximum number of four primary nuclei at late telophase of mitosis. The primary nucleoli apparently follow a definite pattern of fusion which is coupled to the progression of the cells through the cell cycle. The maximum number of 4 nucleoli per nucleus, i.e. the 'primary' number (Heitz 1931), corresponds to the number of nucleoli expected from the presence of 2 nucleolar SAT chromosome pairs with 4 NORs. After the formation of 4 primary nucleoli, two of them associate and consequently nuclei with 3 nucleoli can be observed later on during telophase. The other two primary nucleoli may also fuse resulting in nuclei with 2 larger nucleoli. During interphase, all the nucleoli may be found associated and the cells then contain a single, large nucleolus. Nucleolar fusion is, in all probability, a process which occurs regularly and according to a definite pattern in time (Anastassova-Kristeva 1977; Nicoloff et al. 1977).

Due to chromosome structural changes nucleolus organizer regions may become translocated from their original sites. This was observed to result, in certain cases at least, in modifications of the normal formation and morphology of nucleoli. Combination by means of translocations of all four nucleolus organizing regions (NORs) into a single barley chromosome pair (either in opposite arms or tandemly in one chromosome arm of chromosome 6 or 7) was found to result in complete or partial repression of the transposed RNA cistrons, as is inferred from impaired nucleolus formation by two of the NORs (Nicoloff et al. 1977; Anastassova-Kristeva et al. 1979). Though the mechanism underlying this phenomenon is presently un-

0040-5752/79/0055/0247/$ 1.00

known, the cases so far studied represent what may be called position effects with respect to the transcription of rDNA.

Reconstructed karyotypes of barley with three instead of two chromosome pairs with nucleolus organizing activity due to interchanges with one translocation point inside the NOR revealed quantitative relationships between the size of the secondary constriction (the amount of rRNA cistrons) and the number and size of nucleoli being formed (Anastassova-Kristeva et al. 1977; Nicoloff et al. 1977). In none of these cases, however, was suppression of NOR activity observed.

In the present paper we wish to report on additional evidence for partial or complete inactivation of transposed NORs when all NORs are combined in a single chromosome pair. This phenomenon is analogous in effect to what has been termed 'nucleolar dominance' or 'differential amphiplasty' first observed by Navashin (1928, 1934) in the progeny of certain interspecific *Crepis* crosses in which the formation of nucleoli and nucleolar constrictions occurred at only one of the parental chromosome sets. Subsequent investigations supported this observation for a wide variety of both plant and animal interspecific hybrids (cf. Durica and Krider 1978; Gerstel et al. 1978).

Material and Methods

In the present paper, four translocation lines of *Hordeum vulgare* (T 505, T 548, T 506, and T 571 from the Gatersleben collection of such lines) have been studied. These lines have all four NORs combined in opposite arms of a single nucleolar SAT chromosome pair, either chromosome 6^7 (e.g., T 506) or 7^6 (e.g., T 505). Each of the two homologous SAT chromosomes contains two secondary constrictions and two satellites (Fig. 1). In addition to the diploid lines homozygous for the interchange, structurally heterozygous lines with the SAT chromosome constitution 6, 7, 6^7 or 6, 7, 7^6 and structurally homozygous autotetraploid lines were investigated with respect to nucleolus formation.

Feulgen-stained chromosomes and nucleoli stained with methyl-green-pyronine according to the method of Unna were studied in squash preparations of root tip meristems. Root tips (6 to 8 mm in length) were fixed for 18 h at 4°C in Serra's fixation mixture. After rinsing in distilled water and maceration in Bistrin (Nicoloff and Daskaloff 1966) squashes were made permanent by the dry ice method of Conger and Fairchild (1953).

Silver staining of the nucleolus organizing regions of barley was obtained by adapting the method of Goodpasture and Bloom (1975) as simplified by Varley (1977): Air-dried squash preparations of pectinase-digested root tips were incubated for 12.5 h at 65°C in $AgNO_3$ (Merck) solution (5g/5ml, i.e., double the concentration used by Varley). Then the slides were rinsed in distilled water, again air-dried, dipped into Xylol and mounted in cedax. By this method interphase nuclei and metaphase chromosomes stained yellow. The nucleoli of interphase cells and the secondary constrictions (NORs) of metaphase chromosomes became dark brown (cf. Schubert et al. 1979). The Ag-technique thus allows specific detection of transcriptionally active NORs. The chromosomal component stained by this technique is probably an acidic protein (Howell 1977) or RNA.

Results

The following observations refer to nucleolus formation in the standard barley karyotype and in diploid homo- and heterokaryotypes for the interchanges mentioned above (Material and Methods) as well as in the tetraploid standard and reconstructed karyotypes. All data obtained show that after combination of all NORs into a single chromosome pair the transposed NORs are of a more or less severely reduced activity in nucleolus formation as evident from the production of only micronucleoli.

Nucleolus Organizer Activity in Diploid Homokaryotypes

Four translocation lines (T 505, T 506, T 571 and T 548) with all four NORs in opposite arms of chromosomes 6 (6^7) or 7 (7^6) were studied with respect to nucleolus formation. In all cases 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) (Fig. 1). The two micronucleoli are the product of the transposed NORs which become translocated from chromosome 6 to 7 (e.g., T 505) or vice versa (e.g., T 506) and are indicative of impaired rDNA transcription from these NORs (Table 1). The same effect, i.e. suppression of activity of transposed NORs, was observed when the NORs were tandemly arranged in the same chromosome arm (Nicoloff et al. 1977). During late telophase and early interphase the micronucleoli are of low pyroninophyly, again indicating a severe repression of synthetic activity of the NORs in question. Due to nucleolus association in the course of cell cycle progression, only 5% of the nuclei studied showed the maximum number of four primary nucleoli of the two different size classes.

Nucleolus Organizer Activity in Diploid Heterokaryotypes

The heterokaryotypes resulted from crossings as exemplified by the combinations T 505 × T 506 on the one hand, and T 505 × standard karyotype on the other (Table 1). The first combination gives rise to karyotypes with a pair of differently reconstructed SAT-chromosomes (6^7 and 7^6) with four NORs in opposite arms (Fig. 1). As expected from the behaviour of the homokaryotypes, the maximum number or primary nucleoli is again two of standard size and two micronucleoli. The SAT chromosome constitution of hybrids due to the second route of crossing is characterized by the presence of three SAT chromosomes: standard 6, standard 7 (each with one NOR), and chromosome 7^6, involved in translocation and bearing two NORs. In this karyotype the maximum num-

Table 1. Nucleolus formation in various barley karyotypes

Karyotype	SAT chromosome constitution	Maximum number of nucleoli per nucleus	Standard size nucleoli	Micronucleoli
Homokaryotypes (2n)				
Standard	four standard SATs (6, 6, 7, 7)	4	4	–
T 505[a]	two SATs with two NORs each (7^6, 7^6)	4	2	2
T 506	to SATs with two NORs each (6^7, 6^7)	4	2	2
Heterokaryotypes (2n)				
T 505 X standard	three SATs (two standard, one with two NORs)	4	3	1
T 506 X standard	three SATs (two standard, one with two NORs)	4	3	1
T 505 X T 506[b]	two SATs both with two NORs	4	2	2
Homokaryotypes (4n)				
Standard	eight standard SATs	8	–	–
T 505	Four SATs with two NORs each	8	4	4

[a] Similar observations for T 548 and T 571

[b] Similar observations for hybrids from T 505 X T 548 or T 571, T 506 X T 548 or T 571

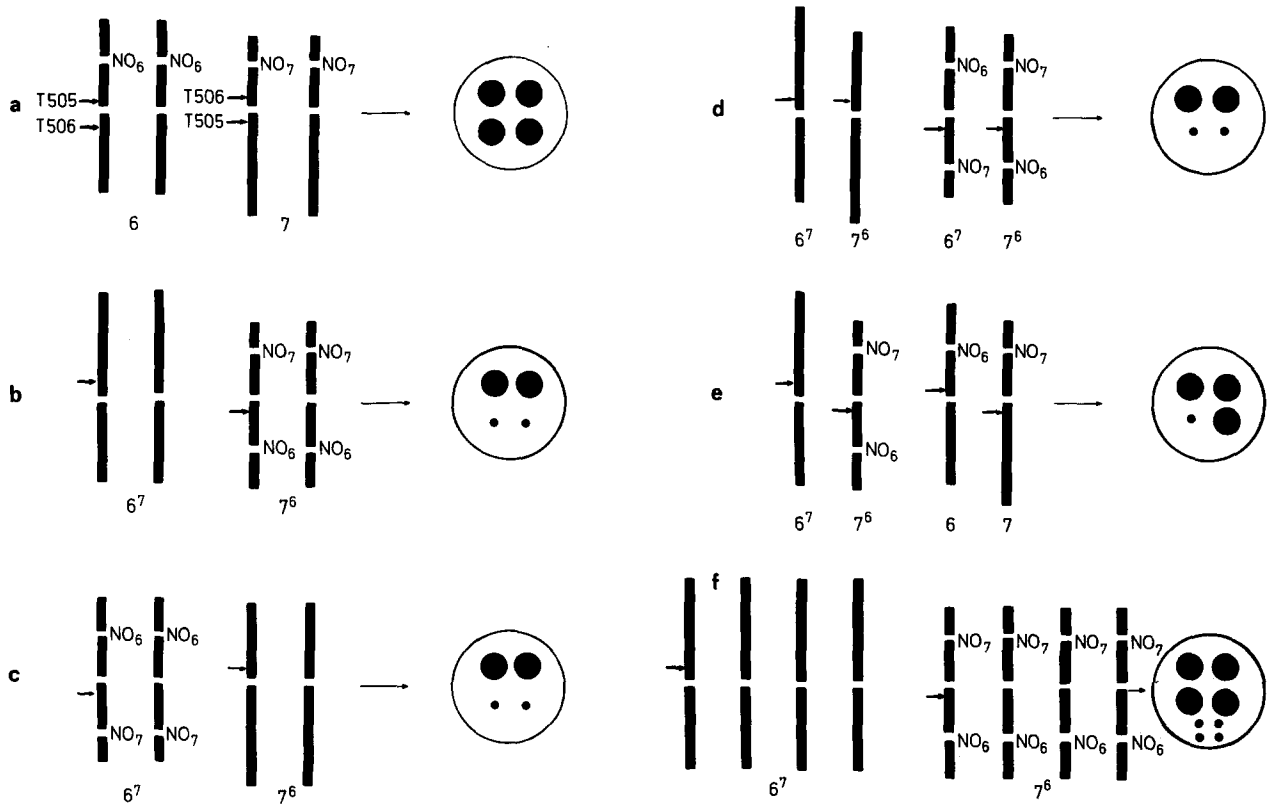

Fig. 1a-f Schematic representation of SAT chromosome constitution, number and size of primary nucleoli in various karyotypes of *H. vulgare*. a Standard karyotype b Translocation line T 505 with all NORs combined in opposite arms of chromosome 7^6 c Translocation line T 506 with all NORs combined in opposite arms of chromosome 6^7 d Hybrids from T 505 X T 506 (heterokaryotypes 7^6, 6^7) e Hybrids from T 505 X standard (heterokaryotypes with three SAT chromosomes: 6, 7, 7^6) f Autotetraploid translocation line T 505 (four SAT chromosomes 7^6). Arrows = translocation points; NO = nucleolus organizer

ber of primary nucleoli is four. Three of these are of standard size, the fourth represents a micronucleolus (Fig. 1). The former three probably originate from NORs which occupy their original positions in chromosomes 6, 7, and 7^6, the micronucleolus from the NOR transposed from standard chromosome 6 to the reconstructed chromosome 7^6. The positional change accompanied by reduced NOR activity remained uninfluenced by the heterozygous state of the structural change. This is true for all combinations tested (Table 1).

Nucleolus Organizer Activity in Tetraploid Homokaryotypes

The tetraploid standard karyotype of barley with four SAT chromosomes 6 and 7 each is characterized by the presence of a maximum number of eight primary nucleoli per nucleus which are of standard size (diameter about 10 μm). Two of these generally undergo rapid association so that the nucleolus frequency observable per late telophase nucleus is frequently only six. Homokaryotypes with four structurally reconstructed SAT chromosomes 7^6 (NORs in opposite arms) also show a maximum number of eight primary nucleoli. Four of these are of standard size, the other four represent micronucleoli (diameter about 1 μm). This observation is in conformity with the expectation based on the situation in the corresponding diploid homokaryotype described above. Similar to the situation found in the tetraploid standard karyotype, this maximum number was relatively seldom observed. Also in these lines, nucleolar fusion followed a definite pattern in time and standard size nucleoli associated rather quickly. As in the diploid homo- and heterokaryotypes, the secondary constrictions are clearly visible in tetraploids and the NORs, independent of their being fully active or of reduced activity (formation of micronucleoli), proved to be stainable by the Ag-technique (Fig. 2).

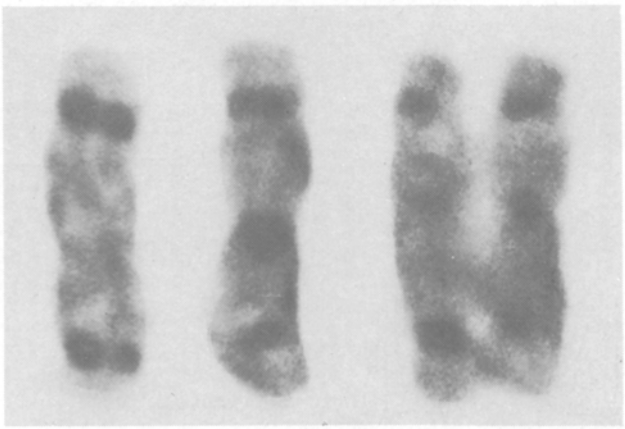

Fig. 2. Silver staining (Ag technique) of the nucleolus organizer regions of the four SAT-chromosomes (7^6) with NORs in opposite arms as observed in tetraploids of translocation line T 505 (x2250)

Discussion

From the observations reported in this paper, it is evident that the combination by interchange of all four barley NORs into a single SAT chromosome pair (either in opposite arms or tandemly in one arm) affects nucleolus formation in the sense that the transcription activity of transposed NORs becomes more or less suppressed. This specific chromosomal combination of NORs results in the production of only tiny micronucleoli by transposed NORs. No such effect occurs if NORs are transposed to former non-SAT chromosomes (cf. Anastassova-Kristeva et al. 1977), i.e. the phenomenon is bound to the presence of all NORs in one chromosome pair. The data obtained for homo-, hetero- and autotetraploid karyotypes are completely consistent with this deduction (Table 1) and suggest that suppression of the activity of transposed NORs represents a rather specific intra-chromosomal position effect in which 'correctly positioned' NORs take influence on the activity of NORs transposed to the same chromosome. In all diploid and tetraploid homokaryotypes, as well as in the heterokaryotypes, nucleolus suppression remained confined to the reconstructed chromosomes and even numerical changes of the chromosomes in question were found to be without influence on the modified pattern of nucleolus formation.

Suppressed nucleolus organizer activity remains without influence on the presence of the secondary constriction involved; in the reconstructed chromosome pair with 4 nucleolus organizing regions all four secondary constrictions are regularly identifiable. The same is true for the bands recognizable in the secondary constriction by application of the silver staining technique. Since Ag staining is indicative of transcriptional activity of rDNA, i.e. of the presence of functional NORs (Miller et al. 1977; Croce et al. 1977), the production of micronucleoli by those NORs which show reduced capability of nucleolus formation after transposition to one of the nucleolar SAT chromosomes of barley is in conformity with the expectation on the basis of Ag band formation. Thus, if the absence of Ag staining of NORs is in fact indicative of a complete shut-off of NOR activity, then reduced activity, as observed in the barley translocation lines, is not recognizable by this technique. No differential silver staining of NORs showing normal or reduced activity, respectively, was observed in these cases.

The effects of nucleolus suppression in structurally homo- and heterozygous barley translocation lines are analogous to the phenomenon of 'differential amphiplasty' or 'nucleolar dominance' observed in the progeny of certain interspecific crosses (cf. Gerstel et al. 1978; Durica and Krider 1978). This phenomenon involves the consistent disappearance of the satellite of one of the parental nucleolar chromosomes accompanied more or less by the complete suppression of nucleolus formation at the NOR concerned. In those cases where the parental RNA species can be discriminated from one another this cytological

observation has been correlated with the transcription of only one class of rRNA (Honjo and Reeder 1973; Croce et al. 1977). Honjo and Reeder (1973) proposed two hypothetical mechanisms which may result in nucleolar dominance: 1) the suppression of one species of rRNA by the products of another, or 2) the competition between rRNAs for some essential factor which is in limited supply. Experimental evidence in favour of either of these or any other explanation for nucleolar dominance is presently completely lacking. But reasoning along similar lines is not too far-fetched in order to interpret the observations made on the translocation lines of barley.

The main differences with respect to the observations made on nucleolar dominance in the progeny of interspecific hybrids and in barley translocation lines with all NORs combined in one SAT chromosome pair are as follows:

1) nucleolar suppression is, in all probability, an interchromosomal effect in the progeny of interspecific hybrids and a strictly intrachromosomal one in translocation lines;

2) in the progeny of certain interspecific hybrids, nucleolar dominance is coupled with the disappearance of the secondary constriction and the satellite of 'former' satellite chromosomes; in the translocation lines, the secondary constrictions are preserved and observable in metaphase chromosomes;

3) nucleolus formation is, at least in many cases, completely shut off in the case of differential amphiplasty (Keep 1971); residual activity of transposed NORs in barley translocation lines is clearly evident from the formation of micronucleoli by the NORs in question and from silver staining by the Ag technique.

In spite of these differences, we do not hesitate to operationally regard the phenomena observed in barley as cases of nucleolar dominance expressed at the intrachromosomal level. As far as the present evidence goes, there is no indication of 'interchromosomal' nucleolar dominance in barley translocation lines (see Anastassova-Kristeva et al. 1977).

Indications of interchromosomal nucleolar dominance have, however, been obtained by Wallace and Langridge (1970) and Lange and Jochemsen (1976) in hybrids of *Hordeum vulgare* × *bulbosum* in which the satellite chromosome of *Hordeum bulbosum* was invisible and the number of nucleoli appropriately reduced. Taken together, these data and our observations in barley translocation lines allow us to conclude that apparently both inter- and intrachromosomal nucleolar dominance may occur in barley; the former in interspecific hybrids, the latter in translocation lines with all NORs in a single SAT chromosome pair.

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Received February 3, 1979, Communicated by H.F. Linskens

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