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The occurrence of Ds-like sequences in cereal genomes

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Summary. The occurrence of DNA sequences similar to the *Ds*-element of *sh*-m5933 maize (*Ds*-like sequences) was studied in other representatives of the Gramineae. The approximate number of copies of such sequences found under gentle and stringent conditions of washing was determined by dot-hybridization. It was shown that in the maize genome the number of copies of *Ds*-like sequences exceeds about ten-fold the content of such sequences found in wheat, rye and barley genomes. Quantitative differences in *Ds*-like sequences between wheat species with various genomes and ploidies (when estimated per genome) as well as between different *H. vulgare* varieties was not determined. The various melting points (T_m) of DNA-duplexes formed when the *Ds*-element is hybridized with wheat, rye and barley DNA respectively do not show significant differences and are essentially lower than the T_m of the *Ds*-element (by 8°–9°C). Thus, these duplexes have 9–11% of nucleotide substitutions in comparison to *Ds sh*-m5933. The data obtained permit one to suppose the presence of a series of *Ds*-like sequences heterogenous for the length and degree of homology to the *Ds*-element isolated from the *shrunken* locus (*sh*-m5933) of maize DNA.

Key words: *Ds*-element – Cereal genomes – The number of copies – Heterogeneity

Introduction

The abundance of information which has accumulated on the general distribution of mobile genetic elements permits them to be regarded as components of eukaryote genomes.

The best studied family of maize "controlling elements"-CE (McClintock 1951) is that of *Ac*-DS elements in which the *Ac* (activator) is an autonomous element. The *Ac* and *Ds* elements have been isolated from several unstable maize loci. Their length and, in some cases, the insertion site in the locus and their primary structure, have been determined (Behrens et al. 1984; Courage-Tebbe et al. 1983; Fedoroff et al. 1983). The *Ds*-elements have been shown to be much more wide-spread in the maize genome than the *Ac* (Fedoroff et al. 1983), and differ from the latter in the lack of a central region.

Ds-elements isolated from different loci are distinguishable in length but have a number of common properties in primary structure: the presence of identical terminal repeats of 11 bp in length, numerous copies of GGGTTT hexanucleotides, etc (Döring et al. 1984). DNA sites characteristic of *Ds*-elements have also been described in the mobile genetic elements of other eukaryote species. In particular, *Drosophila* FB-transposon contains copies of decanucleotide in which the first five letters coincide with the sequence of the maize *Ds*-element (Döring et al. 1984). Homologous sites on the CE ends of *Cin* I maize and the *Drosophila copia*-element, as well as in the inverted repeats of such unrelated species as maize, soybean and snapdragon have been described (Shepherd et al. 1984). These data imply the evolutionary conservatism of some sites in mobile genetic elements or the possibility of their horizontal transfer.

The aim of the present work was to investigate the occurrence of DNA sequences similar to maize *Ds*-elements (*Ds*-like sequences) in other representatives of the Gramineae. The well-known facts of conservatism in the primary structure of transposons of various eukaryote species have served as a basis for this research.

Materials and methods

DNA isolation

DNA was isolated from 4–5 day old seedlings of the following Gramineae species: maize (*Zea mays*, W-23 line), rye (*S. cere-*

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ale 'Vostochnaya Sibirskaya'), wheat (*Tr. monoccocum, Tr. diccocum, Tr. timopheevi, Tr. aestivum* – 'Novosibirskaya-67'), barley (*H. vulgare* of different varieties: 'Winer', 'Nepolegayushchii', 'Galina', 'Sophia'). The isolation was performed by the procedure described by Bedbrook et al. (1980). The *Ds*-element was excised with *Bam*HI restrictase from recombinant plasmid produced on the basis of pBR 322 and kindly supplied by Dr. P. Starlinger (Institut für Genetik, Köln, FRG). This *Ds*-element is a 2.7 kb long site of *shrunken* locus isolated from the maize mutant line *sh*-m5933 excised from the BamHI-sites of the locus (Geiser et al. 1982).

Determination of the number of copies of Ds-like sequences

The presence of *Ds*-like sequences and the number of their copies in the genomes of the above Gramineae species were determined by the saturating dot-hybridization method (Kafatos et al. 1979). Samples with different contents of cereal DNA, DNA T7 phage, DNA pBR 322 and DNA recombinant plasmid with *Ds*-element were immobilized on nitrocellulose filters (Schleicher & Schüll, FRG). The hybridization mixture contained a labelled (^{32}P)-*Ds*-element and a 500-fold excess and nonlabelled competitive DNA pBR 322. The *Ds*-element was excised from the recombinant plasmid with *Bam*HI ("Enzyme", USSR) and isolated by preparative electrophoresis in 5% polyacrylamide gel. (α-^{32}P)-dNTP ("Isotop", USSR) was introdused into the *Ds*-element in the "nick-translation" reaction (Rigby et al. 1977) with DNA-polymerase I produced by the team headed by A. Romashchenko (Institute of Cytology and Genetics, USSR). The specific radioactivity of the preparations obtained was $5 \cdot 10^7$–$1 \cdot 10^8$ cpm/μg. The filters were washed after hybridization as described by Maniatis et al. (1982). At the last stage (2 h in 0.1 SSC, 0.5% SDS) the conditions of washing were "gentle" (room temperature) and "stringent" (t° = 55 °C and 65 °C).

Melting curves

The melting curves of the formed duplexes of *Ds*-element and of cereal DNA wre determined in 3 SSC with 50% formamide by the percentage of radioactivity release from the filters as the temperature rose from 20 °C to 75 °C. The standard deviation found by Marmur and Doty (1962) for repeated determinations of melting curves is equal to ± 0.4 °C.

Results and discussion

The presence of *Ds*-like sequences in cereal genomes was controlled by dot-hybridization. Two methods of washing the filters was used: 1) under "gentle" conditions and 2) under "stringent" condition (see Materials and methods). The temperature of posthybridization washing of filters is known to be a criterion of stringency (Sim et al. 1979). The genomes of all the cereal species studied contain *Ds*-like sequences which is indicated by the hybridization signals found on filters containing the DNA of wheat, rye and barley: they exceed considerably that found on the filter containing the DNA of T7 phage taken as a control (Fig. 1). The label intensity of the filters washed under the "stringent" conditions is considerably reduced except on those filters where the *Ds*-element is self-hybridized.

The quantitative estimations of these experiments are given in Table 1. As can be seen from this table, the genomes of the cereals studied contain approximately from one to three hundred *Ds*-like sequences, about one order less than found in the maize genome; the differences among the *Triticum, Secale* and *Hordeum* genera being small. When estimated per haploid genome there are no essential differences in the *Triticum* genus among the species with various genomes and ploidies. The similarity of different genomes of *Triticum* in the content of *Ds*-like sequences contrasts with their inequivalence in the content of highly repetitive DNA. According to the results obtained by Flavell et al. (1979) the B genome contains a far larger number of high repeats than do the A and D genomes.

Hordeum vulgare varieties do not practically differ from one another in their content of *Ds*-like sequences either. Barley, just as wheat, belongs to a self-pollinated species.

As has already been noted, transposons of the eukaryote genome are limited by inverted repeats having homologous sites even between remote species. As the genomes of the cereals studied contain sites homologous to inverted repeats of *Ds*-element, the hybridization could take place between these sites only, without any spreading over the internal sequence. This possibility was verified. After hybridization, the filters were treated with S_1-nuclease and more than a half of the label remained on the filters (data not shown). It indicates that the homologous sites are located not only within the limits of the flanking repeats.

More stringent conditions of washing do not practically alter the amount of self-reassociated *Ds*-element (Fig. 1). Under these conditions, however, the number of copies in the genomes of all cereal species decreases considerably (twofold, on the average, Table 1). These results indicate that in the cereal genomes there is a rather large quantity of *Ds*-like sequences with a different degree of homology to *Ds sh*-m5933, including a fairly low one.

A question arises: to what extent does the primary structure of numerous *Ds*-like sequences correspond to that of the *Ds*-element from the *sh*-m5933 locus. The question can partly be answered by a comparison of the melting points of DNA-duplexes formed when the *Ds*-element is hybridized with the DNA of various cereal species. As can be seen from Fig. 2 and Table 1 the melting point (T_m) of maize DNA is 2 °C lower than that of the self-reassociated *Ds*-element. Since a decrease of T_m by 1 °C results from the replacement of 1.0–1.2% of nucleotides (Wang and Kallenback 1971; Appels and Dvorak 1982), the *Ds*-like sequences in the maize genome must contain 2.0–2.5% base substitutions. The melting points of DNA in wheat, rye and barley do not significantly differ from one another and

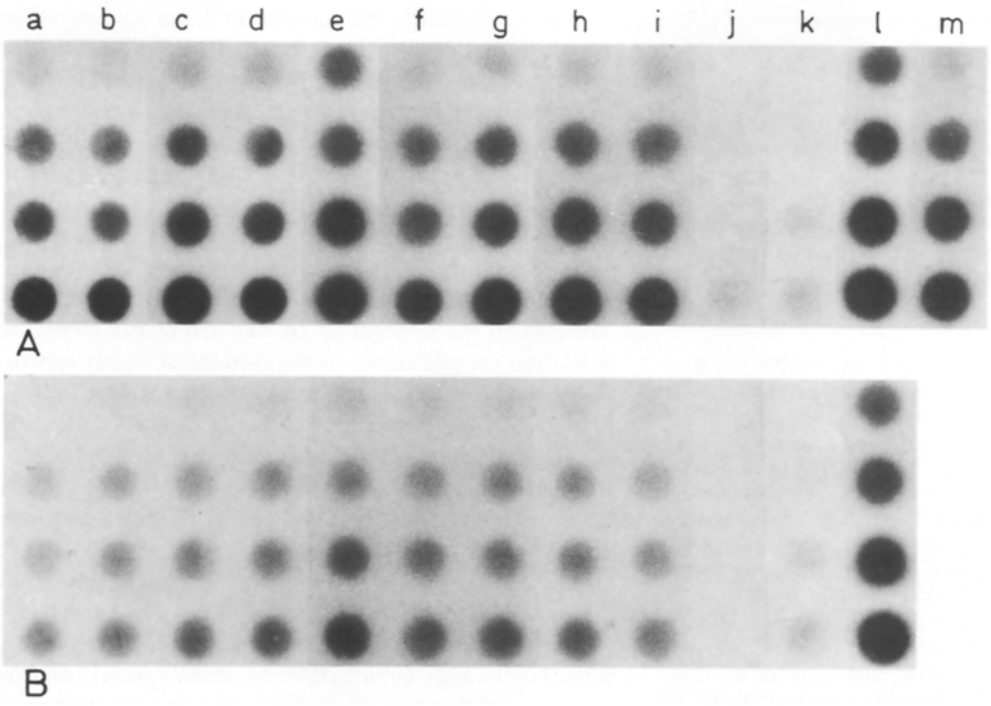

Fig. 1 A, B. Dot-hybridization of (^{32}P)-*Ds sh*-m5933 with DNA of various cereal species. Temperature of washing of filters – 20 °C (**A**), 55 °C (**B**): a) *Tr. monoccocum;* b) *Tr. diccocum;* c) *Tr. timopheevi;* d) *Tr. aestivum;* e) rye; f) barley 'Winer'; g) barley 'Nepolegayushchii'; h) barley 'Galina'; i) barley 'Sophia'; j) phage T7; k) pBR 322; l) pBR 322 with *Ds sh*-m5933; m) maize. DNA concentration on filters from top to bottom: a–j) = 0.1 μg, 0.5 μg, 1.0 μg, 2.5 μg, respectively; k) = 0.018 μg, 0.054 μg, 0.18 μg, 0.54 μg; l) = 0.03 μg, 0.09 μg, 0.3 μg, 0.9 μg; m) = 0.05 μg, 0.1 μg, 0.2 μg, 0.5 μg

Table 1. Number of copies T_m of *Ds*-like sequences in cereal genomes

Species	Genomes	No. of copies[a]			T_m (C°)
		Gentle conditions of washing, t = 20°	Stringent conditions of washing, t = 55°	% of loss	
Tr. monoccocum	A	55	26	47	
Tr. diccocum	AB	119	57	48	
Tr. timopheevi	AG	83	43	52	
Tr. aestivum	ABD	218	94	43	46
S. cereale		337	155	46	45
H. vulgare 'Winer'		160	70	48	45.5
H. vulgare 'Nepolegaiushchii'		185	85	46	
H. vulgare 'Galina'		178	93	52	
H. vulgare 'Sophia'		149	61	41	
Z. mays		1,950	–	–	52
Ds sh-m5933		–	–	10	54

[a] No. of copies represent mean values of 3–5 experiments

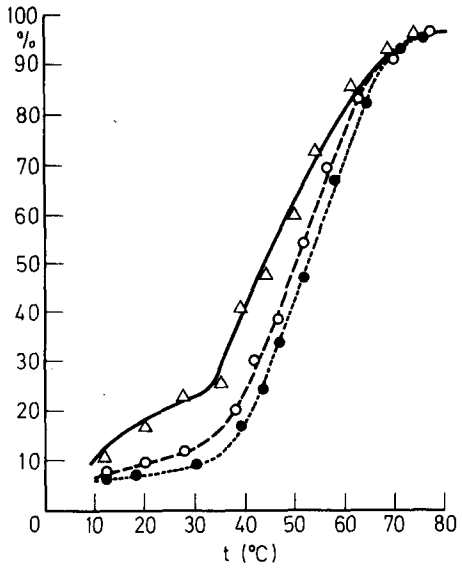

Fig. 2. Melting curves of duplexes formed upon hybridization of *Ds sh*-m5933 with: ----●----●---=*Ds sh*-5933; –○–○–=DNA of maize; —△———△— = DNA of *Tr. aestivum*

are essentially lower than the T_m of the *Ds*-element (by 8°–9 °C). Hence, it can be concluded that the former have 9–11% replacements. The melting curves of *Ds*-like duplexes in DNA of *Tr. aestivum*, rye and barley are similar and rise much more sharply at low temperatures (Fig. 2). Such a curve may result from considerable heterogeneity in the degree of homology of *Ds*-like sequences and/or from polydispersiveness in length (Mandel and Marmur 1968).

Is the reduction in T_m of *Ds*-like sequences of wheat, rye and barley by 8 °C–9 °C essential with respect to the differences in the structure of other sequences between related species? Moore et al. (1981) compared the reduction of T_m in hybridizing 11 cloned repetitive DNA sequences of sea urchin *S. purpuratus* with the DNA of another species, *S. franciscanus*. The reduction value ranged from 1 °C to 6 °C and amounted, on average, to 4 °C. These authors found a correlation between the length of sequences and their divergence within one family of sequences. In the short DNA sequences of *S. purpuratus*, referring to one family of sequences, the reduction in T_m was coinsiderable and reached 25 °C. The results obtained by Appels and Dvorak (1982) while comparing the primary structure of spacer regions of ribosomal DNA in wheat and barley should also be noted: a short sequence of 130 bp in a nontranscribed region of wheat rDNA when hybridized with barley DNA displays a T_m reduction of 17.5 °C whereas a sequence of 750 bp from another region shows no difference in T_m in these species. Thus, the differences found between T_m of *Ds*-like sequences of wheat, rye and barley, and T_m of *Ds sh*-m5933 lie within the limits of interspecific variations revealed for other repetitive sequences.

Summarizing the above data one can assume the existence of a series of *Ds*-like sequences heterogenous for the length and the degree of homology to the *Ds*-element from the *sh*-m5933 locus of maize. In the presence of *Ac*-like and other sequences performing transposition the *Ds*-elements can exert an essential influence on the expression of genes.

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