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Variation in Nucleolar Organiser rRNA Gene Multiplicity in Wheat and Rye

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Abstract. In hexaploid wheat and diploid rye, different varieties have different numbers of ribosomal RNA genes as indicated by rRNA/DNA hybridisation. Wheat has four different chromosomes which carry nucleolar organisers. Analyses of DNA isolated from substitution lines in which each of these nucleolar organiser chromosomes of several varieties has been substituted one at a time into a common genetic background, have indicated that none of the four organiser chromosomes possess an invariant number of ribosomal RNA genes. The ribosomal RNA gene complement of the varieties investigated can be approximately accounted for by the sum of the ribosomal RNA genes on each of the four nucleolar organiser chromosomes.

Introduction

In most eucaryotes the ribosomal RNA (rRNA) genes are multiple and clustered at the sites of the nucleolar organisers (Birnstiel *et al.*, 1971). Studies on related genotypes in *Drosophila* (Ritossa and Spiegelman, 1965), *Xenopus* (Wallace and Birnstiel, 1966) and *Zea mays* (Phillips, Kleese and Wang, 1971) have indicated that for a given species the number of rRNA genes per nucleolar organiser may be constant, although it has been known for many years that under certain circumstances the reduced number of rRNA genes in deletion mutants of *Drosophila* can revert towards the wild type number over several generations (Ritossa, 1968; Tartof, 1973). Recent studies on wheat aneuploids (Flavell and Mohan, 1973; Mohan and Flavell, 1974; Flavell and Smith, 1974), hyacinth aneuploids (Timmis *et al.*, 1973), *Nicotiana* species of differing ploidy (Siegel *et al.*, 1973), related flax genotypes (Timmis and Ingle, 1973) and inbred maize lines (Phillips *et al.*, 1973) have, however, suggested that the number of rRNA genes per nucleolar organiser is not constant in higher plants.

The numerous aneuploid stocks of hexaploid wheat having a common genetic background (Sears, 1954) and the extensive array of stocks having only single chromosome substitutions within a common background (Law, 1968) are particularly attractive for use in a search for, and genetic analysis of, natural variation in rRNA gene multiplicity. We have therefore taken advantage of some of these stocks to investigate rRNA gene multiplicity at each of the four known nucleolar organiser sites (Crosby, 1957) in several hexaploid wheat varieties.

Materials and Methods

Plant Genotypes

All the seed was obtained from stocks maintained at the Plant Breeding Institute, Cambridge. The varieties of hexaploid wheat were as stated in the Results. The substitution lines in which chromosomes 1A, 1B, 6B and 5D of the hexaploid wheat variety Chinese Spring have been replaced one at a time by their homologues from the variety Hope were originally obtained from Dr. E. R. Sears, University of Missouri. The substitution lines, again in a Chinese Spring background, derived from the variety Cheyenne were developed by Dr. Rosalind Morris at the University of Nebraska, whereas the substitution lines from *Triticum spelta* and Cappelle-Desprez were developed at this Institute by Dr. C. N. Law and his colleagues. All the substitution lines have had between 5 and 7 backcrosses to Chinese Spring. It has been assumed in this paper that all the substitution lines have a Chinese Spring genetic background and that the substituted chromosomes have not recombined with their Chinese Spring homologues.

Plants were grown under artificial lighting in a glasshouse maintained at approximately 20° C. When the aerial growth was approximately 9–10 inches high it was harvested and stored in aluminium foil at –20° C. Approximately 10 plants of each genotype were pooled for DNA extractions.

DNA and ^3H rRNA Purification

DNA was purified from frozen aerial plant tissue as described in detail elsewhere (Smith and Flavell, 1974). The method was similar to that described by Bolton *et al.* (1964) followed by treatments with amylase, pancreatic ribonuclease and self-digested pronase. The solution was repeatedly shaken with phenol saturated with 0.2 × SSC (SSC = 0.15 M sodium chloride, 0.015 M sodium citrate) in the presence of 2% sodium lauryl sulphate until no further precipitate appeared at the interface upon centrifugation. The final aqueous layer was dialysed overnight against 0.1 × SSC and the DNA recovered by precipitation with ethanol. All DNA preparations were then further purified on preparative caesium chloride gradients as described by Flamm *et al.* (1966) except those used for the saturation curves. The DNA from the diluted caesium chloride gradient fractions was recovered by ethanol precipitation and centrifugation.

^3H labelled rRNA was purified from germinated wheat embryos (variety Chinese Spring) after incubation in ^3H-uridine exactly as described previously (Mohan and Flavell, 1974). The ^3H labelled rRNA had a specific activity of 24400 cpm.

Hybridisation Procedure

DNA dissolved in 0.1 × SSC was alkali denatured, neutralised and loaded on to HAWP Millipore filters (Mohan and Flavell, 1974). Approximately 25 μg of DNA were loaded per filter. Hybridisation in the presence of 5 μg/ml of radioactive rRNA or as indicated in Results was carried out for 3 hours at 70° C in 6 × SSC. Comparative saturation curves were obtained for several of the DNAs by incubating together four replicate filters of each type of DNA in 6 × SSC with different concentrations of ^3H labelled rRNA. The filters were washed in 2 × SSC, treated with RNase (10 μg/ml) washed further and counted in a scintillation cocktail as described previously (Mohan and Flavell, 1974). The DNA content of each filter was determined after counting by hydrolysing in 1N HCl at 100° C for 20 min and measuring the OD_{260} of the HCl solution (OD_{260} of 27.8 = 1 mg/ml DNA; Brown and Weber, 1968). The percentage hybridisation of rRNA to DNA was calculated from the specific activity of the rRNA and the DNA on the filter. All measurements were

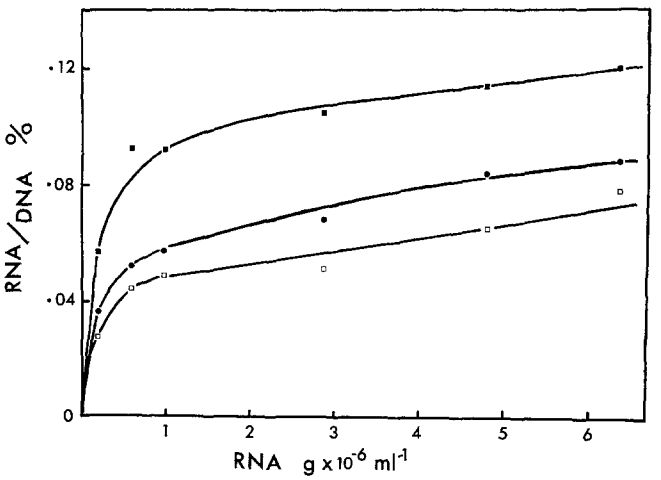

Fig. 1. Saturation curves for hybridisation of rRNA to three wheat varieties. Filters loadedwith DNA were incubated at 70° C in 6 × SSC for 3 hours with different concentrations of rRNA as indicated. • Chinese Spring DNA, ▪ Cheyenne DNA, ▫ Holdfast DNA. Each point is the mean of four replicate filters corrected for the behaviour of filters lacking DNA

corrected for the behaviour of filters lacking DNA. At least six replicate filters containing each DNA were incubated together and in many cases, duplicate DNA preparations also used. Standard errors of the mean percentage of each of the DNAs which hybridised to rRNA were calculated by analysis of variance.

Results and Discussion

The multiplicity of rRNA genes was investigated in DNAs highly purified from seven hexaploid wheat varieties and two rye varieties by rRNA/DNA hybridisation. Some of the wheat varieties were chosen because they are the source of the nucleolar organiser chromosomes substituted into the genetic background of the control variety, Chinese Spring. The hybridisation conditions used (6 × SSC at 70° C for 3 hr in the presence of 5 μg/ml of rRNA) provide a valid comparative estimate of rDNA content (Fig. 1 and 2). Five or six filters loaded with Chinese Spring DNA were included in every hybridisation experiment to serve as internal controls. The mean percentage of Chinese Spring DNA hybridising with rRNA under the conditions described in Materials and Methods was 0.087 ± 0.001. This figure was gained from 10 different hybridisation experiments (80 filters) involving three different preparations of rRNA and five preparations of DNA taken from plant material of different ages, grown under very different conditions.

Table 1. Hybridisation of rRNA to DNA from different wheat and rye varieties

	Variety	% hybridisation (mean[a] × standard error of mean)
A. Wheat		
	Chinese Spring	0.086± 0.004
	Hope	0.072± 0.004
	Koga II	0.073± 0.002
	Cheyenne	0.111± 0.005
	Cappelle-Desprez	0.075± 0.003
	Triticum spelta	0.120± 0.004
	Holdfast	0.055± 0.001
B. Rye		
	Petkus	0.174± 0.002
	King II	0.071± 0.002

[a] Mean of 6 replicate filters.

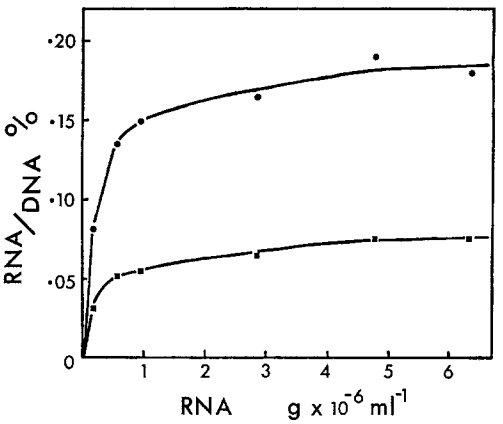

Fig. 2. Saturation curves for hybridisation of rRNA to two rye varieties. Filters loaded with DNA were incubated at 70° C in 6 × SSC for 3 hours with different concentrations of rRNA as indicated. • Petkus DNA, ▪ King II DNA. Each point is the mean of four replicate filters corrected for the behaviour of filters lacking DNA

The percentage of the DNAs of the wheat and rye varieties that hybridised to rRNA are given in Table 1. There is approximately a 2.2 fold variation in the number of rRNA genes within the array of wheat varieties. All the values are significantly different from one another except those for Hope, Koga II and Cappelle Desprez. Approximately

Table 2. Hybridisation of rRNA to DNAs from Chinese Spring lines with different substituted nucleolar organiser chromosomes

Substituted chromosome	Varietal source of substituted chromosome			
	Cappelle-Desprez	*Triticum spelta*	Cheyenne	Hope
1A	0.100± 0.003*	0.115± 0.002***	0.089± 0.002	0.079± 0.002
1B	0.086± 0.002	0.077± 0.002*	—	0.089± 0.002
5D	0.087± 0.003	0.096± 0.002**	0.082± 0.003	0.066± 0.002***
6B	0.044± 0.001***	0.069± 0.002***	—	0.065± 0.003***

Values are mean % ± standard deviation of mean DNA hybridised to rRNA.
* significantly different from internal Chinese Spring control at 5% level of probability,
** significantly different from internal Chinese Spring control at 1% level of probability.
*** significantly different from internal Chinese Spring control at 0.1% level of probability.
— denotes not measured.

2.5 times more rRNA hybridised to Petkus rye DNA than to DNA from the rye variety King II. Saturation curves for wheat rRNA binding to DNAs from three wheat varieties and two rye varieties are shown in Fig. 1 and 2 respectively.

There are at least four different chromosomes in hexaploid wheat, viz. 1A, 1B, 5D and 6B known to be able to form nucleoli (Crosby, 1957). In order to test whether the observed variation in rRNA gene multiplicity in some of the varieties shown in Table 1 could be ascribed to individual nucleolar organisers, the rDNA content was investigated in substitution line stocks derived from the recipient variety Chinese Spring. In these stocks each of the Chinese Spring nucleolar chromosomes 1A, 1B, 5D and 6B has, in turn, been replaced by its homologue from the varieties Cappelle-Desprez, Hope, *Triticum spelta* and Cheyenne. The percentages of DNA from these lines hybridising to rRNA are shown in Table 2. The deviation of the mean value for each genotype from the mean of the internal Chinese Spring control was tested for significance. In no case was the internal Chinese Spring control significantly different from the mean Chinese Spring value determined over many experiments (see above).

At least one example within each group of lines with the same Chinese Spring nucleolar organiser carrying chromosome substituted, was significantly different from Chinese Spring, indicating that within wheat, variation exists in rRNA multiplicity at each of the known nucleolar organiser sites (Flavell and Smith, 1974).

Table 3. The role of chromosomes 1A, 1B, 5D and 6B in determining the rDNA content of Cappelle-Desprez, Hope and *Triticum spelta*

Chromosome donor variety	Sum of effects of substituted chromosomes 1A, 1B, 5D and 6B on rDNA content of Chinese Spring[a]	rDNA of donor variety — rDNA of Chinese Spring
Cappelle-Desprez	−0.027	−0.011
Hope	−0.045	−0.014
Triticum spelta	+0.013	+0.034

[a] Sum of (rDNA substitution lines—rDNA Chinese Spring control) for substitution lines involving chromosomes 1A, 1B, 5D and 6B.

Comparison of the percentage rRNA hybridisation for each of the substitution lines DNAs with the parental control variety Chinese Spring DNA enables the variation on each of the nucleolar chromosomes of the donor varieties to be assessed relative to the homologous Chinese Spring chromosomes. The sum of this variation would be expected to be equivalent to the difference between the rRNA hybridisation values for the donor variety and Chinese Spring, if the total rRNA gene complement of wheat were controlled by only the substituted chromosomes, *i.e.*, 1A, 1B, 5D and 6B, acting additively. The appropriate values derived from the results in Tables 1 and 2 are presented in Table 3. Using the standard errors in Tables 1 and 2, in no case was the sum of the effects of chromosomes 1A, 1B, 5D and 6B significantly different from the difference between Chinese Spring and the donor variety. (For Cappelle-Desprez $P > 0.100$; Hope $P => 0.05 < 0.10$; *Triticum spelta* $P > 0.15$). Thus we can tentatively conclude that chromosomes 1A, 1B, 5D and 6B are the major chromosomes controlling rDNA content and that each of these chromosomes, acting additively, can account for the rRNA gene content of the hexaploid varieties Capelle-Desprez, Hope and *Triticum spelta*. However, it is possible that more extensive studies might uncover a departure from additivity, especially for the variety Hope.

Varietal variation in rRNA gene multiplicity at homologous nucleolar organisers implies that a mechanism exists in the *Triticineae* for altering rRNA multiplicity (Mohan and Flavell, 1974). Such a mechanism may also exist in hyacinth (Timmis *et al.*, 1973), flax (Timmis and Ingle, 1973) and maize (Phillips *et al.*, 1973) in which variation in rDNA content has also been reported.

The finding that different varieties or isolates within a species may have different numbers of rRNA genes emphasizes that in future reports

of rRNA gene multiplicity, the varieties and source of the plant material should be quoted in detail, rather than the species name alone.

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