Chromosoma (Berl.) 71, 135–152 (1979)

CHROMOSOMA

The Genetic Control of Nucleolus Formation in Wheat

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Abstract. The wheat variety Chinese Spring has four pairs of nucleolus organisers of known rDNA content. The genetic control of these has been investigated in root tip cells by cytologically scoring the number of nucleoli per cell in (a) aneuploid derivatives each having a different dosage of a particular chromosome or chromosome arm and (b) in substitution lines where nucleolus organiser chromosomes have been replaced by homologues possessing different amounts of rDNA. It has been assumed that nucleolus organiser activity is correlated with nucleolus size and thus with the presence of a cytologically visible nucleolus. Those nucleolus organisers on chromosomes 1A and 5D, which together possess only 10% of the rDNA form a visible nucleolus only infrequently in the presence of the larger nucleolus organisers on chromosomes 1B and 6B. When a major pair of organisers on chromosomes 1B or 6B is deleted, the smaller nucleolus organisers form a visible nucleolus more frequently. Similarly, when the major nucleolus organisers are replaced by organisers with less rDNA, the smaller nucleolus organisers form visible nucleoli more frequently. When a small nucleolus organiser is replaced by one with much more rDNA, a larger nucleolus is formed. These and other findings lead to the general conclusions that there is a frequently, but not invariably, seen correlation between rRNA gene number and nucleolus size. However the relative size of the nucleolus formed depends principally upon the proportion of the total active rRNA genes in the cell which are localised at the nucleolus organiser in question. Varying the dosage of at least 13 non nucleolus organiser chromosomes also resulted in changes in the number of visible nucleoli per cell. This implies the genetic control of individual nucleolus organisers is complex. Inclusion in the wheat genome of the nucleolus organiser chromosome from *Aegilops umbellulata*, causes suppression of the wheat nucleolus organisers, the *Aegilops umbellulata* organiser remaining active. This suppression is similar to that observed in many interspecific plant and animal hybrids.

Introduction

The ribonucleoprotein precursor particles of ribosomes are assembled in nucleoli. Nucleoli, in most somatic cells form at the chromosomal sites of the genes

0009-5915/79/0071/0135/$03.60

for the principal ribosomal RNA molecules, termed on the basis of their sedimentation constants 25, 18 and 5.8 in higher plants. These chromosomal sites, the nucleolus organisers, consist of tandemly arranged genetic units, each unit containing a 25S, 18S and 5.8S gene separated by "spacer" DNA. In a number of animal species, especially Bufo (Miller and Brown, 1969), *Xenopus* (Miller and Gurdon, 1970), *Ambystoma* (Sinclair et al., 1974), *Plethodon* (Macgregor et al., 1977) and *Drosophila* (Krider and Plaut, 1972a, b; Shermoen and Kiefer, 1975) genetic variants having different numbers of rRNA genes have enabled the relationships between rRNA gene multiplicity, nucleolus organiser activity, nucleolus size (volume) and phenotype to be investigated. The results from these studies can be summarised as follows (1) In natural populations the number of rRNA genes in individual organisms can vary by a factor greater than two. As might be expected with this variation, substantial proportions of the rRNA genes (e.g. 50% in the *Xenopus* lines studied by Miller and Knowland, 1970; Knowland and Miller, 1970; 45% in the *Ambystoma* individual studied by Miller and Brown, 1969) can be deleted without reducing rRNA synthesis or causing noticeable differences in phenotype, i.e., there appears to be some rRNA gene redundancy. Studies on certain lines of *Drosophila* (Shermoen and Kiefer, 1975) and *Xenopus* (Knowland and Miller, 1970), however, show that deletions of rRNA genes can result in reduced rates of rRNA synthesis and altered phenotypes at some developmental stages, presumably those which require high rates of ribosome synthesis. (2) In many instances in mutants with duplications or deletions of rRNA gene clusters, a correlation can be found between the size of the nucleolus constriction in metaphase chromosomes, the number of rRNA genes and the size of the nucleolus (Miller and Knowland, 1970, *Xenopus*; Miller and Brown, 1969, *Bufo*; Sinclair et al., 1974, *Ambystoma*). However, this correlation depends upon the tissue and developmental stage examined, i.e., factors other than the number of rRNA genes must regulate the size of the nucleolus in many cells.

The number of rRNA genes found in insects and mammals is generally in the order of 100 to 1000 (Birnstiel et al., 1971). However, in a survey of 45 higher plant species, Ingle et al. (1975) reported the range to be between 1250 and 32,000 per telophase nucleus. This, together with the considerable intraspecies variation in gene number (Timmis et al., 1972; Phillips et al., 1973; Flavell and Smith, 1974b; Cullis and Davies, 1975; Ramirez and Sinclair, 1975a) suggests that in most higher plant somatic cells, at least, there is a considerable excess of rRNA genes over the number required to maintain ribosome synthesis (Timmis and Ingle, 1975). In maize, where several detailed studies of ribosomal DNA (rDNA) have been accomplished using stocks in which different proportions of the rDNA have been translocated to other chromosomes (McClintock, 1934; Doerschug, 1976; Ramirez and Sinclair, 1975b) it is clear that nucleolus formation is associated with only a small fraction of the rDNA (Givens and Phillips, 1976; Phillips, 1978). Most of the rRNA genes lie in heterochromatin and are normally inactive.

The organisms highlighted so far in genetic studies on rRNA genes, have only one pair of nucleolus organisers. A more complicated situation arises where multiple nucleolus organisers are present. Here "competition" between

organisers as well as gene multiplicity regulates nucleolus formation. Common bread wheat, *Triticum aestivum*, possesses at least four pairs of nucleolus organisers because it is an allohexaploid and carries the nucleolus organisers from its three diploid progenitors. In 1957, Crosby published evidence that wheat chromosomes 1B, 6B, 1A and 5D were able to form nucleoli when assayed individually in micronuclei at meiosis but not all formed nucleoli in hexaploid cells. Since then several cytogenetical investigations have been carried out on nucleolus formation in wheat confirming that the short arms of these four chromosomes carry nucleolus organisers and illustrating that chromosomes 1B and 6B generally form large nucleoli while chromosomes 5D and 1A form only small nucleoli or nucleoli too small to be seen (Longwell and Svihla, 1960; Bhowal, 1972; Darvey and Driscoll, 1972). In the wheat variety Chinese Spring, a complete set of aneuploids affecting the dosage of each of the 21 chromosomes is available (Sears, 1954). Using these stocks, we determined the number of rRNA genes on each of the nucleolus organiser chromosomes (Flavell and Smith, 1974a, 1974b; Flavell and O'Dell, 1976), showing the organisers on 1B and 6B possessed approximately 90% of the total rRNA gene complement (Flavell and O'Dell, 1976). The aneuploid lines have also permitted the production of intervarietal chromosome substitution lines, in which nucleolus organiser chromosomes have been transferred from one genetic background into that of the variety Chinese Spring (Law, 1968). These lines enabled us to show that the number of rRNA genes on homologous chromosomes in different varieties can vary substantially (Flavell and Smith, 1974b). The availability of all these lines, in a relatively constant genetic background, varying in nucleolus organiser dosage, rRNA gene number or in the dosage of chromosomes not known to carry a nucleolus organiser has allowed us to undertake a more detailed genetic investigation of the control of nucleolus formation than is possible with other species (see also Viegas and Mello-Sampayo, 1975). In this paper nucleolus activity has been assayed cytologically. It has been assumed that the transcriptional activity of a nucleolus organiser can be evaluated by whether it makes a nucleolus or not.

Materials and Methods

Genotypes Used. Aneuploid seeds of *Triticum aestivum*, variety Chinese Spring, were originally obtained from Dr. E.R. Sears, University of Missouri, Columbia, Missouri, USA (Sears, 1954) but multiplied at this Institute for many years. Substitution lines in which chromosomes of Chinese Spring have been replaced by their homologues from the variety Hope were also obtained from Dr E.R. Sears. The substitution lines, again in a Chinese Spring background derived from the variety Cheyenne were developed by Dr R. Morris, University of Nebraska while the substitution lines from *Triticum spelta* and Cappelle-Desprez were developed in this Department by Dr C.N. Law and his colleagues (Law, 1968; Law and Worland, 1972). The Chinese Spring lines carrying chromosome $1C^u$ from *Aegilops umbellulata* were developed by Mr V. Chapman of this Department.

Growth of Seedlings. Between five and ten seeds of each genotype to be compared in each experiment were sterilised by soaking for about 10 min in 2% sodium hypochlorite and washed in sterile water. They were then allowed to stand on moist filter paper at 3° C for 7 days to fully imbibe and commence germination. Seeds were then individually planted in wells cut in agar medium

solidified in a dish approximately 20×15 cm. The medium contained a salts solution similar to that known as Hoagland's (see Flavell and MacPherson, 1972 for details) supplemented with 0.5% sucrose and 1% agar. After the seeds were planted, the dishes were covered with foil, sealed in plastic bags containing moist cotton wool and incubated in the dark at 27° C for about 60 h. Root tips were harvested as far as possible from roots growing in the agar.

Estimation of Nucleoli. Root tips from seedlings grown in agar as described earlier were fixed for 24 h in a solution made up of 6.33 ml formaldehyde 12.66 ml of ethanol and 1 ml of glacial acetic acid. The root tips were then hydrolysed in 1N HCl for 2 h at 60° C and stored in 45% acetic acid at 4° C. For cytological examination the root tips were squashed under a cover slip with a drop of acetocarmine stain and the frequency of interphase cells from the meristematic region having 1 to 8 nucleoli was scored. Between 250 and 500 cells in each of four to eight root tips from four to eight different seeds of each genotype were scored. The mean number of nucleoli per cell is given in Results for most genotypes together with the proportion of cells having more than three nucleoli. The latter parameter is a useful one for quantitatively distinguishing genotypes.

When nucleoli are very small, they are more difficult to discern, especially if the nuclear background appears "granular". Care was therefore taken to use only cytologically suitable root tips. This was done to prevent small nucleoli from being omitted from some of the scores.

Determination of Ribosomal RNA Gene Number. This was done by rRNA/DNA hybridisation exactly as described previously (Flavell and Smith, 1974a). The purification of the ^{3}H-labelled rRNA and DNAs was carried out also as described previously (Flavell and Smith, 1974a). For the purposes of this paper only the mean values for five filter determinations of each genotype are given. The rRNA gene contents of many of the genotypes used in this paper have been reported in detail previously (Flavell and Smith, 1974a, b; Flavell and O'Dell, 1976). Gene numbers were calculated assuming the 1C DNA content of hexaploid wheat is 10.65×10^{12} daltons and 25S and 18S rRNA molecules are 1.3×10^6 and 0.7×10^6 daltons respectively.

Results

To estimate nucleolus organiser activity in wheat, the frequency of nucleoli in root tip cells of a wide range of genotypes has been studied. To ensure that the between-genotype comparisons were more meaningful, roots of different genotypes to be compared were grown in the same dish of defined agar medium containing sucrose. This method of growing seedlings improves growth and seedling uniformity (Flavell and McPherson, 1972; Flavell and Barratt, 1977). Seeds of the control variety Chinese Spring were always included in each dish. Before planting, the seeds were imbibed with sterile water for one week to assist uniform germination rates. These precautions were considered desirable since it is possible that nucleolus formation is sensitive to the stage of development and/or nutritional status of the root.

Nucleolus Formation in the Variety Chinese Spring

The frequency of root tip cells in the variety Chinese Spring having between 1 and 6 nucleoli is shown in Figure 1. Cells displaying 5 or more nucleoli are in low frequency even though this variety possesses eight nucleolus organisers. Approximately 58% of the cells display 3 or 4 nucleoli implying Chinese Spring utilises preferentially only four nucleolus organisers (Longwell and Svihla, 1960; Darvey and Driscoll, 1972). This is almost certainly because 90% of the rRNA

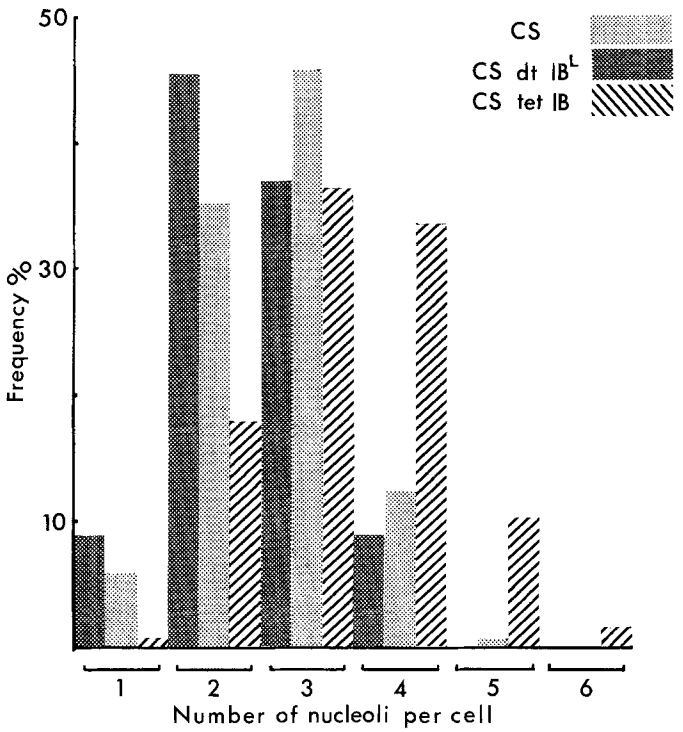

Fig. 1. Nucleolus formation in lines differing in dosage of chromosome 1B nucleolus organiser. *CS* Chinese Spring, *CS dt 1B*^{L} Chinese Spring with the short arm of chromosome 1B deleted, *CS tet 1B* Chinese Spring with two additional 1B chromosomes

genes are associated with two pairs of nucleolus organisers, those on chromosomes 1B and 6B (Flavell and O'Dell, 1976). Most of the remaining 10% are probably on chromosome 5D (Gerlach, personal communication). However, since not all cells displayed four nucleoli (the mean number of nucleoli per cell was approximately 2.6) either some cells fail to use all four major nucleolus organisers or nucleoli fuse to produce larger but fewer nucleoli. Evidence for fusion is well-founded (Darvey and Driscoll, 1972) and dumb-bell shaped nucleoli, the products of fusion, are frequently visible. We therefore assume that essentially all root tip cells of Chinese Spring use the four major nucleolus organisers on chromosomes 1B and 6B, but some if not all, also use the organisers on 5D chromosomes (see later).

The Effect of Varying Major Nucleolus Organiser Chromosome Dosage on the Number of Nucleoli

Deletion of the short arm of chromosome 1B (in ditelosomic 1B^L) deletes a pair of major nucleolus organisers. This was confirmed biochemically by detecting the deletion of approximately 15% of the rRNA genes in DT 1B^L (Flavell

and Smith, 1974a). Loss of these nucleolus organisers significantly reduces the mean number of nucleoli per cell and the percentage of cells with more than three nucleoli (Table 1, Fig. 1). However, 48% of the cells lacking the chromosome 1B nucleolus organisers still had three or four nucleoli cytologically distinct (Fig. 1), indicating that the small nucleolus organisers on chromosomes 5D and/or 1A, must be used to a greater extent when the nucleolus organisers on chromosome 1B are deleted. This illustrates the phenomenon of "nucleolar compensation" and has been observed previously (Longwell and Svihla, 1960; Darvey and Driscoll, 1972).

Duplication of chromosome 1B (in tetrasomic 1B and in the compensated tetrasomic 1B stocks carrying deletions of chromosomes 1A and 1D respectively) resulted in significant increases in nucleoli (Fig. 1 and Table 1). These results illustrate the expected relationship between major nucleolus organiser number and number of nucleoli formed.

Duplication of the other Chinese Spring chromosome carrying a major nucleolus organiser, chromosome 6B, also significantly increased the number of nucleoli (Table 1). However only a small non-significant reduction was observed when these nucleolus organisers were deleted (in DT $6B^L$ and nullisomic 6B tetrasomic 6A). Again nucleolar compensation therefore occurs when a major pair of organisers are deleted.

Deletion of the short arm of chomosome 5D which carries the small nucleolus organiser resulted in a reduction in the mean number of nucleoli per cell and

Table 1. Nucleolus number and variation in nucleolus organiser chromosome dosage

Genotype	No. of [a] rRNA genes per 2C cell	Mean no. nucleoli/cell	% cells with >3 nucleoli/cell
Euploid	9150	2.72±0.05	14.4±2.2
DT $1B^L$	8450	2.41±0.044***	8.1±1.10*
N1B T1A	7100	2.44±0.049**	10.5±0.83
N1B T1D	7700	2.47±0.087*	8.1±1.98*
Tet 1B	13600	3.32±0.035***	47.2±1.68***
N1A T1B	12000	3.42±0.197**	44.8±6.3***
N1D T1B	13000	3.30±0.091**	46.0±2.31***
Tet 6B	15700	3.32±0.08***	38.5±3.5***
DT $6B^L$	3100	2.45±0.11	8.1±2.9
N6B T6A	5750	2.60±0.03	11.4±3.8
Tet 1A	8500	2.59±0.08	11.5±3.0
N1A T1D	9650	2.70±0.02	12.8±2.0
DT $5D^L$	7700	2.49±0.06*	10.6±1.8*

DT = ditelosomic, N = nullisomic, T = tetrasomic

*, **, ***, significantly different from Chinese Spring at the 5%, 1% and 0.1% level of probability respectively

[a] Taken from Flavell and Smith (1974a) and Flavell and O'Dell (1976)

the proportion of cells having 3 or more nucleoli indicating this small nucleolus organiser is active to some extent in Chinese Spring. In previous studies Darvey and Driscoll (1972) showed that it was the very small nucleoli that are deleted in ditelosomic $5D^L$. If the nucleolus organiser on chromosome 1A is too small to form a visible nucleolus (see later) and the major organisers on chromosomes 1B and 6B are active in all cells, compensation for nucleolus number would not be expected in ditelosomic $5D^L$.

Duplication of chromosome 1A in tetrasomic 1A or deletion of chromosome 1A in nullisomic 1A tetrasomic 1D resulted in no significant change in nucleolus number (Table 1).

The Effect of Variation in Ribosomal RNA Gene Number on Nucleolus Formation

It has already been stated that the most likely reason that chromosome 1B and 6B nucleolus organisers promote the formation of the four major nucleoli in Chinese Spring root tip cells is the fact that they carry 90% of the ribosomal RNA genes (Flavell and O'Dell, 1976). We have previously reported that substantial increases in rRNA gene number occur when chromosome 1A of Chinese Spring is substituted by chromosome 1A from certain other varieties (Flavell and Smith, 1974b). These results imply that these varieties have many more rRNA genes in their chromosome 1A nucleolus organisers. To test whether the presence of more rRNA genes results in a larger nucleolus being formed, the numbers of nucleoli were scored in root tip cells of Chinese Spring substitution lines with different 1A chromosomes. It was not necessary to score nucleolus size since the chromosome 1A nucleolar organiser of Chinese Spring does not from a visible nucleolus (Table 1). The results when chromosome 1A of *Triticum spelta* which carries approximately 1500 rRNA genes replaced chromosome 1A of Chinese Spring are illustrated in Figure 2. Clearly, more cytologically visible nucleoli are formed in this genotype. The relationship between rRNA gene number in 1A substitution lines and the mean number of nucleoli per cell for 1A substitution lines involving 1A chromosomes from four varieties is shown in Figure 3. Although other explanations are possible the simplest hypothesis to explain the relationship between rRNA gene number and mean number of visible nucleoli is that the size of nucleolus formed and thus the extent to which it is cytologically visible, is determined by the number of rRNA genes in the chromosome 1A nucleolus organiser.

A similar conclusion can be drawn from studies on lines in which chromosome 5D of Chinese Spring has been substituted by homologous chromosomes from other varieties. Substitution of chromosome 5D of *Triticum spelta* into Chinese Spring increases the number of rRNA genes implying the chromosome 5D organiser is larger in *Triticum spelta* than in Chinese Spring. The number of visible nucleoli in this line is also significantly increased, again suggesting that the number of rRNA genes clustered at a nucleolus organiser determines, at least in part, the size of the nucleolus formed and hence whether it will be visible or not. The correlation between mean nucleolus number and rRNA gene number for some 5D chromosome substitution lines is shown in Figure 4.

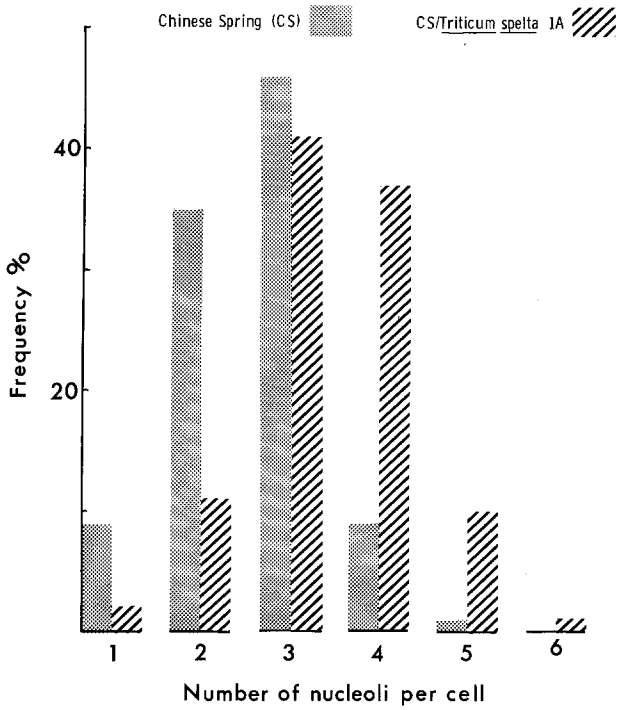

Fig. 2. The effect of chromosome 1A from *Triticum spelta* on nucleolus formation

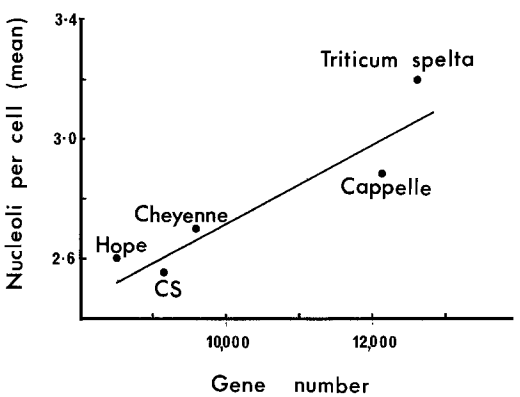

Fig. 3. Nucleolus formation in lines differing in 1A chromosomes carrying different numbers of rRNA genes. The varieties named on the figure are the sources of the 1A chromosomes substituted into the variety Chinese Spring (*CS*). The lines are assumed to be isogenic except for the 1A chromosomes and the variation in rRNA gene number is assumed to be restricted to the 1A nucleolar organisers (Flavell and Smith, 1974b)

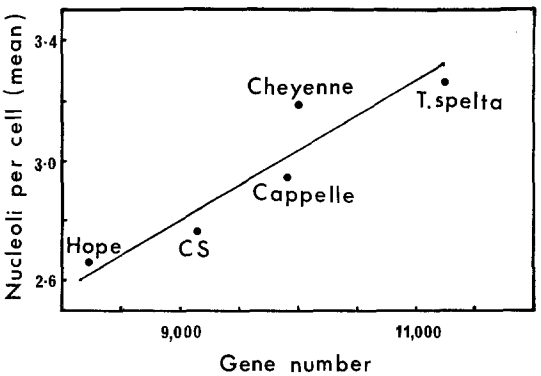

Fig. 4. Nucleolus formation in lines differing in 5D chromosomes carrying different numbers of rRNA genes. The varieties named on the figure are the sources of the 5D chromosomes substituted into the variety Chinese Spring (*CS*). The lines are assumed to be isogenic except for the 5D chromosome and the variation in rRNA gene number is assumed to be restricted to the 5D nucleolar organiser (Flavell and Smith, 1974b)

Table 2. Nucleolus formation in substitution lines differing in chromosome 6B nucleolus organisers

Genotype	No. of rRNA genes per 2C cell[a]	Mean no. of nucleoli per cell	% cells with >3 nucleoli per cell
Chinese Spring Euploid	9150	277 ± 0.06	15.3 ± 1.2
Chinese Spring/6B *T. spelta*	7950	3.35 ± 0.13**	46.8 ± 2.2***
Chinese Spring/6B Cappelle Desprez	4700	3.47 ± 0.04***	47.5 ± 2.8***
Chinese Spring/6B Cheyenne	–	3.36 ± 0.13**	45.9 ± 5.1***

, *, significantly different from Chinese Spring at the 1% and 0.1% level of probability respectively

[a] Taken from Flavell and Smith (1974b)

When chromosome 6B of Chinese Spring is replaced by chromosome 6B from the variety *Triticum spelta*, Cheyenne, or Cappelle-Desprez there is a 20 to 50% reduction in rRNA genes (Table 2) implying the nucleolus organisers on the substituted chromosomes carry considerably fewer rRNA genes than the nucleolus organiser of chromosome 6B of Chinese Spring (Flavell and Smith, 1974b). Root tip nucleoli counts on these chromosome 6B substitution lines show that altering chromosome 6B causes significantly more visible nucleoli to be produced (Table 2). Because $10 \rightarrow 20\%$ of the cells show 5 or 6 nucleoli (results not shown), the nucleolus organisers on chromosomes 5D must be more active in these substitution lines. One explanation for the increased activity of the chromosome 5D nucleolus organisers is that the additional nucleoli are formed to compensate for a smaller nucleolus-organiser and presumably smaller nucleolus on chromosome 6B. Alternatively, (a) Chromosome 6B of Chinese Spring could carry a gene inhibiting the expression of the nucleolus organisers

Table 3. Nucleolus formation in abberant lines with rRNA gene deletions in major nucleolus organisers

Genotype	No. of rRNA genes per 2C cell[a]	Mean No. of nucleoli per cell	% cells with >3 nucleoli per cell
Euploid	9150	2.70±0.07	15.4±1.9
Ditelosomic $1A^L$	5300	3.22±0.13***	34.8±2.6***
Tetrasomic 5D	5400	4.63±0.32***	81.6±2.3***

***, significantly different from Chinese Spring at the 0.1% level of probability respectively

[a] Taken from Flavell and Smith (1974a) and Flavell and O'Dell (1976)

on chromosomes 5D or (b) Chromosomes 6B in the other varieties could carry promotors of the nucleolus organisers on 5D or 1A Chinese Spring chromosomes.

We have observed two other examples where reduction in rRNA gene number is associated with the production of more nucleoli in root tip cells. In the Chinese Spring aneuploid genotype in which the short arm of chromosome 1A has been deleted there is a 40% reduction in rRNA genes (Flavell and Smith, 1974a) and in the Chinese Spring genotype with an additional pair of 5D chromosomes (tetrasomic 5D), there is a 41% reduction in rRNA genes (Mohan and Flavell, 1974). Because the organisers on chromosomes 1A and 5D together possess fewer than 500 genes these reductions in rRNA genes must be due to deletions in the 1B and/or 6B nucleolus organisers. Both the ditelosomic $1A^L$ and tetrasomic 5D stocks have significantly higher numbers of nucleoli in root tip cells (Table 3) again suggesting that deletion of rRNA genes in major nucleolus organisers is compensated by the activation of minor nucleolar organisers. The number of nucleoli in root tips of the tetrasomic 5D stock is especially high – cells containing eight nucleoli were not rare, although all the nucleoli were small. This suggests that in this stock both pairs of nucleolus organisers on chromosomes 1B and 6B as well as the four nucleolus organisers on the four 5D chromosomes are active, i.e., the deletion of rRNA genes has resulted in activation of the 5D organisers which are duplicated in this stock. That the activation of additional organisers in these aneuploids is due to the rRNA gene deletion is supported by the observations that in the stock where chromosome 1A is deleted, (nullisomic 1A tetrasomic 1D) but where significant rRNA gene deletion has not been detected, the number of nucleoli is the same as in the euploid (Table 1).

Regulation of Wheat Nucleolus Organisers by an Alien Nucleolus Organiser Chromosome

The major nucleolus organiser chromosome in the diploid *Aegilops umbellulata* has been designated $1C^u$. It possesses approximately 1,500 genes (Flavell and

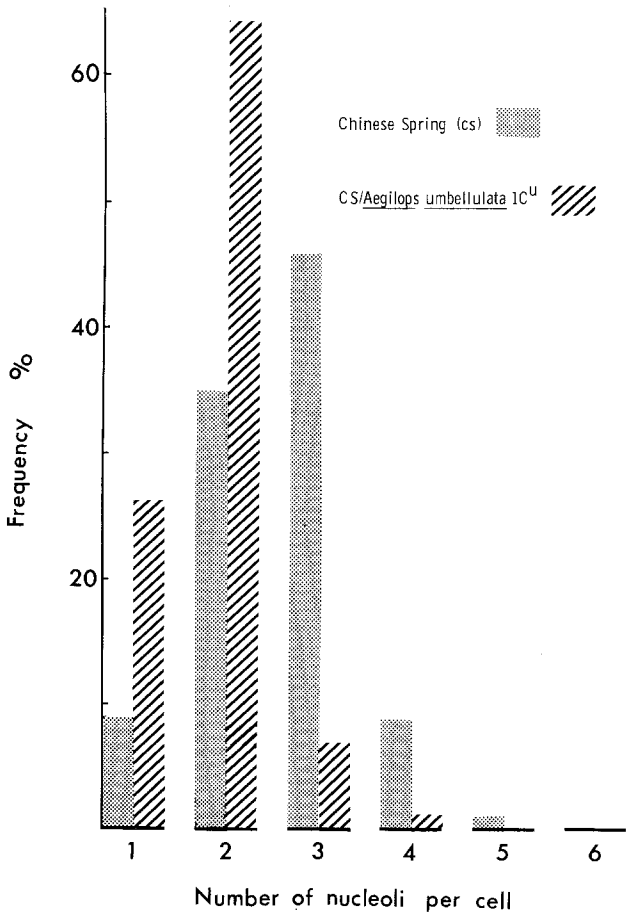

Fig. 5. Suppression of wheat nucleolus formation by chromosome 1C from *Aegilops umbellulata*. Only relatively large nucleoli were included in the results used for this figure (see text)

Smith, 1974a). When this is substituted into Chinese Spring in place of chromosomes 1A or 1D or added to the total complement of Chinese Spring chromosomes, almost all root tip cells possess only one or two large nucleoli (see Fig. 5) and a number of minute, barely visible nucleoli. This pattern is clearly distinguishable from euploid Chinese Spring and is completely at variance from what would be expected from the addition of a pair of large nucleolus organisers to the wheat chromosome complement (see results for tetrasomic 1B in Fig. 1). When chromosome $1C^u$ is substituted for chromosome 1B of Chinese Spring almost all the minute nucleoli disappear implying that the major pair of nucleoli observed in the presence of $1C^u$, form at the *Aegilops umbellulata* nucleolus organiser. This conclusion is also apparent from mitotic metaphase squashes, where in most cells the major constrictions observed are on the $1C^u$ chromosomes; the normally visible constrictions of hexaploid wheat frequently being suppressed. It appears therefore that the nucleolus organising chromosome of

Aegilops umbellulata suppresses the nucleolus organisers of *Triticum aestivum* variety Chinese Spring.

Control of Nucleolus Formation by Non-Nucleolus Organiser Chromosomes

The role of the 17 chromosomes of hexaploid wheat not known to carry a nucleolus organiser in controlling the formation of nucleoli at the nucleolus organisers on chromosomes 1B, 6B, 1A and 5D was first investigated by counting nucleoli in root tips of the aneuploid stocks of Chinese Spring. In these stocks, chromosome arms are deleted or whole chromosomes are duplicated or deleted, the deletions being compensated by the duplication of homoeologous chromosomes. The results are displayed in Table 4 for stocks varying in homoelogous chromosome groups 1, 2 and 3, in Table 5 for stocks varying in homoeologous groups 4 and 5, and Table 6 for stocks varying in homoeologous Groups 6 and 7. Increasing the number of chromosomes 2A, 2B, 2D, 3A, 3B, 3D (Table 4), 4A, 4B and 4D (Table 5) significantly increased the mean number of nucleoli

Table 4. Nucleolus formation in lines aneuploid for non nucleolus organiser chromosomes in homoelogous Groups 1, 2 and 3

Genotype	No. of rRNA genes per 2C cell[a]	Mean no. nucleoli per cell	% cells with >3 nucleoli/cell
Euploid	9100	2.63±0.05	16.0±2.0
DT $1A^S$	–	2.65±0.03	15.2±2.5
Tet 1D	–	2.75±0.06	16.6±0.9
Tet 2A	–	2.96±0.05***	32.3±1.8***
DT $2A^S$	9500	2.49±0.04	14.9±0.64
Tet 2B	–	2.99±0.04***	27.0±2.6***
DT $2B^L$	9700	2.49±0.06	11.9±1.2
Tet 2D	–	3.25±0.07***	33.5±1.7***
DT $2D^L$	9400	2.38±0.04**	12.3±1.9
DT $2D^S$	8000	2.85±0.04*	26.9±1.89**
Euploid	9100	2.76±0.06	16.3±2.1
Tet 3A	8200	3.47±0.17***	45.6±3.7***
DT $3A^{\alpha}$	–	2.71±0.03	16.6±1.4
DT $3A^{\beta}$	–	2.49±0.05**	6.6±0.9**
Tet 3B	10100	3.37±0.14**	45.6±3.7***
DT $3B^L$	–	2.69±0.06	14.7±1.9
Tet 3D	8750	2.98±0.05*	24.1±2.5*
DT $3D^{\alpha}$	–	2.73±0.05	16.0±2.6

DT = ditelosomic, Tet = tetrasomic, – = not measured

*, **, ***, significantly different from Chinese Spring at the 5%, 1% and 0.1% level of probability respectively

[a] Figures are mean values of five determinations with the same batch of DNA

Table 5. Nucleolus formation in lines aneuploid for non-nucleolus organiser chromosomes in homoeologous groups 4 and 5

Genotype	No. of rRNA genes per 2C cell[a]	Mean No. nucleoli per cell	% cells with >3 nucleoli/cell
Euploid	9100	2.86±0.03	18.8 ±1.7
Tet 4A	9100	3.58±0.10***	51.6 ±2.7***
DT $4A^{Lt}$	–	2.75±0.02	16.0 ±0.6
DT $4A^{Rt}$	–	2.56±0.03***	9.9 ±1.1**
Tet 4B	8750	3.76±0.12***	60.0 ±4.8***
DT $4B^{L}$	–	2.80±0.06	18.3 ±0.9
Tet 4D	8700	3.00±0.04*	27.7 ±0.4***
DT $4D^{Rt}$	–	3.30±0.10**	41.3 ±4.5**
Euploid	9050	2.45±0.03	9.7 ±0.88
DT $5A^{L}$	9250	2.31±0.08	5.25±2.14
Tet 5B	9500	2.68±0.05	15.40±2.50
DT $5B^{L}$	9700	2.29±0.02*	3.45±0.55**
DT $5D^{S}$	–	2.86±0.13*	21.7 ±4.2*

DT = ditelosomic, Tet = tetrasomic, – = not measured

*, **, ***, significantly different from Chinese Spring at the 5%, 1% and 0.1% level of probability respectively

[a] Average of 5 determinations using the same batch of DNA

per cell. Increases in the number of visible nucleoli could be due to a reduction in fusion of nucleoli or the activation of nucleolus organisers which are relatively silent in euploid Chinese Spring. Because in all these instances the number of cells showing five or six nucleoli per cell increased substantially it seems reasonable to conclude that six nucleolus organisers are active in these stocks in contrast to four (1B and 6B) in most Chinese Spring root tip cells, i.e., the nucleolus organisers on chromosome 5D or less likely 1A are most active in these tetrasomic stocks.

The finding that increasing chromosomes of the same homoeologous group, e.g. 3A, 3B and 3D, causes a similar change in nucleolus number is an example of the close relationship of the genetic activity of homoeologous chromosomes in wheat.

Removal of the α arm of 3A, the short arm of 2D, the short arm of 5B, the Lt arm of 4A and the long arm of 7A reduced the mean number of nucleoli per cell. Since compensation for deletion of nucleolus organisers can take place (see above for deletion of 1B and 6B chromosomes) it is possible that one pair of organisers is effectively inactivated in these stocks. Indeed, in DT $3A^{\beta}$ and DT $4A^{Rt}$ only a maximum of 4 nucleoli per cell was observed instead of the five or six in euploid and in most of the other genotypes. This strongly suggests that at least chromosomes 3A and 4A regulate the activity of the major nucleolus organisers on chromosomes 1B or 6B in Chinese Spring root tips.

Table 6. Nucleolus formation in lines aneuploid for non-nucleolus organiser chromosomes in homoeologous groups 6 and 7

Genotype	No. of rRNA genes per 2C cell[a]	Mean No. of nucleoli per cell	% cells with >3 nucleoli per cell
Euploid	9000	2.61±0.03	13.9±1.7
Tet 6A	–	2.60±0.11	10.1±2.6
DT $6A^S$	–	2.86±0.02**	22.5±1.2**
Tet 6D	–	2.75±0.08	16.0±0.9
DT $6D^{\alpha}$	–	2.67±0.04	16.4±3.0
DT $6D^{\beta}$	–	2.52±0.04	11.9±2.1
DT $6B^S$	8470	2.63±0.11	10.7±2.04
Euploid	9100	2.63±0.06	15.4±1.1
Tet 7A	10300	2.54±0.06	13.7±1.47
DT $7A^S$	–	2.49±0.04	9.1±0.9**
DT $7A^L$	–	2.97±0.06**	26.1±2.3**
Tet 7B	9000	2.69±0.05	13.3±1.7
DT 7BL	–	2.95±0.08*	21.4±1.9*
DT $7B^S$	–	2.54±0.08	10.0±2.2
Tet 7D	11100	2.63±0.03	14.0±2.1
DT $7D^S$	–	2.63±0.05	13.2±0.3

DT=ditelosomic, Tet=tetrasomic, –=not measured

*, **, significantly different from Chinese Spring at the 5% and 1% level of probability respectively

[a] Average of 5 determinations with same batch of DNA

Because deletion of chromosome arms of at least some group 2, 3 and 4 chromosomes reduced nucleolous number while increasing the dosage of the chromosomes increased nucleolus number, the genetic control of nucleolus organisers by these chromosomes appears to be related to chromosome dosage.

Removal of the short arms of chromosomes 7A and 7B and the long arms of 2D, 5D and 6A increased the number of nucleoli per cell suggesting alleles causing inhibition of nucleolus formation reside on chromosomes 2D, 5D, 6A, 7A and 7B.

Discussion

Formation of a visible nucleolus is a complex process, requiring the transport and assembly of the large number of ribosomal proteins as well as 5S, 25S and 18S RNA molecules. What role the regulation or availability of these molecules plays in development of a visible nucleolus is poorly understood. Recent studies on maize cells lacking the chromosome carrying the 5S genes indicate these molecules are not necessary for visible nucleolus formation (Weber, 1978). There is considerable evidence, however, that nucleolus formation is dependent upon 25S and 18S rRNA transcription.

Many results presented in this paper endorse a correlation between the amount of rDNA and the size of nucleolus formed. These results for wheat support the illustrations in *Xenopus*, (Miller and Knowland, 1970), *Bufo* (Miller and Brown, 1969), and maize (Phillips et al., 1971) of a correlation between rDNA, nucleolus organiser activity and nucleolus volume under certain circumstances. However, the size of a specific nucleolus is not invarient. Reduction of chromosome 1B or 6B rDNA, by chromosomal deletion or substitution, resulted in activation of the 5D organisers to form larger nucleoli. This implies that in euploid cells only a fraction of the rRNA genes on chromosome 5D are used or the rRNA genes are transcribed slowly. However, when the total rRNA gene complement of other chromosomes in the cell is decreased, more chromosome 5D rRNA genes are used or the rate of transcription is increased to maintain an overall regulated level of rRNA synthesis. Conversely, when more or larger nucleolus organisers are added, preexisting organisers are used less and smaller nucleoli are consequently formed. [Although no volumes are reported in this paper, it was almost consistently observed that the average nucleolus size was smaller in cells with additional active organisers, e.g., tetrasomic 1B (Fig. 1).] The conclusion from our cytological scores that not all rRNA genes are transcribed at high frequency in root tip cells is supported by (1) the large number of rRNA genes in plants (Ingle et al., 1975) (2) the intraspecies variation in rRNA gene number (Timmis et al., 1972; Phillips, 1976; Flavell and Smith, 1974b; Cullis and Davies, 1975; Ramirez and Sinclair, 1975a) (3) the constancy of rRNA content in wheat cells with a 5 fold variation in rRNA gene number (unpublished results) and (4) the finding that most of the rRNA genes in maize are localised in heterochromatin (Givens and Phillips, 1976; Phillips, 1978).

The results discussed so far suggest that the probability that a nucleolus organiser will form a visible nucleolus depends not only upon its absolute number of rRNA genes but also on the proportion of the potentially active rRNA genes in the cell contained in the organiser. This conclusion emphasizes the role of *active* RNA gene number in regulating nucleolus formation within the context of an overall control of rRNA synthesis. However, this is only part of the story since at least thirteen non-nucleolus organiser chromosomes in wheat appear to differentially regulate the activity of individual nucleolus organisers (Tables 4, 5 and 6; see also Viegas and Mello-Sampayo, 1975). The genetic control of nucleolus organiser activity in wheat is clearly very complex. We presume that all those non-nucleolus organiser chromosome changes that increased the number of nucleoli either activated the chromosome 5D organisers or partially reduced the activity of the 1B and/or 6B organisers causing nucleolus compensation to occur and that those chromosome changes which decreased the number of nucleoli preferentially inhibited some nucleolus organisers or activated the 1B and/or 6B nucleolus organisers. These interpretations assume that the changes in nucleolus activity occurred in the absence of significant changes in absolute rRNA gene numbers.

The striking inhibition of *Triticum aestivum* nucleolus organisers by chromosome $1C^u$ from *Aegilops umbellulata* also provides strong evidence for an organiser-specific control of nucleolus formation. This appears to be another example

of the frequently observed suppression of nucleolus formation in cells of plant and animals species hybrids. In plant hybrids of *Crepis* (Navashin, 1928; Wallace and Langridge, 1971); *Solanum* (Yeh and Peloquin, 1965); *Salix* (Wilkinson, 1944); *Ribes* (Keep, 1962) and *Hordeum* (Kasha and Sadasivaiah, 1971) the nucleolus constrictions of one particular parent species appear suppressed. In man-mouse hybrid cells which tend to lose human chromosomes, the human nucleolus organisers are suppressed and only mouse 28S rRNA can be detected while in cells which show preferential loss of mouse chromosomes the mouse nucleolus organisers are suppressed (Elicieri and Green, 1969; Bramwell and Handmaker, 1971; Miller and Miller, 1976; Croce and Talavera, 1977). Furthermore it has been shown that in *Xenopus laevis* × *Xenopus mulleri* hybrids, transcription occurs preferentially from the *Xenopus laevis* rDNA (Honjo and Reeder, 1973).

The mechanism of nucleolus organiser suppression in these interspecies hybrids is not known. The suppression in Crepis has been shown to be reversible in that when the nucleolus organiser is backcrossed into a single species background or separated from other chromosomes in aberrant meioses it is active again (Navashin, 1928; Wallace and Langridge, 1971).

The control of ribosomal RNA levels in nucleoli can be at the level of transcription or by turnover of the products of rRNA genes. A biochemical and morphological study or rRNA gene activity during oogenesis of *Triturus* (Scheer et al., 1976) has elegantly shown that as rRNA synthesis increases, more genes are transcribed. Thus control is at the level of transcription and there is a control mechanism regulating the number of genes of a tandem array which are used during developmental stages where full rRNA gene expression is not required. Whether these observations are directly relevant to the variation in nucleolus organiser expression in wheat remains to be studied.

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Received October 2 – November 15, 1978 / Accepted November 15, 1978 by H.E. Macgregor
Ready for press November 22, 1978