Theor Appl Genet (1983) 65:145–147

The identification of the nucleolus organiser chromosomes of diploid wheat

T. E. Miller, J. Hutchinson and S. M. Reader
Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ, England

Summary. The two nucleolus organiser chromosomes of diploid wheat are identified as 1A and 5A by the combination of in situ hybridisation and cytological markers.

Key words: Diploid wheat – Nucleolus organiser – In situ hybridisation – Telocentric markers

Introduction

Using the technique of in situ hybridisation, Gerlach et al. (1980) confirmed the presence of two nucleolus organising chromosomes in the A genome diploid wheats. However, the identity of these chromosomes remained unknown. All of the polyploid wheats have two major and occasionally two minor nucleolus organising sites (Hutchinson and Miller 1982; Miller et al. 1980). The two major sites have been identified on chromosomes 1B and 6B (Flavell and Smith 1974; Flavell and O'Dell 1976; Miller et al. 1980). The two minor sites have been identified, in the hexaploid *Triticum aestivum,* on chromosome 1A and 5D (Miller et al. 1980) but no site has been found on a second A genome chromosome. This paper describes the identification of the two sites in diploid wheat.

Materials and methods

Crosses were made between the *Triticum aestivum* cv. 'Chinese Spring' double ditelocentric lines for each of the A genome chromosomes (2n = 6x = 40 + 4t) and *T. urartu* P.B.I. acc A (Lenningrad K33870) (2n = 2x = 14). The diploid wheat *T. urartu* was chosen as it is known from previous work to produce viable hybrids with 'Chinese Spring' (Chapman et al. 1976; Dvořák 1976) whereas other diploids may not (Miller and Reader 1980). Hybrid embryos were cultured on nutrient agar medium approximately three weeks after pollination and the resulting plants were grown in a controlled environment chamber at 20 °C with continuous illumination.

Anthers at first metaphase of meiosis were fixed in 3 parts absolute alcohol : 1 part glacial acetic acid. Preparations of the pollen mother cells and the in situ hybridisation was carried out as described by Hutchinson et al. (1980). The preparations were hybridised with 100,000 c.p.m. of ^{3}H labelled nucleic acid probe and the autoradiographs exposed for 6 months.

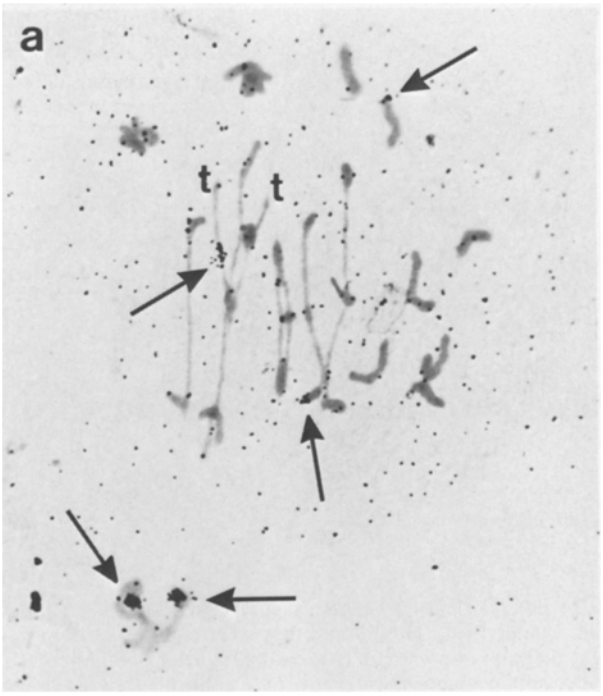
The nucleic acid probe used was tritium labelled RNA transcribed by *E. coli* RNA polymerase from the plasmid pTA71, which contains one copy of each of the wheat ribosomal RNA genes (Gerlach and Bedbrook 1979). These genes lie at the site of the nucleolus organisers.

Pollen mother cells on the developed autoradiographs were scored for meiotic chromosome configurations marked by clusters of silver grains and/or telocentrics (Table 1).

Results

The hybrids produced all had 29 chromosomes including two telocentrics, one for each arm of a single A genome chromosome. In these hybrids, pairing occurs only between the A genome chromosomes of *T. urartu* and the A genome chromosomes of 'Chinese Spring'. The B and D genome chromosomes of 'Chinese Spring' are present as univalents. The two major nucleolus organiser sites on chromosomes 1B and 6B, and the minor site on 5D can be disregarded as they always occur on univalent chromosomes.

There are two critical chromosome pairing configurations that positively indicate that the chromosome being tested carries a nucleolar organiser site: a labelled trivalent involving the two telocentrics (Fig. 1a); or a labelled heteromorphic bivalent. The latter results from failure of one of the telocentrics to



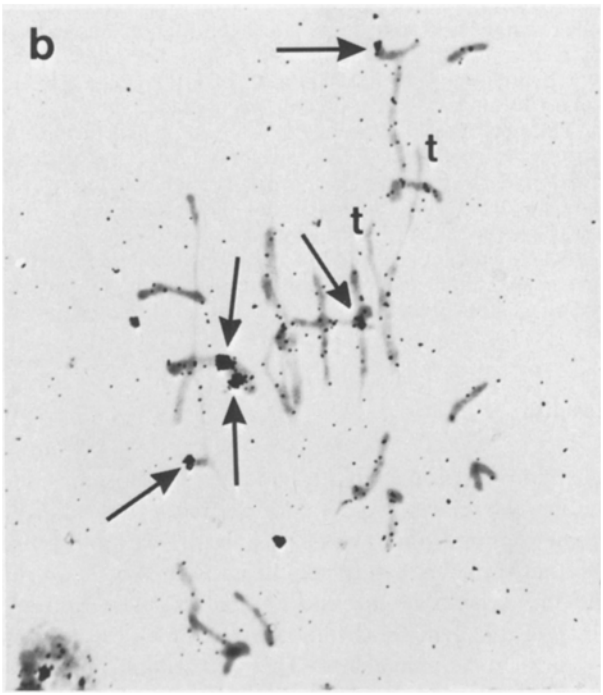


Fig. 1a, b. First metaphase of meiosis of 'Chinese Spring' double ditelocentric × *T. urartu* hybrids after in situ hybridisation with labelled rRNA genes. **a** showing a marked and labelled 5A trivalent, a labelled bivalent and three labelled univalents; **b** showing a marked, unlabelled 6A trivalent, two labelled bivalents and three labelled univalents. (Ribosomal RNA gene sites are arrowed and telocentrics are indicated by t)

pair. In these cases the second diploid wheat site appears on a labelled normal bivalent. On the other hand there are three critical configurations that positively indicate that the chromosome does not carry a site: an unlabelled trivalent involving both telocentrics (Fig. 1 b); an unlabelled heteromorphic bivalent, the result of pairing failure of one telocentric; and two labelled normal bivalents (Fig. 1 b).

Table 1 summarizes the results for six of the seven hybrids. The in situ hybridisation of the 1A combination was unsuccessful. It is clear that only one of the six, chromosome 5A, exhibited configurations indicating the presence of a nucleolus organising site (Fig. 1 a). The other five, 2A, 3A, 4A, 6A and 7A showed configurations that positively indicate that they do not carry a site. In the case of 4A no configurations marked by telocentrics were observed. This was not unexpected as the telocentrics of chromosome 4A of 'Chinese Spring' are known not to pair with the chromosomes of the diploid wheats (Chapman et al. 1976; Dvořák 1976; Miller et al. 1981). However, two complete labelled bivalents were observed, showing that the two sites were on chromosomes other than 4A.

Discussion and conclusions

The results clearly show that chromosome 5A of *T. urartu* carries a nucleolus organiser and that chromosomes 2A, 3A, 4A, 6A and 7A do not. As it is known that the diploid wheats (including *T. urartu*) have two chromosomes with nucleolus organisers (Gerlach et al. 1980) the second site must by deduction be located on chromosome 1A. This conclusion is supported by the presence of a nucleolus organiser region on chromosome 1A of some hexaploid wheats (Miller et al. 1980).

Table 1. Summary of the critical marked (telocentric and/or label) paired chromosome configurations in first metaphase of meiosis of the 'Chinese Spring' A genome double ditelocentric × *T. urartu* hybrids

Chromosome	Marked configurations				
	t/1/t‴ labelled	1/t″ labelled	t/1/t‴ unlabelled	2″ labelled	1/t″ unlabelled
2A	–	–	√	√	√
3A	–	–	√	√	√
4A	–	–	–	√	–
5A	√	√	–	–	–
6A	–	–	√	√	√
7A	–	–	√	√	√
	nucleolus organiser site present		nucleolar organiser site absent		

Table 2. Location of nucleolus organiser regions in some genomes of the *Triticeae*

Species	Genome	Homoeologous group		
		1	5	6
Triticum urartu	A	√	√	
Aegilops umbellulata	U	√	√	
Hordeum vulgare	H^{v}		√	√
Hordeum chilense	H^{ch}		√	√
Agropyron elongatum	E		√	√
Aegilops squarrosa	D		√	
Secale cereale	R^{cer}	√		
Secale montanum	R^{mon}	√		
Triticum B genome donor	B	√		√

Nucleolus organisers may be present on the chromosomes of three homoeologous groups within the *Triticeae*. They occur in various combinations of the group 1, group 5 and group 6 chromosomes in different genomes (Table 2). However in the polyploid species the total number of sites does not always equal the sum of the sites of the donor genomes, e.g. tetraploid and hexaploid wheats (Hutchinson and Miller 1982) and polyploid *Aegilops* (Teoh and Hutchinson, in preparation).

During the evolution of the polyploids some sites must either have been lost or diminished to a level undetectable by in situ hybridisation. This is presumably the case with the A genome sites from the diploid wheats, especially that on 5A which is not found in any of the polyploid wheats.

References

Chapman V, Miller TE, Riley R (1976) Equivalence of the A genome of bread wheat and that of *Triticum urartu*. Genet Res 27:69–76

Dvořák J (1976) The relationship of *Triticum urartu* and the A and B genomes of *Triticum aestivum*. Can J Genet Cytol 18:371–377

Flavell RB, O'Dell M (1976) Ribosomal RNA genes on homoeologous chromosomes of groups 5 and 6 in hexaploid wheat. Heredity 37:377–385

Flavell RB, Smith DB (1974) Variation in nucleolar organizer rRNA gene multiplication in wheat and rye. Chromosoma 47:327–331

Gerlach WL, Miller TE, Flavell RB (1980) The nucleolus organizers of diploid wheats revealed by in situ hybridization. Theor Appl Genet 58:97–100

Gerlach WL, Bedbrook JR (1979) Cloning and characterisation of ribosomal RNA genes from wheat and barley. Nucleic Acid Res 7:1869–1885

Hutchinson J, Chapman V, Miller TE (1980) Chromosome pairing at meiosis in hybrids between *Aegilops* and *Secale* species: a study by in situ hybridisation using cloned DNA. Heredity 45:245–254

Hutchinson J, Miller TE (1982) The nucleolar organisers of tetraploid and hexaploid wheats revealed by in situ hybridisation. Theor Appl Genet 61:285–288

Miller TE, Gerlach WL, Flavell RB (1980) Nucleolus organiser variation in wheat and rye revealed by in situ hybridisation. Heredity 45:377–382

Miller TE, Reader SM (1980) Variation in the meiotic chromosome pairing of hybrids between hexaploid and diploid wheats. Cereal Res Commun 8:477–483

Miller TE, Shepherd KW, Riley R (1981) The relationship of chromosome 4A of diploid wheat to that of hexaploid wheat: a clarification of an earlier study. Cereal Res Commun 9:327–329

Teoh SB, Hutchinson J (in preparation) A comparison of the chromosomal distribution of cloned repetitive DNA sequences in different *Aegilops* species