

Use of biotin-labeled probes to map specific DNA sequences on wheat chromosomes

ABSTRACT: A biotin-labeling technique was used to map a 120 bp rye DNA probe by in situ hybridization to somatic metaphase chromosomes of common wheat. Twenty-four hybridization sites were revealed on 11 chromosomes including 4A, 2D, 3D, 5D, and the seven B-genome chromosomes. The observed results were similar to those of previous studies that used isotope labeling. Biotin labeling was found to be a rapid, consistent, and reliable technique to detect repeated DNA sequences by in situ hybridization in wheat and should be a useful technique for the physical mapping of DNA sequences on plant chromosomes.

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THE TECHNIQUE of in situ hybridization, as originally developed by Gall and Pardue³ and John et al.⁸, involves the annealing of radioactive DNA or RNA probes to cytological preparations and their detection by autoradiography. Recently, biotin-labeled probes have been used to map specific DNA sequences by in situ hybridization in mice¹¹, chickens¹², and *Drosophila*¹⁰. The use of biotin-labeled probes produced higher resolution of the hybridization sites and lower background interference than did conventional in situ hybridization using isotope labeling and autoradiography¹⁰⁻¹². No reports to date have demonstrated the use of biotin labeling for the mapping of DNA sequences by in situ hybridization in plants.

Several repetitive DNA sequences have been mapped on chromosomes of cereal species by conventional in situ hybridization^{1-7,13}. These studies have provided important insights into the problems of origin and evolution of repeated DNAs among genomes of cereal species. However, major limitations of conventional in situ hybridization have been the long exposure times required to detect hybridization sites and poor resolution of autoradiographs.

In this study, biotin-labeled probes were used for mapping specific DNA sequences on wheat chromosomes by in situ hybridization. A 120 bp repeated sequence of rye, which is also found in wheat, was used as the probe and

the distribution of this sequence on chromosomes of common wheat is described.

Materials and Methods

Triticum aestivum (L. em. Thell) cv. Chinese Spring and double ditelosomic lines of all B and D genome chromosomes and 4A of Chinese Spring were analyzed by in situ hybridization experiments. Seed were placed in a moist germination dish for 24 hours at room temperature, at 4°C for 24 hours, and 24 hours at room temperature. The root tips were harvested at 1-2 cm lengths. The root tips were then pretreated in ice water for 24 hours and fixed in 3:1 95 percent alcohol:glacial acetic acid for 2 to 5 days. The squashes were prepared in 1 percent acetocarmine and the slides stored at -70°C.

Clone pSc 119 used in this study was described by Bedbrook et al.¹ and obtained from Dr. R. Flavell of the Plant Breeding Institute in Cambridge, England. The clone consisted of plasmid pBR322 as a vector and contains a 120 bp insert that hybridizes to a minor repeated DNA fraction in rye. This clone also contains sequences that hybridize with wheat DNA both in Southern blot analysis and in situ hybridization.

The chemicals for the nick translation of the probe DNA, available in kit form, were obtained from Enzo Biochem, Inc. (325 Hudson St., New York, NY 10013) as were the

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chemicals for the detection of the biotinylated probe, available as the Detek I-hrp kit.

Probe pSc 119 was nick translated and labeled with biotinylated UTP. The incorporation of biotinylated UTP was monitored by the simultaneous incorporation of ^3H ATP (1–2 million CPM). The remaining nucleotides were 0.03 mM GTP, ATP, and CTP. The nick translation was performed by mixing 400 pg of DNase 1, 12 units of DNA polymerase 1, 1 μg of clone DNA and the nucleotides in a 50 μl reaction mixture of 0.5 M tris(pH 7.5) in 5 mM MgCl_2 . After reacting for 2½ hours at 18°C, the mixture was passed through a Sephadex G-50 spin column to separate the nucleotides from the labeled DNA. Incorporation of 15–30 percent of the ^3H ATP was considered successful labeling.

For in situ hybridization, 0.4 μg of labeled pSc 119 was placed in a mixture of 50 percent formamide, 10 percent dextran, and 30 μg of sheared carrier DNA in 2XSSC (0.6M sodium chloride, 0.06M sodium citrate). The DNA was denatured at 85°C for 10 minutes. The slides, previously stored at –70°C, were placed in 70 percent formamide in 2XSSC at 70°C for 2.5 minutes. The slides were then rapidly dehydrated in an alcohol series (70 percent, 95 percent, and 100 percent) at –20°C. Twenty μl of the probe mixture was applied to each slide and a coverslip was placed over the mixture. The slides were placed in a humidity chamber and incubated for 6 hours at 37°C. After incubation the coverslips were removed and the slides rinsed in 2XSSC at room temperature for 5 minutes, 2XSSC at 37°C for 10 minutes, 2XSSC at room temperature for 5 minutes, and 0.1 percent Triton X-100 in PBS (0.13M sodium chloride, 0.007M sodium phosphate dibasic, 0.003M sodium phosphate monobasic). The slides were then briefly rinsed in PBS.

After the PBS rinse, the slides were allowed to drain but not to dry. One hundred and twenty μl of a complex of strep avidin-biotinylated horseradish peroxidase was placed on each slide and a coverslip was applied. The slides were incubated at 37°C for 30 minutes. The coverslips were removed and the slides rinsed in 2XSSC for 5 minutes and 0.1 percent Triton X-100 in PBS for 2 minutes both at room temperature. After a brief rinse in PBS, 500 μl of a solution of 0.05 percent diaminobenzidine tetrahydrochloride (DAB) and 1 percent hydrogen peroxide was placed on the slides for 5 minutes. The slides were rinsed with PBS and immediately stained with 2 percent Giemsa for 1 minute. The slides were air dried overnight and mounted in Permount.

The hybridization sites were observed and the chromosomes identified by using double

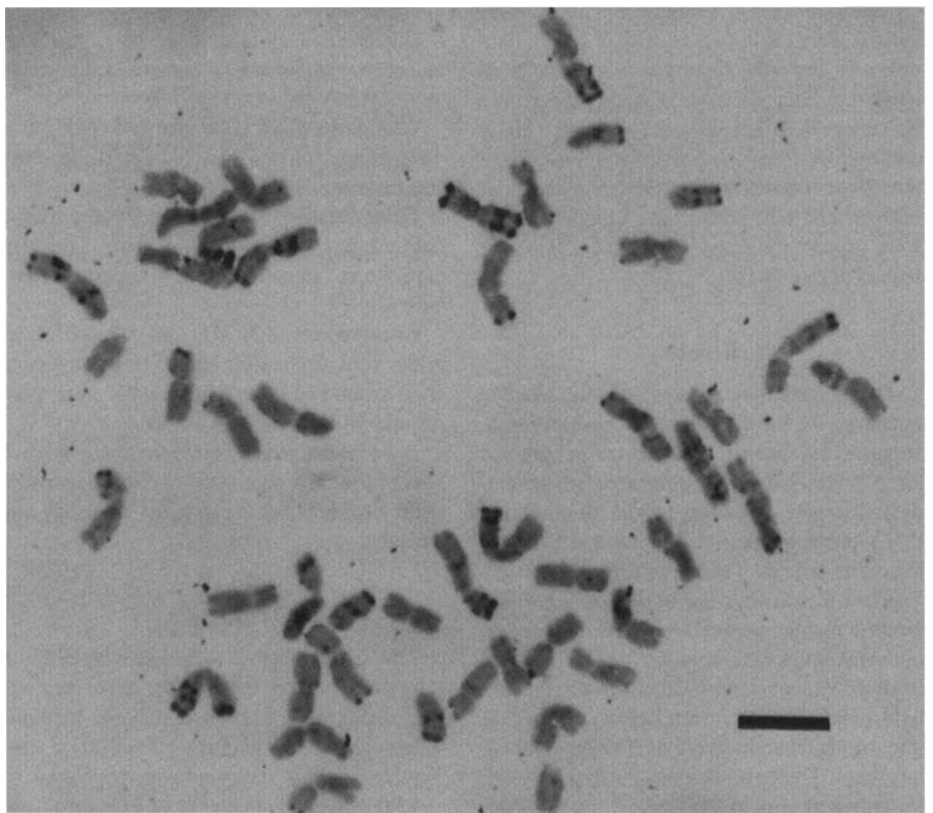


FIGURE 1 In situ hybridization of ditelo(6BS)-tetraploid (6BL) line of Chinese Spring. Bar equals 10 microns.

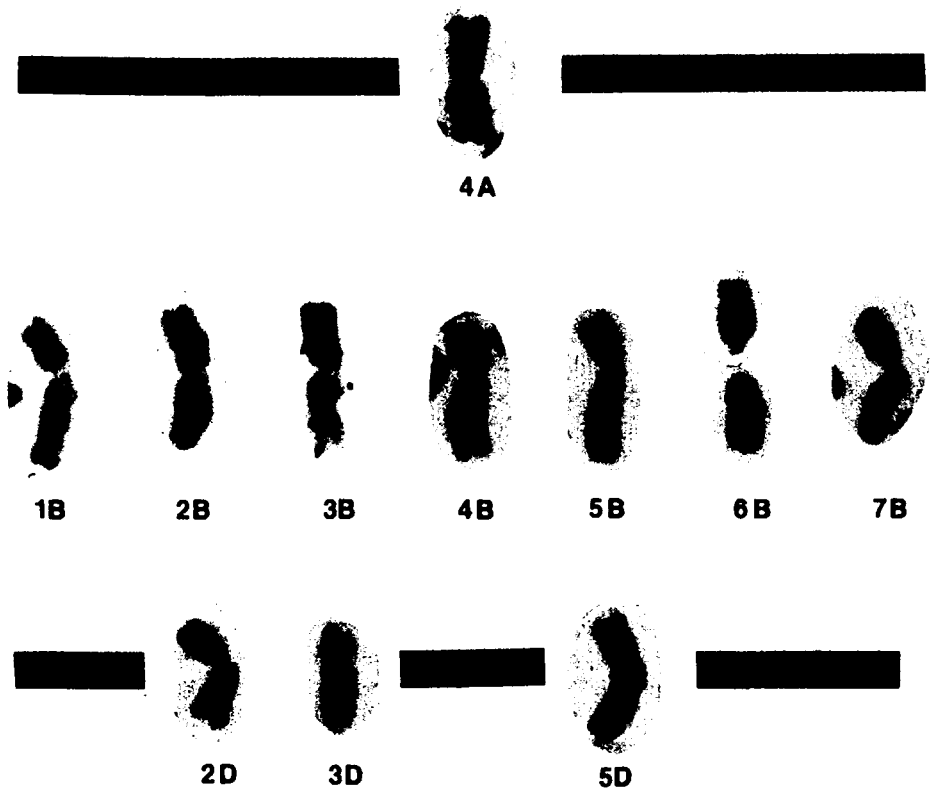


FIGURE 2 Chromosomes of Chinese Spring (verified using double ditelosomic lines) that were observed to have hybridization sites.

ditelosomic lines of Chinese Spring. Observations were initially made under bright field optics in order to distinguish hybridization sites from Giemsa bands that may sometimes be observed after Giemsa staining. Phase contrast was used for routine observations of the chromosomes. Photomicrographs under both bright field and phase contrast optics were taken on a Zeiss photosystem III using technical pan 2415.

Results

The hybridization sites using biotin labeling appeared as brown bands on blue chromosomes. This brown color occurs where the strep avidin-biotinylated horseradish peroxidase complex is bound to the biotinylated DNA probe at the hybridization sites along the chromosomes. The color difference was the result of the brown color of the insoluble precipitate formed from the enzymatic reaction of the peroxidase on the substrate (DAB). This color difference was sufficient to distinguish hybridization sites from Giemsa bands that may result from differential staining of chromosomes. The hybridization pattern revealed for chromosomes of Chinese Spring was consistent from spread to spread and from slide to slide.

In Chinese Spring the patterns of hybridization with probe pSc 119 revealed the labeled chromosomes to be the seven B-genome chromosomes and chromosomes 4A, 2D, 3D, and 5D as determined from the analyzed ditelosomic lines (Figures 1 and 2). Among the 24 sites of the sequence observed in this study, 13 were at the terminal end of the chromosomes. Of the remaining areas, three were very close to the terminus and eight were intercalary.

Chromosome 4A had four major sites, one terminal and two intercalary on the long arm and one terminal on the short arm.

Chromosome 1B long arm contained one major terminal site and one minor intercalary site in the middle of the arm. The short arm showed one minor site on the terminus of the satellite.

Chromosome 2B long arm contained one major intercalary site in the middle of the arm and a major terminal site area in the short arm.

Chromosome 3B contained two major sites, both in the short arm, one terminal area and one subterminal. No sites were revealed on the long arm.

Chromosome 4B had one major site on the terminus and a minor site close to the centromere on the long arm. On the short arm a minor site was observed on the terminal end.

Chromosome 5B long arm contained a major intercalary site. The short arm contained two major sites, one terminal and one subterminal.

Chromosome 6B contained two major sites on the long arm, one intercalary and one at the terminal end.

Chromosome 7B contained one major subterminal site in the short arm. The long arm showed one major site in the middle position.

Chromosomes 2D, 3D, and 5D contained minor sites on the terminus of their respective short arms. The sites on 2D and 5D were more prominent than the site on 3D.

In addition, small sites were observed, but not consistently, on several chromosome pairs; their chromosomal mapping was not attempted.

Discussion

The biotin-labeling technique resulted in a rapid and reliable method for observing hybridization sites on wheat mitotic chromosomes. The detection of the hybridization sites required only a few hours as compared to several days or even weeks of exposure time required with isotope-labeled probes used in conventional *in situ* hybridization. The hybridization sites were more precisely localized by the biotinylated probe since the detection occurs on the chromosome rather than as silver grains in a photographic emulsion overlying the chromosomes. Both the speed and the resolution make biotin labeling an appealing technique for physical mapping of DNA sequences on chromosomes.

Bedbrook et al.¹ carried out *in situ* hybridization with probe pSc 119 using tritium labeling and observed both telomeric and intercalary hybridization sites in tetraploid wheat. The major hybridization sites of their probe were similar to those of the biotin-labeled probe. Several chromosomes such as 1B, 2B, 5B, 6B, and 4A, are easily compared between the two studies.

Hutchinson and Lonsdale⁶ mapped a highly repeated wheat DNA sequence contained in probe pCS(1)C40 (C40) on Chinese Spring chromosomes. They also observed cross hybridization between probe C40 and pSc 119 used in this study. As expected, major patterns of hybridization on most wheat chromosomes with the two probes were similar. Minor differences were noted. These differences may be attributed to nucleotide differences between probes pSc 119 and C40 that contain DNA from rye and wheat, respectively. Differential sensitivity of the two techniques also may explain the detection of additional bands by biotin labeling.

Dennis et al.² observed the localization of a $(GAA)_m(GAG)_n$ satellite sequence on wheat chromosomes. In the majority of the cases, the two sequences, pSc 119 and the $(GAA)_m(GAG)_n$, appeared to reside in different sites on the chromosomes. But for certain sites on chromosome 1B, both sequences appeared to reside in the same areas. In addition, the terminus of the short arm of chromosome 3B and the interstitial region of the short arm of 7B were observed to contain sites where both sequences appear to reside. Although these two sequences are different, it is not surprising to find sites that contain both, since previous studies have shown that rye heterochromatin may contain four different repeated sequences⁹. It is more than likely that additional DNA sequences may be present in areas of chromosomes where other DNA sequences previously have been detected.

When compared to C- or N-banded chromosomes, the majority of the regions of hybridization observed in this study appear to correspond to the lightly banded regions and not the heavily banded regions. These results are consistent with the observation that the major C- and/or N-bands correspond to sites that contain the $(GAA)_m(GAG)_n$ sequence². Moreover, pSc 119 sequence DNA was not observed to be localized around the centromeric regions where C- and N-bands are often observed.

This study demonstrated that biotin-labeled probes can be used for physically mapping DNA sequences to plant chromosomes by the technique of *in situ* hybridization. The technique was rapid and gave consistent and reproducible results. The hybridization sites were accurately determined and the time required to observe them was reduced to two hours. The observed results were consistent with previous reports. Biotin-labeled probes may be used as an alternative to radioactively labeled probes for genomic mapping by *in situ* hybridization in plants.

References

1. BEDBROOK, J. R., J. JONES, M. O'DELL, R. J. THOMPSON, and R. B. FLAVELL. A molecular description of telomeric heterochromatin in *Secal* species. *Cell* 19:545-560. 1980.
2. DENNIS, E. S., W. L. GERLACH, and W. J. PEACOCK. Identical polypyrimidine-polypurine satellite DNAs in wheat and barley. *Heredity* 44:349-366. 1980.
3. GALL, J. G. and M. L. PARDUE. Formation and detection of RNA-DNA hybrid molecules in cytological preparations. *PNAS* 63:378-383. 1969.
4. GERLACH, W. L., R. APPELS, E. S. DENNIS, and W. J. PEACOCK. Evolution and analysis of wheat genomes using highly repeated DNA sequences. *Proc. 5th Int. Wheat Genetics Symposium* 1:81-91. 1978.

5. ——— and W. J. PEACOCK. Chromosomal locations of highly repeated DNA sequences in wheat. *Heredity* 44:269-276. 1980.
6. HUTCHINSON, J. and L. M. LONSDALE. The chromosomal distribution of cloned highly repetitive sequences from hexaploid wheat. *Heredity* 48:371-376. 1982.
7. ———, R. B. FLAVELL, and J. JONES. Physical mapping of plant chromosomes by in situ hybridization. *In Genetic Engineering*. J. Setlow and A. Hollaender, Eds. Plenum Press, NY. 3:207-222. 1981.
8. JOHN, H., M. L. BIRNSTEIL, and K. W. JONES. RNA-DNA hybrids at cytological levels. *Nature* 223:582-587. 1969.
9. JONES, J. D. G. and R. B. FLAVELL. Mapping of highly repeated DNA families and their relationship to C-bands in chromosomes of *Secale cereale*. *Chromosoma* 86:595-612. 1982.
10. LANGER-SAFER, P. R., M. LEVINE, and D. C. WARD. Immunological method for mapping genes on *Drosophila* polytene chromosomes. *PNAS* 79:4381-4385. 1982.
11. MANUELIDIS, L., P. R. LANGER-SAFER, and D. C. WARD. High-resolution mapping of satellite DNA using biotin-labeled DNA probes. *J. Cell Biol.* 95:619-625. 1982.
12. SINGER, R. H. and D. C. WARD. Actin gene expression visualized in chicken muscle tissue culture by using in situ hybridization with a biotinated analog. *PNAS* 79:7331-7335. 1982.
13. TEOH, S. B., J. HUTCHINSON, and T. E. MILLER. A comparison of the chromosomal distribution of cloned repeated DNA sequences in different *Aegilops* species. *Heredity* 51:635-641. 1983.