Theor Appl Genet (1993) 85:825–828

THEORETICAL AND APPLIED GENETICS

Recovery of dissected C-band regions in *Crepis* chromosomes

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Received June 1, 1992; Accepted June 19, 1992
Communicated by F. Mechelke

Summary. Chromosome samples were prepared on a plastic coverslip covered with a polyester membrane and were subjected to the C-banding treatment. The C-band pattern was obtained after Giemsa staining. The C-band positive regions of the *Crepis* chromosomes were identified, dissected out by irradiation with a micro-laser beam and recovered in Eppendorf tubes.

Key words: Laser dissection – Micromanipulation – *Crepis capillaris* – C-banding – Quantitative ideogram

Introduction

Crepis capillaris has only six chromosomes which can be completely identified based on their morphological characteristics. Distinct bands have been revealed on every chromosome by the C-banding method (Tanaka and Taniguchi 1975), by the N-banding method (Kamisugi and Fukui 1989), and by the fluorescent-banding method (Maluszynska and Schweizer 1989). A quantitative chromosome map has also been constructed by the application of imaging methods (Kamisugi and Fukui 1989; Fukui and Kamisugi 1992).

Crepis chromosomes have been extensively used for chromosome research over a long period of time. Various chromosome aberrants were induced by X-rays (Lewitsky 1940) over 50 years ago. Then in 1958 Taylor reported the DNA duplication pattern by using tritium-labelled thymidine. Many reports have described the presence of B chromosomes in *Crepis capillaris* (e.g., Rutishauser 1960; Abraham et al. 1968). Condensation of chromatin in the cell cycle was observed in detail by electron microscopy (Kuroiwa and Tanaka 1971a, b; Kuroiwa 1971) while Sacristán (1971) examined the chromosome variability in cultured tissues derived from haploid and diploid plants.

More recently, transformed Ri plasmid fragments were detected on *Crepis* chromosomes by fluorescent in situ hybridization (Ambros et al. 1986). Jones et al. (1989) have analyzed the ultrastructure of the synaptonemal complex in B chromosome pairing at the meiotic stage. The production of a banding pattern following treatment with restriction endonucleases has also been attempted (Kamisugi and Fukui 1989). Taniguchi and Tanaka (1991) reported the presence of replication bands by BrdU treatment.

The rapid development of micro-opto-mechatronics allows us to manipulate plant chromosomes under the microscope by using a micro-laser beam. Sakai and Fukui (1988) demonstrated the possibility of applying a micro-laser beam for the micromanipulation of plant chromosomes. Barley chromosomes were subsequently successfully dissected by the laster beam (Fukui et al. 1991), while specific regions of barley and rice chromosomes were similarly dissected and the chromosome fragments recovered (Fukui et al. 1992).

In this report, we have applied the C-banding method to *Crepis* chromosomes prepared on a polyester membrane and then dissected the C-band regions under the microscope and recovered the fragments in Eppendorf tubes.

Materials and methods

Root tips of *Crepis capillaris* (L.) Wallr. plants (Compositae, $2n = 6$) were excised and pretreated in an 0.05% colchicine

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solution for 2 h at 18 °C. Then they were fixed in ethanol:acetic acid (3:1) for more than 3 days. After root tips were thoroughly washed, they were macerated using an enzyme cocktail (2% Cellulase Onozuka RS, 0.3% Pectolyase Y-23, 1.5% Macerozyme R200 and 1 mM EDTA, pH 4.2; Kamisugi and Fukui 1990) on coverslips coated with a polyester membrane in a humid chamber for 20 min at 37 °C. The root tips were then washed again to completely remove the enzyme solution. They were tapped with a forceps into almost invisible fragments by the addition of a small amount of fresh fixative and then air-dried.

The chromosome samples on the coverslip were analyzed by the C-banding method (Fukui and Kamisugi 1993). They

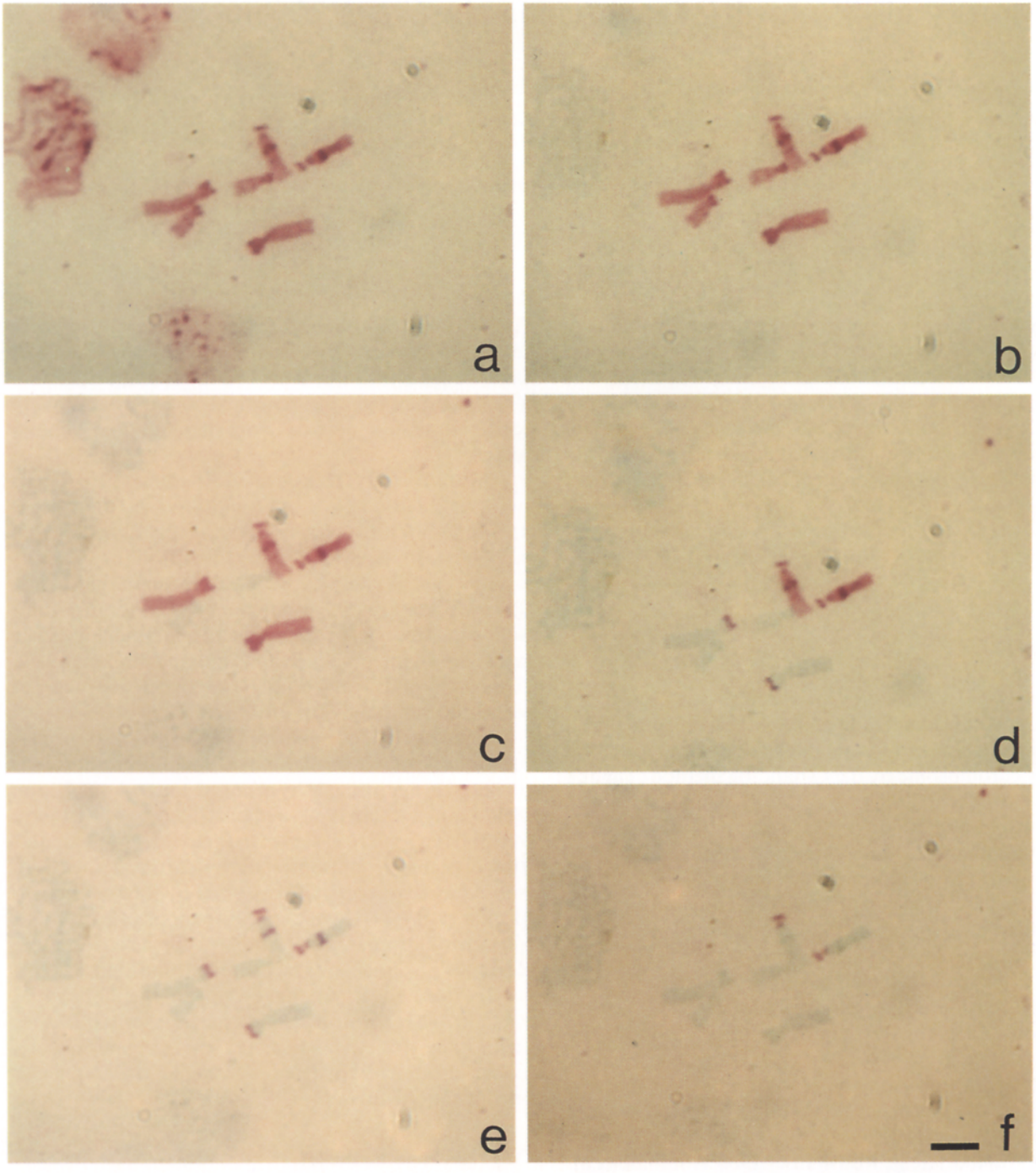

Fig. 1. Sequential representation of the microdissection process of the C-band positive regions of *Crepis* chromosomes. The 1Cp2.3 and 2Cp1.4–q1.1, 2Cq2.1 regions with a C-band pattern were dissected out by the application of a laser beam. Bar, 5 μm

were incubated in an acid solution (0.2 N HCl : 45% acetic acid = 2:1) for 3 min at 55 °C followed by treatment with 5% $Ba(OH)_2$ for 5 min at room temperature. The slides were dipped in 2 × SSC for 30 min at 55 °C. The chromosome samples were stained with a 2% Giemsa solution (pH 6.8) for 30 min and air-dried and then subjected to laser dissection under the microscope by the method previously reported (Fukui et al. 1991, 1992). A cell work station, ACAS 470 (Meridian Instruments Inc., Okemos, Mich, USA), was used for the laser dissection.

Results and discussion

Figure 1 shows the sequential process of the laser dissection of the C-banded *Crepis* chromosomes. All the chromosomes could be clearly identified on the basis of their unique morphology and specific band pattern (Fig. 1a). Two interphase nuclei and one nucleus at prophase were located around the target complement. These nuclei were first removed by laser irradiation so that only the target complement remained on the membrane (Fig. 1b). Then the shortest 3C pair of chromosomes was eliminated by the laser beam (Fig. 1c).

A fairly strong laser beam with an AOM output of 60% under 40 mW laser power was used to eliminate the larger area. After the elimination of the 3C chromosomes, a weaker laser beam with an AOM output of 15% under 50 mW laser power was employed for the microdissection of the 1C chromosomes. These chromosomes showed a distinct C-band positive region at the telomeric region of the short arm. Previously, the finest laser beam of the system was used to cut the chromosomes with a width of 0.5 μm (Fukui et al. 1991). Such laser beam was also used under the same conditions in the present study. The 2C chromosomes are satellited and three conspicuous C-band positive regions were located in the satellite, the centromeric regions of the short and long arms, and the interstitial region of the long arm (Fig. 2). These C-band positive regions could only be dissected out by the fine laser beam (Fig. 1e).

In the current system, the laser beam was focused very accurately on the target regions by the computer, and the laser beam being controlled by the mouse. The irradiation point of the laser beam was monitored by the monitor display. Thus control of the laser beam was conveniently achieved and the total time required to dissect the chromosomes from the stages represented in Fig. 1a–e was about 10 min.

Figure 1f illustrates the last stage of chromosome dissection based on the quantitative chromosome map of the *Crepis* chromosomes (Fukui and Kamisugi 1993). Only the p1.1–p1.4 and q1.1 regions of the 2C chromosomes were dissected out on the membrane while the other regions with a C-band positive pattern were eliminated. Figure 2 shows a summary of the laser dissection of the *Crepis* chromosomes. The p2.3 (◀) chromosomal regions were dissected out from the 1C chromosomes. The regions from p1.4 to q1.1 (◁), and q2.1 (◀) were dissected out from the 2C chromosomes while for 3C, the entire chromosomes were eliminated. The chromosomal fragments were recovered by the application of a stronger laser beam which was able to dissect the polyester membrane on the coverslip. The cut pieces of the membrane with chromosomal fragments on it were then picked up with a fine forceps under a stereo-microscope.

The results obtained in this study show that chromosome dissection is likely to become a standard cytological technique. The combination of chromosome dissection with the PCR method may enable the cloning of site-specific DNA sequences from specific regions of the chromosomes. Indeed, this convenient laser dissection method appears to be suitable for a wide range of chromosome research.

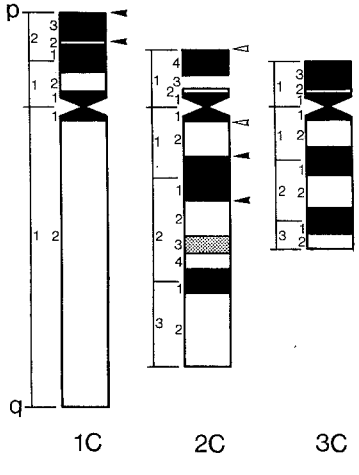

Fig. 2. Quantitative map of *Crepis* chromosomes (Fukui and Kamisugi 1993) with an indication of the dissected regions (*arrow heads*). The regions with a C-band pattern that were dissected out corresponded to the chromosome fragments shown in Fig. 1e

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