Analysis of Drosophila chromosome 4 using pulsed field gel electrophoresis

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Abstract. Previous estimates of the size of Drosophila melanogaster chromosome 4 have indicated that it is 1% to 4% of the genome or ~6 Mb. We have used pulsed field gel electrophoresis (PFGE) to separate megabase-sized molecules of D. melanogaster chromosomal DNA. Southern blots of these gels were probed with DNA fragments from the cubitus interruptus and zJh-2 genes, which are located on chromosome 4. They each identify the same-sized distinct band that migrates at approximately 5.2 Mb in DNA preparations from the Kc cell line. We interpret this band to be intact chromosome 4. In DNA obtained from embryos of various D. melanogaster wild-type strains, this chromosome band showed strain-specific size variation that ranged from 4.5 to 5.2 Mb. The D. melanogaster chromosome 4 probes also identified a single, 2.4 Mb band in embryonic DNA from Drosophila simulans. We conclude that D. simulans chromosome 4 is substantially smaller than that of D. melanogaster, presumably owing to differences in the amount of heterochromatic DNA sequences. Our simple DNA preparation from embryos and PFGE conditions should permit preparative isolation of chromosome 4 DNA and will facilitate the molecular mapping of this chromosome.

Introduction

Chromosome 4 in Drosophila melanogaster is considerably smaller than the X, Y, second or third chromosomes. In standard metaphase spreads chromosome 4 appears only as a dot and is thought to be an acrocentric chromosome (Hochman 1973; Roberts 1972). In the much larger, more specialized polytene chromosomes of the salivary gland, chromosome 4 is a small segment that extends from the chromocenter with about 50 distinct bands, or 1% of the total number of polytene bands. The polytene banded region represents euchromatic DNA only and does not include the centromeric heterochromatin present on chromosome 4. Size estimates obtained from ultraviolet absorbance measurements of metaphase chromosomes suggest that chromosome 4 is 3.5% of the genome (Hochman 1973; Rudkin as cited in Kavenoff and Zimm 1973), or ~6 Mb, based on a total genome size of 170 Mb. This size estimate of chromosome 4 is subject to uncertainty and has not yet been confirmed by other means.

Chromosome 4 is an atypical Drosophila chromosome. There is normally no crossing over on this chromosome in either sex. This has hampered genetic analysis of chromosome 4 genes. Unlike the other much larger chromosomes (the Y chromosome excepted), aneuploidy for chromosome 4 can be tolerated even though relatively mild phenotypic alterations do accompany such mutations (see Lindsley and Zimm 1992). Another unusual feature of this chromosome is that the euchromatic regions of chromosome 4 contain a higher frequency of repeated DNA sequences than the corresponding regions of other Drosophila chromosomes (Miklos et al. 1988). These repeated sequences appear not to be limited to the centromeric heterochromatin as on the other chromosomes but are dispersed in the euchromatic region of chromosome 4. There have been at least 37 essential loci plus at least 6 other loci with recessive visible alleles mapped to chromosome 4, and statistical estimates indicate that the total number of mutable loci is 70–80 (Hochman 1973).

The lack of recombinational mapping makes molecular mapping of this chromosome essential for Drosophila studies. Its small size and the presence of interspersed repeated sequences (as well as centromeric repeated sequences) also make chromosome 4 an ideal model for the study of normal higher eukaryotic chromosome structure. At the maximum estimated size of 6 Mb, this chromosome should be amenable to separation and isolation by pulsed field gel electrophoresis (PFGE). The use of low voltage and long switching times has permitted the separation of whole chromosomes from Schizo-
Saccharomyces pombe \((3.5-5.7 \text{ Mb})\), as well as from other lower eukaryotes such as *Neurospora*, whose largest chromosomes are > 10 Mb (Fan et al. 1989; Orbach et al. 1988). In *Drosophila*, a minichromosome of 1.3 Mb, containing centromeric and terminal regions of the X chromosome, has been resolved (Karpen and Spradling 1990), but to our knowledge there has been no investigation of chromosome 4 using PFGE.

We have obtained chromosome sized DNA from *D. melanogaster* cultured cells and embryos using simple isolation techniques. Using PFGE, Southern blotting, and probes for chromosome 4 specific gene sequences we have separated and identified chromosome 4. This is the first description of *Drosophila* chromosome 4 using PFGE. A survey of different strains showed that the size of chromosome 4 varies with strain specificity from 4.5 to 5.2 Mb.

Materials and methods

*Drosophila* stocks. The *D. melanogaster* strains used in this study are described in Lindsley and Zimm (1992) and grown at 22° or 25° C on standard sucrose medium. Stocks were acquired from the Bowling Green, Umel, or Indiana University *Drosophila* Stock centers except where noted.

Preparation of chromosome sized DNA. *Drosophila* Kc cells (Echandler 1976) were grown at room temperature in Shields and Sang M3 insect medium (Sigma) supplemented with 0.25% Difco Bactopeptone, 0.05% Difco Bacto-yeastolate and 5% heat-inactivated calf serum. Chromosome sized genomic DNA was obtained from these cells by standard methods used for yeast and vertebrate cell lines (Schwartz and Cantor 1984).

Chromosome sized DNA was also obtained from embryos. Flies were permitted to lay eggs on grape agar plates for the times indicated (usually 6-18 h). Eggs were collected and washed with ice-cold 0.1% Triton-X100 solution and then dechorionated with bleach. After additional washing to remove the bleach, samples of 10–100 μl of eggs were gently disrupted using a plastic micro tissue homogenizer (no. 749520-0000, Kontes Scientific Glassware/Instrument, Vineland, N.J.) in 150 μl of Hanks' balanced salt solution (Gibco). This was quickly mixed with an equal volume of molten 1% Incert agarose (FMC) and poured into a plug forming mold (BioRad). After 10-20 min at 4°C the solidified plug was placed in 2-4 ml of ESP (0.1 M EDTA, 1% SDS, and 50 μg/ml Proteinase K) and incubated with gentle shaking at 45°–50°C for 48–72 h, after which plugs were stored at 4°C in this solution. Before loading into the pulsed field gel well, each plug was washed twice with the appropriate PFGE running buffer.

PFGE Procedures. Southern blots, and DNA probes. DNA was separated using a CHEF DR II apparatus (BioRad). A number of different conditions were used (see Results), however all gels were 0.7% agarose and run at 50 V at 14°–15°C. For 1 x TBE running buffer the run time was generally 7 to 8 days at the switch times indicated in Results. For a decreased run time, we used larger pore sized Chromosomal Grade Agarose (BioRad), 0.5 x TAE, and a shorter switch time. Bands were sized using a CHEF DR II apparatus (BioRad). A number of bands were separated using a CHEF DR II apparatus (BioRad). A number of bands were separated using a CHEF DR II apparatus (BioRad).

Prior to vacuum transfer to GeneScreenPlus membrane (DuPont), DNA in the gels was depurinated (0.5 M HCl, 5-10 min) to facilitate transfer of large fragments. Probes were 32P labeled by the oligo-labeling procedure of Feinberg and Vogelstein (1983, 1984) using DNA fragments isolated in low gel temperature agarose. Two probes from chromosome 4 were used: a 2.0 kb BglII fragment 5' to the *cubitus interruptus* (ci) gene (Orenic et al. 1990; Locke and Tartof 1993) which will be referred to as the ci probe and a 3.6 kb EcoRI fragment containing sequences of a cDNA clone of the zinc finger homolog-2 (zfh-2) gene (Lundell and Hirsh 1992; Fortini et al. 1991), which will be referred to as the zfh-2 probe. Sequences for the ci and zfh-2 are located at 101EF and 102C, respectively, on chromosome 4. For a control, non-chromosome 4 probe, an 8.5 kb SalI fragment containing the yellow gene and flanking sequences (Geyer and Corces 1987) was used and will be referred to as the yellow probe. Yellow is located near the tip of the X chromosome, at 1B. The rosy gene sequences were used were the 7.2 kb HindIII fragment from Carnegie 20 (Rubin and Spradling 1983). rosy is located at 87D on chromosome 3.

These probe sequences are all single copy in the genome. When the same blot was hybridized sequentially to several probes, the previous probe was always removed first by treatment with 0.5 M NaOH at 42°C.

Results

**Chromosome 4 from Drosophila Kc cell line DNA**

The methodology for isolating chromosome sized DNA from yeast and mammalian cultured cells was used to prepare agarose-embedded DNA from a *D. melanogaster* Kc cell line. The separation of DNA from Kc cells, embedded at different concentrations, is shown in Fig. 1a. These PFGE conditions showed clear separation of the three *S. pombe* chromosomes (3.5, 4.6, and 5.7 Mb), which spans the estimated size of chromosome 4 (lane 5). No visible band corresponding to chromosome 4 was apparent in the Kc lanes of the ethidium bromide stained gel (lanes 3 and 4), although the small amount of sheared DNA present may have obscured a diffuse, faint band.

Probing a Southern blot of this gel with *ci* sequences (located on chromosome 4) showed hybridization to a non-resolving zone of compression (zc) directly below the well and to a band at 5.2 Mb (Fig. 1b). Under certain PFGE conditions, a distinct zone of compression was not seen. When the zone of compression was absent, the *ci* probe hybridized to only the well and a 5.2 Mb band (data not shown). Reprobing the blot in Fig. 1 with the chromosome 4 zfh-2 sequences produced a pattern that could be superimposed and was indistinguishable in size and morphology from that of the *ci* probe (Fig. 1c). There were also several smaller faint bands evident, which were probably degradation artifacts. To show that hybridization to this 5.2 Mb band was specific for chromosome 4 probes, the blot was probed with yellow gene sequences from the *X* chromosome (Fig. 1d). Although hybridization signal was seen in the wells, zone of compression and diffused along the lane, no hybridization to the 5.2 Mb band was seen with the yellow probe. Preliminary experiments have indicated that a probe for the rosly locus on chromosome 3 also does not hybridize to the 5.2 kb band (data not shown). Because different amounts of DNA were loaded in adjacent lanes, the effect of concentration on mobility could be