Mapping Chromosome Rearrangement Breakpoints to the Physical Map of *Caenorhabditis elegans* by Fluorescent *in Situ* Hybridization

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ABSTRACT

A scheme for rapidly mapping chromosome rearrangements relative to the physical map of *Caenorhabditis elegans* is described that is based on hybridization patterns of cloned DNA on meiotic nuclei, as visualized by fluorescent *in situ* hybridization. From the nearly complete physical map, DNA clones, in yeast artificial chromosomes (YACs), spanning the rearrangement breakpoint were selected. The purified YAC DNAs were first amplified by degenerate oligonucleotide-primed polymerase chain reaction, then reamplified to incorporate fluorescein dUTP or rhodamine dUTP. The site of hybridization was visualized directly (without the use of antibodies) on meiotic bivalents. This allows chromosome rearrangements to be mapped readily if the duplicated, deficient or translocated regions do not pair with a normal homologous region, because the site or sites of hybridization of the probe on meiotic prophase nuclei will be spatially distinct. The pattern, or number, of hybridization signals from probes from within, or adjacent to, the rearranged region of the genome can be predicted from the genetic constitution of the strain. Characterization of the physical extent of the genetically mapped rearrangements places genetic landmarks on the physical map, and so provides linkage between the two types of map.

THE success of combining genetic and molecular approaches to the study of the normal and abnormal biology of an organism has highlighted the need for a physical map of the organism's genome and ultimately its entire DNA sequence. For a number of organisms cytological and genetic maps exist along with partial or complete physical maps, and from comparison of these maps, it is clear that the proportion relating genetic and physical (or cytogenetic) maps is not constant across the genome (LINDSLEY and SANDLER 1977; DONIS-KELLER *et al.* 1987; COULSON *et al.* 1991). In general, it appears that the distal portions of autosomes are regions where recombination is frequent, leading to genetic map expansion between distal loci. Therefore, for the physical map to be fully utilized, it is necessary to establish the correspondence between the genetic and physical maps across the entire genome. Linking of the two types of map can be accomplished by molecularly cloning genetically characterized genes, by identifying mutations in physically mapped genes, by identifying genetically informative DNA polymorphisms, or by the physical and genetic characterization of chromosomal rearrangements or breakpoints.

In the nematode *Caenorhabditis elegans*, the nearly complete physical map (COULSON *et al.* 1991) has been tied to the genetic map by molecular cloning of genetically identified genes. This has involved direct transposon tagging of a gene (GREENWALD 1985) or mapping of closely linked DNA polymorphisms (BAILLIE and BECKENBACH 1985). Genetic mapping of DNA polymorphisms has also contributed genetic and physical landmarks on the maps (HIRSH *et al.* 1979; ROSE *et al.* 1982; FILES, CARR and HIRSH 1983), and recently, a genetic mapping system involving sequence-tagged sites has been described (WILLIAMS *et al.* 1992) that not only provided genetic landmarks on the physical map, but also contributed to the construction of the map by locating previously unassigned DNA contigs.

The chromosomes of *C. elegans* are holocentric (ALBERTSON and THOMSON 1982) and therefore chromosome rearrangements involving translocations, deficiencies, or small pieces of chromosomes, called free duplications, are readily isolated and maintained [for a review, see HERMAN and KARI (1989)]. These also provide a source of genetic markers delimiting regions of the genome that can link the physical and genetic maps, but only limited use has been made of chromosome rearrangements for this purpose. Using cytogenetics, DNA clones have been mapped relative to chromosome rearrangement breakpoints in order to identify corresponding genetic loci (ALBERTSON 1985) and, for the cloning of particular genes, the extents of small deficiencies (KRAMER *et al.* 1988) or duplications (H. BROWNING, personal communication) have been mapped relative to the physical map.

Since genes are often mapped relative to rearrangements, it seemed appropriate to map a number of the rearrangements with respect to the physical map, thereby providing more genetic landmarks on the physical map, and at the same time physical char-

acterization of some of the chromosome rearrangements. One way of doing this is to use the physical map and the available genetic data to select DNA clones mapping near the rearrangement and to map these by *in situ* hybridization to the rearranged chromosomes. Both breakpoints and the extent of duplicated regions, for example, can be mapped relative to the physical map in this way. However, to map genes cytologically in *C. elegans*, DNA probes are hybridized *in situ* to the very few mitotic chromosomes obtained by squashing embryos from large numbers of animals (ALBERTSON 1984a, 1985). Since chromosome rearrangements are often lethal or unstable, they are maintained as heterozygotes, and therefore metaphase karyotypes of embryos from these mothers will be of several different constitutions and the number of metaphases obtained from squashes too small for analysis of a number of rearrangements. Therefore a different strategy has been adopted. Since a large number of meiotic prophase nuclei can be obtained from an individual animal, DNA clones have been mapped to these chromosomes obtained from a few selected individuals of a specific genotype. Although the meiotic bivalents are so small that the position of hybridization along the chromosome can only be determined at very low resolution, it is only necessary to score for the presence or absence of hybridization signals on a specific number of bivalents depending on the particular genotype. The analysis makes use of the existing resource of purified genomic DNA cloned in yeast artificial chromosomes (YACs) used to construct the physical map (COULSON *et al.* 1988). Since *in situ* hybridization typically requires as much as 0.1 μg of DNA per slide (ALBERTSON 1984), the isolated YACs, stored as gel slices, were amplified by the degenerate oligonucleotide primed-polymerase chain reaction (DOP-PCR) (TELENIUS *et al.* 1992a,b) and then reamplifed incorporating directly fluorescently labeled deoxynucleotides for use as probes for *in situ* hybridization. These YACs, labeled by DOP-PCR, were mapped on metaphase chromosomes, demonstrating that DOP-PCR can be used to generate probes for *in situ* hybridization in organisms lacking the interspersed repeats often used to prime labeling by techniques such as *Alu*-PCR (BREEN *et al.* 1992; LENGAUER, GREEN and CREMER 1992). The large hybridization signals from these directly fluorescently labeled YAC probes renders characterization of chromosome rearrangements relatively easy in an organism with otherwise "difficult" cytogenetics.

MATERIALS AND METHODS

Nematode strains: The strains used were all derived from *C. elegans* var. Bristol and maintained as described by BRENNER 1974). The strain CB1517 (HODGKIN 1980), with the genetic constitution *eDf2/eDf2 III;eDp6(III;f)* was obtained from J. HODGKIN. The *mnDp33(X;IV)* rearrangement (HERMAN, MADL and KARI 1979) in strain SP309 (genetic constitution: *mnDp33/+ IV; unc-20(e112) X*) was obtained from R. K. HERMAN and the *mnT2(X;II)* translocation (HERMAN, KARI and HARTMAN 1982) in strain SP400 (genetic constitution: *mnT11(X;II)/+ II;dpy-3(e27) X;mnDp11(X;II;f)*) was obtained from the Caenorhabditis Genetics Center.

Labeling YACs by DOP-PCR: Purified YAC DNAs (COULSON *et al.* 1988) were heated at 65° and a 1-μl aliquot was taken for amplification by DOP-PCR as described by TELENIUS *et al.* (1992b). Theoretically, priming is predicted at every 4 kb (TELENIUS *et al.* 1992a) and the amplified products, when electrophoresed through short agarose gels typically displayed more than 12 bands. A 5-μl aliquot was subjected to a second amplification of 30 cycles, omitting the low temperature annealing cycles used in the first amplification. The amplified YAC DNA was labeled in the second reaction by the addition of 30 μM fluorescein dUTP (Fluorescein-11-dUTP, Amersham; Fluorescein-12-dUTP, Boerhinger-Mannheim) or rhodamine-4-dUTP (FluoroRed, Amersham) and reduction of the dTTP concentration to 160 μM. The labeled product was used for *in situ* hybridization without purification by adding 0.5 μl of the PCR product to 20 μl of hybridization solution consisting of 50% formamide, 0.01 M piperazine-*N*,*N*′-bis[2-ethane-sulfonic acid] (PIPES), 0.001 M EDTA, pH 7.0, 10% dextran sulfate and 0.3 M NaCl (ALBERTSON 1984a).

Cytology: Individual animals were picked and cut to release the gonad, as described previously (ALBERTSON 1984b), then fixed in ethanol:acetic acid 3:1 for 30–60 min before processing for *in situ* hybridization as described previously for embryonic squashes (ALBERTSON 1984a).

Hybridization: The probe (20 μl) was denatured, as described previously (ALBERTSON 1984a) and then applied to the slide and covered with a 12 × 12-mm piece of Parafilm. Slides were incubated at 37° for 1–16 hr in a moist chamber, then washed as described previously (ALBERTSON 1984a). For visualization of rhodamine labeled YACs, the chromosomes were stained with 0.3 μg/ml 4′,6-diamidino-2-phenylindole (DAPI) and for visualization of fluorescein-labeled YACs, with 100 μg/ml propidium iodide. The slides were rinsed briefly in 10 mM Tris, pH 9.5 and mounted in 1,4-diazobicyclo[2,2,2]octane (DABCO) to reduce fluorescence fading (JOHNSON *et al.* 1982).

Microscopy: The hybridization signals from fluorescein-labeled YACs on propidium iodide-stained chromosomes were visualized using a Bio-Rad MRC600 confocal microscope, as described previously (ALBERTSON, SHERRINGTON and VAUDIN 1991). The site of hybridization of rhodamine-labeled YACs was visualized using conventional fluorescence microscopy and an intensified CCD camera, as described previously (ALBERTSON, SHERRINGTON and VAUDIN 1991), using Zeiss filter sets 00 and 2.

RESULTS

Mapping YACs on embryonic metaphase chromosomes: In the absence of interspersed repeats to specifically prime labeling of the genomic DNA inserted in the YAC, it was necessary to use purified YAC DNA for the generation of DNA probes. A source of purified YACs was available, as these had been produced in the course of construction of the physical map and stored as frozen gel slices (COULSON *et al.* 1988). In order to conserve this resource, a 1-μl aliquot of the slice was amplified by DOP-PCR (TELENIUS *et al.* 1992a,b). A second amplification reaction was carried out to incorporate either fluorescein dUTP or rhodamine dUTP. This labeled DNA was

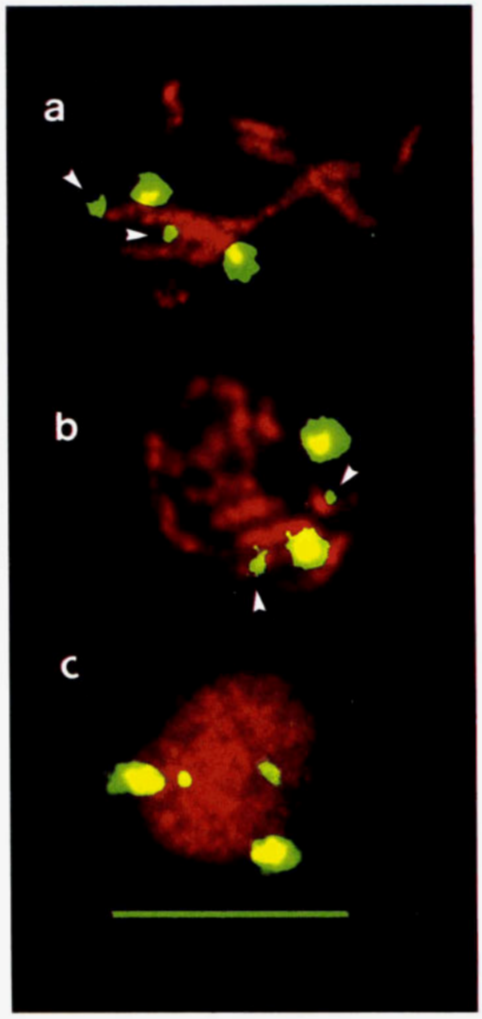

FIGURE 1.—**Hybridization of fluorescein dUTP-labeled YACs to embryonic metaphase chromosomes. Hybridization of fluorescein** dUTP-labeled YACs and the ribosomal DNA probe to embryo squashes from wild-type animals was visualized by confocal microscopy. The microscope was operated in the dual channel mode, the images from the two channels were merged and differentiated in false color (red, propidium iodide; green, fluorescein). (a) Hybridization of Y75A4 (arrowheads) and the ribosomal probe (larger signals) to opposite ends of linkage group I. There are approximately 100 copies of the 7-kb ribosomal DNA repeat, and the size of the YAC, Y75A4, from mobility on pulsed field gels is estimated to be 100 kb. Therefore, the genomic target size of the YAC probe is approximately one-seventh that of the ribosomal probe. (b) Hybridization of Y40B6 (arrowheads) and the ribosomal probe (larger signals) to linkage group I. (c) Hybridization of Y40B6 (smaller signals) and the ribosomal probe (larger signals) to interphase nuclei from embryo squashes. Scale bar is 10 μm.

shown to be a suitable probe for *in situ* hybridization by mapping a YAC, Y75A4, already positioned on the left end of the physical map of linkage group I. Figure 1a shows the hybridization of the YAC and the ribosomal DNA probe to a metaphase spread from a wild-type *C. elegans* embryo. Since the chromosomes are indistinguishable, either morphologically distinct rearrangements or a second gene probe (in this case the ribosomal genes) is used to label the chromosome, and the site of hybridization is assigned as the percentage distance from the genetic left end of the chromosome (ALBERTSON 1985). As expected, the YAC hybridized to the left end of chromosome *I*, identified by the hybridization of a ribosomal DNA probe to the right end of linkage group I (ALBERTSON 1984a). A second, unmapped YAC, Y40B6, was then mapped to the middle of linkage group I (Figure 1b). The position of this YAC on the physical map of chromosome *I* was independently assigned by physical methods (A. COULSON and R. SHOWNKEEN, personal communication). The distribution of the hybridization signals from the YACs along metaphase chromosomes is comparable to that obtained when using cosmids. Thus, these YACs, directly fluorescently labeled by DOP-PCR, are suitable probes for *in situ* hybridization on both chromosomes and interphase nuclei (Figure 1c).

Mapping chromosome rearrangements: Mapping of genes to *C. elegans* chromosomes by cytogenetics has generally involved the assignment of hybridization signals to positions on embryonic metaphase chromosomes identified by a second marker, as described above. The use of these metaphase chromosomes to map chromosome rearrangements presents several problems, since few chromosomes are obtained in this way and most rearrangements are maintained as heterozygotes so that embryonic metaphases will have different karyotypes. A more uniform and abundant supply of chromosomes is available however in meiotic prophase nuclei. In adult hermaphrodites, meiotic stages are linearly disposed along each of two arms of the gonad with many pachytene nuclei in the distal portion of the gonad in which chromosomes are visible but not usually individually distinct. Proximally, the nuclei arrest in diakinesis prior to fertilization and, typically, in three or more nuclei, depending on age and genotype, the meiotic bivalents can be distinguished. The pattern of hybridization to meiotic bivalents in these nuclei can be predicted from the genotype of the animal, while confirmation of a duplication, for example, might be obtained from observation of multiple hybridization signals over the early prophase nuclei where individual bivalents are not visible. Since each animal contains a number of nuclei suitable for mapping by *in situ* hybridization, only a few animals are required. For each YAC to be mapped with respect to a rearrangement, approximately five animals of the desired genotype were picked to a glass microscope slide and processed for *in situ* hybridization. In order to determine the physical extents of the rearrangements, the number of fluorescent hybridization signals on pachytene nuclei and the pattern of hybridization signals on meiotic bivalents (univalents, in the case of a single *X* chromosome) were scored.

The eDf2 and eDp6 breakpoint: Due to the holocentric nature of *C. elegans* chromosomes, breakage of a

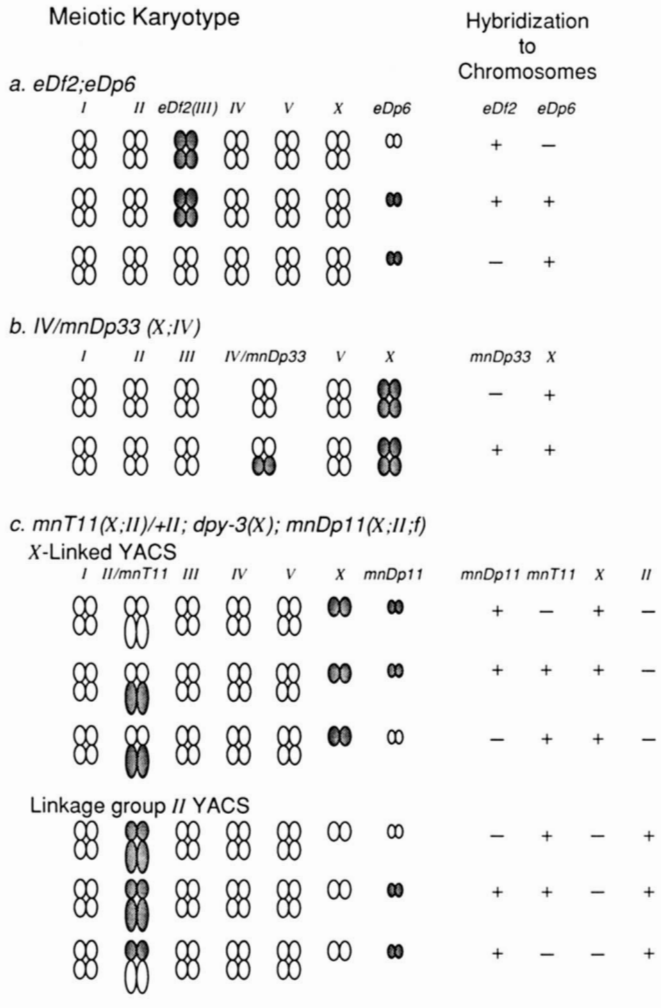

FIGURE 2.—Meiotic karyotype at prophase I and hybridization pattern of YACs relative to rearrangement breakpoints. Idiograms of meiotic chromosome constitutions of strains carrying chromosome rearrangements. Since the chromosomes are holocentric, centromeres are absent and the homologs are held together in an end-to-end association, possibly through terminalized chiasmata. Either genetic end of the homologs can be involved in the end-to-end association, such that hybridization of a probe from one end of a chromosome can be observed at the inside or the outside of the bivalent in different nuclei (ALBERTSON and THOMSON 1993). Therefore for a probe mapping near the end of a chromosome, some bivalents will be oriented such that hybridization of the probe is to the outside of the bivalent and in animals heterozygous for that probe hybridization to only one half bivalent will be observed as in Figure 7. Although all four chromatids have been drawn, they are rarely distinguishable in these squash preparations. However, a constriction is usually visible, that indicates the site of association of the two half bivalents. The pattern of hybridization of YACs to meiotic bivalents (filled) has been drawn. The inclusion (+), or absence (−) of the YAC on a chromosome is indicated by these hybridization patterns. The number of hybridization signals over early meiotic prophase nuclei will be determined by the number of different bivalents to which the YAC hybridizes. (a) *eDf2;eDp6*. Linkage group III is rearranged such that the *eDf2*-bearing chromosome appears as a normal bivalent. The *eDp6* chromosome is easily distinguished from the six bivalents by its small size. (b) *mnDp33(X;IV)*. Animals heterozygous for *mnDp33* display six bivalents in meiotic prophase I. Since the duplicated portion of the *X* has been translocated to linkage group IV, this bivalent is composed of one normal linkage group IV half bivalent and the linkage group IV half bivalent carrying *mnDp33*. (c) *mnT2(X;II)*.

chromosome followed by recovery of both products is possible, as acentric fragments are rarely produced. Breakage of linkage group III to generate a chromosome deficient for approximately 30% of the chromosome and recovery of the remainder as a free duplication in one animal has been described and well characterized genetically (HODGKIN 1980). These animals are viable and their meiotic chromosome constitution is drawn in Figure 2a. Of the six linkage groups of *C. elegans*, only the third linkage group is altered in this strain. Since the majority of linkage group III is intact, it appears cytologically as a normal bivalent, referred to here as the *eDf2*-bearing chromosome. The other small piece of linkage group III can exist in addition to normal linkage group III chromosomes as a small free chromosomal duplication and so is named *eDp6*. Thus, rather than the normal complement of six bivalents, meiotic nuclei in diakinesis in these animals contain an additional small chromosome. As shown in Figure 2a, YACs that map to the left of the breakpoint can be identified by hybridization to the *eDf2*-bearing chromosome, while those to the right of the breakpoint should hybridize to the *eDp6* chromosome.

Since the breakpoint of *eDf2* and *eDp6* has been well mapped genetically, only four YACs with minimal overlap were selected and labeled. The YAC Y53F7 covers the genes *unc-50* and *unc-69*, known to map to the left of the breakpoint (Figure 3), and therefore, as expected, this YAC mapped on the *eDf2*-bearing chromosome, while the YAC Y39F2, covering *pha-1* and *tra-1*, mapping to the right of the breakpoint hybridized to the *eDp6* chromosome (Figures 3b and 4c). The hybridization pattern of the remaining two YACs showed that Y40C2 maps to the left of the breakpoint (Figures 3b and 4a) and that since Y39A1 mapped to both the *eDf2*-bearing chromosome and *eDp6* (Figures 3b and 4b), the breakpoint must lie within the genomic DNA cloned in this YAC.

Mapping breakpoints to provide genetic markers on the left end of the X: For the leftmost 25% of the current physical map of the *X* chromosome, only two genetic markers have been linked to the physical map. A number of chromosomal rearrangements involving this region have been described and mapping of two of these is described here.

The duplication *mnDp33* is homozygous lethal and includes the *X*-linked genes *osm-5*, *unc-20*, *unc-78* and *lin-18* translocated to linkage group IV (HERMAN,

This reciprocal translocation is made up of the two half translocations *mnT11(X;II)* and *mnDp11(X;II;f)*. Since the *mnT11* half translocation pairs with and disjoins from a normal linkage group II chromosome, this bivalent is composed of the normal linkage group II half bivalent and the slightly longer appearing *mnT11* half bivalent. A single *X* chromosome is present in these animals and this univalent is morphologically distinct. The *mnDp11* half translocation can also be distinguished as a small chromosome.

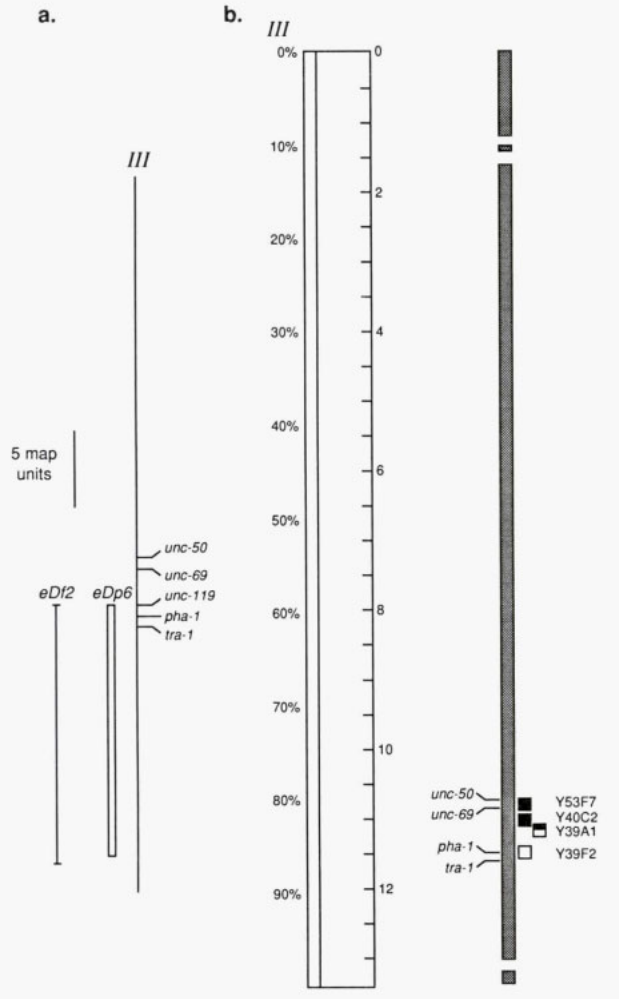

FIGURE 3.—The breakpoint of *eDf2* and *eDp6*. (a) The genetic map of linkage group III with the genetic left end up and showing genetic markers mapping close to the breakpoint. (b) A physical map of linkage group III. This physical map representation of linkage group III was redrawn from the "Physical Chromo Map" display in the *C. elegans* database ACEDB (R. DURBIN and J. THIERRY-MIEG, personal communication). The contiguous regions of the physical map are shown as hatched boxes, with arbitrary small gaps between contigs. Two scales are shown; one indicates the percentage length along the chromosome from the left end and the other scale, in megabases, is calculated from the number of *Hin*dIII bands after fingerprinting clones (COULSON *et al.* 1986). The YACs mapping to the *eDf2*-bearing chromosome (■) or to the *eDp6* chromosome (□) are shown to the right of the physical map at their physical map locations. The YAC Y39A1 (◧) mapped to both the *eDf2*-bearing chromosome and *eDp6*. Several molecularly cloned genes, mapping near the breakpoint, are shown to the left of the physical map.

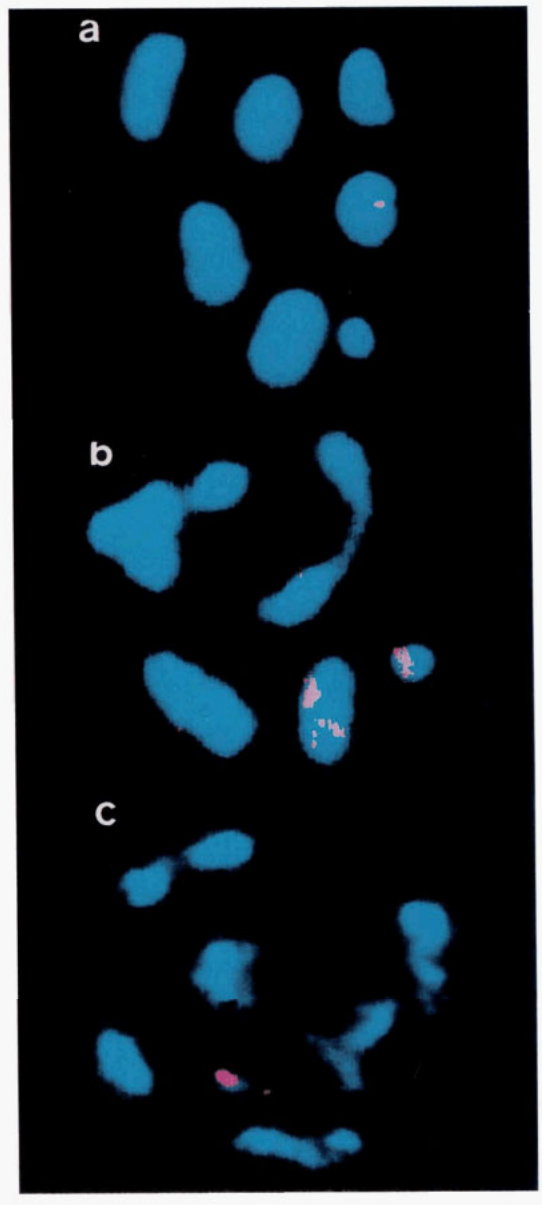

FIGURE 4.—Hybridization of YACs to bivalents from *eDf2;eDp6* hermaphrodites. The site of hybridization of rhodamine dUTP-labeled YACs (red false color) was visualized on DAPI-stained chromosomes (blue false color). The separate images from the two fluorochromes were aligned, then merged and displayed in false color. (a) Hybridization of Y40C2 to one bivalent identified as the *eDf2*-bearing chromosome. The *eDp6* chromosome is easily distinguished from the bivalents by its small size and has not hybridized. (b) Hybridization of the YAC Y39A1 to a meiotic bivalent (*eDf2*) and *eDp6*. (c) The YAC Y39F2 hybridized to the *eDp6* chromosome. No signal is observed on any of the six bivalents.

MADL and KARI 1979). For mapping, animals of genotype *mnDp33/+;unc-20*, which are phenotypically wild type hermaphrodites, were picked and processed for hybridization with a set of YACs from the left end of the *X* chromosome. Since few genetic and physical markers were available to select the set of YACs, the YACs were chosen based on previous *in situ* hybridization mapping of cosmids covering the *X*-linked genes *vit-3,4*, *gpd-2,3*, *vit-5* and *vit-2* (see Figure 5b) to *mnDp33(X;IV)* chromosomes along with a cosmid containing *unc-22(IV)*. All these genes, except *vit-2* were found to be linked to *unc-22* on *mnDp33* chromosomes (data not shown). Therefore the right breakpoint of *mnDp33* was located between *vit-2* and *vit-3,4*. Figure 2b shows the meiotic karyotype of these animals. The duplication, carried on one linkage group IV chromosome is too small to visibly alter the chromosome cytologically. Therefore these animals appear to have six normal bivalents. Inclusion of a

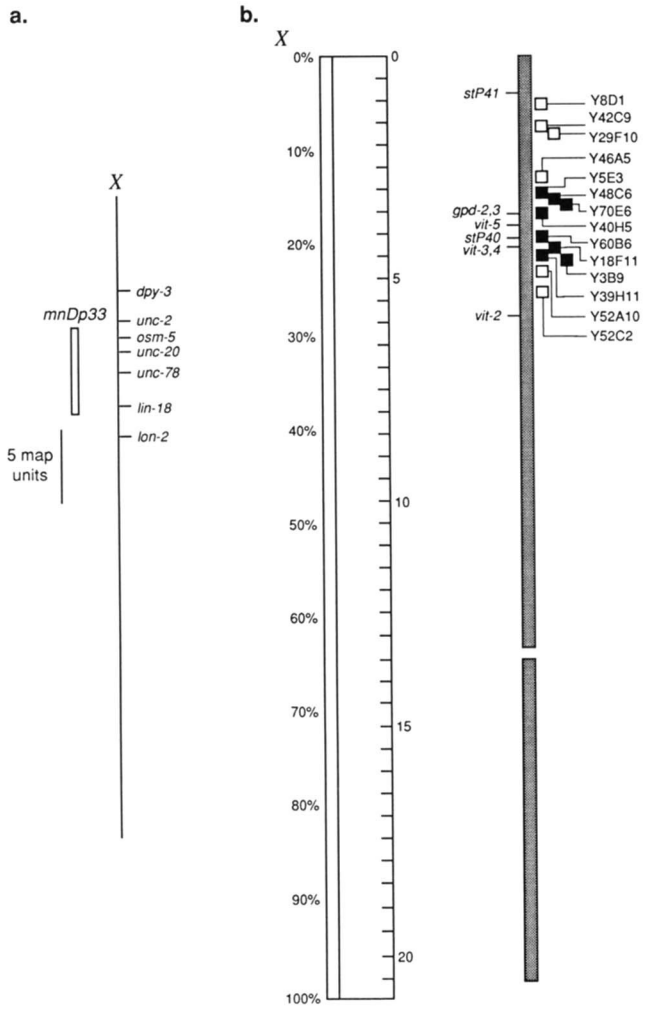

FIGURE 5.—The region of the *X* duplicated by *mnDp33(X;IV)*. (a) The *X*-linked genetic markers included in *mnDp33*. (b) Physical map of the *X* chromosome, drawn as in Figure 3. The YACs included (■) in *mnDp33*, or tested, but not included (□) are shown to the right of the physical map. The positions of some molecularly cloned genes are shown to the left of the physical map, together with two sequence-tagged sites, *stP40* and *stP41*.

YAC in the duplicated portion of the genome was indicated by hybridization of the YAC to two sites in pachytene nuclei, or to two bivalents at diakinesis. The assignment was confirmed by observing "symmetric" hybridization of the *X*-linked YAC to the *X* meiotic bivalent, but "asymmetric" hybridization, that is, to only the *mnDp33* half bivalent of linkage group *IV*. The YACs included in *mnDp33* are shown in Figure 5.

The reciprocal translocation, *mnT2* (HERMAN, KARI and HARTMAN 1982) is composed of the two half translocations called *mnT11(X;II)* and *mnDp11(II;X;f)*, as shown in Figure 6. The meiotic karyotype of the animals selected for mapping is shown in Figure 2c. Phenotypically wild type hermaphrodites carried, in addition to normal linkage groups I, III, IV and V, one normal linkage group II chromosome that pairs with and disjoins from *mnT11*, a single *X* chromosome and the free duplication *mnDp11*. As shown in Figure 2c, the *X*-linked breakpoint of the translocation was determined by mapping *X*-linked YACs to these animals and looking for hybridization signal not only on the univalent *X*, but also, on either *mnDp11* or asymmetrically to the *mnT11* half bivalent. Similarly, the breakpoint on linkage group II was determined from hybridization of YACs symmetrically to the linkage group II and *mnT11* half bivalents or asymmetrically to the normal linkage group II half bivalent and *mnDp11* (Figures 2c and 7). The results of these hybridizations are shown in Figure 6. The *X*-linked breakpoint falls between Y8D1 and Y42C9, while the breakpoint on linkage group II is within the region of genomic DNA cloned in Y70H7, since this YAC hybridized to both *mnT11* and *mnDp11*, as well as to the normal linkage group II chromosome.

DISCUSSION

A scheme for rapidly mapping chromosome rearrangements relative to the physical map of *C. elegans* has been described that is based on hybridization patterns of YACs on meiotic nuclei. From the nearly complete physical map (COULSON *et al.* 1991), YACs potentially covering a rearrangement breakpoint can be predicted, selected and then labeled for *in situ* hybridization. In the absence of interspersed repeats to prime labeling, DOP-PCR was used first to amplify, then incorporate directly fluorescently labeled nucleotides into purified YACs. This PCR product was suitable for *in situ* hybridization without purification and hybridization times as short as 60 min resulted in visible hybridization signals. The analysis was speeded up further by direct visualization of the fluorescence from the rhodamine or fluorescein molecule coupled to the dUTP incorporated in the probe DNA. This not only saved time in processing samples, because antibody incubations are omitted, but also allows the use of cytological material that is apt to give significant background fluorescence from antibody binding. The many meiotic nuclei in the gonad of the organism provide a source of large numbers of chromosomes, difficult to obtain from asynchronous populations of embryos. The hybridization pattern on the meiotic nuclei from as few as five animals can indicate the location of the YAC relative to the chromosomal rearrangement breakpoint. Since the aim of this work was to develop a means of mapping a number of rearrangements, YACs, and often the larger YACs, were chosen as probes in order to cover a chromosomal region efficiently. However, the same labeling and mapping strategy can be used with cosmids as probes, although this is slightly more difficult because smaller probes are used.

In some cases YACs spanning the breakpoint could be identified when hybridization signals on both halves of a translocation, for example, were observed (Figure 7). Although the breakpoints have not been

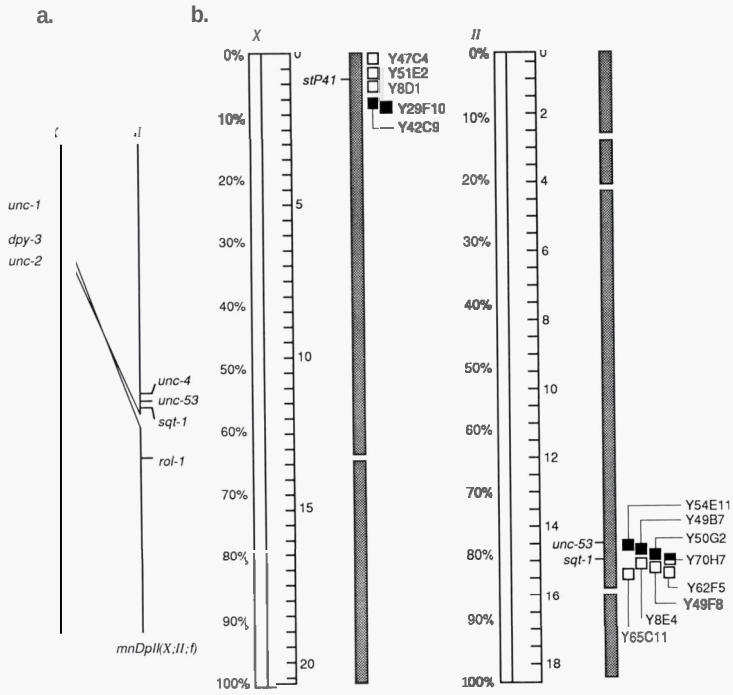

FIGURE 6.—The breakpoints of *mnT2(X;II)* on linkage groups *II* and *X*. (a) Genetic map of the breakpoints of the two half translocations, *mnT11* and *mnDp11*. (b) The physical map of the *X* and chromosome *II* drawn as in Figure 3. The YACs included in *mnDp11* (□) or *mnT11* (■) are shown to the right of the physical maps of the two chromosomes. The YAC, Y70H7 (◧) from chromosome *II* mapped to both *mnT11* and *mnDp11*. Several genetic markers that have been molecularly cloned are shown to the left of the physical map.

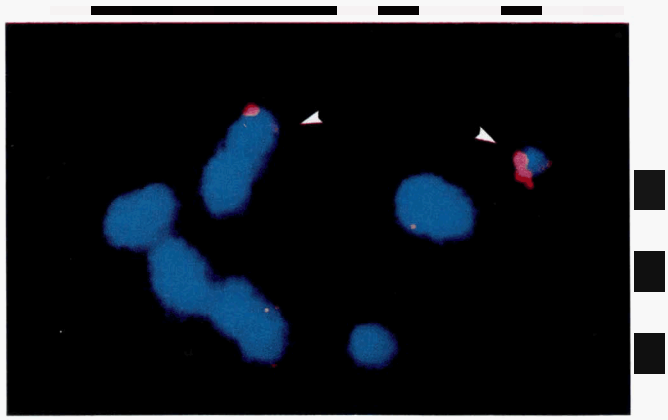

FIGURE 7.—Hybridization of Y65C11 to one half bivalent and to *mnDp11* (arrows). The site of hybridization of the rhodamine labeled YAC (red) was visualized on DAPI-stained chromosomes (blue) displayed here in false color after aligning and merging the two separate images of the two different fluorochromes. The two hybridization signals, one on each chromatid, identify this half bivalent as linkage group II. The failure of the YAC to hybridize to both half bivalents indicates that Y65C11 is not included on the portion of linkage group II carried on *mnT11*, but is included on *mnDp11*, as seen by the hybridization signal on this small chromosome.

localized to cosmids, it appears from these YACs that reliable hybridization signals can be seen when half a YAC (approximately 100 kb) is included on the rearranged chromosome. However, it is also likely that the nature of the breakpoint itself will greatly influence the hybridization characteristics of the YACs, since in the formation of the rearrangement small duplications, deletions or inversions may have occurred locally.

The extent of deficiencies can also be mapped cytogenetically in this way, although not reported here. It would be necessary to score more animals if the deficiency were maintained in a heterozygous strain, since the mapping would require distinguishing asymmetric hybridization to bivalents at diakinesis. Observations of asymmetric hybridization to the *mnDp33*, *mnT11* or linkage group II (Figure 7) half bivalents are examples of this type of analysis.

Since genes are often genetically mapped relative to chromosome rearrangements, the assignment of breakpoints to the physical map should facilitate the molecular cloning of genes. In some instances, chromosome rearrangements will serve as markers to delimit a region of the physical map within which a particular genetic locus will be found. In a few cases, breakpoints might identify a gene. This appears to be true for the breakpoint of *eDf2* and *eDp6*. Although homozygous-viable, the *eDf2;eDp6* animals are uncoordinated. A gene, *unc-119*, with a similar phenotype has been mapped very close to, or within, the breakpoint, since this gene is neither included in *eDf2*, nor is it covered by *eDp6* (D. PILGRIM and J. HODGKIN, personal communication). Location of the breakpoint within Y39A1 should facilitate the molecular cloning of this gene.

The breakpoint of *eDf2* and *eDp6* had been quite well mapped genetically and only four YACs were required to cover genetic markers mapping to either side of the breakpoint. The linkage group II breakpoint of *mnT2(X;II)* had also been well mapped genetically, close to and to the right of *sqt-1* (previously called *rol-5*), by demonstrating that *mnDp11(X;II;f)* did not carry a functional *sqt-1* gene, although it was not possible to demonstrate the presence of a functional *sqt-1* gene on *mnT11* (HERMAN, KARI and HARTMAN 1982). The breakpoint of *mnT2* on linkage

group II was mapped to Y70H7, since this YAC mapped to both *mnT11* and *mnDp11*. The genetic location of the breakpoint near *sqt-1* is confirmed, since Y70H7 covers *sqt-1*. Also consistent with these genetic data were the observations that the YACs (Y49F8 and Y62F5) physically mapped near *sqt-1* and to the right of *sqt-1* both hybridized to *mnDp11*, while the YACs, Y50G2 and Y54E11, physically mapped to the left of *sqt-1* hybridized to *mnT11*. However, an additional YAC covering *sqt-1* (Y8E4) mapped only to *mnDp11*. From physical mapping of cDNA clones (WATERSTON *et al.* 1992) to both Y54E11 and Y70H7, but not Y8E4, it appears that Y70H7 extends further to the left than Y8E4. Therefore the breakpoint is more likely to be near *sqt-1* and to the left, rather than to the right of this gene. This apparent contradiction could be explained if, in the creation of *mnDp11*, multiple breaks or rearrangements occurred, that have rendered *sqt-1* inactive. Since the initial description of *mnT2* (HERMAN, KARI and HARTMAN 1982), several small deficiencies and lethal mutations have been isolated and mapped to this region (SIGURDSON, SPANIER and HERMAN 1984) and genetic complementation tests between some of these and *mnDp11* might help to characterize the breakpoint.

Aside from characterizing the rearrangements themselves, the mapping of rearrangement breakpoints places genetic landmarks on the physical map. The left end of the *X* chromosome is one area of the genome where few genetic markers have been placed on the physical map, yet genetic studies suggest that analysis of DNA sequences in this region would be interesting. For example, it is within this region that DNA sequences likely to be important for the homologous pairing of the *X* chromosome in meiosis are thought to reside (HERMAN and KARI 1989). Previously, two sequence-tagged sites were mapped to the left end of the *X* (WILLIAMS *et al.* 1992). Now, by mapping the breakpoints of the two chromosomal rearrangements, *mnT2* and *mnDp33*, as reported here, three more landmarks have been added to this region of the physical map. From the physical map it is possible to locate the breakpoint of *mnT2* on *X*, previously mapped between *unc-1* and *dpy-3*, to the interval between *stP41* and *dpy-3*, since Y8D1, which maps to the right of *stP41* was included on *mnDp11* (Figure 6b). For the region covered by *mnDp33*, the location of *stP40* in Y60B6 provides a physical and genetic marker to subdivide the duplicated region. In addition to the genes genetically mapped to the region covered by *mnDp33*, a number of genes identified by molecular cloning or by tagged sequencing of cDNAs (WATERSTON *et al.* 1992) have been mapped to this region of the physical map. Assignment of physical endpoints to *mnDp33* should facilitate design of genetic screens for identification of mutations in some of these genes.

The extent of *mnDp33* is entirely contained within a contiguous portion of the physical map. An estimate of the physical size of the duplicated region of the *X* is therefore possible from the physical map. As shown in Figure 5b, *mnDp33* spans approximately 1.75 Mb of DNA and approximately 6 map units on the genetic map. Therefore, for this region of the *X* chromosome, 1 map unit corresponds to 300 kb. This is similar to an average rate calculated over the genome as a whole, and provides a contrast to the suppression of recombination that has been observed on several autosomes, where the ratio of the physical and genetic metrics can be up to 1–2 Mb/map unit (GREENWALD *et al.* 1987; PRASAD and BAILLIE 1989; STARR *et al.* 1989). It has been noted previously that the *X* differs from the autosomes in that it is largely devoid of the genetic clusters that characterize the autosomes (BRENNER 1974).

The analysis of chromosome rearrangements described here relies on prior knowledge of the genetic constitution of the rearrangement. It cannot therefore detect unexpected alterations in the genome outside the region covered by the selected YACs. Small deficiencies or additions, such as telomeric sequences at the ends of free duplications, cannot be identified. In the analysis of translocations in human cell lines, a more complete picture of the composition of a rearranged chromosome has been obtained by labeling flow sorted chromosomes (TELENIUS *et al.* 1992a,b), or microdissected chromosomes (MELTZER *et al.* 1992) and "painting" these back onto normal human metaphases by *in situ* hybridization. In this way unexpected sequences contained in the rearrangement may be visualized. In plants and animals where somatic metaphase chromosomes may be difficult to obtain in large numbers, these methods for characterizing chromosomal rearrangements may be less useful, since microdissection and flow sorting may be difficult. In *C. elegans*, as in many other organisms, when metaphase chromosomes are obtained they are small, and identification of the chromosomes in normal metaphases requires a set of *in situ* hybridization markers in addition to the specific chromosomal painting probes. Often meiotic tissues provide a richer source of chromosomes, and use of these chromosomes for *in situ* hybridization mapping (MOENS and PEARLMAN 1990; ALBINI and SCHWARZACHER 1992) appears to be a good alternative to metaphases, in some cases allowing high resolution cytogenetic mapping. Even at relatively low resolution, cytogenetics offers an efficient method for mapping chromosome rearrangements to unite physical and genetic maps.

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