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Localization of the ribosomal genes in *Caenorhabditis elegans* chromosomes by *in situ* hybridization using biotin-labeled probes

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The site of the ribosomal gene cluster on embryonic metaphase chromosomes of *Caenorhabditis elegans* has been mapped by *in situ* hybridization using probe DNAs that have been nick-translated to incorporate biotin-labeled UTP. The hybridized probe DNA was detected by a double-layer fluorescent antibody technique. Since chromosomes from wild-type *C. elegans* embryos are indistinguishable, *in situ* hybridization was carried out with chromosomes from *C. elegans* strains carrying cytologically distinct translocation or duplication chromosomes in order to identify the right end of linkage group I as the site of the ribosomal genes. Chromosomes carrying a lethal mutation, *let-209 I* displayed smaller hybridization signals than wild-type, suggesting that these chromosomes carried a partial deficiency of the ribosomal gene cluster. A duplication of the ribosomal genes, *eDp20(I;II)* rescued *let-209* homozygotes. Chromosomes carrying the alterations in the ribosomal genes were combined with *mnT12(IV;X)* to facilitate the mapping of genes in *C. elegans* by *in situ* hybridization. Linkage groups I and II are then labeled by the distinctive hybridization signals from the ribosomal probes, linkage groups IV and X are together distinguishable morphologically and linkage group V is labeled by hybridization to a 5S gene probe.

Key words: ribosomal genes/*Caenorhabditis elegans*/*in situ* hybridization/biotin labeling

Introduction

The arrangement of the genes coding for the 18S, 5.8S and 26S rRNAs of the nematode *Caenorhabditis elegans* (var. Bristol) was described from analysis of the clone pCe7, which hybridizes to *C. elegans* rRNA (Files and Hirsh, 1981). The ribosomal genes, present in the genome in 55 copies (Sulston and Brenner, 1974) form a tandem array. The repeating units are ~7000 bp in length and are very homogeneous since only a single deletion of 2900 bp was found in one of the repeats. The non-transcribed spacer between the ribosomal genes is composed of 1000 bp and is therefore short in comparison with other eukaryotic ribosomal gene spacers. Both the coding sequences and the spacers are conserved between the Bristol and Bergerac strains of *C. elegans*, showing no differences in restriction endonuclease cleavage patterns.

The conservation of the ribosomal gene sequences between the Bristol and Bergerac strains of *C. elegans* has frustrated attempts to assign the ribosomal genes to a linkage group using restriction enzyme polymorphisms as has been done for other *C. elegans* genes (Rose *et al.*, 1982; Files *et al.*, 1983). In many organisms the ribosomal genes have been mapped by *in situ* hybridization to metaphase chromosomes (Henderson, 1982). Metaphase chromosomes obtained from *C. elegans* embryos are similar in size and morphology and therefore display few cytological characteristics to differentiate them, one from another. However, because these chromosomes are holocentric it has been possible to obtain free chromosomal duplications and translocations that are small enough or large enough to be clearly distinguished from the unrearranged chromosomes by light or fluorescence microscopy (Albertson and Thomson, 1982). The assignment of the ribosomal genes to the right arm of linkage group I, reported here, was accomplished by *in situ* hybridization of the cloned ribosomal DNA (pCe7) to *C. elegans* strains carrying genetically defined and cytologically visible chromosomal translocations and duplications.

Mapping genes using chromosomal polymorphisms as markers for linkage groups may require that *in situ* hybridization be performed on several strains, each carrying different chromosomal rearrangements. This type of gene mapping has been greatly facilitated by the use of DNA probes in which biotin-labeled UTP has been incorporated. The site of hybridization may then be detected by a double layer fluorescent antibody technique, rather than by autoradiography.

Results

Hybridization of pCe7 to N2 chromosomes

There are six linkage groups in *C. elegans* (Figure 1a). Hermaphrodites have a pair of X chromosomes, while males have a single, unpaired X chromosome. Figure 1b shows a set of 12 Hoechst-stained chromosomes from wild-type hermaphrodite embryos that were hybridized with biotin-labeled pCe7 probe DNA. The rhodamine (TRITC) fluorescence of the antibodies bound to this spread is shown in Figure 1c. Super-position of the two images from Figure 1b and c showed that the probe bound to the end of two chromosomes (Figure 1d), and therefore the ribosomal genes are located on a single linkage group.

Hybridization of pCe7 to translocation and duplication chromosomes

The linkage group to which pCe7 hybridized was identified by *in situ* hybridization of pCe7 to chromosome spreads from *C. elegans* strains carrying genetically defined chromosome rearrangements that result in cytologically distinct chromosomes. In this way linkage groups II, IV and X were eliminated as the chromosomes to which pCe7 hybridized. Figure 2 shows the results of *in situ* hybridization of the pCe7 probe DNA to chromosomes from embryos carrying *mnT12(IV;X)*. Since the probe failed to hybridize to the large translocation chromosome, linkage groups IV and X did not carry the ribosomal genes. In a similar manner it was also concluded that linkage group II did not carry the ribosomal genes, since pCe7 did not hybridize to *mnT11(II;X)* or to the free duplications *mnDp35(II;f)*, *mnDp36(II;f)* and *mnDp11(II;X;f)*.

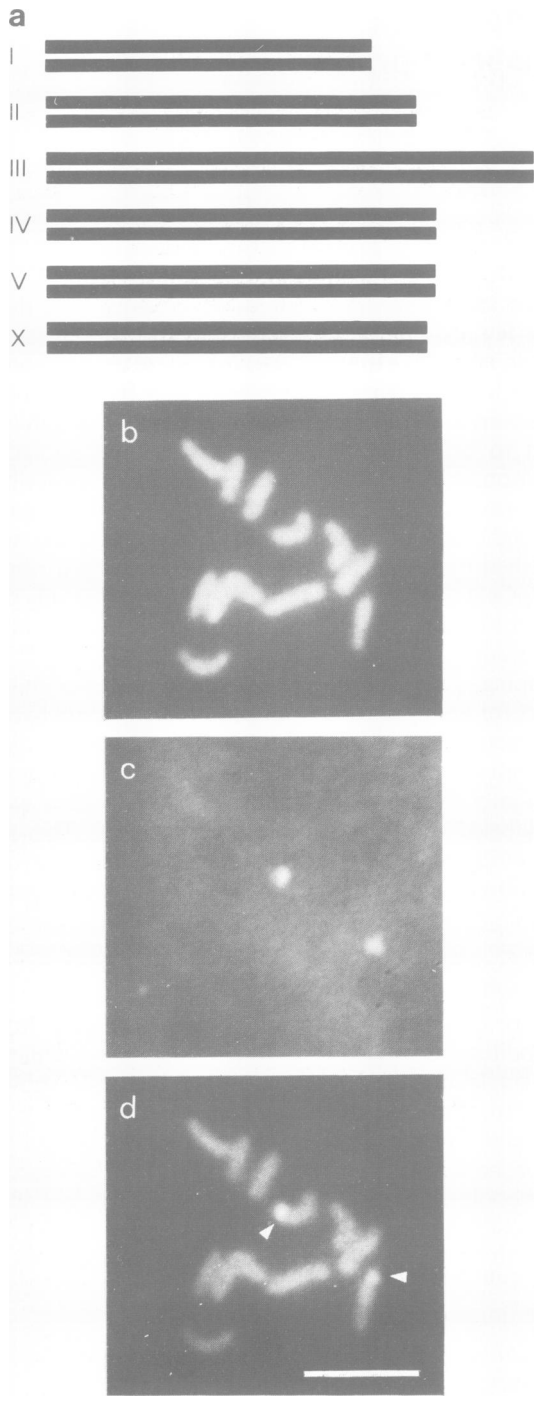

Fig. 1. Wild-type chromosomes hybridized *in situ* with pCe7 DNA. **(a)** Schematic diagram of the N2 karyotype. The relative lengths of the chromosomes are taken from the genetic map (Riddle and Swanson, 1982) and therefore may not represent the relative lengths of the metaphase chromosomes. **(b)** Hoechst-stained spread of chromosomes from wild-type embryo. **(c)** TRITC immunofluorescence indicating site of hybridization of biotin-labeled pCe7 DNA. **(d)** Superposition of negatives from **b** and **c** to show the site of hybridization of pCe7 on N2 chromosomes. The bar = 5 μm.

The ribosomal genes were mapped to the right arm of linkage group I. Figure 3 shows the hybridization of pCe7 to a spread of chromosomes from a strain carrying *mnDp10(X; I)*. The probe hybridized to the two longest chromosomes in the spread, thereby identifying linkage group I as the chromosome carrying the ribosomal genes. Furthermore, the ribo-

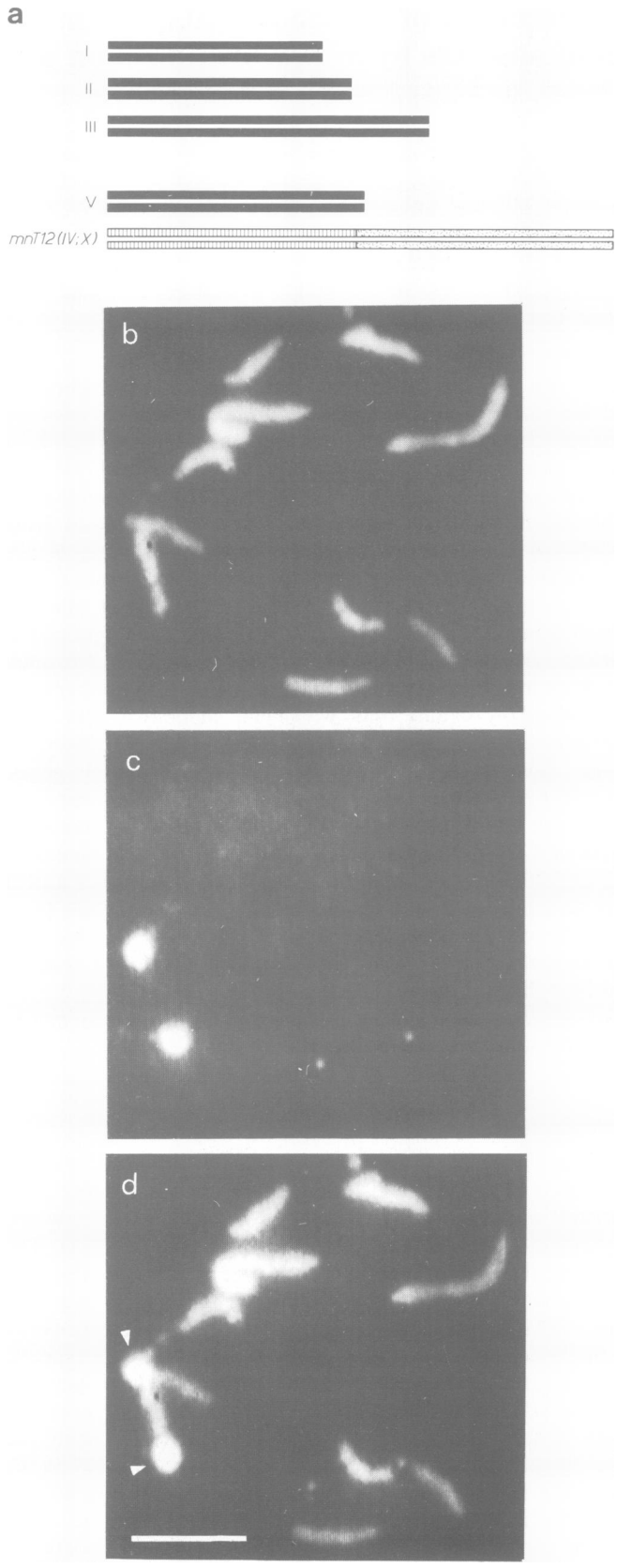

Fig. 2. Hybridization *in situ* of the probe pCe7 to chomosomes from embryos carrying *mnT12(IV;X)*. **(a)** Schematic diagram of the karyotype of embryos carrying *mnT12(IV;X)* chromosomes. **(b)** Hoechst 33258 fluorescence. **(b)** TRITC fluorescence. **(d)** Superposition of the negatives from **b** and **c**. The bar = 5 μm.

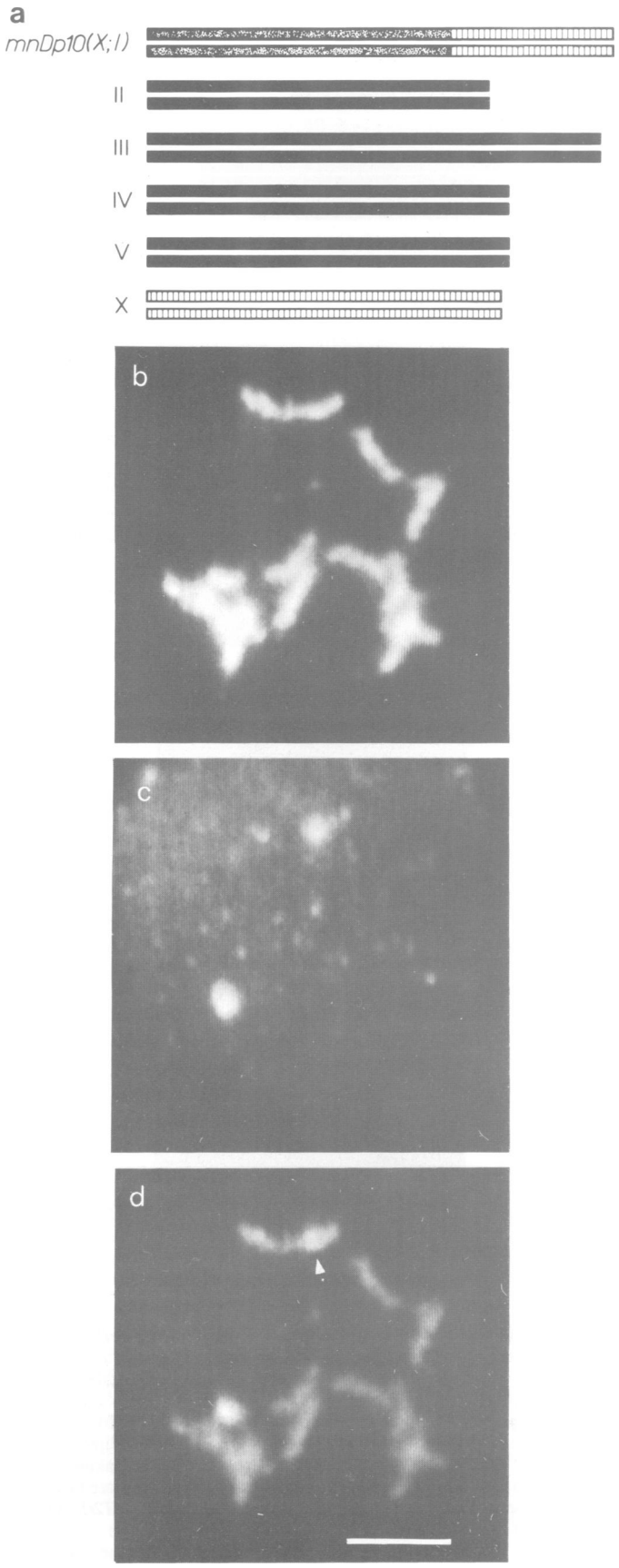

Fig. 3. Hybridization *in situ* of pCe7 to embryos carrying *mnDp10(X;I)*. **(a)** Schematic diagram of the karyotype of embryos carrying *mnDp10(X;I)*. **(b)** Hoechst fluorescence. **(c)** TRITC fluorescence. **(d)** Superposition of the negatives from **b** and **c**. The arrow indicates the site of hybridization on one *mnDp10(X;I)* chromosome. The bar = 5 μm.

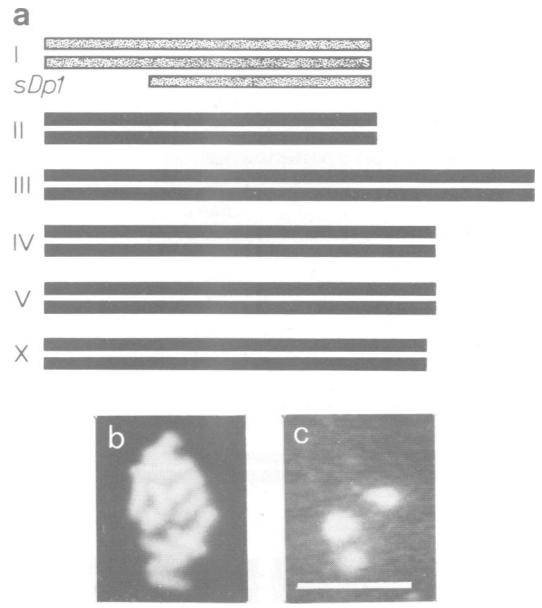

Fig. 4. Hybridization *in situ* of pCe7 to chromosomes from *lin28;sDp1* embryos. **(a)** Schematic diagram of the karyotype of *lin28;sDp1* embryos. **(b)** Hoechst fluorescence. **(c)** TRITC fluorescence. The bar = 5 μm.

somal genes must be located close to the end of the right arm of linkage group I, since the site of hybridization is no longer at the end of the chromosome but at an interior site due to the addition of the X chromosome DNA on to the right arm of I.

A free duplication of linkage group I, *sDp1(I;f)* carries genes on the right half of linkage group I, including *unc-54* which maps near the right-most end of linkage group I. Embryos that carried *sDp1(I;f)* showed three sites of hybridization with pCe7 (Figure 4). Therefore *sDp1(I;f)* also carries the ribosomal genes.

To locate the ribosomal genes more precisely, *in situ* hybridization with pCe7 was carried out with a deficiency, *eDf3* that deletes *unc-54* and genes to the left (Figure 5a). The deficiency is held over a balancer chromosome *let-209*. The results of the hybridization of pCe7 to squashed embryos from *eDf3/let-209* hermaphrodites are shown in Figure 5. Two sites of hybridization over nuclei and/or chromosome spreads were seen, however the hybridization signal from one of these chromosomes was very small. The *let-209* chromosome appeared to carry the smaller signal since embryos from *unc-54/let-209* hermaphrodites also showed a normal and a decreased signal similar to the signals observed with *eDf3/let-209* embryos. In *unc-54/let-209* embryos the fluorescence intensity of the small pCe7 hybridization signal was 7.0 ± 0.7% of the large signal. Therefore the ribosomal genes are not covered by *eDf3* and the *let-209* mutation, which is the right most marker on LG I, appears to be a deficiency in ribosomal genes as judged from the size of the hybridization signal.

A duplication of the ribosomal genes

In the course of this work the strain SP400 *mnT2(II;X);dpy-3(e27)* was used as a source of the linkage group II marker chromosomes *mnT11(II;X)* and *mnDp11(II;X;f)*. Embryos from *mnT2(II;X);dpy-3(e27)* hermaphrodites displayed two (30.6%), three (51.6%), or four (14.5%) sites of hybridization with pCe7. All sites of hybridization were at the ends of chromosomes other than *mnT11(II;X)* and *mnDp11(II;X;f)*. Hybridization of pCe7 to oocyte chromosomes at diakinesis

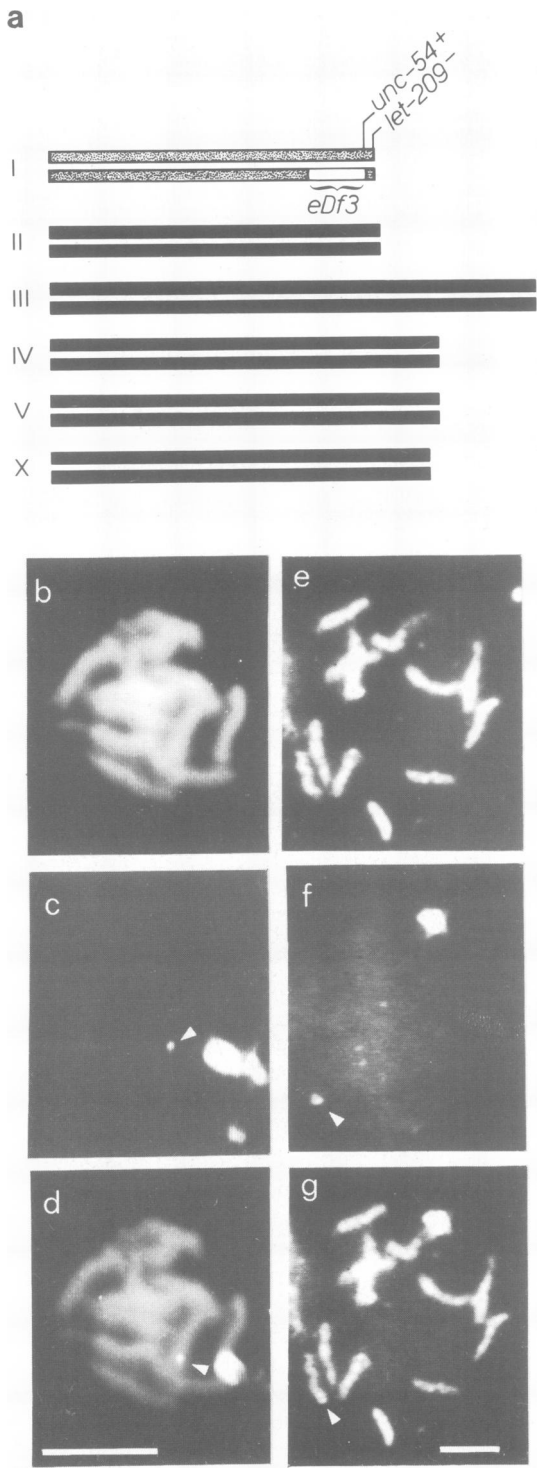

Fig. 5. Hybridization *in situ* of pCe7 to embryos heterozygous for *let-209*. **(a)** Schematic diagram of the karyotype of *eDf3/let-209* embryos. **(b)** Hoechst fluorescence of chromosomes from an *eDf3/let-209* embryo. **(c)** TRITC fluorescence indicating the site of hybridization of biotin-labeled pCe7 DNA to chromosomes in **b**. **(d)** Superposition of negatives from **b** and **c**. The bar indicates the magnification of **b**–**d**; bar = 5 μm. **(e)** Hoechst fluorescence of chromosomes from an *unc-54/let-209* embryo. **(f)** TRITC immuno-fluorescence indicating the site of hybridization of biotin-labeled pCe7 DNA to chromosomes in **e**. **(g)** Superposition of the negatives from **e** and **f**. Note that the right arm of the *unc-54* chromosome has been stretched in this squash. The bar indicates the magnification of **e**–**g**; bar = 5 μm. The arrows indicate the hybridization signal on *let-209* chromosomes.

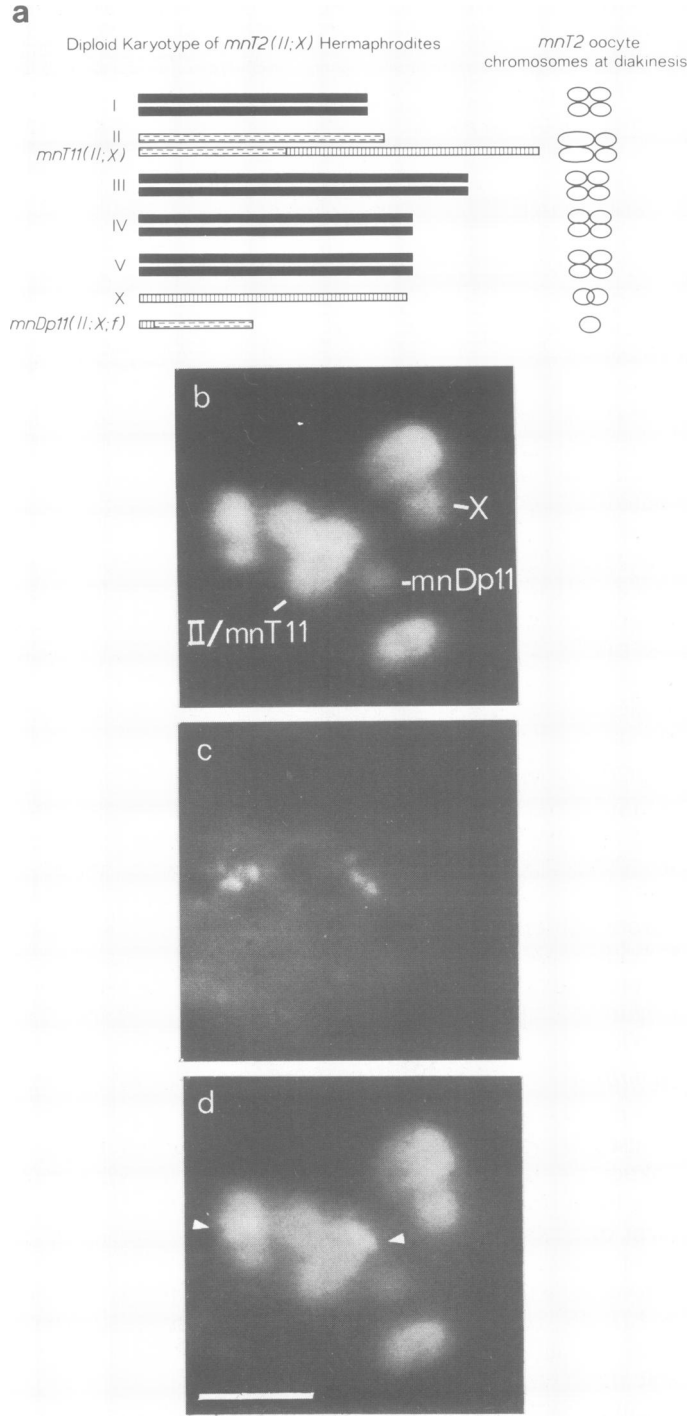

Fig. 6. Hybridization of pCe7 to oocytes in diakinesis from *mnT2(II;X); dpy3-(e27)* hermaphrodites. Hermaphrodites were cut behind the pharynx to allow the gonad to be extruded. These were transferred to subbed slides, covered with a cover slip and frozen on dry ice. The cover slip was pried off and the gonads fixed by immersing the slides in methanol for 2 min, followed by acetone for 4 min. **(a)** Schematic diagram of the diploid karyotype of *mnT2(II;X);dpy-3* hermaphrodites. Oocytes at diakinesis in *mnT2* hermaphrodites have seven chromosomes, five of these are bivalents and are composed of four chromatids. The rearrangement *mnT2(II;X)* is composed of two parts *mnT11(II;X)* and *mnDp11(II;X;f)*. The *mnT11(II;X)* chromosome pairs with chromosome II to give the larger bivalent. These hermaphrodites have a single X that may be distinguished as the univalent, and the smallest chromosome is *mnDp11*. **(b)** Hoechst fluorescence. **(c)** TRITC fluorescence. **(d)** Superposition of negatives from **b** and **c**. The sites of hybridization of pCe7 to two bivalents is indicated by the arrows. Bar = 5 μm.

from *mnT2* hermaphrodites demonstrated that pCe7 did not hybridize to the univalent X chromosome or *mnDp11(II;X; f)*, but to the homologue paired with *mnT11(II;X)* and to another autosomal bivalent (Figure 6). Thus, the strain SP400 carries a duplication of the ribosomal genes that probably arose spontaneously. The duplication has been designated *eDp20(I;II)* and as shown below is on linkage group II.

From SP400, *dpy-3* hermaphrodites were cloned. These carry two linkage group II chromosomes and no *mnT11(II;X)* chromosome. When pCe7 was hybridized to embryos from the *dpy-3* hermaphrodites four sites of hybridization were seen at the ends of four chromosomes. Therefore the duplication is homozygous viable. The dumpy marker was removed, generating phenotypically wild type hermaphrodites that carry a duplication of the ribosomal gene cluster (Figure 7).

To determine the linkage of *eDp20, eDp20* males were mated to *dpy-10(e128)unc-52(e444)* hermaphrodites and recombinants that were dumpy or unco-ordinated were picked. Hybridization of pCe7 to three dumpy recombinant clones and three unco-ordinated recombinant clones demonstrated that *eDp20(I;II)* is linked to *unc-52*, a gene at the right end of linkage group II, since the dumpy recombinants consistently displayed four sites of hybridization, while only two sites of hybridization were seen in embryos of the unco-ordinated recombinants.

eDp20 rescues let-209

A double mutant *let-209(e2000);eDp20(I;II)* was constructed that appeared phenotypically wild type. Embryos from the *let-209(e2000);eDp20(I;II)* hermaphrodites were squashed and pCe7 was hybridized *in situ* to the embryonic chromosomes. Two chromosomes displayed the small hybridization signal characteristic of *let-209* and two chromosomes showed normal sized hybridization signals (Figure 8). Therefore *eDp20(I;II)* rescued *let-209*. The duplication, *eDp20(I;II)* does not cover *unc-54*.

Identification of linkage groups using reiterated gene probes

The distinctive hybridization signals obtained with pCe7 can be used as markers for the chromosomes corresponding to linkage groups I and II in experiments involving gene mapping by *in situ* hybridization. To facilitate further the assignment of cloned DNAs to a linkage group the *mnT12(IV;X)* chromosome was combined with the *let-209* and *eDp20(I;II)* chromosomes, generating a strain in which linkage groups I and II can be identified unambiguously by hybridization with pCe7 and linkage groups IV and X together are distinguishable morphologically (Figure 9).

Linkage groups III and V are not labeled with pCe7. In order to distinguish which of the remaining, unmarked chromosomes corresponded to linkage group III or V, *in situ* hybridization was carried out with cloned DNA probes for genes that have been mapped to linkage group V. Both the cluster of three actin genes (Files *et al.*, 1983) and the 5S genes (D.W. Nelson and B.M. Honda, personal communication) have been mapped to linkage group V using restriction fragment length polymorphisms. The 5S genes are reiterated 110 times in the genome (Sulston and Brenner, 1974), and therefore should provide a suitable marker for linkage group V. Figure 10 shows the hybridization of pCe7 and a 5S gene probe to chromosomes from *let-209;eDp20(I;II); mnT12(IV;X)* embryos. The 5S probe did not hybridize to

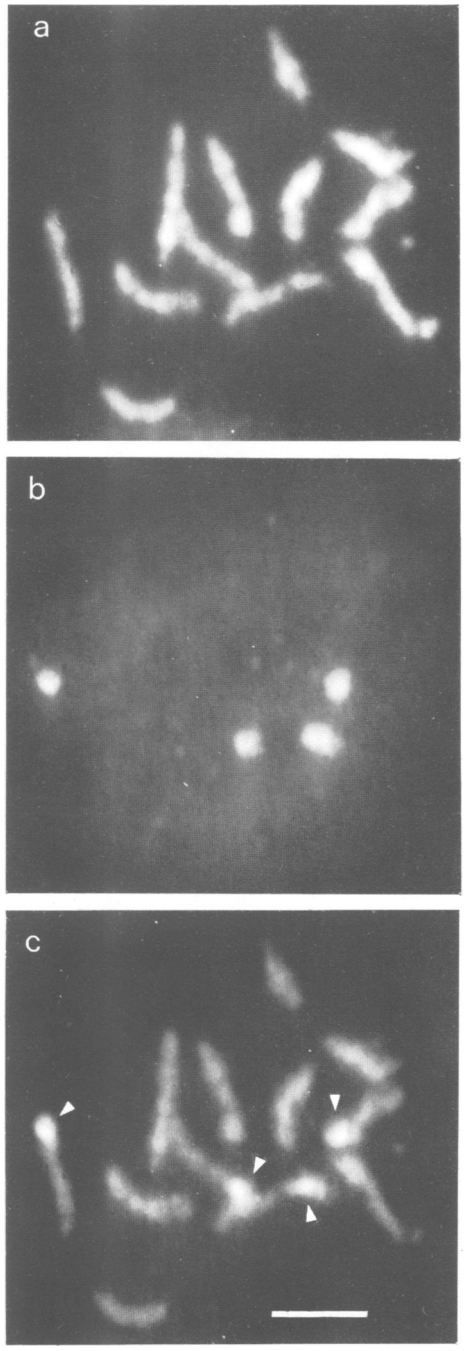

Fig. 7. Hybridization of pCe7 to chromosomes from *eDp20(I;II)* embryos. **(a)** Hoechst fluorescence. **(b)** TRITC fluorescence. **(c)** Superposition of negatives from **a** and **b**. The four sites of hybridization at the ends of four chromosomes are indicated by the arrows. Bar = 5 μm.

any of the marker chromosomes (I, II, IV or X), consistent with the restriction fragment length polymorphism map data. Since the 5S genes appear to be located at the Hoechst dark band on chromosome V and not at the end of the chromosome, the site of hybridization of the 5S gene probe is easily distinguished from the site of hybridization of pCe7 to *let-209*. Therefore the 5S genes provide a suitable marker for linakge group V. Using both the ribosomal probe and the 5S probe all the chromosomes from *let-209;eDp20(I;II); mnT12(IV;X)* embryos can be identified.

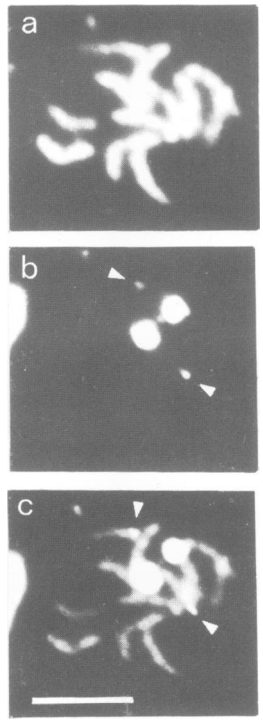

Fig. 8. Hybridization *in situ* of pCe7 to chromosomes from *let209; eDp20(I;II)* embryos. **(a)** Hoechst fluorescence. **(b)** TRITC fluorescence. **(c)** Superposition of **a** and **b**. The hybridization signal from the *let-209* chromosome is indicated by the arrows. Bar = 5 μm.

Discussion

Hybridization *in situ* using a cloned probe DNA has been used to locate the ribosomal genes on the genetic map of *C. elegans*. The availability of chromosomal polymorphisms, translocations, duplications and deficiencies permitted first the linkage group to which the probe hybridized to be identified, and then a more precise region on the linkage group to be identified. Finally it was possible to show that *let-209* is a lethal mutation that affects the ribsomal genes. In the future it should be possible to assign genes to linkage groups more readily since a *C. elegans* strain has been constructed in which the chromosomes can be marked by hybridization with reiterated gene probes and a chromosomal polymorphism. Therefore hybridization need only be performed on chromosomes from one strain, rather than from several different strains, as was necessary to map the ribosomal genes.

Immunofluorescence was used to localize biotin-labeled probes hybridized to chromosomes *in situ*. The method readily detected the 55 copies of ribosomal DNA present on each linkage group I chromosome. If *let-209* is a deficiency in the number of copies of the ribosomal genes as the small signal size suggests, then it is possible to detect fewer than 55 copies. The number of ribosomal genes present on *let-209* chromosomes may be estimated from the relative fluorescence of the two hybridization signals obtained with *unc-54/let-209* embryos assuming that fluorescence intensity increases linearly with gene number. If there are 55 copies of the ribosomal genes on the *unc-54* chromosomes, then the *let-209* chromosomes may carry as few as four copies. The fluorescent signal from biotin-labeled pCe7 probe DNA hybridized to *let-209* chromosomes is easily seen by eye. Thus it is possible to detect the hybridization of a probe DNA to 28 kb of sequence in the chromosome without special equipment. The hybridization signal from 'single copy' genes can be detected by means of an image intensifying camera. In this way it has been possible to record the chromosomal locations of the actin gene cluster on linkage group V and the fourth, unlinked actin gene.

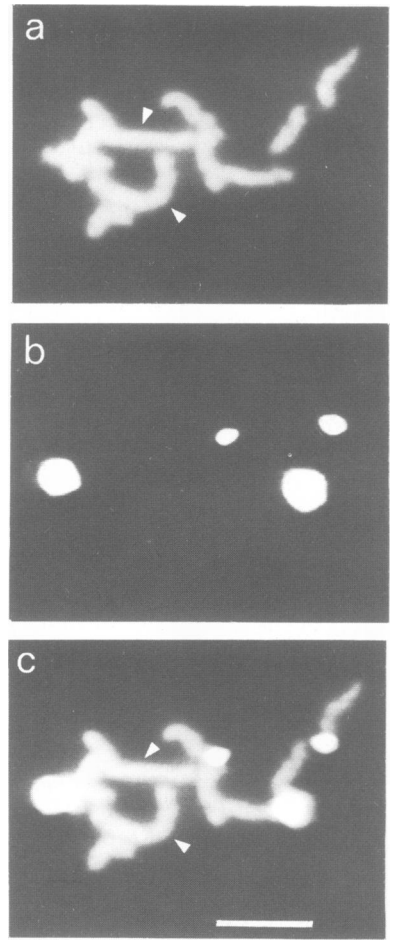

Fig. 9. Hybridization of pCe7 to chromosomes from *let-209;eDp20(I;II); mnT12(IV;X)* embryos. The *mnT12(IV;X)* chromosome is indicated by the arrows. **(a)** Hoechst 33258 fluorescence. **(b)** TRITC fluorescence. **(c)** Superposition of **a** and **b**. Bar = 5 μm.

The fluorescence measurements indicate that *let-209* embryos may contain as little as 7% of the ribosomal DNA complement of wild-type embryos. The *let-209* embryos complete morphogenesis, but frequently fail to hatch completely. In *Drosophila* (Ritossa *et al.*, 1966) and *Xenopus* (Miller and Knowland, 1970) reduction of the ribosomal DNA to $<25-50\%$ of the haploid quantity is lethal. Thus, it is likely that the lethality of the *let-209* mutation is due to an insufficient number of ribosomal genes. Since *eDp20(I;II)* rescues *let-209* homozygotes, it suggests that the translocated genes may be functional.

Materials and methods

Nematode strains and growth

Wild-type (N2) and mutant strains were maintained as described by Brenner (1974). The genes and alleles *unc-54(e190)I, dpy-10(e128)II* and *unc-52(e444)II* were obtained from the Cambridge collection. The lethal mutation, *let-209(e2000)I* (Riddle and Swanson, 1982) was isolated by P. Anderson, after X-ray mutagenesis (P. Anderson, personal communication).

The following chromosomal rearrangements were used to identify a linkage

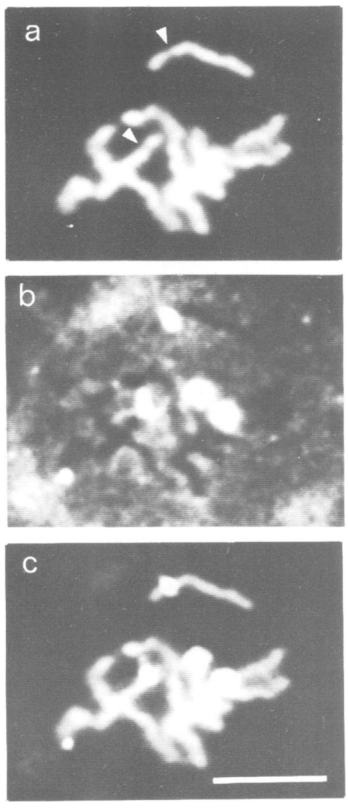

Fig. 10. Hybridization of pCe7 and the 5S gene probe to chromosomes from *let-209;eDp20(I;II);mnT12(IV;X)* embryos. The hybridization signal from one *let-209* chromosome is missing. It is likely that the large signal from one of the *eDp20* chromosomes has obscured the *let-209* signal. The spread shows a particularly good example of the Hoechst dark band (arrows) to which the 5S probe hybridized. **(a)** Hoechst fluorescence. **(b)** TRITC fluorescence. **(c)** Superposition of **a** and **b**. Bar = 5 μm.

group (LG) or part of a LG. LG I: *mnDp10(X;I)* (Herman *et al.*, 1979); for the right end, *eDf3* (Riddle and Swanson, 1982) and *sDp1* (Riddle and Swanson, 1982). LG II: for the left end, *mnT11(II;X)* (Herman *et al.*, 1982); for the right end, *mnDp11(X;II;f)* (Herman *et al.*, 1982), *mnDp35(II;f)* and *mnDp36(II;f)* (Herman *et al.*, 1979). LGs IV and X: *mnT12(IV;X)* which is a translocation of the left end of IV onto the right end of X (Sigurdson and Herman, personal communication). All chromosomal rearrangements with the prefix *mn* were obtained from R.K. Herman. The deficiency *eDf3* was obtained from the Cambridge collection. *sDp1(I;f)* held over *lin-28 I* was obtained from V. Ambros. Chromosomal rearrangements that were helpful in mapping genes, have been drawn schematically and these drawings have been included in the figures that show the results of the *in situ* hybridization to these chromosomes.

Preparation of squashed embryos for in situ hybridization

Embryos were collected from gravid adults and aliquots of embryos were pipetted onto subbed slides as described previously (Albertson and Thomson, 1982). An 18 x 18 mm coverslip was placed over the drop of embryos on the slide, the slide was inverted and gentle pressure was applied to squash the embryos. Slides were immediately frozen on dry ice for at least 10 min, then the cover slip was pried off and the slides were placed in ethanol:acetic acid (3:1) fixative overnight.

After fixation the slides were air dried, rinsed in 2 x SSC (0.3 M NaCl, 0.03 M Na citrate, pH 7) and then incubated at 37°C for 1 h in 20 μg/ml ribonuclease in 2 x SSC. Following ribonuclease digestion slides were rinsed in 2 x SSC and passed through two changes each of 70% and 95% ethanol, then air dried. The slides were then incubated for 1.5 min in 0.7 M NaOH, immediately passed through alcohols as before and air dried.

Recombinant DNA clones

The recombinant plasmid pCe7 contains a randomly cloned *Bam*HI fragment (Emmons *et al.*, 1979) that has been identified as a single ribosomal repeat (Files and Hirsh, 1981). The probe for the 5S RNA genes was isolated from a lambda 1059, partial *Sau*3A library by R. Waterston. The phage carries multiple copies of the tandemly repeated 1058-bp sequence that contains the coding sequence for 5S RNA (D.W. Nelson and B.M. Honda, personal communication).

Preparation of biotin-labeled probe DNAs

The recombinant DNA was labeled by an *in vitro* nick translation reaction in which 5-allylaminobiotin-labeled deoxyuridine triphosphate (Langer *et al.*, 1981) was used in place of thymidine triphosphate according to the protocol described by Langer-Safer *et al.* (1982). Generally 4 μg of plasmid DNA were labeled in a 100 μl reaction. Nucleotide triphosphates were present at 12.5 μM. After incubation at 15°C for 20–40 min the reaction was terminated by the addition of EDTA to a final concentration of 20 mM and incubation at 65°C for 10 min. Sonicated salmon sperm DNA was added to a final concentration of 60 μg/ml and the DNA precipitated with ethanol. The precipitated DNA was pelleted by centrifugation, washed twice with 70% ethanol, and dried.

The biotin-labeled probe DNA was dissolved in the appropriate amounts of 100 mM 1,4-piperazinediethanesulfonic acid (Pipes), 10 mM EDTA pH 7.0, water and formamide that would give a final DNA concentration of 10 μg/ml in the hybridization mixture described below. The dissolved DNA was stored at −20°C.

Hybridization of biotin-labeled DNA to squashed embyros

Several protocols for hybridization *in situ* were modified (Gall and Pardue, 1971; Henikoff and Meselson, 1977; Livak *et al.*, 1978). Hybridization was carried out in 0.01 M Pipes, 0.001 M EDTA, pH 7.0, 0.3 M NaCl, 50% formamide (Fluka), and 10% dextran sulfate. Prior to the addition of NaCl the DNA was denatured by heating at 70°C for 10 min. It was then rapidly chilled on ice and a 10 μl aliquot of the hybridization mixture was pipetted onto a slide containing squashed embryos. An 18 x 18 mm cover slip was placed over the drop and the edges of the cover slip were sealed with Carter's rubber cement. The slides were placed in a covered container over 50% formamide in 2 x SSC and then incubated at 37°C for 17–20 h. After this time the rubber cement was peeled off the slides, and the cover slips floated off by immersing the slides in 50% formamide in 2 x SSC. Slides were rinsed two times in 50% formamide-2 x SSC for 30 min at 37°C, then in 1.5 l of 2 x SSC and finally in phosphate buffered saline (PBS, 12 mm NaCl, 16 mM Na_2HPO_4, 8 mM NaH_2PO_4).

Detection of hybridization

Excess PBS was removed by blotting the slides, leaving a 100 μl drop of liquid covering the squashed embryos. Bovine serum albumin (100 μg) was added to the drop of liquid along with goat anti-biotin (IgG fraction, 2.4 mg/ml, Enzo) at a final dilution of 1:1000. Slides were incubated in humidified chambers for 2 h at room temperature, then rinsed in two changes of PBS and tetramethyl rhodamine (TRITC) labeled anti-goat IgG (Miles-Yeda, Ltd.) was added. Slides were incubated at room temperature for 20–45 min then rinsed in PBS. Just before viewing the slides were incubated for 5 min in PBS containing 1 μg/ml Hoechst 33258 to strain the chromosomes. The slides were rinsed briefly and mounted in PBS for microscopy.

Fluorescence microscopy

Slides were viewed with a Universal R microscope [Zeiss (Oberkochen) Ltd.] equipped with epi-fluorescence optics. Rhodamine, rather than fluorescein was chosen as the fluorochrome for the immunofluorescent detection of the hybridization signal because a lower background may be obtained with rhodamine, thereby giving a higher signal-to-noise ratio. High selectivity rhodamine filter set 15, green excitation H546 was used. The chromosomes were viewed using the Hoechst 33258 filter set 5, blue-violet excitation 400-440 [Carl Zeiss (Oberkochen) Ltd.]. For each strain at least two slides containing ~150 embryos were examined. A signal was detected over the nuclei of at least 90% of the embryos when *in situ* hybridization was carried out with pCe7. Since these embryos have not been arrested by drugs that disrupt microtubules, nuclei are in all stages of the cell cycle and embryos are also of different ages. As development progresses in the embryos the size of the cells, the nuclei, and the metaphase chromosomes decreases. Therefore chromosome spreads are best obtained from embryos with <50 cells. At least 10 chromosome spreads were scored for each strain. In those embyros carrying a duplication of a portion of a linkage group the number of sites to which the probe hybridized on interphase nuclei may also be distinguished in ~75% of the embryos. If embryos are too old then the individual hybridization signals appear as a single spot in the small nuclei.

Although the fluorescent signals were clearly visible by eye, initially fluorescent images were recorded on video tape by means of an RCA ISIT camera with two stages of intensification. The use of video tape recording resulted in a degradation of the image. Better pictures were obtained by passing the camera output directly to an Intellect 100 image processing system (Micro Consultants, UK Ltd.), which permitted images to be time averaged for noise reduction and stored digitally on magnetic disk. The processed images were then photographed from the video monitor with a 35 mm camera.

To increase the magnification of the chromsomes on the video screen a lens was placed in the camera tube. Over the course of this work various combinations of lenses and objectives were tried. Initially, the 100 x neofluor objective and a 12.5 x eyepiece placed at various levels in the camera tube were used, but more recently the planapo 63/1.4 objective has been used in combination with an auxiliary 4 x lens [Carl Zeiss (Oberkochen) Ltd., Cat. No. 477909] in the camera tube. This combination gave a high level of mangification (7500 x on the video monitor) with maximum light gathering capability. The Hoechst and rhodamine filter sets are positioned in the microscope such that the images seen with these filter sets are displaced from each other by ~1 μM. This displacement was calibrated on the videomonitor using very brightly red fluorescent objects in the field. For the purpose of illustration the negatives of the two fluorescent images were super-imposed and aligned in order to make the prints showing the position of the hybridization signal on the chromosome.

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