Genetic Analysis of Chromosomal Rearrangements in the *cyclops* Region of the Zebrafish Genome

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Manuscript received June 24, 1997
Accepted for publication October 7, 1997

ABSTRACT

Genetic screens in zebrafish have provided mutations in hundreds of genes with essential functions in the developing embryo. To investigate the possible uses of chromosomal rearrangements in the analysis of these mutations, we genetically characterized three gamma-ray induced alleles of *cyclops* (*cyc*), a gene required for development of midline structures. We show that *cyc* maps near one end of Linkage Group 12 (LG 12) and that this region is involved in a reciprocal translocation with LG 2 in one gamma-ray induced mutation, cyc^{b213}. The translocated segments together cover approximately 5% of the genetic map, and we show that this rearrangement is useful for mapping cloned genes that reside in the affected chromosomal regions. The other two alleles, cyc^{b16} and cyc^{b229}, have deletions in the distal region of LG 12. Interestingly, both of these mutations suppress recombination between genetic markers in LG 12, including markers at a distance from the deletion. This observation raises the possibility that these deletions affect a site required for meiotic recombination on the LG 12 chromosome. The cyc^{b16} and cyc^{b229} mutations may be useful for balancing other lethal mutations located in the distal region of LG 12. These results show that chromosomal rearrangements can provide useful resources for mapping and genetic analyses in zebrafish.

THE zebrafish (*Danio rerio*) has become an important model organism for the study of vertebrate biology (Kimmel 1989; Driever *et al.* 1994; Eisen 1996; Felsenfeld 1996; Grunwald 1996; Holder and McMahon 1996). Large-scale mutagenesis screens have provided mutations in hundreds of genes essential for normal development, physiology, and behavior (Riley and Grunwald 1995; Driever *et al.* 1996; Gaiano *et al.* 1996; Haffter *et al.* 1996; Henion *et al.* 1996). Moreover, the optical clarity and accessibility of the embryo allow detailed phenotypic characterization with techniques such as cellular transplantation and lineage tracing (Ho and Kane 1990; Hatta *et al.* 1991; Melby *et al.* 1996). Thus, the cellular functions of genes defined by mutations in zebrafish can be studied in great detail. Molecular genetic analysis of these mutant loci is now an important goal, and development of genetic tools and genomic resources that facilitate the cloning of these genes is therefore a high priority (Postlethwait *et al.* 1994; Johnson *et al.* 1996; Knapik *et al.* 1996; Postlethwait and Talbot 1997).

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Chromosomal rearrangements induced by gamma-rays and X-rays, including deletions, duplications, translocations, and inversions are important tools in the genetic analysis of a number of model organisms. In Drosophila, for example, chromosomal rearrangements are used to distinguish amorphic from hypomorphic alleles, to balance lethal and sterile mutations, to map mutations, to investigate gene dosage effects, and to identify mutated genes through breakpoint mapping (Ashburner 1989). By comparison, little has been done to characterize gamma-ray induced mutations in zebrafish, although gamma-ray induced deficiencies have been used in zebrafish to establish null phenotype (Talbot *et al.* 1995; Schier *et al.* 1997; Halpern *et al.* 1997) and to remove chromosomal segments containing specific cloned genes (Fritz *et al.* 1996).

We set out to determine the genetic nature of three gamma-ray induced mutations involving *cyclops* (*cyc*), a gene essential for development of the embryonic midline (Hatta *et al.* 1991). We found that two of these alleles, cyc^{b16} and cyc^{b229}, have deletions in the distal region of Linkage Group 12 (LG 12), and that both suppress recombination between markers in a segment of LG 12, thereby functioning as balancer chromosomes for this chromosomal region. The third allele, cyc^{b213}, is a reciprocal translocation between LG 12 and LG 2. These results suggest that the systematic collection and analysis of gamma-ray induced mutations in

other parts of the genome will provide important tools for genetic analysis in zebrafish.

MATERIALS AND METHODS

Fish strains: The AB strain (Chakrabarti *et al.* 1983) was used in the screens that identified *cyc*^{b213} and *cyc*^{b229}. Other strains used to produce hybrids for mapping include DAR, Tü, and TL (Postlethwait *et al.* 1994; Haffter *et al.* 1996).

Nomenclature: We followed previous linkage group designations (Postlethwait *et al.* 1994; Johnson *et al.* 1996). Each linkage group corresponds to a different chromosome because each has been assigned a centromere (Johnson *et al.* 1996).

Following guidelines for Drosophila rearrangements, the *b213* reciprocal translocation is described as *T(LG2;LG12)*^{b213}, and the two elements of the translocation are termed *T(LG2;LG12)*^{b213}, $2^{P}12^{D}$ (for the rearranged chromosome with the centromere-proximal segment of LG 2 and distal segment of LG 12) and *T(LG2;LG12)*^{b213}, $12^{P}2^{D}$. Segregation of these rearranged chromosomes and their normal order counterparts results in euploid and aneuploid meiotic products (see Figure 7). For convenience of discussion, we refer to the haploid genotype with a normal order LG 2 and a *T(LG2;LG12)*^{b213}, $12^{P}2^{D}$ chromosome as *cyc*^{b213} and the genotype with a normal order LG 12 and a *T(LG2;LG12)*^{b213}, $2^{P}12^{D}$ chromosome as *nec*^{b213}, referring to the cyclopic and necrotic phenotypes characteristic of aneuploid haploid embryos with these genotypes. We emphasize that the *cyc*^{b213} and *nec*^{b213} phenotypes result from the loss and duplication of chromosomal segments, not individual genes.

Isolation of new *cyclops* alleles: The identification of *cyc*^{b16} and *cyc*^{m294} has been described (Hatta *et al.* 1991; Schier *et al.* 1996). *cyc*^{b213} was identified in a screen for gamma-ray induced mutations causing morphological defects in the embryo (Kimmel 1989). The *cyc*^{b213} mutation fails to complement *cyc*^{b16}. The *cyc*^{b229} mutation was identified in a screen for gamma-ray induced mutations that fail to complement *cyc*^{b16}. To examine the possibility that *cyc*^{b229} was an inadvertent reisolate of *cyc*^{b16}, we compared alleles of PCR-based genetic markers present on the two mutant chromosomes. The *cyc*^{b16} and *cyc*^{b229} chromosomes have different alleles of a simple sequence length polymorphism (SSLP) marker (z1400) in the region where recombination is suppressed by both mutations (see Figure 2), suggesting that *cyc*^{b229} is a newly generated *cyc* allele. The observation that *cyc*^{b229} recombines more frequently with proximal LG 12 markers, *e.g.*, z3801, (see Table 1) than *cyc*^{b16} provides further evidence for the independent origin of the two alleles.

Genetic mapping and markers: Generation of haploid embryos, genomic DNA preparation, and PCR conditions have been described (Postlethwait *et al.* 1994; Johnson *et al.* 1996). Polymorphisms were detected either by agarose gel electrophoresis and ethidium bromide staining or by acrylamide gel electrophoresis of ^{32}P-labelled PCR products.

The LG 12 markers shown in Figure 1C were mapped in at least one of two haploid mapping panels. One panel was produced from 48 haploid progeny of a TL/Tü female and the other was produced from 48 haploid progeny of an AB/Tü female. Maps were compiled with Map Manager (K. Manley and R. Cudmore, http://mcbio.med.buffalo.edu/mapmgr.html). Maps produced from these two panels were integrated with each other by reference to markers scored in both panels.

Primers for SSLP markers (Knapik *et al.* 1996) were obtained commercially (Research Genetics, Birmingham, AL). The primers used to amplify *ptc1* (Concordet *et al.* 1996) were PTCF1 (5′-AGCAAGGAGCTACGCTACAC-3′) and PTCR1 (5′-GCAGGGGAAAAGCTTATCAA-3′). The primers for other genes shown in Figure 7 will be described elsewhere (J.H.P. *et al.*, in preparation). The randomly amplified polymorphic DNA (RAPD) markers 18AF.1190, 15T.750, and 12R.360 (Postlethwait *et al.* 1994; Johnson *et al.* 1996; Halpern *et al.* 1997) were converted to sequence-tagged site (STS) markers by deriving locus-specific primers from the sequences of the cloned RAPD fragments. The primers for the 18AF.1190 STS were 18AF.1190F (5′-CCCTCTGCACAGAACTGAAACCTC-3′) and 18AF.1190R (5′-CCGTTTCCTGTGAAGACAGGAAG-3′). The primers for the 15T.750 STS were 15T.750F (5′-CTGTCT GGAGAAAGTCTTATTTG-3′) and 15T.750R (5′-GGATGC CACTGGTACTAATTGATA-3′). The primers for the 12R.360 STS were 12R.360F (5′-ACAGGTGCGTCCAATAGCTCC-3′) and 12R.360R (5′-ACAGGTGCGTGATCAAATGTT-3′). These loci were scored as STS markers in some experiments (such as those shown in Figures 1, 3, 5, and 6) and as RAPDs in others (including some experiments shown in Table 1).

RESULTS

Genetic mapping of *cyclops*: Previous work demonstrated that *cyclops* is linked to randomly amplified polymorphic DNA (RAPD) markers in the distal region of Linkage Group 12 (LG 12; Postlethwait *et al.* 1994). This assignment employed *cyc*^{b16}, which we show below to be a rearrangement that suppresses recombination over part of LG 12 and therefore obscures the exact position of *cyc*. To avoid this difficulty, we analyzed LG 12 markers in mapping crosses constructed with *cyc*^{m294}, which was induced with N-ethyl-N-nitrosourea (ENU) and thus is likely to be a point mutation (Schier *et al.* 1996). Scoring LG 12 markers, including RAPDs, simple sequence length polymorphisms (SSLPs), and sequence-tagged sites (STSs) derived from cloned RAPDs, in individual haploid progeny of DAR x *cyc*^{m294} F1 females confirmed the assignment of *cyc* to LG 12 (Figure 1). The *cyc*^{m294} mutation is linked to 18AF.1190 (1 recombinant among 44 haploid individuals), a RAPD that has been cloned and converted into an STS (Figure 1A). Analysis of markers proximal to 18AF.1190, such as SSLP z1400, in the same *cyc*^{m294} mapping crosses indicated that *cyc* is distal to 18AF.1190 (Figure 1, B and C). LG 12 markers were also scored in haploid embryos from wild-type, *i.e.*, *cyc*$^{+}$, reference mapping panels (Postlethwait *et al.* 1994; Johnson *et al.* 1996; this work, see materials and methods). Distances between LG 12 markers do not differ significantly in *cyc*$^{+}$ and *cyc*^{m294} mapping panels, indicating that *cyc*^{m294} maps as a point mutation or other small lesion, as would be expected for an ENU-induced mutation. For example, there were 6 recombinants between markers 18AF.1190 and z1400 among 44 individuals in a wild-type mapping panel and 4 recombinants among 44 individuals in a *cyc*^{m294} mapping panel (Figure 1 and Table 1).

***cyc*^{b16} and *cyc*^{b229} delete markers near *cyc*:** The *cyc*^{b16} and *cyc*^{b229} mutations are gamma-ray induced *cyc* alleles that segregate as Mendelian recessives (Hatta *et al.* 1991; Halpern *et al.* 1997; Table 2, this work). To investigate the possibility that these mutations involve deletions in

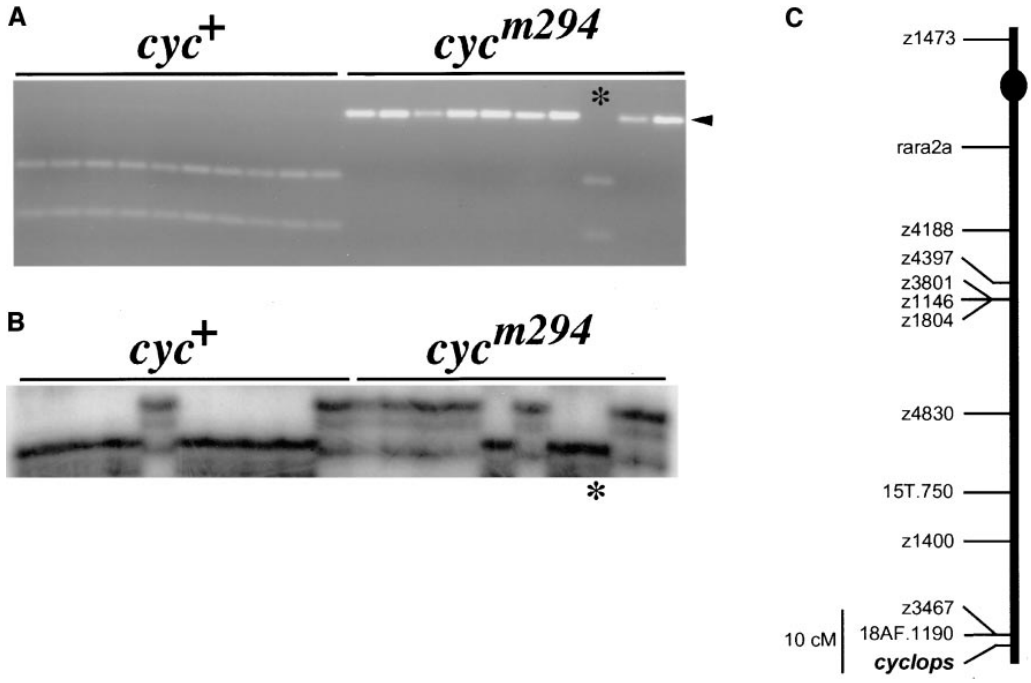

Figure 1.—Genetic mapping of *cyclops*. (A) The marker 18AF.1190 is linked to *cyc*. Ten wild-type and 10 cyc^{m294} haploid siblings were analyzed with an STS marker derived from the 18AF.1190 RAPD. An *Mnl* I site polymorphism reveals that the 18AF.1190 locus is linked to *cyc*, and one recombinant (*) is shown. This was the only recombinant identified among 44 haploid progeny of a DAR x cyc^{m294} female. The uncleaved 18AF.1190 fragment is 360 bp. The arrowhead marks the 300-bp fragment that is linked in coupling to the mutant chromosome; the other allele is marked by 190-bp and 110-bp *Mnl* I fragments. (B) The same 20 embryos were analyzed with the SSLP marker z1400. Five recombinants are shown, including the recombinant identified with 18AF.1190 (*) in A. This places z1400 and 18AF.1190 on the same side of *cyc*. Five recombinants for z1400 and *cyc* were identified among 44 haploid progeny of the DAR x cyc^{m294} female. (C) Genetic map of Linkage Group 12, showing 18AF.1190, z1400, and *cyc*. The markers shown were scored in a wild-type, *i.e.*, cyc^{+}, haploids, and a map was generated (see materials and methods). The position of *cyc* was inferred from the analysis of z1400 and 18AF.1190 in the cyc^{m294} mapping cross. The centromere location (dot) has been reported previously (Johnson *et al.* 1996). The map order shown is consistent with previous maps (Postlethwait *et al.* 1994; Johnson *et al.* 1996; Knapik *et al.* 1996).

the vicinity of *cyc*, we assayed LG 12 markers on genomic DNA isolated from cyc^{b16} and cyc^{b229} haploid embryos and their wild-type siblings. Figure 2 shows that one of the markers nearest to *cyc*, the SSLP z3467, amplifies from the wild-type samples, but not from cyc^{b16} or cyc^{b229} DNA. This result, together with our observations that 18AF.1190 and other nearby markers give similar results (data not shown), indicates that both cyc^{b16} and cyc^{b229} contain deletions in the vicinity of *cyc*. The SSLP z1400, which lies about 10 cM from z3467 and 18F.1190 (Figure 1C), amplifies from both wild-type and mutant samples (Figure 2), indicating that neither cyc^{b16} nor cyc^{b229} involves a deletion extending beyond this marker.

***cyc*^{b16} and *cyc*^{b229} suppress recombination on LG 12:** During the analysis of cyc^{b16} and cyc^{b229}, we noted that both of these mutations suppress recombination between markers in the distal region of LG 12 (Figure 3 and Table 1). For example, 15T.750 frequently recombines with cyc^{m294} but only rarely, if at all, recombines with cyc^{b16} (Figure 3 and Table 1). Analysis of LG 12 markers in cyc^{b16} mapping crosses revealed that recombination is suppressed in a region stretching from *cyc* to beyond marker z4830 (Table 1). Similarly, the cyc^{b229} mutation suppresses recombination on LG 12 but over a smaller region than cyc^{b16} (Figure 3 and Table 1). The cyc^{b229} mutation failed to recombine with 15T.750 in 113 haploid individuals, whereas there were 8 recombinants for these loci among 44 individuals in cyc^{m294} mapping crosses. More proximal markers, such as z3801, recombine frequently with *cyc* in cyc^{b229} mapping crosses (Table 1), demarcating the region of recombination suppression by this mutation.

TABLE 1

Genetic distance between *cyc* and LG 12 markers in *cyc* mutant mapping crosses

	Percent recombination with *cyc* (No. rec./total)			
cyc allele	z1400	15T.750	z4830	z3801
m294	11 (5/44)	18 (8/44)	26 (11/42)	36 (15/41)
b16	0 (0/94)	0 (0/96)	0 (0/47)	2 (1/66)
b229	0 (0/72)	0 (0/113)	3 (2/69)	22 (15/69)

***cyc*^{b213} involves a reciprocal translocation between LG 2 and LG 12:** All three alleles discussed so far segregate according to Mendel among haploid offspring of heterozygous $cyc^{+/-}$ mothers; in these cases, about 50% of haploid embryos showed the mutant phenotype (Table 2). In contrast, the haploid offspring of $cyc^{b213/+}$ mothers display distinctly non-Mendelian segregation. Twenty-five percent (156/624) of the haploid progeny of $cyc^{b213/+}$ females had a cyclopic phenotype, whereas the Mendelian expectation is 50% (Table 2). Moreover, 25.8% (161/624) of the embryos in these clutches dis-

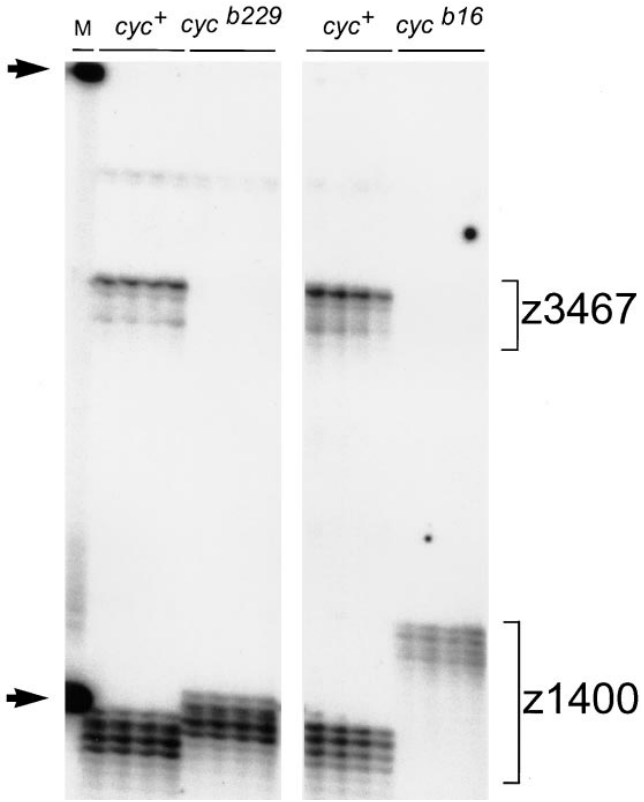

Figure 2.—Markers near *cyc* fail to amplify from cyc^{b16} and cyc^{b229} genomic DNA. Individual haploid embryos obtained from mothers heterozygous for the cyc^{b16} and cyc^{b229} mutations were assayed with the SSLP markers z3467 and z1400. The z3467 fragment amplifies from the wild-type, but not the mutant, genomic DNA samples. Primers for both markers were included in the same PCR assays, so that z1400 serves an internal control for the mutant samples. Note that the two mutant chromosomes have different alleles of z1400, providing evidence that cyc^{b229} is not an inadvertent reisolate of cyc^{b16}. Arrows indicate size standards of 100 nucleotides and 200 nucleotides in the marker lane (M).

played a characteristic necrotic mutant phenotype, which we have termed nec^{b213} (Table 2 and Figure 4C; see materials and methods). This 2:1:1 segregation is characteristic of reciprocal translocations (Morgan *et al.* 1925), suggesting that the *b213* mutation may be a chromosomal rearrangement of this sort.

TABLE 2

Inheritance of *cyclops* alleles in haploid embryos

cyc allele	% wild-type (wild-type/total)	% cyc^- (cyc^-/total)	% nec^{b213} (nec^{b213}/total)
b16	51.6 (262/508)	48.4 (246/508)	0
b229	48.4 (134/277)	51.6 (143/277)	0
m294	47.1 (98/208)	52.9 (110/208)	0
b213	49.2 (307/624)	25.0 (156/624)	25.8 (161/624)

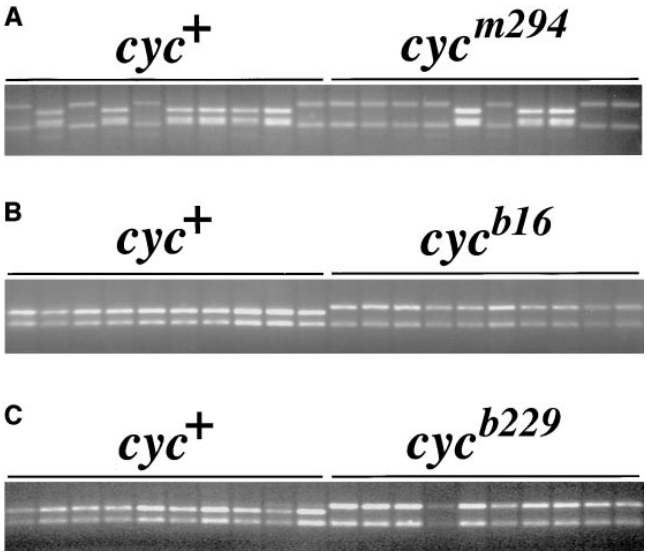

Figure 3.—Suppression of recombination by cyc^{b16} and cyc^{b229}. An STS marker derived from RAPD 15T.750 was analyzed in haploid mapping crosses for (A) cyc^{m294}, (B) cyc^{b16}, and (C) cyc^{b229}. In each case, 10 wild-type and 10 mutant siblings are shown. The primers amplify allelic fragments of different sizes in all three crosses, but all fragments were nevertheless cleaved with the restriction enzyme *Mnl* I to generate fragments of optimal size for detecting the polymorphism with agarose gel electrophoresis.

If *b213* involves a reciprocal translocation with a breakpoint proximal to the *cyc* locus, then the cyclopic phenotype would result from the absence of the region of LG 12 that contains the cyc^+ gene. As predicted by this model, markers from the distal region of LG 12 amplified from the genomic DNA of wild-type and nec^{b213} haploid embryos, but not from their cyc^{b213} siblings (Figure 5). The region of LG 12 proximal to z4830 is present in cyc^{b213} individuals, whereas the markers in the segment distal to 15T.750 fail to amplify, indicating that the breakpoint on the rearranged chromosome lies 32 ± 6 cM proximal to *cyc*. This result indicates that the loss of cyc^+ function in cyc^{b213} mutants stems from the absence of cyc^+ and other loci in the distal region of LG 12 rather than a breakpoint that disrupts the cyc^+ gene.

If cyc^{b213} involves a reciprocal translocation as suggested by the segregation data, and then the distal region of some linkage group other than LG 12 should be present in two copies in cyc^{b213} embryos but absent in individuals with the nec^{b213} phenotype. A systematic screen for markers absent from the DNA of nec^{b213} mutants identified LG 2 as the translocation partner. Markers over a large segment of LG 2 (extending distally from *twhh*) did not amplify from nec^{b213} embryo DNA (Figure 5 and data not shown). The proximal marker 12R.360 is present in these embryos, indicating

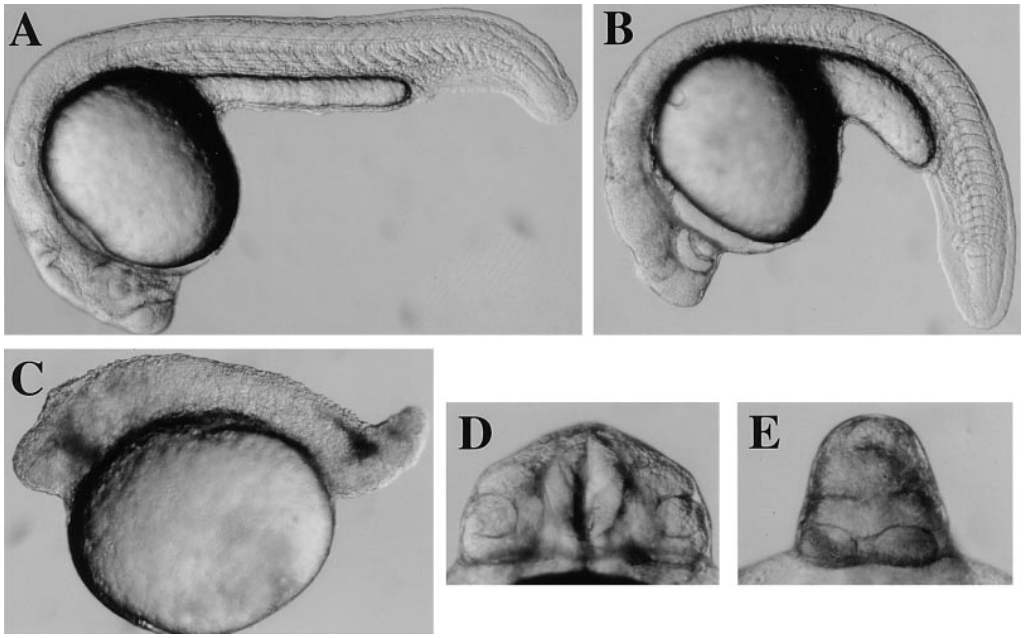

Figure 4.—Wild-type (A, D) *cyc*^{b213} (B, E) and *nec*^{b213} (C) diploid embryos at 22 hours after fertilization. Side (A–C) and frontal (D, E) views are shown.

that the LG 2 breakpoint lies between 12R.360 and *twhh*.

Analysis of LG 2 and LG 12 markers in the haploid progeny of a *b213*/+ heterozygous female confirmed that these linkage groups are involved in a reciprocal translocation in the *b213* mutation (Figures 6 and 7).

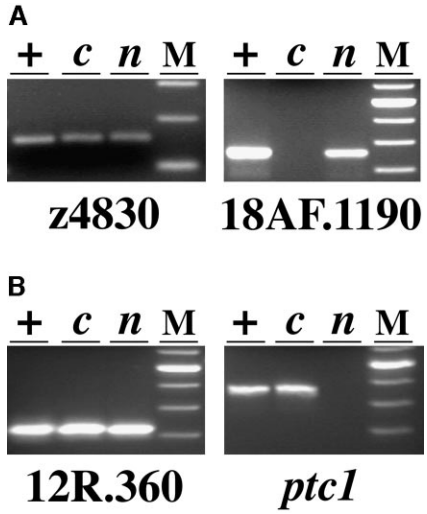

Figure 5.—Analysis of LG 12 (A) and LG 2 (B) markers in haploid progeny of a *b213*/+ female. Pools of DNA from wild-type (lanes +), *cyc*^{b213} (lanes *c*), and *nec*^{b213} (lanes *n*) haploid embryos were tested with the SSLP z4830, STS markers derived from the RAPDs 18AF.1190 and 12R.360 and the gene *patched1 (ptc1)*. z4830 and 12R.360 amplify from all three DNA samples, whereas 18AF.1190 fails to amplify from *cyc*^{b213} DNA and *ptc1* fails to amplify from *nec*^{b213} DNA. Size standards (100-bp ladder, Life Technologies, Gaithersburg, MD) are shown in lanes M; fragment sizes are 160 bp for z4830, 360 bp for 18AF.1190, 330 bp for 12R.360, and 480 bp for *ptc1*.

The 350-bp allele of the LG 12 marker 15T.750 cosegregates with the 330-bp allele of the LG 2 marker 12R.360 (arrowheads in Figure 6), demonstrating that these loci lie together on a rearranged chromosome, *T(LG2; LG12)*^{b213}, *2*^{P}*12*^{D}, comprised of the centromeric region of LG 2 and the distal region of LG 12 (Figure 7). In contrast, the 320-bp allele of the LG 12 marker 15T.750 (arrow in Figure 6) segregates independepently of the 350-bp allele of the same locus in the *b213* mapping panel, as would be expected for markers on different chromosomes, but not for alleles of the same locus. The wild-type individuals inherited one (never zero or two) 15T.750 allele, which is expected for allelic segregation in haploid embryos. However, the *cyc*^{b213} animals inherited neither 15T.750 allele and the *nec*^{b213} animals inherited both, as expected for loci on different chromosomes. In similar experiments (summarized in Figure 7A), markers from the proximal region of LG 12, including z4830, cosegregate with markers from the distal region of LG 2, including *eng3*, confirming that the *b213* mutation involves a reciprocally rearranged chromosome, termed *T(LG2;LG12)*^{b213}, *12*^{P}*2*^{D}, comprised of the centromeric region of LG 12 and the distal region of LG 2 (Figure 7). The combined results demonstrate that the diploid mother of the *b213* haploid mapping family was a balanced translocation heterozygote, possessing a normal order LG 2, a normal order LG 12, and two reciprocally rearranged LG 2-LG 12 chromosomes (Figure 7). Analysis of the inheritance of the rearranged chromosomes and the resulting phenotypes indicates that the wild-type embryos are euploid, inheriting both or neither rearranged chromosomes (schematized as wild type* and wild type, respectively, in Figure 7B) and that the *cyc*^{b213} and *nec*^{b213} embryos are aneuploid, having a deletion of either distal LG 12 or

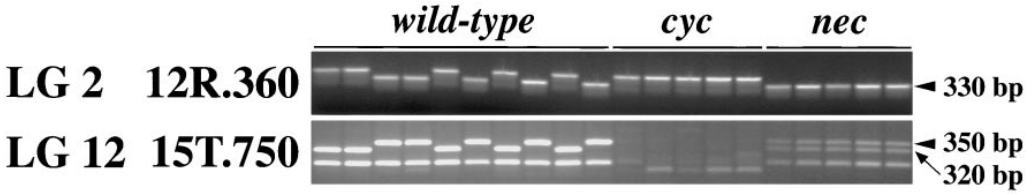

Figure 6.—LG 2 and LG 12 markers cosegregate in a *b213* mapping cross. Individual haploid progeny of a DAR x *b213* F1 female were analyzed with STS markers derived from the RAPDs 12R.360 (LG 2) and 15T.750 (LG 12). Both markers amplify allelic fragments of different sizes. The 12R.360 alleles are 360 bp and 330 bp (arrowhead). The 15T.750 fragments were cleaved with the restriction enzyme *Mnl* I to generate fragments of optimal size for detecting the polymorphism with agarose gel electrophoresis. The allele-specific fragments are 320 bp (arrow) and 350 bp (arrowhead); the common fragment is 270 bp. *nec*^{b213} haploids inherit both alleles of 15T.750, whereas *cyc*^{b213} embryos inherit neither allele; the faint bands in the *cyc*^{b213} samples are nonspecific amplification products.

distal LG 2 and a duplication of the reciprocal fragment.

DISCUSSION

Recombination suppression by *cyc*^{b16} and *cyc*^{b229}: The results show that the gamma-ray induced *cyc*^{b16} and *cyc*^{b229} mutations contain deletions in the distal region of LG 12. Interestingly, both mutations suppress recombination between genetic markers spanning more than 30 cM of LG 12, including a region far removed from the deletion. It is possible that both *cyc*^{b16} and *cyc*^{b229} are complex rearrangements, with other aberrations accounting for the recombination suppression. For example, each allele could bear an inversion for a segment of LG 12 in addition to a deletion in the vicinity of *cyc*.

An intriguing alternative is that the *cyc*^{b16} and *cyc*^{b229} deletions affect a chromosomal site that is required for recombination of markers at a distance. Precedent for this derives from work on meiotic pairing and crossing over in *Caenorhabditis elegans* (McKim *et al.* 1988; Villeneuve 1994; Wicky and Rose 1996). In this species, analysis of the effects of chromosomal rearrangements on recombination has led to the proposal that a region near one end of each chromosome functions as a *cis*-acting pairing site required for homolog recognition, recombination, and disjunction in meiosis. The parallel between the present study and the *C. elegans* pairing sites is striking, as in both cases removal of a region near one end of a chromosome reduces recombination between markers elsewhere on the chromosome. The *cyc*^{b16} and *cyc*^{b229} mutations suppress recombination to different extents (Table 1), suggesting that if these effects are caused by deletion of a site required for crossing over, then activity of the site is distributed over a region that is differentially affected by the two mutations. Whatever the mechanism of recombination suppression, the *cyc*^{b16} and *cyc*^{b229} mutations may be useful for balancing other lethal mutations located in the distal region of LG 12.

Translocations in zebrafish: We have shown that the *T(LG2;LG12)*^{b213} mutation is a reciprocal translocation between LG 12, where *cyc* resides, and LG 2. This con-

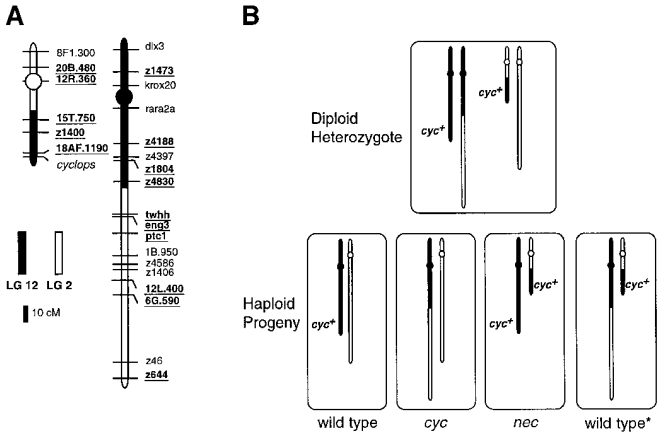

Figure 7.—Reciprocally rearranged chromosomes in *T(LG2;LG12)*^{b213}. (A) The positions of the breakpoints were determined by analyzing LG 2 and LG 12 markers (underlined) in pooled or individual DNA samples from the *b213* haploid mapping family shown in Figure 6. Marker distances from previous wild-type maps (Postlethwait *et al.* 1994; Johnson *et al.* 1996) were used to determine the lengths of segments involved in the rearrangements. Markers not underlined were not analyzed in the *b213* mapping panel; their positions are inferred from the previous maps cited above. Centromere positions (circles) have been reported (Johnson *et al.* 1996). (B) Schematic showing haploid progeny derived from a balanced translocation heterozygote. Half of the haploid embryos inherit the rearranged LG 12 (proximal LG 12-distal LG 2) that lacks *cyc*$^{+}$. Half of these individuals (wild type*) also inherit the rearranged LG 2 (proximal LG 2-distal LG 12), and so they have a *cyc*$^{+}$ gene and are euploid. These animals have a wild-type phenotype, indicating that neither breakpoint results in a phenotype morphologically detectable in haploid embryos. The other animals inheriting the rearranged LG 12 (*cyc*) also inherit a normal order LG 2, which does not carry the *cyc*$^{+}$ gene. The *nec*^{b213} phenotype occurs in embryos inheriting a rearranged LG 2 and a normal order LG 12.

clusion derives from the segregation of the *cyc*^{b213} and *nec*^{b213} mutant phenotypes and also from demonstration of rearranged *T(LG2;LG12)*^{b213}, *12*^{P}*2*^{D} and *T(LG2;LG12)*^{b213}, *2*^{P}*12*^{D} chromosomes by analysis of genetic markers. The *cyc*^{b213} and *nec*^{b213} mutant phenotypes result from aneuploidy for the distal regions of LG 12 and LG 2 that measure ~33 ± 6 cM and ~125 ± 20 cM, respectively. Thus *cyc*^{b213} mutants are deficient for approximately 1% of the 3000 cM genome (Postlethwait *et al.* 1994), a region that surely contains many genes. Despite this, the *cyc*^{b213} mutant phenotype during embryogenesis is similar to that caused by the ENU-induced allele *cyc*^{m294} (Schier *et al.* 1996), and homozygotes for both *cyc* alleles survive until just before the end of embryogenesis. This suggests that *cyc* function is essential earlier in development of the zygote than other genes in the region. In contrast, the phenotype of *nec*^{b213} mutants is quite severe, and these animals do not typically survive beyond 30 hr. The large size of the deficient segment in *nec*^{b213} mutants and the nature of the phenotype suggest that multiple genes with early, essential functions are deleted in these animals. Although these aneuploid phenotypes cannot be attributed to the functions of individual genes in the deficient segments, these rearrangements are useful in concert with single-gene mutations in the definition of the amorphic phenotypes of genes in the translocated segments.

Translocations have important applications in mapping cloned genes and mutations. For example, *patched1 (ptc1*; Figure 5) and other genes (Figure 7 and data not shown) were assigned to the region of LG 2 deleted from the *T(LG2;LG12)*^{b213}, *2*^{P}*12*^{D} chromosome by virtue of their failure to amplify from genomic DNA of the aneuploid animals lacking this segment. Thus a panel of translocations covering the genome would be useful for mapping cloned genes without the need to identify linked polymorphisms. The resolution of translocation panel mapping would be significantly less than traditional genetic mapping (Postlethwait *et al.* 1994) and radiation hybrid approaches (Cox *et al.* 1990), and perhaps comparable to mapping with somatic cell hybrids, which also assigns genes to chromosomes or large chromosomal segments (Ekker *et al.* 1996). However, a translocation panel has the important additional advantage that it can be applied to the mapping of mutations. By crossing heterozygotes to a panel of translocation stocks, a mutation can be mapped to a deleted chromosomal segment by failure of complementation.

A number of other gamma-ray induced rearrangements have been described previously (Fritz *et al.* 1996; Fisher *et al.* 1997), and these mutations together with the *T(LG2;LG12)*^{b213} translocation collectively cover more than 500 cM of the genetic map, about 17% of the total. For comparison, 10–15% of the mouse genome is covered by large deletions (Brown and Peters 1996). Thus significant progress toward assembling a translocation panel has already been achieved. Methods that couple the use of haploid embryos for simplifying segregation analysis and the use of PCR (Fritz *et al.* 1996) for targeting specific regions of the genome marked by cloned genes or SSLPs should enable the construction of an expanded panel of translocations.

We thank Scott Hawley, Ruth Lehmann, Alexander Schier, and the members of our laboratories for helpful discussions; Wolfgang Driever for support during the screen that identified the *cyc*^{m294} allele; and Tim Carl for technical assistance. This work was supported by grants R01 RR12349 (to W.S.T.), P01 HD22486 (to J.H.P. and C.B.K.), R01 RR10715 (to J.H.P.), and R01 HD29761 (to W. Driever) from the National Institutes of Health.

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Communicating editor: N. A. Jenkins