The transient receptor potential (trp) mutant in Drosophila is known to manifest retinal degeneration and involves defects in the intermediate steps of visual transduction. The chromosome walking technique has been conducted at the cytogenetic location of trp. Overlapping phage clones that cover a DNA stretch of 73 kb were obtained. Southern blotting studies detected a 2.3 kb DNA deletion within this region in the mutant. The deleted DNA fragment contains exons of three transcripts with lengths of 3.5, 1.5 and 0.8 kb. The transcription of the 3.5 and 1.5 kb RNAs is completely abolished in the mutant, whereas that of 0.8 kb RNA appears to be modified. The impairment in production of these RNAs, due to the DNA deletion, offers a possible molecular basis of the multiple defects displayed by the trp mutant. DNA structure changes associated with the trp mutation have previously not been determined. This article describes a 2.3 kb DNA deletion in the trp mutant within a 73 kb chromosomal walking region at the cytogenetic location of trp. The deleted DNA fragment appears to retain coding sequences for three distinct mRNAs. Our findings suggest that the multiple effects of the trp mutant may reflect the impairment of multiple transcripts from the trp locus.

**Materials and Methods.** A Drosophila genomic DNA library was probed with DNA fragments labeled with $^{32}$P-dCTP (3,000 Ci/m mole, Amersham, Arlington Heights, IL). The procedure known as chromosome walking was used to isolate genomic DNA adjacent to specific DNA fragments or probes which were previously isolated. Plaques (approximately 30,000 per 150 mm Petri plate) were transferred to nitrocellulose. The filters were baked for 2 hr at 80°C under vacuum. They were then prehybridized for 1-2 hr at 65°C with 0.6 M NaCl, 8 mM EDTA, 0.12 M Tris-Cl (pH 8), and 2X Denhardt’s solution, and finally hybridized with the $^{32}$P-labeled probes in prehybridization buffer plus 0.1 mg/ml sheared salmon sperm DNA at 65°C for 16-24 hr. The filters were washed once for 10 min duration at 20°C with 0.15 M NaCl, 0.17 M Na citrate (pH 7), and 0.1% SDS, then three times for 30 min durations at 65°C in 0.45 M NaCl, 0.09 M Tris-Cl, 6 mM EDTA (pH 8) and 0.2% SDS and finally for 30 min at 65°C with 0.15 M NaCl, 0.03 M Tris-Cl, 2 mM EDTA (pH 8) and 0.2% SDS. Following air drying the filters were exposed to Kodak XAR-5 film with an intensifying screen for 3-24 hr at -70°C.

Genomic DNA for blotting was prepared from adult flies according to the procedure described in Kuner et al. Restriction endonuclease digests of genomic DNA and cloned DNA were electrophoresed, along with size markers (restriction digests of lambda DNA), for 16 hr at 1.1 V/cm in 0.7% agarose gels in TAE buffer. Each lane contained 1-10 $\mu$g of DNA. The DNA was transferred to nitrocellulose. The labeled DNA probes were hybridized to the DNA blots as described above for plaque filter hybridization.

DNA fragments for subcloning were separated by electrophoresis in agarose gels, followed by electrophoretion in TAE buffer. The eluate was passed through an Elutip-d column (Schleicher and Schuell, Keene, NH) and the DNA was precipitated with ethanol. The DNA fragments were then cloned into the appropriate restriction sites in pBR322.

Drosophila RNA was prepared using the procedure described in Wong et al. Poly(A)$^+$RNA was purified with an oligo(dT) column. RNA was electrophoresed for 16 hr at 1.1 V/cm in 1.4% agarose gels containing 2.2 M formaldehyde. Mouse liver and E. coli...
ribosomal RNA were used as size markers. The separated Drosophila RNA was transferred to nitrocellulose, blotted, and hybridized with labeled probes. In order to calibrate the amount of RNA in each lane, signals resulting from hybridization using a DNA probe which contains an actin sequence were compared to those obtained with other DNA probes.

**Results.** A genomic clone 559 was initially mapped to 99C 5-6 on the right arm of the third chromosome of Drosophila. Our unpublished in situ hybridization results show that it resides in the cytogenetic location of trp, which is defined by a pair of duplication-deletion strains. Chromosomal walk steps from both sides of clone 559 have been characterized (see Fig. 1). By using clone 559 as probe, eight clones that partially overlap with clone 559, including clones 7A1, 1F2 and 1A1, were initially obtained from the genomic library. Restriction fragments from clones 7A1 and 1F2, both of which were most distant from clone 559, were used as probes in subsequent repetitions of the procedure to obtain, respectively, clones including 4A and 14. Appropriate fragments obtained from these “steps,” in turn, were used as probes and they yielded respectively clones 52 and T2. The extent of overlap of neighboring clones was determined by cross-hybridizations.

For comparison, total genomic DNA extracted from homozygous mutant and from normal flies was digested with a set of restriction enzymes, electrophoresed, blotted, and hybridized, using as probes individual clones obtained from chromosome walking. In most cases identical hybridization patterns were observed between the genomic DNA of mutant and the normal flies. However, when clone 7A1 was used to hybridize EcoRI digested genomic DNA blots, different hybridization patterns were detected. The genomic DNA blot from normal flies yields six DNA bands with sizes of 1.8, 2.7, 3.5, 5.0, 5.1 and 11.6 kb (Fig. 2a, lane 1), whereas the blot from the mutant gives five identical bands as those of the normal fly and an aberrant sixth band of 9.3 kb (Fig. 2a, lane 2). This DNA structure change identified by probe 7A1 suggests a deletion in the trp mutant, as no additional bands were detected. This DNA structure change was also observed when four other enzymes were used to digest the genomic DNA. One of them in particular, BamHI, revealed a deletion of a single 2.3 kb band in the trp genomic DNA (Fig. 2b).

The 2.3 kb deleted DNA was isolated from clone 4A of the normal flies and subcloned asymmetrically as two BamHI-SalI fragments (Fig. 1). The resulting subclones, designated BS32 (1.0 kb insert) and BS60 (1.3 kb insert), were used as probes to hybridize to BamHI digested genomic DNA from the mutant and from the normal flies. The resulting patterns confirmed that the 2.3 kb band was missing from the trp mutant (Fig. 3). The probes also identified other bands which were present in normal as well as trp flies. These observations suggest that there is homology between the 2.3 kb DNA sequence and other DNA fragments in and outside the trp locus.

The two probes BS32 and BS60 were subsequently

![Fig. 1. A schematic diagram of the relationship of a set of eight overlapping genomic clones generated by EcoRI restriction (vertical lines) that spans 73 kb of DNA in the trp region. B, BamHI site; S, SalI site. The 2.3 kb deletion identified by probe 4A was subcloned as two BamHI-SalI fragments: BS60 (1.3 kb) and BS32 (1.0 kb).](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933133/ on 09/20/2018)

![Fig. 2. DNA structure change identified by different probes on genomic DNA blots. (a) One of the six EcoRI restriction fragments identified by probe 7A1 shows DNA structure change, 11.6 kb in normal flies (Oregon-R strain, lane 1) versus 9.3 kb in the mutant (lane 2). (b) Probe 4A identified seven BamHI restriction fragments of 1.6, 2.1, 2.3, 3.0, 4.1, 9.0 and 10 kb in normal flies (lane 1). One of the seven DNA bands (2.3 kb) is missing in the mutant (lane 2).](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933133/ on 09/20/2018)
right arm of the third chromosome. Clone 559 which overlaps with the cytogenetic location of *trp* was used as probe for chromosome walking, in such a way that partially overlapping DNA clones that cover a DNA stretch of 73 kb were obtained (Fig. 1). The Southern blotting study of the 73 kb chromosomal walking region reveals a DNA deletion in the mutant (Fig. 2). Furthermore, subclones BS32 and BS60, derived from the deleted DNA fragment, hybridize to a DNA band in normal flies that is absent in the mutant DNA, suggesting that the 2.3 kb fragment is a simple deletion and that other complications on the DNA

![Fig. 3](image3.png)

**Fig. 3.** The identified 2.3 kb deleted DNA band was subcloned from clone 4A as two fragments. These fragments were individually used as probes to hybridize *BamHI* digested genomic DNA blots: BS60 (lanes 1 and 2) and BS32 (lanes 3 and 4). BS60 identified the 2.3 kb band in the normal fly (lane 1), but not in the mutant (lane 2). The same patterns were observed by using BS32 as probe to hybridize the normal fly (lane 3) and mutant DNA (lane 4). The probes identified other bands suggesting homology between the 2.3 kb DNA and the additional DNA such as the 2.1 kb band.

![Fig. 4](image4.png)

**Fig. 4.** RNA bands from the normal fly identified by probes BS60 (lane 1) and BS32 (lane 2). The sizes of the mRNAs are indicated in kbs.

used to hybridize to RNA extracted from normal flies (Fig. 4). Probe BS60 identified one RNA band of 0.8 kb (lane 1) and probe BS32 identified three RNA bands of 3.5, 1.5 and 0.8 kb (lane 2).

**Discussion.** The *trp* mutation in *Drosophila melanogaster* is recessive. Although the final products for the *trp* gene(s) are not known, the cytogenetic location of *trp* has been mapped to 99C 5–6 on the

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structure are not involved (Fig. 3). This observed DNA structure change probably defines the primary defect associated with the mutation. The deleted fragment identifies three RNAs on northern blots of RNA of normal flies (Fig. 4). Some coding sequences in these transcripts are probably missing in the mutant (Fig. 4). It is concluded that the protein products they encode are also affected. Specifically, two proteins are probably missing because two mRNAs (3.5 and 1.5 kb respectively) are missing and the protein encoded by the 0.8 kb transcript may be altered because some of its exons have been deleted in the mutant. The abnormality of more than one transcript, due to the deletion, may result in the numerous defects associated with the trp mutant.

The 3.5, 1.5 and 0.8 kb RNAs are independent stable transcripts, since we have obtained cDNA clones complementary to each of these RNAs. We have also hybridization-selected these mRNAs from normal fly total RNA and translated them in vitro into three distinct proteins of 107, 66 and 33 kD (data not shown). Therefore, these transcripts are not precursors or degraded products of each other. The deletion in the mutant did not significantly alter the size or the expression of the 0.8 kb transcript as the corresponding band appears in similar amounts and at the same position as in that of the normal fly. A full length cDNA clone complementary to the 0.8 kb transcript was used to hybridize EcoRI and BamHI digested blots of genomic clones obtained from chromosome walking (unpublished results). Exons of 0.8 kb RNA were determined to be localized within a genomic DNA stretch of 9 kb. The cDNA clone hybridizes to the deleted fragment along with several other DNA bands that flank the 2.3 kb band. Since the cDNA clone identifies the deleted fragment and the 2.1 kb adjacent DNA band, it suggests that both DNA fragments contain exon(s) for the 0.8 kb transcript. Furthermore, subclone BS60 identifies the 0.8 kb transcript as the sole RNA band in the northern blot (Fig. 4), strongly indicating that the deleted DNA fragment contains exon(s) for the 0.8 kb transcript.

Earlier attempts were made to study the mutational defects of trp by looking for alterations in molecular weight and isoelectric point of proteins in the mutant. The major difficulty associated with this approach is that a simple mutation may cause changes in many proteins, often making it impossible to distinguish the primary effect of the mutation from the secondary ones. Identification of any alterations in DNA of the mutant is simpler to interpret. As alterations in DNA can be identified, the mutant phenotype can be specifically ascribed to modifications in the ultimate gene product, the protein. Although there could still be multiple changes resulting from the mutation, a direct causal relationship for the primary effect can be established. For example, the sequence of these mRNA transcripts can be determined so that the corresponding amino acid sequences can be identified. The amino acid sequences derived can be compared with all known protein sequences. If it is revealed that the proteins have homologous peptides with some familiar proteins, they may also have analogous functions. The sequence information would also allow the production of antibodies targeted by design at unique sites of the proteins for studying the localization, function and developmental expression of the proteins.

Key words: visual transduction, retinal abnormality, DNA deletion, mutational defect, multiple transcripts

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