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A TRUNCATED P ELEMENT IS INSERTED IN THE TRANSCRIBED REGION OF THE Cu, Zn SOD GENE OF AN SOD "NULL" STRAIN OF *DROSOPHILA MELANOGASTER*

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The decreased Cu,Zn SOD activity (less than 5%) in a "null" SOD^{CA1} *Drosophila melanogaster* strain isolated in our laboratory is due to the insertion of a truncated P element into the transcribed region of the Cu,Zn SOD gene.

Using a cDNA Cu,Zn SOD probe from a wild type *D. melanogaster* (*F* allele) we isolated an EcoRI Cu,Zn SOD clone from an EMBL3 genomic library of the SOD^{CA1} strain, subcloned it, restriction-mapped and partially sequenced it. The 2.5 kb clone consists of a wild-type 1.84 kb EcoRI fragment containing the Cu,Zn SOD gene previously isolated in our laboratory, with an insertion of 0.68 kb derived (by an internal deletion) from an autonomous, 2.9 kb P element. The insertion starts 21 bp upstream from the coding sequence and causes an 8 bp target site duplication characteristic of P elements.

A point mutation in the second exon results in a replacement of Asn by Lys at position 96, confirming that the mature protein encoded by the SOC^{CA1} is the same one encoded by the *S* allele, commonly found in natural populations. The diminished expression of SOD^{CA1} allele is most possibly due to a reduction of the rate of transcription attributable to the insertion of the P element.

KEY WORDS: Null mutation, DNA transposition, transcription, fitness, population genetics.

INTRODUCTION

P elements are a class of transposable DNA elements that invaded *D. melanogaster* populations some 3 decades ago causing a phenomenon collectively known as hybrid dysgenesis, which is related to many profound changes in gene expression.[1]

The class consists of a 2.9 kb autonomous element and smaller ones, heterogenous in size, derived from the 2.9 kb fragment by internal deletion.[2] The 2.9 kb element encodes a transposase responsible for the mobility of the family,[3] which also requires the 31 bp inverted repeats present at both ends of P elements. P elements are known to insert preferentially near the transcription start of several genes[4,5] and diminish the level of transcription of a particular gene.[5,6]

Cu,Zn SOD is present in two allelic forms (*S* and *F* allele) in natural *Drosophila melanogaster* populations. Both allelic enzyme products have been purified, characterized[7] and one of them (*F*) sequenced.[8] It was also shown that a single replacement of Asn by Lys at position 96 of the mature protein gives rise to the *S* allele.[9] Both cDNA[10] and genomic DNA[11,12] sequences for the *F* allele have been obtained; as well

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as a partial 97 bp sequence of the *S* allele, which would account for the difference in amino acid sequence between the *S* and *F* allozymes.[13] Recently a *Drosophila melanogaster* strain with Cu,Zn SOD activity decreased to less than 5% (SOD^{CA1}) was isolated in our laboratory.[14] The clone may prove to be helpful in elucidating the effects of low SOD activity on several physiological features such as longevity, sensitivity to certain oxygen radical generators, radiation, and so on. We have cloned and partially sequenced the Cu,Zn SOD gene region of the SOD^{CA1} strain in order to investigate the molecular mechanism of Cu,Zn SOD inactivation.

MATERIALS AND METHODS

Construction of the Library

Genomic DNA of high molecular weight from flies of *D. melanogaster* SOD^{CA1} strain was extracted according to,[15] with modifications. 0.3 g of adult flies was ground with liquid nitrogen and then suspended in 3 ml of lysis buffer. Lysis buffer contained 50 mM Tris-HCl, pH 8, 50 mM EDTA, 50 mM NaCl, 1% SDS, 5% sucrose and 150 μg/ml proteinase K. Lysis was performed for 1.5 h at 68°C and for 1 h at room temperature. The resulting suspension was extracted with phenol, phenol/chloroform and chloroform and precipitated with ethanol. To obtain ligatable fragments of about 10–20 kb in length, DNA (60 μg in 0.6 ml) was partially digested with Sau3A restriction enzyme. The restricted genomic DNA was cloned into the phosphorylated BamHI site of lambda EMBL3 phage (Promega).

Library Screening

The library was screened using the *in situ* plaque hybridization technique.[16,17] 2.5×10^5 phages were arrayed on 10 plates and transferred to duplicate nitrocellulose filters, lysed and baked for two hours in vacuum. The filters were prehybridized for 8 hours at 42°C in solution containing: 50% formamide, 5 × Denhardt's solution, 5 × SSC, 100 μg/ml denaturated herring sperm DNA, 10 mM sodium phosphate pH 7.5 and 0.1% SDS. The same solution was used for hybridization with the addition of 10^7 cpm/ml of ^{32}P-labeled cDNA of Cu,Zn SOD from *D. melanogaster* (strain Oregon R).[10] The cDNA was obtained from Dr. G.M. Tener as an insert in plasmid pUC 13. The DNA of the plasmid was prepared by the liquid lysis method and purified by cesium chloride gradient centrifugation.[16,17] The insert was obtained by digestion with Eco RI restriction enzyme and subsequent excision of the DNA band from low-melting agarose gel (Seaplaque) and was used for random priming labeling with ^{32}PdCTP.[18] Hybridization was performed at 42°C for 24 h. Filters were washed 5 times for 30 min at 60°C; the first at 2 × SSC and the final one in 0.2 × SSC, 0.1% SDS[16,17] and subjected to autoradiography for 16 hr at −70°C with two intensifying screens. The clones were purified in two subsequent steps of rescreening.

Identification of SOD Sequences

DNA from the purified clones was obtained by the plate lysate method,[16] digested with EcoRI enzyme, electrophoresed and subjected to Southern blot transfer[19] to identify the DNA fragment carrying the SOD gene. The fragments hybridizing to the SOD probe were subcloned into plasmid pGEM4Z for further analysis. Plasmid DNA was then purified by the small scale alkaline lysis method[16] and subjected to restriction enzyme analysis

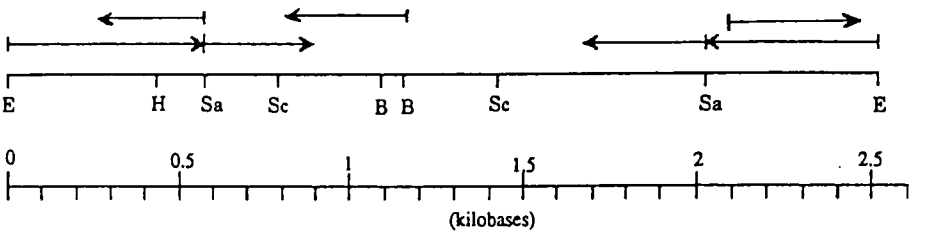

FIGURE 1 Partial restriction map and sequencing strategy of the EcoRI SOD^{CA1} clone. Arrows indicate the direction and the length of sequencing; E, EcoRI; H, HindIII; Sa, SalI; Sc, ScaI; B, BstBI.

Sequence Analysis

The plasmid DNA fragments for sequencing were released by appropriate restriction enzymes and subcloned into pUC19. Additional clones were obtained by unidirectional nested deletions with exonuclease III[20] using Promega's Erase-a-Base System according to the manufacturer. Double stranded plasmid templates from DNA mini-preparations[16,17] were used after denaturation directly for sequencing by the dideoxynucleotide chain-termination method[21] according to a manufacturer's protocol (United States Biochemical Corporation) using modified bacteriophage T7 DNA polymerase (Sequenase).[22]

RESULTS

We isolated 4 positive EMBL3 clones, which after digestion of their DNA with EcoRI proved to contain a 2.5 kb EcoRI fragment hybridizing to a *D. melanogaster* SOD cDNA probe. This fragment was subcloned into pGEM4Z plasmid and restriction mapped (Figure 1). The clone exhibits a 0.7 kb-long foreign DNA stretch starting at position around 400, when compared to a wild type SOD region of *D. melanogaster*. The Southern blot of an EcoRI digest of genomic DNA of the "null" strain gives upon hybridization with labeled wild type Cu,Zn SOD cDNA one band corresponding to the clone (not shown).

Comparison of partial sequences of the SOD null clone reveals no differences between the SOD locus of SOD^{CA1} and wild type SOD from Canton S strain[12] outside the insertion, except one. This point mutation (not shown), a replacement of cytosine in the wild type gene by adenine in the "null" is in the translated region of the second exon and gives rise to a replacement of Asn by Lys at position 96 of the mature protein. Thus the protein encoded by the SOD^{CA1} is the same one encoded by the *S* allele, commonly found in natural populations.

The 0.68 kb insert in the SOD locus of the SOD^{CA1} strain proved to be the truncated P element derived from an autonomous 2.9 kb P element by internal deletion, as identified by computer-assisted sequence analysis (Figure 2). It contains 31 bp inverted repeats at both ends and creates an 8 bp duplication of a target sequence. The deletion apparently starts and ends at positions 163 and 2389, respectively, of a complete, 2.9 kb P element, bringing together the first and the fourth exons of transposase (Figure 3). However, a deletion event within the fourth (TGC) codon of the transposase changes it into a stop codon (TGA), leaving the last open reading frame

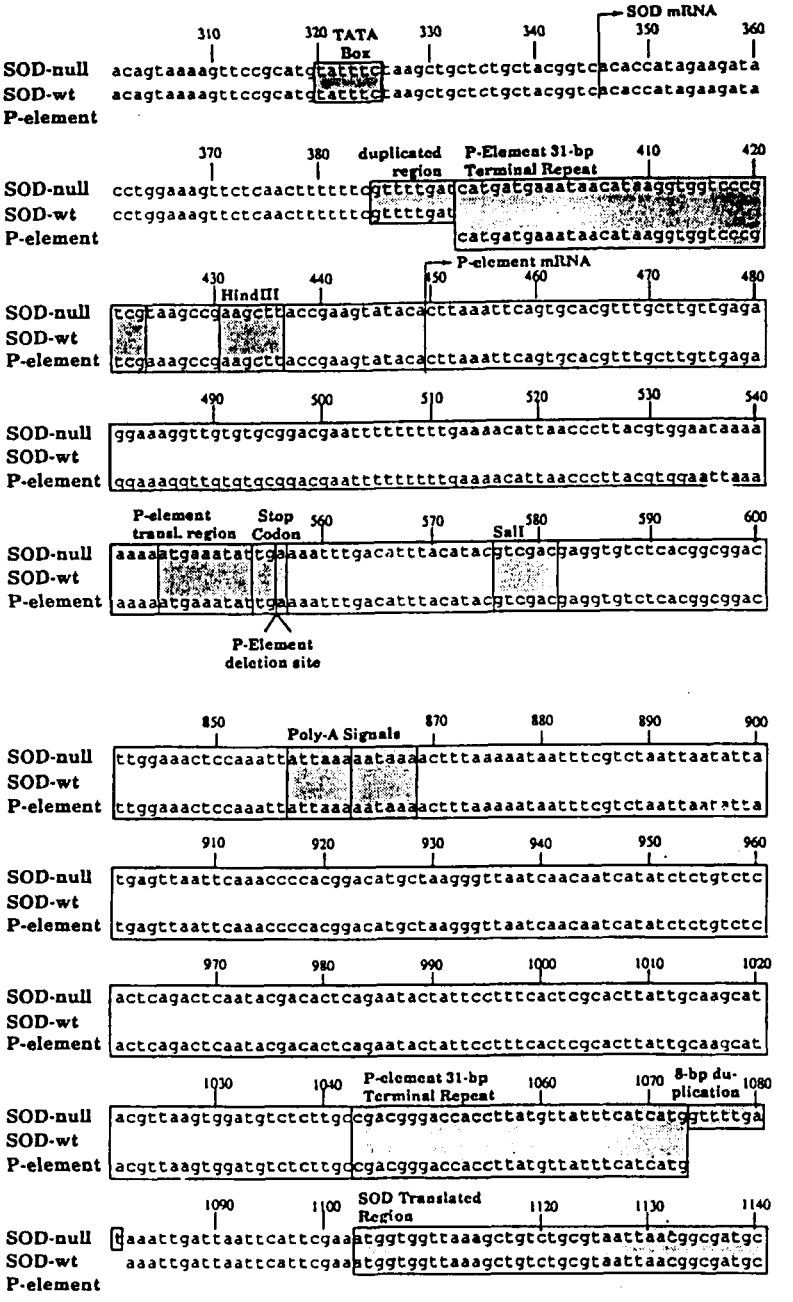

FIGURE 2 The sequence of the SOD^{CA1} locus adjacent to the insertion of the P element. The picture shows the alignment of SOD^{CA1}, and respective regions of the P element[2] and Cu,Zn SOD[12] sequences of *D. melanogaster*. The 8 bp target site duplications as well as the site of the internal deletion within the P element are indicated.

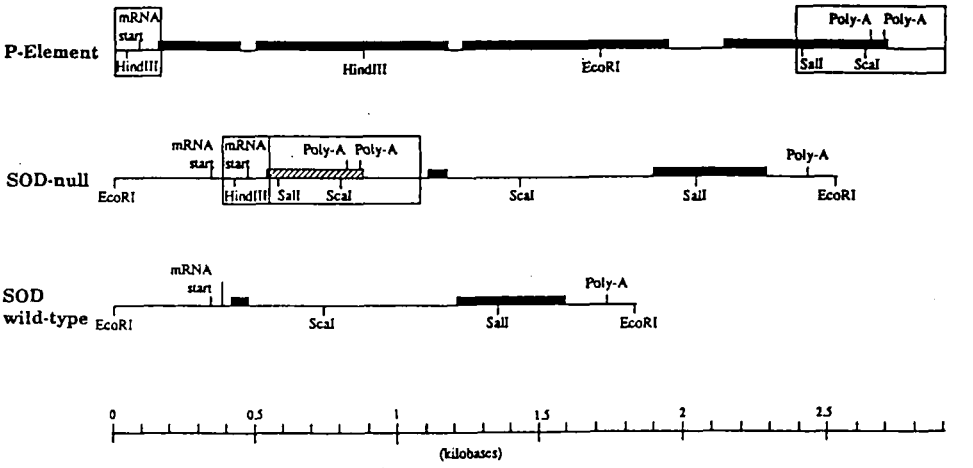

FIGURE 3 The organization of the Cu,Zn SOD locus in the SOD^{CA1} strain of *D. melanogaster*. Dark boxes represent coding regions. The cross-hatched bar is the portion of the 4-th P element ORF. Transcription start points and polyadenylation signals are indicated.

without ATG as a starting codon and thus possibly preventing the translation of a truncated transposase.

DISCUSSION

The effects of P element insertion have been extensively analyzed for a number of *D. melanogaster* genes. The insertion placed usually in the 5′ untranslated region of a gene abolishes or grossly reduces the gene transcription, most probably by providing polyadenylation signals at the end of the P element.

The sequence analysis of the Cu,Zn SOD gene of *D. melanogaster* SOD^{CA1} strain indicates that the truncated P element is inserted in the 5′ untranslated portion of the gene, 21 bp upstream from the translation start. The level of expression of Cu,Zn SOD in this strain is only about 3.5% of normal, with respect to activity as well as protein content.[14] Northern blot analysis of total RNA from the SOD^{CA1} strain reveals two weak bands hybridizing to a Cu,Zn SOD cDNA probe.[23] A larger, stronger band is 1.5–1.6 kb long and a weaker one is 0.7–0.8 kb long. In the light of the results provided here, the larger band comes most probably from a transcription of the Cu,Zn SOD gene, initiated by either the Cu,Zn SOD gene promoter, P element promoter, or both; which transcription of the SOD gene occurs as a result of "leaking" polyadenylation signals in the P element insert. Such readthroughs have been reported for P element transcripts.[24] The primary transcript would then be spliced and translated properly, giving rise to an about 1.4 kb mRNA, but its level would be reduced severely because most of the transcripts would be terminated within the insert. The presence of these smaller transcripts could also account for the presence of a smaller hybridizing RNA band, since they would be able to hybridize owing to the presence of 46 bp of the SOD transcript (Figures 2, 3).

The sequence data provided herein account well for the diminished level of Cu,Zn SOD expression in the SOD^{CA1} strain of *D. melanogaster*. The postulated mechanism

seems to involve a decrease in transcription by providing additional polyadenylation signals upstream from the translated region of the SOD gene due to the insertion of the truncated P element. This mechanism will be further investigated in our laboratory by hybridization analysis of mRNA from the SOD "null" strain.

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References

1. W.R. Engels (1983) the P family of Transposable elements in *Drosophila. Annual Reviews in Genetics*, **17**, 315–344.
2. K. O'Hare and G.M. Rubin (1983) Structures of P transposable elements and their sites of insertion end excision in the *Drosophila melanogaster* genome. *Cell*, **34**, 25–36.
3. D.C. Rio, F.A. Laski and G.M. Rubin (1986) Identification and immunochemical analysis of biologically active *Drosophila* P element transposase. *Cell*, **44**, 21–32.
4. M.R. Kelley, S. Kidd, R.V. Berg and M.W. Young (1987) Restriction of P-element insertions at the notch locus of *Drosophila melanogster. Molecular and Cellular Biology*, **7**, 1545–1548.
5. G. Howes, M. O'Connor and W. Chia (1988) On the specificity and effects on transcription of P-element insertion at the *yellow* locus of *drosophila melanogaster. Nucleic Acids research*, **16**, 3039–3052.
6. R. Levis, K. O'Hare and G.M. Rubin (1984) Effects of transposable element insertion of RNA encoded by the white gene of *Drosophila. Cell*, **38**, 471–481.
7. Y.M. Lee, H.P. Misra and F.J. Ayala (1981) Superoxide dismutase in *Drosophila melanogaster*: Biochemical and structural characterization of allozyme variants. *Proceedings of the National Academy of Sciences of the United States of America*, **78**, 7052–7055.
8. Y.M. Lee, D.J. Friedman and F.J. Ayala (1985) Complete amino acid sequence of copper-zinc superoxide dismutase from *Drosophila melanogaster. Archives of Biochemistry and Biophysics*, **241**, 577–589.
9. Y.M. Lee and F.J. Ayala (1985) Superoxide dismutase in *Drosophila melanogaster*. Mutation site difference between two electromorphs. *FEBS Letters*, **179**, 115–119.
10. N.O.L. Seto, S. Hayashi and g.M. Tener (1987) *Drosophila* Cu-Zn superoxide dismutase cDNA sequence. *Nucleic Acids Research*, **15**, 5483.
11. N.O.L. Seto, S. Hayashi and G.M. Tener (1987) The sequence of the Cu-Zn superoxide dismutase gene of *Drosophila. Nucleic Acids Research*, **15**, 10601.
12. J. Kwiatowski, M. Patel, F.J. Ayala (1989) *Drosophila melanogaster* Cu,Zn superoxide dismutase gene sequence. *Nucleic Acids Research*, **17**, 1264.
13. K.C. Kirkland and J.P. Phillips (1987) Isolation and chromosomal localization of genomic DNA sequences coding for cytoplasmic superoxide dismutase from *Drosophila melanogaster. Gene*, **61**, 415–419.
14. J-D. Graf and F.J. Ayala (1986) Genetic variation for superoxide dismutase level in *Drosophila melanogaster. Biochemical Genetics*, **24**, 153–168.
15. T. Jowett (1986) Preparation of nucleic acids. In *Drosophila a practical approach* (ed. D.B. Roberts), IRL Press, Washington, pp. 275–286.
16. T. Maniatis, E.F. Fritsch and J. Sambrook (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
17. F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.A. Smith, J.G. Seidman and K. Struhl (1987) *Current Protocols in Molecular Biology*, John Wiley and Sons, New York.
18. A.P. Feinberg and B. Vogelstein (1984) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Analytical Biochemistry*, **137**, 266–276.
19. E. Southern (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology*, **98**, 503–517.
20. S. Henikoff (1984) Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. *Gene*, **28**, 351–359.
21. F. Sanger, S. Miklen and Coulson, A.R. (1977) DNA sequencing with chain-terminating inhibitors.

Proceedings of the National Academy of Sciences of the United States of America, **74**, 5463–5467.
22. S. Tabor and C.C. Richardson (1987) DNA sequence analysis with a modified bacteriophage T7 DNA polymerase. *Proceedings of the National Academy of Sciences of the United States of America*, **84**, 4767–4771.
23. N.O.L. Seto, S. Hayashi and G.M. Tener (1989) Cloning, sequence analysis and chromosomal localization of the Cu-Zn superoxide dismutase gene of *Drosophila melanogaster*. *Gene*, **75**, 85–92.
24. F.A. Laski, D.C. Rio and G.M. Rubin (1986) Tissue specificity of *Drosophila* P element transposition is regulated at the level of mRNA splicing. *Cell*, **44**, 7–19.

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