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IDENTIFICATION OF ANTIOXIDANT GENE IN *Caenorhabditis elegans* THROUGH COMPUTATIONAL APPROACHES

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ABSTRACT

Antioxidants are the substances when present in the low concentration compared to that of oxidizable substrate significantly delay or prevent oxidation of that substrate. In human total >100 genes known that have antioxidant properties but despite of being 60% similarities of genomes of *Caenorhabditis elegans* with Homo sapiens only five gene named Prdx-3, Y38, CIAA, clk-1, gro-1 are known which shows antioxidant properties in *Caenorhabditis elegans*. Such similarity index shows that some other unknown genes of *Caenorhabditis elegans* must also have antioxidant properties that are still not known. For identification of other unknown genes in *Caenorhabditis elegans* that attains antioxidant properties, BLAST of known antioxidant genes in *Homo sapiens* against genome of *Caenorhabditis elegans* at Worm database was performed, significant results are found, identification of a locus in *Caenorhabditis elegans* named KO8D12 that showed good homology with JUND gene present in Homo sapiens whose gene ID 3727 that is present on chromosome 19p13.2. JunD activates transcription of the human ferritin H gene through an antioxidant response element during oxidative stress. Identification of such new antioxidant gene in *Caenorhabditis elegans* will boost the research areas as this worm in current time is used as a unique model organism for research.

KEY WORDS: Antioxidant, *Caenorhabditis elegans*, BLAST, EST, contig

Interest in *Caenorhabditis elegans* as a target for drug discovery has grown considerably, particularly in the pharmaceutical and agro-chemical industries. Several neurodegenerative disorders have been modeled in *C. elegans* including Huntington's disease, Alzheimer's disease, Parkinson's disease, and cancer. A significant study has been performed in a view to prove that a great similarity index is present in between Homo sapiens and *C. elegans.* Considering, this at the genomic level there must be similarity at gene level in *C. elegans with Homo sapiens* (Pennisi, E. 1998). JUND (official name) originally named jund D proto-oncogene present in *Homo sapiens,* geneID 3727, on chromosome number 19, its location is 19p13.2. The protein encoded by this intron less gene is a member of the JUN family, and a functional component of the AP1 transcription factor complex. It has been proposed that it protects cells from p53-dependent senescence and apoptosis. JunD is another ARE regulatory protein for transcriptional activation of the human ferritin H gene and probably other antioxidant genes containing the conserved ARE sequences by which JunD may confer cytoprotection during oxidative stress (Yoshiaki Tsuji., 2005). Keeping this in view it is an attempt to identify similarity between the JUND, which is an antioxidant gene of *Homo sapiens* with *C. elegans.* Proceeding towards approach BLAST analysis of known antioxidant gene i.e. JUND of *Homo sapiens* against the genome of *C. elegans at worm database was performed.* Result was very fascinating, it was identified that, gene. A good similarity indexes was found in-between JUND gene of *Homo sapiens* with locus *Caenorhabditis elegans* cosmid K08D12.

MATERIALS AND METHODS

Selection of a sequence and Genome analysis

Expressed Sequence Tag (EST) databases are valuable resources for discovering novel genes through *in silico* cloning (Rinner et al., 2002). In this current study repeated EST searching, multiple sequence comparisons, and other data-mining techniques were employed to obtain the *antioxidant* JUND gene by using NCBI database. *C.elegans* database i.e. WORMBASE (http://www.wormbase.org) and hierarchical database i.e ace DB (http://www.acedb.org) system are used for displaying genomic data of *C. elegans* in this study (Schultz et al., 2000).

In order to analyze the sequence of the C.elegans BLAST analysis of JUND gene (Gene Id: 3727 & Acc No: NM_005354.2**)** of Homo sapiens was performed with the whole genome of *C.elegans* in Wormbase ans acDB

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database. Sequence of interest was obtained from worm base database through data mining techniques. In continuation Intron and Exon has been identified by using gene finder tool (http://wwwbioscience.org/urllists/genefind.htm) and genscan (http://genes.mit.edu/GENSCAN.html) for the identification of coding sequences CDS. CpG Island is important in gene identifications and predicted by WebGene. (Http://125.itba.mi.cnr.it/cgi-bin/wwwcpg.pl) (Milanesi and Rogozin, 1999)

RESULTS AND DISCUSSION

Nucleotide sequence analysis

Sequence of interest is retrieved from wormbase database through data mining techniques specifically by analysis frequent pattern, sequential patterns and structured patterns. Association and correlation methods are also adopted during data mining. Further Selection of a known antioxidant gene i.e. JUND of *Homo sapiens* is selected due to its significant role as potent antioxidant expression as well as showing importance in different metabolic regulations. Due to the experimental attributes of *C. elegans* that make it successful in research laboratories also make it a favorable model organism in current time (Gelain, et al., 2009). The selected gene i.e. JUND shows 55% identities with KO8D12 of *Caenorhabditis elegans* after BLAST analysis and the score obtained for first HSP are in given table 1.

Table1: Score obtained for first HSP

Plus Strand HSP	Score: 429 (70.1 bits)	Expected Threshold: 2.9e-10
Identities = 55%	Positives = 50%	Strand = Plus /Plus

There are four plus strands HSP, first HSP have 55% identities where as second also has identities of 50%. The first HSP start from 26768 and end at 27757, the second HSP starts from 27092 and end at 27878bps.The map generated through BLAST at there expected threshold value 0.01(default) from *C.elegans* database are shown fig1.

Through frequent pattern, sequential patterns data mining techniques locus AC006672 named K08D12 was identified showing 55% identities against JUND and indicates that this locus shows antioxidant properties similar to that of JUND gene of *Homo sapiens* in Fig1. So Cosmid K08D12 sequence (Acc no: AC006672.2, Locus: AC006672) selected as a target sequence and identified there CDS with help of online server GENSCAN. Further CpG Island was predicted by Web Gene. The predicted CDS shows two island first start from 3107 and end at 3328 bps. Where as the second island start from 3396 and end at 3646 base pairs. The G+C % of first Island is 50% and the observed value is 9 where as the second island shows the percentile less than it i.e. approximately 49% and its observed value is 6.

CpG_island 26586.26812
/note="CpG island: %C+G=" 65; % CpG= 6, Obs./Exp.= 0.62

CpG_island 26880.27838
/note="CpG island: %C+G=" 65; %CpG= 6, Obs./Exp.= 0.63

The predicted site of the coding region for the gene, predicted by gene scan is showing in Fig2. Although JunD gene of different organisms has been identified, but there is no report on JUND gene structure and its function, as key electron donor in the defense against oxidizing agents and in reductive biosynthetic reactions in *C. elegans* till now (Rees et al, 1997). As steps are going ahead in area of functional genomics scientist are trying to get new and more appropriate models for genomic studies. *C. elegans* which is a good model organism is used for structural and functional studies.

Identification of new gene for antioxidant in *Caenorhabditis elegans* will boost the research work because this worm, approximately 1 mm long, has a short generation time and is transparent, which made it a unique model organism for research (Gelain et al. 2009). During the course of study identification of novel *C.elegans* JunD gene through bioinformatics approaches was carried out. It was found that this gene located within contig K08D12 of *C.elegans* genome. Similar kind of findings for gene G6PD has already been done in *C.elegans* by adopting the same protocol. (Jitendra et al., 2007). This comparative structural genomic study may lead to a better approach for *Caenorhabditis elegans* as a model organism for oxidative stress mediated studies.

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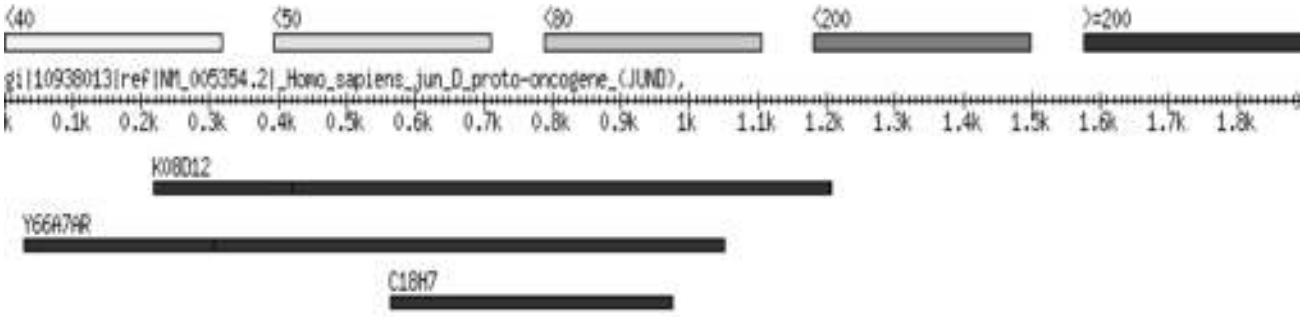

Fig1: The Blast result of JUND gene of *Homo sapiens*

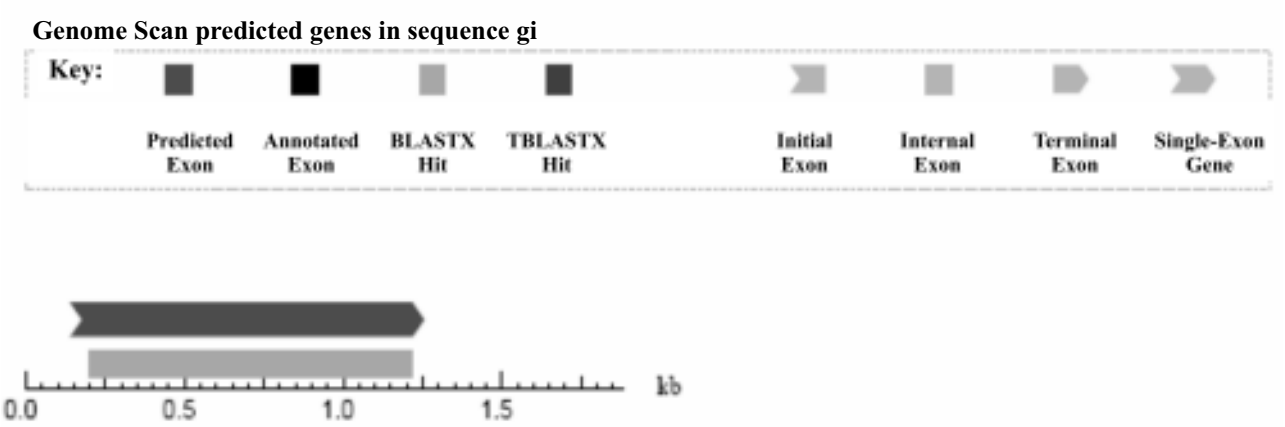

Fig2: Predicted coding region by Gene Scan server

REFERENCES

Clement P. M., Henderson C. A., Jenkins Z. A., Smit McBride Z., Wolff E. C., Hershey J. W., Park M. H. and Johansson H. E.; 2003. Identification and characterization of eukaryotic initiation factor 5A-2. Eur. J. Biochem., **270**: 4254-4263.

Florea L., Hartzell G., Zhang Z., Rubin G. M. and Miller W.; 1998. A computer program for aligning a cDNA Sequence with a genomic DNA sequence. Genome Res., **8**: 967-974.

Gardiner Garden M. and Frommer M.; 1987. CpG islands in vertebrate genomes. J. Mol. Biol., **196**: 261-282.

Gelain D.P., Dalmolin R. J., Belau V.L., Moreira J. C., Klamt F. and Castro M.A.; 2009. A systematic review of human antioxidant genes. Frontiers in Bioscience, **14**: 4457-4463.

Jitendra N., Prachi S., Alpana V. and Shakti S.;2007. In Silico Identification of Antioxidant gene G6PD in *Caenorhabditis elegans*. Online Journal of Bioinformatics, **8 (1):** 61-73.

Milanesi L., Dangelo D. and Rogozin I. B.; 1999. Gene Builder: interactive in silico prediction of gene structure. Bioinformatics, **15**: 612-621.

Pennisi E.; 1998. Worming secrets from the *C. elegans* genome. Science, **282**: 1972-1974.

Reese M. G., Eeckman F. H., Kulp D. and Haussler D.; 1997. Improved splice site detection in Gene. J. Comp. Biol., **4**: 311-323.

Rinner O. and Morgenstern B.; 2002. AGenDA: gene prediction by comparative sequence analysis. In Silico Biol., **2**: 195-205.

Schultz J., Doerks T., Ponting C. P., Copley R. R. and Bork, P.; 2000. More than 1,000 putative new human signaling proteins revealed by EST data mining. Nat. Genet., **25**: 201-204.

Yoshiaki Tsuji.; 2005. JunD activates transcription of the human ferritin H gene through an antioxidant response element during oxidative stress. Oncogene, **24(51)**: 7567–7578**.**