Genome Wide Survey of the Entire *C. elegans* *alpha*-Tubulin Gene Family: cDNA Cloning, Expression, and Structure-Function Analysis

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1 Introduction

With the aim of molecularly and functionally characterizing each and every member of the *alpha*-tubulin gene family in a metazoan, previously we have cloned and characterized four *alpha*-tubulin genes in *Caenorhabditis elegans*. In a genome wide search, we have now discovered four novel *alpha*-tubulin genes. Using a size selected *C. elegans* cDNA library, we have identified cDNA clones corresponding to three new *alpha*-tubulin isotypes. The new *alpha*-tubulin genes, *tba-5*, *tba-6*, *tba-7* and *tba-8* are transcribed at a low level. The expression pattern of the *C. elegans alpha*-tubulin genes are quite complex. To study tubulins at a high molecular resolution, a comparative study between the crystal structure of the pig brain *alpha*-tubulin [1] was done, using computational mutagenesis to mutate the pig *alpha*-tubulin to *C. elegans* isotypes and we analysed the changes in the 3D structures using MIDAS graphic package. We use CLUSTALW program to determine the homology between *C. elegans* and other *alpha*-tubulin from bacteria, fungi, insects, and mammalians. We also constructed a phylogeny tree for the *alpha*-tubulin families. Our study of the promoter region of the tubulin genes show divergence in the frequency and distribution of regulatory sequences. These data provide strong evidence that although the *alpha*-tubulin appear to have very similar protein structure, nevertheless, their expression is highly regulated during animal development. Based on these data we propose, that the genomic architecture of tubulins and the regulation of its expression could directly contribute to the specificity of tubulin function during animal development.

2 Results and discussion

To isolate the novel *alpha*-tubulin cDNA clones, a *C. elegans* cDNA library in the lambda ZAP vector (Y. Kohara) was screened at a low stringency using the *tba-1* cDNA probe [2], 27 positive clones were isolated, majority of which encoded the *tba-1* gene. However, nucleotide sequence of the 5' upstream region of the three positive clones revealed three new *alpha*-tubulin cDNAs, which were mapped on *C. elegans* chromosomes, in *silico*, i.e. by computer based homology search, and were assigned to three different cosmid clones. The sequence of the tubulin cDNA clones was also compared with the set of EST tagged clones in the *C. elegans* cDNA library (Y. Kohara). The genomic sequence of the *alpha*-tubulin cDNA clones was obtained from the nematode sequencing consortium [3]. No full length cDNA clone corresponding to the *tba-5* (encoded by the cosmid F16D3.1), *tba-7* (encoded by the cosmid T28D6.2), and *tba-8* (encoded by the cosmid ZK899.4) has been obtained, but two clones for the *tba-6* (encoded by the cosmid F32H2.9) isotype, namely yk450g5, and SQ#CBG32 have been isolated, and partially sequenced. The tba-5 and *tba-6* genes are located on the chromosome I, *tba-7* is located on chromosome III, and *tba-8* on chromosome X. How much homology exist among the new *C. elegans alpha*-tubulin and the homologous ones from other species? The CLUSTALW program shows that the homology ranges between 63the nematode), and 82the nematode). In our search for motifs, we find that key features of the *alpha*-tubulin such as the GTP nucleotide binding site GGGTGSG, is highly conserved in all *C. elegans* isotypes. Similarly, the cell attachment RDG and the amidation site, YGKK, which are specific only to the alpha, but not beta-tubulin, are present. Using computational mutagenesis we mutate the TBA_PIG to the new C. elegans *alpha*-tubulin proteins and analysed the changes in the 3D structures, in the active site, and the morphological changes in the secondary structures, and correlated these changes in the structure and functionality of the TBA_PIG with the change in the parent gene. The computational mutagenesis was done in the following way: we used MIDAS graphic package [4] to replace the aminoacids from the TBA_PIG with those of our isotypes, then we added the hydrogen atoms and performed a minimisation of the energy with the molecular mechanics force field of AMBER [5].

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