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Nucleotide Sequence of β-tubulin Gene from the Soft Coral *Scleronephthya gracillimum* (Kükenthal)

Seungshic Yum[1], Seonock Woo[1], Man Chang[1], Taek-Kyun Lee[1]*, and Jun-Im Song[2]

[1]*Southern Coastal Environment Research Division, South Sea Institute, KORDI, Geoje 656-830, Korea*

[2]*Department of Biological Science, College of Natural Sciences, Ewha Womans University, Seoul 120-750, Korea*

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Abstract – We cloned the complete cDNA of the β-tubulin from the soft coral, *Scleronephthya gracillimum* (Kükenthal) (Alcyonacea, Octocorallia, Anthozoa, Cnidaria), via the random sequencing of a cDNA library and the 5'-rapid amplification of cDNA end (RACE) technique. The full-length cDNA of the *S. gracillimum* β-tubulin comprised 1541 bp, not including the poly (A)$^+$ stretch, also contained a complete open reading frame, which codes for a total of 445 amino acids. The amino acid residues 16-402 appeared to be in a state of conservation in a variety of animals. Northern blot analysis clearly demonstrated that the sequence we have obtained is, indeed, the full-length cDNA of the β-tubulin gene in *S. gracillimum*.

Key words – *Scleronephthya gracillimum*, soft coral, β-tubulin, cDNA library, 5'-RACE

1. Introduction

Microtubules are filamentous proteins in the cytoskeleton, which are involved in a variety of cellular processes, such as chromosome movement during cell division, cellular polarization, mRNA targeting, and intracellular transportation. They are assembled by the heterodimerization of α- and β-tubulin, both of which exhibit 50% homology with respect to amino acid sequencing. Tubulins manifest some of their specific functions after polymerization, which is controlled by tubulin's GTPase activity (Erickson and O'Brien 1992; Mejillano *et al.* 1996). Cysteine residues in the β-tubulin monomer play important roles in the assembly of tubulin into microtubules, by influencing the catalysis of GTP hydrolysis, and, ultimately, by affecting tubulin's intrinsic GTPase activity. Mutation in β-tubulin cysteine residues results in a dramatic decline in microtubule dynamics in yeast (Gupta *et al.* 2001, 2002), and leads to abnormal spindle orientation in the early embryonic stages of *Caenorhabditis elegans* (Wright and Hunter 2003). Thus, the determination of the primary structure of β-tubulin is essential and necessary, in order to predict alterations in the behavior and functioning of the microtubules, in terms of 'dynamic instability' (Mitchison and Kirschner 1984).

The filamentation temperature-sensitive protein Z (FtsZ), a crucial component of the cell division machinery found in Archaea, has been theorized to be an evolutionary counterpart of the tubulin found in eukaryotes (Baumann and Jackson 1996; Margolin *et al.* 1996). Despite its low amino acid sequence identity with the tubulins (Amos *et al.* 2004), there are some similarities with regard to three-dimensional structures (Erickson 1998; van den Ent *et al.* 2001), and similar mechanisms underlying the GTP-dependent polymerization of both proteins (de Boer *et al.* 1992; Erickson 1998; RayChaudhuri and Park 1992), raising the possibility that eukaryotic tubulins are, in fact, derived from Archaea FtsZ. Therefore, additional homologues of eukaryotic tubulin proteins can be useful in the further understanding of tubulin's evolutionary aspects in the cytoskeleton.

The soft coral, *Scleronephthya gracillimum* (Alcyonacea, Octocorallia, Anthozoa, Cnidaria) is predominantly distributed at depths of 15~40 m, in the subtropical ocean zone near Seogwipo, Jeju, Korea, and contributes to the species diversity of this area, which was designated Natural Monument # 421 by the Korean government on July 18, 2000, due to the unique faunal and floral characteristics inherent to the region. However, the high biodiversity of the soft coral community has been challenged by environmental changes and human impact, such as global warming, habitat destruction, and marine pollution. Thus, recently, we have promoted a policy of extensive expressed gene profiling, in order to develop a biomonitoring system for continuous knowledge of the circumstances of the coral species with

*Corresponding author. E-mail: tklee@kordi.re.kr

respect to environmental factors, and also to establish effective and practical protocols for maintenance and/or restoration at a molecular level, according to the results of this monitoring. During the screening of the cDNA library, we cloned a cDNA from polyp tissue encoding the *S. gracillimum* β-tubulin gene via random sequencing and the 5'-rapid amplification of the cDNA end (RACE) technique.

2. Materials and Methods

Animal, RNA preparation and cDNA library construction

Polyps of the *Scleronephthya gracillimum* colonies were cut into pieces about 5×5×5 mm in size, then these pieces were quickly frozen in liquid nitrogen and stored at -80 ℃. Total RNA was extracted by following the method of Woo *et al.* (2004). One microgram of poly A^+ RNA, which was purified by using an Oligotex direct mRNA

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          TTGCCATTCCTCTATTGCGAAGCATTGTGTTCAGATATTGCAGCTGTTCTTCCGTAAAAACTTTTTAAAA   70
ATG AGA GAA ATC GTT CAT CTT CAA GCT GGC CAG TGC GGA AAT CAA ATC GGA GCG AAG TTC  130
 M   R   E   I   V   H   L   Q   A   G   Q   C   G   N   Q   I   G   A   K   F   20
TGG GAA GTC ATC TCG GAC GAA CAT GGC GTG GAT CCG ACT GGC ACA TAT CAC GGC GAT TCA  190
 W   E   V   I   S   D   E   H   G   V   D   P   T   G   T   Y   H   G   D   S   40
GAC CTT CAA TTG GAA AGA ATA AAC GTT TAT TAC AAC GAA GCG ACC GGC GGG AAA TAT GTT  250
 D   L   Q   L   E   R   I   N   V   Y   Y   N   E   A   T   G   G   K   Y   V   60
CCG AGA GCA GTT TTG GTC GAT TTG GAG CCA GGA ACG ATG GAT TCC GTT CGC TCT GGA CCT  310
 P   R   A   V   L   V   D   L   E   P   G   T   M   D   S   V   R   S   G   P   80
TTT GGA CAG ATT TTC AAA CCG GAC AAT TTT ATA TTT GGC CAG AGC GGA GCT GGA AAT AAC  370
 F   G   Q   I   F   K   P   D   N   F   I   F   G   Q   S   G   A   G   N   N  100
TGG GCT AAA GGA CAT TAC ACA GAA GGG GCT GAA CTT GTA GAT TCT GTT CTC GAT GTT GTT  430
 W   A   K   G   H   Y   T   E   G   A   E   L   V   D   S   V   L   D   V   V  120
AGA AAA GAA TCT GAA GGG TGT GAT TGC TTA CAA GGT TTT CAA CTC ACA CAT TCT CTT GGT  490
 R   K   E   S   E   G   C   D   C   L   Q   G   F   Q   L   T   H   S   L   G  140
GGT GGA ACT GGC TCT GGA ATG GGC ACC TTG TTA ATT TCG AAA ATC CGT GAA GAA TAT CCA  550
 G   G   T   G   S   G   M   G   T   L   L   I   S   K   I   R   E   E   Y   P  160
GAC AGA ATA ATG ACC ACA TTC AGC GTG GTA CCA TCA CCT AAA GTT TCA GAC ACA GTT GTT  610
 D   R   I   M   T   T   F   S   V   V   P   S   P   K   V   S   D   T   V   V  180
GAG CCA TAC AAT GCA ACC CTG TCA GTA CAT CAA CTG GTT GAA AAC ACA GAT GAA ACC TTC  670
 E   P   Y   N   A   T   L   S   V   H   Q   L   V   E   N   T   D   E   T   F  200
TGT ATT GAC AAC GAA GCT TTG TAT GAT ATC TGT TTC CGA ACC TTG AAG CTT ACC ACA CCC  730
 C   I   D   N   E   A   L   Y   D   I   C   F   R   T   L   K   L   T   T   P  220
ACC TAT GGT GAC TTA AAC CAT CTT GTT TCT GCT ACC ATG AGT GGT ATT ACA ACC TGC CTA  790
 T   Y   G   D   L   N   H   L   V   S   A   T   M   S   G   I   T   T   C   L  240
CGA TTC CCT GGT CAG TTG AAT GCA GAT TTG CGA AAG CTT GCT GTC AAC ATG GTA CCA TTC  850
 R   F   P   G   Q   L   N   A   D   L   R   K   L   A   V   N   M   V   P   F  260
CCT CGA CTT CAT TTC TTC ATG CCT GGC TTT GCC CCG CTT ACC AGC CGT GGT TCA TCC CAA  910
 P   R   L   H   F   F   M   P   G   F   A   P   L   T   S   R   G   S   S   Q  280
TAC CGT GCA CTC ACT GTG CCA GAA CTC ACA CAA CAA ATG TTC GAT GCC AAA AAC ATG ATG  970
 Y   R   A   L   T   V   P   E   L   T   Q   Q   M   F   D   A   K   N   M   M  300
GCA GCT TGT GAT CCT CGT CAT GGT CGA TAT CTT ACT GTT GCT GCA ATG TTC CGT GGC CGT 1030
 A   A   C   D   P   R   H   G   R   Y   L   T   V   A   A   M   F   R   G   R  320
ATG TCT ATG AAG GAA GTT GAC GAA CAG ATG TTG AAT GTC CAG AAC AAG AAC AGC TCC TAC 1090
 M   S   M   K   E   V   D   E   Q   M   L   N   V   Q   N   K   N   S   S   Y  340
TTT GTG GAG TGG ATT CCA AAC AAC GTG AAG ACC GCT GTC TGC GAT ATC CCA CCA AGA GGT 1150
 F   V   E   W   I   P   N   N   V   K   T   A   V   C   D   I   P   P   R   G  360
TTG AAG ATG TCT GGT ACC TTT ATT GGA AAC AGC ACA GCA ATT CAA GAA TTG TTC AAA CGA 1210
 L   K   M   S   G   T   F   I   G   N   S   T   A   I   Q   E   L   F   K   R  380
ATC AGT GAG CAG TTC ACC GCT ATG TTC CGT CGC AAA GCT TTC CTT CAT TGG TAC ACT GGT 1270
 I   S   E   Q   F   T   A   M   F   R   R   K   A   F   L   H   W   Y   T   G  400
GAA GGC ATG GAT GAA ATG GAA TTC ACG GAG GCT GAA TCA AAC ATG AAT GAC TTG GTT TTT 1330
 E   G   M   D   E   M   E   F   T   E   A   E   S   N   M   N   D   L   V   F  420
GAG TAT CAA CAA TAC CAA GAA GCA ACA GCA GAG GAG GAA GGA GAA TTT GAA GAG GAG GAA 1390
 E   Y   Q   Q   Y   Q   E   A   T   A   E   E   E   G   E   F   E   E   E   E  440
GAA GAA GAG GAA GCT TAA TTTGAAAATAAAACTCTTGAACTGCTTGATTTTTAAAACATTTTATAAAGTTACT 1463
 E   E   E   E   A   *                                                          445
TTGTGTCTGTTGTGCTGATTGTGAAGCAAAATTGTGAACAATGCCTGGTTCGACAGTAGATAACAAATAAAATTTTGA 1541
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Fig. 1. Nucleotide and deduced amino acid sequence of the *Scleronephthya gracillimum* β-tubulin gene. Numbers on the right side refer to the positions of the nucleotides and amino acids. The complete sequence was determined by the 5'-RACE technique.

mini kit (Qiagen, USA), and the SMART cDNA library construction kit (BD Biosciences, USA) were used for cDNA library construction following the manufacturer's direction. The detailed description of the cDNA construction will be published elsewhere. By random sequencing of the cDNA library, 992 base pairs of β-tubulin cDNA missed at the 5'-flanking region obtained.

5'-rapid amplification of the cDNA end (RACE)

The first-strand cDNA, which was synthesized by using a SMART PCR cDNA synthesis kit (BD Biosciences, USA), an oligonucleotide primer (5'-GCATTGTATGGCTCAACAAC-3') corresponding to nucleotide numbers 624 to 605 of the β-tubulin sequence of *S. gracillimum* (DDBJ/EMBL/GenBank database accession number AY703828), and the

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Sgr  IGAKFWEVISDEHGVDPTGTYHGDSDLQLERINVYYNEATGGKYVPRAVLVDLEPGTMDSVRSGPF
Sfu  ............I.....................................................
Nve  ............I.....................................I...............
Aau  ............I.....................................................
Mle  ............I.....................................................
Cin  ............I.....................................................
Hsa  ............I.....................................................

Sgr  GQIFKPDNFIFGQSGAGNNWAKGHYTEGAELVDSVLDVVRKESEGCDCLQGFQLTHSLGGGTGSGM
Sfu  ....R....V.................................A.S.....................
Nve  ....R....V.................................A.S.....................
Aau  ....R....V.................................A.S.....................
Mle  ....R....V.................................A.S.....................
Cin  ....R....V.................................A.S.....................
Hsa  ....R....V.................................A.S.....................


Sgr  GTLLISKIREEYPDRIMTTFSVVPSPKVSDTVVEPYNATLSVHQLVENTDETFCIDNEALYDICFR
Sfu  ................N.................................Y.............
Nve  ................N.................................Y.............
Aau  ................N.....................................................
Mle  .....................................................A.............
Cin  ................N.................................Y.............
Hsa  ................N.................................Y.............


Sgr  TLKLTTPTYGDLNHLVSATMSGITTCLRFPGQLNADLRKLAVNMVPFPRLHFFMPGFAPLTSRGSS
Sfu  ....................V.........................................Q
Nve  ....................V.........................................Q
Aau  ....................V.............................I...........Q
Mle  ....................V.........................................Q
Cin  ....................V.........................................Q
Hsa  ....................V.........................................Q


Sgr  QYRALTVPELTQQMFDAKNMMAACDPRHGRYLTVAAMFRGRMSMKEVDEQMLNVQNKNSSYFVEWI
Sfu  .....................................................................
Nve  .....................................................................
Aau  .....................................................................
Mle  .....................................................................
Cin  .....................................................................
Hsa  ..................................V..................................


Sgr  PNNVKTAVCDIPPRGLKMSGTFIGNSTAIQELFKRISEQFTAMFRRKAFLHWYTGEG
Sfu  ................A..V.....................................
Nve  ................A.......................................
Aau  ................A.......................................
Mle  ................A.......................................
Cin  ................A.......................................
Hsa  ................A.......................................
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Fig. 2. Multiple alignment of amino acid residues 16-402 of the β-tubulin from various animals. Dots represent conserved residues with *Scleronephthya gracillimum* β-tubulin. Sgr, *Scleronephthya gracillimum* (Anthozoa, Cnidaria, AY682093); Sfu, *Suberites fuscus* (Demospongiae, Porifera, AY226062); Nve, *Nematostella vectensis* (Anthozoa, Cnidaria, AY226067); Aau, *Aurelia aurita* (Scyphozoa, Cnidaria, AY226068); Mle, *Mnemiopsis leidyi* (Ctenophora, AY226069); Cin, *Ciona intestinalis* (Ascidiacea, Chordata, AK116810) and Hsa, *Homo sapiens* (Vertebrata, Chordata, BC024038).

SMART IV oligonucleotide (BD Biosciences, USA), 5'-AAGCAGTGGTATCAACGCAGAGT-3', were used for 5'-RACE. PCR conditions were as follows: 25 cycles at 94 °C for 30 seconds, 55 °C for 30 seconds, and 72 °C for 60 seconds. The 663 bp cDNA fragment was amplified (data not shown), which overlapped with the 992 bp cDNA clone, and was assembled into the full-length cDNA of the β-tubulin gene.

Northern blot analysis

The total RNA (10 μg) was fractionated on a 1.2% formaldehyde-agarose gel, and blotted onto a Hybond N^+ nylon membrane (Amersham). A β-tubulin cDNA (992 bp long), obtained by the random sequencing of the *S. gracillimum* cDNA library, was labeled [α-^{32}P]dCTP. A rapid hybridization buffer (Amersham) was employed in this procedure. Hybridization and washing were performed according to the manufacturer's instructions. The membrane was exposed for 6 hours to an X-ray film (Agfa), and then the film was developed.

3. Results and Discussion

The complete nucleotide sequence of the cDNA and the deduced amino acid sequence of the *S. gracillimum* β-tubulin, are shown in Fig. 1. In the 1541 bp sense strand, not including the poly $(A)^+$ stretch at the 3'-end, the methionine codon starts at nucleotide number 71, and contains an open reading frame (ORF) coding for a predicted protein composed of 445 amino acid residues. Two putative domains involved in polymer formation were found in the predicted protein. One is the GTPase domain, which corresponds to amino acid residues 45-244, and the other is the C-terminal domain, which corresponds to amino acid residues 246-383. The coding gene and deduced amino acid sequences were submitted to the DDBJ/EMBL/GenBank nucleotide sequence database (AY703828). This is the only full-length cDNA information of the cnidarian β-tubulin gene available. Using the tblastn algorithm of the NCBI server, a homology search was performed. Since the full-length cDNA information were available only in a few species of the diploblastic animal group, including Porifera, Cnidaria and Ctenophora, the amino acid residues 16-402 were selected for comparison. *S. gracillimum* β-tubulin exhibited a high degree of similarity with various animals from phylum Porifera to phylum Chordata (Fig. 2). In brief, *S. gracillimum* β-tubulin shared 92% identities and 93% positives with *Ciona intestinalis* (Ascidiacea, Chordata, AK116810) and *Mnemiopsis leidyi* (Ctenophora, AY226069); 91% identities and 93% positives with *Nematostella vectensis* (Anthozoa, Cnidaria, AY226067), *Aurelia aurita* (Scyphozoa, Cnidaria, AY226068), *Suberites fuscus* (Demospongiae, Porifera, AY226062) and *Homo sapiens* (Vertebrata, Chordata, BC024038). The amino acid sequence of *S. gracillimum* β-tubulin showed a more unique primary structure than those of other animals (Fig. 2). This may indicate that the *S. gracillimum* β-tubulin gene reported in this paper is a new type of β-tubulin. Numerous isotypic forms of β-tubulin encoded by different genes were found in many organisms (Hall *et al.* 1983; Lewis *et al.* 1985; Yoshikawa *et al.* 2003) and in differentiating or developing tissues (Aniello *et al.* 1991; Bieker and Yazdani-Buicky 1992; Burgoyne *et al.* 1988; Joshi and Cleveland 1989). Northern blot analysis confirmed that the β-tubulin gene manifested as a single transcript, approximately 1.6 kb in length (Fig. 3). This indicates that the β-tubulin sequence shown in Fig. 1 corresponded to the full-length cDNA of *S. gracillimum*.

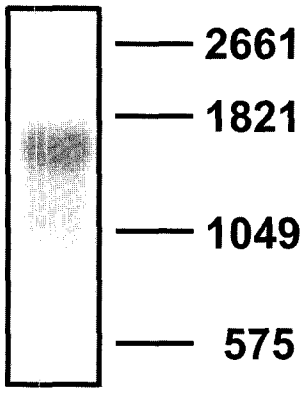

Fig. 3. Northern blot analysis of the β-tubulin transcript. The sizes of the markers in the bases are indicated at the right.

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