

## Gene cloning and characterization of pearl oyster *Pinctada fucata* $\alpha$ -tubulin

Tomoyuki Miyashita<sup>1</sup>, Akiko Takami<sup>1</sup>, Yui Sugimoto<sup>1</sup>, Ayumi Sakai<sup>1</sup> and Ryosuke Takagi<sup>1</sup>

### Abstract

We have isolated a full-length *Pinctada fucata*  $\alpha$ -tubulin gene that is one of the genes obtained in the process of selecting the BMP (Bone Morphogenetic Protein) type II receptor gene. The 1678 base pair (bp) length of  $\alpha$ -tubulin cDNA encodes 451 amino acids and the calculated mass is 42 kDa. The gene belongs to one of the *Pinctada fucata*  $\alpha$ -tubulin family and was designated  $\alpha$ -tubulin 2. The amino acid sequence of *Pinctada fucata*  $\alpha$ -tubulin 2 contains the GGGTGSG domain that is a Guanosine triphosphate (GTP) nucleotide-binding site required for  $\alpha$ - $\beta$ -tubulin polymerization, and also contains putative posttranslational modification sites that are the phosphorylation residues at arginine (R) 79 and lysine (K) 336, and an acetylation residue at lysine (K) 40. Phylogenetic analysis revealed that bivalvia *Pinctada fucata*  $\alpha$ -tubulin 2 is closer to a gastropoda (*Ilyanassa obsoleta*, *Patella vulgata*)  $\alpha$ -tubulin than that of a bivalvia (*Scrobicularia plana*, *Crassostrea gigas*, *Mytilus galloprovincialis*). RT-PCR analysis showed that  $\alpha$ -tubulin 2 messenger RNA was expressed in mantle edge, gill, adductor muscle and liver but not in mantle pallial or foot. It is expected that other members of the  $\alpha$ -tubulin family are expressed in mantle pallial and foot.

**Key words:**  $\alpha$ -tubulin, cDNA, *Pinctada fucata*, bivalvia

### 1. Introduction

The microtubule cytoskeleton function has essential roles in eukaryotic cell homeostasis as the most important components of the mitotic spindle and the interphase network that contribute to a cellular structure formation<sup>(1)</sup>. All microtubules are assembled from tubulin heterodimers composed of monomers of  $\alpha$ -tubulin and  $\beta$ -tubulin, and have organelle-specific properties and functions. During mitosis, mitotic spindles are essential for chromosome segregation and congression through the cycles of polymerization-depolymerization of microtubules termed dynamic instability of MTs (Microtubules)<sup>(2)</sup>. In the interphase network, microtubules take part in a large number of various cellular phenomena including cell division, cellular morphogenesis, intracellular rearrangements, cell differentiation, and vesicle transport. Furthermore, they participate even in signal transduction.

Tubulin heterodimers undergo cycles of phenomenon of polymerization-depolymerization that is the process by which microtubule polymers alternate between elongation and shortening<sup>(3)</sup>. The dynamic instability is strictly regulated during the cell cycle<sup>(1)</sup> and requires an energy source that depends on hydrolysis of GTP to GDP<sup>(4)</sup>. Two molecules of GTP bind to two sites termed N and E. The GTP on the N site of  $\alpha$ -tubulin, that is located between the two monomers, does not hydrolyze. On the other hand, the GTP on the E site of  $\beta$ -tubulin, that is exposed to the surface, is hydrolyzed to GDP. Under the GTP-bound stable form, a microtubule maintains polymerized form. But conversely, the form of GDP-tubulin is tended to depolymerization<sup>(5)</sup>.

It is still not clear enough that how various microtubule structures with organelle-specific properties and functions are formed. The many results obtained from various experiments suggest a hypothesis that post-translational modifications (PTMs) of tubulin attribute to generate functional diversity of microtubules. Expression of different tubulin isotypes and PTMs such as detirosination, acetylation, polyglutamylation (Glu-tubulin) and polyglycylation generates distinct microtubule subtypes<sup>(6-12)</sup>.

In the current study, we have cloned the complete  $\alpha$ -tubulin cDNA that belongs to  $\alpha$ -tubulin family of *Pinctada fucata* and characterized by comparing the amino acid sequences of the various  $\alpha$ -tubulin genes, and also performed phylogenetic analysis in order to clarify the systematic and evolutionary position.

---

received 24 Nov 2012

This study was supported by the Project Research of the school of Biology Oriented Science and Technology No. 09-IV-21, 2010.

1. Department of Genetic Engineering, Faculty of Biology-Oriented Science and Technology, Kinki University, 930 Nishimitani Kinokawa City, Wakayama 649-6493, Japan

## 2. Materials and methods

### 2.1 cDNA cloning of $\alpha$ -tubulin

The  $\alpha$ -tubulin gene is one of the genes that was taken in the process of isolating the *Pinctada fucata* BMP type II receptor gene. The procedure is as follows:

Total RNA was extracted from the mantle with Micro-to-Midi Total RNA Purification system (Invitrogen) according to the instruction manual. To obtain the first strand cDNA, a reverse transcription was performed using a standard Oligo dT-3sites Adaptor Primer [5'-poly(dT)-CTGATCTAGAGGTACCGGATCC-3'(TAKARA BIO INC)]. Subsequently 3'-RACE-PCR was performed using the adaptor primer that has 3 restriction sites as the reverse primer [5'-CTGATCTAGAGGTACCGGATCC -3'(TAKARA BIO INC)] and a specific degenerated primer as the forward primer. A degenerated primer ATGCGGCCGCGT(T/C/A/G) GG(T/C/A/G) AC(T/C/A/G) CT(T/C/A/G) CG(T/C/A/G) TA(T/C) ATG was designed based on the highly conserved sequences VGTLYRM of *Crassostrea gigas* BMP-IIIR. The sequence ATGCGGCCGC is the linker sequence containing the recognition sequence of *NotI*. The amplification reaction conditions were as follows: 5 rounds of 10 s at 98°C, 1 min at 44°C, and 1 min at 72°C; followed by 30 rounds of 10 s at 98°C, 1 min at 55°C, and 1 min at 72°C. Amplified DNA was cloned into the *NotI*-*XbaI* site of pBluescript II SK(+). After transformation, plasmids that contained the expected size (about 110 bp) of the insert DNA were selected, and then the nucleotide sequence of the insert was determined. Although the deduced amino acids sequence was not equivalent to that of the BMP type II receptor, a homology search showed that the amino acids sequence has an extensive identity with human and rat  $\alpha$ -tubulin. Therefore, the PCR amplified cDNA is the gene of the *Pinctada fucata*  $\alpha$ -tubulin, although incomplete (GenBank accession No. AB575964). The incomplete  $\alpha$ -tubulin cDNA was designated  $\alpha$ -tubulin 1. To obtain the complete cDNA, we have screened the cDNA library using the PCR amplified incomplete  $\alpha$ -tubulin 1 cDNA as the probe. Screening cDNA library was performed as described previously<sup>(13)</sup>.

### 2.2 DNA sequencing

Sequencing was performed by the dideoxynucleotide chain termination method using an ABI 377 automated DNA sequencer (Applied Biosystems, Foster City, CA).

### 2.3 Phylogenetic analysis

Multiple sequence alignments were generated using the site ClustalW (<http://clustalw.ddbj.nig.ac.jp/index.php?lang=ja>)<sup>(14)</sup>. The generated alignments were subjected to phylogenetic analysis by using the neighbor-joining (NJ) method<sup>(15)</sup> with 1000 replicate runs of bootstrap values followed by visualized with the program Treeview 1.6.6<sup>(16)</sup>.

### 2.4 Gene expression analysis by RT-PCR

Tissue expression patterns of the  $\alpha$ -tubulin 2 mRNA were examined by RT-PCR. Total RNA isolation from various tissues (mantle edge, mantle pallial, liver, foot, gill, and adductor muscle) and the cDNA synthesis by reverse transcriptase were the same as those described above. The generated cDNA was used as the template for PCR. The following primer pairs were used for PCR amplification of the  $\alpha$ -tubulin and the  $\beta$ -actin gene fragments: Forward primer of the  $\alpha$ -tubulin, 5'-CAGATGGGCAATGCATGCTGGGAG-3'; Reverse primer of the  $\alpha$ -tubulin, 5'-CGAAAGAGCTAAATCCGTATCCGT-3'; Forward primer of the  $\beta$ -actin, 5'-TgTAYgCCTCTggYcYACC-3'; Reverse primer of the  $\beta$ -actin, 5'-CVACRTCRCACTTCATgATgS-3'. Primers for the  $\beta$ -actin are described in Suzuki et al.<sup>(17)</sup>. The amplification reaction conditions were as follows: initial denaturation at 94°C for 3 min; followed by 30 rounds of 30 s at 94°C, 30 s at 50°C, and 1 min at 72°C.

## 3. Results and discussion

### 3.1 DNA cloning and sequence

To isolate the *Pinctada fucata* BMP type II receptor gene, we performed PCR amplification described in Materials and methods. In this experiment, we have isolated the incomplete  $\alpha$ -tubulin gene (GenBank accession No. AB575964). To isolate the complete  $\alpha$ -tubulin gene, we have screened the cDNA library using the incomplete  $\alpha$ -tubulin cDNA as the probe.

About  $5 \times 10^5$  recombinant phages of cDNA library were screened. Hybridization and detection were performed as described in Materials and methods. Positive clones were visualized with a BCIP/NBT reagent in accordance with the manufacturer's instructions (Boehringer Mannheim Germany). Three clones producing strong signals were isolated. These cDNA inserts were subcloned into the pBluescript sequencing vector. Sequence analysis reveals that one of the inserts has the proper open reading frame and poly (A) tail. The open reading frame of the cDNA starts at nucleotide 96 (ATG) and extends to the stop codon (TAA) at 1450 (Fig. 1). The cDNA is 1678 base pairs long and encodes 451 amino acids that have extensive homology with various  $\alpha$ -tubulins (Fig. 2). The calculated mass is 50 kDa. A putative polyadenylation signal TAATTT is present at the 1544th nucleotide. Concerning this signal, human sequence is consulted. Although most typical polyadenylation signal is TTATTT in human genes, TAATTT is an allowed variant of this sequence<sup>(18)</sup>. Therefore, we concluded that the sequence TAATTT is a variant of polyadenylation signal.

Pairwise Sequence Alignment suggested that few differences exist between the sequence of the probe cDNA ( $\alpha$ -tubulin 1) and 1678 bp cDNA, suggesting that these two  $\alpha$ -tubulins are a family. Therefore, the  $\alpha$ -tubulin gene which has been isolated newly was designated  $\alpha$ -tubulin 2. We found the GGGTGS domain that is a GTP nucleotide-binding site required for  $\alpha$ - $\beta$ -tubulins polymerization<sup>(19)</sup>. Moreover posttranslational modification sites were also found. These are the putative phosphorylation residues at R79 and K336, and an acetylation residue at K40.

```

CCACGGCTCCGGCTCATCACAGTCAGACGGAGAACGGACAATCCCTTTCAATCCCGGATTATATTCTTCTTAAGACTCATCACCAAGTGTTCAAAATGCGTGAATGTATCAGTGTCCATG 120
                                     M R E C I S V H V 9
TEGGCCAGGCCGGAGTTTCAGATGGGCAATGCATGCTGGGAGTTGTACTGTTTAGAGCATGGTATCCAGCCCAGTGGACAGATGCCAGTGGACAAAATATTGGGGTGTGACGACTCCT 240
G Q A G V Q M G N A C W E L Y C L E H G I Q P D G Q M P S D [K] T I G G G D D S F 49
TCAACACCTTCCTCAGTGAGACAGGGGCAAGGCAACATGTACCTAGGGCTGTATTGTAGACCTCGAACCAACTGTTGTAGATGAAGTCCGAACTGGTACATACAGACAACGTGTCCATC 360
N T F F S E T G A G K H V P R A V F V D L E P T V V D E V [R] T G T Y R Q L F H P 89
CTGAACAACCTGCTCACCGGGAAGGAAAGATGCTGCCAACAACTACGCTCGTGTCACTACACAATTTGGTAAAGAAATTGTTGACCTTGTCCCTTGGCCGTATCCGTAAGAATGCTGACCAAT 480
E Q L V T G K E D A A N N Y A R G H Y T I G K E I V D L Y L D R I R K H A D Q C 129
GTACAGGGCTGCAGGGTTTCTTATCTTCCACAGTTTGGTGAGGAACTGGTCTGGTTTACCTCCCTGCTCATGGAAAGCCCTCAGTGTGACTATGGCAAGAAATCCCAAGCTGGAGT 600
T G L Q G F L I F H S F [G G G T G S G] F T S L L N E R L S V D Y G K R S K L E F 169
TTGCCATCTACCCAGCCCAACAGGATGACAGCTGTTTGGAAACCTACAACTCCATTCTGACCACCACACCACACTAGAGCACTCTGACTGTGCCITCATGGTGACAATGAGGCTA 720
A I Y P A P Q V S T A V L E P Y N S I L T T H T T L E H S D C A F N V D M E A I 209
TCTACGATATCTCGCGAGCTAACCTTGACATTGAGAGACCCACCTACACCAACTGAACCGTCTGATTGGTCAGATTGTCAGCTCCATCACTGCCCTCTCTCAGATTGATGGTGCCTGGA 840
Y D I C R R N L D I E R P T Y T H L N R L I G Q I V S S I T A S L R F D G A L N 249
ATGTGGATCTGACAGATTCAGACCAACTGGTGGCCCTACCCAGGATGCACTTCCCTCTGGCTACATATGTTCTTGTCTCTCTGCTGAAAAGGCCCTACATGAACAACCTTACCGTTG 960
V D L T E F Q T N L V P Y P R I H F P L A T Y V L V I S A E K A Y H E Q L T V A 289
CTGAATAACCAATTCCTGCTTCGAGCCAGCAATCAGATGGTGAATGGCCAGCCAGTCAAGGCAAAATACATGGCTTGTGTATGTTGTACAGAGGAGATGTGGTGGCCCATGAGGCTCA 1080
E I T N S C F E P A N Q M V K C D P R H G K Y M A C C M L Y R G D V Y P M D Y N 329
ATGCAGCCATTGCCACATTAGAGCAAGAGGACCAATCAGTTTGTGGACTGGTGTCCACAGGGTCAAGGATGGCATCAACTACAGCCACCCACCCGATGATACCAGGTGGTGAACCTGG 1200
A A I A T I [K] T K R T I Q F V D W C P T G F K D G I N Y Q P P T V V P G G D L A 369
CCAAGGTACAGAGGGCGGTGTGTATGTTGAGTAACACCACAGCCATTGCTGAAGACTGGGCAAGTCTGGACCACAAGTTTGACCCTGATTATGCCAAGCGAGCTTTGTACACTGGTACG 1320
K V Q R A V C N L S N T T A I A E D M A R L D H K F D P D Y A K R A F V H W Y V 409
TAGGAGAGGGGTATGGAGGAAAGGAGAAATTCCTCCGAGGCTCGTGAAGATTGGCTGCTTTGGAGAAAGATTACGAGGAAAGTGGGTGTGACTCTGTGGAGGAGAGGGTGAAGAGAGGGGG 1440
G E G M E E G E F S E A R E D L A A L E K D Y E E V G Y D S Y E A E G E E E G D 449
ATGAATATAAATCTTCTCTATCTACAGAAGTGTACTTTTGCACCTTGAGACGGATACGGATTAGCTCTTTCGATGTGCAATTGTGTTCCATGAACAAGTAATTTTATTGTTTATT 1560
E Y *
ATTATATATTTTCGCAAGCGAATCAAAATTTGAATACGTTGTTATATTTACTGATAAAATTTTGTGCAATAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 1678

```

Fig. 1. Nucleotide and deduced amino acid sequences of *Pinctada fucata*  $\alpha$ -tubulin 2. A number of nucleotide and amino acid positions are indicated in the right margin. The stop codon is marked with star. Polyadenylation signal TAATTT is indicated as gray box shading. The thick line box represents the guanosine triphosphate nucleotide binding site (GGGTGS). The thin line box represents the MRECI sequence. Putative phosphorylation residues (R79 and K336) and putative acetylated residue K40 are indicated by a thin dashed line box. Genebank accession number:AB764096.

### 3.2 The multiple sequence alignment of *Pinctada fucata* $\alpha$ -tubulin 2 with different kinds of homologs and functional domain.

We assessed the amino acid multiple sequence alignment of *Pinctada fucata*  $\alpha$ -tubulin 2 gene obtained this time against the known other animal species  $\alpha$ -tubulin gene products and its homologs (Fig. 2). The *Pinctada fucata*  $\alpha$ -tubulin 2 showed about 85%, 95%, 96%, 96%, and 80% identity to those of *Homo sapiens*, gastropoda *Ilyanassa obsoleta*, bivalvia *Crassostrea gigas*, gastropoda *Patella vulgata*, and *Xenopus laevis*, respectively. As described in the previous chapter, we found the GGGTGS domain, and various posttranslational modification sites were also found. These are identical to all of them of the  $\alpha$ -tubulin 2 except an acetylation residue at K40 as shown in Fig. 1 (Fig. 2).

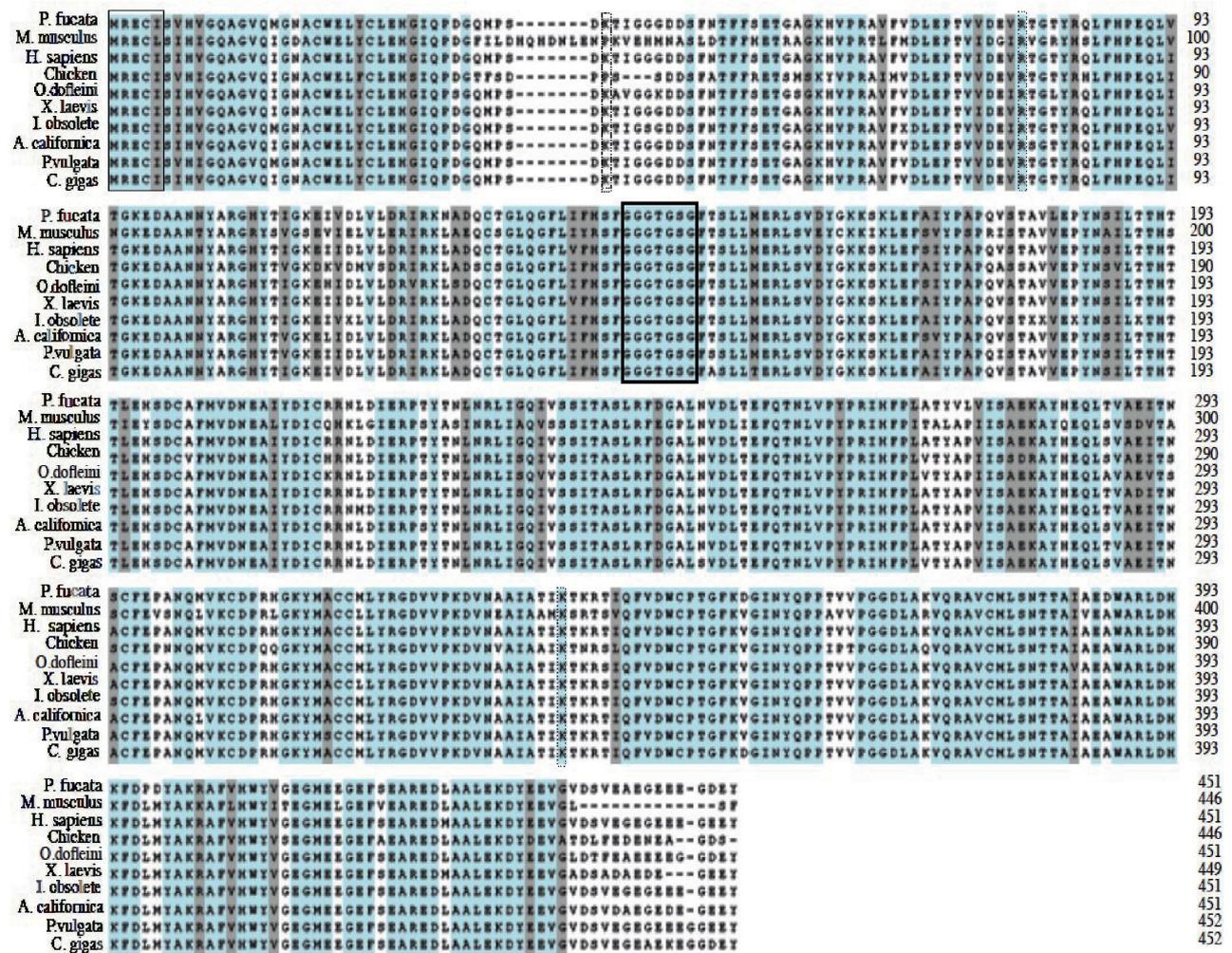


Fig. 2. Multiple amino acid sequence alignment of  $\alpha$ -tubulin. An analysis of sequence alignment of  $\alpha$ -tubulin of different vertebrate and invertebrate species was performed using Multiple Align Show ([http://www.bioinformatics.org/sms/multi\\_align.html](http://www.bioinformatics.org/sms/multi_align.html)). Identical amino acids are indicated by lightest blue shading. Similar amino acids are indicated by light gray shading. A number of amino acid positions are indicated in the right margin. Functional domain and putative posttranslational modification sites are shown completely similarly to Fig. 1. Abbreviations are as follows: *Homo sapiens*, *H. sapiens*; *Pinctada fucata*, *P. fucata*; *Mus musculus*, *M. musculus*; *Octopus doylei*, *O. doylei*; *Xenopus laevis*, *X. laevis*; *Ilyanassa obsoleta*, *I. obsoleta*; *Aplysia californica*, *A. californica*; *Petella vulgata*, *P. vulgata*; *Crassostrea gigas*, *C. gigas*.

It is well known that the synthesis of  $\alpha$ - and  $\beta$ -tubulin is autoregulated by a posttranscriptional mechanism. That is, a fundamental quantity of these proteins are automatically adjusted according to the unassembled tubulin subunit concentration via selectively altering  $\alpha$ - and  $\beta$ -tubulin mRNA levels by degradation<sup>(20)</sup>. In the  $\beta$ -tubulin case, the amino-terminal tetrapeptide MR(E/D)I takes part in autoregulated post-transcriptional mechanisms<sup>(21)</sup>. Previous report showed that the amino-terminal tetrapeptide MR(E/D)I specifies cotranslational degradation of  $\beta$ -tubulin mRNA but not that of  $\alpha$ -tubulin mRNA<sup>(22)</sup>. Therefore, although  $\alpha$ -tubulin of various species have almost the same amino acids sequence MRECI at the aminoterminal region (Fig. 2), this sequence does not take part in the regulation of  $\alpha$ -tubulin mRNA degradation.

### 3.3 Phylogenetic analysis

In order to clarify the systematic and evolutionary position of *Pinctada fucata*  $\alpha$ -tubulin 2, we have constructed a phylogenetic tree. The amino acid sequences of the  $\alpha$ -tubulin were aligned using the CLUSTALW, and a phylogenetic tree was constructed using Treeview as described in Materials and methods. The result is shown in Fig. 3.

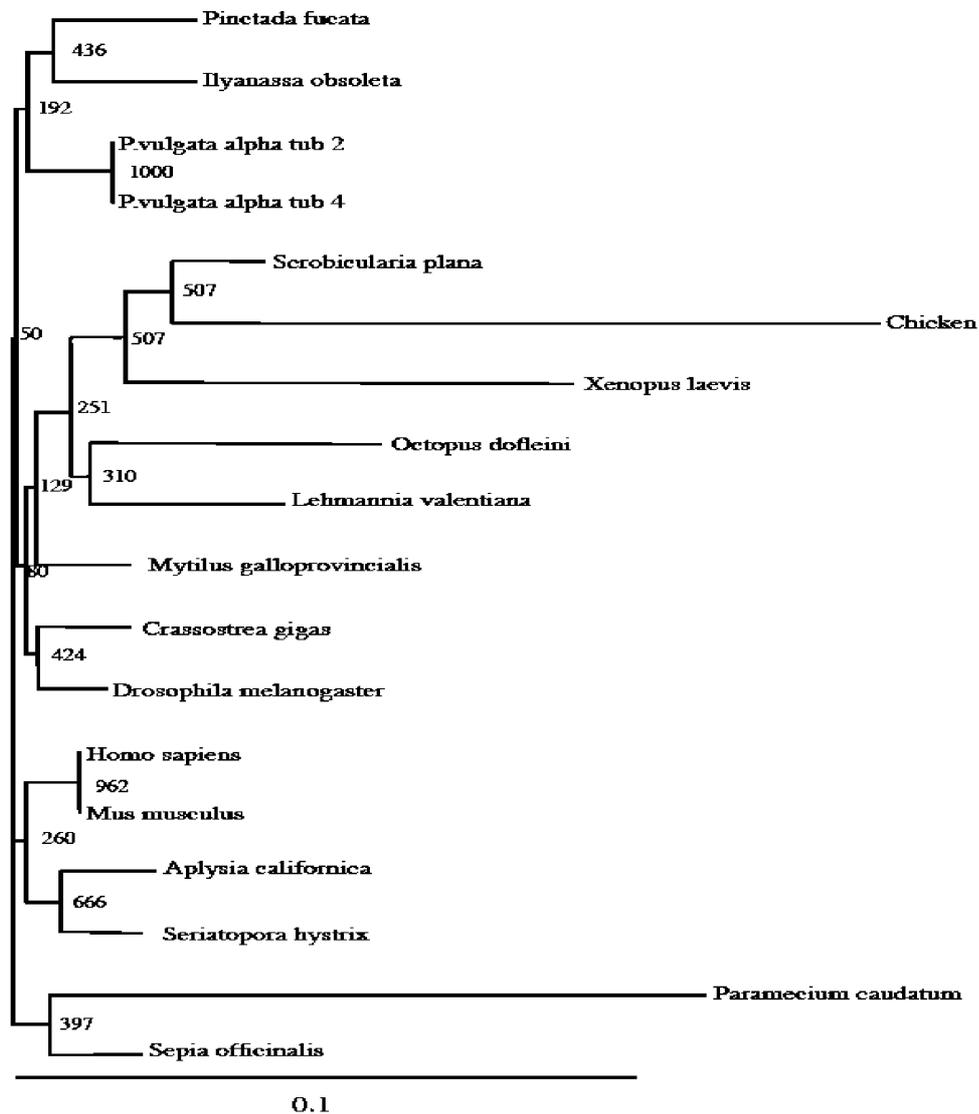


Fig. 3. Phylogenetic tree constructed by the NJ Method based on the amino-acid sequences. Numbers at the tree nodes indicate the bootstrap values from 1,000 replicates. All of these sequences used to make multiple sequence alignments were obtained from the EMBL/GenBank data bases. Genebank accession numbers are as follows: *Scrobicularia plana* alpha tubulin, JF274234; *Ilyanassa obsoleta* alpha tubulin-like protein, EU087595; *Lehmannia*

*valentiana* alpha-tubulin, partial, AB099707; *Sepia officinalis* alpha-tubulin, HE687194; Chicken testis-specific alpha-tubulin, M16030; *Seriatopora hystrix* alpha-tubulin, HM147129; *Paramecium caudatum* alpha-tubulin, AB035413; *Crassostrea gigas* alpha-tubulin, AB196533; Homo sapiens alpha 1a tubulin, NM\_006009; *Mus musculus* alpha 1A tubulin, NM\_011653; *Xenopus laevis* alpha 1a tubulin, NM\_001091468; *Aplysia californica* alpha tubulin 1, AF481055; *Octopus dofleini* alpha-tubulin, L10110; *P. vulgata* alpha tubulin 2, X79468; *Mytilus galloprovincialis* alpha-tubulin, partial, HM537081; *P. vulgata* alpha tubulin 4, X77618; *Drosophila melanogaster* alpha-1 tubulin; M14643. Branch length indicates evolutionary distance and the scale bar represents an evolutionary distance of 0.1 amino acid substitution per protein.

In this Phylogenetic tree, bivalve *Pinctada fucata*  $\alpha$ -tubulin 2 and gastropoda *Ilyanassa obsoleta*  $\alpha$ -tubulin split from the same node, suggesting that these  $\alpha$ -tubulin belong to the same clade. As well, two gastropoda *Patella vulgata*  $\alpha$ -tubulins split from the same node and have the same root as that of *Pinctada fucata* and *Ilyanassa obsoleta*. Therefore these four  $\alpha$ -tubulins have a common ancestor. The  $\alpha$ -tubulin of other mollusk bivalves *Scrobicularia plana*, *Crassostrea gigas*, and *Mytilus galloprovincialis* belong to another clade different from that of the *Pinctada fucata*. These results suggested that bivalvia *Pinctada fucata*  $\alpha$ -tubulin 2 is closer to a gastropoda snail  $\alpha$ -tubulin than that of a bivalvia. Furthermore, the phylogenetic tree produced by the UPGMA method using the site (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>) was also the same result, and was close to a gastropoda snail  $\alpha$ -tubulin than that of bivalvia. These results were unexpected although the reason is unknown. The elucidation of the evolutionary relationship of a gastropoda snail  $\alpha$ -tubulin and a bivalvia  $\alpha$ -tubulin is a future subject.

### 3. 4 Expression of $\alpha$ -tubulin 2 gene

The  $\alpha$ -tubulin 2 is transcribed actively in mantle edge, gill, and adductor muscle. The expression was also observed in the liver at a low level. On the other hand, almost no expression of the mRNA was observed in mantle pallial and foot (Fig. 4). The  $\alpha$ -tubulin gene unexpressed in certain tissues is generally to be observed. In such a case, other members of the  $\alpha$ -tubulin family are expressed<sup>(23, 24)</sup>. Therefore, it is expected that other members of the  $\alpha$ -tubulin family of *Pinctada fucata* are expressed in mantle pallial and foot. Such a phenomenon is known well.

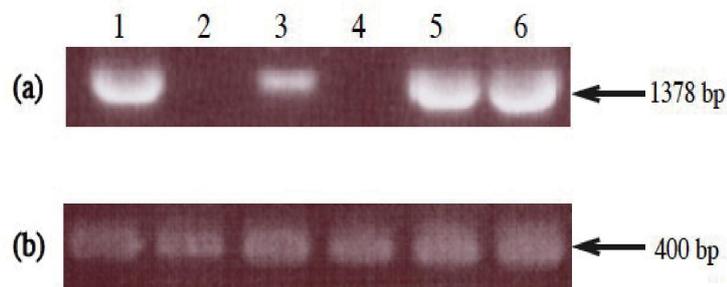


Fig. 4. RT-PCR analysis of the transcripts. (a) *P. fucata*  $\alpha$ -tubulin 2 gene. (b)  $\beta$ -actin gene. lane 1, mantle edge; lane 2, mantle pallial; lane 3, liver; lane 4, foot; lane 5, gill; lane 6, adductor muscle. The housekeeping gene  $\beta$ -actin was included as a positive control.

### 4. References

- (1) Nogales, E., (2000) Structural insights into microtubule function, *Annu Rev Biochem*, vol. 69, pp. 277-302.
- (2) Gadde, S., and R. Heald, R., (2004) Mechanisms and molecules of the mitotic spindle, *Curr. Biol*, vol. 14, pp. R797-805.
- (3) Desai, A., and Mitchison, T.J., (1997) Microtubule polymerization dynamics, *Annu Rev Cell Dev Biol*, vol. 13, pp. 83-117.
- (4) Erickson, H.P., and O'Brien, E.T., (1992) Microtubule Dynamic Instability and GTP Hydrolysis. *Annual Review of Biophysics and Biomolecular Structure*, vol. 21, pp. 145-166.
- (5) McNally, F.J., (1999) Microtubule dynamics: Controlling split ends, *Curr Biol*, vol. 9, pp. R274-276.
- (6) Alliegro, M.C., and Alliegro, M.A., (2005) Differential expression of tyrosinated tubulin in *Spisula Spisulasoli solidissima* polar bodies, *Dev Dyn*, vol. 232, pp. 216-220.
- (7) Verhey, K.J., and Gaertig, J., (2007) The tubulin code, *Cell Cycle*, vol. 6, pp. 2152-2160.

- 
- (8) Hammond, J.W., Cai, D., and Verhey, K.J., (2008) Tubulin modifications and their cellular functions, *Curr Opin Cell Biol*, vol. 20, pp. 71-76.
  - (9) Janke, C., and Kneusse, M., (2010) Tubulin post-translational modifications: encoding functions on the neuronal microtubule cytoskeleton, *Trends in Neurosciences*, vol. 33, pp. 362-372.
  - (10) Wloga, D., and Gaertig, J., (2010) Post-translational modifications of microtubules, *J Cell Sci*, vol. 123, pp. 3447-3455.
  - (11) Wloga, D., and Gaertig, J., (2011) Post-translational modifications of microtubules, *J. Cell Sci*, vol. 123, pp. 3447-3455.
  - (12) Garnham, C.P., and Roll-Mecak, A., (2012) The chemical complexity of cellular microtubules: tubulin post-translational modification enzymes and their roles in tuning microtubule functions, *Cytoskeleton (Hoboken)*, vol. 69, pp. 442-463.
  - (13) Miyashita, T., Hanashita, T., Toriyama, M., Takagi, R., Akashika, T., and Higashikubo, N., (2008) Gene cloning and biochemical characterization of the BMP-2 of *Pinctada fucata*, *Biosci Biotechnol Biochem*, vol. 72, pp. 37-47.
  - (14) Thompson, J.D., Higgin, D.G., and Gibson, T.J., (1994) Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice, *Nucleic Acids Research*, vol. 22, pp. 4673-4680.
  - (15) Perriere, G., and Gouy, M., (1996) WWW-Query: an online retrieval system for biological sequence banks, *Biochimie*, vol. 78, pp. 364-369.
  - (16) Page, R.D., (1996) TreeView: an application to display phylogenetic trees on personal computers, *Comput. Appl. Biosci*, vol. 12, pp. 357-358.
  - (17) Suzuki, M., Sakuda, S., and Nagasawa, H., (2007) Identification of chitin in the prismatic layer of the shell and a chitin synthase gene from the Japanese pearl oyster, *Pinctada fucata*, *Biosci Biotechnol Biochem*. Vol. 71, pp. 1735-1744.
  - (18) Beaudoin, E., Freier, S., Wyatt, J.R., Claverie, J.M., and Gautheret, D., (2000) Patterns of variant polyadenylation signal usage in human genes, *Genome Res*, vol. 10, pp. 1001-1010.
  - (19) Kirschner, M., and Mitchison, T., (1986) Beyond self-assembly: from microtubules to morphogenesis, *Cell*, vol. 45, pp. 329-342.
  - (20) Theodorakis, N.G., and Cleveland, D.W., (1993) Translationally coupled degradation of tubulin mRNA, In Belasco, J., and Brawermann, G., (ed.), *Control of messenger RNA stability*, Academic Press, San Diego. pp. 219-328.
  - (21) Cleveland, D.W., (1989) Autoregulated control of tubulin synthesis in animal cells, *Current Opinion in Cell Biology*, vol. 1, pp. 10-14.
  - (22) Bachurski, C.J., Theodorakis, N.G., Coulson, R.M., and Cleveland, D.W., (1994) An amino-terminal tetrapeptide specifies cotranslational degradation of beta-tubulin but not alpha-tubulin mRNAs, *Mol Cell Biol*, vol. 14, pp. 4076-4086.
  - (23) Lewis, S.A., Lee, M.G., and Cowan, N.J., (1985) Five mouse tubulin isotypes and their regulated expression during development, *J Cell Biol*. Vol. 101, pp. 852-861.
  - (24) Theurkauf, W. E., Baum, H., Bo, J., and Wensink, P.C., (1986) Tissue-specific and constitutive alpha-tubulin genes of *Drosophila melanogaster* code for structurally distinct proteins. *Proc Natl Acad Sci U S A*. vol. 83, pp. 8477-8481.

## 和文抄録

## アコヤ（真珠）貝アルファチューブリン遺伝子の単離およびその特徴

宮下 知幸<sup>1</sup>、高見 晶子<sup>1</sup>、杉本 唯<sup>1</sup>、酒井 亜由美<sup>1</sup>、高木 良介<sup>1</sup>

真珠貝であるアコヤ貝アルファチューブリン遺伝子を単離した。この遺伝子の長さは1678 bpで、451個のアミノ酸から構成される大きさ42 kDaのタンパク質をコードしており、アルファチューブリン2と命名した。アミノ酸配列中には、 $\alpha$ - $\beta$ -チューブリンの重合に必要なグアノシン3リン酸が結合する部位が存在する。さらに、翻訳後修飾部位として、リン酸化を受ける79番目と336番目の残基であるアルギニンとリジンが、また、アセチル化を受ける40番目の残基であるリジンが存在する。近隣結合法（NJ法）による系統樹は二枚貝であるアコヤ貝より単離したこのアルファチューブリンは二枚貝より巻貝のアルファチューブリンに近いことを示した。RT-PCR法でmRNAの発現部位を解析した結果、外套膜縁、貝柱、鰓および肝臓で発現が観察されたが、足と外套縁膜では発現していなかった。これら二つの組織ではファミリーの他のアルファチューブリン遺伝子が発現していると考えられる。

キーワード：アルファチューブリン、cDNA、アコヤ貝、二枚貝

---

1. 近畿大学生物理工学部遺伝子工学科、〒649-6493 和歌山県紀の川市西三谷930