

Molecular characterization of tubulin genes of the pacific oyster *Crassostrea gigas*

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Abstract

We have isolated two cDNAs encoding α - and β -tubulin from mollusc *Crassostrea gigas*. Both of the conceptual proteins exhibit significant sequence similarity to α - and β -tubulins in metazoan respectively. In contrast to overall conservation of the sequences, amino acid substitutions are largely clustered at the carboxyl terminus, which is most similar to that of mollusc *Patella vulgata*. Sequence identity between α and β is 40%, which suggests that the α and β tubulin genes have diverged before the separation of the mollusc line.

1. Introduction

Microtubules are not randomly distributed in cells but exist as highly ordered arrays and involved in a number of cellular functions including flagellar motility, chromosome segregation, mitosis and intracellular transport⁽¹⁾. Tubulin, the subunit of microtubules, is a heterodimer of two polypeptides, designated α and β which are the most abundant in the eukaryotic cell and have been studied most extensively⁽²⁾. The α β subunit assembles with tissue-specific microtubule-associated proteins (MAPs) to form microtubules⁽³⁾. Thus, functional specialization reflects in the structures composed of α - and β -tubulins and MAPs. Studies of tubulin proteins and genes from a wide variety of species indicate that the primary structure of tubulin is highly conserved among species. Each tubulin subunit contains a single binding site for GTP and its hydrolysis on β -tubulin is associated with tubulin polymerization and appears to play an important role in regulating microtubule assembly⁽⁴⁾. These sites for GTP binding are found in regions of high similarity between α - and β -tubulin and have been conserved throughout evolution. The carboxyl-terminal region of both α - and β -tubulin varies among different tubulin gene products^(2, 5, 6), suggesting that tubulin genes encode functionally distinct protein isotypes in most eukaryotes. In addition to heterogeneity of tubulin genes, tubulin proteins undergo a number of post-translational modifications that include tyrosination, acetylation of α -tubulin, phosphorylation of β -tubulin, and glutamination of both α and β ⁽⁷⁾.

Sequence analyses of tubulin genes have been done in animals, plants, fungi and protists⁽⁷⁾. These analyses enable estimation of the evolutionary process of tubulin genes and phylogenesis in eukaryotes. In vertebrates, tubulin genes are reported in many species including human, rat, chicken, *Xenopus*, and mouse, in which the most extensive data are obtained. In invertebrates, *Drosophila* is best studied on the structure and function of tubulins^(8, 9), and in addition, echinoderm has been one of the useful system in pursuing the behavior of tubulin in the process of early cleavage⁽¹⁰⁾.

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Mollusca, the second animal phylum after Arthropoda, contains over 50,000 described living species and 70,000 known fossils, of which size ranges from microscopic bivalves to giant clams that reach 1 m in length, to giant squids reaching 20 m in overall length. In this work, we report the cloning and sequencing of α - and β -tubulin genes from the bivalve pacific oyster, *Crassostrea gigas*. The both tubulin genes are very similar to other counterpart of tubulins in animals, indicating that α - and β -tubulin genes was duplicated prior to at least bivalve evolution.

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1  AGTTTGAATTTGTGCGAGACGCGTTACACATCTCAGTGCAAAGTCCATATTTATCCGTCACCTTCTTCCAAGGATTTAAAAAAGAAATTC
91  TGTCAGAATGCGTGAGTGTATATCTATTCTATGTTGGACAAGCTGGTGTCCAGATTGGAAATGCCTGTCTGGGAGCTGTACTGCTTGGAGCA
    M R E C I S I H V G Q A G V Q I G N A C W E L Y C L E H      28
181  CGGCATTACGCCAGATGGGCAGATGCCAAGTGACAAGACAATGGAGGGGAGATGATTCATTCAACACCTTTTTCAGTGAAGCTGGTGC
    G I Q P D G Q M P S D K T I G G G D D S F N T F F S E T G A      58
271  TGGCAAGCATGTACCAAGGGCTGTATTGTAGACCTGGAGCCCAGTGTGTTGATGAGGTGCGCACAGGGACGTACCGCCAACTTTTCCA
    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      88
361  TCCAGAACAGCTGATAACTGGAAAAGAGGATGCTGCCAACACTATGCAAGAGGTCACCTACACCATTGGAAAGGAAATTTGTGGACTTGGT
    P E Q L I T G K E D A A N N Y A R G H Y T I G K E I V D L V      118
451  TTTGGATCGCATCAGAAAATTTGGCTGACCAATGCATGGTCTTCAAGGGTTCCTGATTTTTCACAGCTTTGGAGGAGGAAGCTGGTTCGG
    L D R I R K L A D Q C T G L Q G F L I F H S F G G G T G S G      148
541  ATTTGCCTCCCTTCTGACGGAGAGACTGTCTGTTGATTATGAAAGAAGTCCAAGCTTGAATTTGCCATTTACCTGTCTCTCAGGTATC
    F A S L L T E R L S V D Y G K K S K L E F A I Y P A P Q V S      178
631  TACAGCAGTAGTTGAGCCATAACAATTCATCCTTACCACACACTACTCTGGAGCATTCCGACTGTGCCTTCATGGTGTGACAATGAGGC
    T A V V E P Y N S I L T T H T T L E H S D C A F M V D N E A      208
721  TATCTATGATATTTGCCGAAGGAACCTGGACATTGAGAGGCCAACATACCCAACCTGAAACCGCCTGATTGGTCAGATTGTTAGTTCAT
    I Y D I C R R N L D I E R P T Y T N L N R L I G Q I V S S I      238
811  CACAGCCTCCCTTCGATTTGATGGAGCCTTGAGTGTGGACCTGACAGAGTTCAGACAAATCTTGTACCTTACCACGATTCACCTTTCC
    T A S L R F D G A L S V D L T E F Q T N L V P Y P R I H F P      268
901  ATTGGTGACCTACGCTCTGTCTATCTGTCAGAGAAGGCCTACCATGAACAGCTGTGAGTAGCGGAAATTACCAATGCGTGCTTTGAACC
    L V T Y A P V I S A E K A Y H E Q L S V A E I T N A C F E P      298
991  AGCAAAATCAAAATGGTGAATGTGATCCAGACATGGCAAGTACATGGCTTGTGTCATGTGTACAGAGGGGATGTTGTACCTAAGGATGT
    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 V P K D V      328
1081  CAATGCTGCCATTGCCACCATCAAGACAAAGAGAACCATTAGTTTGGGATGGTGTCTACTGGTTTCAAAGATGGCATCAACTACCA
    N 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      358
1171  GCCACCAACTGTTGTTCTGGAGGTGACCTTGCCAAGGTACAGAGAGCTGTCTGCATGTTGAGTAACACCACTGCCATTGCTGAGGCGCTG
    P P T V V P G G D L A K V Q R A V C M L S N T T A I A E A W      388
1261  GGCTCGTCTGGATCACAAGTTTGACCTGATGATGCCAAGCGTGCCTTTGTTCACTGGTATGTGGAGAGGGAATGGAAGAAGGTGAATT
    A R L D H K F D L M Y A K R A F V H W Y V G E G M E E G E F      418
1351  CTCTGAGGCCCGTGAAGATCTGGCTGCCTTGGAGAAGGATTATGAAGAAGTGGGTGTGGATTCTGTTGAGGGAGAGGCTGAAAAGGAAGG
    S E A R E D L A A L E K D Y E E V G V D S V E G E A E K E G      448
1441  TGGTGATGAGTACTAAAGACTTGGTTGTTAATTTGGATCTTCAAAGACGTTTATAATTTGTATGTTAATCAGTTTACAAAATATTTTG
    G D E Y *
1531  GCAACTTTGATTCCTCAACTGTTCACTGTTAAAGAGAGAAAATAAATTTCTGATATTACTGTGT

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Fig. 1 Nucleotide and deduced amino acid sequence of α -tubulin of *C. gigas*.

The asterisk indicates a stop codon. A conceptual polyadenylation signal is underlined. DDBJ/EMBL/GenBank accession number: AB196533

2. Materials and Methods

Sequencing. The nucleotide sequences of cDNAs cloned into pT7Blue vector were determined with a Big-Dye terminator kit and ABI 377 DNA sequencer (PE Biosystems). Each sequence was translated into amino acid sequence in six frames and subjected to search for similarity against protein database using the BLAST program.

Construction and screening of a cDNA library. 2 μ g of poly(A)⁺ RNA obtained from the mantle was used for the cDNA library construction using a SMART cDNA library Construction Kit (Clontch). No.15 and 391 cDNA fragments were used to screen the cDNA library as probes.

5'-RACE. RACE method is performed according to the manufactures instructions (Clontech) using 2 μ g of mantle poly(A)⁺RNA. As specific primers in the nucleotide sequences, an antisense primer 5'-CTGTGTAGTGGCCCTTGGCCAGTT-3' for No.15 and 5'-CTGATGCGATCCAAAACCAAGTCCA-3' for No.391 were used. The amplified products after PCR were cloned into the pGEM T easy TA cloning vector.

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1  AGTTATCTTAATTCATTAACAACTAAGGGAGGGCTAGGGCCGGATTCTGTGAATTTACAACAAACACACCAACAAAATGAGGGAAATTTGTGCA
                                     M R E I V H 6
91  TATGCAAGCTGGCCAGTGCGGAAACCAGATTGGTGCTAAATTTCTGGGAAGTGATATCTGATGAACACGGCATTGACCCAACAGGAACCTA
M Q A G Q C G N Q I G A K F W E V I S D E H G I D P T G T Y 36
181  TCATGGAGACTCAGACTTGCAGTTAGAAAAGAATTAATGTCTACTACAATGAAGCAACAGGTGAAAAATGTACCTCGTAGCATTCTTAT
H G D S D L Q L E R I N V Y Y N E A T G G K Y V P R S I L I 66
271  CGATCTTGGACCCAGGAACCATGGACTCAGTCCGATCAGGCCCATTCGGACAAAATTTTCAGACCAGACAACCTCGTGTTCGGACAAAGCGG
N L E P G T M D S V R S G P F G Q I F R P D N G F R P Q S G 96
361  AGCAGGAAACAACCTGGGCCAAGGGCCACTACACAGAGGGAGCCGAATTTGATCGACTCAGCTTTGGATGTTGTCAGAAAAGGAGGGCGAAAG
A G N N W A K G H Y T E G A E L I D S A L D V V R K E A E S 126
451  CTGTGACTGTATTACAGGGATTTCAACTTACACACTCATTGGGCGGGGGCCTGGTGTGGTATGGGAACACTACTCATCAGCAAAATCCG
C D C I Q G F Q L T H S L G G G T G A G M G T L L I S K I R 156
541  CGAGGAATACCCCGACAGAAATCATGAACACTTTTCCGTTGCCCCATCTCCAAAAGTATCCGACACCGTGGTGGAAACCTTACAACGCTAC
E E Y P D R I M N T F S V V P S P K V S D T V V E P Y N A T 186
631  CCTCTCTGTTACCAACTTGTGAGAACACCGACGAAACATACTGCATTGATAACGAGGCTCTATATGACATCTGCTTCCGTACTACTCAA
L S V H Q L V E N T D E T Y C I D N E A L Y D I C F R T L K 216
721  ACTTACCACCCCAACATACGGGACCTCAACCATCTCATCTCAGCTACCATGTCGGAGTCACAACATGATGGCTGCCATCCCTCAAT
L T T P T Y G D L N H L I S A T M S G V T T C L R F P G Q L 246
811  GAACGCTGACTTAAGAAAGATCGCTGTCAACATGGTCCCTTCCCTCGTCTCCACTTCTCATGCTGGATTGCTCCATTGACATCAG
N A D L R K I A V N M V P F P R L H F F M P G F A P L T S R 276
901  TGGTAGCCAGCAGTACAGGGCTCTGACCGTCCAGAACTGACCCAGCAGATCTTCGATGCCAAGAACATGATGGCTGCCATCCAG
G S Q Q Y R A L T V P E L T Q Q I F D A K N M M A A C D P R 306
991  TCACGGAAGATACTTAACCTGTCAGCGCCCTCTTCCGTGGACGATGTCAATGAAAGAGTTGACGAACAGATGTTGAACGTCAGAACAA
H G R Y L T V S A L F R G R M S M K E V D E Q M L N V Q N K 336
1081  GAACAGCAGCTACTTCGTGGAAATGGATCCCAACACGTCGAAGACCGCGTCTGTGACATCCCAACCGTGGTCTGAAAATGTCCGCCAC
N S S Y F V E W I P N N V K T A V C D I P P R G L K M S A T 366
1171  CTTCTCGGAAACACAACCTGCCATCCAGGAATCTTCAACACGGTGTCTGAACAATTCAGTCCATGTTCCGTCGTAAGGTTTTCTTACA
F V G N T T A I Q E L F K R V S E Q F T A M F R R K A F L H 396
1261  TTGGTACACTGGTGAAGGATGGACGAGATGGAGTTTACTGAGCCGAGTCCCAACATGAACGATTTGGTGTGGGTACCAACAGTACCA
W Y T G E G M D E M E F T E A E S N M N D L V S G Y Q Q Y Q 426
1351  GGACGCCACCGCCGAGGAGGGGGAGTTTGGAGGGAAGAGGGAGGAGGCGCAATAAACATTAATTAACGCAGCAATTTTGTAG
D A T A E E E G E F E E E G E E A Q * 446
1441  GTCATCCGTCATTTATATTTACTAGTTACACTGAAAATTTATAGAGATATTAGGACAATCTAATTAACAATTTGTAATACGCAATGAG
1531  CTGTTGACATGTCATCTACAGTACACAAATTTCTTGTAGCAAAAGCTCTGAACATAAAATAAACAGGATATCAAATACC

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Fig. 2 Nucleotide and deduced amino acid sequence of β -tubulin of *C. gigas*.

The asterisk indicates a stop codon. A conceptual polyadenylation signal is underlined. DDBJ/EMBL/GenBank accession number: AB196534

3. Results and Discussion

The expression profile of the mantle of mollusc is largely unknown. Then, the poor understanding of molecular events responsible for shell formation, which is regulated by proteins produced from the mantle, prompted us to identify tissue specific genes expressed in the mantle of pacific oyster

Crassostrea gigas⁽¹¹⁾. Results from this analysis identified several cytoskeletal genes, in which No. 15 and No. 391 cDNAs were both similar to tubulin genes. To gain information about the primary structure of the two genes, we screened a cDNA library constructed from the mantle of *C. gigas*. To know the whole structure of 5'-end, we isolated the 5'-cDNAs by the 5'-RACE method using oligonucleotides specific to the sequence of No.15 and 391 cDNAs as primers.

The entire sequence of No. 391 was 1593 bp in length and followed by poly-A tail. Untranslated sequences of 97 bp in 5'-end and 140 bp in 3'-end were found, and conceptual translation indicates that there is a single open reading frame which encodes a predicted 452 amino acid protein. The sequence was shown to be highly homologous to various known α -tubulins in metazoans by the FASTA program. To mouse and *Drosophila*, similar identity (94-98%) was found, and as reported, amino acid substitutions are largely clustered at the carboxyl terminus. The C-terminal sequence (DYEEVGVDSEGEAEKEGGD) was most similar to that of mollusc *Patella vulgata*⁽¹²⁾.

The cDNA clone No. 15 was 1593 bp in length and followed by poly-A tail. A single open reading frame of 446 amino acids was found in this cDNA. The sequence of 446 amino acid protein was highly similar to those of β -tubulin proteins in animals. The predicted protein sequence of No. 15 was most similar to β -tubulins in mouse, *Xenopus*, and *P. vulgata*, which showed 94% identity. The C-terminal sequence (EEEEFEFEFEFEAEQ) was most similar to that of mollusc *P. vulgata*.

In contrast to the above sequence conservation of No. 391 and 15 in α and β orthologous tubulin genes, identity between No. 391 and 15 was lower (40%), suggesting that the α and β -tubulin genes diverged before the separation of the mollusc line. Analysis of the tubulin gene family in invertebrates has indicated the presence of different forms of tubulin. *Drosophila* have four genes for α -tubulin and four for β -tubulin, which are expressed in different tissues or cell types^(8,9). Similarly, the No. 391 and 15 genes may be a member of multi-gene family and function differently in various tissues including the mantle of molluscs.

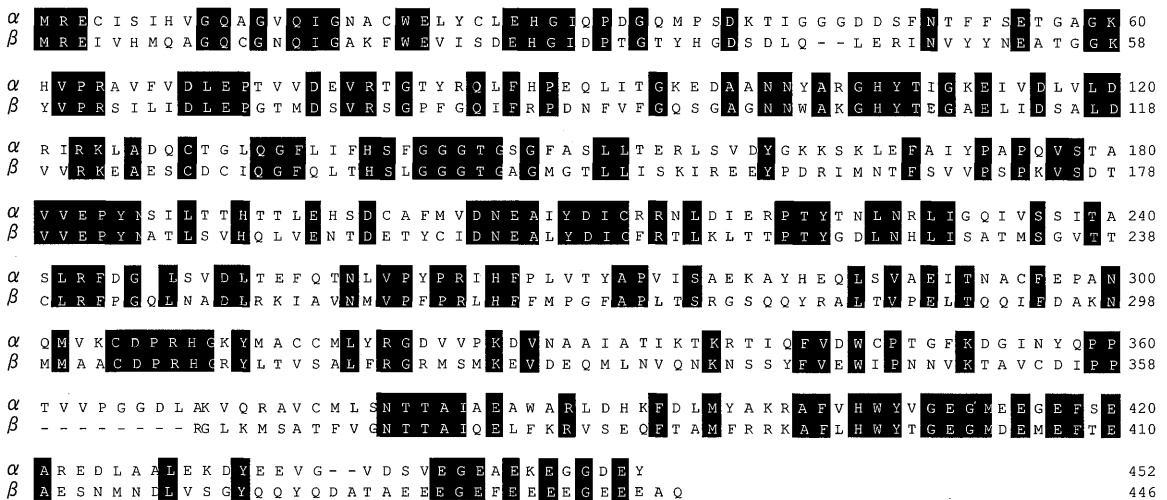


Fig. 3 Comparison of the deduced protein sequences of α - and β -tubulin of *C. gigas*.

The residues are boxed when conserved between the sequences. A dash indicates a gap added to the sequence.

4. Acknowledgments

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5. References

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和文抄録

マガキチューブリン遺伝子の解析

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牡蛎 (*Crassostrea gigas*) の外套膜の cDNA ライブラリーから単離した cDNA No. 15 と 391 はそれぞれ、チューブリン遺伝子との相同性が見られた。完全長の cDNA の単離、塩基配列の決定後、各種後生動物のチューブリン遺伝子との相同性検索を行ったところ、No. 15 は β -チューブリンと、No. 391 は α -チューブリンとそれぞれアミノ酸レベルで 90%以上の一致を示すことが明らかとなった。No. 15 と No. 391 の間ではアミノ酸レベルで 40%の一致であり、この二つの遺伝子は、軟体動物の分岐以前に分かれたと推測される。