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Characterization of a tropomyosin cDNA from the hydrozoan *Podocoryne carnea**

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A cDNA clone from the hydrozoan *Podocoryne carnea* was characterized. It consists of an open reading frame of 726 nt flanked by a 84 nt 5′ and a 307 nt 3′ untranslated region. The corresponding gene exists apparently as a single copy. The transcript is ubiquitously expressed in the polyp and the medusa stage. Several features of the predicted peptide sequence indicate a relationship to tropomyosins. At the amino acid level it shares 26–30% identical residues with other invertebrate and vertebrate tropomyosin sequences.

Tropomyosin; cDNA; Hydrozoa; *Podocoryne carnea*

1. INTRODUCTION

Tropomyosins are a highly conserved family of actin filament binding proteins found in all eukaryotic cells [1]. Cell type specific expression is achieved by a combination of multiple genes, which either contain alternative promoters or exhibit alternative splicing patterns of primary RNA transcripts. So far, several tropomyosins encoded by 4 highly conserved genes have been described from various vertebrate species. Presumably they evolved by duplication from a common ancestor [1]. In contrast, only a few invertebrate tropomyosins are known. Recently, we succeeded in isolating a tropomyosin cDNA clone from the coelenterate *Podocoryne carnea* (PcTpm1). *Podocoryne* is a marine hydrozoan (Phylum Cnidaria) with a biphasic life cycle, involving an asexually reproducing, sedentary polyp and a sexually reproducing, planktonic medusa stage [2].

2. MATERIALS AND METHODS

Cultures of *Podocoryne carnea* were maintained as described by Schmid [3]. Molecular biology methods were applied as suggested by Sambrook et al. [4], if not particularly mentioned. 1 μg of poly(A)$^+$ RNA from polyps, medusa buds and medusae was used to construct an oligo(dT) primed cDNA expression library in the vector lambda ZAP II according to the manual of the supplier (Stratagene, Heidelberg, Germany). Approximately one million recombinant clones were obtained and amplified. Whole mount in situ hybridization analysis of polyps and medusae was done according to the slightly modified method of Tautz and Pfeifle [5,6].

Computer sequence analyses were performed using the GCG program package [7]. Multiple sequence alignment of peptide sequences was generated by the CLUSTAL V package [8].

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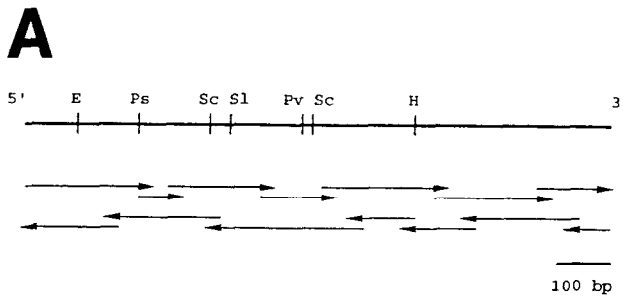
*The nucleotide sequence data reported in this paper have been deposited in the EMBL, GenBank and DDBJ nucleotide sequence databases under the accession number X71418.

3. RESULTS AND DISCUSSION

A cDNA clone with an insert of approximately 1.1 kb further referred to as PcTpm1 was obtained by random selection. Both strands of PcTpm1 were sequenced according to the strategy shown in Fig. 1A. The PcTpm1 insert has a length of 1120 nt. It apparently contains a full coding region with ATG as a putative start codon at position 85–87, and a TAA stop codon at position 811–813. The 726 nt long open reading frame, coding for a polypeptide of 242 amino acids, is flanked by a 84 nt 5′ and a 307 nt 3′ untranslated region including a 20 nt long poly(A) stretch (Fig. 1B).

A comparison of PcTpm1 at the nucleic acid level did not show any specific homology to other sequences. Therefore, it was not possible to identify the sequence at this level. However, a comparison of the complete deduced amino acid sequence revealed a 26–30% identity over its whole length to various types of known tropomyosins from several vertebrate and invertebrate species. Tropomyosins can be identified by a repeating 7-residue pattern of non-polar and polar or charged residues, characteristic for the stabilization of coiled-coil α- helical proteins [9]. A similar characteristic distribution of amino acid residues exists for PcTpm1 (Table I). However, the heptapeptide repeat structure is disrupted at several positions.

The predicted amino acid sequence of PcTpm1 was aligned to other tropomyosin sequences (Fig. 1C). The



B

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                                                                  TCCAGGTCC      9
CTGAGTTTAAGACGATAAAAACTTTCTTACTTTTTCTGTCATATTTGGTTGTTAACCTCACTACAAGAATTCAAA    84
ATGGACGCAATTAAAAAGAAAATGAGTGCAATGAAAACGAAACTTGAAGAAGCAGATAAACAAGCTCAGGACGCT   159
M  D  A  I  K  K  K  M  S  A  M  K  T  K  L  E  E  A  D  K  Q  A  Q  D  A     25

GAGGATGAATTAACAGCTACCCTTGAGAAGGCTGCTGAGACTGAACAAACTGCAGACGAATTGCAGAAAACTTTG   234
E  D  E  L  T  A  T  L  E  K  A  A  E  T  E  Q  T  A  D  E  L  Q  K  T  L     50

GCAGATTTGGAGGATGAGTTAGATGCTGCTGAATCCAGACTTACCTCACTTACAGAAAAATATAATGAAGAAGAA   309
A  D  L  E  D  E  L  D  A  A  E  S  R  L  T  S  L  T  E  K  Y  N  E  E  E     75

AAGAAAGCTGAGGAAGGAAGAAGAGCTCACAAAGAACTCGAAAATCGAGGCCAAACAGATTATAGTCGACTGAAT   384
K  K  A  E  E  G  R  R  A  H  K  E  L  E  N  R  G  Q  T  D  Y  S  R  L  N    100

AGGCTCGAAACTGAACTTGCGGAAATTACAGAACAAAACGAAGTAGTTGTTGAAAAACTTTCAGAACTCTCCTCA   459
R  L  E  T  E  L  A  E  I  T  E  Q  N  E  V  V  V  E  K  L  S  E  L  S  S    125

CAACTAGAAGAAAATGAAAGAATATTGGACGAAGAAGAAGAAAGATGTGCAACAGCTGACGCACAAGTCAAAGAG   534
Q  L  E  E  N  E  R  I  L  D  E  E  E  E  R  C  A  T  A  D  A  Q  V  K  E    150

CTCGAAGTTGATGTTGTTCAAGTTGGCAATCAACTGAGAAGCATGGAAATAAACGAAGAAAAAGCATCGAAAAGT   609
L  E  V  D  V  V  Q  V  G  N  Q  L  R  S  M  E  I  N  E  E  K  A  S  K  S    175

AACGACCAATCAGCGAATAAGTTAGAAGATACAATCGAAAAATACAACACTATAAAAGACCGTGCGGACGATGCG   684
N  D  Q  S  A  N  K  L  E  D  T  I  E  K  Y  N  T  I  K  D  R  A  D  D  A    200

GAAGCAAGATCAAGAGACTTGGAAGCAGAGTTGAACGAATGCGACGACGAACTAGCAGCGGCGAAAGAAGCTTAT   759
E  A  R  S  R  D  L  E  A  E  L  N  E  C  D  D  E  L  A  A  A  K  E  A  Y    225

GGTCAATCGAAAGCTGATATGGACGAATTACTGTTGGAACTAGCGTCAATGTAATGGAGGAAAAACGAGCAGAGA   834
G  Q  S  K  A  D  M  D  E  L  L  L  E  L  A  S  M  *                         242

CTTTGTATGGGTAAAATATACGCAAAATAAAAAATCAAATCTAAATGAAAGGGGTCGGGGTAATTTTTTTGAATA   909
ATATAAATTTATATGAGATACATTTATAATTTTTTTCCTCTGTTATTTAAATTATGTGAATACAAAGTAACGAGG   984
ACATCGTGTTAGTTGAAGAGTACGCTCTATATACATGTGACGCATCTTTCTTCCTGTATTTTGTTCGGTTTTTAC  1059
ATATGCTAACTTTTGTAGAAAAAACTTCAGCTTTTGTTTTTAAAAAAAAAAAAAAAAAAAA               1120
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C

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1   M------DAIKKKMSAMKTKLEEADKQAQD
2   M------DKIREKLSNLKLEAESWQEKYEE
3   M------DAIKKKMQAMKIEKDNALDRADA
4   M------DAIKKKMLAMKMEKENAIDRAEQ
5   M------DGIKKKMIAMKLEKENAMERAVQ
6   M------DAIKKKMQAMKLEKDNAIDKADT
7   M------DAIKKKMQAMKVDKDGALERALV
8   M------DAIKKKMQMLKLDKENALDRAEQ
9   M------DAIKKKMQMLKLDKENALDRAEQ
10  MAGISSIDAVKKKIQSLQQVADEAEERAEH
11  M------DAIKKKMQMLKLDKENALDRAEQ
12  M-----MEAIKKKMQMLKLDKENALDRAEQ
    *          *
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Fig. 1. (A) Partial restriction endonuclease map and the sequencing strategy used for the PcTpm1 cDNA. Restriction enzymes are: E, *Eco*RI; H, *Hin*dIII, Ps, *Pst*I; Pv, *Pvu*II; Sc, *Sac*I; Sl, *Sal*I. (B) Nucleotide and deduced amino acid sequence of PcTpm1. The sequences are numbered on the right of each line. The initiator ATG codon is in bold. The terminator codon is underlined twice and the AATAAA polyadenylation signal once. (C) Alignment of the first 24 amino acids deduced from the PcTpm1 cDNA (1) to several tropomyosins from other species; (2) tropomyosin from *Saccharomyces cerevisiae* (yeast), (3) tropomyosin from *Trichostrongylus colubriformis* (nematode); (4) tropomyosin from *Biomphalaria glabrata* (blood fluke); (5) tropomyosin from *Schistosoma mansoni* (blood fluke); (6) tropomyosin 1 and (7) tropomyosin 2 from *Drosophila melanogaster*; (8) tropomyosin a chain, skeletal muscle, and (9) skin fibroblast, from *Coturnix coturnix japonica* (Japanese quail); (10) tropomyosin β3, fibroblast, from *Gallus gallus* (chicken); (11) tropomyosin γ, smooth muscle, from *Coturnix coturnix japonica*; (12) tropomyosin, skeletal muscle, from *Homo sapiens*. (*) Identical amino acid residues, (.) conserved amino acid exchanges.

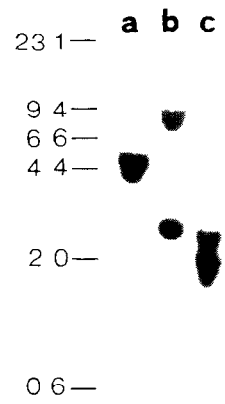

Fig. 2. Southern blot of *Podocoryne carnea* DNA digested with *Eco*RI (lane a), *Hin*dIII (lane b) or both enzymes in combination (lane c).

high conservation of the first 8 amino acid residues reported earlier [10] proved true for PcTpm1 as well. This part of the peptide sequence is involved in both the head-to- tail association of the protein to form filaments [9] and the binding of tropomyosin to actin and troponin T [11]. The remaining conserved residues are regularly distributed over the whole length of the sequences.

For Southern blot analysis, 3 μg of *Podocoryne* DNA were digested with *Eco*RI, *Hin*dIII or both enzymes in combination, fractionated by agarose gel electrophoresis and transferred onto a nylon membrane. The filters were hybridized with a ^{32}P-labelled PcTpm1 probe at 60°C (Fig. 2) and 50°C (not shown). Both temperatures gave identical results. Whereas after *Eco*RI digestion a single signal lit up at a size corresponding to 4.5 kb, two bands were obtained with *Hin*dIII (7.2 and 2.7 kb) and both enzymes in combination (2.6 and 2.1 kb). These results suggest that (1) the PcTpm1 probe is gene specific, and (2) there are no related genes which cross-hybridize at low stringency conditions. This observation is in contrast to the situation in vertebrates, where the tropomyosin diversity is based on a complement of 4 different, but highly related genes. However, up to now, only two tropomyosin genes have been identified in *Drosophila melanogaster* [12,13] and only one per genome for other invertebrates as the trematode *Trichostrongylus colubriformis* [14] or *Caenorhabditis elegans* [15].

In order to investigate the life stage and tissue specific expression of the transcript, Northern blot analysis and in situ hybridization studies were performed. A formaldehyde RNA gel was run with 4.5 μg of total RNA from gasterozoids (feeding polyps) and medusae and transferred onto a nylon membrane. High stringency hybridization (60°C) with a ^{32}P-labelled PcTpm1 probe revealed the presence of a corresponding mRNA of about 1200 nt in both gasterozoids and medusae (Fig. 3). Polyps and medusae were prepared for whole mount in situ hybridization with a digoxigenin labelled PcTpm1 probe. As a control a striated muscle specific myosin heavy chain probe was used [6]. Whereas the control probe specifically stained the striated muscle cell layer in medusae, an overall expression of the PcTpm1 message could be detected in both polyps and medusae (results not shown). Therefore, a life stage or cell type specific function of PcTpm1, as has been reported for skeletal muscle tropomyosins involved in the Ca^{2+} mediated regulation of muscle contraction [16] can not be assumed.

The relationship of PcTpm1 to other tropomyosins (shown in Fig. 1C) was investigated by means of a multiple alignment of peptide sequences [8]. With 73–76% of divergence, the *Saccharomyces cerevisiae* tropomyosin is far apart from the tropomyosins of other organisms. The PcTpm1 peptide is slightly less distant from the other sequences compared (65–68%). However, except for the *S. cerevisiae* tropomyosin, all other tropomyosins included in this analysis are much more

Table I

Distribution of nonpolar, polar, acidic and basic residues in the repetitive heptapeptide deduced from the PcTpm1 cDNA

Amino acid	Position in heptapeptide						
	a	b	c	d	e	f	g
Nonpolar	**28**	7	6	**24**	6	7	3
Polar	5	11	9	8	6	9	9
Acidic	1	13	18	3	**16**	11	8
Basic	1	4	2	0	6	4	**14**

The 7 residues are designated a to g. Nonpolar residues found in a high proportion in positions a and d and acidic and basic residues accumulated in positions e and g, respectively, are shown in bold.

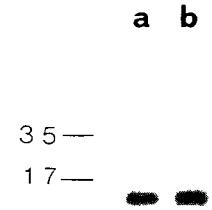

Fig. 3. Northern blot with total RNA from gasterozoids (lane a) and medusae (lane b).

related to each other than they are to the PcTpm1 sequence. Considering the phylogenetic distance of the cnidarians to other animals [17,18,19], this result is not surprising.

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