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Acanthamoeba healyi: Molecular cloning and characterization of a coronin homologue, an actin-related protein ☆

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Abstract

Coronin, described in organisms from yeasts to humans, has been found to be involved in various actin-associated activities. It has yet to be described in *Acanthamoeba*, medically significant as the causative agent of granulomatous amebic encephalitis and amoebic keratitis and used extensively in actin-related studies. We isolated and characterized a cDNA encoding a coronin-like protein in *A. healyi* by sequence analysis and demonstrated intracellular localization of the gene product by transient transfection. Named Ahcoronin, the gene is composed of 454 amino acids which contain the characteristic WD repeats of coronin and coronin-like proteins. The C-terminal region of the gene was also predicted to have a high tendency of forming a coiled-coil, another structural characteristic of coronin. The gene showed a 50% homology to coronins. Ahcoronin occurs as a single copy and expressed as a transcript of approximately 1.4 kb in *A. healyi*. Results of transfection showed that *Ahcoronin* was localized in the cell's periphery and in the leading edge consistent to that of actin. The fusion protein has also been observed to localize around phagocytic cups but was disassembled later during phagocytosis. Sequence analysis of Ahcoronin homologue of *A. healyi* showed numerous potential for further studies and is sure to contribute in the growing interest toward the properties and functions of coronin and coronin-like proteins.

Keywords: Actin-binding protein; Locomotion; Phagocytosis; Transfection

1. Introduction

Since coronin was first reported in *Dictyostelium discoideum* (de Hostos et al., 1991), it has been described in various organisms from yeasts to humans with a high degree of homology (de Hostos, 1999). Coronin have been found to play important roles in various actin-associated activities such as cell motility, cytokinesis, and endocytosis and phagocytosis (de Hostos, 1999). Furthermore, coronin gene-disrupted cells showed defective phenotypes more pronounced than mutants lacking more familiar actin-binding proteins such as ABP 120, α-actinin, and severin (Andre et al., 1989; Brink et al., 1990; Wallraff et al., 1986). In *D. discoideum*, coronin has been observed to localize in areas of the cell where actin is also most abundant such as the crowns, transient macropinocytic structures from which the protein derived its name, phagocytic cups, and the leading edge (de Hostos et al., 1991; Gerisch et al., 1995; Maniak et al., 1995). Mutants lacking coronin were found to have slowed locomotion and defective cytokinesis (de Hostos et al., 1993). Phagocytosis was also slowed down in these mutants (Maniak et al., 1995). However, there is still much to investigate on its roles and specific mechanisms.

The most conspicuous structural characteristic of coronin is a WD-repeat motif at the center of the protein which forms a β-propeller structure that allows its interaction with several proteins by the formation of

☆ The nucleotide sequence of cDNA of coronin homologue of *Acanthamoeba healyi* was submitted to GenBank, and allocated the Accession No. AY525781.

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complexes (Smith et al., 1999). The N-terminus and the WD repeats are highly conserved in organisms. At the end of the WD repeats is a very diverse unique region and the last 25–40 amino acids in the C-terminal that is predicted to have a tendency to form a coiled-coil (de Hostos, 1999). The family of WD-repeat proteins is thought to assume many functions related to regulating cellular functions including cell division, cell-fate determination, signal transduction, RNA processing, gene transcription, gene regulation, transmembrane signaling, and vesicular traffic (Smith et al., 1999).

Coronin has not yet been characterized in *Acanthamoeba* which has been extensively used for actin-related studies due not only to its well developed cytoskeleton but also dynamic functions including motility and phagocytosis that require actin and actin-related protein interactions. *A. healyi* is one of the most recently isolated species of the genus (Moura et al., 1992).

In this study, a cDNA encoding a coronin-like protein was characterized in *A. healyi* by sequence analysis and intracellular localization of the EGFP fusion protein was confirmed in live amoeba by transient transfection techniques.

2. Materials and methods

2.1. Amoeba cultivation

Acanthamoeba healyi OC-3A strain (ATCC #30886) originally isolated from a GAE patient (Moura et al., 1992) was obtained from the American Type Culture Collection (ATCC) and grown axenically in peptone–yeast extract–glucose (PYG) medium at 25 °C (Kong et al., 1995). At the end of logarithmic growth, the trophozoites were harvested and used for further analysis.

2.2. Sequence analysis of cDNA clone encoding coronin homologue

The cDNA used in this study was derived from a previously constructed cDNA library of *A. healyi* (Kong et al., 2001). The UNI-ZAP XR vector containing the cDNA was extracted from its XL1-blue MRF strain host using a plasmid DNA purification kit (DNA-spin Plasmid Purification Kit, Intron, South Korea). The DNA inserts were ascertained by digestion with *Eco*RI and *Xho*I and the size was estimated by electrophoresis in agarose gel. The extracted plasmid was sent to an outside company for sequencing (Macrogen, Korea). Homology search was conducted using the Basic Local Alignment Search Tool (BLAST) program of the National Center for Biotechnology Information, National Institute for Health. Sequence alignment with other coronins in the database was performed using the CLUSTALW 1.7 program of the European Bioinformatics Institute Server. Predicted motifs and secondary structures were obtained through Predictprotein, a service for sequence analysis and protein structure prediction from the Columbia University Bioinformatics Center (Rost, 1996). The molecular mass and isoelectric point were computed from the deduced amino acid sequence from the MWCALC program (Infobiogen).

2.3. Southern and Northern analysis

Genomic DNA of *A. healyi* for Southern blot was prepared by the method described previously (Hong et al., 2004). After determining the concentration with a spectrophotometer, equal amount of the isolated genomic DNA was digested with the following enzymes: *Bam*HI, *Eco*RI, *Sca*I, and *Xho*I. The digests were electrophoresed in an agarose gel and transferred to a nylon membrane by upward capillary action. DNA was cross-linked the following day by exposure to 1.5 J/cm of UV. Total RNA was isolated from trophozoites of *A. healyi* OC-3A, *A. castellanii* Castellani, and *A. castellanii* Neff according to procedures specified for extracting total RNA by Trizol reagent (Gibco-BRL, USA). The probe used for both Southern and Northern analysis was derived by digesting the cDNA clone with *Sal*I and *Hin*dIII, resulting in a fragment with 432 bp. The fragment was eluted using Jetsorb (Genomed, Germany) and radiolabeled with [^{32}P]dCTP using a random primed DNA labeling kit (Roche, Switzerland). Hybridization of the membranes followed using procedures specified for the ExpressHyb Hybridization Solution (Clontech, USA). A 300 bp fragment of 18s rDNA from *Acanthamoeba* was derived using PCR method. This was used as loading standard for expression level for Northern blot. The RNA transferred membrane was deprobed and rehybridized with the probe of 18s rDNA.

2.4. Construction of expression vectors

Transfection vectors were constructed by ligating the full-length cDNA or fragments of the cDNA with a pUb-EGFP expression vector which contains an *Acanthamoeba* ubiquitin promoter (Kong and Pollard, 2002). The full-length cDNA and cDNA fragments corresponding to the conserved region (the WD repeats and the conserved region of the C-terminal) and the last sequences of the C-terminal region (including the unique and supposed coiled-coil regions) were utilized (Fig. 1). Primers that include an *Spe*I site at the 5′ end and an *Xba*I site at the 3′ end were constructed to amplify the cDNA fragments which were then inserted after EGFP. Constructs inserting the N-terminal region and a C-terminal deleted fragment were also prepared. Ligated inserts and plasmids were transformed in *Escherichia coli* and plasmid DNA was extracted from transformed colonies.