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Review

The spectraplakins of *Caenorhabditis elegans*: Cytoskeletal crosslinkers and beyond

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ABSTRACT

Spectraplakins are evolutionary conserved cytolinkers with characteristics of both the spectrin and the plakin family proteins. *Caenorhabditis elegans* possesses two categories of spectraplakin isoforms encoded by a single locus termed *vab-10*. Here we summarize the structure, homology, expression and functions of these spectraplakin family proteins in the nematode. We particularly focus on the diverse roles of VAB-10 isoforms in a number of organs and tissue types, as well as the similarities and distinctions of the underlying mechanisms. We also discuss the functional partners of VAB-10 in different contexts, plus the involvement of VAB-10 isoforms in physiological processes beyond cytoskeletal integration. We emphasize the importance of spectraplakin-related studies using *Caenorhabditis elegans* as the model, and their contributions to our understanding of spectraplakins across species.

Contents

Abbreviations: ABD, actin-binding domain; *abf*, antibacterial factor related; AC, anchor cell; ACF7, actin crosslinking family protein 7; ALML, anterior lateral microtubule cell; AMPK, AMP-activated protein kinase; B-LINK, basement membrane-LINKage; BM, basement membrane; BPAG1, Bullous pemphigoid antigen 1; CED, cell death abnormality; CeHD, *C. elegans* hemidesmosome; *C. elegans*, *Caenorhabditis elegans*; CH, calponin-homology; *clec*, C-type LECtin; *cnc*, CaeNaCin (Caenorhabditis bacteriocin); co-IP, co-immunoprecipitation; CRISPR, clustered regularly interspaced short palindromic repeats; DA, dopaminergic; *dod*, downstream of DAF-16; DTC, distal tip cell; EB1, end-binding protein 1; ECM, extracellular matrix; EFF-1, epithelial fusion failure 1; EPS, EPS (human endocytosis) related; FBD, fusogen binding domain; Fer, feline encephalitis virus-related kinase; GABA, gamma-aminobutyric acid; GAS2, growth-arrest-specific protein 2; GAS2L3, growth arrest specific 2 like 3; GIT, mammalian G protein-coupled receptor kinase InTeractor; GSK-3, glycogen synthase kinase-3; HIM, high incidence of males; IF, intermediate filament; INA-1, INtegrin alpha; kb, kilobase; LET-805, LEThal; *lys*, LYSozyme; MACF1, microtubule-actin crosslinking factor 1; MAX, motor AXon guidance; MT, microtubule; MUA, MUscle attachment abnormal; MUP, MUscle positioning; *nlp*, neuropeptide-like protein; PAK, P21-activated kinase family; PAT, paralysed arrest at two-fold; PD, Parkinson's disease; PIX, Pak (p21-activated kinase) interacting eXchange factor; PLML, posterior lateral microtubule cell; PR, plectin repeat; RACK1, the receptor for activated C kinase 1; RNAi, RNA interference; RT-PCR, reverse transcription-PCR; SAGE, serial analysis of gene expression; SH3, Src homology 3; Shot, short stop; SR, spectrin repeat; STA, STAT transcription factor family; UNC, UNCoordinated; utse, uterine seam cell; VAB, variable ABnormal morphology.

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1. Introduction

Spectraplakins are enormous multi-domain cytoskeletal proteins named after their features resembling both the spectrin and the plakin family. Since the initial discovery of the spectraplakin superfamily in the 1990s, members of this family have been identified and described in model organisms throughout evolution, including *Caenorhabditis elegans* (*C. elegans*), *Drosophila*, zebrafish and rodents [1–4]. Numerous works about spectraplakins have demonstrated their pivotal roles in a vast variety of developmental, physiological and pathological-related processes [5–7]. On a mechanistic basis, the functions of spectraplakins are categorized into four groups: crosslinking different cytoskeletal networks, tethering cytoskeletons to the plasma membrane proteins, defining membrane subdomains and scaffolding signaling proteins [8]. As the simplest multicellular model organism, *C. elegans* harbors a single spectraplakin locus, which produces multiple spectraplakin isoforms capable of fulfilling all the functions mentioned above (Fig. 1). The organismal simplicity and the powerful genetic tools enable the *C. elegans* model to offer comprehensive and in-depth knowledge about the spectraplakins in multicellular organisms. In this review, we provide the basic facts and summarize recent advances about *C. elegans* spectraplakins. In particular, we describe and compare the roles and underlying mechanisms of *C. elegans* spectraplakins in different physiological systems, namely the physical barrier, the alimentary system, the reproductive system, the nervous system and the locomotor system. We also discuss the involvement of *C. elegans* spectraplakins in processes beyond cytoskeletal integration.

2. The genomic organization and protein structures of VAB-10

The Variable ABnormal morphology 10 (*vab-10)* gene, once predicted as three genes (ZK1151.1, ZK1151.2 and ZK1151.3), was recognized as the sole *C. elegans* spectraplakin locus according to homology analysis [1,8,9]. The *vab-10* locus is positioned at *C. elegans* chromosome I and spans 44.7 kilobase pairs of genomic sequence. It contains a total of 32 exons and generates multiple isoforms by alternative splicing (Fig. 1A). These isoforms are categorized into two subsets, *vab-10A* and *vab-10B,* based on their distinct 3′ regions [1]. These two isoform subsets share a common region from exon 1 to exon 15. Exon 16 and exon 17 are unique to the *vab-10A* isoforms, while *vab-10B* contains exon 18–32 instead of exon 16–17. Reverse transcription-PCR (RT-PCR) identifies additional alternative splicing events affecting exon 5, 9, 16, 21, 22, 23, or 27, although it is not clear whether all the isoforms exist in vivo [1]. The presence of VAB-10A and VAB-10B isoforms is further confirmed by Western blots using antibodies specific for either VAB-10A (MH5, 4F2) or VAB-10B (K22, K32) [1,10] (Fig. 1). There are also isoforms with or without the N-terminal 170 amino acid region encoded by exon 1–5 in both VAB-10A and VAB-10B subsets. The isoforms containing exon 1–5 are defined as VAB-10A1 and VAB-10B1, and those without are termed VAB-10A2 and VAB-10B2 [10] (Fig. 1). RT-PCR and Western blots failed to detect the existence of an isoform containing all exons [1] (Fig. 1A).

In the common region of VAB-10A and VAB-10B, there is a pair of calponin-homology (CH) domains encoded by exons 2–8 and six spectrin repeats (SRs) encoded by exons 9–15 (Fig. 1B). The N-terminal CH-domain containing region of VAB-10 fused with GFP co-localizes nicely with actin filaments in vivo and be used as a fluorescent probe to visualize actin organization in *C. elegans* [11]. It confirms that the two calponin-homology domains serve as an actin-binding domain (ABD) in VAB-10. However, the CH domains of VAB-10 are not only capable of binding to actin. The second CH domain expressed in epidermis appears to be localized in the hemidesmosomes [10]. This phenomenon may reflect the abilities of the CH domains to bind to adhesion receptors [12]. The six spectrin repeats shared by both VAB-10A and VAB-10B constitute a plakin domain. Among these spectrin repeats, the SR3 of VAB-10 contains an Src Homology 3 (SH3) domain like the SR5 of vertebrate plectin, which is a feature shared by all plakin members [5,13,14] (Fig. 1B). It was proposed that the accessibility of the SH3 embedded in a SR domain is determined by intramolecular interaction with other SR domains, indicating complex regulation of protein interactions within these domains [14–16].

The VAB-10A-specific exon 16 and 17 encode sixteen plectin repeats (PRs) following a small coiled-coil domain [1,5]. Among the PR domains, the tighter arrangement of PR11-15 is similar as the PR cluster binding intermediate filaments (IFs) in rat plectin and human desmoplakin [17,18] (Fig. 1B). The VAB-10B-specific domains contain 20 spectrin repeats, an EF-Hand domain and a microtubule (MT) −binding domain called Growth-arrest specific protein 2 (GAS2)-related homology. VAB-10B also harbors a potential EB1-binding site and a potential GSK-3beta phosphorylation site at the C-terminus [1,5,10] (Fig. 1B). Based on the overall domain features, VAB-10A is more similar to vertebrate plectin/BPAG1e. On the other hand, the protein structure of VAB-10B is similar to that of MACF1a/BPAG1a [1,5,8]. Therefore, the predicted function of VAB-10A might be to crosslink actin and intermediate filament cytoskeletons, whereas VAB-10B might mediate interaction between actin and microtubules. Since an isoform containing both the plectin repeats and the GAS2 domain like BPAG1b/MACF1b is not identified in *C. elegans*, such a giant spectraplakin interacting with all three cytoskeletons could be the evolutionary result toward functional integration.

3. The expression patterns of VAB-10 isoforms

C. elegans spectraplakins are widely distributed in the alimentary, locomotor, reproductive, nervous systems and the physical barriers in multiple developmental stages (Fig. 2). Immunostaining results show that VAB-10A is present in the epidermal cells, the pharyngeal epithelium and along the mechanosensory neurons, while VAB-10B is distributed in the epidermis, pharynx, intestine, nerve ring, somatic gonad including distal tip cells (DTCs) and body wall muscles [1,10,19]. The VAB-10A:GFP fusion made by Clustered regularly interspaced short palindromic repeats (CRISPR) knock-in not only verifies the localization of VAB-10A in the epidermis and mechanosensory neurons but also reveals the presence of VAB-10A in the anchor cell (AC) and the uterine seam cell (utse) [5,20]. A florescent reporter with a 3-kb promoter upstream of *vab-10* exon 1, which drives the transcription of VAB-10A1 and VAB-10B1, is expressed in the DTCs, pharynx and body wall muscles. On the other hand, a reporter with *vab-10* intron 5 as the promoter, which drives the transcription of VAB-10A2 and VAB-10B2, is expressed mainly in the epidermis [10]. Serial analysis of gene expression (SAGE) also indicates that *vab-10* could be transcribed in the ciliated neuronal cells [21] (Fig. 2).