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Review

Drosophila Short stop as a paradigm for the role and regulation of spectraplakins

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ABSTRACT

Spectraplakins are evolutionarily well conserved cytoskeletal linker molecules that are true members of three protein families: plakins, spectrins and Gas2-like proteins. Spectraplakin genes encode at least 7 characteristic functional domains which are combined in a modular fashion into multiple isoforms, and which are responsible for an enormous breadth of cellular functions. These functions are related to the regulation of actin, microtubules, intermediate filaments, intracellular organelles, cell adhesions and signalling processes during the development and maintenance of a wide variety of tissues. To gain a deeper understanding of this enormous functional diversity, invertebrate genetic model organisms, such as the fruit fly *Drosophila*, can be used to develop concepts and mechanistic paradigms that can inform the investigation in higher animals or humans. Here we provide a comprehensive overview of our current knowledge of the *Drosophila* spectraplakin Short stop (Shot). We describe its functional domains and isoforms and compare them with those of the mammalian spectraplakins dystonin and MACF1. We then summarise its roles during the development and maintenance of the nervous system, epithelia, oocytes and muscles, taking care to compare and contrast mechanistic insights across these functions in the fly, but especially also with related functions of dystonin and MACF1 in mostly mammalian contexts. We hope that this review will improve the wider appreciation of how work on *Drosophila* Shot can be used as an efficient strategy to promote the fundamental concepts and mechanisms that underpin spectraplakin functions, with important implications for biomedical research into human disease.

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1. Introduction

The cytoskeleton comprises actin, intermediate filaments and microtubules and is essential for most, if not all, cellular processes and functions, including cell division, shape, dynamics, force generation, intracellular transport, membrane dynamics, organelle function, adhesion, signalling, cell maintenance and processes of cell death [1]. Accordingly, a high percentage of regulators of the cytoskeleton (and here we refer to components which constitute the cytoskeleton, or directly bind or associate with it) has close links to human diseases [2], and many more can be expected to be discovered in future studies.

One of the most complex and versatile protein families of cytoskeletal regulators are the spectraplakins [3,4]. They comprise VAB-10 in the worm *Caenorhabditis*, Short stop (Shot; also known as Kakapo or Groovin) in the fruit fly *Drosophila* and, in vertebrates, dystonin (also known as Bullous Pemphigoid Antigen 1/BPAG1, BP230, BP240) and Microtubule-Actin Crosslinking Factor 1 (MACF1; also known as Actin Crosslinking Family 7/ACF7, Marcrophin 1, Tabeculin α, Magellan).

Spectraplakin genes encode 7 major functional domains (Fig. 1). Through generating alternative isoforms with different combinations of these domains, spectraplakins provide a modular tool set and have been referred to as the "cytoskeleton's Swiss army knife" [5]: they can interact with actin, intermediate filaments and microtubules alike, establish numerous structural or regulatory links between cytoskeleton components, or from cytoskeleton to other molecules or cell compartments [3,4]. To illustrate the enormous versatility of this family, spectraplakins can be classed as true members of three important protein families:

1. the plakins (e.g. plectin, desmoplakin, envoplakin, periplakin, epiplakin) which are cytoskeleton-associated scaffold proteins maintaining tissues under mechanical stress primarily at cell junctions [6];
2. the spectrins (e.g. α-/β-spectrin, α-actinin, dystrophin, utrophin) which primarily form links between proteins at the cell cortex [7,8];
3. the Gas2-like proteins (Gas2, Gas2-like 1–3) which act as linkers between MTs, end binding (EB) proteins and F-actin, important for cytoskeletal dynamics in cell division and development [9–12].

Through their modular nature, spectraplakins functionally contribute in all three of these contexts, making them active members of those protein families. Accordingly, they have been discovered as players in a wide range of disorders or conditions. In humans, they include skin blistering of the epidermolysis bullosa simplex type (OMIM #615425) [13–16], hereditary sensory and autonomic neuropathy type VI (HSAN6; OMIM #614653) [17–19], Parkinson's disease [20,21], neuro-developmental disorders [22–24], different forms of cancer [25–27] and the infection process of *Herpes* virus [28,29]. Mouse models lacking *dystonin* functions have revealed additional defects in glial cells potentially linking to multiple sclerosis [30–32], and neuromuscular junction defects associated with intrinsic muscle weakness [33–35]. Mouse models lacking ACF7/MACF1 show early developmental aberrations relating to Wnt signalling [36], impaired heart and gut physiology [37,38], aberrations of the brain and of hair follicle stem cells both relating to cell migration defects [39–41], and defective axonal and dendritic growth [40,42,43].

To make sense of this enormous breadth of functions and their underlying mechanisms, invertebrate model organisms, in particular the worm *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*, provide important experimental strategies capable of deciphering complex aspects of biology [44,45]. These invertebrate model organisms have a long and important history of pioneering fundamental concepts and delivering understanding of molecular and biological functions, which can then be used as facilitating or instructive paradigms for related studies in higher organisms including humans [46,47].

As an important prerequisite for applying this strategy, spectraplakins are evolutionarily well conserved (protein domains displaying up to 79% identity; Fig. 1). In particular, the *Drosophila* spectraplakin Shot is expressed in almost every tissue and has been studied in a broad spectrum of biological contexts, revealing enormous variation in domain requirements and functional mechanisms. In a previous review, we used Shot as an example to explain the methodological and experimental strategies available in *Drosophila* to decipher gene functions [45]. Here we will provide an overview of important understanding derived from such work and discuss whether and how it applies to related contexts in higher animals and humans.

2. A detailed comparison of the *shot* gene and its mammalian homologues

Currently, GENCODE (release 25, comprehensive gene annotation) and flybase.org (release FB2016_05) list 22 annotated isoforms for the *Drosophila shot* gene which includes 7 major, evolutionarily conserved functional domains or motifs, some containing smaller sub-motifs (Fig. 1). Different isoforms can contain stark variations regarding the presence or absence of these domains. These domains include an actin-binding domain (ABD), a plakin domain (PD), a plakin repeat region (PRR), a spectrin repeat rod (SRR), two EF-hand motifs (EFH), a Gas2-related domain (GRD) and a Ctail. Most functional studies in *Drosophila* have been carried out with the Shot-PE and −PC isoforms (often referred to as Shot-LA/-LC) and deletion derivatives of these. These isoforms contain all domains except a PRR, and vary at the N-terminus (different lead sequences and a complete versus incomplete ABD; Fig. S1) [45].

The same release version of GENCODE lists 17/15 partial, overlapping and potential full length protein coding isoforms for human/mouse MACF1 and 18/14 protein coding isoforms for human/mouse dystonin (Fig. S1) which differ in part from the isoforms released by UniProt [MACF1 5 human (Q9UPN3), 4 mouse (Q9QXZ0); DST 8 human (Q03001), 7 mouse (Q91ZU6)]. GENCODE and UniProt are curated and validated databases, but the isoforms they list significantly deviate from the current literature [3,17]. Even in NCBI RefSeq (which lists many more automated predictions