Tissue integrity: **Hemidesmosomes and resistance to stress**

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How do animal tissues resist the shearing forces to which they are exposed during locomotion or harsh encounters with the environment? Genetic analysis in *Caenorhabditis elegans* is furthering our understanding of the nature and function of the attachments that preserve tissue integrity.

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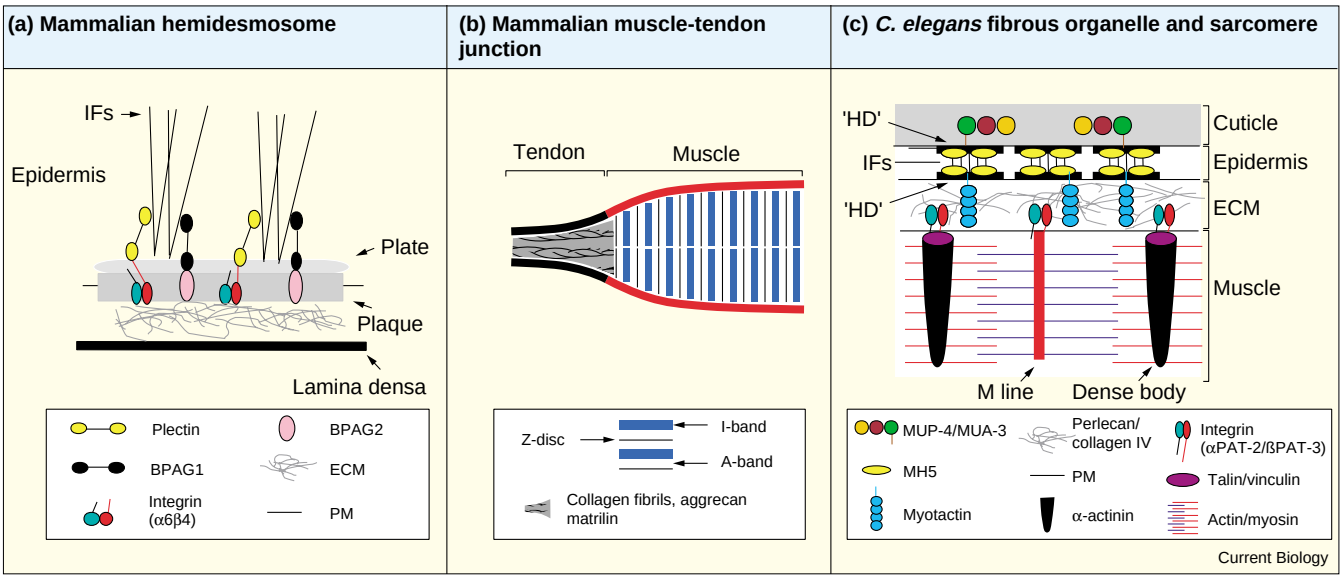
We are becoming increasingly familiar with genetic diseases, such as muscular dystrophies or epidermolysis bullosa, that affect the integrity of muscles or the epidermis, two tissues which are highly exposed to mechanical stress. Rupturing then occurs at the level of specialised attachments that anchor these tissues. Biochemical and genetic analysis in vertebrates has revealed the molecular nature of those attachments. Recent studies have identified the functionally homologous structures in the nematode *Caenorhabditis elegans*. A comparison of vertebrate and invertebrate attachments uncovers an unexpected level of conservation, implying that the much simpler worm, with its powerful genetic tools, can help us to understand how the attachments are assembled and function.

Anchoring structures in vertebrates

In vertebrates, the epidermis is anchored to the basal lamina and dermis through specialised attachments called hemidesmosomes because of their appearance as half-desmosomes in the electron microscope. Hemidesmosome components were identified biochemically in the 1970s and 1980s. As shown in Figure 1, two sets of integral membrane proteins, BPAG2 and the $\alpha_6\beta_4$ integrin dimer which provides adhesion to the basal lamina, are connected to the network of keratin intermediate filaments via two members of the plakin family, BPAG1 and plectin [1]. Plakins are very large proteins with two globular terminal heads linked by a central rod domain, which bridge together different cytoskeletal networks [2]. It was the molecular cloning in the 1990s of the genes responsible for the diseases mentioned above, and the development of mice knock-out models, that demonstrated the essential role of hemidesmosomes as epidermis attachments [1,2]. Mutations in hemidesmosome components lead to severe skin blistering as a result of rupturing of the basal epidermal layer at or near the hemidesmosome [1,2].

Figure 1



Structure and components of (a) the vertebrate hemidesmosome (adapted from [2]), (b) the myotendonous junction (adapted from [17]), and (c) *C. elegans* fibrous organelle with the underlying muscle sarcomere (adapted from [3]). ECM, extracellular matrix; PM, plasma membrane; the MH5 antibody recognises a homologue of a vertebrate hemidesmosome component (our unpublished data). The respective thicknesses of the cuticular, epidermal and muscle layers is not accurate.

Similarly, the structure and function of vertebrate skeletal muscle relies on strong mechanical connections to the basal lamina. In this case, however, the attachments occur in regions between sarcomeres, at the basement membrane (through the dystroglycan complex), and at myotendonous junctions which transfer the longitudinal forces of muscle to tendons. Proteins that preserve muscle and epidermis integrity are generally distinct, with the notable exception of plectin found at the level of Z-discs. Mutations of mouse or human plectin lead to epidermolysis bullosa as well as to a late onset muscular dystrophy, which is characterised by a disappearance of Z-discs [1,2].

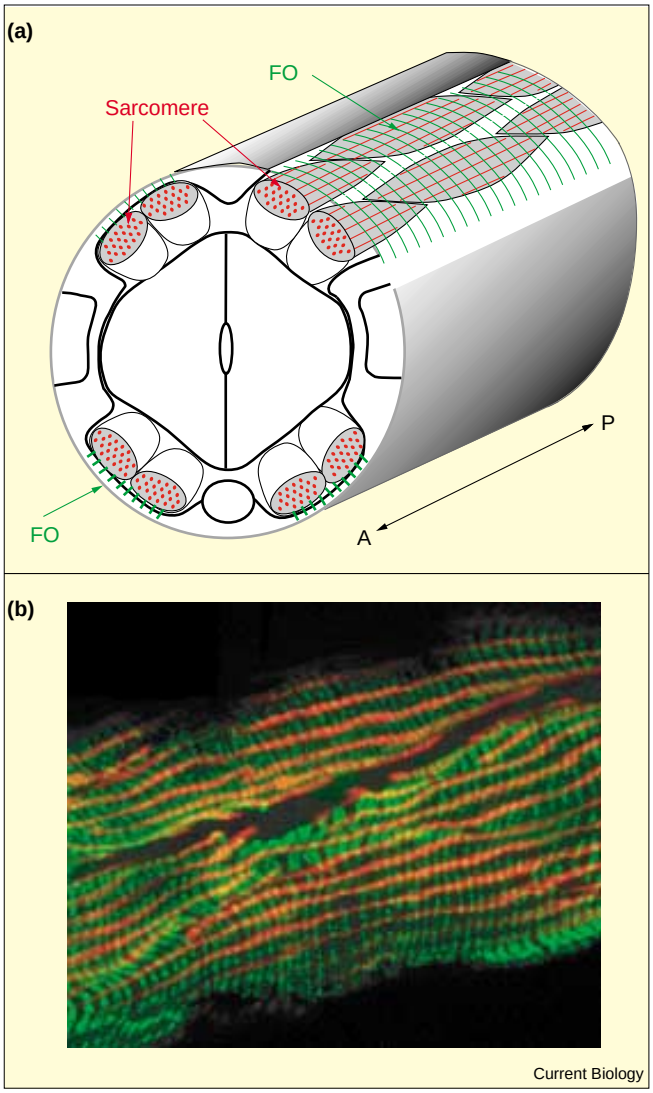
Anchoring structures in *C. elegans*

The *C. elegans* body mainly consists of two concentric tubes, with an external epidermal layer and an internal intestine. Muscles cells are organised in four quadrants located between the intestine and the epidermis (Figure 2). The external cuticle laid by the epidermis acts as an exoskeleton onto which muscle forces are exerted. In regions of muscle contact, epidermal cells are strongly flattened (their thickness is less than 100 nm) because they are compressed by a continuous structure, beginning at the muscle–epidermis basement membrane, spanning the epidermis and continuing into the cuticle (Figure 1). Thus, in *C. elegans* a single structure links muscles, the epidermis and the cuticle.

Muscle–cuticle attachments in *C. elegans* consist of two modules [3]. Within the epidermis are fibrous organelles, which appear as two electron-dense plaques, one located at the basal membrane facing the extracellular matrix and muscles, the other at the apical membrane facing the cuticle (Figures 1,2). Fibrous organelles are reminiscent of vertebrate hemidesmosomes, as both plaques are linked by intermediate filaments. Within muscles, two electron-dense structures can be distinguished which anchor sarcomeres to the muscle membrane: dense bodies at the level of thin filaments and the M-line at the level of thick filaments.

Pioneering work from Bob Waterston's laboratory has led to the initial characterisation of the fibrous organelles and dense bodies in *C. elegans*. Waterston and colleagues [3] initially isolated a remarkable series of monoclonal antibodies against proteins that remain associated with the cuticle. Three of these antibodies, MH4, MH5 and MH46, reveal a regular pattern of parallel bands in the epidermis, strongly suggesting that they recognise fibrous organelle components. Indeed, MH4 recognises an epitope shared by several intermediate filaments [3,4]. Most other monoclonal antibodies identify dense body components. In parallel, Waterston's laboratory isolated several embryonic lethal '*pat*' mutations, characterised by paralysis of the embryo [5,6]. All of the corresponding genes turned out to encode dense body components, the extracellular matrix proteoglycan perlecan, or proteins that regulate muscle contractions.

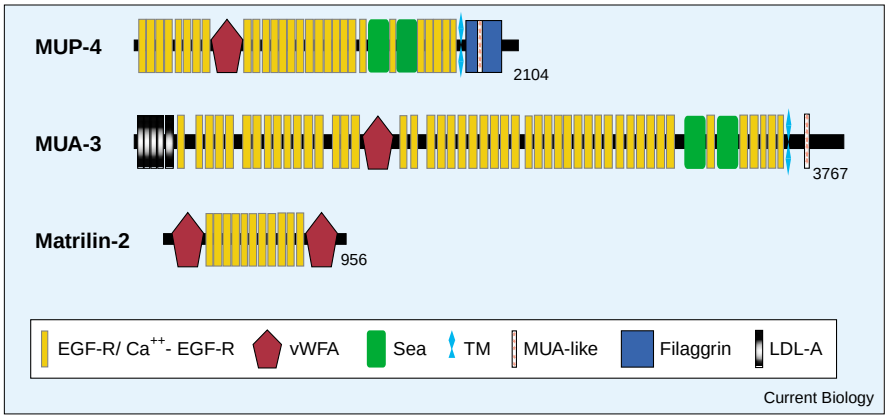
Figure 2



(a) A section through *C. elegans* along with part of the body in which the cuticle has been removed to show fibrous organelles and the underlying muscles by transparency. Note that the circumferential orientation of fibrous organelles is orthogonal to the anterior–posterior (A–P) oriented muscle fibres, which ensures proper anchoring.
(b) Fibrous organelles (green) and thick filaments (red), as visualised by immunostaining with specific antibodies.

The molecular characterization of fibrous organelles came from different directions. In 1999, Chelley Hresko [7] in the Waterston laboratory identified the gene encoding the epitope recognised by the MH46 antibody, by screening an expression library. The corresponding protein, which was dubbed myotactin, is a single-pass transmembrane protein located at the basal epidermal surface. Its extracellular region contains 32 fibronectin type III (FNIII) repeats, while its cytoplasmic domain does not contain any obvious known motifs. In *C. elegans* myotactin mutants,

Figure 3



Schematised structures of MUP-4 and MUA-3, along with one of their closest vertebrate homologues, matrilin-2. EGF-R/Ca^{2+}-EGF-R, epidermal growth factor repeat or calcium-binding EGF-R; vWFA, von Willebrand factor A-domain; Sea, sea urchin enterokinase module; TM, transmembrane domain; Filaggrin, domain with homology to human filaggrin; LDL-A, low-density lipoprotein type A repeat.

muscles detach when they start contractions, but fibrous organelles (as revealed by the antibodies MH4 and MH5) remain initially surprisingly well organised [7].

Hresko *et al.* [7] suggested that myotactin is required to maintain the association between fibrous organelles and muscles, and plays a role analogous to that of BPAG2 in vertebrates, even though the proteins have quite different sequences. From its number of FNIII repeats, myotactin can probably contact the muscle membrane, where one potential receptor is integrin. But as myotactin remains associated with muscle cells in embryos lacking integrins, it has been suggested that another protein is the myotactin receptor.

The laboratories of John Plenefisch [8] and Elizabeth Bucher [9] have now reported the identification of two other putative fibrous organelle components: MUA-3 and MUP-4, respectively. The genes *mup-4* and *mua-3* were identified in a genetic screen for essential genes, which showed that they act in the epidermis and yet affect muscle–epidermis interactions [10]. The *mua-3* gene was independently identified in a directed screen for mutants with fragile muscle attachments [11]. As shown by electron microscopy and immunostaining, the epidermis detaches from the cuticle in *mup-4* or *mua-3* mutants, but muscles remain attached to the epidermis [8–10]. Both proteins are expressed in epidermal cells, in a pattern similar to that revealed by MH4 or MH5.

Interestingly, MUA-3 and MUP-4 are homologous transmembrane proteins containing an association of modules not found elsewhere (Figure 3), except in part in the matrilins, a recently identified family of proteins found mainly in connective tissues, including tendons, ligaments and bone epiphysis, where they interact with collagens and aggrecan [12]. The cytoplasmic domain of MUP-4 has weak but significant homology to the filaggrins, which are known to bind intermediate filaments [13]. Thus, as suggested by the authors [8,9], MUP-4 and MUA-3 should act at the epidermal apical surface, binding on the one hand to cuticle collagens via their extracellular domain, and on the other hand to intermediate filaments through their cytoplasmic tail.

At the same time, the laboratory of Klaus Weber, with a long-standing interest in *C. elegans* intermediate filaments [14], reported the characterisation of their expression patterns and performed RNA interference against individual intermediate filament genes [4]. Although it is well documented that intermediate filaments form a major cytoskeletal network involved in transmitting mechanical tension in animal cells, their great diversity in vertebrates (more than 50 genes in mammals) makes the genetic analysis of their function a challenge [15]. As shown by Weber and colleagues [14], *C. elegans* has only 11 intermediate filament genes, the analysis of which should simplify an understanding of intermediate filament functions.

Weber's laboratory [4] showed that four of the *C. elegans* intermediate filaments are essential for development: the isotypes A1, A2, A3 and B1, among which A2 and A3 are recognised by the antibody MH4 and are expressed in the epidermis. Interestingly, DIC microscopy suggests that muscle cells detach from the body wall in embryos and larvae lacking the A2 and A3 intermediate filaments, resulting in paralysis, although it is not yet known at which level this detachment takes place [4]. Their study is therefore consistent with the notion that intermediate filaments transmit mechanical tension from muscles to the cuticle.

The identification of fibrous organelle components is thus unexpectedly revealing the presence of proteins related to hemidesmosome components. The strong homology observed between MUP-4/MUA-3 and matrilins is

intriguing. One wonder if fibrous organelles might represent an ancestral attachment structure combining properties and molecules found in vertebrate hemidesmosomes and tendons.

Future directions

The identification of myotactin, MUP-4, MUA-3 and intermediate filaments, and the ongoing efforts in several laboratories to characterise further fibrous organelle components — in particular, the products of the *mua* genes, which promise to be a gold mine [11] — is just a beginning. With a more complete picture at hand, it will be possible to probe molecular interactions among individual components, to understand how fibrous organelles are assembled and whether they have additional roles beyond their function in mechanical transmission. Given the conservation of many players, *C. elegans* can provide a fast and efficient system to complement biochemical studies of hemidesmosomes in vertebrates, where issues of assembly and signalling are at the core of current efforts [1,2]. There is, for instance, some evidence suggesting that fibrous organelles could be involved in cell signalling. When muscle precursors are ablated with a laser microbeam during early embryogenesis, the expression or stability of the proteins recognised by MH4 and MH5 is strongly affected [7].

Above all, *C. elegans* will provide an invaluable genetic model to analyse the importance of mechanical coupling during embryonic development, a problem that would be very difficult to approach in vertebrates. Another recent example of an important contribution to this field is the identification of hemicentin, a novel conserved extracellular matrix protein required to organise fibrous organelles in regions where the uterus and mechanosensory axons contact the epidermis [16].

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