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Mechanobiology: forging a strong matrix at tendons

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Summary:

Nature faces the challenge of stably attaching soft muscles to a stiff skeleton. A new study combines live imaging and fly genetics to reveal that mechanical tension and a putative intracellular chaperone assist in assembling the gigantic extracellular matrix protein Dumpy at fly tendon-skeleton interfaces.

Bilaterian animals including nematodes, arthropods and chordates share a common strategy to effectively power movements: muscles produce mechanical forces to displace parts of their skeleton, making animals move. Hence, animal survival relies on effective force transmission from muscles to the skeleton. This is a biophysical challenge as stably connecting hard (skeleton) – soft (muscle) material interfaces is demanding¹. Evolution solved this problem by placing tendons between muscle and skeleton, and by assembling specialised strong extracellular matrices (ECMs) that durably attach muscles to tendons and tendons to the skeleton.

Vertebrate tendons are a dense connective tissue, which is connected to the hard ossified bone by a transitional zone with graded material properties called fibrocartilaginous enthesis²⁻⁴. One key tendon component is the triple helical collagen I, which assembles into large collagen fibers in a force dependent manner^{5,6}, whereas the fibrocartilage contains more collagen II. Finally, on the bone surface, mineralised calcium phosphate (Apatite) is deposited in a graded manner between the collagen I fibrils². Proper assembly of these different ECM components into higher order arrays is essential for their correct physical properties and thus for effective linkage of tendon to bone. We know that active muscle forces are needed for proper maturation of tendon ECM and the tendon-bone interface^{7,8}. However, little is known about the molecular mechanisms by which these specialised ECMs assemble to acquire these defined properties.

In this issue of *Current Biology*, Chu and Hayashi⁹ make a step towards deciphering these molecular mechanisms by using the *Drosophila* muscle-tendon system to investigate tendon attachment to the skeleton. The authors chose the adult flight muscle, a beautiful system whose development can be recorded by whole mount live imaging in fly pupae¹⁰. Developing flight muscles attach to the basal side of a specialised epithelial cell monolayer, the fly tendon cells (Figure 1A). Tendon cells connect on their apical side to the chitin exoskeleton, which

they produce themselves by extruding chitin polysaccharide chains¹¹. Integrin-dependent attachment of the forming muscles to the basal side of tendons generates a high mechanical force^{10,12,13} that tendons need to resist by their solid attachment to the rigid chitin skeleton. To decipher the mechanisms by which fly tendons manage to effectively attach to the cuticle, Chu and Hayashi turned to one of the first fly mutants identified by Thomas Morgan in 1910 called *dumpy* (initially called ‘*truncate*’ because of its stubby wings)¹⁴.

Dumpy is a gigantic 2.5MDa protein, the largest in *Drosophila*. It contains 308 EGF, 185 DPY repeats and one zona pellucida (ZP) domain at its C-term followed by a transmembrane domain that can be cleaved, resulting in an extracellular secreted form of Dumpy^{15,16}. Dumpy has multiple roles in anchoring the apical surface of various epithelia to the rigid chitin skeleton. One prominent phenotype of *dumpy* mutants is the ‘comma’ phenotype, indentations of the adult cuticle at positions where the flight muscles are attaching to the tendons, suggesting that muscle forces are producing these indentations because of an unstable tendon-skeleton set-up¹⁷. Hence, Chu and Hayashi used Dumpy-YFP protein fusion and beautifully combined it with live imaging to show that indeed forces produced by the basally attached flight muscles are required to generate and order long Dumpy filaments on the apical side of the tendons connecting to the overlaying chitin skeleton (Figure 1). Interestingly, similar to the ordering of the collagen filaments in vertebrate tendons the authors also find that Dumpy-YFP is assembled and ordered into periodic structures with step-length of less than 1 μm fitting with the estimated size of the folded Dumpy-YFP protein (Figure 2)¹⁶.

To unravel the mechanism of Dumpy ordering, Chu and Hayashi turned to a second fly mutant that results in similar deformations of the thorax cuticle, hence called *quasimodo* (*qsm*)¹⁸. Double mutant analysis suggests that *dpy* and *qsm* operate in one pathway, as *qsm* null mutants enhance the cuticle indentation phenotype of hypomorphic *dpy* alleles. Strikingly, live imaging shows that Dumpy filaments in the *qsm* mutants do not assemble into ordered

structures, but become overstretched and eventually break under muscle generated tension (Figure 2). This suggests that Qsm is needed to somehow allow the high order assembly of rigid Dumpy filaments upon force.

Qsm is a short protein with a signal sequence, and interestingly also contains a single ZP domain followed by a protease-cleavage site and a putative transmembrane domain. Impressive live imaging by Chu and Hayashi showed that Dumpy-YFP and Qsm-Cherry are present together in intracellular vesicles and both get secreted. However, while Dumpy assembles into stable filaments, Qsm localises diffusely in the extracellular space showing high mobility (Figure 2). Hence, Qsm does not stably bind to assembled Dumpy filaments, so how does it affect Dumpy?

The authors turned to biochemical experiments in cells expressing a mini-Dumpy only containing the Dumpy ZP domain, protease cleavage site and transmembrane domain, together with Qsm. These experiments suggest that Qsm is helping Dumpy to effectively assemble into multimers, likely already within the intracellular vesicles. This ordered Dumpy can then be secreted and incorporated into periodic Dumpy filaments in the extracellular space.

It is known that ZP domains can form homo and hetero-dimers using their multiple disulfate bridges¹⁶, hence the authors speculate that Qsm may act as a molecular chaperon helping the conformational maturation of Dumpy's ZP domain. Where does this modification happen? Chu and Hayashi suggest it should happen within the vesicles before Dumpy is cleaved and secreted, as Qsm only binds effectively to non-cleaved mini-Dumpy (Figure 2). On the other hand, they also provide data that Qsm can act non-cell autonomously if either expressed in neighbouring epithelial cells or even in basally attached muscle cells, rescuing the *qsm* mutant phenotype. This argues that Qsm does not need its transmembrane domain and could rather modify Dumpy in the extracellular space. However, Chu and Hayashi find that muscle produced Qsm can be taken up into the tendon cells and colocalise with Dumpy in

intracellular vesicles which are then secreted apically. Thus, the role of Qsm as a chaperon for Dumpy might be limited to intracellular compartments, although its ability to chaperon secreted Dumpy in the extracellular space needs to be further investigated.

Taken together, this study is a fantastic illustration of how *Drosophila*, with its powerful genetics combined with high resolution live imaging, can help dissect a molecular mechanism. Chu and Hayashi identify a dynamic mechanism of matrix maturation involving mechanical force and a molecular co-factor, providing insight into how a gigantic extracellular protein complex can assemble into a strong organised matrix. As ZP domain proteins are present in multiple mammalian organs, deciphering the mechanism by which these various proteins assemble into higher ordered structures is an exciting topic. There are a few other examples of such dynamic maturation of ECM components, including the force dependant assembly of collagen I and fibronectin fibers in vertebrates^{4,5,19}, and the co-secretion dependent assembly of NOAH proteins in *C. elegans*²⁰. This study by Chu and Hayashi provides new evidence that mechano-chemical mechanisms are at play in specialised ECM maturation processes, opening new avenues of research. Such investigations might have important implications in regenerative medicine, and in particular in the field of interfacial tissue engineering¹, to respond to the challenge of reproducing the complex 3D organisation and mechanical properties of biological materials at soft-hard interfaces.

Figure legends.

Figure 1. Force and attachment order Dumpy filaments at tendons.

Attachment initiation and maturation of *Drosophila* flight muscle myotubes (green) to epithelial tendon layer (orange). Dumpy protein is produced in tendon vesicles (magenta) and secreted to the apical space facing the chitin exoskeleton (grey). Upon an increase in mechanical tension the myotube assembles its myofibrils (green-yellow) and in turn the Dumpy filaments become stretched and ordered showing a periodic spacing (magenta-green). This results in stable muscle-tendon-chitin attachment.

Figure 2. Quasimodo assists Dumpy maturation.

Wild-type (left) and *qsm* mutant (right) tendon cells secreting Dumpy containing vesicles at the apical surface (ovals). Qsm (blue) binds to membrane bound Dumpy (magenta) in wild type, which might facilitate the conformational maturation of Dumpy, and assists its ordering into larger filaments which become secreted. These Dumpy filaments mature further in the extracellular space to periodic filaments (magenta-green) in wild type but not in *qsm* mutants. As a consequence, the chitin – tendon interface breaks upon mechanical load in *qsm* mutants (right).

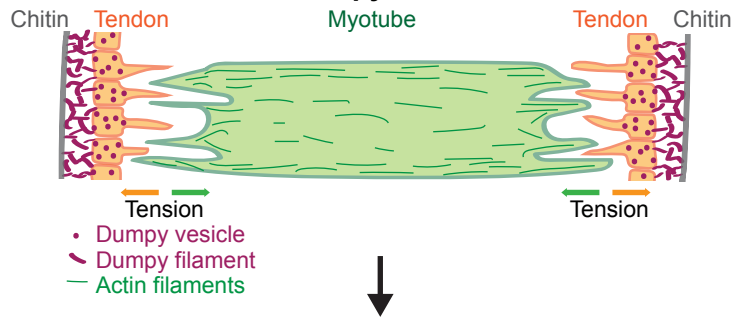
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Force-dependent Dumpy matrix assembly

Attachment initiation, Dumpy secretion



Attachment maturation, Dumpy ordering

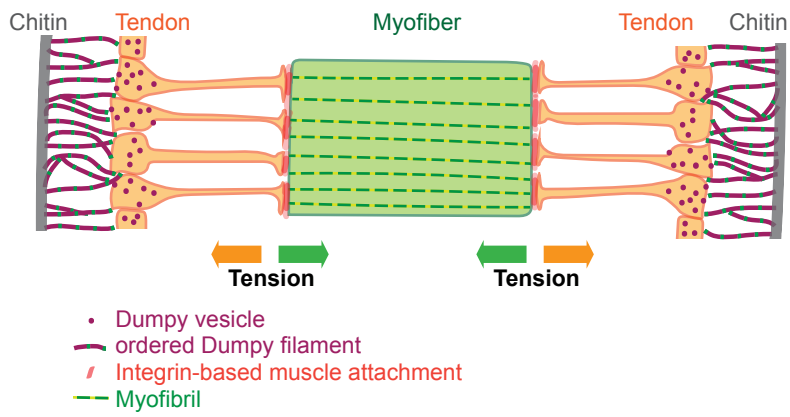


Figure 1

Quasimodo assists Dumpy maturation

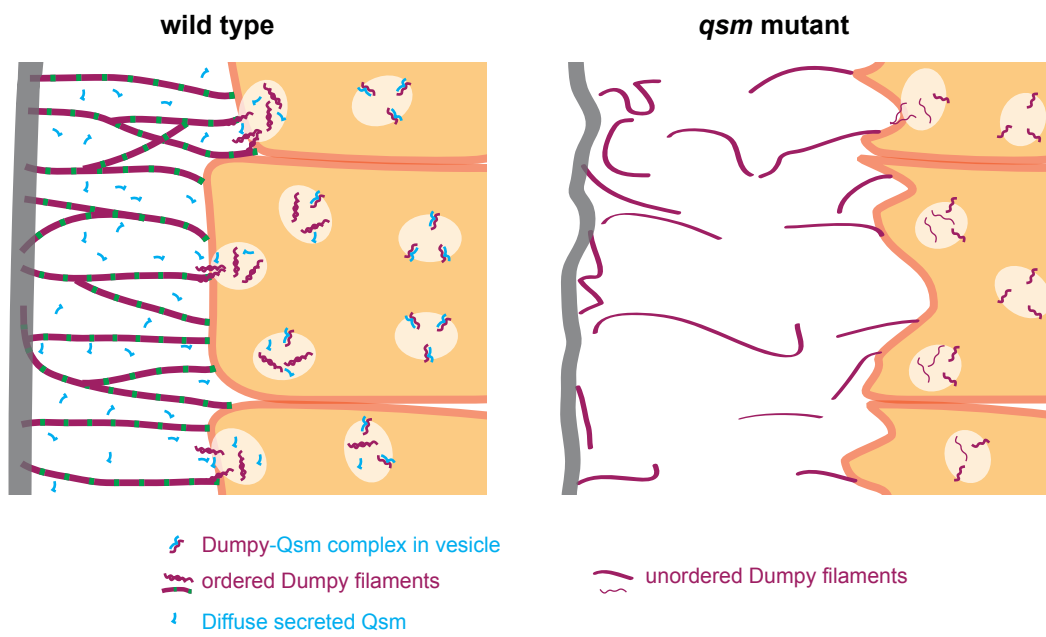


Figure 2