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Editorial

## Tendon—bridging the gap

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### 1. Introduction

Tendon, like fine art, is easy to recognize but difficult to define—indeed, this volume covers a variety of morphologies, from linearly arrayed tendon to sheet-like myoseptal tendons (Gemballa and Vogel, 2002). Furthermore, in contrast to the common perception of a static, inert, uniform tissue that connects muscle to bone, the manuscripts contained herein reveal tendon to be reactive, biochemically complex, and functionally diverse. Tendon bridges the functional gap between muscle and environment, and the papers from this symposium span gaps both in scale, from molecular to organismal, and in discipline from biochemistry to biomechanics and from paleontology to evolutionary biology.

Tendons are dense, well-organized, fibrous constructs composed primarily of collagen fibrils that transmit the forces generated by muscle fibers to the skeletal system (Benjamin et al., 2002; Purslow, 2002). We include in this description a variety of forms that contain tendinous tissue and vary in fiber organization and overall structure. The long tendons intrinsic to tetrapod limbs, particularly the digital flexor and extensor tendons are the most familiar form of tendon and naturally bias our perceptions of form and function. However, tendons first evolved as thin, sheet-like structures (myosepta) that deliver force over a broad area (Summers and Koob, 2002). Myosepta are the visible boundary between segmented axial musculature in fishes, and these stiff walls are a key component of the sinuous undulation these longitudinal muscles generate (Azizi et al., 2002; Long and Root, 2002). Some fish also possess linear tendons connecting the myomeres to the tail. These differ from mammalian tendons in

fascicle organization, yet they exhibit similar mechanical properties (Shadwick et al., 2002). Challenging our notion of tendon as a purely fibrous tissue, some tendons will mineralize, particularly in birds and dinosaurs (Hutchinson, 2002). These mineralizations are emphatically different from bone, the more familiar mineral/collagen amalgam (Landis and Silver, 2002). In a more subtle way, unmineralized linear tendons vary among themselves in morphology and material properties even within a single individual mammal (Alexander, 2002).

Tendons function primarily to transmit the force of muscle contraction, a function that requires a suite of integrated properties. The collagen fibrils in tendons are strong and stiff in tension. The organization in fibers and fiber bundles and the inter-fibrillar interactions of these fibrils allows appropriate deformation of the tendon, but at some length imparts sufficient stiffness to deliver the force of muscle with efficiency. The tensile strength of tendon must be far higher than the maximum force that can be generated by the muscle of origin. Tendon is also able to withstand intermittent low to high frequency repetitive loading, as well as constant loads, without attenuation of its tensile properties, fatigue, or irreversible elongation.

How do tendons achieve these properties? The collagen fiber system is responsible for the bulk properties of the tissue. The organization of these fibers constrains the magnitude of overall deformation, and their strength and stiffness dominate the tensile mechanics. Other extracellular matrix macromolecules likely contribute to the force—deformation properties of the tissue, and may play a significant role in mediating responses to dynamic loads and fatigue (Ker, 2002; Smith et al.,

2002). While these conclusions seem justified on the basis of a large body of empirical studies, it is safe to say that the exact molecular and physico-chemical bases for the mechanical properties of tendons have not been clearly delineated. This is an area ripe for future research.

Mammalian and avian tendons have been the focus of targeted research for the past half century. Interest in mammalian tendons stemmed in large part from four distinct motivations—(1) mammalian tendons are readily available, (2) since tendon is over 90% collagen, no better tissue could be found to delineate collagen fibrillar structure. The highly ordered arrays of collagen make tendon an ideal subject for studies using X-ray diffraction and electron microscopy. (3) The digital and extensor tendons play a clear, well defined biomechanical role in large mammals and are a superb material for mechanical testing, and (4) biomedical research has sought fundamental answers to why tendons fail to repair adequately after rupture or laceration, and what techniques and approaches are amenable to improving the repair process. In contrast, avian tendons have been exploited primarily as model systems *in vitro* to explore biosynthesis of collagen molecules and the assembly of collagen fibrils.

Much less attention has been garnered by tendons in non-model organisms. The functional properties of specific tendons as they relate to locomotion or other muscular activity have been examined in only a few species (Alexander, 2002; Roberts, 2002). Sporadic reports on particular functional attributes have pointed to the diversity and adaptation of tendons. However, there have been no systematic attempts to understand the biology of tendon. We are at a loss to explain why these potentially interesting and informative systems have not been explored, other than a general bias that tendon is an inert, homogeneous tissue with an obvious and well-understood function. Our intent here is to provide a brief overview of biochemical, structural and mechanical properties of tendon. We also highlight unresolved aspects of tendon biology, and point out areas we feel are important for future research. Our overall thesis is that tendons are dynamic structures that adapt to functional demands of the musculoskeletal system and have evolved diverse structural and functional properties to allow vertebrates to maneuver the skeletal system while applying directed loads of appropriate magnitude.

### *1.1. The cell biology of tendon*

During growth and development tendon fibroblasts synthesize, assemble and construct complex extracellular supramolecular arrays with long range integrated architecture. The mechanism of construction and the factors that regulate the process are unknown. The phenotypic expression of extracellular matrix macromolecules seems very likely to be under epigenetic control but the degree to which this plasticity is assimilated into the genome has not been explored.

Tendons attain an appropriate size and strength in relation to muscle mass and distance from insertion during growth and development, therefore tendon fibroblasts (tenocytes) must continually monitor the magnitude and direction of load. The mechanism of load sensing remains a mystery; however, empirical work on isolated tendon fibroblasts has shown that they respond to mechanically induced strains (Banes et al., 1999a,b). While cells appear as separate, dispersed units in tendon, they are in fact interconnected by extensive processes and gap junctions and can communicate local conditions to neighboring cells (McNeilly et al., 1996).

Several distinct tissues, each with their own cell types, are associated with tendons: the tendon proper which consists of the collagen fascicles and inter-fascicular domains, a thin bounding layer of cells with little matrix called the epitenon, and the sheath or peritenon. The development, properties and function of the cells that inhabit these tissues remains largely unexplored even in mammalian models (Koob, 2002), and nothing is known about differentiated tendon cells in lower vertebrates.

### *1.2. Tendon collagen*

Tendons are composed predominantly of collagen organized into fibrils, fibers, fiber bundles and fascicles, an organization presumed to be at the root of the mechanical properties of tendon (but see Summers and Koob, 2002). The fundamental unit is the type I collagen molecule, a triple helix composed of two  $\alpha_1$  and one  $\alpha_2$  chains. The  $\alpha_1$  and  $\alpha_2$  chains are products of different genes rather than post-translational modifications of a single molecule. Collagen molecules crystallize in specific linear arrays forming multi-molecular fibrillar aggregates easily visualized in electron micrographs as periodically banded fibrils. Neighboring

molecules are enzymatically polymerized after assembly in the extracellular matrix via lysine derived crosslinks. The extent and nature of crosslinking is variable, as pointed out by Shadwick et al. (2002), but the taxonomic and functional variation is largely unexplored.

A commonly measured parameter of tendon collagen is fibril diameter, usually obtained from transmission electron micrographs. Fibrils vary in diameter as a function of age, anatomical site and exercise. Tendons from young mammals have relatively small fibrils that fall within a unimodal distribution. As animals age, fibril diameters increase and generally segregate in a bimodal distribution (Parry et al., 1978). Derwin and Soslowsky (1999) also found a relationship between material properties and fibril diameter. However, it is important to interpret these results with caution since there are probably coincidental changes in the matrix. The causal mechanism of fibril diameter changes is likely rooted in changes in the non-collagenous macromolecules of the matrix, and it is possible that material property changes have their basis here as well. In other words, until we have more information on the effects of fibril diameter and the extent of changes it is possible that it is an epiphenomenon of changes in the matrix.

The length and the shape of the collagen fibril are also important parameters to be measured. Implicit in reports of fibril diameter distributions from tendon is the assumption that fibrils are nearly uniform cylinders. If this assumption is correct, then a fibril's measured diameter is a valid descriptor, and distributions indicate that cylindrical fibrils of varying diameter comprise tendon fibers. If instead the collagen fibrils have a spindle shape, as has been proposed by Trotter and Wofsy (1989), then diameter distribution is an artifact of sectioning different fibrils at different points along their tapering length. Surprisingly, after half a century of study on tendon fibril structure, the answer remains obscure, though the implications, functionally and organizationally, are clearly important (Trotter, 2002).

A question related to the shape of the fiber, and no less difficult to address, is the issue of length. Do collagen fibrils span the entire tendon between the muscle and the point of insertion, or do fibrils end in the body of the tendon? There is little evidence to substantiate either hypothesis. However, Trotter and Wofsy (1989) reported that col-

lagen fibrils in rat tail tendon taper and end within the body of the tendon. The answer to this question has immediate implications for the predominant mode of force transmission in tendon. Tendons are regarded as predominantly tensile structures, and if the fibers are full length then they are certainly tensile structures. However, if the fibrils do not run the length of the tendon then force must be transmitted from one fibril to another. If the fibrils do not taper then tensile force can be transmitted through an abutting joint between fibrils, or through shear between adjacent fibrils. On the other hand, if the fibrils are tapering then the only possibility is that shear is the dominant mode of force transmission in the tendon. This would have implications for the natural mode of repair, the mechanics of failure, and the mechanism of fatigue.

### *1.3. Non-collagenous matrix macromolecules of tendon*

In spite of their low relative abundance, non-collagenous extracellular macromolecules, either intimately associated with collagen fibrils or dispersed in the inter-fibrillar matrix or inter-fascicular domains, play important roles in tendon biology. We do not even have a complete catalog of the molecules present in tendon from different vertebrates, let alone a clear idea of their function. However, empirical evidence suggests a role for at least a few of the more prevalent molecules.

Decorin, a dermatan sulphate-rich proteoglycan with a single glycosaminoglycan side chain, is so called because it 'decorates' the assembled collagen fibrils in tendon. Decorin appears to wrap around the collagen at discrete locations, as a rubber band might hold together a sheath of pasta. Evidence from knockout mice and from in vitro collagen assembly experiments suggests that decorin in some way mediates the formation of the collagen fibril. A controversial, but plausible hypothesis, is that it allows fibrils to increase in diameter up to a point and then prevents further self-assembly (Canty and Kadler, 2002). It is worth mentioning that in spite of clear morphological evidence that decorin spans collagen fibrils (Scott, 2001), there is no experimental evidence at all that they form cross links or have any effect on the material properties of tendon. This is an important area of research as the degree of cross

linking has been imputed to affect stiffness (Buchanan and Marsh, 2001, 2002).

Aggrecan, a very large proteoglycan, is found in high concentration in regions of tendon subjected to compressive loading. This molecule has a high fixed negative charge density and is responsible for much of the resilience in cartilage. Presumably it is playing a similar role here, though the mechanism of induction is not known. Other proteoglycans including biglycan, fibromodulin, lumican, and versican, are found in tendon matrix, but essentially nothing is known about their function or their regulation. In addition to proteoglycans, tendon contains Type VI collagen in microfibrillar arrays, fibronectin associated with cells, and cartilage oligomeric matrix protein. Again, we know little about the functional significance of these macromolecules, although they vary in abundance with respect to tendon type and regional specializations, age and exercise (e.g. see Smith et al., 2002).

#### 1.4. The development of tendon

Tendon arises from the lateral plate mesoderm, the same cells that give rise to endoskeletal cartilage. Though this tissue has biomedical significance there is very little research directed towards understanding the very early embryology of tendon. We know a great deal more about both muscle and bone formation than we do about the tissue between them, though this area is ripe for a molecular investigation, and there are some good early data on signaling molecules (D'Souza and Patel, 1999). The involvement of TGF $\beta$ , and scleraxis imply that there are similarities between the pathways that pattern the skeleton and those at work in tendon, but the particulars are far from clear (D'Souza and Patel, 1999; Edom-Vovard et al., 2002).

Surprisingly there is no experimental evidence on the most obvious question of tendon development, namely 'how does a tendon get longer and thicker during ontogeny'? It is not clear how a rope under continuous tension can be lengthened, yet that is essentially the situation that obtains during the growth to adulthood. If collagen fibers run the length of the tendon then length could be added by appositional growth, though width is a more difficult process to imagine. If fibers run only part way then new ones can be added in the middle, but there is a requirement that bonds

between fibers break and reform as the tendon grows in length. Neither possibility has been experimentally addressed, though in a ligament it appears that the latter situation applies (Wood et al., 1998).

#### 1.5. Bridging the gap

This symposium represents the very first time that a gathering of biologists from different disciplines has come together specifically to discuss the biology of tendon. We feel the symposium was held at a key juncture in the study of this tissue. It is a time when our knowledge of tendon is maturing to the point where there can, and will, be exciting and productive collaborations between scientists working at very different organizational levels. Functional morphology will inform development, biochemistry will inform biomaterial research, and paleontology and evolutionary biology are beginning to have enough basic information to commence fruitful investigations of tendon.

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