Understanding the extraocular muscles: connective tissue, motor endplates and the cytoskeleton

We constantly direct our eyes to the object of interest with the help of the extraocular muscles, and thereby use foveal fixation to attain the best possible visual acuity. The muscles around the eye are rather different from other skeletal muscles, being, for example, simultaneously the fastest muscles in the body and impossible to exhaust. The most exciting property of the extraocular muscles is their unique response to disease, as they often remain unaffected in muscle conditions which lead to severe handicap and premature death. Understanding the coping strategies that allow the extraocular muscles to remain unaffected may provide clues for the future treatment of severe diseases such as muscle dystrophies.

The extraocular muscles are crucial for vision and have unique properties

Each eye is equipped with six extraocular muscles (EOMs) that are fundamental for the execution of the different types of eye movements required for optimal vision. With our eyes, we are, for example, able to fixate, to smoothly follow an object in motion, to converge or to rapidly redirect our gaze. We constantly direct our eyes to the object of interest and use foveal fixation to attain the best possible visual acuity, as illustrated in Figure 1. Disturbed eye movement coordination leads to ocular misalignment (squint, strabismus) and double vision (diplopia). These have negative effects not only on sight, but also on self-esteem, social relations, employment and driving. Furthermore, untreated strabismus in children leads to permanent low visual acuity (lazy eye, amblyopia).

The complexity of actions performed by the EOMs is reflected by how these muscles are built. For example, the characteristics and molecular composition of their cells, the muscle fibres, differ substantially from those of the muscle fibres of ordinary skeletal muscles. Indeed, the gene expression profile of the EOMs differs fundamentally from that of the limb muscles with respect to metabolic pathways, structural components and developmental and regeneration markers by over 330 genes. For example, the EOMs strikingly lack glycogen storage and rely on direct supply of glucose from the blood circulation. This has very important physiological implications, making it metabolically possible for the EOMs to be among the fastest muscles in the body and, at the same time, fatigue resistant like no other. Imagine being able to run a marathon forever at a speed higher than that of a 100 m sprinter without getting tired!

Despite this knowledge of differences in gene expression profile, our understanding of some fundamental properties of the EOMs is still rather limited. The most remarkable property of the EOMs is their distinct response to disease. The EOMs are selectively affected in autoimmune disorders such as myasthenia gravis, Miller-Fisher syndrome and Graves’ ophthalmopathy. In contrast, they are surprisingly spared in various muscular dystrophies such as Duchenne, Becker, limb-girdle and congenital muscular dystrophies that are devastating for the other muscles in the body, causing severe weakness, loss of ambulation and early death. The EOMs are also particularly resistant to amyotrophic lateral sclerosis (ALS), being capable of maintaining intact neuromuscular junctions until the end of the disease. In other words, the EOMs remain unaffected by very severe muscle diseases that we lack effective treatment for.

Our research is focused on understanding the cellular and molecular basis of the unique properties of the EOMs, such as their particular resistance to diseases that affect all...
other muscles in the body. Understanding how the EOMs cope with gene defects that lead to muscle fibre rupture, degeneration and loss of function may provide helpful insights on how to develop new therapies for muscle dystrophies and myopathies that lead to severe handicap and death. It is well known that muscle fibre integrity relies on an intact molecular link from the connective tissue, in particular, the basement membrane on the surface of the muscle fibre, across the cell membrane (the sarcolemma) to the cytoskeleton inside the muscle fibre. Furthermore, the cytoskeleton is highly specialized at the neuromuscular junctions. In other words, understanding the connective tissue, the motor endplates and the cytoskeleton is of crucial importance to understand the EOMs and their unique properties.

**Fibre types in the EOMs**

The muscle fibres in the EOMs are rather small and have a very complex composition with regard to the major proteins determining contraction force and velocity (myosin heavy chains [MyHC]), and fibre relaxation rate. Skeletal muscle fibres are usually divided into slow-twitch and fast-twitch fibre types containing either slow or fast MyHC isoforms. In contrast, the muscle fibres in the EOMs usually co-express several MyHC isoforms including very unusual isoforms, such as MyHC extraocular or MyHC slow tonic, or isoforms that are typically present during muscle development but that are down-regulated in mature skeletal muscle, such as MyHC embryonic and MyHC fetal. The co-expression of a variable number of MyHC isoforms in varying proportions indicates that the fibre types in the EOMs are very finely tuned, forming a continuum that most likely reflects small differences among motor units. Another special characteristic of the EOMs is the small size of their motor units, which is around 10 muscle fibres only, in contrast to a typical motor unit comprising hundreds of myofibers.

In conclusion, the cellular and molecular composition of the EOMs is far more complex than that of other...
muscles in the body and seems well adapted to the special functional requirements of moving the eye so precisely.

## Connective tissue

A particular feature that distinguishes the EOMs is their abundant connective tissue bed. In fact, generally, each myofiber in the EOMs is surrounded by connective tissue rather than being in immediate contact to adjacent fibres, as it is typically seen in other skeletal muscles (Figure 2). This gives the EOM fibres a round contour, whereas in other muscles, the adjacent fibres typically get a polygonal contour, dictated by the close apposition of the neighbouring muscle fibres. We have established that the connective tissue around individual muscle fibres in the EOMs is continuous with that between myofibers (the endomysium) and around muscle fascicles (the perimysium) and finally with that forming a cover around the whole EOM (the epimysium). There is also physiological evidence for the lateral dissipation of force within the EOMs, indicating a strong interconnectedness from the individual myofiber to the whole muscle level. Furthermore, this lateral transmission of force is most likely to be of functional importance, given that the muscle fibres in the EOMs do not run the full length of the muscles. Altogether, these findings indicate that separate movement of individual parts of the EOMs is not possible.

The connective tissue among muscle fibres in the EOMs contains fibroblasts that show distinct properties from those of other skeletal muscles and that likely contribute to the unique response of the EOMs to inflammatory disease, such as their particular involvement in Graves' ophthalmopathy. On the other hand, important differences in the composition of the basement membrane are likely to protect the EOMs in congenital muscle dystrophy caused by gene defects on the major laminin α-chain isoform, Lna2-deficient congenital muscular dystrophy, also known as merosin-deficient congenital muscular dystrophy. The basement membrane is located on the immediate surface of skeletal muscle fibres, and the laminins are the major non-collagenous components of the basement membrane. Laminins are linked to receptors that span the thickness of the cell membrane and are thereby anchored to the cytoskeleton inside the muscle fibre. We have shown that the basement membrane around the muscle fibres in the human EOMs contains both the typical laminin α-chain isoform present in adult skeletal muscle fibres and, in addition, other laminin chain isoforms that are only present during fetal development and are normally down-regulated thereafter. In summary, the EOMs remain unaffected by congenital defects on Lna2 because they normally have additional isoforms of the same laminin chain. The maintained presence of developmental isoforms that are down-regulated in other adult skeletal muscles is a common trait of the adult EOMs and it may indeed be a protective strategy in disease.

## Motor endplates

In general, each muscle fibre has one motor endplate where a single axon delivers the chemical signal, acetylcholine, that will command the muscle fibre to twitch. The invaginated muscle fibre membrane in this area is specialized, displaying acetylcholine receptors which can easily be visualized by using the snake venom α-bungarotoxin and which are composed of several subunits. The subjacent cytoplasm is also specialized, particularly at the cytoskeleton.

![Figure 2. By using scan electron microscopy, we can easily visualize the connective tissue sheaths around the muscle fibres. On the left, a rather thin sheath separates adjacent muscle fibres in a limb muscle (a). In contrast, on the right, you see the impressive sleeve of connective tissue surrounding each individual muscle fibre in the EOMs (b). Please note that the scale bars are different between the two pictures, as the muscle fibres in the EOMs are much smaller than those in limb muscles.](image-url)
The typical motor endplates described above, seen in muscle fibres all over the body, are also present in all major types of muscle fibres in the EOMs, where they are called ‘en plaque’ endplates. In addition, the special muscle fibres of the EOMs that contain MyHC slow tonic are multiply innervated and display rows of small endplates, the so-called ‘en grappe’ motor endplates. These muscles fibres have a tonic mode of contraction, in contrast to the twitch mode of contraction. We have recently described a novel type of innervation in the human EOMs, consisting of multiple en plaque endplates closely located on the same muscle fibre. Adjacent en plaque endplates on the same muscle fibre may contain different isoforms of acetylcholine receptors, which can be interpreted as an evidence of poly-innervation, that is, that different axons supply a single muscle fibre, in contrast to the dogma that mature muscle fibres are innervated by a single axon. Altogether, at least one-third of the muscle fibres in the human EOMs are multiply or poly-innervated. This has physiological implications for the function of this so finely tuned muscles and may be protective in diseases that lead to the loss of axons, such as ALS.

**The cytoskeleton and clues to understanding other muscles**

The cytoskeleton of muscle cells plays an important role anchoring the contractile filaments inside the muscle fibre to each other and to the cell membrane, and thereby to the basement membrane on the surface. It is of utmost importance in dictating the cell shape and length and for the transmission of changes in cell shape, that is, muscle contraction, to the surrounding connective tissue between adjacent muscle fibres and to the tendon. Desmin has been considered ubiquitous in muscle fibres and the most important cytoskeletal protein with this role. Labeling for desmin has been routinely used to identify muscle fibres and to localize their motor endplates, as desmin is particularly enriched on the muscle side of the synapse (Figure 3). Although muscle fibres can be formed in the absence of desmin, gene defects in this protein lead to a myopathy particularly evident in highly used fast muscles. However, it turns out that a subgroup of healthy, intact muscle fibres in the human EOMs lack desmin, which is a true paradigm shift and indicates that desmin is not ubiquitous in muscle. When we first reported this, we were rather puzzled by the fact that the lack of desmin was not seen on all muscle fibres of a given type, and we have since then been able to show that there is a very complex relation between desmin and the motor endplates on the human EOMs. In fact, there are motor endplates that lack desmin (Figure 3), raising important questions regarding signalling between the extracellular matrix on the surface and the interior of the muscle fibres. We have also been able to determine that the lack of desmin in muscle fibres of the EOMs is not a fault, but rather a conserved feature across species, all the way to zebrafish (Figure 4). This is a very exciting finding.

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**Figure 3.** The top row shows typical muscle fibres in a limb muscle (a–c) containing desmin, which is more abundant at the motor endplate (arrow). In the lower row, we see two muscle fibres in an EOM (d–f). The upper muscle fibre is labeled green, indicating that it contains desmin, whereas the lower muscle fibre lacks desmin and has four motor endplates close by, the so-called multiple ‘en plaque’ endplates (arrowheads). α-Btx is the abbreviation of α-bungarotoxin, a snake venom used to label the motor endplates.
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as it provides us with a sophisticated model to further investigate the roles of desmin and, in particular, the importance of the cytoskeleton for the transmission of information inside the muscle cell. In addition, and you can almost guess by now, the EOMs remain unaffected in desmin myopathy.

Gaining insight on how these muscles are able to keep muscle fibre integrity independently of such a crucial cytoskeletal protein (desmin) may provide useful clues for future treatment of the other muscles. The opportunity to find clues about how to successfully deal with gene defects that affect all muscles in the body is, in fact, what makes the EOMs irresistible!

Summary

We constantly direct our eyes to the object of interest with the help of the EOMs, and thereby use foveal fixation to attain the best possible visual acuity. The muscles around the eye are rather different from other skeletal muscles, being, for example, simultaneously the fastest muscles in the body and impossible to exhaust. The most exciting property of the EOMs is their unique response to disease, as they often remain unaffected in muscle conditions which lead to severe handicap and premature death. Understanding the coping strategies that allow the EOMs to remain unaffected may provide clues for the future treatment of severe diseases such as muscle dystrophies.
Jing-Xia Liu was born in China and got her PhD at Umeå University in Sweden. After her postdoc in the USA, she moved back to Umeå University where she investigates the extraocular muscles using different approaches to understand how their unusual molecular composition contributes to their resistance to disease.

Nils Dennhag is doing his PhD on muscle diseases using the zebrafish model. Since he is the zebrafish expert in our lab, he has been given a lot of responsibility and freedom in terms of designing experiments, which he enjoys and appreciates a great deal.

Fatima Pedrosa Domellöf comes from Portugal and is a professor of ophthalmology at the Department of Clinical Science, Umeå University. She combines being the PI for the dream team working on the extraocular muscles with teaching medical students and working as a clinician at the Eye Clinic at Umeå University Hospital. Email: fatima.pedrosa-domellof@umu.se