An Introduction to Smooth Muscle Mechanics (2nd Edition)

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By

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To Nancy and Kathryn

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PREFACE

This book is an updated version of a book with the same title that I published in 2016. New materials were added in chapters 2, 3, and 5. The following is the preface I wrote for the first edition; it is just as relevant to the current edition.

The need for an introductory level textbook on smooth muscle mechanics became obvious when I started to teach the subject in a graduate level course and parts of some undergraduate courses at the University of British Columbia many years ago. The students who took the courses came from diverse backgrounds (with a wide range of departmental affiliations: Pathology, Physiology, Pharmacology, Mechanical Engineering, Biochemistry, Human Kinetics, Physiotherapy, Physical Education, and Nursing). A challenge I faced was to make this subject understandable to these students who possessed limited knowledge of muscle physiology, and many of them also lacked general knowledge about the physics of solids and fluid mechanics. Ironically, many of them were not interested in learning the latest developments in the field of smooth muscle research, although most of them used smooth muscle preparations in their thesis research. Smooth muscle to them was a tool. For example, a pharmacology student studied the interaction of drugs with receptors on the cell membrane of smooth muscle, and the force produced by the muscle was taken as the final outcome of that interaction.

Questions from the class were usually related to the methodology, instrumentation, and data analysis needed for a smooth muscle experiment where mechanical properties of the tissue were measured. Available textbooks on muscle physiology or biomechanics unfortunately fall into two categories, one with too little information (e.g., general physiology textbooks), and the other with too much (e.g., books dealing with advanced and sometimes controversial topics of muscle physiology, intended for smooth muscle researchers). Neither of them is helpful to my class.

This book is therefore written for students who are interested in the measurement and analysis of basic mechanical properties of smooth muscle. To understand and interpret the data from their experiments, it is necessary that the students know about the essentials of smooth muscle function and structure, and also some fundamentals in physics, specifically mechanics. To properly understand muscle mechanics, which encompasses elements of

solid and fluid mechanics, mathematics is an essential tool. It may be possible to explain the mechanical properties and behaviors of smooth muscle in non-mathematical terms, but it will be superficial and imprecise.

The use of mathematical formulation and analysis in this book is based on easily understood basic principles and is intended to foster the ability of the students to gain not only qualitative but also quantitative understanding of a subject through their own derivation based on relatively simple facts and assumptions. It is also intended to cultivate a habit of systematic approach in obtaining solutions from understanding the fundamentals underlying the problem. The detailed, step-by-step mathematical derivation is therefore a unique feature of this book. I believe that some smooth muscle characteristics, such as the exponential time course of shortening against a constant load, and the kinetics of the actomyosin cross-bridge cycle and its mechanical manifestation as a hyperbolic force-velocity relationship, can only be truly understood through mathematics.

Although the book is written for graduate level courses, the prerequisite for this book is elementary physics, biology, and mathematics at the college freshman level. Advanced topics in muscle mechanics, physiology, biochemistry, and pharmacology are avoided in this book.

I would like to thank Drs Peter Paré, Lu Wang, and Pasquale Chitano, who have read the manuscript that was the precursor of this book, for their invaluable comments and suggestions for improvement. I am grateful to the authors and publishers of previously published material for granting me permission to adapt from their work in many of the figures in this book.

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Vancouver, Canada
2021

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LIST OF SYMBOLS

α_1-AR	α_1 adrenergic receptor
AC	adenylate cyclase
β_2-AR	β_2 adrenergic receptor
CICR	calcium-induced calcium release
CE	contractile element
CM	calmodulin
ΔL	isotonic shortening
ΔL_{max}	maximal isotonic shortening
DG	diacylglycerol
EFS	electric field stimulation
ER	endoplasmic reticulum
f_{APP}	rate of (cross-bridge) attachment
F_{max}	maximal isometric force
F_{rest}	resting force
F_{total}	total force ($F_{max} + F_{rest}$)
g_{APP}	rate of (cross-bridge) detachment
GPRC	G-protein coupled receptor
IP_3	inositol 1,4,5-trisphosphate
L_{ref}	reference length
M_3R	muscarinic 3 receptor
MLCK	myosin light chain kinase
MLCP	myosin light chain phosphatase
PEC	parallel elastic element
PIP_2	phosphatidylinositol 4,5-biphosphate
PLC	phospholipase C
PKC	protein kinase C
P_{max}	maximal power
PSS	Physiological saline solution
RLC	myosin regulatory light chain
RyR	ryanodine receptor
SEC	series elastic element
SR	sarcoplasmic reticulum
V_{max}	maximal shortening velocity

CHAPTER 1

INTRODUCTION

Anatomy and Physiological Function of Smooth Muscle

Muscle cells are specialized to perform mechanical work by shortening against a load. There are three types of muscle: skeletal, cardiac, and smooth. Skeletal muscle attaches to the skeleton through tendons and is responsible for actions such as locomotion, maintenance of posture, respiration, and speech. Synchronized contraction of cardiac muscle is responsible for the pumping action of the heart. Both skeletal and cardiac muscles are categorized as striated muscle because of the transverse striations seen in these cells under a phase-contrast or interference light microscope. The striations reflect the highly organized contractile filaments of the sarcomeric structure within these cells. Smooth muscle cells, on the other hand, do not possess cross-striations, due to the lack of uniformly aligned arrays of contractile filaments. The absence of a well-organized filamentous structure in smooth muscle does not mean that the contractile filaments are missing. In fact, the myosin-containing thick filaments and the actin-containing thin filaments are abundantly present in smooth muscle, and it is believed that the sliding-filament, cross-bridge theory explaining striated muscle contraction (Hanson and Huxley, 1953; Huxley and Niedergerke, 1954; Huxley 1957) is also applicable for smooth muscle contraction.

However, there are some fundamental differences between striated and smooth muscles in the structure of the contractile apparatus, especially in the degree of malleability and plasticity of the cytoskeletal structures of smooth muscle (not seen in striated muscles) that deform in response to external stress and strain, and in smooth muscle's ability to function over a large length range (Seow and Solway, 2011).

Smooth muscle cells line the walls of hollow organs and serve as the driving force that alters organ volume and wall stiffness. Changes in organ dimension and wall stiffness have direct consequences for the organs' physiological function. The diverse functions of smooth muscle in

hollow organs include maintenance of blood pressure through regulation of blood-vessel diameter, moving contents along the digestive tract through peristalsis, and emptying urine by reducing the bladder volume, just to mention a few. Smooth muscle cells usually work as a group, not as individual cells. Fig. 1-1 illustrates a *smooth muscle bundle* made of individual cells embedded in an **extracellular matrix**.
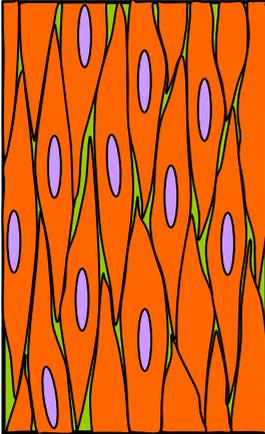
Fig. 1-1: *Schematic drawing of a longitudinal section of a bundle of smooth muscle cells. Smooth muscle cells (orange with purple nuclei) are embedded in the extracellular matrix, which is largely made of collagen and elastin fibers (green).*

In hollow organs that regularly undergo large changes in volume, such as the urinary bladder, the functional length-range of the smooth muscle is correspondingly large. This is expected because the function of the organ requires the muscle to be able to generate force over its entire working length-range. However, in many tissues (e.g., airway and blood vessel), even though a small working length-range of the smooth muscle is sufficient for their proper function, the muscle nevertheless still possesses a large functional length-range (Pratusevich et al, 1995). Unlike the relatively small functional length-range of skeletal muscle (limited by the skeleton), the large length-range of smooth muscle, if not properly regulated, could become a source of pathological conditions underlying organ or tissue dysfunction, such as that in an excessively narrowed blood vessel or airway.

Unlike the cartoon depiction shown in Fig. 1-1, actual smooth muscle cells *in situ* are long and thin, and are sometimes referred to as fibers. Many of the cells have tapered ends and each cell contains a centrally located nucleus. The diameter of a smooth muscle cell varies inversely with the cell length because the cell volume is largely conserved at different lengths (Kuo et al, 2003). For **cell dimension**, the diameter of

the central segment of the cell ranges from 2 to 10 μm, and the cell length is typically 100-200 μm long, but can be as long as 600 μm. Smooth muscle cells are usually found in bundles, and the cells within a bundle work as a mechanical syncytium (Kuo and Seow, 2004). Within each bundle, the cells are mechanically connected to one another directly or indirectly through the extracellular matrix in which the cells are embedded. The cells in a bundle are aligned in such a way that their long axes are parallel to one another (Figure 1-1). The long axes of the cells are also in parallel with the axis of force transmission through the cell bundle under *in vivo* working conditions.

Because smooth muscle cells *in situ* can only effectively generate or transmit force along their long axes, the cell orientation within an organ wall is important for proper function of the organ. In **tubular vessels** such as arteries, veins, and bronchi, smooth muscle cells are usually arranged circumferentially so that shortening of the muscle can be effectively converted to narrowing of the vessel (Fig. 1-2). Exceptions can be found, especially in small blood vessels and bronchi, where smooth muscle bundles are arranged obliquely along the tube length.
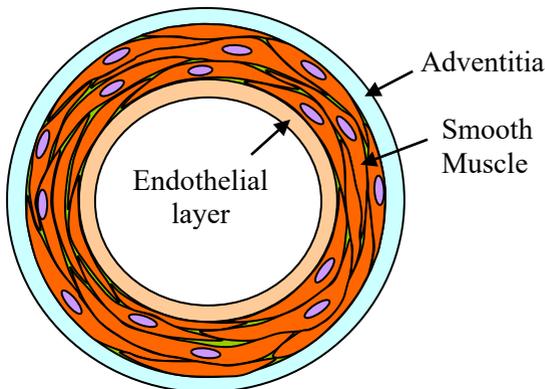
Fig. 1-2: *Schematic drawing of a cross-section of a small artery.*

In organs with a **saccular** structure, such as the urinary bladder and stomach, there are usually many layers of smooth muscle cells. In different layers, the muscle bundles are oriented in different directions. This allows the organ to reduce its wall area in all directions when the cells contract, and therefore effectively reduce organ volume or increase pressure inside the organ.

Peristalsis, observed in many **tubular organs** — such as the colon and ureter — requires coordinated contraction of circumferential, longitudinal, and even obliquely oriented layers of smooth muscle cells. Fig. 1-3 shows a part of a cross-section of a pig ureter. Muscle bundles of different orientations within the ureter wall are evident. The circumferentially arranged muscle cells appear as an elongated bundle, whereas the longitudinally arranged cells (along the tube length) appear as a rounded bundle (Fig. 1-3).
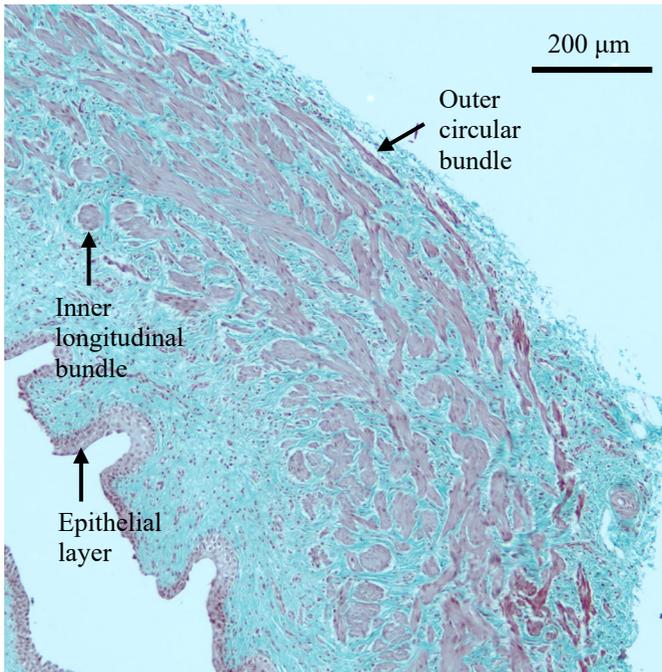
Fig. 1-3: *A part of a pig ureter cross-section, showing smooth muscle bundles (red) embedded in the ureter wall, which is made up of mostly collagen and elastin fibers (green-blue). In between the outer circular muscle layer (with cell bundles appear to be elongated) and the inner longitudinal muscle layer (with cell bundles appear to be rounded), there are layers of muscle cells with oblique orientations. The tissue was fixed with formaldehyde and paraffin embedded. The section was stained with Masson's trichrome.*

Activation of Smooth Muscle

Smooth muscle can be activated electrically or with agonists such as neurotransmitters or inflammatory mediators. *Electrical stimulation* may lead to the generation of action potentials in some smooth muscles (such as the visceral smooth muscles), but only results in depolarization in others (such as the vascular smooth muscles).

Depending on the pattern of innervation and the spread of electrical signals, smooth muscles can be categorized as either single unit or multiunit. In **single unit** smooth muscle, the cells are bundled together as an electrical syncytium. This is made possible by the presence of **gap junctions** on the cell membrane, which provide low resistance pathways for electrical signals between adjacent cells. Gap junctions also allow the diffusion of small molecules between cells. In **multiunit** smooth muscle, the cells are innervated individually and there is no electrical connection between cells; activation of one cell does not lead to activation of the adjacent cells. The broad classification of smooth muscles into single unit and multiunit is an over-simplification, because most smooth muscles possess both characteristics of the single-unit and the multiunit types. The classification simply groups smooth muscles into one of the extremes of the spectrum, which varies from purely single unit behavior to that of multiunit.

Another system of classification divides smooth muscles into phasic and tonic. This classification is also an over-simplification for the same reason as the classification of smooth muscle as single-unit and multiunit. The terms phasic and tonic are commonly used to describe the general behavior of smooth muscles in terms of the pattern of their maintained state of activation. Transient activation is seen in **phasic smooth muscles**, such as those lining the walls of the stomach, intestines, and urinary bladder. Sustained (and often partial) activation is seen in **tonic smooth muscles**, such as those lining the blood vessels and sphincters. There are smooth muscles that do not fall neatly into these two categories. Airway smooth muscle, for example, sometimes exhibits phasic characteristics, while at other times it behaves like tonic smooth muscle. This perhaps reflects the fact that the behavior of many smooth muscles depends on the immediate micro-environments within which they reside. In the chronic presence of contractile agonists and/or the absence of agonist inhibitors, airway smooth muscle may behave more like tonic smooth muscle, while in the absence of agonists and/or the presence of inhibitors it may behave more like phasic smooth muscle.

Most smooth muscles are innervated by the autonomic nervous system; many receive both sympathetic and parasympathetic innervations. Smooth muscle in blood vessels is dominated primarily by the **sympathetic innervation**, whereas smooth muscle in the airways is dominated by the **parasympathetic innervation**. The neurotransmitters associated with the sympathetic stimulation are norepinephrine and epinephrine; these agonists act on **α₁ adrenergic receptors** to cause contraction, and on **ß₂ adrenergic receptors** to cause relaxation. The neurotransmitter associated with parasympathetic stimulation is acetylcholine. The **muscarinic receptor** mediates cholinergic transmission in this type of smooth muscle. Binding of acetylcholine to the receptors leads to contraction. Many smooth muscle tissue preparations contain autonomic nerve endings. Upon **electrical stimulation** neurotransmitters are released from the nerve endings and through diffusion bind to receptors on smooth muscle cell membrane to cause contraction. Activation by electrical stimulation in an airway smooth muscle bundle can be abolished by atropine, a muscarinic receptor antagonist, demonstrating the fact that electrical stimulation does not directly activate smooth muscle cells in this preparation.

Table 1-1 lists the major neurotransmitters and receptors that are expressed on the cell membrane of some smooth muscles, in addition to several common pharmacological agonists and antagonists for the receptors.

Table 1-1: Agonists and antagonists for adrenergic and cholinergic receptors in some smooth muscles

Agonist	Receptor	Muscle Response
Nor(epinephrine)	α_1-AR	Contraction
	β_2-AR	Relaxation
Isoproterenol	β_2-AR	Relaxation
Phenylephrine	α_1-AR	Contraction
Acetylcholine	M_3R	Contraction
Methacholine	M_3R	Contraction
Antagonist	**Receptor**	**Muscle Response**
Phentolamine	α_1-AR	Relaxation
Propranolol	β_2-AR	Contraction
Atropine	M_3R	Relaxation

Note: Norepinephrine and epinephrine act on both α and β receptors: α₁-AR: α₁ adrenergic receptor; β₂-AR: β₂ adrenergic receptor; M₃R: M₃ receptor (muscarinic).

Besides the receptors for neurotransmitters, smooth muscle cells also possess many receptors for hormones and inflammatory mediators, as well as local factors, such as angiotensin II, vasopressin, endothelin, histamine, and adenosine. Binding of mediators to their receptors directly modulates the state of activation of the smooth muscle.

As in striated muscle, Ca^{2+} is central to the activation of smooth muscle (Fig. 1-4). An increase in the intracellular (cytoplasmic) concentration of Ca^{2+} ($[Ca^{2+}]_i$) is the result of influxes of Ca^{2+} into the cytoplasm of smooth muscle through calcium channels. On the sarcolemma, there are voltage-dependent, store-operated, and receptor-activated Ca^{2+} channels. On the sarcoplasmic reticulum (SR), there are **inositol 1,4,5-trisphosphate (IP$_3$)**-gated channels through which Ca^{2+} can enter the cytoplasm. Ca^{2+} entry can also occur through calcium-induced calcium-release (CICR) via the **ryanodine receptor (RyR)**. Voltage-dependent Ca^{2+} channels are activated by membrane depolarization, store-operated channels are activated by depletion of intracellular calcium stores, and receptor-activated channels are activated by direct binding of an agonist to the channel-receptor. In resting smooth muscle, $[Ca^{2+}]_i$ is normally maintained at less than 10^{-7} M by the action of Ca^{2+} pumps and Na^+-Ca^{2+} exchangers on the sarcolemma, as well as Ca^{2+} pumps on the SR. A Na^+-Ca^{2+} exchanger is an antiporter which uses the energy from the electrochemical gradient of Na^+ to extrude Ca^{2+} to the extracellular space.
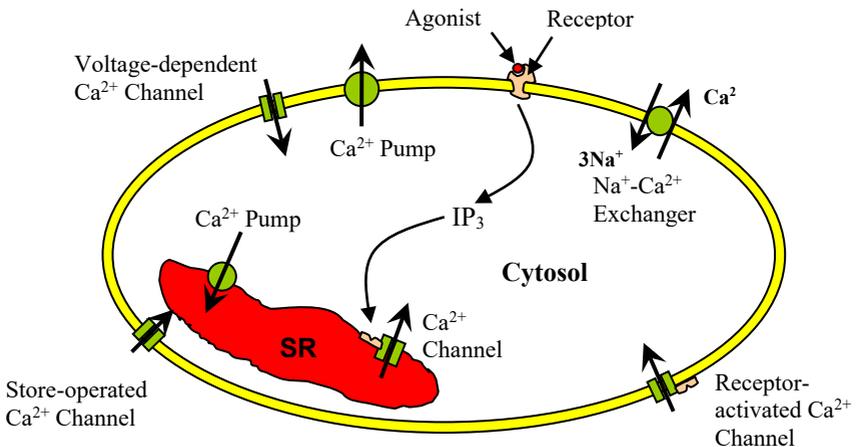

Fig. 1-4: *Major players of Ca^{2+} handling in smooth muscle.*

Unlike in striated muscle, where the binding of Ca^{2+} to the troponin-tropomyosin complex leads to activation of the actin-containing thin filaments, in smooth muscle, activation is through phosphorylation of the **myosin regulatory light chain (RLC)** (Sobieszek, 1977; Ikebe et al, 1977) on the myosin molecule. When the RLC is phosphorylated by Ca^{2+}-activated **myosin light chain kinase (MLCK)**, the myosin head (also called the cross-bridge) is able to interact with thin filaments to generate force or filament sliding: i.e., muscle contraction. Contraction in striated muscle is thin-filament regulated, whereas contraction in smooth muscle occurs mainly through regulation of the myosin-containing thick filaments.

The signaling pathways that lead to smooth muscle contraction are complex and not entirely understood. However, it is believed that the main pathway is associated with calcium binding to **calmodulin (CM)**; the $Ca^{2+} \cdot CM$ complex then binds to MLCK and activates the enzyme complex. The activated enzyme complex ($Ca^{2+} \cdot CM \cdot MLCK$) phosphorylates RLC, which enables the cross-bridges to interact with the thin filaments to cause muscle contraction.

A parallel pathway, which inhibits **myosin light chain phosphatase (MLCP),** is often activated in agonist-induced smooth muscle contraction. Binding of ligands to **G-protein-coupled receptors (GPCRs)** can lead to activation of MLCK (Fig. 1-5, the $G\alpha_q$ pathway); it can also lead to inhibition of MLCP (Fig. 1-5, the $G\alpha_{12/13}$ pathway). G-proteins are guanine nucleotide binding proteins, which normally exist as heterotrimeric (αßγ) complexes. The different isoforms of the subunits can assemble into a large number of combinations, which allow the G-protein to interact with many receptors and effectors, thus generating a wide variety of signals with the same agonist stimulation (Fig. 1-5).

Ligands that activate GPCR coupled to $G\alpha_q$ stimulate **phospholipase C (PLC)**, which in turn converts **phosphatidylinositol 4,5-biphosphate (PIP$_2$)** to IP$_3$ and diacylglycerol (DG). IP$_3$ stimulates Ca^{2+} release from the SR (Fig. 1-4).

Protein kinase C (PKC) is also activated in the $G\alpha_q$ pathway; PKC inhibits MLCP. In parallel to the $G\alpha_q$ pathway, activation of GPCR coupled to $G\alpha_{12/13}$ results in the activation of the small G-protein **RhoA** (Rho·GTP), which is facilitated by guanine nucleotide exchange factors (GEFs). The subsequent stimulation of **Rho kinase** inhibits the MLCP activity, thus enhancing RLC phosphorylation by MLCK. The $G\alpha_s$ pathway also acts in parallel with the $G\alpha_q$ and $G\alpha_{12/13}$ pathways. Stimulation of $G\alpha_s$ leads to the activation of **adenylate cyclase (AC)**, which converts ATP to cyclic AMP (cAMP). cAMP stimulates MLCP and also facilitates Ca^{2+} reuptake into the SR.
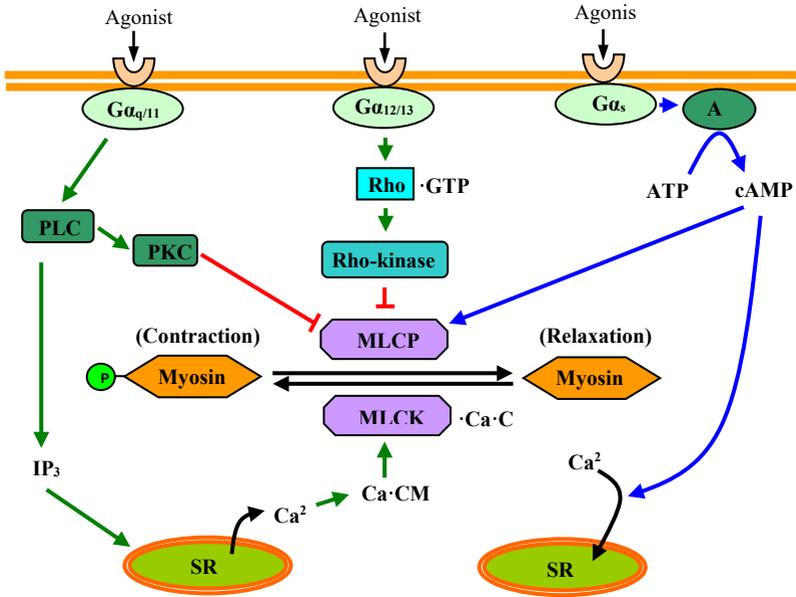
Fig. 1-5: *Overview of canonical pathways of smooth muscle activation. Agonist stimulation of G-protein coupled receptors (GPCRs) leads to simultaneous activation of MLCK and inhibition of MLCP in smooth muscle. Myosin-P represents myosin with phosphorylated RLC. $G\alpha_{12/13}$, $G\alpha_q$, $G\alpha_s$: heterotrimeric G-proteins with different α subunits; Rho·GTP: small G proteins, also called RhoA. PLC: phospholipase C; PKC: protein kinase C; CM: calmodulin; AC: adenylate cyclase; SR: sarcoplasmic reticulum; and cAMP: cyclic AMP. The green and red pathways lead to contraction; the blue pathways lead to relaxation.*

Activation of PKC and Rho kinase leads to enhancement of RLC phosphorylation and results in increased Ca^{2+} sensitivity in the activation of myosin cross-bridges, and underlies the mechanism of **calcium sensitization** of the myofilaments in smooth muscle. That is, fewer Ca^{2+} ions are required than it normally would to activate a certain number of myofilaments. On the other hand, activation of the $G\alpha_s$ pathway leads to **calcium desensitization** (i.e., more Ca^{2+} ions are needed to activate a certain number of myofilaments) because of the antagonistic action of cAMP on MLCP and the calcium reuptake by the SR. (For a review, see Puetz et al, 2009).

In addition to Ca^{+2}-calmodulin dependent RLC phosphorylation, activation of smooth muscle is also regulated by other mechanisms not

mentioned above. One of the signaling pathways for activation includes an integrin-linked kinase (ILK) whose activation is calcium-calmodulin independent. There is also evidence for thin-filament linked regulation of activation, which involves thin filament associated proteins, caldesmon and calponin. Actin polymerization and stabilization of the cytoskeleton are regulated by agonist-induced activation through Rho-kinase associated pathways (Puetz et al, 2009).

Similarities and differences between smooth and striated muscles

The most obvious common property shared by smooth and striated muscles is the ability to produce force and shortening. The muscles also share the same fundamental mechanism, i.e., actomyosin interaction, with which the chemical potential energy of ATP is converted to mechanical energy in the form of sliding contractile filaments. The contractile filaments in smooth muscle are similar, but are not the same as those in striated muscle. The actin-containing thin filaments in smooth muscle do not contain the troponin complex found in the thin filaments of striated muscle. Instead, the thin filaments in smooth muscle contain caldesmon and calponin.
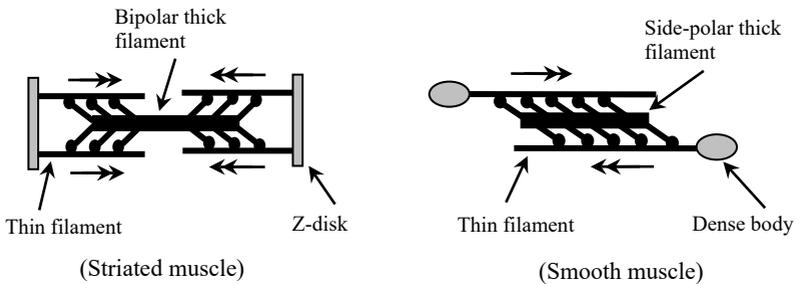
Fig. 1-6: *Schematic illustration of contractile units in striated and smooth muscles. Double arrows indicate the directions of thin filament movement relative to the thick filament in contracting muscles.*

       In striated muscle, the thin filaments are anchored to the Z-disks (Fig. 1-6). Thin filaments attached to the opposite faces of the Z-disks possess opposite polarities, so that when interacting with myosin heads, the thick filaments always slide towards the Z-disks anchoring the thin filaments. A similar arrangement is thought to be present in smooth

muscle, with dense bodies replacing Z-disks as the anchorage sites for the thin filaments in smooth muscle (Bond and Somlyo, 1982).

The myosin-containing thick filaments in smooth muscle are different from their counterparts in striated muscle in the way the myosin II molecules are assembled into filaments and the stability of the filamentous structure. Smooth muscle thick filaments are described as "side-polar" (Xu et al, 1996), meaning that the myosin heads on one side of the filament can only interact with thin filaments possessing a certain polarity, and the myosin heads on the other side of the thick filament can only interact with thin filaments with the opposite polarity (Hodgkinson et al, 1995); this ensures that a thick filament always slides towards the anchoring point of the thin filament with which it interacts (Fig. 1-6). Myosin monomers can be added or subtracted from a side-polar thick filament; this feature may allow the thick filaments in smooth muscle to rapidly alter their length through reversible polymerization (Liu et al, 2013).

As mentioned, in striated muscle, activation is initiated by calcium binding to the troponin complex located on the thin filaments; it is therefore regarded as *thin-filament regulated*. In smooth muscle, activation occurs mainly through RLC phosphorylation, and it is thus *thick-filament regulated*. In addition to activating the myosin ATPase associated with the myosin heads, RLC phosphorylation also facilitates thick filament formation *in vitro* (Kendrick-Jones et al, 1987; Trybus et al, 1987), and possibly *in vivo* (Qi et al, 2002).

Compared with those in striated muscle, the thick filaments in smooth muscle are known to be less stable structurally, especially when the filaments are not phosphorylated. This unique attribute may be important for reversible polymerization of smooth muscle thick filaments, which is likely crucial for plastic structural adaptation of the contractile apparatus to accommodate large changes in cell length while maintaining an optimal overlap between the contractile filaments (Seow, 2005). The "smoothness" or the lack of highly ordered and permanent structures (akin to the sarcomeres in striated muscle) may reflect the need of smooth muscle to constantly alter its intracellular organization to adapt to large changes in cell and organ geometry.

CHAPTER 2

STRUCTURAL BASIS OF SMOOTH MUSCLE CONTRACTION

The development of phase-contrast and interference light microscopes between 1900 and 1950 led to a significant advance in our understanding of the structure of living cells of skeletal and cardiac muscles. A common feature shared by these cells is the cross-striations, which are now known to stem from the highly organized and precisely aligned sarcomeres within the cells. Smooth muscle, on the other hand, lacks such cross-striations.

Fine structures of biological tissues were first investigated using electron microscopy in the mid 1900s. The thick and thin filaments of striated muscle could be seen clearly with an electron microscope. However, with the same preparative technique used to chemically fix striated muscles, studies of smooth muscle's ultrastructure often failed to see the thick filaments, although the thin and intermediate filaments could be readily observed. Since the 1960s, with the use of glutaraldehyde as the primary fixative and epoxy resins for embedding, the thick filaments of smooth muscle have been regularly seen in electron micrographs. However, it is generally believed that the thick filaments of smooth muscle are less stable than those of striated muscle. The evanescent nature of myosin filaments in smooth muscle cells likely plays an important role in the cells' adaptation to externally imposed changes in cell geometry and the maintenance of their ability to generate force over a large length range.

Ultrastructure - transverse views

Individual smooth muscle cells are surrounded by an extracellular matrix and they often aggregate into bundles of cells. The cells possess organelles — such as **nuclei**, **mitochondria**, smooth and rough **endoplasmic reticulum**, and the **Golgi apparatus** — as typically seen in other cell types (Figs. 2-1 to 2-4).

What separates differentiated smooth muscle cells from non-muscle cells in ultrastructural appearance is that the bulk of the cytoplasm

in smooth muscle is occupied by the actin-containing **thin filaments** and the myosin containing **thick filaments**. This reflects the fact that the muscle is specialized to carry out the mechanical function of contraction.
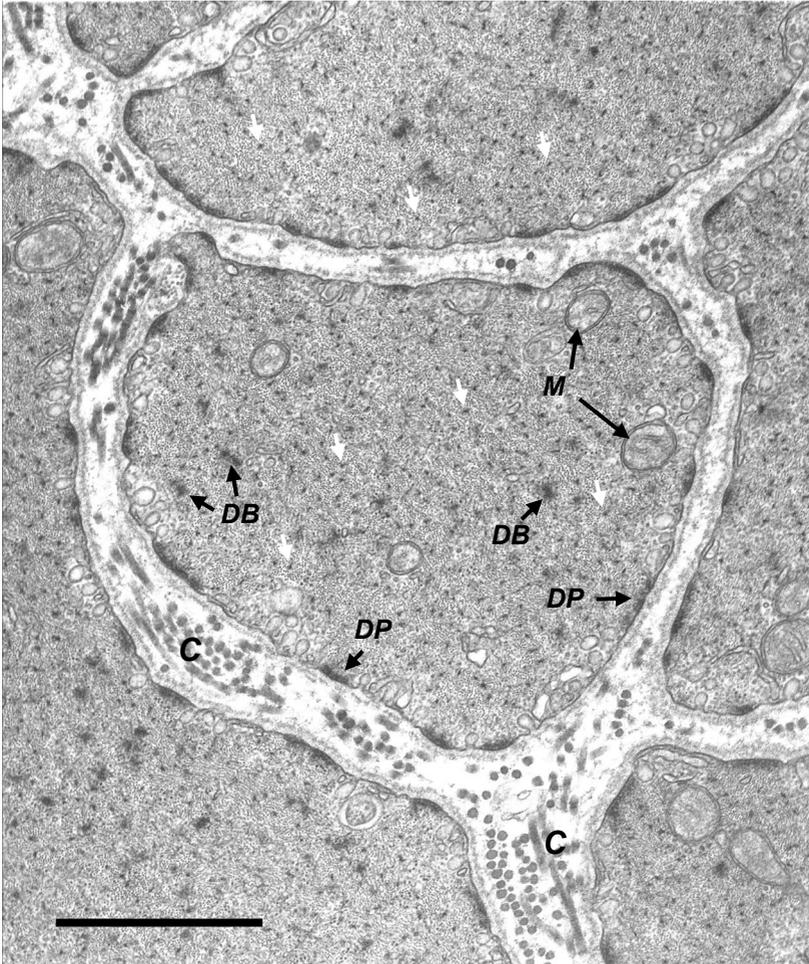

Fig. 2-1: *Electron micrograph of a transverse section of porcine tracheal smooth muscle. **C**, collagen fibers (note that some of the fibers are perpendicular to and some are in parallel with the cross-section); **M**, mitochondria; **DB**, dense body; **DP**, dense plaque. White arrows point to thick filaments surrounded by thin filaments. Note that the thin filaments greatly outnumber the thick filaments. Scale bar: 1 μm.*
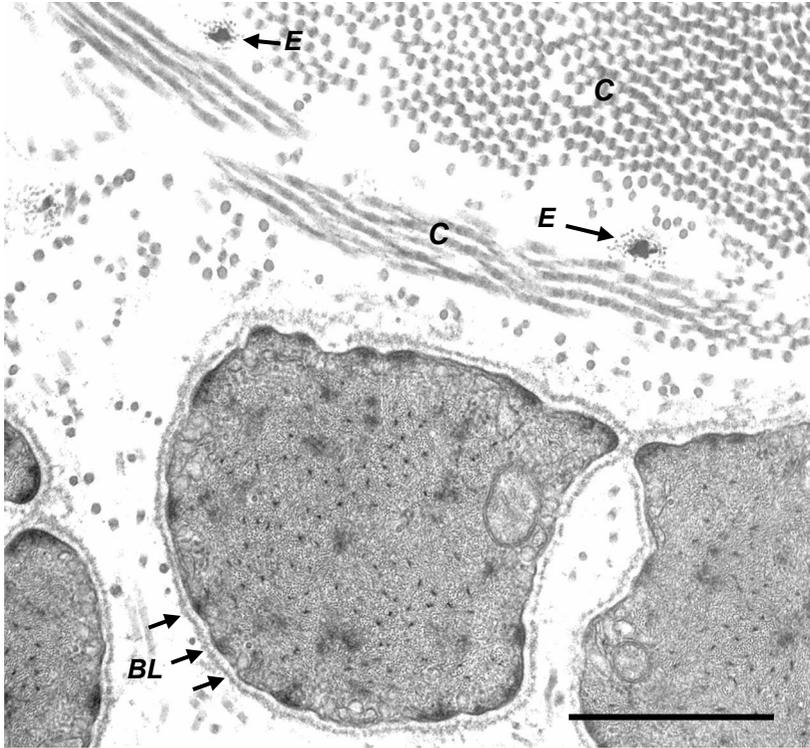
Fig. 2-2: *Electron micrograph of a transverse section of porcine tracheal smooth muscle, showing cells surrounded by basal lamina (**BL**). The extracellular matrix is mostly made up of collagen (**C**) and elastin (**E**) fibers. Note that the orientation of the collagen fibers is not always in parallel with the long axis of the muscle cells. Calibration bar: 1 μm.*

      The presence of contractile filaments (the thin and thick filaments) suggests that the sliding-filament, cross-bridge mechanism of contraction found in striated muscle (Huxley and Niedergerke, 1954; Huxely, 1957) is likely operative in smooth muscle as well.

      Basal lamina is found to envelope static structure cells such as smooth muscle and epi- or endothelial cells. Basal lamina is not associated with mobile cells such as fibroblasts and immune cells. Basal lamina is part of the extracellular matrix. It is secreted by the cells within the laminar envelope or the nearby fibroblasts. Basal lamina consists of two layers: **lamina lucida**, which is electron-lucid (transparent) and contains glycoprotein laminin. This layer of the basal lamina is found in between

the sarcolemma of smooth muscle cells and the electron-dense layer of the basal lamina, **lamina densa**, which is composed of type IV collagen and is the visible part of the basal lamina seen with electron microscopy (e.g., Fig. 2-2).
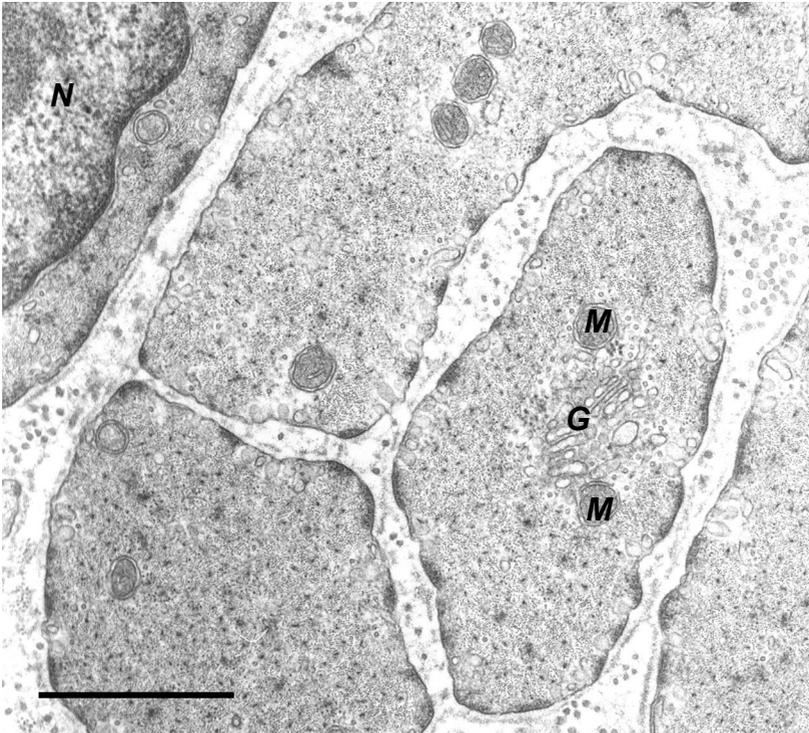

Fig. 2-3: *Electron micrograph of a transverse section of porcine tracheal smooth muscle cells. The nucleus (N) of the cell at the upper left is partially visible. A Golgi apparatus (G) in between two mitochondria (M) is seen in another cell. Calibration bar: 1 μm.*
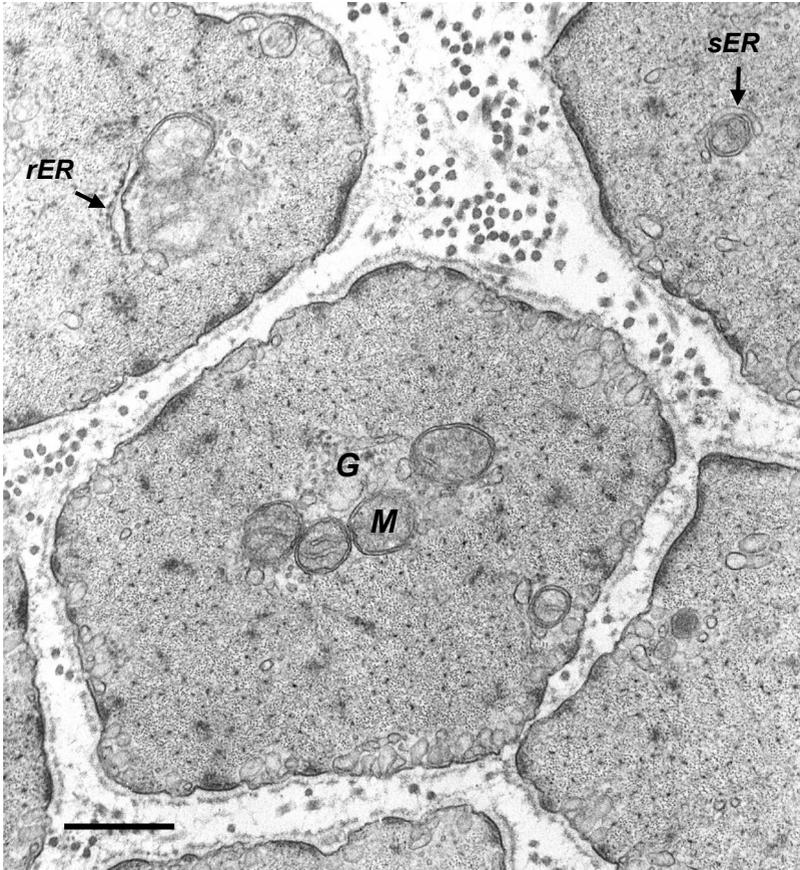
Fig. 2-4: *Electron micrograph of a transverse section of porcine tracheal smooth muscle cells. The cell in the center contains a cluster of centrally located mitochondria (**M**) with a partially visible Golgi apparatus (**G**). This usually indicates that the section is not too far from the nucleus. The cell at the upper right corner contains a smooth endoplasmic reticulum (**sER**), wrapping around a mitochondrion. The cell at the upper left corner contains a rough endoplasmic reticulum (**rER**) with ribosomes (dark-staining granules) on the outer surface of the organelle. Mitochondria are almost always surrounded by endoplasmic reticulum. Calibration bar: 0.5 µm.*

Basal lamina is sometimes confused with **basement membrane**. Basal lamina is part of the basement membrane; but besides the lamina lucida and lamina densa, basement membrane contains a third layer called *lamina reticularis*, with fibronectin as an important part of its constituents. Type VII collagen plays an important role in connecting basal lamina to lamina reticularis. Basement membrane is visible in light microscopy, whereas basal lamina is only visible with electron microscopy.

The collagen fibers seen in between smooth muscle cells (Figs. 2-1 to 2-4) have a triple-helix structure made of collagen fibrils, which in turn are mostly made of type I, II and III collagen molecules.

Ultrastructure - longitudinal views

Smooth muscle cells in a bundle normally lie parallel to one another, with their long axes oriented in one direction. The orientation of the long axes also coincides with the axis of force transmission through the cells. The cell arrangement within a bundle is best appreciated in longitudinal views (Fig. 2-5 to 2-8). As seen in the transverse views, the extracellular space surrounding smooth muscle cells is mostly occupied by collagen and elastin fibers. These fibers form a network, which provides mechanical support (as a scaffold) for the cells. This network is known as the **extracellular matrix**. Physical connections between a muscle cell and the extracellular matrix are seen in both transverse and longitudinal sections shown in this chapter. There are also cell-to-cell junctions that mechanically couple neighboring cells.

Although the intracellular space is mostly occupied by the contractile filaments, in some cell segments the organelles predominate. The central segment of a smooth muscle cell is occupied by a relatively large nucleus (Fig. 2-5). Clusters of mitochondria and other membranous organelles, such as the **sarcoplasmic reticulum** in smooth muscle cells (or endoplasmic reticulum in cells in general), are often seen accumulating near the two poles of the cigar-shaped nucleus, but they can also be found in other locations within the cell (Fig. 2-5). Dense bodies and dense plaques, as well as the myosin thick filaments, can also be recognized in longitudinal sections (Figs. 2-6 to 2-8).

It is clear from the longitudinal views that the central cell segment, which contains the nucleus, has little cytoplasmic space for myofilaments. To avoid non-uniform force-transmission throughout the entire cell length, the cells within a bundle form a mechanical syncytium. This is elaborated later in this chapter.
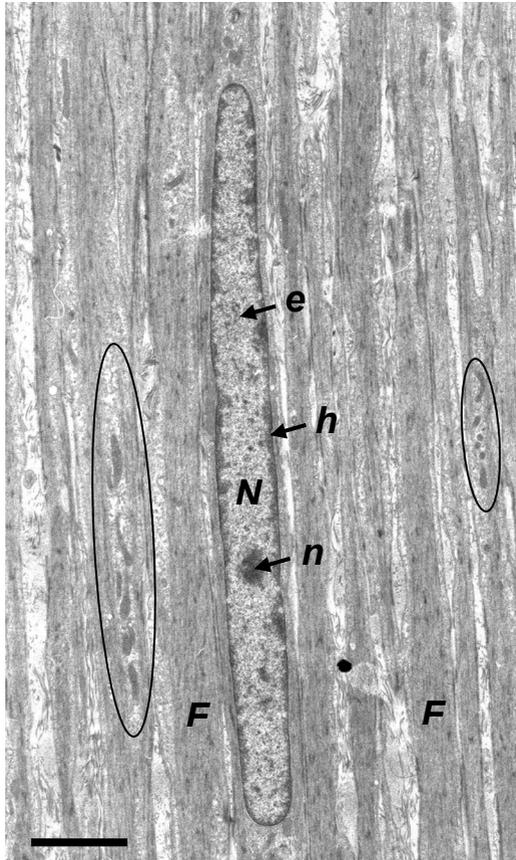
Fig. 2-5: *Electron micrograph of a longitudinal section of a bundle of porcine tracheal smooth muscle cells. A nucleus is prominent in the middle cell. Although the cell interior is mostly filled with myofilaments, clusters of mitochondria mixed with other membranous organelles can be seen near the poles of the nucleus (**N**) and other intracellular locations, as highlighted by the ovals. The dark-staining patches (most of them located near the inner surface of the nuclear membrane) are the condensed heterochromatin (**h**), whereas the light staining part of the nucleus is the euchromatin (**e**). **n**: nucleolus; **F**: myofilaments. Scale bar: 3 μm.*