We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Chapter 3

Role of Membrane Cholesterol in Modulating Actin Architecture and Cellular Contractility

Barbara Hissa and Bruno Pontes

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76532

Abstract

Atherosclerosis is a chronic inflammatory process that initiates with accumulation of apolipoprotein B containing lipoproteins (LPs) in the subendothelium (intima), especially in areas where the laminar flow is disturbed. LP retention triggers an inflammatory response leading to activation of endothelial and vascular smooth muscle cells that culminates with recruitment of leukocytes. Atherosclerosis is the leading cause of vascular disease worldwide being its major clinical manifestations ischemic heart disease, ischemic stroke, and peripheral arterial disease. Even though a lot has been done to unravel the role of turbulent flow and mechanotransduction for atherosclerosis development, little is known about the role of plasma membrane (PM) cholesterol in this process. This chapter is going to be focused on exploring what has been done so far to decipher the role of PM cholesterol in regulating actin architecture, cellular mechanical properties, and cellular contractility in muscle and nonmuscle cells.

Keywords: cholesterol, actin, myosin, cytoskeleton, contractility

1. Introduction

The role that cholesterol plays in cardiovascular diseases is widely known and studied [1]. However, less appreciated is the importance that cholesterol has in orchestrating other important cellular functions, such as cellular contractility and cytoskeleton organization. Cellular contractility is the ability of a cell to exert mechanical work on a substrate or on a neighboring cell due to actomyosin cytoskeleton enzymatic activity [2]. In muscle and nonmuscle cells, most of the cholesterol content is localized at the plasma membrane [3] where it can partition into microdomains called lipid rafts. Lipid rafts are highly dynamic regions of the plasma membrane

IntechOpen

© 2018 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

that contain sphingolipids and cholesterol and are responsible for compartmentalizing and regulating several intracellular signaling events [4–6]. One way of studying the importance of cholesterol for a specific cellular function is to decrease its concentration by either interfering directly with its synthesis, through the mevalonate pathway, or by chelating the molecule directly through the use of cyclodextrins [7]. However, depending on how one does the cholesterol depletion, the effects on cellular contractility can be opposite. Differences in muscle versus nonmuscle cell contractile behavior are observed upon cholesterol depletion using cyclodextrins. Muscle cells get impairment in their contractile machinery [8] whereas nonmuscle cells get more contractile [9]. This book chapter gives an overview about how cholesterol is organized at the plasma membrane and how its depletion changes cellular contractile properties.

2. Lipid rafts and membrane heterogeneity

Even though the Cell Theory started to be developed in the nineteenth century [10], it was not until the first quarter of the twentieth century that the idea of a membrane encompassing the cell was experimentally observed. In 1924, the Dutch physiologists Gorter and Grendel elegantly demonstrated, for the first time, the existence of a lipid bilayer surrounding red blood cells of various animals [11]. They isolated erythrocytes from humans and different mammals (rabbit, dog, guinea pig, sheep, and goat), extracted their lipids using acetone, and let those lipids spread on an air-water interface of a Langmuir-Adam apparatus. By knowing the number and area of the erythrocytes used in their experiment, they concluded that those cells were surrounded by a layer of lipids whose thickness was equivalent to two molecules [11], hence a lipid bilayer. The Gorter and Grendel model for cellular plasma membrane considered only the lipid nature of this cellular component and, because of that and due to other experimental and theoretical inaccuracies, it failed in explaining satisfactorily experimental results for membrane thickness [12], membrane tension [13], membrane electrical capacitance [14], and membrane permeability [15].

In order to explain those membrane properties, another model, called the paucimolecular model, was proposed by Danielli and coworkers in 1935 [12, 16, 17]. By examining the surface tension of a single drop of mackerel egg oil, Danielle and Harvey found that the value they measured was lower than the equivalent obtained for nonliving pure water-oil systems. They hypothesized that the difference observed for surface tension in living versus nonliving water-oil systems was due to the fact that the plasma membrane not only contained lipids but also proteins adsorbed in the lipid bilayer [16]. In the same year, Danielli and Davson [17] extended the paucimolecular model in order to explain permeability experiments. In that model, the layer of proteins adsorbed on top of a lipid film was able to discern size of molecules and charge of ions that were penetrating the membrane. This lipid film containing adsorbed proteins was considered to be relatively stable with mosaics consisting of practically impenetrable regions and hydrated areas where anions could move through [17].

For the next 30 years, the paucimolecular model was the most accepted one among the scientific community. However, with the advancement of microscopy techniques and structural studies, a new and more robust model, named the fluid mosaic model, was proposed in 1972 by Singer and Nicolson [18]. According to that model, integral transmembrane proteins are

http://dx.doi.org/10.5772/intechopen.76532

arranged in the plasma membrane of living cells such that the polar regions are facing the aqueous phase and the hydrophobic regions are embedded on a viscous phospholipid bilayer and those proteins are able to move freely on that two-dimensional, approximately homogeneous fluid "sea" of phospholipids [18]. One year later, in 1973, Bretscher published a Science paper in which he discusses overall membrane organization based on evidences collected from experiments performed in red blood cells [19]. According to that paper, the plasma membrane of mammalian cells was not as simple as depicted by the fluid mosaic model. Some of the integral proteins span the membrane and their glycosylation is responsible for locking them at the membrane impeding their migration to the cytoplasm. Another important contribution from this paper is that proteins not only interact with the outer layer of the plasma membrane but also with the inner layer, and membrane proteins are a subtype of cytoplasmic proteins that are not secreted [19]. In the same year, Yu and collaborators, also performing experiments in red blood cells, showed that when those cells are incubated with the nonionic detergent Triton X-100, there are some fractions of the cellular proteins that are resistant to the detergent extraction and seem to form oligomeric complexes with some of the lipid components, which were preferentially composed by nonglycosylated proteins and sphingolipids [20].

2.1. Lipid rafts

The idea of possible membrane microdomains started to be speculated in the early 1970s [20, 21] and experimentally demonstrated in 1982 by Karnovsky [22], who showed that there were multiple phases in the lipid environment of a membrane. One type of microdomain can be formed by cholesterol and sphingolipids [23]. These microdomains were already shown to be present in cell membranes [24]. In 1988, after several experimental demonstrations, Simons and van Meer called these microdomains as lipid rafts [25]. Thus, lipid rafts are defined as small, heterogeneous, and highly dynamic microdomains enriched in cholesterol, glycosphingolipids, and proteins that are much more organized than the surrounding lipid bilayer [26]. These membrane microdomains serve as organizing clusters capable of influencing several cellular processes such as membrane trafficking and neurotransmission [26].

The most striking difference between lipid rafts and the plasma membrane from which they are derived from is the lipid composition. Experiments have shown that rafts contain much more cholesterol than the surrounding bilayer [27, 28]. Cholesterol, therefore, works as a sort of "dynamic glue" that maintains the raft together [29], serving as a molecular spacer and filling the empty spaces between sphingolipids [30]. One of the main challenges when studying lipid rafts in living cells is their size. They are small microdomains ranging from 10 to 200 nm, below the classical diffraction limit of the optical microscope [28]. The first studies in the field considered methods to extract and separate rafts from the surrounding membrane. The procedure would take advantage of lipid raft resistance to nonionic detergents. When detergents are added to cells, the fluid membrane will dissolve while the lipid rafts may remain intact and could be extracted [31]; however, the validity of this methodology has been called into question due to ambiguities in the lipids and proteins obtained after extraction [32]. Other methods, based on synthetic membranes, were also used, however with many drawbacks. Firstly, synthetic membranes either lack or have lower protein concentration when compared to cell membranes [26]. Secondly, it is very difficult to simulate, in synthetic membranes, the membrane-cytoskeletal interactions that occur in cell membranes, although

some recent studies have been able to overcome these limitations [33–35]. Finally, another problem includes the lack of natural asymmetry between the bilayer leaflets [36].

Although lipid rafts present sizes below the classical diffraction limit of the optical microscope, fluorescence microscopy has been extensively used in the field. For example, fluorophores conjugated to cholera-toxin B-subunit, which binds to the raft constituent ganglioside GM1, is used. Also, lipophilic membrane dyes (such as Laudran) that either partition between rafts and the surrounding membrane or change their fluorescent properties in response to membrane phase are used. Finally, lipid rafts can also be fluorescently labeled in cells after genetic expression of fluorescent fusion proteins [35].

Another methodology, which has been widely used in the study of lipid rafts, is the manipulation of cholesterol contents in membranes. Sequestration (using filipin, nystatin, or amphotericin), depletion and removal (using methyl-β-cyclodextrin, MβCD), or inhibition of cholesterol synthesis (using 3-hydroxy-3-methyl-glutaryl-coenzyme A, HMG-CoA, reductase inhibitors) are great examples of how cholesterol can be manipulated in lipid raft studies [26]. Several questions, however, have been raised against the effectiveness of the experimental design when disrupting lipid rafts. Acute methods of cholesterol depletion, which disrupt the rafts, can also disrupt another lipid, called PI(4,5)P2, which plays an important role in cytoskeletal regulation [37]. Thus, the loss of a particular cellular function after cholesterol depletion cannot necessarily be attributed only to raft disruption, since other processes are also being affected.

Despite these limitations, more sophisticated methods have been applied in order to fight against the problems of small size and dynamic nature of lipid rafts. These methods include single particle and molecule tracking using very sensitive CCD cameras together with total internal reflection microscopy. These combined techniques provide information of the diffusion coefficient of particles in the membrane and also reveal membrane corrals, barriers, and sites of confinement [38]. Finally, other optical techniques have been used to elucidate other features of lipid rafts: fluorescence correlation spectroscopy, to gain information of fluorophore mobility in the membrane [39]; fluorescence resonance energy transfer, to detect when fluorophores are in close proximity [40], and optical tweezers, to give information about the membrane mechanical parameters [8, 41]. In the future, it is expected that other super-resolution microscopy techniques, such as stimulated emission depletion microscopy [42] or various forms of structured illumination microscopy may overcome the problems imposed by the diffraction limit.

Apart from the different imaging methods, research over the last decades have demonstrated the existence of two types of rafts: (1) planar lipid rafts (also known as noncaveolar or glycolipid rafts) and caveolae. Planar rafts are known to be continuous with the plane of the plasma membrane (not invaginated) and contain flotillin proteins. Caveolae are flask shaped invaginations of the plasma membrane that contain caveolin proteins. Both types are enriched in cholesterol and sphingolipids. Flotillin and caveolins can either recruit or separate other molecules from lipid rafts and caveolae, respectively, thus playing an essential role in signal transduction [43].

2.2. Caveolae

Caveolae are plasma membrane invaginations with a diameter ranging from 60 to 80 nm and were first identified in the early 1950s by electron microscopy [44]. These invaginations are

expressed in various cell types such as smooth muscle, fibroblasts, endothelial cells and adipocytes, among several others. Their functions are diverse and include endocytosis, calcium signaling as well as regulation of various cell signaling pathways [45].

The major constituent of caveolae is caveolin1 [46], followed by two other isoforms: caveolin2 [47] and the muscle-specific caveolin3 [48]. All three caveolin proteins share a common topology with both their N and C terminal domains in the cytoplasm and a long hairpin transmembrane domain. All three types of caveolin are formed inside the cells, more precisely in the Golgi apparatus, as monomers [49]. However, as soon as they enter in the secretory pathway, they start to be structured as oligomers [50]. For caveolin1, for example, its exit from the Golgi apparatus is accelerated upon addition of cholesterol [49]. The oligomerization ability of caveolin1 is crucial for caveolae formation [51]. Caveolin2 has also been implicated in caveolae formation [52], and although caveolin1 null mouse shows a significant decrease in caveolae assembly, they are still present in the caveolin2 mouse [53]. In muscle, caveolin3 is crucial for caveolae formation. Mutations or loss of caveolin3 result in dystrophic phenotypes [54, 55].

Caveolin expression at the plasma membrane is not the only inducer of caveolae formation. Cholesterol extraction has been extensively shown to disrupt caveolae at the plasma membrane [46] since it is required for caveolin incorporation into raft domains at the plasma membrane, a critical event for caveolae formation [56].

Although caveolins and cholesterol were initially thought to be necessary and sufficient for caveolae formation, several studies have shown additional molecular players called cavins. This protein family has four different members already described: cavin-1 (also called PTRF) [57], cavin-2 (also called SDPR) [58], cavin-3 (also called SRBC) [59], and the muscle-specific cavin-4 (also called MURC) [60]. These four proteins are essential to caveolae formation and functions. Thus, caveolae formation is a highly complex and regulated cellular process. It has been estimated that ~150–200 caveolin monomers are necessary to associate with ~50–60 cavins in order to form a single caveola [61, 62]. Moreover, caveolae architecture was recently proposed to be a dodecahedron formed by cavins aligned with their vertices and also in the caveolin oligomers located at each of the pentagonal faces [61, 62].

As already mentioned, caveolae represent a subdomain of lipid rafts [43]. Confocal microscopy has shown that the distribution of GM1, a well characterized raft marker, do not merge with caveolin1 [63]. Another raft marker, flotillin, defines noncaveolar rafts and merges with GM1 [63]. Thus, rafts exhibit a heterogeneous distribution over the plasma membrane changing between caveolar (invaginated) and noncaveolar (planar) regions.

3. Actomyosin cytoskeleton: the contractile machinery of muscle and nonmuscle cells

The cytoskeleton constitutes a dynamic network of filaments that exists in the inner space of a cell. This network not only provides scaffolding but is also responsible for transporting organelles, generating and transducting mechanical forces. The cytoskeleton maintains cellular organization by linking together several cellular components in such a way that it

mediates communication across the entire cell and, therefore, has a tremendous impact on cellular functions [64]. Three main filaments constitute the cytoskeleton, each one with its distinct protein composition and function: the microtubules, intermediate filaments, and microfilaments.

Microfilaments, also known as actin filaments, are ~7 nm in width. They are primarily composed of actin, the most abundant protein in cells. Actin filaments can create a huge number of arrays, such as bundles, two-dimensional networks, and three-dimensional gels. These different structural organizations are controlled by several actin-binding proteins and are found, for example, at the leading edge of a moving cell, particularly in filopodia and lamellipodia (**Figure 1B**), which causes the actin filaments to be the primary cytoskeletal component to drive cell motility [64]. Actin filaments also allow the cell to probe or sense its microenvironment. More stable networks of actin filaments, known as stress fibers, allow cells to brace against the underlying surface [65]. Thus, microfilaments can either be alone, as simple filaments, or together with the myosin filaments, which are part of the actomyosin contractile apparatus, in muscle and nonmuscle cells. Myosin filaments, associated with actin filaments, use ATP hydrolysis to exert forces against stress fibers during cytoskeletal contractility [65].

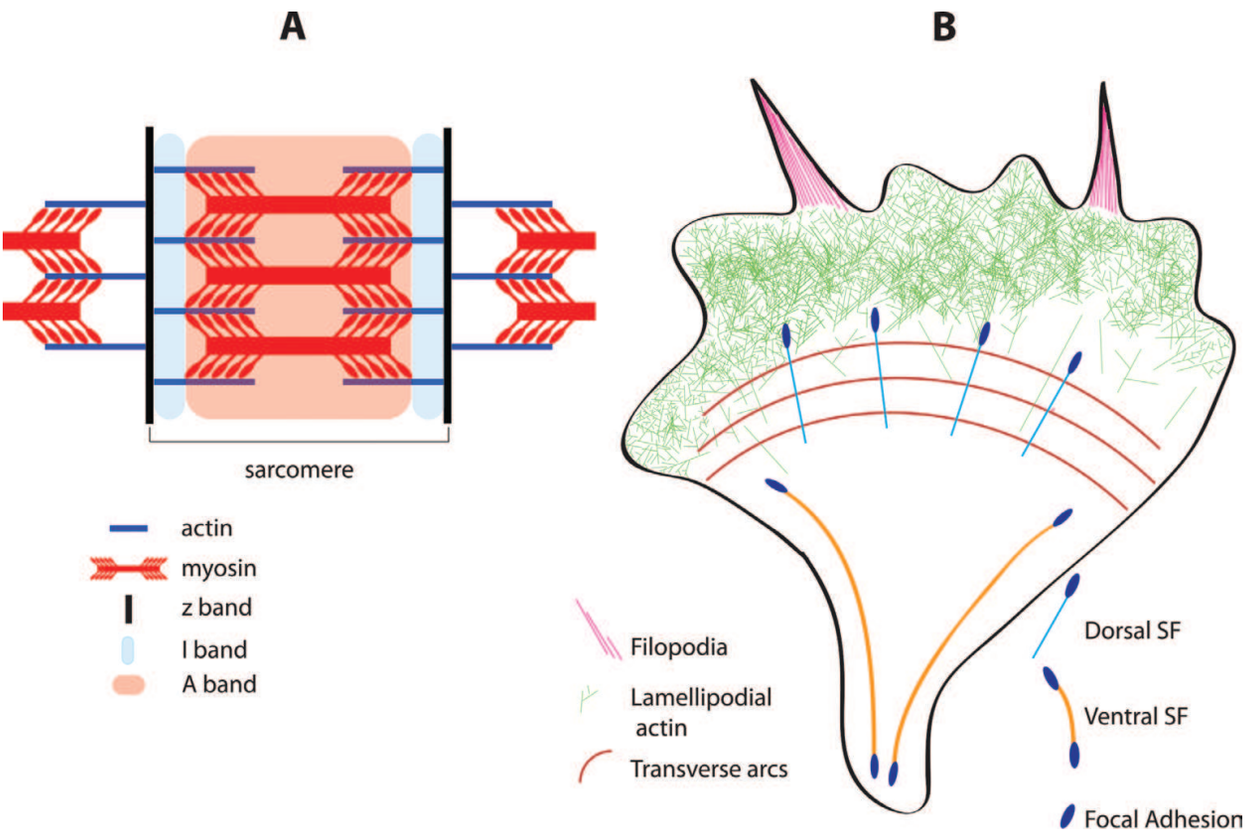


Figure 1. Actomyosin cytoskeleton schematic of striated muscle cell (A) and smooth muscle/nonmuscle cells. Striated muscle cells have the sarcomeric organization, which is shown in A and consists of actin and myosin filaments overlapping. Smooth muscle and nonmuscle cells (B) have different populations of actin stress-fibers that can be found in distinct parts of the cell. There are stress fibers that form filopodia (pink), lamellipodia (green), contractile transverse arcs (red), dorsal stress fibers (light-blue) and ventral stress fibers (orange) that terminate into one or two focal adhesions (navy-blue).

The actomyosin contractile machinery is relatively well conserved, despite some differences in organization and dynamics among different cell types. Actin filaments are polarized as barbed ends (fast-growing end) and pointed ends (slow-growing ends) and serve as scaffold for myosin filaments, which vary in size ranging from a few heads, in nonmuscle cells, to hundreds of heads in thick filaments of striated muscle cells. The myosin filaments drive the translocation of actin filaments toward their barbed ends. This event can trigger either the contraction or extension of two actin filaments [64].

The most well understood example of a contractile actomyosin apparatus is found in striated muscles and is called sarcomere. The sarcomeres are what give the striated muscles their appearance. It is known that a sarcomere is defined as a segment between two neighboring Z-lines. The Z-lines (from the German "Zwischenscheibe") are "dark" lines that appear in both extremities of a sarcomere (**Figure 1A**). They act as anchoring points for actin filaments. Surrounding two Z-lines, there are two regions called I-bands, regions of actin filaments that are not superimposed by myosin filaments (**Figure 1A**). Between the two I-bands is an A-band, which contains the entire length of myosin filaments and part of actin filaments that extend from I-bands (**Figure 1A**). The barbed ends of actin filaments are localized at the Z-line. The myosin filaments are segregated toward the pointed ends of actin filaments. Several other proteins are present and allow the stability of a sarcomere. The interaction between actin and myosin filaments in the A-band of a sarcomere is responsible for muscle contraction (**Figure 1A**) [64].

The contraction starts when a motor neuron releases acetylcholine, a neurotransmitter that binds to a postsynaptic nicotinic acetylcholine receptor on the muscle fiber, causing a change in receptor conformation and allowing an influx of sodium ions followed by postsynaptic action potential initiation. The action potential travels through T-tubules until it reaches the sarcoplasmic reticulum, where it activates voltage-gated L-type calcium channels. The initial inward flow of calcium from the L-type calcium channels activates ryanodine receptors, which releases a huge amount of calcium ions from the sarcoplasmic reticulum toward the cytoplasm of muscle cells. This mechanism is called calcium-induced calcium-release [64]. Inside muscle cells, the protein tropomyosin covers the myosin binding sites of actin filaments in the sarcomere. In order to allow contraction, tropomyosin must be moved from its original place. Initially, tropomyosin is attached to the actin filaments, covering myosin binding sites. When calcium ions enter in the muscle cell cytoplasm, they immediately bind to troponin-C and trigger a change in the structure of tropomyosin. This change in conformation forces tropomyosin to reveal the myosin-binding sites on actin filaments and allows myosin filaments to pull antiparallel actin filaments together. Muscle contraction ends when calcium ions are pumped back from the muscle cell cytoplasm into the sarcoplasmic reticulum, allowing the contractile machinery to relax [64].

The actomyosin cytoskeleton in nonmuscle and smooth muscle cells is organized in similar ways, both different from sarcomeres of striated muscles. Nonmuscle and smooth muscle cells use myosin to generate contractility during migration, cytokinesis, as well as cell-cell and cell-matrix junctions, for example [66]. Nonaligned actomyosin networks, with actin filaments and clusters of bipolar myosin filaments interacting with each other at their ends, represent the simplest contractile machinery in nonmuscle and smooth muscle cells, especially

in cytoskeletal regions that do not have stress fibers [67, 68]. Smooth muscle and nonmuscle cells also contain more organized actomyosin bundles, such as transverse arcs, radial stress fibers, peripheral bundles, and ventral stress fibers (**Figure 1B**) [65]. Transverse stress fibers or arcs (**Figure 1B**) are formed after reorganization of lamellipodial actin filaments [69] during lamellipodium retraction [70]. This process is driven by myosin filaments, which become co-aligned with actin filaments and form stacks separated by alfa-actinin [68]. Radial stress fibers (**Figure 1B**), on the other hand, are anchored to focal adhesions in one end. Myosin molecules are recruited to the tips of focal adhesions, where nascent radial stress fibers start to form [71]. Contractility of the radial-transverse-stress fiber network leads to the formation of ventral stress fibers (**Figure 1B**) attached to focal adhesions on both ends [72]. Also, actomyosin filaments from nonmuscle and smooth muscle cells are highly dynamic when compared to striated muscle cells. Both actin and myosin can frequently undergo turnover or cycles of assembly/disassembly [73, 74].

Based on all above-described features, the most striking differences between stress fibers and sarcomeres are: (1) the molecular composition is cell-specific [75], (2) stress fiber contraction is regulated by phosphorylation of myosin light chain (pMLC) (see pMLC regulation in Section 4), while sarcomere contraction is regulated by troponin switching [76], (3) stress fibers are approximately one order of magnitude thinner, less organized (with different directions and lengths) and with less coordinated contraction when compared to sarcomeres [75], (4) the magnitude of the force they produce is different, while the stress fiber contractile forces come from individual cells and are applied, through focal adhesions, to the extracellular environment in which these cells are located [77], the sarcomeres from striated muscles can transmit contractile forces over macroscopic lengths. Finally, (5) striated muscles can rapidly contract and relax based on action potentials and Ca^{2+} release [64, 78] while stress fibers from smooth muscle and nonmuscle cells respond much slower and do not depend solely on electrical pulses [78].

While striated muscle cells present different actomyosin organization and features when compared to nonmuscle and smooth muscle cells, these three cell types share a common actomyosin structure: the actin cortex, also known as cell cortex or actomyosin cortex. This is a thin and highly disordered contractile actomyosin network underling the plasma membrane of cells [79]. It was first discovered in large cells, like amoeba and animal eggs and subsequently, extrapolated to all animal cells [79]. Non-adherent cells [80], cells during mitosis [81], or cells performing amoeboid-like migration [82] present a well distributed and uniform actin cortex. Cells spread over flat surfaces, although more difficult to be observed, also present a cortical layer of actomyosin, as shown by electron microscopy [83]. The actomyosin appears as an isotropic network parallel (and some perpendicular) to the plasma membrane with a width of 20–250 nm [83, 84]. Numerous actin-binding proteins have already been described to be part of the actin cortex [84], most of them are classical actin-binding proteins; however, little is known about how actin cortex is assembled.

The actin cortex plays a major role in cell mechanics as the main determinant of cell surface tension [79]. Biophysical methods like micropipette aspiration and membrane tether pulling assays (using either optical tweezers or atomic force microscopy) have been used to measure cell surface mechanics [85]. Micropipette aspiration is a suitable technique to measure

the overall cellular tension, which is a combination of the tension in the plasma membrane together with the tension in the underlying actin cytoskeleton [86]. Moreover, membrane tether pulling assays also bring information about the membrane itself and its attachment with the cortical cytoskeleton [86, 87]. During bleb formation, for example, a momentary separation between the plasma membrane and the actin cortex occurs [88].

Biophysical methods show that the membrane-cortex attachment is the major determinant in cell surface tension [89, 90] and that different cells have different surface tension values, indicating that there may have different mechanisms to maintain surface tension homeostasis among cells [90]. Modifications of specific cross-linking proteins, whose function is to link the plasma membrane to the actin cortex, can induce changes in cell surface tension [91–94]. Also, actin filament disruption or myosin inhibition can reduce cell surface tension [90, 95–97]. Changes in membrane composition, particularly in cholesterol content, have also been shown to influence cell surface tension. MβCD causes an increase in tension in embryonic kidney cells [98], fibroblasts [9] and cardiomyocytes [99]. This increase is not only due to changes in membrane composition, but it also affects the actomyosin cytoskeleton. In fibroblasts, MβCD treatment shows an increase in stress fiber formation [9] whereas cardiomyocytes show sarcomeric disorganization together with contraction abnormalities [99].

4. Cellular contractility in nonmuscle cells: the role of Rho and pleiotropic effects of statins

In order to divide, migrate, and undergo tissue morphogenesis, cells change shape and exert forces either on the substrate that they are attached to or on the neighboring cells. Nonmuscle cells generate contractile stresses via molecular motors, such as myosin, that are able to convert chemical energy into mechanical work [2]. Myosin activity is controlled through phosphorylation of its light chain via myosin light chain kinase (MLCK) [100, 101] which, in turn, is activated by Rho kinase ROCK and the small GTPase Rho A upstream. In 1985, the Rho gene was first isolated, from abdominal ganglia of the Aplysia, and identified as a member of the Ras family [102]. In 1990, after injecting a constitutively active form of Rho (Vall4rho), Paterson and collaborators verified that active Rho is able to cause changes in cellular morphology inducing formation of stress fibers [103]. In 1992, Ridley and Hall showed that active RhoA induces formation of stress fibers and focal adhesions upon growth factor stimulation [104]. Being a GTPase, Rho can switch back and forth between its active state, when bound to GTP, and its inactive state, when bound to GDP. The switching process is finely regulated by guanine nucleotide exchange factors (GEFs), which promote activation, and GTPase-activating proteins (GAPs), which promote inactivation. There are approximately 60 GEFs and 70 GAPs that were already identified in the human genome [105]. In order to be activated, Rho goes through some posttranslational modifications that are essential to induce Rho migration toward the plasma membrane, where it gets activated. Prenylation is a posttranslational modification that is known to be pivotal for Rho translocation to the membrane [106]. Protein prenylation is essentially an insertion of a prenyl group, which is a hydrophobic group, to the c-terminal of a protein. That way, the protein has a lipid anchor that allows

it to stay membrane bound. There are two types of prenylation: farnesylation and geranyl-geranylation, which are regulated by farnesyltransferase and geranylgeranyltransferase I, respectively [107]. In the case of RhoA, the protein gets geranyl-geranylated before it goes to the membrane to get activated and trigger downstream effectors [108, 109] (**Figure 2**).

Both isoprenoids, farnesyl pyrophosphate and geranylgeranyl pyrophosphate, are synthetized by the mevalonate pathway. Interestingly, cholesterol is the end product of this pathway [110] (**Figure 3**). Therefore, by manipulating the mevalonate pathway, one can perturb both cholesterol synthesis and prenylation of important target proteins such as RhoA, and, as a consequence, cellular contractility (**Figure 3**).

The rate-limiting step of the mevalonate pathway is regulated by the enzyme HMG-CoA reductase. The activity of HMG-CoA reductase is precisely governed by the amount of cholesterol available. There are basically two different sources of cholesterol in the body: the exogenous one (obtained through intestinal absorption of cholesterol from the diet) and the endogenous one (through the *de novo* synthesis via the mevalonate pathway), being the endogenous source down regulated when enough cholesterol is obtained from nutrition [111]. During the early 1970s, a lot of effort was put into identifying pharmacological candidates that were able to reduce the HMG-CoA reductase activity especially in patients with high LDL cholesterol. In 1984, on a National Institutes of Health (NIH) Consensus Conference for Coronary Primary Prevention Trial, it was demonstrated the importance of a balance diet and drug treatment in order to lower LDL-cholesterol to prevent coronary heart disease [112]. After 1987, statins, that are essentially very specific drug inhibitors of HMG-CoA reductase activity, started to be prescribed for patients with high cholesterol

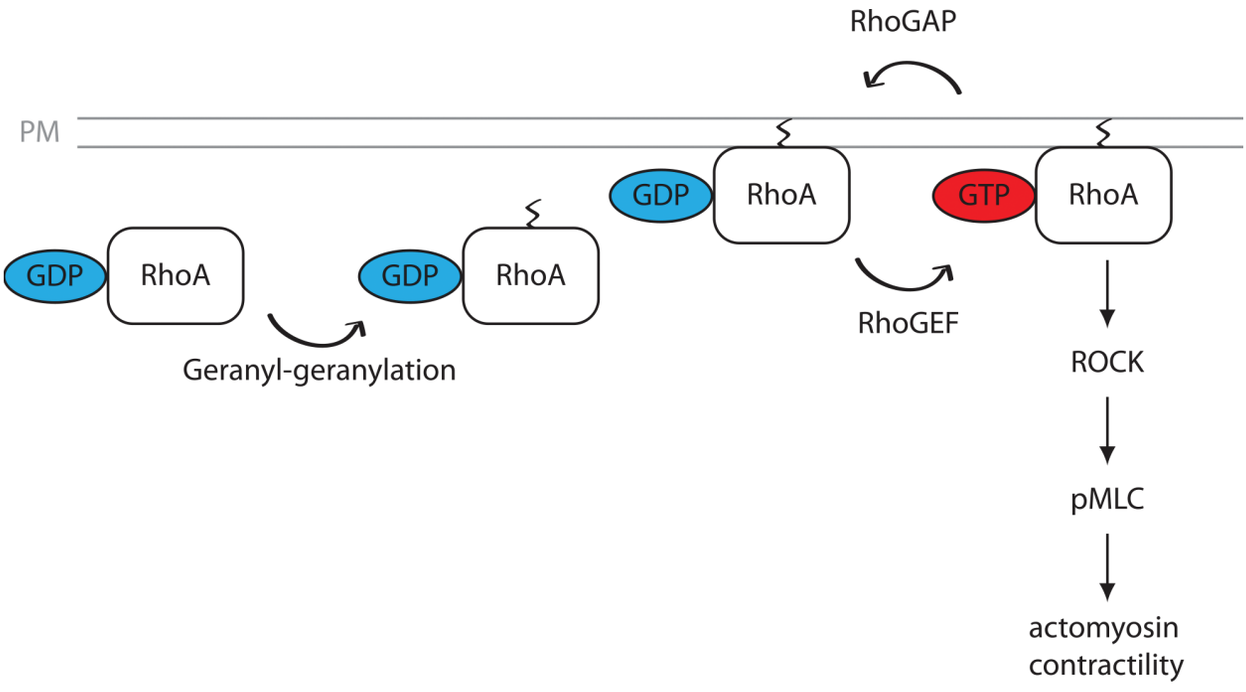


Figure 2. RhoA requires geranyl-geranylation in order to go to the membrane and be activated by RhoGEFs. Active RhoA triggers actomyosin contractility by inducing ROCK phosphorylation of myosin light chain (pMLC).

http://dx.doi.org/10.5772/intechopen.76532

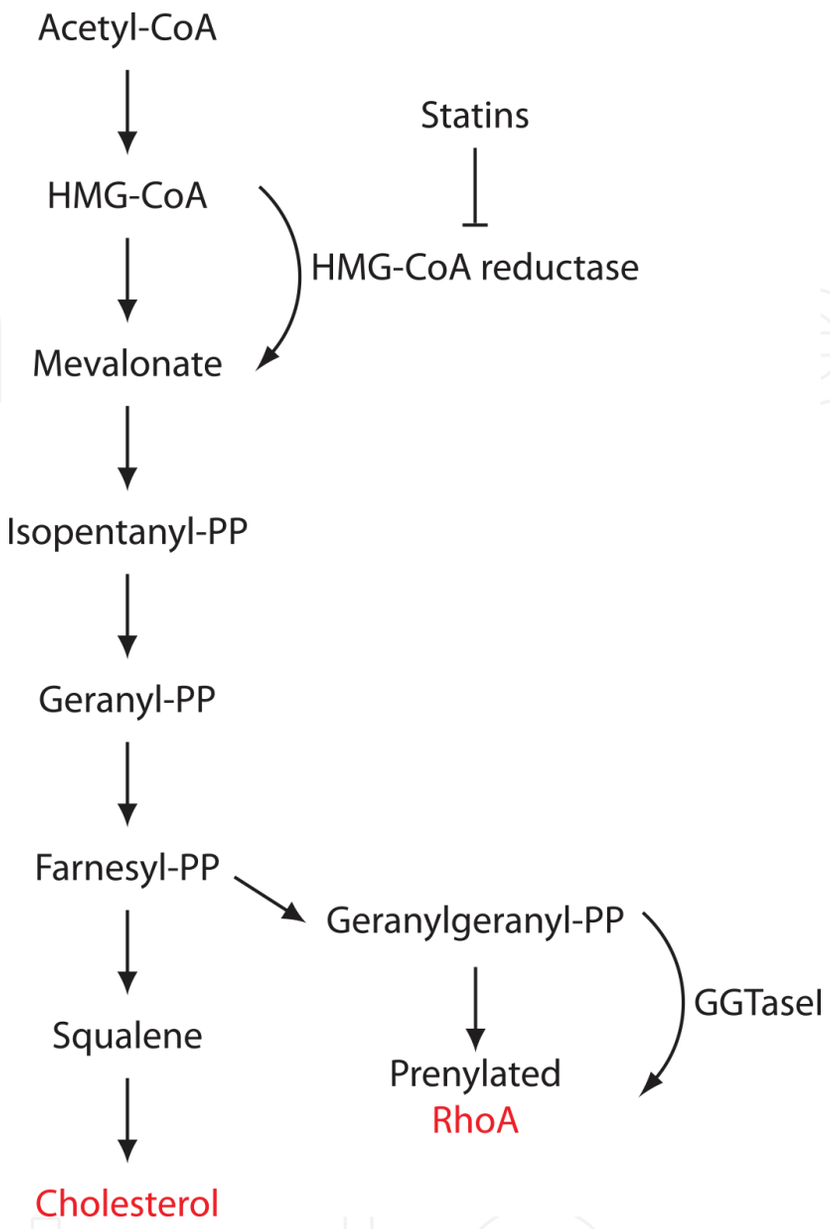


Figure 3. Simple schematic of the mevalonate pathway showing that posttranslational modifications pivotal for RhoA (red) activation and cholesterol (red) synthesis are part of the same intracellular pathway.

values [113]. By reducing the activity of HMG-CoA reductase, statins not only lower the amount of circulating cholesterol but also the amount of isoprenoid, both synthesized the mevalonate pathway, as one can see in **Figure 3**. Active RhoA as well as RhoA downstream effectors, such as ROCK, are inhibited upon statin treatment [114]. In endothelial cells, for example, a combination of flow and simvastatin exposure led to cell rounding and disorganization of the actin cytoskeleton [115]. In order to mimic atherosclerosis and aging effects on vessel walls, endothelial cells were plated in a series of substrates with low (physiological) and high stiffness values. High stiffness substrates increased both RhoA and ROCK activities. However, upon simvastatin incubation, contractility was abrogated in those cells [116].

Interestingly, when cholesterol is directly depleted by MβCD, an opposite trend is observed regarding nonmuscle cellular contractility. Human skin fibroblasts, after MβCD treatment, showed a reduction in the mobility of plasma membrane proteins being that reduction in motion a direct result of cytoskeleton reorganization [117]. It was also shown, for bovine aortic endothelial cells, that MβCD-dependent cholesterol depletion increased cortical stiffness [118]

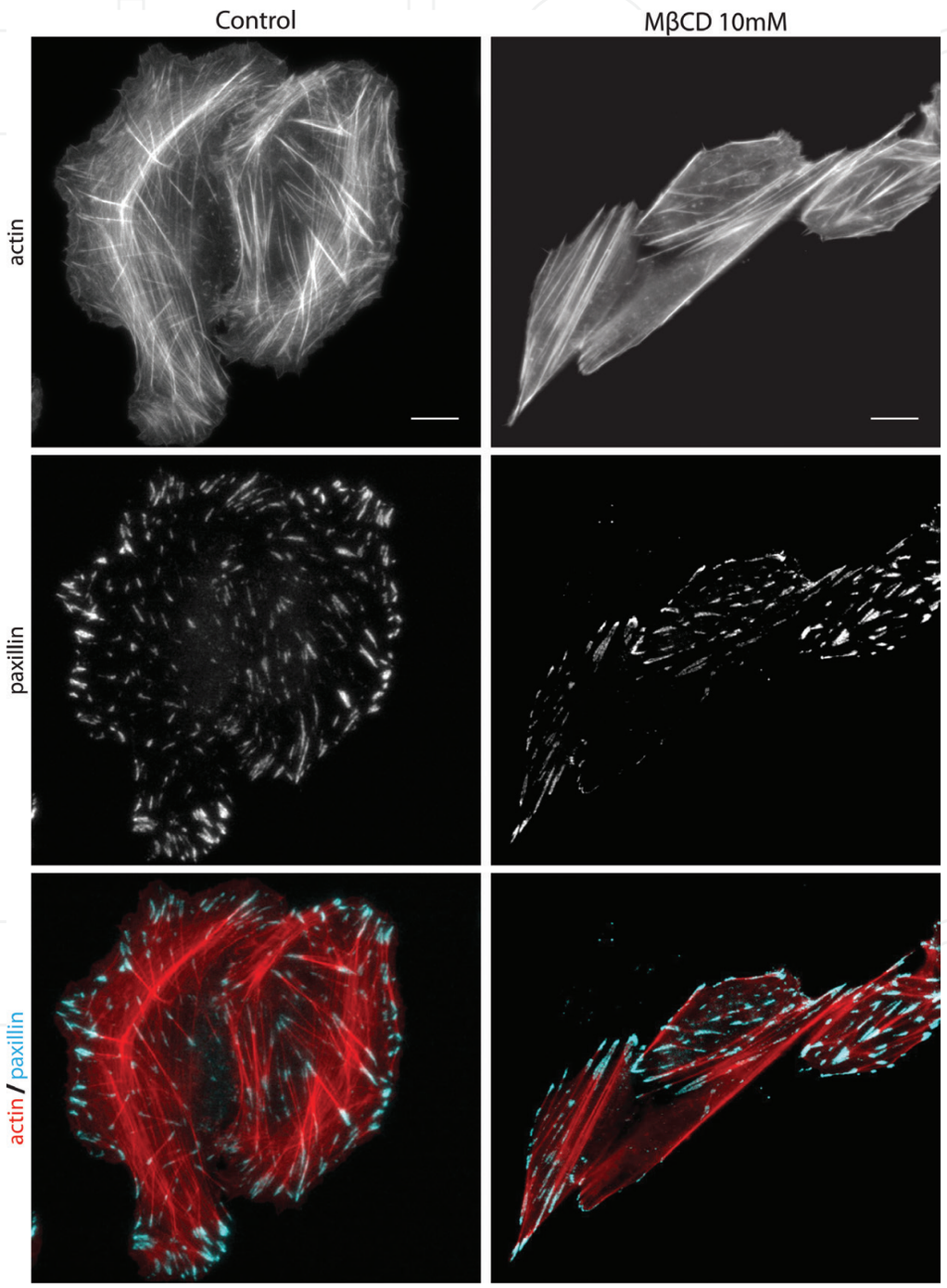


Figure 4. Fixed human osteosarcoma cell line U2OS, labeled for actin (red) paxillin (cyan), a focal adhesion protein. Notice the change in stress fibers between control and cholesterol depleted cells and how aligned the stress fibers get in the latter. Scale bar 10 μm.

as well as adhesion energy between membrane and cytoskeleton, which decreased the lipid diffusion coefficient [119]. Serum starvation followed by cyclodextrin-mediated cholesterol depletion increased stress fiber formation and RhoA activation in an osteoblast cell line [120]. Later on, those results were also corroborated by our group in a murine fibroblast cell line [41]. Similar features can also be observed in an osteosarcoma cell line U2OS (**Figure 4**) in which cholesterol depletion led to stress fiber formation and reorganization of actin cytoskeleton. More studies need to be performed in order to understand why different manipulations in cholesterol content trigger opposite results regarding cellular contractility.

5. Cellular contractility in muscle cells: interplay among Ca^{2+}, sarcomeres and cholesterol

Even though statins had been shown to be relatively safe and to promote health benefits to patients with high risks of cardiac diseases, there are some side effects and risks associated with statin therapy. Myotoxicity is one of the most adverse side effects, being the most common clinical outcomes: myosite, myalgia, and rhabdomyolysis [121]. In vitro studies performed on single muscle fibers isolated from rat skeletal muscle showed that fluvastatin and pravastatin led to contractility impairment and vacuolization of the muscle after 72 h of treatment and cell death after 120 h. Those changes in cellular morphology and contraction were proven to be dependent on geranyl-geranylation of GTPases since concomitant incubation of fluvastatin and geranylgeranyl pyrophosphate attenuated the deleterious effects of statins [122]. In vivo and in vitro treatment with simvastatin also led to contractile dysfunction, actin cytoskeleton disruption and apoptosis of smooth muscle cells [123].

Regarding the effects of direct cholesterol depletion mediated by MβCD on muscle cells, our group demonstrated, using primary cell culture of neonatal rat cardiomyocytes, that a lower cholesterol content increased the contraction rate of those cells and also led to defects in cell relaxation [8]. Moreover, cholesterol depletion increased the Ca^{2+} cytoplasmic concentration and Ca^{2+} sparks during contraction. This phenotype can be attributed to changes in caveolin3 and L-type Ca^{2+} channels distribution across the plasmalemma and hyperactivation of cAMP-dependent PKA activity. Cholesterol-depleted cardiomyocytes also present aberrant myofibrils due to calpain (a Ca^{2+} sensitive protease) activation. By using high-quality confocal microscopy and quantitative data analysis, this work has set in stone the role of cholesterol in regulating cardiomyocyte contractile behavior [8]. Other groups have also shown, for adult rat cardiomyocytes, that cholesterol depletion due to MβCD incubation changed localization of caveolin-3 from a raft to a nonraft membrane fraction changing MAPK signaling and increasing contractility and intracellular Ca^{2+} concentration [124]. Adult murine cardiomyocytes treated with MβCD also presented impairment in the T-tubule system and intercalated discs, which reinforces the role of cholesterol in regulating cardiac contractility [125]. More studies need to be performed in order to understand why MβCD-driven cholesterol depletion in nonmuscle cells increase contractile behavior whereas in muscle cells the same treatment tend to abrogate cellular contractility in several levels.

6. Conclusions

Cholesterol is a very important lipid that controls several cellular processes. This chapter describes how cholesterol is organized in cellular membranes and how it regulates and orchestrates the contractile machinery in muscle and nonmuscle cells. Cholesterol and RhoA protein prenylation share the same synthetic route: the mevalonate pathway. By lowering cholesterol concentration, using either chelating agents, such as MβCD, or inhibitors of HMG-CoA reductase, such as statins, one can observe opposite effects on actin cytoskeleton organization and contractile behavior. Cellular treatments with statins lead to a less-contractile profile, since this drug depletes the amount of prenylated RhoA, which, in turn, is the main upstream regulator of contractility in nonmuscle cells. On the other hand, MβCD-mediated cholesterol depletion induces RhoA activation, stress fiber formation, and increase in cortical stiffness pointing toward a more contractile behavior. In muscle cells, the results are even more intriguing: treatments with either statins or MβCD lead to myofibril disorganization, increase of contraction rate and defects in cell relaxation and in the ability of cells to handle intracellular Ca^{2+}. The reason why muscle and nonmuscle cells behave differently regarding cholesterol depletion is not completely understood and further investigation needs to be performed in order to elucidate this paradigm.

Acknowledgements

This work was supported by the Brazilian agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de Amparo à Pesquisa do Rio de Janeiro (FAPERJ).

Conflict of interest

The authors declare that they have no conflict of interest.

Author details

Barbara Hissa[1*†] and Bruno Pontes[2*†]

*Address all correspondence to: barbarahissa@uchicago.edu and bpontes@icb.ufrj.br

1 The University of Chicago, James Franck Institute, Institute for Biophysical Dynamics and Physics Department, Chicago, IL, USA

2 LPO-COPEA, Federal University of Rio de Janeiro, Institute of Biomedical Sciences, Rio de Janeiro, Brazil

†Both authors contributed equally to the chapter.

References

[1] Pirillo A, Bonacina F, Norata GD, Catapano AL. The interplay of lipids lipoproteins, and immunity in atherosclerosis. Current Atherosclerosis Reports. Springer Nature. 2018;**20**: 12-21. DOI: 10.1007/s11883-018-0715-0

[2] Murrell M, Oakes PW, Lenz M, Gardel ML. Forcing cells into shape: The mechanics of actomyosin contractility. Nature Reviews Molecular Cell Biology. Springer Nature. 2015;**16**:486-498. DOI: 10.1038/nrm4012

[3] Breidigan JM, Krzyzanowski N, Liu Y, Porcar L, Perez-Salas U. Influence of the membrane environment on cholesterol transfer. Journal of Lipid Research. American Society for Biochemistry and Molecular Biology (ASBMB). 2017;**58**:2255-2263. DOI: 10.1194/jlr.m077909

[4] Simons K, Sampaio JL. Membrane organization and lipid rafts. Cold Spring Harbor Perspectives in Biology. Cold Spring Harbor Laboratory. 2011;**3**:a004697-a004697. DOI: 10.1101/cshperspect.a004697

[5] Quest AFG, Leyton L, Párraga M. Caveolins caveolae, and lipid rafts in cellular transport, signaling, and disease. Biochemistry and Cell Biology. Canadian Science Publishing. 2004;**82**:129-144. DOI: 10.1139/o03-071

[6] Head BP, Patel HH, Insel PA. Interaction of membrane/lipid rafts with the cytoskeleton: Impact on signaling and function. Biochimica et Biophysica Acta (BBA)—Biomembranes. Elsevier BV. 2014;**1838**:532-545. DOI: 10.1016/j.bbamem.2013.07.018

[7] López CA, Vries AH de, Marrink SJ. Molecular mechanism of cyclodextrin mediated cholesterol extraction. In: Berkowitz M, editor. PLoS Computational Biology. Public Library of Science (PLoS). 2011;**7**:e1002020. DOI: 10.1371/journal.pcbi.1002020

[8] Hissa B, Oakes PW, Pontes B, Juan GR-S, Gardel ML. Cholesterol depletion impairs contractile machinery in neonatal rat cardiomyocytes. Scientific Reports. Springer Nature. 2017;**7**:43764. DOI: 10.1038/srep43764

[9] Hissa B, Pontes B, Roma PM, Alves AP, Rocha CD, Valverde TM, et al. Membrane cholesterol removal changes mechanical properties of cells and induces secretion of a specific pool of lysosomes. PLoS One. 2013;**8**:e82988

[10] Lombard J. Once upon a time the cell membranes: 175 years of cell boundary research. Biology Direct. Springer Nature. 2014;**9**:32-67. DOI: 10.1186/s13062-014-0032-7

[11] Gorter E, FJEM G. ON bimolecular layers of lipoids on the chromocytes of the blood. Journal of Experimental Medicine. Rockefeller University Press. 1925;**41**:439-443. DOI: 10.1084/jem.41.4.439

[12] Danielli JF. The thickness of the wall of the red blood corpuscule. The Journal of General Physiology. Rockefeller University Press. 1935;**19**:19-22. DOI: 10.1085/jgp.19.1.19

[13] Cole KS. Surface forces of the arbacia egg. Journal of Cellular and Comparative Physiology. Wiley-Blackwell. 1932;**1**:1-9. DOI: 10.1002/jcp.1030010102

[14] Fricke H. The electric capacity of suspensions with special reference to blood. The Journal of General Physiology. Rockefeller University Press. 1925;**9**:137-152. DOI: 10.1085/jgp.9.2.137

[15] Woodhouse DL, Pickworth FA. Permeability of vital membranes. Biochemical Journal. Portland Press Ltd. 1932;**26**:309-316. DOI: 10.1042/bj0260309

[16] Danielli JF, Harvey EN. The tension at the surface of mackerel egg oil with remarks on the nature of the cell surface. Journal of Cellular and Comparative Physiology. Wiley-Blackwell. 1935;**5**:483-494. DOI: 10.1002/jcp.1030050408

[17] Danielli JF, Davson H. A contribution to the theory of permeability of thin films. Journal of Cellular and Comparative Physiology, Wiley-Blackwell. 1935;**5**:495-508. DOI: 10.1002/jcp.1030050409

[18] Singer SJ, Nicolson GL. The fluid mosaic model of the structure of cell membranes. Science. 1972;**175**:720-731

[19] Bretscher MS. Membrane structure: Some general principles. Science. American Association for the Advancement of Science (AAAS). 1973;**181**:622-629. DOI: 10.1126/science.181.4100.622

[20] Yu J, Fischman DA, Steck TL. Selective solubilization of proteins and phospholipids from red blood cell membranes by nonionic detergents. Journal of Supramolecular Structure. Wiley-Blackwell. 1973;**1**:233-248. DOI: 10.1002/jss.400010308

[21] Stier A, Sackmann E. Spin labels as enzyme substrates heterogeneous lipid distribution in liver microsomal membranes. Biochimica et Biophysica Acta (BBA)—Biomembranes, Elsevier BV. 1973;**311**:400-408. DOI: 10.1016/0005-2736(73)90320-9

[22] Karnovsky MJ. The concept of lipid domains in membranes. The Journal of Cell Biology. Rockefeller University Press. 1982;**94**:1-6. DOI: 10.1083/jcb.94.1.1

[23] Estep TN, Mountcastle DB, Barenholz Y, Biltonen RL, Thompson TE. Thermal behavior of synthetic sphingomyelin-cholesterol dispersions. Biochemistry. American Chemical Society (ACS). 1979;**18**:2112-2117. DOI: 10.1021/bi00577a042

[24] Goodsaid-Zalduondo F, Rintoul DA, Carlson JC, Hansel W. Luteolysis-induced changes in phase composition and fluidity of bovine luteal cell membranes. Proceedings of the National Academy of Sciences of the United States of America. 1982;**79**:4332-4336

[25] Simons K, Meer GV. Lipid sorting in epithelial cells. Biochemistry. American Chemical Society (ACS). 1988;**27**:6197-6202. DOI: 10.1021/bi00417a001

[26] Sezgin E, Levental I, Mayor S, Eggeling C. The mystery of membrane organization: Composition regulation and roles of lipid rafts. Nature Reviews Molecular Cell Biology. Springer Nature. 2017;**18**:361-374. DOI: 10.1038/nrm.2017.16

[27] Anchisi L, Dessì S, Pani A, Mandas A. Cholesterol homeostasis: A key to prevent or slow down neurodegeneration. Frontiers in Physiology. Frontiers Media SA. 2013;**3**:486-498. DOI: 10.3389/fphys.2012.00486

[28] Pike LJ. The challenge of lipid rafts. Journal of Lipid Research. American Society for Biochemistry and Molecular Biology (ASBMB). 2008;**50**:S323-S328. DOI: 10.1194/jlr.r800040-jlr200

[29] Korade Z, Kenworthy AK. Lipid rafts cholesterol, and the brain. Neuropharmacology. Elsevier BV. 2008;**55**:1265-1273. DOI: 10.1016/j.neuropharm.2008.02.019

[30] Fantini J, Garmy N, Mahfoud R, Yahi N. Lipid rafts: Structure function and role in HIV Alzheimers and prion diseases. Expert Reviews in Molecular Medicine. Cambridge University Press (CUP). 2002;**4**:1-22. DOI: 10.1017/s1462399402005392

[31] Fivaz M, Abrami L, Goot FG van der. Landing on lipid rafts. Trends in Cell Biology. Elsevier BV; 1999;**9**:212-213. DOI: 10.1016/s0962-8924(99)01567-6

[32] Heerklotz H. Triton promotes domain formation in lipid raft mixtures. Biophysical Journal. Elsevier BV. 2002;**83**:2693-2701. DOI: 10.1016/s0006-3495(02)75278-8

[33] Honigmann A, Sadeghi S, Keller J, Hell SW, Eggeling C, Vink R. A lipid bound actin meshwork organizes liquid phase separation in model membranes. eLife. 2014;**3**:e01671

[34] Mueller V, Ringemann C, Honigmann A, Schwarzmann G, Medda R, Leutenegger M, et al. STED nanoscopy reveals molecular details of cholesterol- and cytoskeleton-modulated lipid interactions in living cells. The Biophysical Journal. 2011;**101**:1651-1660

[35] Klymchenko AS, Kreder R. Fluorescent probes for lipid rafts: From model membranes to living cells. Chemical Biology. 2014;**21**:97-113

[36] Contreras F-X, Sánchez-Magraner L, Alonso A, Goñi FM. Transbilayer (flip-flop) lipid motion and lipid scrambling in membranes. FEBS Letters. Wiley-Blackwell. 2009;**584**:1779-1786. DOI: 10.1016/j.febslet.2009.12.049

[37] Caroni P. Actin cytoskeleton regulation through modulation of PI(4,5)P2 rafts. The EMBO Journal. Wiley-Blackwell. 2001;**20**:4332-4336. DOI: 10.1093/emboj/20.16.4332

[38] Ritchie K, Shan X-Y, Kondo J, Iwasawa K, Fujiwara T, Kusumi A. Detection of non-brownian diffusion in the cell membrane in single molecule tracking. Biophysical Journal. Elsevier BV. 2005;**88**:2266-2277. DOI: 10.1529/biophysj.104.054106

[39] Macháň R, Wohland T. Recent applications of fluorescence correlation spectroscopy in live systems. FEBS Letters. 2014;**588**:3571-3584

[40] Martinac B. Single-molecule FRET studies of ion channels. Progress in Biophysics and Molecular Biology. 2017;**130**:192-197

[41] Hissa B, Pontes B, Roma PMS, Alves AP, Rocha CD, Valverde TM, et al. Membrane cholesterol removal changes mechanical properties of cells and induces secretion of a specific pool of lysosomes. In: Seaman M, editor. PLoS ONE. Public Library of Science (PLoS). 2013;**8**:e82988. DOI: 10.1371/journal.pone.0082988

[42] Eggeling C, Ringemann C, Medda R, Schwarzmann G, Sandhoff K, Polyakova S, et al. Direct observation of the nanoscale dynamics of membrane lipids in a living cell. Nature. Springer Nature. 2008;**457**:1159-1162. DOI: 10.1038/nature07596

[43] Allen JA, Halverson-Tamboli RA, Rasenick MM. Lipid raft microdomains and neurotransmitter signalling. Nature Reviews Neuroscience. Springer Nature. 2006;**8**:128-140. DOI: 10.1038/nrn2059

[44] Classic Pages. Circulation research. Ovid Technologies (Wolters Kluwer Health). 1970;**27**: 482-482. DOI: 10.1161/01.res.27.3.482

[45] Parton RG, Simons K. The multiple faces of caveolae. Nature Reviews Molecular Cell Biology. Springer Nature. 2007;**8**:185-194. DOI: 10.1038/nrm2122

[46] Rothberg KG, Heuser JE, Donzell WC, Ying YS, Glenney JR, Anderson RG. Caveolin, a protein component of caveolae membrane coats. Cell. 1992;**68**:673-682

[47] Scherer PE, Okamoto T, Chun M, Nishimoto I, Lodish HF, Lisanti MP. Identification, sequence, and expression of caveolin-2 defines a caveolin gene family. Proceedings of the National Academy of Sciences of the United States of America. 1996;**93**:131-135

[48] Tang Z, Scherer PE, Okamoto T, Song K, Chu C, Kohtz DS, et al. Molecular cloning of caveolin-3, a novel member of the caveolin gene family expressed predominantly in muscle. The Journal of Biological Chemistry. 1996;**271**:2255-2261

[49] Pol A, Martin S, Fernández MA, Ingelmo-Torres M, Ferguson C, Enrich C, et al. Cholesterol and fatty acids regulate dynamic caveolin trafficking through the Golgi complex and between the cell surface and lipid bodies. Molecular Biology of the Cell. 2005;**16**:2091-2105

[50] Ren X, Ostermeyer AG, Ramcharan LT, Zeng Y, Lublin DM, Brown DA. Conformational defects slow Golgi exit, block oligomerization, and reduce raft affinity of caveolin-1 mutant proteins. Molecular Biology of the Cell. 2004;**15**:4556-4567

[51] Parton RG, Hanzal-Bayer M, Hancock JF. Biogenesis of caveolae: A structural model for caveolin-induced domain formation. The Journal of Cell Science. 2006;**119**:787-796

[52] Fujimoto T, Kogo H, Ishiguro K, Tauchi K, Nomura R. Caveolin-2 is targeted to lipid droplets, a new membrane domain in the cell. The Journal of Cell Science. 2001;**152**:1079-1085

[53] Drab M, Verkade P, Elger M, Kasper M, Lohn M, Lauterbach B, et al. Loss of caveolae, vascular dysfunction, and pulmonary defects in caveolin-1 gene-disrupted mice. Science. 2001;**293**:2449-2452

[54] Galbiati F, Volonte D, Chu JB, Li M, Fine SW, Fu M, et al. Transgenic overexpression of caveolin-3 in skeletal muscle fibers induces a Duchenne-like muscular dystrophy phenotype. Proceedings of the National Academy of Sciences of the United States of America. 2000;**97**:9689-9694

[55] Minetti C, Sotgia F, Bruno C, Scartezzini P, Broda P, Bado M, et al. Mutations in the caveolin-3 gene cause autosomal dominant limb-girdle muscular dystrophy. Nature Genetics. 1998;**18**:365-368

[56] Hailstones D, Sleer LS, Parton RG, Stanley KK. Regulation of caveolin and caveolae by cholesterol in MDCK cells. The Journal of Lipid Research. 1998;**39**:369-379

[57] Hill MM, Bastiani M, Luetterforst R, Kirkham M, Kirkham A, Nixon SJ, et al. PTRF-Cavin, a conserved cytoplasmic protein required for caveola formation and function. Cell. 2008;**132**:113-124

[58] Hansen CG, Bright NA, Howard G, Nichols BJ. SDPR induces membrane curvature and functions in the formation of caveolae. Nature Cell Biology. 2009;**11**:807-814

[59] McMahon KA, Zajicek H, Li WP, Peyton MJ, Minna JD, Hernandez VJ, et al. SRBC/cavin-3 is a caveolin adapter protein that regulates caveolae function. EMBO Journal. 2009;**28**:1001-1015

[60] Bastiani M, Liu L, Hill MM, Jedrychowski MP, Nixon SJ, Lo HP, et al. MURC/Cavin-4 and cavin family members form tissue-specific caveolar complexes. Journal of Cell Biology. 2009;**185**:1259-1273

[61] Stoeber M, Schellenberger P, Siebert CA, Leyrat C, Helenius A, Grünewald K. Model for the architecture of caveolae based on a flexible net-like assembly of Cavin1 and Caveolin discs. Proceedings of the National Academy of Sciences. 2016;**113**:E8069-E8078. DOI: 10.1073/pnas.1616838113

[62] Ludwig A, Nichols BJ, Sandin S. Architecture of the caveolar coat complex. Journal of Cell Biology. 2016;**129**:3077-3083

[63] Lajoie P, Kojic LD, Nim S, Li L, Dennis JW, Nabi IR. Caveolin-1 regulation of dynamin-dependent, raft-mediated endocytosis of cholera toxin-B sub-unit occurs independently of caveolae. Journal of Cellular and Molecular Medicine. 2009;**13**:3218-3225

[64] Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Molecular Biology of the Cell. 5th ed. New York: Taylor & Francis Group; 2007

[65] Tojkander S, Gateva G, Lappalainen P. Actin stress fibers-assembly, dynamics and biological roles. The Journal of Cell Science. 2012;**125**:1855-1864

[66] Heissler SM, Manstein DJ. Nonmuscle myosin-2: Mix and match. Cellular and Molecular Life Sciences. 2013;**70**:1-21

[67] Verkhovsky AB, Svitkina TM, Borisy GG. Myosin II filament assemblies in the active lamella of fibroblasts: Their morphogenesis and role in the formation of actin filament bundles. Journal of Cell Biology. 1995;**131**:989-1002

[68] Svitkina TM, Verkhovsky AB, McQuade KM, Borisy GG. Analysis of the actin-myosin II system in fish epidermal keratocytes: Mechanism of cell body translocation. Journal of Cell Biology. 1997;**139**:397-415

[69] Hotulainen P, Lappalainen P. Stress fibers are generated by two distinct actin assembly mechanisms in motile cells. Journal of Cell Biology. 2006;**173**:383-394

[70] Burnette DT, Manley S, Sengupta P, Sougrat R, Davidson MW, Kachar B, et al. A role for actin arcs in the leading-edge advance of migrating cells. Nature Cell Biology. 2011;**13**:371-381

[71] Pasapera AM, Plotnikov SV, Fischer RS, Case LB, Egelhoff TT, Waterman CM. Rac1-dependent phosphorylation and focal adhesion recruitment of myosin IIA regulates migration and mechanosensing. Current Biology. 2015;**25**:175-186

[72] Tojkander S, Gateva G, Husain A, Krishnan R, Lappalainen P. Generation of contractile actomyosin bundles depends on mechanosensitive actin filament assembly and disassembly. eLife. 2015;**4**:e06126

[73] Luo W, Yu CH, Lieu ZZ, Allard J, Mogilner A, Sheetz MP, et al. Analysis of the local organization and dynamics of cellular actin networks. Journal of Cell Biology. 2013;**202**: 1057-1073

[74] Wilson CA, Tsuchida MA, Allen GM, Barnhart EL, Applegate KT, Yam PT, et al. Myosin II contributes to cell-scale actin network treadmilling through network disassembly. Nature. 2010;**465**:373-377

[75] Livne A, Geiger B. The inner workings of stress fibers-from contractile machinery to focal adhesions and back. Journal of Cell Science. The Company of Biologists. 2016;**129**:1293-1304. DOI: 10.1242/jcs.180927

[76] Huxley HE. The mechanism of muscular contraction. Science. American Association for the Advancement of Science (AAAS). 1969;**164**:1356-1366. DOI: 10.1126/science.164.3886.1356

[77] Trichet L, Le DJ, Hawkins RJ, Vedula SR, Gupta M, Ribrault C, et al. Evidence of a large-scale mechanosensing mechanism for cellular adaptation to substrate stiffness. Proceedings of the National Academy of Sciences of the United States of America. 2012;**109**:6933-6938

[78] Wood AW. Physiology, Biophysics and Biomedical Engineering. Boca Raton, FL: CRC Press, Taylor & Francis Group; 2012. p. 782

[79] Salbreux G, Charras G, Paluch E. Actin cortex mechanics and cellular morphogenesis. Trends in Cell Biology. Elsevier BV. 2012;**22**:536-545. DOI: 10.1016/j.tcb.2012.07.001

[80] Tooley AJ, Gilden J, Jacobelli J, Beemiller P, Trimble WS, Kinoshita M, et al. Amoeboid T lymphocytes require the septin cytoskeleton for cortical integrity and persistent motility. Nature Cell Biology. 2009;**11**:17-26

[81] Kunda P, Baum B. The actin cytoskeleton in spindle assembly and positioning. Trends in Cell Biology. 2009;**19**:174-179

[82] Wolf K, Mazo I, Leung H, Engelke K, von AUH, Deryugina EI, et al. Compensation mechanism in tumor cell migration: Mesenchymal-amoeboid transition after blocking of pericellular proteolysis. Journal of Cell Biology. 2003;**160**:267-277

[83] Morone N, Fujiwara T, Murase K, Kasai RS, Ike H, Yuasa S, et al. Three-dimensional reconstruction of the membrane skeleton at the plasma membrane interface by electron tomography. Journal of Cell Biology. 2006;**174**:851-862

[84] Charras GT, Hu CK, Coughlin M, Mitchison TJ. Reassembly of contractile actin cortex in cell blebs. Journal of Cell Biology. 2006;**175**:477-490

[85] Moeendarbary E, Harris AR. Cell mechanics: Principles, practices, and prospects. Wiley Interdisciplinary Reviews: Systems Biology and Medicine. 2014;**6**:371-388

[86] Pontes B, Monzo P, Gauthier NC. Membrane tension: A challenging but universal physical parameter in cell biology. Seminars in Cell and Developmental Biology. Elsevier BV. 2017;**71**:30-41. DOI: 10.1016/j.semcdb.2017.08.030

[87] Nussenzveig HM. Cell membrane biophysics with optical tweezers. The European Biophysics Journal. 2017;**46**:1-16

[88] Charras GT, Coughlin M, Mitchison TJ, Mahadevan L. Life and times of a cellular bleb. The Biophysical Journal. 2008;**94**:1836-1853

[89] Dai J, Sheetz MP. Membrane tether formation from blebbing cells. The Biophysical Journal. 1999;**77**:3363-3370

[90] Pontes B, Ayala Y, Fonseca AC, Romão LF, Amaral RF, Salgado LT, et al. Membrane elastic properties and cell function. PLoS One. 2013;**8**:e67708

[91] Liu Y, Belkina NV, Park C, Nambiar R, Loughhead SM, Patino-Lopez G, et al. Constitutively active ezrin increases membrane tension, slows migration, and impedes endothelial transmigration of lymphocytes in vivo in mice. Blood. 2012;**119**:445-453

[92] Rouven BB, Pietuch A, Nehls S, Rother J, Janshoff A. Ezrin is a major regulator of membrane tension in epithelial cells. Scientific Reports. 2015;**5**:14700

[93] Nambiar R, McConnell RE, Tyska MJ. Control of cell membrane tension by myosin-I. Proceedings of the National Academy of Sciences of the United States of America. 2009;**106**:11972-11977

[94] Gérard A, Patino-Lopez G, Beemiller P, Nambiar R, Ben-Aissa K, Liu Y, et al. Detection of rare antigen-presenting cells through T cell-intrinsic meandering motility, mediated by Myo1g. Cell. 2014;**158**:492-505

[95] Pontes B, Viana NB, Salgado LT, Farina M, Moura NV, Nussenzveig HM. Cell cytoskeleton and tether extraction. The Biophysical Journal. 2011;**101**:43-52

[96] Chen C, Tao T, Wen C, He WQ, Qiao YN, Gao YQ, et al. Myosin light chain kinase (MLCK) regulates cell migration in a myosin regulatory light chain phosphorylation-independent mechanism. The Journal of Biological Chemistry. 2014;**289**:28478-28488

[97] Ayala YA, Pontes B, Hissa B, Monteiro AC, Farina M, Moura-Neto V, et al. Effects of cytoskeletal drugs on actin cortex elasticity. Experimental Cell Research. 2017;**351**:173-181

[98] Khatibzadeh N, Gupta S, Farrell B, Brownell WE, Anvari B. Effects of cholesterol on nanomechanical properties of the living cell plasma membrane. Soft Matter. 2012;**8**:8350-8360

[99] Hissa B, Oakes PW, Pontes B, Ramírez-San JG, Gardel ML. Cholesterol depletion impairs contractile machinery in neonatal rat cardiomyocytes. Science Reports. 2017;**7**:43764e

[100] Citi S, Kendrick-Jones J. Regulation of non-muscle myosin structure and function. BioEssays. Wiley-Blackwell. 1987;**7**:155-159. DOI: 10.1002/bies.950070404

[101] Chrzanowska-Wodnicka M. Rho-stimulated contractility drives the formation of stress fibers and focal adhesions. The Journal of Cell Biology. Rockefeller University Press. 1996;**133**:1403-1415. DOI: 10.1083/jcb.133.6.1403

[102] Madaule P, Axel R. A novel ras-related gene family. Cell. Elsevier BV. 1985;**41**:31-40. DOI: 10.1016/0092-8674(85)90058-3

[103] Paterson HF. Microinjection of recombinant p21rho induces rapid changes in cell morphology. The Journal of Cell Biology. Rockefeller University Press. 1990;**111**:1001-1007. DOI: 10.1083/jcb.111.3.1001

[104] Ridley AJ, Hall A. The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. Cell. Elsevier BV. 1992;**70**:389-399. DOI: 10.1016/0092-8674(92)90163-7

[105] Etienne-Manneville S, Hall A. Rho GTPases in cell biology. Nature. Springer Nature. 2002;**420**:629-635. DOI: 10.1038/nature01148

[106] Maltese WA. Posttranslational modification of proteins by isoprenoids in mammalian cells. The FASEB Journal. FASEB. 1990;**4**:3319-3328. DOI: 10.1096/fasebj.4.15.2123808

[107] Rowell CA, Kowalczyk JJ, Lewis MD, Garcia AM. Direct demonstration of geranylgeranylation and farnesylation of Ki-Ras in vivo. Journal of Biological Chemistry. American Society for Biochemistry and Molecular Biology (ASBMB). 1997;**272**:14093-14097. DOI: 10.1074/jbc.272.22.14093

[108] Katayama M, Kawata M, Yoshida Y, Horiuchi H, Yamamoto T, Matsuura Y, et al. The posttranslationally modified C-terminal structure of bovine aortic smooth muscle rhoA p21. The Journal of Biological Chemistry. 1991;**266**:12639-12645

[109] Waiczies S, Bendix I, Prozorovski T, Ratner M, Nazarenko I, Pfueller CF, et al. Geranylgeranylation but not GTP loading determines rho migratory function in T cells. The Journal of Immunology. The American Association of Immunologists. 2007;**179**:6024-6032. DOI: 10.4049/jimmunol.179.9.6024

[110] Goldstein JL, Brown MS. Regulation of the mevalonate pathway. Nature. Springer Nature. 1990;**343**:425-430. DOI: 10.1038/343425a0

[111] Siperstein MD, Fagan VM. Feedback control of mevalonate synthesis by dietary cholesterol. The Journal of Biological Chemistr. 1966;**241**:602-609

[112] Lowering blood cholesterol to prevent heart disease. NIH Consensus Development Conference statement. Arteriosclerosis, Thrombosis, and Vascular Biology. 1985;**5**:404-412. Originally published July 1, 1985. DOI: 10.1161/01.atv.5.4.404

[113] Tobert JA. Lovastatin and beyond: The history of the HMG-CoA reductase inhibitors. Nature Reviews Drug Discovery. Springer Nature. 2003;**2**:517-526. DOI: 10.1038/nrd1112

[114] Oesterle A, Laufs U, Liao JK. Pleiotropic effects of statins on the cardiovascular system. Circulation Research. Ovid Technologies (Wolters Kluwer Health). 2017;**120**:229-243. DOI: 10.1161/circresaha.116.308537

[115] Dick M, Jonak P, Leask RL. Statin therapy influences endothelial cell morphology and F-actin cytoskeleton structure when exposed to static and laminar shear stress conditions. Life Sciences. Elsevier BV. 2013;**92**:859-865. DOI: 10.1016/j.lfs.2013.03.002

[116] Lampi MC, Faber CJ, Huynh J, Bordeleau F, Zanotelli MR, Reinhart-King CA. Simvastatin ameliorates matrix stiffness-mediated endothelial monolayer disruption. In: Komarova Y, editor. Plos ONE. Public Library of Science (PLoS). 2016;**11**:e0147033. DOI: 10.1371/journal.pone.0147033

[117] Kwik J, Boyle S, Fooksman D, Margolis L, Sheetz MP, Edidin M. Membrane cholesterol lateral mobility, and the phosphatidylinositol 4,5-bisphosphate-dependent organization of cell actin. Proceedings of the National Academy of Sciences. 2003;**100**:13964-13969. DOI: 10.1073/pnas.2336102100

[118] Byfield FJ, Aranda-Espinoza H, Romanenko VG, Rothblat GH, Levitan I. Cholesterol depletion increases membrane stiffness of aortic endothelial cells. Biophysical Journal. Elsevier BV. 2004;**87**:3336-3343. DOI: 10.1529/biophysj.104.040634

[119] Sun M, Northup N, Marga F, Huber T, Byfield FJ, Levitan I, et al. The effect of cellular cholesterol on membrane-cytoskeleton adhesion. Journal of Cell Science. The Company of Biologists. 2007;**120**:2223-2231. DOI: 10.1242/jcs.001370

[120] Qi M, Liu Y, Freeman MR, Solomon KR. Cholesterol-regulated stress fiber formation. Journal of Cellular Biochemistry. Wiley-Blackwell. 2009;**106**:1031-1040. DOI: 10.1002/jcb.22081

[121] Hilton-Jones D. Statin-related myopathies. Practical Neurology. British Medical Journal. 2018;**18**(2):97-105. DOI: 10.1136/practneurol-2017-001738

[122] Sakamoto K, Honda T, Yokoya S, Waguri S, Kimura J. Rab-small GTPases are involved in fluvastatin and pravastatin-induced vacuolation in rat skeletal myofibers. The FASEB Journal. 2007;**21**:4087-4094

[123] Kang S, Kim K, Noh JY, Jung Y, Bae ON, Lim KM, et al. Simvastatin induces the apoptosis of normal vascular smooth muscle through the disruption of actin integrity via the impairment of RhoA/Rac-1 activity. The Journal of Thrombosis and Haemostasis. 2016;**116**:496-505

[124] Haque MZ, McIntosh VJ, Samra ABA, Mohammad RM, Lasley RD. Cholesterol depletion alters cardiomyocyte subcellular signaling and increases contractility. In: Hsieh YH, editor. Plos One. Public Library of Science (PLoS). 2016;**11**:e0154151. DOI: 10.1371/journal.pone.0154151

[125] Zhu Y, Zhang C, Chen B, Chen R, Guo A, Hong J, et al. Cholesterol is required for maintaining T-tubule integrity and intercellular connections at intercalated discs in cardiomyocytes. Journal of Molecular and Cellular Cardiology. Elsevier BV. 2016;**97**:204-212. DOI: 10.1016/j.yjmcc.2016.05.013

IntechOpen

IntechOpen