

Review Article

The Cytoskeleton of the Cardiac Muscle Cell

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Key words:

Cardiac myocyte,
intercalated disc,
sarcolemma,
sarcomere, Z-disc.

Manuscript received:
December 3, 2010;

Accepted:
June 19, 2012.

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The cytoskeleton of the cardiomyocyte has a special place among the subcellular biological structures, due to its complexity, its exceptional organisation, and to the multiple roles it plays. Cytoskeleton proteins and interactions among them are constantly being discovered. It has been demonstrated that mutations of these proteins, of different type and importance, are involved in the pathogenesis of cardiomyopathies, the heart's participation in several muscular dystrophies, and cardiac arrhythmias.¹⁻⁵ The volume of new information added to this field is huge and concerns a large variety of cytoskeleton proteins. The purpose of this review is to present a picture that is analytical, as far as possible, but coherent and simplified, of how the parts of the cytoskeleton are structured and, finally, how they are integrated into a unique, effective and functional total.

Basic morphology of the cardiac muscle cell

In order to describe the sophisticated subcellular organisation of the cardiomyocyte, is useful to simplify and classify its structure. For that purpose, it is necessary to divide the cardiomyocyte into separate subcellular areas, as follows (Figure 1):

- a. The *intercalated disc*, which is an exclusive feature of the cardiomyocyte.^{6,7}
- b. The *lateral sarcolemma*. This name indicates the different topography of the

lateral sarcolemma from the intercalated disc, which is a separate area, as regards both structure and function.

- c. The *cytoskeleton* and, finally,
- d. the *cellular organelles*.

The cardiomyocyte (Figure 1) is cylindrical, with a diameter of about 10-15 μm and a length of about 100 μm .⁸ Usually, it branches and is connected to its adjacent cardiomyocytes along the longitudinal axis, through the intercalated discs. It is striated and has one or two centrally located, non-dense, slightly elongated nuclei.⁶⁻⁹

The cardiomyocytes' arrangement leads to the formation of a grid, or *syncytium*, which constitutes the cardiac muscle. However, in the myocardium a variety of other cell types are found, such as fibroblasts—which represent the majority of cells in the normal heart—endothelial cells, and smooth muscle cells.^{6,10,11} The connective tissue that surrounds the cardiomyocytes includes, from the outermost layer to the inner one, the *epimysium*, the *perimysium* and the *endomysium*. The endomysium contains the extracellular matrix, which plays a key role in the dynamic interaction between cardiomyocytes. The normal extracellular matrix in the heart (cardiogel) is produced both by the myocardial cells themselves and by the myocardium fibroblasts. It consists of I, III and IV-type collagen, lamin, fibronectin and proteoglycans. It seems that it plays an important role in the generation of the

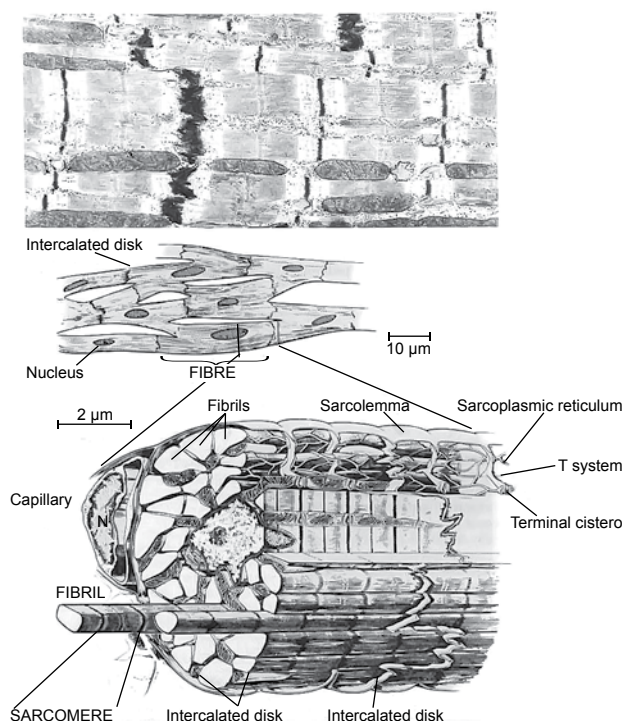


Figure 1. The basic morphology of the cardiac muscle cell. Top: The cardiac muscle as seen in the electron microscope. Middle: An illustration of the cardiac muscle grid. Bottom: An illustration of part of a cardiac muscle cell in higher magnification.

myofibrils and the intercalated disc, during the differentiation and maturation of the cardiac stem cells to myocardial cells.¹¹⁻¹⁶

Intercalated disc

The intercalated disc (Figure 1) represents the area where the adjacent myocardial cells are connected along the longitudinal axis, and it appears as a specialised part of the cell membrane.^{7,9,17} It is present as an intercalated structure, and consists of two parts: the *transverse part*, which extends across the longitudinal axis of the muscle fibres, and the *lateral part*, which goes along the axis of the muscle fibres. In the area of the intercalated disc, the membrane forms leafing protrusions. The intercalated disc presents certain differences in morphology among the several types of myocardial cells (atrial, ventricular, conduction system cells).^{6,18} In fact, the intercalated disc is a synaptic complex with a specific functional mission: to provide proper connection and communication between myocardial cells. According to its functional mission, the intercalated disc consists of:^{6,7,9,19}

- i. The *desmosomes*, or *maculae adherentes*, which prevent the detachment of cells under the normal contraction activity.^{6,7,17} The desmosomes are formed by desmoglein-2 and desmocollin-2 transmembrane proteins, which establish a transcellular connection. The following cytoplasmic proteins also participate: desmin, desmoplakin, plakophilin-2, p0071 and plakoglobin, to which the intermediate desmin filaments are attached.^{7,9,14,20}
- ii. The *adherens junctions* or *fasciae adherens*,^{18,21} which serve as attachment points for the terminal sarcomeres' actin filaments. In this way they represent a key-post for the power transmission between neighbouring myocardial cells.^{6,17} The main proteins of the adherens junctions include N-cadherin, which is a transmembrane protein and ensures the transcellular connection,²² β_1 D integrin (also a transmembrane protein), and the following cytoplasmic proteins: α - and β -catenins, to which thin actin filaments are attached, vinculin, metavinculin, plakoglobin, spectrin, and ARVCF (Armadillo repeat gene deleted in Velo-cardio-facial syndrome), SPAL (Spa-1-like protein), a GTPase-activating protein (GAP family protein), ALP (actinin-associated LIM Protein), and N-RAP (a LIM domain-containing protein), which possibly function as tension sensors.^{9,14,17,20,23}
- iii. The *gap junctions* or *nexus* or *maculae communicantes*,¹⁸ which are key structures for ion transportation between adjacent cardiomyocytes. Gap junctions are formed by connexins. In this type of intercellular communication, the non-muscular cells of myocardium may also participate.^{6,10} As regards connexins, in the ventricular myocardium connexin-43 predominates, while connexin-40 and connexin-45 are present in different quantities. Six connexin molecules per cell membrane are necessary for the formation of a gap junction between two myocardial cells. It is not yet been confirmed whether there is a fourth type of connection in the intercalated disc, called *tight junction*.^{9,23,24}

Lateral sarcolemma

The lateral sarcolemmatic area is the total remaining sarcolemma (the myocardial cell's membrane) apart from the intercalated disc. In this part of the membrane, specialised areas of the cardiomyocyte are recognised that are very important for its homeostasis. These sites are called *costameres* (Figure 2). Costa-

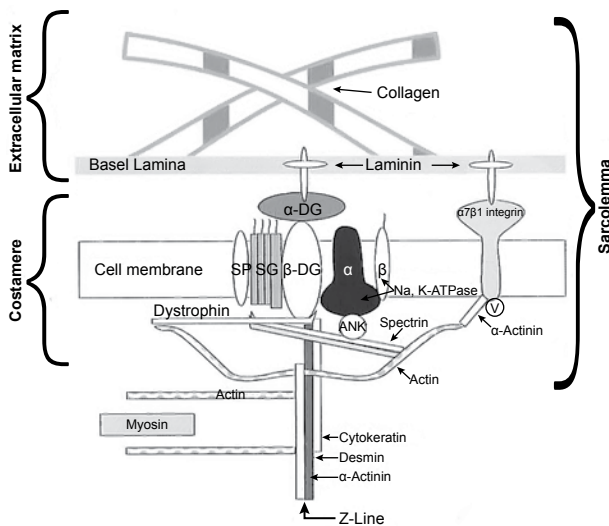


Figure 2. Schematic illustration of the lateral sarcolemma containing a costamere. Costameres consist of the dystrophin-glycoprotein complex, the focal adhesion complex and the spectrin complex (not shown here), and play an important role in connecting the extracellular matrix with the rest of the cytoskeleton. DG – dystroglycan; SP – sarcospan; SG – sarcoglycan complex; α , β – syntrophins; V – vinculin; ANK – ankyrin 3. (From reference 26. Reproduced with permission from Wolters Kluwer Health.)

meres are sub-sarcolemmatic structures that are connected to the myocardial cell's contractile elements, creating a pericellular grid.^{9,25,26} Costameres represent sites of attachment for several cytoskeleton networks, forming a “communication centre” between the extracellular matrix, the sarcolemma and the Z-disc.^{9,25,27} It is strongly believed that they serve as posts of connection with the extracellular matrix in order to stabilise the sarcolemma, as well as to complete the signal transduction pathways involved in the mechanical power transmission.^{7,9,17,28-31}

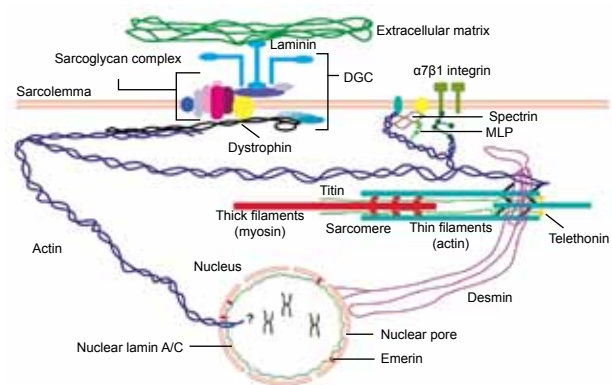
Costameres consist of: a) *dystrophin and the dystrophin-associated glycoprotein complex* and b) the *focal adhesion complex*. The sarcomere's third constituent is the spectrin complex (Figures 2 & 3).^{9,11,13,29}

A distinctive feature of the lateral sarcolemmatic area, present at the Z-disc level, is the *transverse tubular system*, or *T-system* (Figure 1).⁷ The T-system consists of folds of the sarcolemma entering deep into the cell, allowing fast and uniform transmission of the cell membrane's action potential to the muscle fibre. In the T-system, potassium channels and their subunits, minK, are present.^{17,28} Moreover, several of the proteins of the lateral sarcolemma described above are found in the T-system providing protein support.²³

Cytoskeleton

The cytoskeleton is the structure that provides mechanical support to the cell, also contributing to the spatial arrangement of the other subcellular elements. The cytoskeleton preserves the structural and functional integrity of the myocardial cell. Moreover, parts of the cytoskeleton participate in various cell procedures, such as cell increase and division, cell migration, intracellular transfer of vesicles, proper arrangement and function of the cell organelles, allocation of membrane receptors, and intercellular communication. It is also believed to play an important role in the cell's mechanical signal transduction.^{14,29}

The cytoskeleton, as regards morphology and topography, is divided into *sarcomeric*, *extra-sarcomeric*, *membrane-submembrane* and *nuclear* cytoskeleton (Figure 3).⁹ This division constitutes a simplification, since the cytoskeleton is literally a complicated, but united and inextricable entity, so the purpose of the simplification is to facilitate the study and understanding of this entity. On the other hand, there is the functional division of the cytoskeleton, according to which it consists of the *contractile* part, exclusively regarding the thin and thick filaments of the sarcomere, and the *non-contractile* part, which serves the transmission of the produced power, the signal transduction and the preservation of the cell's structural integrity.²³



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Figure 3. The cytoskeleton as a total entity. In the centre, the sarcomeric contractile cytoskeleton is composed of thin and thick filaments. The extra-sarcomeric cytoskeleton connects the sarcomere to the costameres and to the nuclear cytoskeleton. (From reference 4. Adapted and reproduced with permission from the authors and Cambridge University Press.)

Sarcomeric cytoskeleton

The sarcomere in general

The sarcomeric cytoskeleton consists of *thin filaments*, *thick filaments* and the *Z-discs* (Figures 3 & 4).^{4,9} A sarcomere is limited by two *Z-discs*.^{4,9,17,32,33} Sarcomeres are approximately 2.2 μm long and they are connected serially in order to form a *myofibril*.^{8,32} Consequently, the myocardial cell's inner part consists of many fascicles of myofibrils, each of which has a diameter of approximately 1-2 μm .^{4,9} In the two sarcomere ends, *Z-discs* are located. The thin actin filaments are attached to the *Z-discs* and extend in the opposite direction from both sides of the *Z-disc*.^{4,32} Among the actin filaments, there are thick myosin filaments. The thin and thick filaments are the most

important elements for the execution of the contraction cycle. A thick myosin filament is surrounded by six actin filaments circularly forming a hexagon.^{6,34} The sarcomere is divided into the following bands (or zones): the *A-band* is the area where thin and thick filaments overlap; the *I-band* consists only of thin filaments; and the *H-band* consists only of thick filaments. The *H-band* partition is made by the *M-line*, an area where neighbouring thick filaments are laterally connected (Figure 4).^{9,17,32}

Thin filaments

The thin filaments (length $\sim 1.0 \mu\text{m}$) consist of cardiac actin, α -tropomyosin and C-, I- and T- troponins (Figure 4).^{4,9,34} Actin is formed by two chains, each

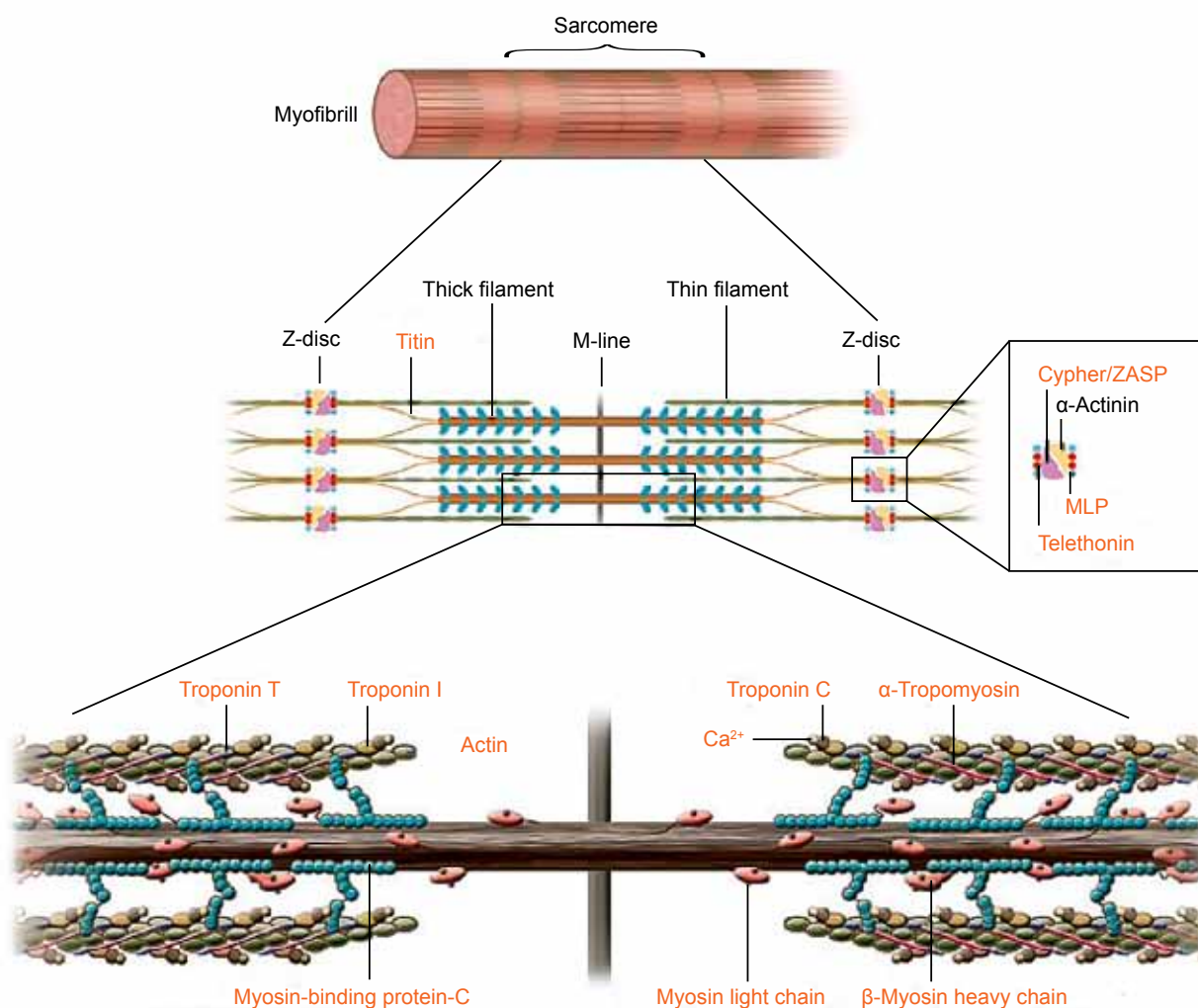


Figure 4. The sarcomere from lower to higher magnification. The arrangement of thick and thin filaments is illustrated. The M-line partitions the H-band, which consists of thick filaments only. (From reference 33. Reproduced with permission from the American Society for Clinical Investigation.)

of which is formed by ball-shaped G-actin monomers. These two chains twine around each other, forming a double helix. In this way, a filiform polymer occurs, the sarcomeric F-actin.³⁵

Each actin G-monomer has a binding area for myosin and has a remarkable feature, which is its structural asymmetry. When G-actin monomers are polymerised, they are connected on their sides, creating a filament with distinctive polarity.⁶ One end of the thin filament is tied to the Z-disc and the other reaches the A-zone. In the A-zone, the ends of the G-actin polymerised molecules remain connected, with the assistance of tropomodulin, which plays an important role in the preservation of the thin filaments' proper length.^{34,36,37} Regulatory proteins, including tropomyosin and a hetero-tripartite complex of cardiac troponins, are connected to the actin.³⁸

Tropomyosin is a large, thin molecule, 40 nm in length, that consists of two polypeptide chains in the form of a helix. These two polypeptide chains are twisted one around the other. Tropomyosin molecules are aligned, head to tail, creating two tropomyosin polymers per actin filament.³⁴ The two tropomyosin molecules run on the actin subunits, along the fissure resulting from the twisted actin bands. Each tropomyosin molecule covers a distance of seven G-actin molecules and has a troponin complex united on its surface. In this way, the stoichiometry of the actin, tropomyosin and troponin complex molecules is 7:1:1.^{6,34,39} The tropomyosin's role, especially when it interacts with tropomodulin, is to stabilise the thin filament, preventing the thin filament's de-polymerisation.^{34,40}

Cardiac troponin is a complex of three subunits: T-troponin (cTnT), which serves the tight connection of troponin complex with tropomyosin, I-troponin (cTnI), which suppresses the actin-myosin interaction, and C-troponin (cTnC), on which calcium is bound. The troponin complex is tied to a specific site on each tropomyosin molecule.^{4,6,8}

Thick filaments

The thick filament (~1.6 μm in length) consists mainly of myosin and C-, H- and X-myosin binding proteins, the first of which (MyBP-C) plays the most important role, since it seems to have a structural and regulatory character (Figure 4).^{4,9,34,41} In more detail, MyBP-C is connected to the actin only in the sarcomere's A-band. Consequently, MyBP-C is connected to myosin, actin and titin, thus providing stability to the sarco-

meric structure.^{4,14} Myosin is a rather large complex with a molecular weight of ~500 kDa. Myosin may be divided into two similar heavy chains (MHC) and two pairs of light chains (MLC1 and MLC2). Myosin heavy chains are thin baculiform molecules, 150 nm long and 2-3 nm thick, which are composed of two chains twisted one around the other. The small ball-shaped domains on the one end of each chain create the heads, which can be connected to the actin and have ATP binding sites. The same heads have enzymic capacity to carry out ATP hydrolysis. The four light chains are connected to the head. After a short proteolysis, heavy chains break into two parts, the light and the heavy meromyosin. Light meromyosin is the largest part of the myosin molecule's baculiform part, while heavy meromyosin is mostly the ball-shaped projection and a small part of the rod.^{4,6,11}

Several hundreds of myosin molecules are necessary in order to form a thick filament. The myosin molecules are arranged on each thick filament with their baculiform part covered and their ball-shaped heads pointing to one of their ends. As has been already mentioned, the H-zone is a myosin overlapping area, without heads, which consists only of the molecule's baculiform part. Between the thin and thick filaments, cross bridges are identified. These bridges are formed by the myosin molecules' heads and a small portion of the buliform part. These bridges are deemed to be directly responsible for the transformation of chemical into mechanical energy.⁴

Titin, myomesins and nebullette

It goes without saying that thin and thick filaments cannot "stand" alone in the right position, having the right arrangement, in order to serve their functional purpose, which is contraction. The sarcomere is a unique example in biology, in which hundreds of protein molecules are gathered and form a sub-molecular structure that has exceptional stability and organisation.⁴² A "scaffold" is necessary to support and, probably, to regulate the thin and thick filaments' function. Titin, myomesins and nebullette are among the most important proteins that play such a role in the sarcomere.⁹

Titin (also known as connectin) is a giant protein, with a molecular weight more than 3000 kDa, and is the largest protein molecule known today. Its composition is also completely known. It traverses the half sarcomere, from the Z-disc, where it is connected to the α -actinin, until the M-line. It is connected to myosin

through MyBP-C, MyBP-H, 1- and 2-myomesin, and the baculiform part of the myosin.^{4,9,14,17,25,34,42,43} Titin consists of a flexible part in the I-zone and a more inflexible part in the A-zone.¹⁷ It interacts with the thin filaments in the Z-disc through the α -actinin and with the T-cap (see below), but there is also the opinion that titin may be connected to the thin actin filaments outside the Z-disc. Interestingly, a titin of smaller size (700 kDa) has been discovered in the cardiac muscle.^{25,34}

The role of titin is multiple. It contributes to the sarcomeric organisation by serving as a molecule ruler, “centring” in some way the thick filaments inside the sarcomere.^{9,17,25,34} It gives flexibility to the myofibril, since it actually serves as a spring.^{4,9,34} Moreover, it takes part in the transfer of the energy produced, in the recovery of the sarcomere’s initial length after the contraction,^{17,25,32} and in the sarcomere genesis.^{4,14,42} Other data suggest that protein complexes that interact with titin function as sensors that “realise” the myocardial tension, serving the normal functional unity between myocardial cells.^{17,25,44}

Nebulette (107 kDa) is the analogous protein of the skeletal muscle’s nebulin, with the difference that cardiac muscle’s nebulette is shorter and it does not cover the total length of actin filament.^{25,34,42} While the skeletal muscle’s nebulin regulates the arrangement of the actin, it seems that nebulette in the heart cannot have a similar role, mostly because of its much smaller size.^{25,42} However, according to other reports, it participates in both the structure and the function of the Z-disc. It does that, on the one hand, by organising myofibrils inside the Z-disc and regulating the disc’s thickness and, on the other hand, by assisting the thin filament’s integrity. However, nebulette, in contrast to actin and titin, does not cross the entire length of the Z-disc, but is limited to its surface. Moreover, it seems to participate in the binding of the sarcomeric actin with the α -actinin, and it may possibly play some role in the signal transduction routes.^{25,42}

Z-disc

There are several questions arising beyond the structure and organisation of thin and thick filaments. On what domain are the thin filaments tied up? How is the produced mechanical energy transferred? The answer to these questions is given by the structure called Z-disc. It is the structure that “collects” the mechanical energy produced by the interaction between myosin and actin, and transfers it to costameres. The

myofibrils are connected to each other at the level of the Z-disc, creating an absolutely arranged three-dimensional grid (Figure 5) in which the sarcomere, the extra-sarcomeric cytoskeleton and the sarcolemma meet. The Z-disc is about 100 nm thick and is located at the centre of the I-zone.²⁵ The complexity of the Z-disc’s construction is a remarkable example of molecular architecture, even though the complete structure and function have not been totally clarified.⁴⁵

On either side of a Z-disc there are two sarcomeres. Actin filaments begin from one side to create the thin filaments of the one sarcomere, and similarly, from the other side, to create the thin filaments of the neighbouring sarcomere.⁸ Two antiparallel actin filaments in the area of the Z-disc present a different polarity and are connected between them and stabilised in the Z-disc area, through α -actinin (Figures 5 & 6).^{13,34,38} The protein that binds and stabilises the actin on the Z-disc, and more specifically on the α -actinin, is called CapZ.³⁴ The β_1 -subunit of CapZ, which is identified in the Z-disc, and the β_2 -subunit, which is identified in the intercalated disc and the cell periphery, prevail in the myocardial cell. The role of CapZ is to suspend the thin filaments’ elongation and de-polymerisation.^{25,34} On the other hand, α -actinin is a crucial protein of the Z-disc that plays an important role in the orientation of the counter-parallel end of different-polarity actin filaments, which are oriented towards the two sarcomeres beside the Z-disc. Other proteins, among which are titin and nebulette, seem to participate in this orientation regulation.²⁵

The N-end of titin enters the Z-disc, where it is stabilised with the help of a protein called telethonin (also known as T-cap).^{17,25} Titin crosses the Z-disc and organises the connection between α -actinin 2 and actin (Figures 5 & 6).⁴² The domain of titin, which interacts with actin, consists of 45 amino acids and is called z-repeats.²⁵ The C-end of titin is inside the sarcomere, on the M-line, as mentioned above, and it interacts with the CARP (cardiac restricted ankyrin protein) transcription agent and the ankrd2, DARP and myopalladin proteins.^{17,46} In addition, the C-end of titin has a protein kinase action.^{25,42}

The molecular weight of T-cap (titin-cap) equals 19 kDa; it has a unique structure and, until now, it has only been identified in the cardiac and skeletal muscle.^{25,42} It seems that it functions as a tension sensor, being connected to the titin’s N-end on the Z-disc and connecting the sarcomere with the T-system.^{17,32,46}

Obscurin, with a molecular weight equal to ~800

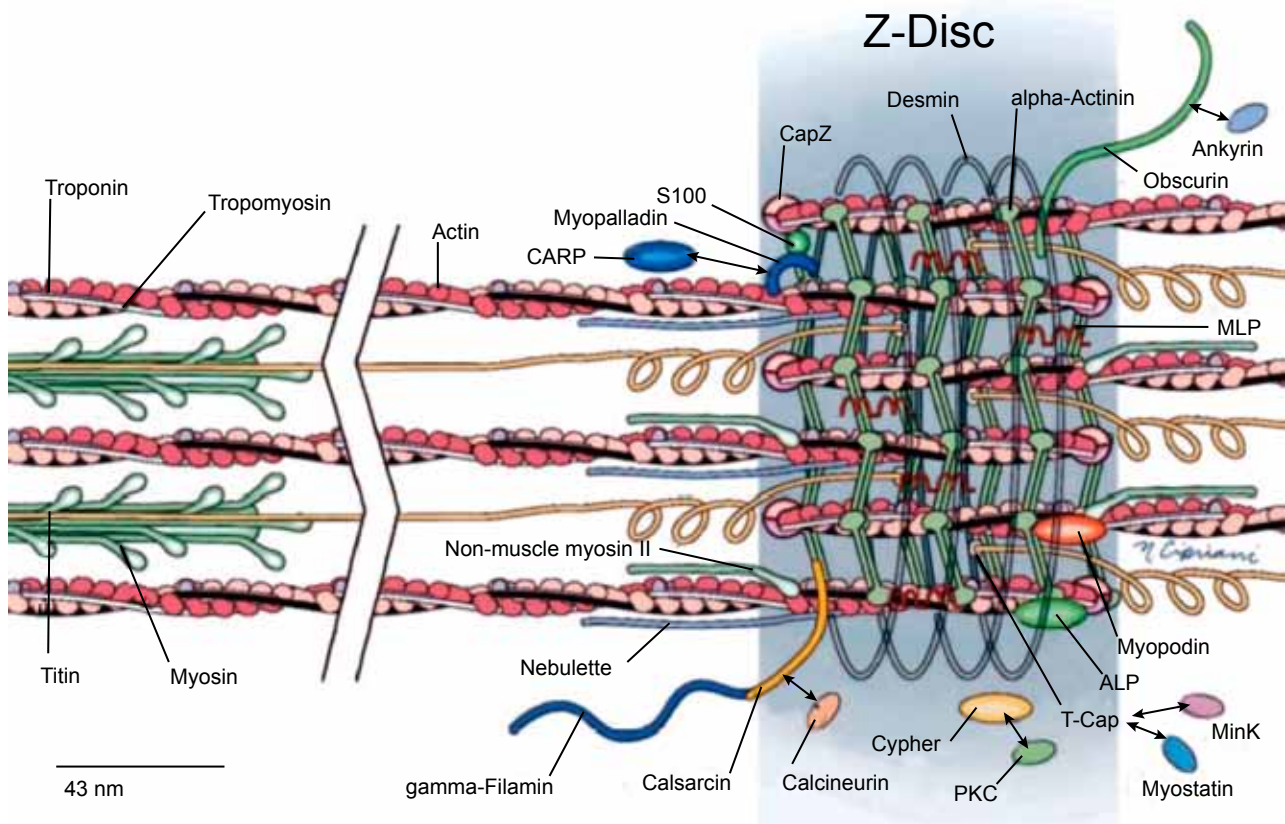


Figure 5. Schematic illustration of the Z-disc, emphasising the relationship between the proteins interacting in this area. (From reference 19. Reproduced with permission from Wolters Kluwer Health.)

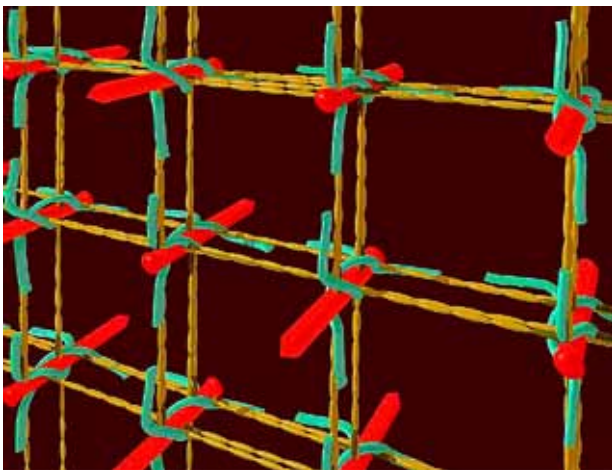


Figure 6. A three-dimensional model of the Z-disc illustrating the topology of α -actinin (yellow), F-actin (red) and the N-term of titin (green). (From reference 20. Reproduced with permission from John Wiley and Sons.)

kDa, is a recently discovered protein that presents similarities with titin, in that it has a flexible area. This area interacts with both the titin's N-terminal on

the Z-disc and C-terminal in M-line. Apart from its structural role, this protein has the capacity to participate in signal transduction pathways.^{25,47}

Myotilin (myofibrillar protein with titin-like Ig domains) is a protein in the Z-disc's periphery that has domains analogous to those of titin. It is connected to α -actinin and γ -filamin.^{9,32,45}

Filamins (proteins of connection with actin) belong to a family of bipartite proteins that cross actin filaments. Gamma-filamin (specific to striated muscle) is also identified in the disc's periphery. It is connected to α -actinin and myotilin, and after that it is bound on the cell membrane and the dystrophin-sarcoglycan complex, and more specifically on the γ - and δ -sarcoglycans (but not on the α - and β - sarcoglycans).^{29,45}

Everything stated above about the Z-disc concerns the arrangement and interaction of some very basic proteins of the disc. It seems, though, that the role of Z-disc is not only structural, aiming only at the connection of the sarcomere elements and the transmission of the energy produced. It is strongly believed that, through several proteins and mechanisms, it is

involved in a variety of functions regarding the cell, in general, and its homeostasis.

More and more data involve the Z-disc in signal transduction pathways, regulated by the changes in the tension received by the myocardial cell. These changes, in turn, result from the different haemodynamic requirements, whatever they may be. Starling's law is a globally accepted axiom of cardiovascular physiology. However, the molecular basis of Starling's law is largely unclear. The attractive theory that involves the Z-disc in the related cases has titin at its core. The idea is that a sidelong arrangement of titin's flexible part is capable of transmitting power with radial direction to the thick and thin filaments, modifying the space between them and increasing the chances that a following contraction will begin. The theory sets as prerequisite that titin is bound on the Z-disc.^{25,32}

An additional function of the Z-disc is that it serves as a centre of signal transduction, in which kinases, phosphatases and Ca^{++} also participate. It achieves that by providing binding sites for transcription factors, signal transduction proteins related to Ca^{++} , kinases and phosphatases, elements that affect the function and the expression of genes. Moreover, the Z-disc is a station for proteins that regulate the transcription and which move between the Z-disc and the cell nucleus. These proteins include the following: LIM proteins, namely the MLP (Muscle LIM Protein) and the ALP (actinin associated LIM Protein), myopalladin, myopodin, ZASP (also known as Cypher or Oracle), calsarcin, also known as FATZ or myozenin, CARP, and the calcium binding protein, S-100.^{25,45}

A- and C-protein kinases (PKA, PKC), PKD, PKG, Pak1 and Pho-dependent kinases are identified in the Z-disc. CapZ and ZASP in particular contribute to the binding of PKC on the disc.⁴⁶ Recent data have shown that these kinases participate in the phosphorylation/dephosphorylation of the cTnI and cTnT in the area near the Z-disc (I-Z-I area), affecting cardiac function.^{25,38,39,45,48,49} Calsarcin is connected to α -actinin and γ -filamin,^{25,38,50} and it is the binding site on the Z-disc for calsineurin, a phosphatase that dephosphorylates the NFAT nuclear factor (nuclear factor of activated T cells).⁴⁶ The dephosphorylated NFAT enters the nucleus and participates in the transcription. Myopodin (85 kDa) is one of the proteins that move from the Z-disc to the nucleus. There, it assists the binding of the extra-sarcomeric actin and, possibly, plays some role in the mRNA trans-

fer.²⁵ Myopalladin (145 kDa) is bound on nebulin and α -actinin and interacts with CARP.^{25,45} CARP is a nuclear protein that modifies the expression of several genes, some of them encoding C-troponin, the myosin light chain, and atrial natriuretic factor.²⁵ Finally, another function of the Z-disc is its participation in the mechanoelectrical feedback, with ultimate purpose to regulate the function of several ion channels. Telethonin, titin and the β -subunit (MinK) of the K^+ slow channels also participate.²⁵

Extra-sarcomeric cytoskeleton

The extra-sarcomeric cytoskeleton consists of *intermediate desmin filaments*, *actin micro-filaments* and *microtubules*.^{9,51} It connects the sarcomere with the sarcolemma and the extracellular matrix through the Z-disc and the sub-membrane cytoskeleton.^{29,52} In this way, the transmission of the power produced by the sarcomeres is ensured.⁹ It also provides support to the subcellular structures, organises the cytoplasm, regulates the topography of the cell membrane and, finally, transmits mechanical and chemical signals both inside the cell and between cells.^{9,51}

Intermediate desmin filaments

Desmin (molecular weight 53 kDa) is the main protein of the intermediate filaments and has a diameter of about 10-12 nm.^{23,35} Desmin is deemed to be necessary, apart from everything else, for the myocardial cell's structural integrity, the allocation and functionality of its mitochondria, the nucleus position and the sarcomeres' genesis.^{13,52} The mechanism through which the cytoskeleton and the desmin filaments affect the function of the mitochondria remains largely unknown.⁵² It is very possible that other proteins, such as paranemin, are necessary for the organisation of the desmin and the formation of the intermediate filament network.⁵³

The intermediate desmin filaments create a three-dimensional skeleton that covers the cytoplasm over its entire extent (Figure 7). They play an important role, not in the development of the myofibrils, but in their preservation.⁵⁴ They envelop Z-discs, extending from one Z-disc to another,^{9,23,25} and it seems that they are related to other cell organelles as well, such as the sarcoplasmic reticulum and the T-system. The desmin filaments extend from the Z-disc to the costameres, where they are bound through plectin and dysferlin (Figure 7).⁵⁵ They also extend to the

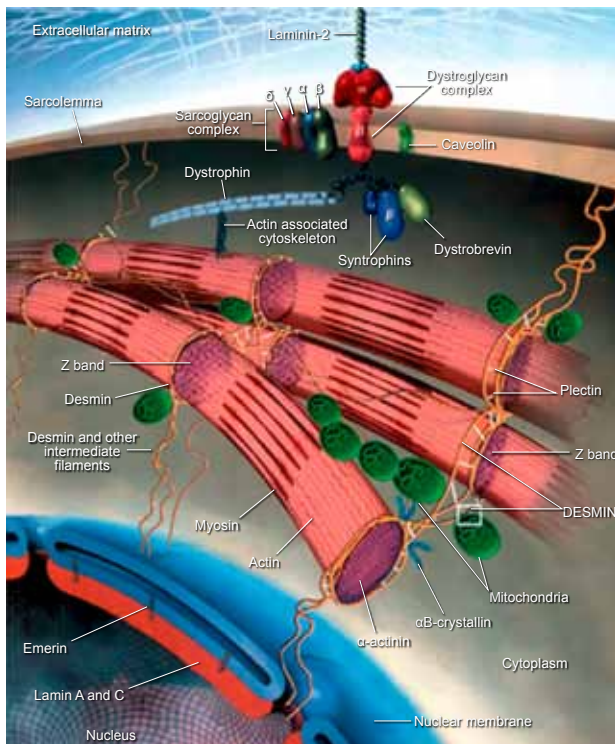


Figure 7. Another illustration that reveals the interconnections between the parts of the cytoskeleton, placing emphasis on the network of the intermediate desmin filaments that envelops the sarcomeres at the level of Z-disc, extending from the lateral sarcolemma to the nucleus. Desmin filaments also interact with intracellular organelles such as mitochondria. (From reference 13. Reproduced with permission from Elsevier.)

intercalated disc and they emerge from the Z-discs of the perinuclear myofibrils to the nuclear membrane.^{55,56} The angle of interaction with the nuclear membrane depends on the sarcomere's length.⁵⁷ A small number of desmin filaments seem to create an elongated network that envelops myofibrils like a cage.⁵² The desmin filaments are connected to the actin filaments and the microtubules through the plectin.⁵⁵ Based on the above observations, it has been proposed that desmin filaments unify the contraction movements, by connecting the individual myofibrils both among themselves and to the Z-discs' periphery and, following that, by connecting the Z-discs to the cell membrane, the nuclear membrane and the cell organelles.^{13,23,52} The external mechanical tension modifies the arrangement of the desmin network and as a result it affects the nuclear functions, including gene transcription.²⁵ However, there are some other intermediate protein filaments in the myocardial cells, such as nestin, peripherin and vimentin.⁵⁵

Actin filaments

The existence of actin microfilaments in the myocardial cell's skeleton has been questioned, given that the separation of this cytoskeleton element from the extremely dense network of sarcomeres has been difficult. However, studies have shown that microfilaments are also present in the myocardial cells. They consist of sarcomeric (β - and γ -) actin that, similarly to the intermediate desmin filaments, form a three-dimensional grid connecting the Z-disc (through α -actinin) with the several parts of the costameres and the intercalated disc (Figure 7). In this way, an additional connection is established between the intracellular and the extracellular environment.^{23,35} In contrast to other cells, the role of the non-sarcomeric actin in the organisation of the myocardial cell's cytoskeleton is not clear.^{9,29}

Several proteins are located on sites where the actin microfilaments are attached to the costameres' complexes. Examples of such proteins, which seem to regulate the function and organisation of the actin microfilaments, are α -actinin, the Rho GTPases, and muscle LIM protein.^{9,23,58,59} In addition, a new protein, migliffin, seems to interact with actin at the site where the latter is bound with the adhesion zones.⁶⁰

Microtubules

Microtubules (molecular weight 55 kDa) are tubular structures created by the polymerisation of α - and β -tubulin heterodimers. Their diameter is about 25 nm and they cross the cell mostly along its longitudinal axis.²³ They are very dynamic elements because of their capacity to de-polymerise and re-polymerise rapidly. In this way, they are capable of altering the cytoskeleton's flexibility and, consequently, the myocardial cell's contraction capacity. In the myocardial cell, only 30% of the total tubulin is polymerised in the form of microtubules, while the remaining 70% exists in the form of a non-polymerised protein in the cytoplasm.³⁵

With the electronic microscope the microtubules can be identified in the perinuclear area, where the contractile fibrils are absent. The microtubules' functional mission is multiple, but has not been completely clarified. They transmit chemical and mechanical stimuli within the cell and among cells.^{14,23,51} They contribute to the cell's stability by being connected to subcellular structures, such as the mitochondria, the Golgi apparatus and the myofibrils. They are also ca-

pable of altering the response of the myocardial cell to β -adrenergic agonists.^{23,51} The proteins connected to the microtubules (MAPs) are bound on α - and β -tubulin and play an important role in the stabilisation of microtubules, so that the latter can interact with the other cell organelles.^{14,23,29,35}

Membrane–submembrane cytoskeleton

The membrane–submembrane cytoskeleton consists of the *dystrophin–glycoprotein complex*, the *focal adhesion complex* and the *spectrin complex* (costamere) (Figures 2 & 3).^{9,13,14} These structures are deemed to belong to the cytoskeleton, but are usually referred to as a separate group of proteins, because they are located in the cell membrane and have a specific functional role. These proteins serve the connection of sarcomeres with the lateral sarcolemma area and the stabilisation of the T-system. They are also used as interveners in the connection between the intracellular environment and the extracellular matrix.¹⁴

Dystrophin–glycoprotein complex

The dystrophin-associated glycoprotein complex (DGC) (Figure 2) is a structure that has a key role both in the normal function of the cell and in pathological situations caused by mutation or absence of the dystrophin gene and/or the other proteins of the complex.^{23,61} No dystrophin has been observed in the intercalated disc.⁶¹

Dystrophin and the DGC complex consist of the cytoskeleton protein dystrophin, which is bound both on the actin filaments and on the DGC.^{4,23} Dystrophin is identified in ventricular, atrial and specialised Purkinje cells.²³ The associated DGC includes α - and β -dystroglycans, α -, β -, γ -, δ -, ϵ -, and ζ -sarcoglycans, α - and β -dystrobrevins, α - and β -syntrophins and caveolin-3.⁶² DGC has a cytoplasmic, a transmembrane and an extracellular part. The cytoplasmic part is connected to the dystrophin COOH-terminal while the extracellular part is connected to laminin-2.⁹

Dystroglycans are the core of the DGC and in fact they establish the transmembrane connection between laminin-2 and dystrophin. α -dystroglycan is connected to laminin-2 and β -dystroglycan is connected to dystrophin.^{4,62} Sarcoglycans are also transmembrane proteins. Sarcospan has four transmembrane areas. α -dystrobrevin-2 is connected to dystrophin but also to the sarcospan/glycoprotein complex.^{4,62,63} Syntrophins are proteins that attract complex pro-

teins having a signal transduction role, such as the Na^+ channels, NO synthetase, and caveolin-3.^{62,63} Syncoilin is a protein that interacts with desmin and provides connection between DGC and the intermediate filament network.^{62,63}

Taken together, the main role of DGC is to connect the actin cytoskeleton with the extracellular matrix (laminin-2), thus stabilising the sarcolemma during repeated cycles of contraction.^{4,62,64} In addition, it is the structure through which the power produced by the sarcomeres is transmitted to the extracellular matrix.^{31,43} There is also evidence that it is involved in the transmission of mechanical signals to the Z-disc.⁴

Focal adhesion complex

The focal adhesion complex is characterised by the presence of the transmembrane integrin proteins (α - and β - subunits) (Figure 2). Integrins serve the transduction of mechanical signals caused by biochemical change and play an important regulatory role as regards the elongation and the tension in the cell, since they represent connection sites between the cell and the extracellular matrix.^{17,30,46,65} Their intracellular part interacts with talin, tensin, vinculin, ankyrin, paxillin, zyxin, FAK (focal adhesion kinase), melusin,⁴⁶ and α -actinin proteins, and these, in turn, interact with the actin filaments that connect the focal adhesion complex with the Z-discs.^{46,65} In this way, changes in the formulation of integrins are transmitted to the Z-disc, α -actinin and sarcomeric actin.^{9,11,13,14,17,25,29} The extracellular part of integrins interacts with the collagens, lamin and fibronectin of the extracellular matrix.^{25,66}

More specifically, vinculin (M.W 116 kDa) connects the cell membrane to the actin filaments, through talin, paxillin and α -actinin. Contrary to what was believed in the past, vinculin is not identified in the Z-disc but in the T-system.¹⁴

Metavinculin, a larger vinculin isomorph protein, interacts with the thin filaments and is identified at the same sites as vinculin: in the intercalated discs, in the costameres of the lateral sarcolemma area and in the T-system.^{14,43,59}

Spectrin complex

This consists of a complex of spectrins, ankyrin and 4.1 proteins. These proteins cross each other, creating a submembrane scaffold that is connected to the actin and desmin filaments, as well as to the transmem-

brane proteins (Figure 3).^{9,17} This complex's role in the preservation of the cell morphology is known. The spectrin complex is connected to the Z-disc (through the actin and desmin filaments) and in this way the transmission of the power to the cell membrane is assisted. Recently it has been proved that complex constituents are identified, apart from the costameres, in the inner membranes of the cell, the intercalated disc, the M-line and the Z-disc.^{9,17,67}

Nuclear cytoskeleton

Finally, there is the skeleton, which is directly below the inner nuclear membrane where lamins play the central role. The nuclear cytoskeleton (nuclear lamina) (Figures 3 & 8) constitutes a dense network of filaments that supports the nuclear periphery through its interaction with both the nuclear envelope and the subjacent chromatin.^{68,69} Lamins belong to the family of intermediate filaments and are the main constituents of the nuclear cytoskeleton.⁷⁰

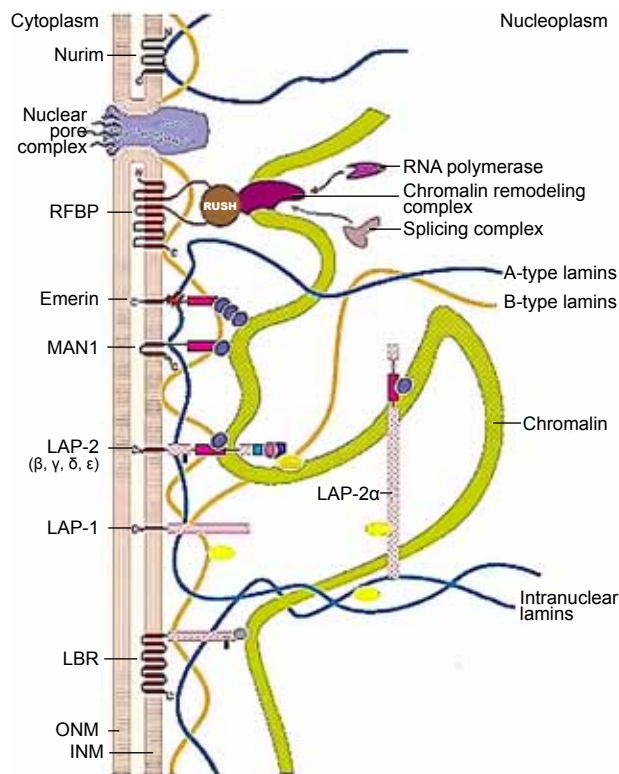


Figure 8. The nuclear cytoskeleton. Interactions with the nuclear membrane and chromatin. ONM – outer nuclear membrane; INM – inner nuclear membrane. (From reference 4. Reproduced with permission from Cambridge University Press.)

Nuclear lamin filaments exist inside the nucleoplasm as tubular structures and their identification depends on the phase of the cell cycle. The variable identification of lamins has led to hypotheses that involve them in several cellular functions, such as the growth of the nucleus and the preservation of its shape, DNA replication, regulation of transcription, chromatin organisation, nuclear pore stabilisation, RNA recombination, cell differentiation, apoptosis, and control of the nuclear architecture, depending on the phase of the cellular cycle. Lamin filaments consist of monomers that are polymerised. Lamins are divided into A/C- and B-types.⁶⁸⁻⁷⁰

There are four known A/C-type lamins and all come from a different recombination of the LMNA gene. The main A/C-type lamins are A-lamin and C-lamin. The other two are the C₂ and A-D10 lamins. In contrast, B-type lamins come from two genes, LMNB1 and LMNB2, which codify B1 and B2 lamins, respectively. An additional difference between the A/C-type and B-type lamins is that, in all cells, B-type lamins are constantly expressed during development, while the expression of A/C-type lamins is only observed in differentiated cells.^{68,69}

The proteins discovered in the inner nuclear membrane and related to the subjacent skeleton now total more than 67, but only 12 of them are regarded as basic. Seven among them belong in two groups, LAP-1 and LAP-2 (lamina associated proteins). LAP-1 proteins include the A, B and C isoforms, while LAP-2 include the β, γ, δ and ε isoforms.^{31,69} The other five proteins of the inner nuclear membrane do not belong in one of the above groups; they are: emerin, MAN1, nurim, LBR (lamin B receptor) and RFBP (ring-finger binding protein). Among these, nurim, LBR and RFBP cross the nuclear membrane several times, MAN1 crosses it twice, and emerin, LAP1 and LAP2 only once. Generally, it is considered that the main role of the inner nuclear membrane's proteins is to connect the nuclear membrane to chromatin and to the nuclear proteins, thus regulating the way chromatin is organised and the expression of genes.^{68,69} At the same time, this whole complex that exists below the inner nuclear membrane is connected to the T-system and the actin microfilaments of the extra-sarcomeric cytoskeleton.⁶⁹ However, there are data suggesting that emerin is also found in the intercalated discs.³¹

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