DOI 10.26724/2079-8334-2024-1-87-208-213
UDC 611.12+611.12.018.1

G.E. Zagoruyko, V.P. Martsynovskyi, V.L. Filatova[1], T.M. Matvienko[1], O.D. Sargosh[1], T.M. Gusakovska
Rivne State University of Humanities, Rivne[1]; [1]Poltava State Medical University, Poltava

CHANGES IN CARDIOMYOCYTE NUCLEI LOCATION AND CONFORMATION DURING POSTNATAL DEVELOPMENT OF WISTAR RATS

e-mail: prof.zagoruykoGE@gmail.com

The study examines the characteristics of changes in the location and causes of deformation of cardiomyocyte nuclei in the left ventricle of Wistar rats. The analysis was conducted on a series of electron microscopic images of rat myocardium from birth to 45 days of postnatal development. The study identified four zones of nucleus location in cardiomyocytes: central, marginal, under the sarcolemma, and in the sarcolemma protrusion into the interstitial space. The location of the nucleus was most frequently determined in the *marginal* zone, which is biologically appropriate as it reduces the distance for chemical transport from blood capillaries to the sarcoplasm and nucleus. Occasionally, under normal conditions of rat myocardial functioning, protrusion of the nucleus and sarcolemma into the interstitium is observed, which does not affect the contractile function of the cardiomyocyte. The nucleus located in the protrusion of the sarcolemma is inactive state. When metabolic processes intensify, the nucleus moves from the sarcolemmal protrusion into the "body" of the cardiomyocyte, where its functional properties become activated. During the 'contraction ↔ relaxation' process of cardiomyocytes, short-term impulse pressures from myofibrils and mitochondria cause deformation and reorganization of the 3D genome of the nucleus, which may lead to changes in gene expression.

Key words: cardiomyocyte, nucleus, conformation, localization, apoptosis.

Г.Є. Загоруйко, В.П. Марциновський, В.Л. Філатова, Т.М. Матвієнко, О. Д. Саргош, Т.М. Гусаковська

ЗМІНИ ЛОКАЦІЇ І КОНФОРМАЦІЇ ЯДЕР КАРДІОМІОЦИТІВ У ПРОЦЕСІ ПОСТНАТАЛЬНОГО РОЗВИТКУ ЩУРІВ ЛІНІЇ WISTAR

У роботі досліджено особливості зміни локації і причини деформації ядер кардіоміоцитів у лівому шлуночку щурів лінії Вістар. Проведено аналіз серії електронно-мікроскопічних зображень міокарда щурів від народження до 45-ти діб постнатального розвитку. У кардіоміоцитах виявлено чотири зони локації (розташування) ядра: центральна, крайова, під сарколемою, та у випинанні сарколеми в інтерстиційний простір. Найбільш часто визначалося крайове розташування ядра, що біологічно доцільно, оскільки суттєво зменшується відстань для транспорта хімічних речовин з кровоносних капілярів в саркоплазму та ядро. В нормальних умовах функціонування міокарда щурів, іноді виявляється протрузія (випинання) ядра разом з сарколемою в інтерстицій, яка не перешкоджає скорочувальної функції кардіоміоцита. Ядро в випинанні сарколеми знаходиться в неактивному стані. В умовах інтенсифікації метаболічних процесів, ядро з випинання сарколеми переміщується у «тіло» кардіоміоцита де відбувається активація його функціональних властивостей. В процесі «скорочення ↔ розслаблення» кардіоміоцитів утворюються короткочасні імпульсні тиски міофібрил і мітохондрій на ядро, під дією яких відбувається його деформація, реорганізація 3D-геному та, ймовірно, зміна експресії генів.

Ключові слова: кардіоміоцит, ядро, конформація, локалізація, апоптоз.

This study is a fragment of the research project "Educational and Scientific Innovations in the Field of Biology and Problems of Human Health", state registration No. 0122U000563.

In vertebrates' somatic cells, the nucleus is typically round and located in the central zone [4]. However, in cylindrical cells of the mammalian stomach and intestine, the nucleus is situated at the base, and in hepatocytes, it is in the central zone. Recent evidence suggests that the conformation, size, and location of the nucleus are dependent on metabolic and physiological processes in the cytoplasm of cells and may change with aging [12]. Additionally, it has been established that the size of cell nuclei undergoes cyclic fluctuations. The study determined the periodicity of fluctuations in cell nucleus size. [13]. In pathological conditions, the size and shape of cell nuclei may change. Currently, researchers are investigating the molecular mechanisms behind changes in the size and shape of nuclei in various cells [1].

In some mammalian and human cardiomyocytes (CMC), the location of the nucleus under the sarcolemma has been identified, and localized areas of sarcolemma protrusion with the nucleus (nucleus protrusion) into the interstitial space have been reported [8, 9]. *Abnormal* locations of the nucleus, not in the central zone of the CMC, are believed to be mainly characteristic of pathologically altered human myocardial muscle cells [8]. In the available professional literature there is no scientific explanation for changes in the location and conformation of CMC nuclei under conditions of age-related normality, pathology, and experiment.

The shape of the CMC nucleus in vertebrates and humans is described as oval, convex, elliptical, rod-shaped, and of complex configuration [3]. The location and conformation of nuclei change significantly during the cell cycle [12]. Changes in the size of cardiomyocyte nuclei occur during early postnatal development of rats [5]. Pathological conditions can cause morphofunctional changes in the nucleus, such as karyopyknosis, karyorexis, and karyolysis [9].

Morphometric studies approximate the shape of cell nuclei using geometric shapes such as spheres, ovals, ellipses, and cylinders [3]. The reasons for the formation of complex nucleus shapes in somatic cells are unknown. This raises the question of how changes in the location and conformation of the nucleus affect its functional state and the functional properties of the cell.

The purpose of the study was to identify the causes of changes in the location and conformation of left ventricular cardiomyocyte nuclei during the normal postnatal development of Wistar rats.

Materials and methods. This study analysed previously obtained images of the ultrastructure of the left ventricular myocardium of Wistar rats aged from birth (n/a) to 45 days of postnatal development [5]. In each of the 12 animal age groups, 50 myocardial images were analyzed, with a total area of 12500 μm^2. The rats used in the study were from the Institute of Biology nursery at the Faculty of Biology of Kharkiv National University (Kharkiv) and were kept in standard vivarium conditions. All manipulations with the rats were performed in compliance with the European Convention.

Results of the study and their discussion. The analysis of left ventricular myocardium images from rats of varying ages revealed four zones of nucleus localization in the sarcoplasm of CMC: central, marginal, under the sarcolemma, and in the sarcolemma protrusion into the interstitial space.

In the electron micrograph (Fig. 1A), the ellipsoidal nucleus is located in the central zone of the CMC. The nucleus has a slightly wavy nucleolemma, which forms numerous small invaginations towards the sarcoplasm.

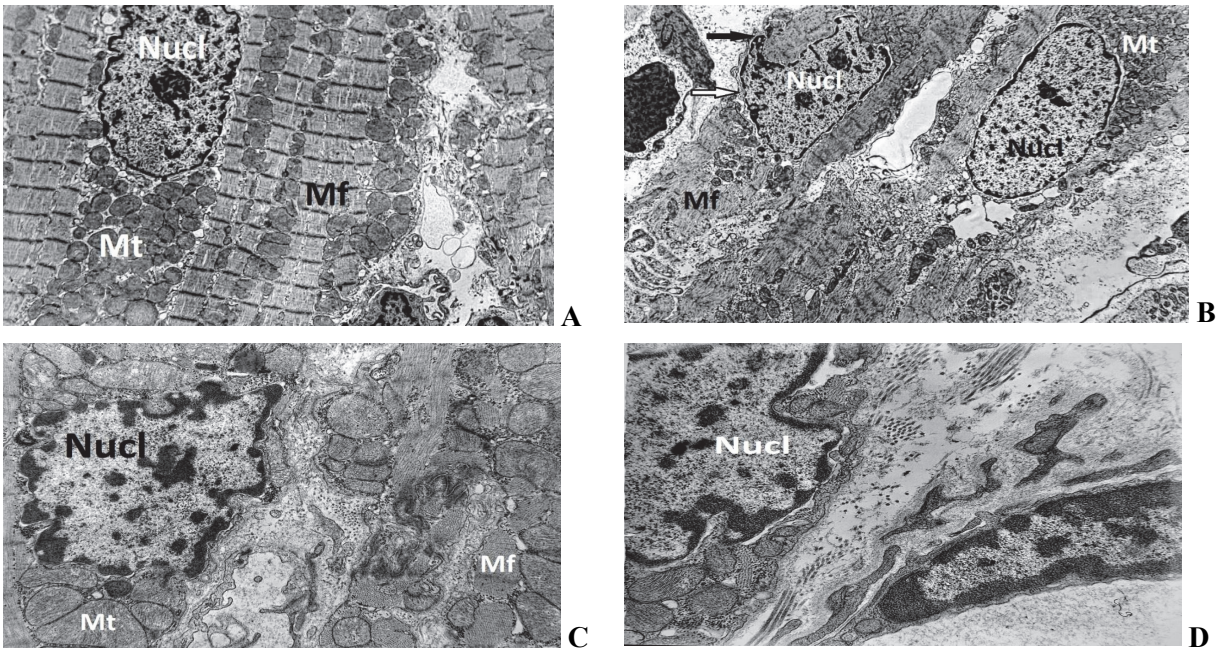

Fig. 1. Ultrastructure of juvenile rat myocardium. A – the nucleus is located in the central zone of the CMC; B – the marginal location of the nucleus and its protrusion. C – the deformed nucleus is located under the CMC sarcolemma. D – the malformed nucleus has a large protrusion in the form of a wide "mushroom leg". Designations: myofibrils (Mf), mitochondria (Mt), and nucleus (Nucl). Magnification — 7000 x.

Clusters of mitochondria, elements of the Golgi apparatus, and vesicles of the smooth endoplasmic reticulum (SER) are detected at both poles of the nucleus. The nucleolus is located in the central zone of the nucleus, and the nucleoplasm is filled with euchromatin elements. A thin strip of peripheral heterochromatin is localized along the inner surface of the karyolemma. Myofibrils are found along the outer surface of the nuclear envelope. In the myocardial parenchyma of newborn rats, numerous cardiomyocytes (CMC) are detected, in which the nucleus is located in the marginal zone (Fig. 1B).

Some large protrusions of deformed nuclei are localized directly under the sarcolemma. The marginal location of the protrusion of the deformed nucleus is characteristic of most myocardial muscle cells of juvenile rats. In Fig. 1B, on the left, there is a CMC with ultrastructural manifestations of the initial

stage of physiological apoptosis. The deformation of the CMC is indicated by their long "splitting" along the lateral surface of the nucleus **(⇨)**, and the formation of apoptotic bodies due to fragmentation of the peripheral zone of the CMC. The deformed nucleus forms deep and large protrusions into the sarcoplasm. On the left, the distorted nucleus protrusion is in direct contact with the inner surface of the CMC sarcolemma (□). On the right, there is a CMC with morphological manifestations of physiological apoptosis. Its nucleus has a regular elliptical shape. However, at the lower pole of the nucleus, the sarcoplasm contains a cluster of small, light vesicles and optically dense apoptotic bodies caused by mitochondrial destruction. A large bright zone of sarcoplasm is detected at the site of the destroyed organelles. To the right of the nucleus, numerous small vesicles are detected, likely formed from sarcolemma fragments. Cellular detritus, which consists of decay products from organelles, moves into the interstitial space through the damaged areas of the CMC sarcolemma.

Figure 1C shows the deformed CMC nucleus of a 15-day-old rat located under the sarcolemma with numerous invaginations towards the sarcoplasm. The nucleolemma of the nucleus is in contact with the sarcolemma on the right, and mitochondria are identified around the nucleus. The nuclear pores are outlined in the nuclear envelope. Morphological manifestations of hyperchromatosis and fragmentation of heterochromatin, located along the inner surface of the nuclear envelope, are detected. Electron-dense fragments of heterochromatin, which have a rounded shape, appear in the caryoplasm. The location of these 'nuclear bodies' suggests that they gradually move towards the nuclear envelope in contact with the sarcolemma. The large nucleolus has a complex shape. The area of the sarcolemma in the zone of its contact with the nuclear envelope lacks a basement membrane. Vesicles are found near the sarcolemma on the interstitium side. Some vesicles are in contact with the basal surface of the capillary endothelial cell. The intercellular fluid comes into contact with the nuclear envelope through the fragmented sarcolemma.

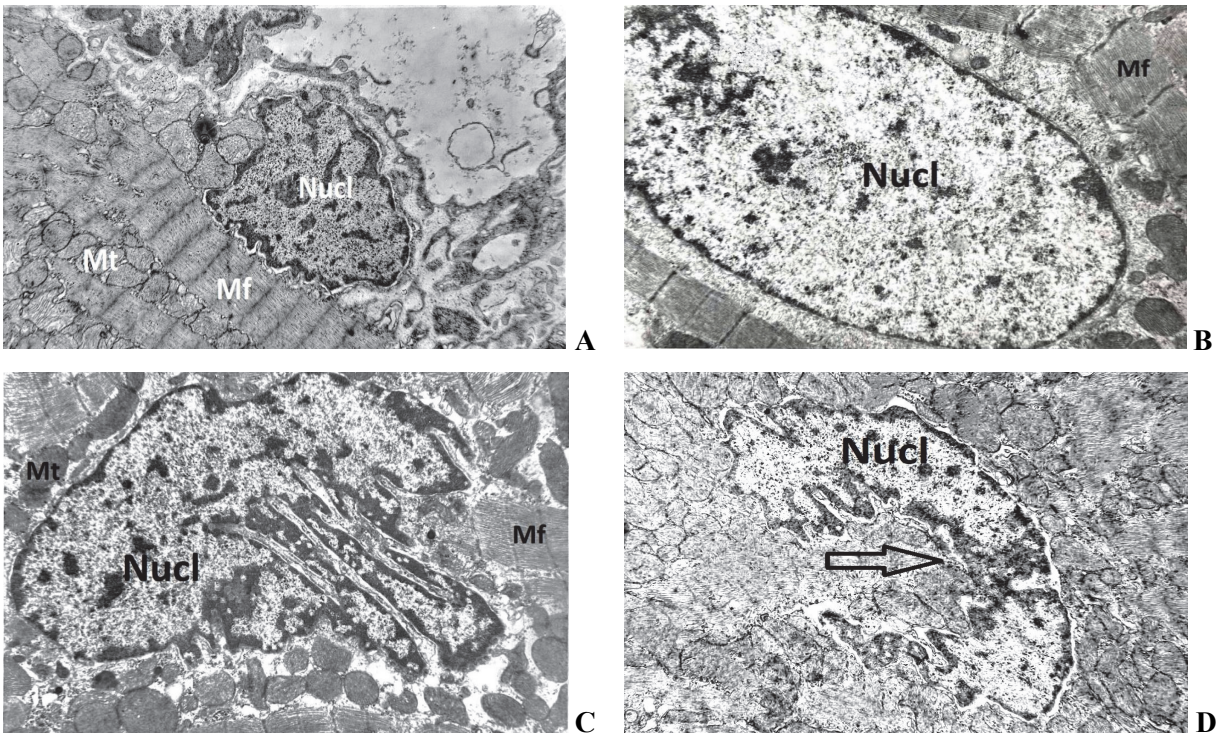

Fig. 2. Ultrastructure of juvenile rat myocardium. A – location of the nucleus in the deep protrusion of the CMC sarcolemma. B – CMC nucleus of elliptic shape; C – CMC nucleus of complex shape with extended invaginations into sarcoplasm; D – CMC nucleus of "convex-concave" shape with invaginations into sarcoplasm; ⇨ direction of mechanical action on the CMC nucleus. Designation: myofibrils (Mf), mitochondria (Mt), and nucleus (Nucl). Magnification — 7000 x.

Figure 1D shows the deformed CMC nucleus of a 45-day-old rat with a large protrusion resembling a wide "mushroom leg" located directly under the sarcolemma. "Nuclear bodies" are found inside the protrusion. A thin layer of sarcoplasm, containing the elements of the SER, is present between the nucleus protrusion and the sarcolemma. The nucleoplasm of the nucleus and its protrusion contain a large number of microfragments of chromatin. An expanded perinuclear space is formed in the upper part of the nucleus. The protrusion of the nucleus leads to a disordered arrangement of collagen fibers in the interstitium. There

is a loosening of collagen fibril bundles and a disturbance in the relative orientation of individual fibers. Thin, tortuous microfibrils appear in the interstitium. The protrusion of the CMC nucleus affects the intercellular substance and leads to a shift of fibroblast processes and amorphous interstitial matrix toward the basal surface of the capillary endothelial cell. In the myocardial parenchyma of 15- and 45-day-old rats, we detected single CMC with nuclei located in the middle of deep sarcolemmal protrusions into the intercellular space (Fig. 2A).

The coiled nuclear envelope is in contact with the deep protrusion of the sarcolemma. The opposite part of the sarcolemma is in contact with the myofibril, which separates the nucleus from the CMC body. The ultrastructure of the myofibril indicates that protrusion of the CMC nucleus does not interfere with the cardiac myocyte's contractile function, as evidenced by the same size of sarcomeres in the myofibril. The quantity of heterochromatin along the inner surface of the nuclear envelope decreases in the CMC nucleus. In the central zone of the nucleus, there are distinct extended clusters of heterochromatin directed towards the nuclear envelope. The density of the nucleolus is significantly reduced. The protrusion of the nucleus and sarcolemma into the intercellular space results in a bend in the peripheral zone of the endothelial cell at the nearest capillary. In a series of electron micrographs of rat myocardium of different ages, we observed varying shapes of sections of CMC nuclei. Depending on the complexity of the shape of the nuclei sections, they can be placed between two polar images: an ellipse and a complex shape. The contours of nuclei sections in the form of an ellipse or an oval are formed in the absence of pressure on the nucleus from the side of myofibrils and mitochondria (see Fig. 2B). Under these conditions, a layer of sarcoplasm often separates myofibrils and mitochondria from the nucleus, creating a space between the elliptical nucleus and the CMC organelles. The elliptical shape of the nucleus is observed in the sarcoplasm of relaxed CMC.

Figure 2C displays the complex cross-sectional shape of the CMC nucleus. The upper part of the nuclear envelope appears smoothed, while a thin strip of marginal heterochromatin is located along the inner surface of the caryolemma, which contains single pores. The lower part of the caryolemma forms extended invaginations of various sizes and shapes into the sarcoplasm. The surface of the caryolemma invaginations clearly outlines nuclear pores. The density of nuclear pores in different areas of nuclear envelope invaginations varies significantly. An increase in the number of nuclear pores is observed in the central areas of cariolemma invaginations and in the zone of wide protrusion of the nucleus. Figure 2D shows an electron micrograph of CMC in which the deformed nucleus has a 'convex-concave' shape. This shape is formed when the components of the myofibrillar and mitochondrial apparatus of a muscle cell in a state of contraction exert directed mechanical action on the nucleus.

The obtained data raise several questions regarding the deformation of cardiomyocyte nuclei, including: the causes of such deformation? the biological significance of this phenomenon? the duration of nucleus protrusion and nuclear envelope invaginations? and the causes of changes in the location of the nucleus in the sarcoplasm of CMC? Additionally, the biological significance of nucleus protrusion in native CMCs is also a topic of interest. The following questions will aid in the objective description of morphological changes in the functional state of CMCs, their nuclei, and organelles under normal, experimental, and pathological conditions.

Causes of cardiomyocyte nuclei deformation. Unlike stationary somatic cells (e.g. hepatocytes), CMCs are in a state of active dynamic mobility throughout vertebrate life. During the continuous cycles of “contraction ↔ relaxation” of CMCs (300–500 cycles/min in rat myocardium), the spiral orientation of myofibrils results in intricate spatial movements of the muscle layers of the ventricular myocardium [11]. Short-term contacts and impulsive mechanical pressures on the nucleus during cardiac contractions from the side of myofibrils and mitochondria constitute up to 80 % of the total relative volume in mature CMC [5]. The literature provides limited information on the contact interactions between the nucleus and surrounding myofibrils and mitochondria. During contact interactions of the “nucleus + organelles” type, the nucleus and nuclear envelope undergo deformation. This suggests that the CMC nucleus is mechanosensitive to the physical pressure of surrounding organelles and adjusts its conformation accordingly. Myocardial electron micrographs reveal varying cross-sectional contours of CMC nuclei at the moment of cardiac arrest. The contractile function of CMC ceases rapidly at different phases of the cardiac cycle, resulting in the appearance of deformed nuclei with various shapes in the electron micrographs of the CMC. After cardiac extirpation, most of the CMC in the left ventricular myocardial parenchyma of Wistar rats have deformed nuclei of varying shapes.

Biological significance of deformation in cardiomyocyte nuclei. Short-term cycles of "contraction ↔ relaxation" cause changes in the conformation of the nucleus and mechanically stretch the nuclear envelope. The inner nucleolemma is physically connected to the nuclear lamina, which is the site of attachment of interphase chromosomes. During the interphase stage of somatic cells, each decondensed chromosome is "anchored" to the nuclear lamina by local heterochromatin sites, which are attached to the inner nuclear membrane [1]. When there is no mechanical pressure on the nucleus, the decondensed chromosomes maintain a fixed position within the nucleus and form a 3D spatial organization of the genome. Mechanical deformation of the nucleus causes spatial reorientation of decondensed chromosomes, leading to the reorganization of the 3D genome and affecting gene expression [7, 10]. Changes in the spatial shape of decondensed chromosomes are responsible for the development of gene expression, which does not require biochemical signalling and occurs much faster (≈1 ms vs. 5–10 s) [14]. Deformations of the nucleus lead to gene expression and transcription of different types of RNA, which are then exported through nucleopores to the sarcoplasm where the biosynthesis of various structural components of the cell takes place [9].

Duration of nuclei protrusion and invaginations of the nuclear envelope. In the series of electron micrographs studied, protrusions of the nucleus and elongated invaginations of the nuclear envelope are rarely detected. This suggests that the formation of nucleolemma protrusions and invaginations of various sizes and shapes in CMC is a transient phenomenon. The formation of nuclear envelope invaginations is necessary to increase the nucleolemic area and the number of nucleopores in the short term, without significantly changing the nucleus volume. The prolonged protrusion of the nucleus and invasion of the caryolemma induce gene expression and enable the "import-export" of substances into specific cellular compartments.

Causes of changes in the location of the nucleus in the sarcoplasm of CMC. The electron micrographs indicate that the optimal marginal location of the nucleus in the sarcoplasm of the CMC is found in the myocardial parenchyma of rats of different ages, from a micrologistics perspective. The marginal placement of the nucleus is biologically feasible due to two reasons. Firstly, it reduces the distance for the transport of chemicals from blood capillaries to the sarcoplasm and the nucleus of the CMC. Secondly, the marginal placement of the nucleus does not interfere with the contractile function of CMC myofibrils. Thus, for each CMC, changes in the nucleus location are necessary to ensure optimal logistics and coordination of the transport, information, synthesis, and energy systems, as well as the efficient functioning of the contractile apparatus of the heart muscle.

The biological significance of nucleus protrusion in native CMC. Nucleus protrusion and extrusion into the interstitium are believed to be successive stages of the morphological process of apoptotic death of CMC. However, under normal conditions of myocardial functioning, nucleus protrusion may indicate a special state of CMC. In some cases, the information activity of one nucleus is sufficient for the function of binuclear CMC. Currently, the second nucleus is located in the sarcolemma protrusion and is inactive, not affecting the contractile function of CMC. Upon activation of the cardiovascular system, the nucleus moves from the sarcolemma protrusion to the "body" of the CMC. The second nucleus undergoes deformation under the influence of mechanical impulses from myofibrils and mitochondria, activating its chromosomal apparatus. The export of information molecules from the first and second nuclei to the sarcoplasm increases. This leads to the biosynthesis of ultrastructures and affects the energy metabolism of CMCs [3]. This phenomenon is observed in skeletal muscles during extended physical activity on the musculoskeletal system. In skeletal muscles, myosatellite cells are activated and become embedded in muscle fibres, increasing the number of nuclei [6].

Conclusions

1. Four zones of nucleus location were found in CMC: central, marginal, under the sarcolemma, and in the middle of the local sarcolemmal protrusion at the interstitium.

2. Under age-related conditions, in the left ventricular myocardial parenchyma of Wistar rats, most CMC contain deformed nuclei of different conformation.

3. During myocardial contraction, contact interactions "nucleus + organelles" occur in the sarcoplasm of the CMC. Mechanical pressures on the nucleus by myofibrils and mitochondria are formed, which leads to deformation of muscle cell nuclei.

4. The marginal placement of the nucleus in the CMC is biologically appropriate because it significantly reduces the distance for the transport of chemicals from the blood capillaries to the sarcoplasm and CMC nucleus and does not interfere with myofibril function.

5. Normally, the location of one of the CMC nuclei in a localized sarcolemmal protrusion provides it with relative functional rest. Under these conditions, the information load on CMC biological systems is reduced.

6. In mammalian and human myocardial histopreparations, the appearance of a freely located nucleus outside the CMC is not always due to its extrusion. If the cut passes through the nucleus protrusion and sarcolemma but does not "engage" the CSC body, a false impression of nuclear extrusion into the extracellular space is created.

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Стаття надійшла 20.03.2023 р.