# NUCLEAR SHAPE IN MUSCLE CELLS

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## INTRODUCTION

Every student of muscle ultrastructure is familiar with the frequently convoluted appearance of the muscle cell nucleus (e.g. Figs. 1–3). It is a reasonable assumption that this particular shape of the nucleus can be correlated with the contracted state of the muscle fiber. Lane (12) was able to show that in contracting intestinal smooth muscle cells the nucleus undergoes a change from an unfolded, elongated state to an ovoid and invaginated one (compare also references 9 and 15). Although observations on smooth muscle cells cannot be easily extended to striated ones, this established correlation of nuclear shape and state of contraction should be kept in mind in this connection.

In order to elucidate whether the infoldings of the nuclear membrane in muscle cells are merely a result of extranuclear forces exerted by the contractile elements or whether they are stabilized by structural modifications within the nucleus itself, an investigation of muscle nuclei isolated by different methods was undertaken.

The present article provides evidence that the invaginations of skeletal muscle nuclei are maintained in the isolated state even for several days.

## MATERIALS AND METHODS

Small pieces of the femoral musculature of fasted albino rats were fixed simultaneously at 0°C either in a freshly prepared mixture consisting of 2% glutaraldehyde and $OsO_4$, buffered with 0.05 M cacodylate to pH 7.0, for 1 hr, or with 1% glutaraldehyde at the same temperature for 30 min. After several washings with the same buffer, the materials from both fixations were postosmicated for 2 hr in the cold with cacodylate-buffered 2% $OsO_4$. After washing and dehydration through an ethanol series, the material was embedded in Araldite and sectioned on a Reichert Ultramicrotom OmU2 (Reichert, Vienna, Austria). The sections were double stained with uranyl acetate and lead citrate and observed with a Siemens Elmiskop IA.

Nuclei were isolated from the same material by using either the procedure described by Edelman et al. (5), who employed sucrose media (0.32 M and 2.15 M) with the addition of 1 mM $MgCl_2$, buffered with phosphate to pH 6.7–6.8, or by using a modification of the method which was introduced by Kuehl (11). This method is routinely used in our laboratory for isolating nuclei from plant and animal material (7, 8, 20, 21). The muscle pieces were incubated for 6 hr in a medium consisting of 0.4 M sucrose, 4% gum arabic, 4 mM *n*-octanol and 10 mM Tris-buffer, adjusted to pH 7.0 (medium A). Homogenization was performed with a high-speed rotating knive homogenizer (Fa. Bühler, Tübingen, Germany) at 25,000 rpm for 7 sec. The homogenate was filtered through nylon and flannel cloth and centrifuged for 12 min at 1,000 *g*. The supernatant then was discarded and the pellet was resuspended in a medium of the same composition but with a higher sucrose concentration (2.2 M). This suspension was centrifuged in swinging buckets (WKF Ultracentrifuge, Brandau, Germany) for 2 hr at 55,000 *g*. The pellet was resuspended in medium A, centrifuged at 1,000 *g*, and further purified by ultracentrifugation at a density boundary at 70,000 *g* according to the procedure of Birnstiel et al. (3). The nuclei were collected from the density boundary by conventional methods and suspended in medium A. All steps were carried out in the cold. The nuclear fractions obtained were observed with phase contrast microscopy or prepared for electron microscopy by using the methods described above. In some cases,

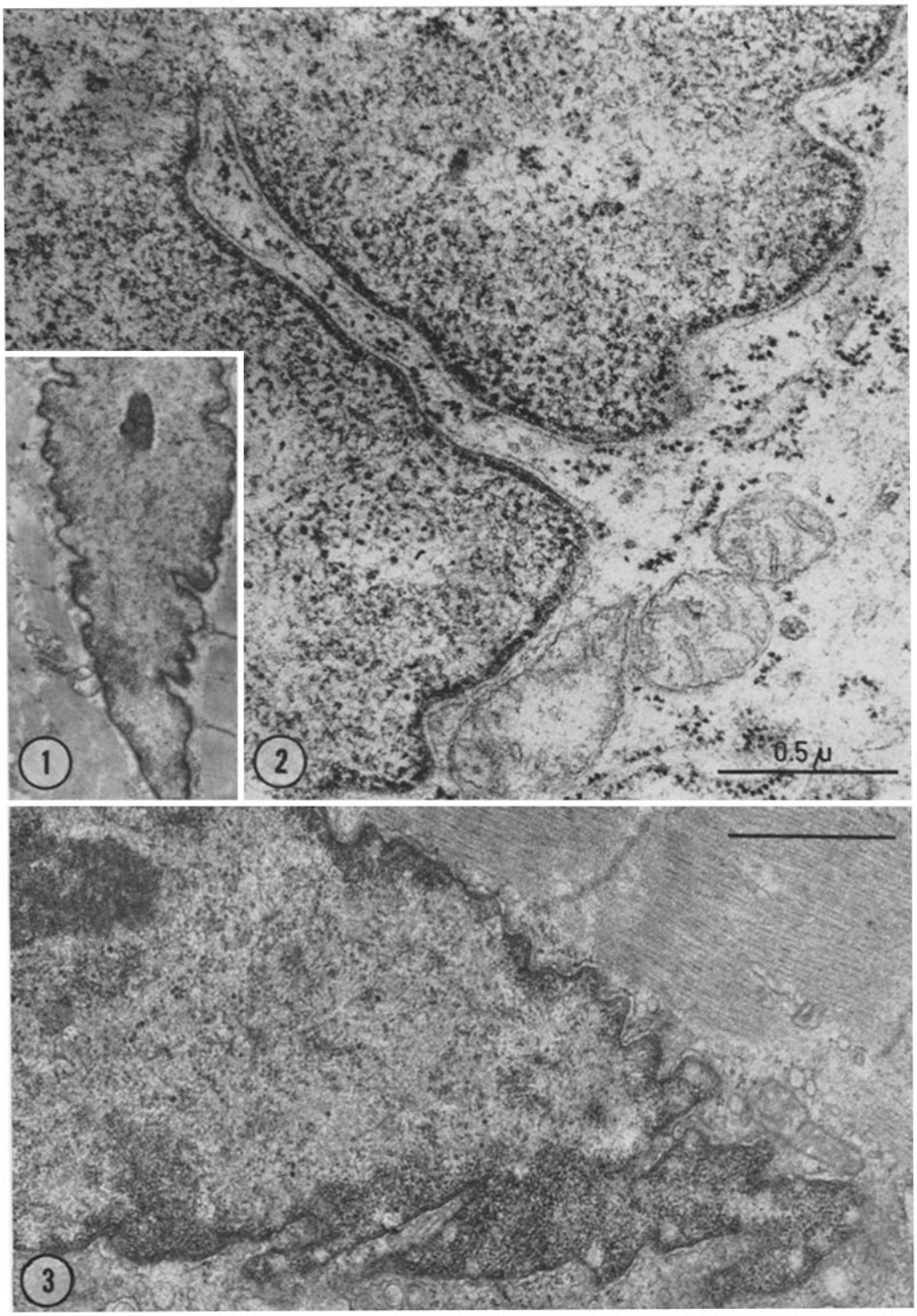


FIGURE 1 Typical rat skeletal muscle nucleus showing the greatly invaginated shape. × 8,000.

FIGURE 2 A nuclear invagination at higher magnification. The inner membrane is lined by the layer of peripheral chromatin that can be revealed as consisting of distinct globules. × 54,000.

FIGURE 3 Part of another invaginated muscle nucleus in which a different aspect of chromatin distribution is present. The chromatin material is not limited to a narrow layer but is still more concentrated in the peripheral part of the nucleus. Scale indicates 1 μ. × 25,000.

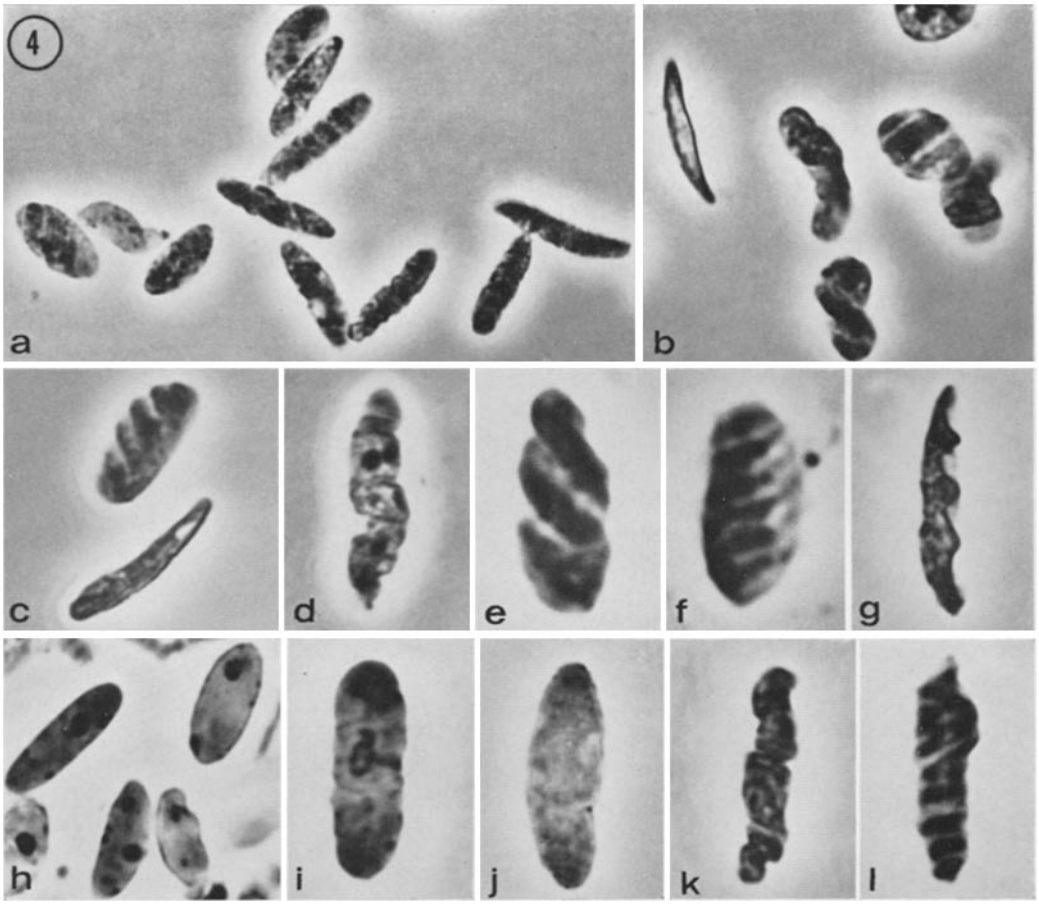


FIGURE 4 Phase contrast micrographs of nuclei isolated from muscles of the rat femur (*a–j*), of the rat heart (*k*) and of the pigeon breast (*l*). While muscle nuclei isolated by the method of Edelman et al. appear swollen and smoothly rounded in shape (*h*), most of the nuclei isolated by the procedure described in this article are plate-like and show invaginations which frequently can be revealed as a more or less regular coiled furrow (*a–g*). The invaginated nuclear shape disappears partially when the nuclei are incubated in water (*i*) and totally when they are incubated in 1 mM EDTA (*j*). *a*, *b*, × 1,000; *c*, × 1,200; *d*, × 900; *e*, *f*, × 2,200; *g*, × 1,500; *h*, × 800; *i*, *j*, × 1,300; *k*, × 1,500; *l*, × 2,000.

the isolated nuclei were suspended in twice-distilled water or EDTA and the swelling process was observed. Similar isolations of muscle nuclei were performed from rat heart musculature and from pigeon breast muscles.

## RESULTS AND DISCUSSION

When isolated after the procedures described above, the nuclei surprisingly maintain the characteristic invaginated shape (Figs. 4 *a–g*), in contrast to the nuclei isolated by the method of Edelman et al. which appear swollen and rounded (Fig. 4 *h*; see also Figs. 2 and 3 in reference 15). The same swollen appearance can be seen in the illustrations of nuclei isolated from rat heart and uterus as published by Widnell et al. (19). More than 90% of our nuclear fraction, which usually contained also a small quantity of nuclei from non-muscle cells like fibroblasts, endothelial and blood cells, reveal this "contracted" appearance. The muscle nuclei possess one or two nucleoli and exhibit generally a more or less elongated plate-like shape, with lengths in the range of 11–18 μ, widths of 4–7 μ, and a depth of about 1 μ. This configuration is most likely a consequence of the

peripheral location of the nucleus within the muscle fiber. Edge-on views present the isolated nuclei as somewhat comma-shaped and do not reveal indentations (Figs. 4 *b*, *c*, *g*), while the other views show the invaginations as a furrow, sometimes as deep as half the nuclear width. These invagination indentures often can be observed to be distributed as coils around the nucleus (Figs. 4 *b–f*). Sometimes four or more turns of such a coiled invagination can be discerned (e.g. Fig. 4 *f*). The gyres of the coil frequently are regularly spaced and have a rather constant angle with respect to the long axis of the nucleus (e.g. Figs. 4 *c*, *f*, *l*). Such nuclear invaginations or coiled furrows are not only limited to rat femoral musculature, but can also be observed in nuclei isolated from rat heart (Fig. 4 *k*) and from pigeon breast (Fig. 4 *l*) as well. The nuclei maintain this invaginated shape for several weeks when kept in cold isolation medium. They lose this appearance to a certain degree when incubated in distilled water (Fig. 4 *i*). There occurs a total disappearance of the invaginations, concomitant with swelling, when the nuclei are incubated in a 1 mM solution of EDTA (Fig. 4 *j*). A survey electron micrograph of the isolated nuclei with preserved invaginations is presented in Fig. 5. For comparison, a nucleus isolated by the method of Edelman et al. is also shown (Fig. 6). Nuclei isolated by the latter procedure reveal a somewhat extracted nucleoplasm as can be seen in the micrographs presented in the original article by those authors (5).

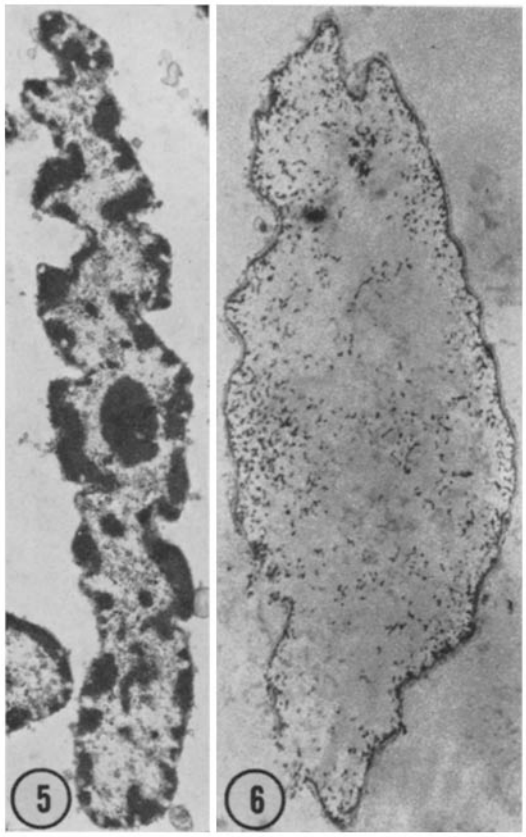


FIGURE 5 Typical electron microscopic appearance of a muscle cell nucleus isolated by the method described in this article. Nucleoplasmic components and the invaginations are well preserved in the isolated state. × 10,000.

FIGURE 6 Typical electron microscopic appearance of a nucleus isolated by the procedure of Edelman et al. The nucleus seems to be somewhat extracted and no remarkable indentations are present. × 12,000.

Particular attention was directed toward which structural component of the nucleus might be responsible for the maintenance of the contracted shape. Our first thoughts that the integrity of both nuclear membranes, or at least of the inner one, were necessary for this structural preservation could be ruled out, since in nuclei which reveal the invaginations described the outer or even both of the nuclear membranes not infrequently have disappeared (Fig. 8). After isolations by the method of Edelman et al., such nuclear indentations are minimal, but the nuclear envelope, on the other hand, can be widely preserved (Figs. 6, 7). That the preservation of the nuclear membranes is not a prerequisite for the preservation of a great many internal structures of the muscle cell nucleus can also be concluded from the remarks made by Nayler and Merrillees (14) who investigated the structural alterations of the muscle fiber during glycerol extraction.

A second possibility has been suggested that the electron-opaque, peripheral layer frequently seen in nuclei fixed *in situ* (Fig. 1, compare e.g. reference 18) stabilizes the particular nuclear structure. Such layers of electron-opaque material which underlie the inner nuclear membrane and which either resemble chromatin or stain differently, as the latter is the case in the so-called fibrous lamina (e.g. references 6, 16), occur in many kinds of cells. They have been repeatedly discussed as being possibly concerned with a nucleo-

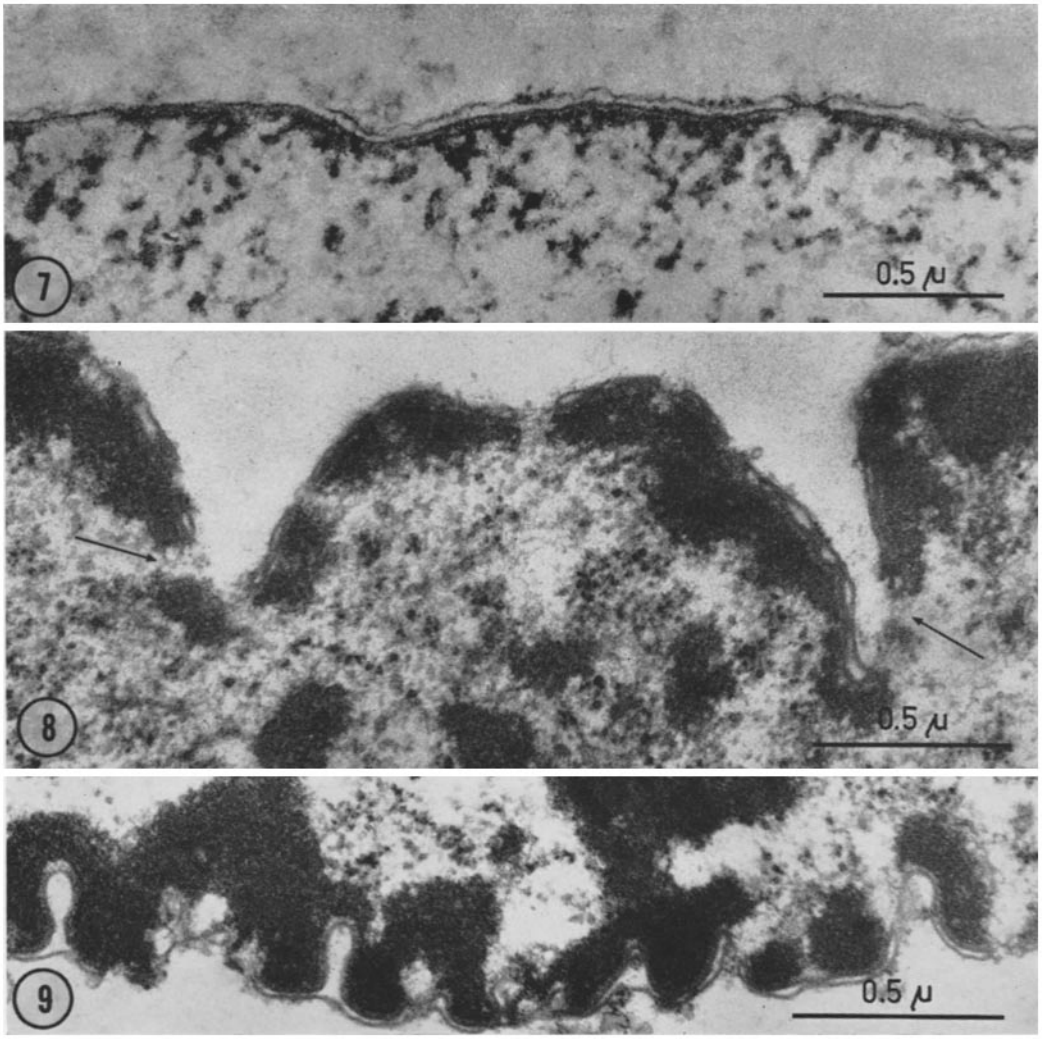


FIGURE 7 Peripheral part of a muscle nucleus isolated by the procedure of Edelman et al. Nuclear membranes are widely preserved as well as the peripheral chromatin layer at the inner nuclear membrane. × 52,000.

FIGURE 8 Peripheral part of a muscle nucleus isolated by the method described in this article. Invaginations are present, but at several sites the nuclear membranes have disappeared during the isolation (arrows). Nucleoplasm, particularly the peripheral chromatin, is fairly well preserved. × 52,000.

FIGURE 9 Nucleus from the same isolation as that shown in Fig. 8. The inner nuclear membrane is frequently indented while the outer one is not. × 47,000.

skeletal function (e.g. references 6, 10, 13). Our result of the comparison of the nuclei having preserved invaginations and nuclei which were isolated by a method that does not preserve them, does not support this speculation. Even if a remarkable amount of the nuclear material, including parts of the chromatin, has obviously been extracted from muscle nuclei isolated by the procedure of Edelman et al., the peripheral supporting material is still well preserved (Fig. 7). In nuclei isolated by the modified Kuehl method, this peripheral layer cannot be further distin-

guished from the bulk of the chromatin (Figs. 5, 8). Thus, it can not be the peripheral material which is necessary for preserving the contracted shape of the muscle nuclei. From the micrographs obtained, one has the impression that the presence of the bulk of the chromatin, probably somewhat stabilized and condensed by a sort of clumping induced by the decreased ionic strength of the media used, is a prerequisite for the observed stability of the invaginations characteristic for the contracted state of the muscle cell nucleus. The pattern of the chromatin distribution found in our isolated nuclei resembles that of certain muscle nuclei as can be seen in muscle fibers fixed in the tissue (Fig. 3). Such muscle nuclei do not reveal the sharply limited peripheral layer. The finding that preservation of the main part of the chromatin framework is necessary for the preservation of this particular invaginated nuclear shape leads one to the suggestion that, possibly in correlation with the muscle contraction, changes in the state of the chromatin occur which can be kept stable under proper conditions. Frequently, it can be encountered that only the inner membrane of the nuclear envelope is indented, while the outer one is not (Fig. 9). This phenomenon might be tentatively explained by a relatively tight connection of the inner membrane to the framework of the peripheral layer. In our opinion, it is hard to imagine how the nuclear shape could follow directly the action of the contracting myofilaments. It is not likely that the changes in nuclear shape correlated with contraction, as reported for smooth muscle cells, occur also in skeletal muscles since deeply indented nuclear outlines can also be observed in denervated rat leg muscles (17). On the other hand, it is well-known that changes in the ionic environment, especially of bivalent cations, can cause changes of nuclear shape (4) as well as of the appearance of the chromatin (e.g. references 1, 2). Thus, one should preferentially consider the possibility that the invaginated shape of the muscle cell nuclei might be induced by intracellular ion changes rather than by the mechanism of the muscle contraction itself.

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