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Research Articles

Structural characteristics and distribution of satellite cells along crayfish muscle fibers

M. Novotová and B. Uhrík

Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Vlárska 5, 833 34 Bratislava (Czechoslovakia)
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Abstract. The distribution of satellite cells (sc) in long-sarcomere muscle fibers from the carpopod extensor muscle of the crayfish (*Astacus fluviatilis*) has been studied electron-microscopically. The sc are spindle-shaped and are oriented parallel to the long axis of a fiber. The mean lengths of sc nuclei (17.00 μm) and that of myonuclei (18.35 μm) differ non-significantly. In older animals, the mean ratio of the number of sc nuclei to the total number of nuclei (sc nuclei + myonuclei) is 0.0716, 0.0848, and 0.034 for the tendon, central and shell segments, respectively. The corresponding values for younger animals are 0.158, 0.166, and 0.081. The mean numbers of sc nuclei per mm of a fiber are 94, 117, and 47 (older animals), and 164, 117, and 94 (younger animals) for the tendon, central and shell segments, respectively. The high incidence of sc per unit fiber length in crayfish may be related to the fact that crayfish muscle fibers have a much larger diameter than vertebrate muscle cells.
Key words. Crayfish muscle; satellite cells.

One of the most important problems in the study of muscle regeneration concerns the origin of myogenic cells. The results obtained from vertebrate skeletal muscle[1–4] have shown that myogenic cells originate from activated satellite cells. Satellite cells are mononuclear cells located between the external lamina and the plasma membrane of muscle fibers[5]. Their number, size and form vary in the course of muscle development[6, 7], and they are assumed to represent the stage of dormant or resting myoblasts. It is known that after local damage to muscular tissue in vertebrates mitotic activity of satellite cells is induced, with migration of the dividing cells from undamaged regions to the site of injury[8]. The mechanism of activation of satellite cells is as yet unknown.

In addition to satellite cells, a supplementary source of myogenic cells has been suggested in a study on regeneration of crustacean (crayfish) muscle fibers following mechanical injury[9]. Hemocytes, after penetration into damaged areas, undergo gradual transformation manifested in cell apposition, degranulation and the appearance of contractile filaments. The transformation of hemocytes has been assumed to be induced by activated satellite cells present in degenerated material.

To test this hypothesis, and to study systematically the process of myogenic transformation of satellite cells, basic data concerning structural characteristics and distribution of satellite cells along crayfish muscle fibers are necessary. The findings of the present study have already been reported in a preliminary form[10].

Materials and methods

For quantitative estimation of satellite cells, long-sarcomere muscle fibers from the carpopod extensor muscle of the crayfish (*Astacus fluviatilis*) were used. Two groups of animals of different ages were investigated: a) 12-cm-long crayfish (approx. 5 years old) and b) 6-cm-long crayfish (approx. 2 years old).

Following amputation of the first cheliped and opening of the shell, the muscle was exposed for 2 × 3 min to the fixative solution, 2% glutaraldehyde and 0.05% OsO_4 in 0.15 mol/l sodium cacodylate (pH 7.4)[11], and then fixed for 60 min in 2% glutaraldehyde in cacodylate buffer. The muscle fibers were then teased apart in a buffer solution to obtain single fibers. After post-fixation in 1% OsO_4 for 30 min and staining with 2% aqueous uranyl acetate overnight, the fibers were dehydrated in an

ethanol series and embedded in Durcupan. Ultrathin sections were cut with a Porter-Blum MT-2 ultramicrotome, picked up on formvar-coated single-slot copper grids, stained with lead citrate [12] and examined in a JEOL JEM 1200/EX electron microscope at an accelerating voltage of 80 kV.

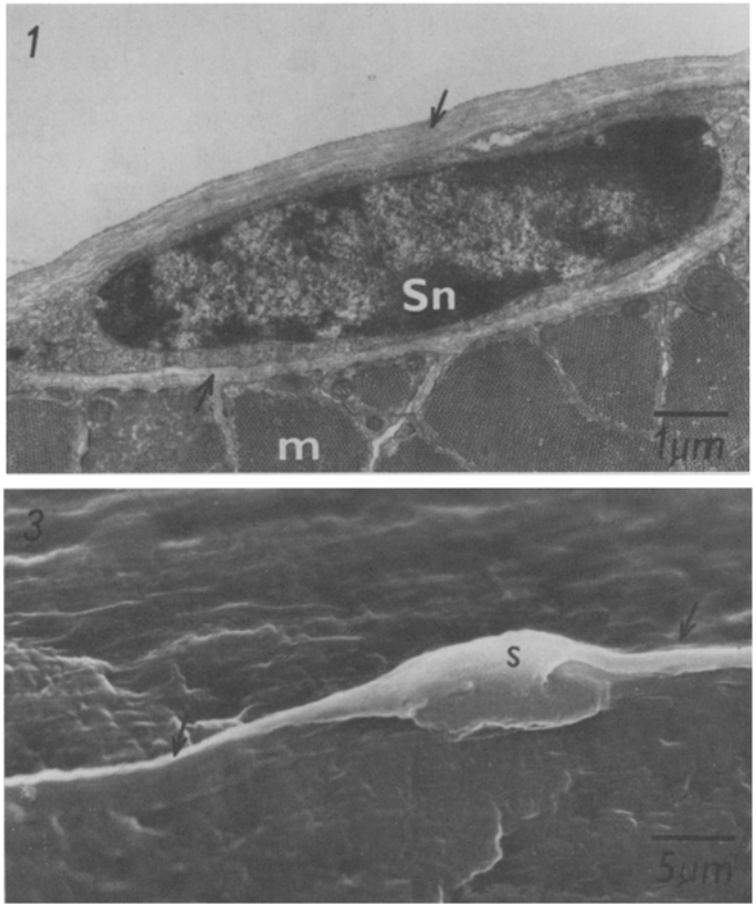
Results

Cells located between the plasma membrane and the external lamina, or within the external lamina of muscle fibers, were identified as satellite cells (sc) (fig. 1). Sporadically, they could also be found within sarcolemmal invaginations (clefts) (fig. 2).

Sc are spindle-shaped with a nucleus in the central part and with most of the cytoplasm in elongated ends or tails. They are oriented parallel to the long axis of the fiber (fig. 3). The nucleus is elliptical in shape in both longitudinal and transverse sections. Heterochromatin is accumulated predominantly along the nuclear membrane. Myonuclei are situated not only at the periphery and under the plasma membrane of sarcolemmal clefts but also in central regions of the sarcoplasm (fig. 4a and b). In most cases they are angular in shape.

Quantitative analysis

Size of nuclei. The length of the sc nuclei was measured on electronmicrographs obtained from longitudinally-sectioned muscle fibers. To minimize underestimation, only sections encompassing both tails of a satellite cell were evaluated. Ten nuclei from different segments of muscle fibers of older animals were measured. The resultant mean value was 17 μm ± 0.96 (mean ± SEM). The same mean value was measured also in the group of younger animals. The length of myonuclei (mn) was 18.35 ± 1.2 (n = 10). The difference was not significant. The depth of the nuclei of sc was measured at three points, at 1/4, 2/4 and 3/4 of their length. The average value as determined from 10 nuclei was 2.65 ± 0.21



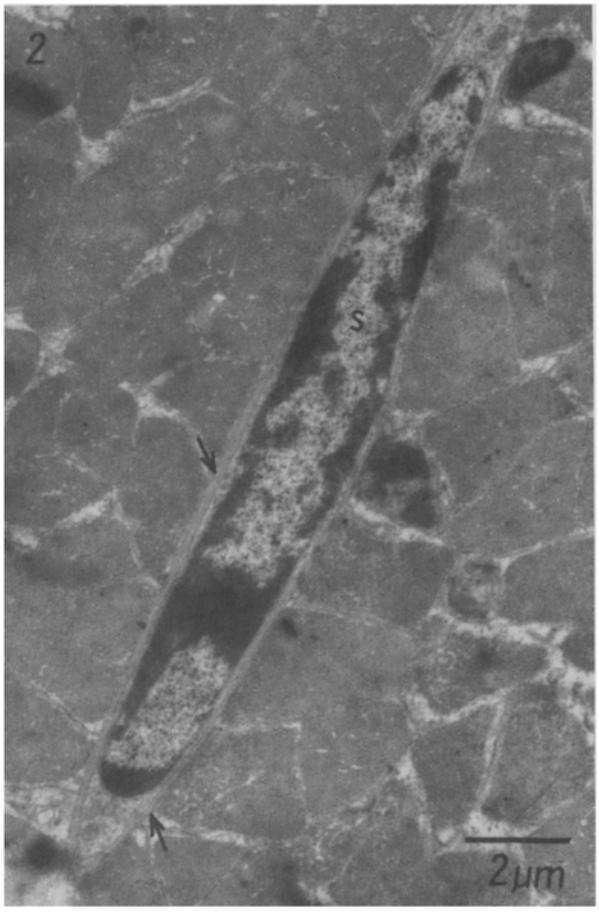


Figure 1. Transverse section of a satellite cell in carpopod extensor muscle of the crayfish, situated within the external lamina (arrows). Sn = satellite cell nucleus; m = muscle fiber.

Figure 2. Satellite cell (s) within a sarcolemmal invagination surrounded by the external lamina (arrows).

Figure 3. Scanning electron microscopic picture of a satellite cell (s) lying on the surface of a muscle fiber and tapering into long fine processes (arrows).

(older animals). The mean maximal depth was 3.49 μm ± 0.25.

Number of satellite cells

The number of sc was expressed as the ratio of the number of sc nuclei (scn) to the total number of nuclei (mn + scn)[6, 13]. Five fibers in each group of animals were evaluated. Each fiber was represented by three transverse sections taken from tendon, central and shell segments. The entire area of a section was used to count mn and scn under the electron microscope. The results are summarized in tables 1 and 2, and their comparison shows higher numbers of satellite cells (scn/(mn + scn)) in younger animals.

The mean number of nuclei of satellite cells per unit fiber length (N) was calculated from the equation[14]:

$$N = \frac{A}{L_n + M}$$

where A = number of scn in a transverse section (see tables 1 and 2);
M = section thickness (about 100 nm, corresponding to sections of gold color);
L_n = mean length of scn.

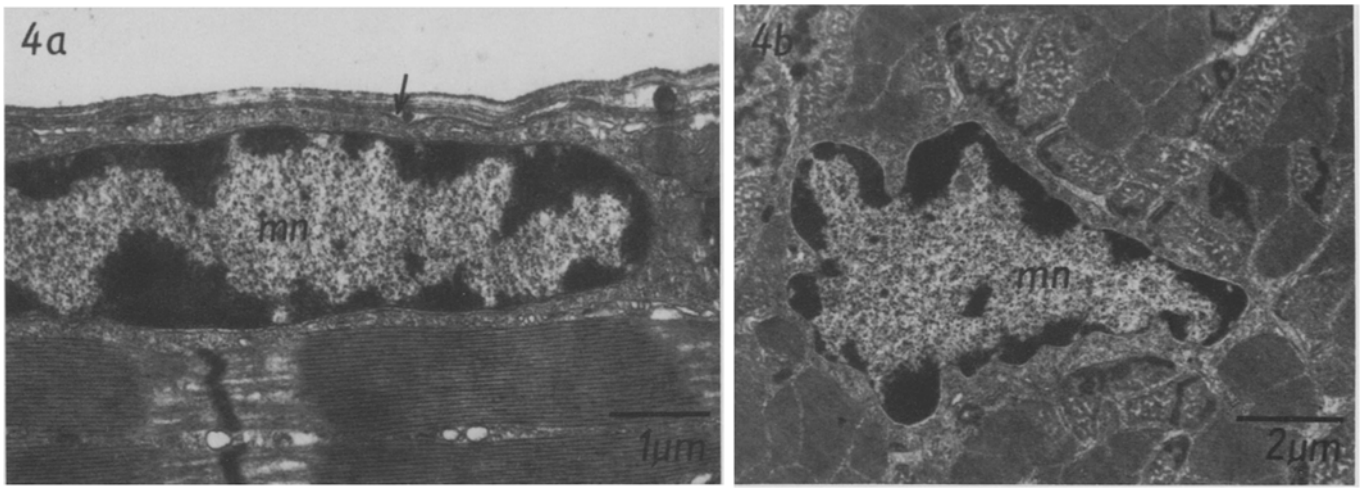


Figure 4. *a* Longitudinal section of a crayfish muscle fiber with the myonucleus (mn) situated at the periphery of the fiber below the plasma membrane (arrow). *b* Transverse section of the central region of the sarcoplasm. mn = myonucleus of angular shape.

Table 1. Average counts of satellite cell nuclei: adult animals (approx. 5 years old)

Animal No.	Tendon segment mn	scn	scn/(scn + mn)	Central segment mn	scn	scn/(scn + mn)	Shell segment mn	scn	scn/(scn + mn)
1	21	2	0.087	24	3	0.111	25	1	0.038
2	15	1	0.062	20	3	0.130	20	1	0.048
3	27	1	0.036	22	2	0.088	31	1	0.031
4	20	1	0.048	17	1	0.055	14		
5	21	3	0.125	24	1	0.040	18	1	0.053
mean	20.8	1.6	0.071	21.4	2	0.084	21.6	0.8	0.034
± SEM			0.016			0.017			0.009

mn, myonuclei; scn, satellite cell nuclei.

Table 2. Average counts of satellite cell nuclei: young animals (approx. 2 years old)

Animal No.	Tendon segment mn	scn	scn/(scn + mn)	Central segment mn	scn	scn/(scn + mn)	Shell segment mn	scn	scn/(scn + mn)
1	22	5	0.185	21	2	0.086	23	1	0.041
2	20	1	0.047	12	1	0.076	12	2	0.142
3	15	2	0.117	16.75	2	0.107	16	2	0.111
4	5	1	0.166	5	1	0.166	6		
5	13	5	0.277	23	4	0.148	16	2	0.111
mean	15	2.8	0.158	15.5	2	0.166	14.6	1.4	0.081
± SEM			0.038			0.017			0.026

mn, myonuclei; scn, satellite cell nuclei.

For older animals the values of N for the tendon, central and shell segments were 94 mm^{-1}, 117 mm^{-1} and 47 mm^{-1}, respectively. The corresponding values for younger animals were 164 mm^{-1}, 117 mm^{-1} and 94 mm^{-1}.

These values, as well as those shown in tables 1 and 2, demonstrate a higher occurrence of sc in the tendon and central segments of fibers in comparison with the shell end.

Discussion

A quantitative analysis of satellite cells in long-sarcomere crayfish muscle fibers has shown a high incidence of sc. However, the scn/(mn + scn) ratio is similar to that in other animal species (table 3). Following experimental lesions these cells become activated and are involved in the regeneration process (preliminary reports [15, 16] and a paper in preparation).

The high incidence of sc in the crayfish is due to the large number of sc per unit fiber length. This parameter in frog sartorius muscle is 13.45 mm^{-1} [14], which is about 6–8 times less than in crayfish. The relatively high numbers of sc per unit fiber length may result from a much larger diameter (300–500 µm) [17] and volume of muscle fibers in crayfish than in vertebrates.

The distribution of sc along crayfish muscle fibers is non-uniform, with lower incidences of sc in the shell segment. The reason for this non-uniformity is unknown. The possibility of increased numbers of sc in regions of synaptic nerve endings as described in vertebrates [18–20] should be tested in further studies. This question is especially interesting in relation to crustacean muscles because of the presence of different synaptic types along a single fiber [21] (own unpublished observations). The non-uniform distribution of sc could also be related to preferential growth of fibers at one end only [22].

The higher incidence of satellite cells in younger animals found in the present study has also been reported for other animal species [6, 23], and may be assumed to be typical of young developing muscles. The reduction in the number of sc in adult animals could result from fusion of sc with muscle fibers [24]. On the other hand Takahama [25], in his study of tadpole muscles during metamorphosis, described a gradual separation of sc from muscle fibers, with the resultant appearance of free cells similar to pericytes [26].

In conclusion, the main result of our study is the demonstration that satellite cells are present along the muscle fibers of arthropod as well as vertebrate muscle, and that if the incidence is related to the total number of nuclei it is similar to that found in vertebrates.

However, if the incidence of satellite cells in crayfish is related to the unit length of a muscle fiber it is very high. Crayfish muscle could therefore be useful in studies of activation and myogenic transformation of sc during muscle regeneration, since a satellite cell may be expected at the level of each transverse section.

Table 3. Average counts of satellite cell nuclei in various animal species (Modified from Maruenda [14])

Animal	Muscle	scn/(scn + mn)	Reference
Shark	axial r.	0.032	Kryvi [27]
	axial w.	0.016	
Frog	gastrocnemius	0.016	Trupin [28]
	sartorius	0.100	Maruenda [14]
Mouse	lumbricalis	0.040	Schultz [6]
	gastrocnemius	0.060	Cardasis [29]
Rat	EDL	0.027	Kelly [18]
	soleus	0.048	Kelly [18]
	soleus	0.058	Hanzlíková [30]
Crayfish	ext. carpopoditi	0.099	this study

mn, myonuclei; scn, satellite cell nuclei.

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