Echinoderms: Potential Model Systems for Studies on Muscle Regeneration

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Abstract: Organisms of the phylum Echinodermata show some of the most impressive regenerative feats within the animal kingdom. Following injury or self-induced autotomy, species in this phylum can regenerate most tissues and organs, being the regeneration of the muscular systems one of the best studied. Even though echinoderms are closely related to chordates, they are little known in the biomedical field, and therefore their uses to study pharmacological effects on muscle formation and/or regeneration have been extremely limited. In order to rectify this lack of knowledge, we describe here the echinoderm muscular systems, particularly the somatic and visceral muscle components. In addition, we provide details of the processes that are known to take place during muscle regeneration, namely dedifferentiation, myogenesis and new muscle formation. Finally, we provide the available information on molecular and pharmacological studies that involve echinoderm muscle regeneration. We expect that by making this information accessible, researchers consider the use of echinoderms as model systems for pharmacological studies in muscle development and regeneration.

Keywords: Echinoderms, muscles, dedifferentiation, myogenesis, coelomic epithelium, regeneration.

INTRODUCTION

Echinoderms

Scientists working in the biomedical field are confronted with a paradox. As the range and precision of scientific techniques and tools available to probe for the solution to research questions increases, the number of model organisms where to apply these techniques seems to be decreasing. Thus, nowadays most research is done in what have been termed "classical model systems", which include mainly animals that are amenable to genetic manipulations [1]. This has created a distorted view, where researchers are more familiar with the fruit fly *Drosophila melanogaster* or with the nematode *Caenorhabditis elegans* than with other animal species that are evolutionarily closer to humans. Moreover, researchers have ignored animal groups, whose particular properties might provide important insights into the molecular bases of cellular processes and diseases [2]. Such is the case of the echinoderms.

The phylum Echinodermata comprises five extant classes. These are the Echinoidea (sea urchins), Asteroidea (sea stars, commonly called starfish), Holothuroidea (holothurians, commonly called sea cucumbers), Ophiuroidea (brittle stars) and Crinoidea (sea lilies). Although a few species from this phylum, particularly sea urchins, have been widely used in studies of fertilization and embryogenesis, only limited biomedical studies have focused on adult echinoderms. These animals show amazing regenerative capabilities. Some regenerative phenomena are subtle, such as the regeneration of spines in the sea urchin. However, others can be rather striking, such as the regeneration of a new organism from a sea star arm.

In view that adult echinoderms might not be well-known to biomedical investigators, it is imperative to describe their phylogenetic relationship to vertebrates and some of their structures, in particular those associated with the muscular system, prior to the discussion of their regenerative processes.

Echinoderm Phylogenetic Tree

Adult echinoderms show pentaradial symmetry and lack a clear anterior cephalized structure. These morphological characteristics are probably the reasons why many investigators view them as primitive animals. Their embryological development, however, shows strong similarities to those of more advanced animals and have served to place them among the Deuterostomia, the same evolutionary branch where vertebrates are found. In fact, echinoderms together with tunicates, cephalochordates and hemichordates are the only deuterostome invertebrates (Fig. **1**). (All other invertebrates are grouped in the Protostomia, a different branch of the evolutionary tree). This close relationship, established using morphological, embryological and fossil data, has been reaffirmed in molecular studies [3, 4, 5]. Nonetheless, there is some discussion on the exact localization of the various groups within the Deuterosomia [5, 6]. The currently accepted phylogenetic relationship groups together the phylum Echinodermata with the phylum Hemichordata into the Ambulacraria and places these as a sister group to the phylum Chordata which contains the Urochordata (tunicates), Cephalochordata and the Chordata (Fig. **1**). It has been proposed that the common ancestor of chordates and the echinoderms existed around 600-700 Myr ago [5]. Nonetheless, they still share many developmental processes and with these, the genetic machinery that controls these events [7, 8]. This close relationship to chordates, in particular when compared to other invertebrates

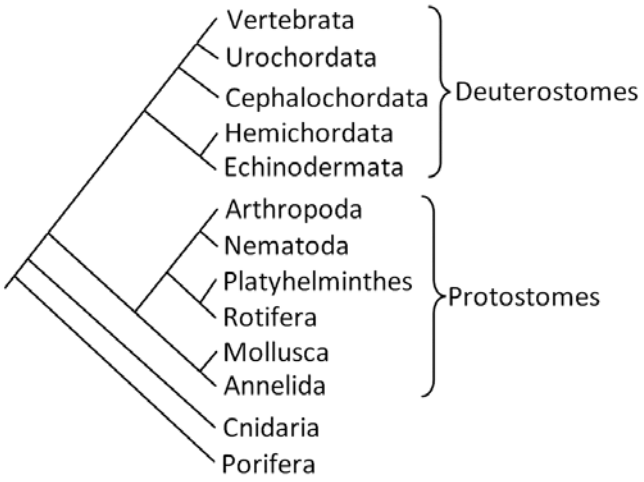

Fig. (1). Phylogenetic tree showing the relationship of echinoderms to other animal groups.

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that have provided important information on cellular and molecular processes, such as *D. melanogaster* and *C. elegans*, is one of the reasons that makes them attractive as upcoming model systems. Thus, echinoderms, with their amazing regenerative properties might provide important clues to the phenomenon of regeneration. In this review we focus on their muscular system and summarize what is known of the process of regeneration of both somatic and visceral muscle.

Morphology of Echinoderm Muscles

Echinoderms possess well-differentiated, but morphologically simple musculature. Like in vertebrates, their muscle system has been subdivided into two types, i.e., somatic and visceral musculature. However, in echinoderms there seems to be few distinctive differences between these two muscle categories, and muscle types consists more of a range of structures that reflect the evolution of the muscle as can be observed in different organisms and/or different organs. Histologicaly, echinoderm muscle resembles more vertebrate smooth muscle than skeletal, but little is known of the molecular machinery that constitutes its contractile apparatus.

The visceral musculature is composed of a coelomic epithelium or mesothelium (Fig. **2 (A)**). It covers coelomic surfaces of internal organs and lines inner surface of the water-vascular system. Coelomic epithelia are basically composed of myoepithelial and peritoneal cells [9-14].

Each peritoneal cell consists of an extended apical part that faces the coelomic cavity and a slender basal peduncle penetrating the entire depth of the mesothelium and adhering to basal lamina. The apical part of the cell houses a subspherical or irregularly shaped nucleus and bears a cilium and microvilli. Adjacent cells are joined to each other with zonulae adherens. The slender basal peduncle often contains bundles of intracellular filaments, which sometimes reach the apical part of the cell. In some cells the bundles exhibit clear cross striation. In the sea cucumber intestine, where it has been best studied, adjacent peritoneal cells adhere closely to each other, forming groups, which arch over basal lamina, thus delimiting spaces that house myoepithelial cells [15, 16].

Myoepithelial cells are confined to the basal portion of the coelomic epithelium and are connected to basal lamina through hemidesmosomes. Adjacent myoepithelial cells are bound to each other by desmosomes. The bulk of cytoplasm is occupied by myofilaments, which form a powerful contractile apparatus. The myoepithelial cells can be oriented in various directions forming longitudinal, transversal, or oblique muscles of the organ.

In addition, the coelomic epithelium contains much nervous tissue [10, 14, 17-19]. Nerve cells and their processes are located in-between peritoneal and myoepithelial cells. There are no synaptic specializations, such as those that are common to vertebrate motor neuron-skeletal muscle junctions. Nonetheless, in some cases visceral muscle can be observed to extend cytoplasmic prolongations to the area where the nerve fibers are most abundant, indicating some type of muscle specialization that are common to other invertebrate species, such as nematodes [20].

The somatic musculature of echinoderms comprises large muscles having different organization and function [21]. In crinoids and brittle stars it is represented by muscle bundles connecting arm segments to each other and ensuring their mobility [21-23]. In sea urchins the principal musculature is the muscle system of the Aristotle's lantern and takes part in the process of feeding [24, 25]. In holothurians the principal somatic muscles are the body wall longitudinal and circular muscles that provide the contraction of the body wall associated with spatial movements. This musculature includes the five longitudinal muscle bands (LMB), which extend from the anterior end of the body to the posterior one occupying the radial positions [21]. It also includes the circular muscles embedded in their body wall, that although not as conspicuous as the longitudinal muscle probably work in coordination to direct the animal movement. In addition, in holothurians of the Order Dendrochirotida there are five retractor muscles that pull in the oral complex of organs with tentacles (aquapharyngeal complex) into body cavity [21]. In sea stars, no large muscle bundles are present.

Despite differences in anatomical location, echinoderm muscles have similar histological structure. In most echinoderms they consist of individual bundles of smooth muscle fibers embedded in the extracellular matrix of connective tissue [12, 25-29]. The muscles of crinoid arms consist of obliquely striated fibers [10, 23, 30]. Each muscle bundle is composed of several myocytes (usually 8-20) and surrounded by basal lamina (Fig. **2 (B)**). Myocytes are connected to each other by spot desmosomes, and fastened to basal lamina by hemidesmosomes. Almost the entire cytoplasm of the cell is occupied by myofilaments. Nuclei are separated from the contractile apparatus, and most of them are located toward the central part of the bundle. Muscle bundles contain no connective tissue and most of the area is filled with myocyte processes. Basal bodies (kinetosomes) of cilia can sometimes be observed in myocytes [19].

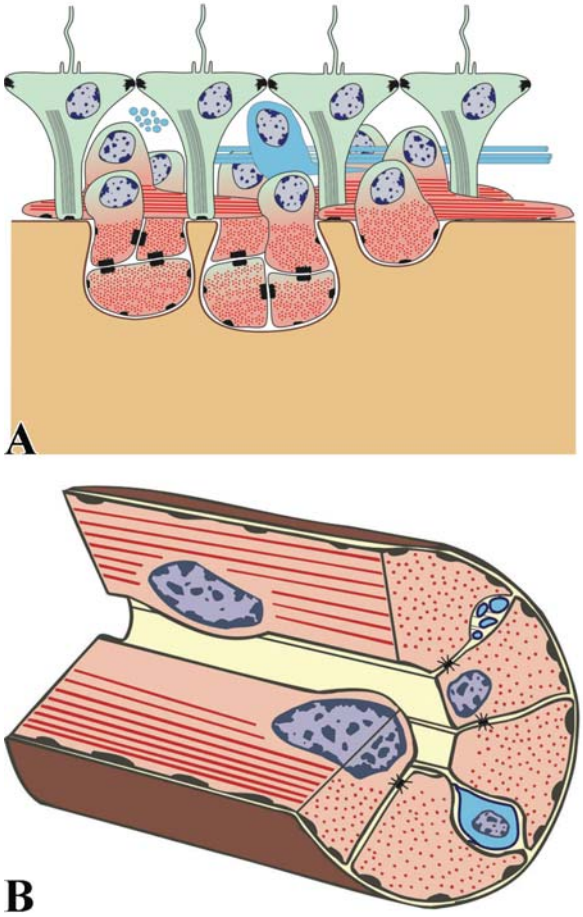

Fig. (2). Schemes of normal organization of muscle systems in echinoderms. A. Visceral musculature (coelomic epithelium) is composed of ciliated peritoneal cells with bundles of intermediate filaments (green) and groups of myoepithelial cells (green-reddish). The latter contain myofilaments (red spots and lines). Moreover the epithelium contains nerve cells and their processes (blue). Peritoneal and myoepithelial cells adhere to basal lamina (deep brown) by hemidesmosomes. The coelomic epithelium is situated on connective tissue layer (brown). B. Muscle bundle of somatic musculature. The muscle bundle is composed of several myocytes (pink) and surrounded by basal lamina (deep brown). The cytoplasm of the cell contains myofilaments (red spots and lines). Besides myocytes, muscle bundles contain putative neurons and their processes (blue).

Besides myocytes, muscle bundles contain putative neurons and their processes. The connective tissue of muscles consists of a network of thick striated (collagenous) and thin unstriated fibers and an amorphous component. Connective tissue also comprises fibroblasts, nerve cells and different coelomocytes. In some muscles of the Aristotle's lantern, juxtaligamental-like cells embedded into the connective tissue between the muscle bundles were observed [19, 25]. Such a combination of contractile cells and mutable connective tissue is very interesting from both physiological and evolutionary points of view.

Peritoneocytes, myoepithelial cells and myocytes are successive stages of specialization of a single cell type, the epithelial cell of coeloms [31]. They differ from each other in the respective level of specialization and, therefore, the level of dedifferentiation during regeneration. The common origin is also reflected in the structure and behavior of these cells. Echinoderm muscles are unique in retaining some epithelial features, which strongly supports the hypothesis that their muscle bundles might have evolved from epithelia [9]. The muscle bundles in these animals are covered with a continuous basal lamina. Myocytes are polarized cells, whose basal surfaces are attached to basal lamina by hemidesmosomes, while the apical portions lie in the central region of bundle. In other words, the echinoderm muscle bundle can be considered as a sheet of myoepithelium rolled up to form a tube [32].

MUSCLE REGENERATION

Several studies of echinoderm muscle regeneration have provided a good understanding of the cellular events that occur during the regenerative process. Muscle regeneration has been mostly studied in holothurians, because they are the echinoderm group with the most developed muscle systems and second, because some of their muscles, in particular the LMB of the body wall are easily dissected or available for experimental manipulations [33, 34]. In addition, some holothurian species can undergo a process of evisceration where most of the internal viscera are eliminated following application of noxious stimuli. Evisceration is then followed by a process of regeneration where viscera are replaced. Muscle regeneration in sea stars, crinoids and ophiuroids has been mainly observed in studies of arm regeneration [35, 36]. In contrast, in sea urchins, due to the presence of external calcified skeleton, the muscles of Aristotle's lantern are not available for studies. Regeneration studies in this group have mainly focused on spine and skeletal (test) regeneration [37, 38]. Therefore, even though some information is available for most echinoderm groups, the most detailed studies have been done on the somatic and visceral muscles of holothurians by the laboratories of the authors and on the arm muscles of crinoids by Candia Carnevali's group. These studies use microscopy, both at the electron and light levels, to dissect the steps by which muscle cells regenerate. What emerges is the description of a process that while showing some species-related differences, appears to be well conserved among the echinoderm groups.

Following injury, evisceration, and amputation, two events have been documented in echinoderm muscular tissues; de-differentiation and myogenesis. De-differentiation occurs early during the regeneration process, starting while the tissue is still undergoing wound healing and continuing at different rates during the regenerative period. Myogenesis, on the other hand is an integral part of the regenerative period and takes place together with many other regenerative events, once the new organ or tissue is being formed.

Dedifferentiation

De-differentiation is observed at the microscopic level as a disorganization of the muscle tissue usually followed by a thinning or complete disappearance of the muscle layer or muscle bundle. De-differentiation proceeds in somewhat different manner in myoepithelial cells of smooth musculature and myocytes of somatic muscles, which is obviously due to the size of the cells, the number of myofilaments and the structural organization of respective muscles. The trademark of muscle de-differentiation is the appearance of spindle-like structures (SLS). Vacuoles containing filaments that were similar in structure with myofilaments were for the first time found among muscle cells in the ampulla of ambulacral foot of the sea star *Astropecten irregularis* [39]. Baccetti and Rosati described similar SLS in the epithelium of the water-vascular system of the holothurian *Holothuria tubulosa* [40]. They supposed these cells to be degenerating and considered SLS as an indicator of the process of cell regeneration in the epithelium. Jensen [41] revealed SLS in coelomic epithelium of dorsal hemal vessel in the holothurian *Parastichopus tremulus.* Later on, SLS were found in the muscles of Aristotle's lantern in the adults of the sea urchin *Strongylocentrotus nudus* [19].

The development of spindle bodies, which were staining exactly as muscles, in the course of regeneration was for the first time registered in the holothurian *Thyonella gemmata* after the amputation of the anterior body end [42]. However, the relationship between SLS and muscle dedifferentiation during regeneration in holothurians was only demonstrated later on [43]. In this case, the regenerating retractor muscles and mesothelium of the aquapharyngeal complex of the holothurian *Eupentacta fraudatrix* was studied following evisceration. Following this finding, de-differentiation was also described during the regeneration of LMB of the body wall of two holothurians (*E. fraudatrix* and *Apostichopus* (*Stichopus*) *japonicus*) following injury [44, 45]. More recently the dedifferentiation process has been described in the LMB of the body wall of a third holothurian species, *Holothuria glaberrima*, following transection of the body wall-muscle-nerve complex [46].

The production of SLS has also been found to occur during the regeneration of any muscle-containing organ of a holothurian. De-differentiated cells forming SLSs were described in the mesothelium of the stomach and stomach-intestinal junctions of transversely cut young specimens of *E. fraudatrix* undergoing regeneration [15]. Similarly SLSs have been found in the muscle of the remaining mesentery following evisceration of the digestive tract in *H. glaberrima* (Fig. **3 (A, B)**) [47]. In this system, the myocytes slowly disappear from the mesentery during the early days following evisceration and the process parallels the appearance of SLSs that appear to be expelled from the mesentery or otherwise phagocyted by amoebocytes within the connective tissue (Fig. **4**). Dedifferentiation of myoepithelial cells and formation of SLS were registered in the cloaca coelomic epithelium in areas where the regeneration of respiratory trees takes place (Fig. **3 (C, D)**) [48].

Holothurians are not the only echinoderms where SLSs and myocyte dedifferentiation occurs during regeneration. In the crinoid *Antedon mediterranea*, after arm autotomy there is little if any dedifferentiation of the muscle stump tissues at the distal-intermediate region [49]. This happens, because the muscles are not damaged during autotomy [36]. However, muscle de-differentiation has been documented in regenerating crinoid arm explants [36]. The description of muscle dedifferentiation closely mimics that of the dedifferentiating holothurian muscle. There are signs of disorganization of the muscle contractile apparatus, disappearance of myocytes, and the remains of the contractile material found in the extracellular space and in phagosomes. It is interesting that the dedifferentiation process appears to be increased in the presence of pseudo-estrogenic pollutants [50]. Exposure to organotin compounds (triphenyltin-chloride) also causes extensive muscle dedifferentiation in regenerating crinoid arms [51]. Likewise, in regenerating crinoids that are treated with environmental contaminants (PCBs), the rate of regeneration increases, and there

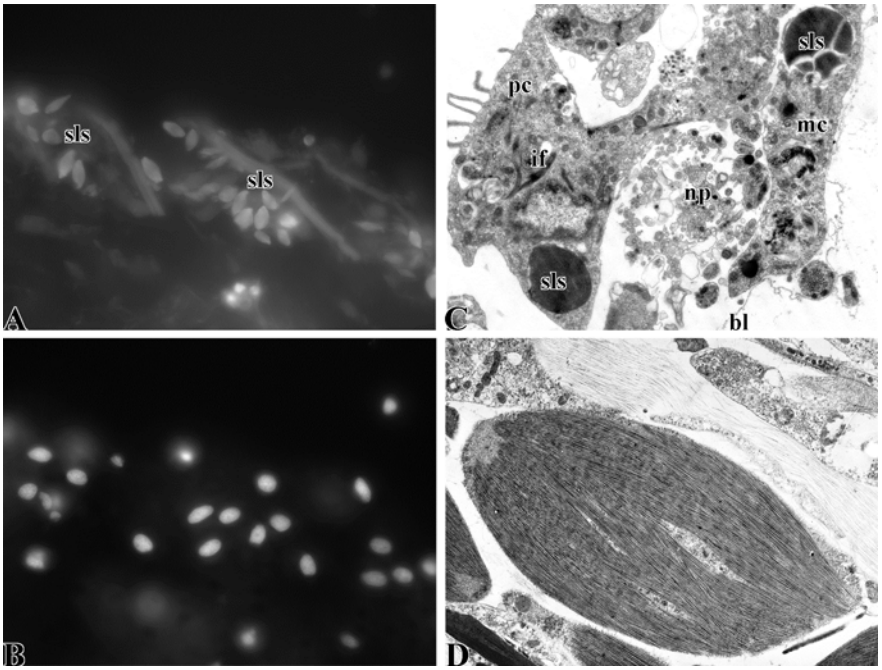

Fig. (3). Dedifferentiation of cell of visceral musculature. A. Spindle-like structures (sls) and fibers labeled with rhodamine-labeled phalloidin in regenerating intestinal mesentery. B. Cell nuclei labeled with DAPI. Note the lack of correlation between SLS and cell nuclei. C. Electron micrograph of regenerating cloacal coelomic epithelium during regeneration of respiratory trees. Note fragmented bundles of intermediate filaments (if) in peritoneal cells (pc). The cells loose connections to the basal lamina (bl). SLS (sls) can be observed in the cytoplasm of both peritoneocytes and myoepithelial cells (mc). There are bundles of nerve processes (np). D. Electron micrograph of SLS.

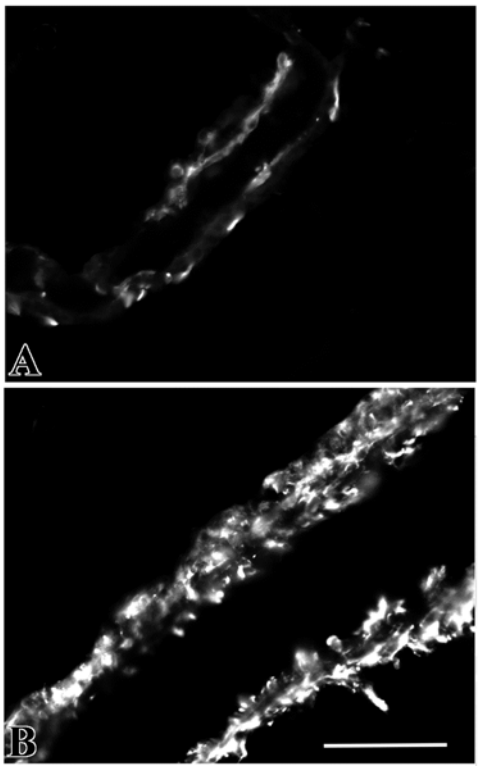

Fig. (4). Disappearance of muscle cells from the mesentery in animals regenerating their intestine. A. Few muscle cells are labeled with a muscle-specific antibody in the mesentery of a specimen undergoing intestinal regeneration one week following evisceration. B. In contrast, the mesentery of a normal non-regenerating animal shows well-organized muscle system labeled with the same antibody.

is abnormal growth of the regenerate [52]. These animals show enhanced rearrangement of the tissues at the stump. In particular, muscle cells were found to loose the contractile apparatus and to dedifferentiate into what seems like migrating coelomocytes. Thus, in crinoids, muscle regeneration might occur via two processes. When arms are distally amputated, most of the regenerative processes appear to involve migrating cells that originate from the coelomic epithelia and radial nerve. However, under highly stressful conditions, such as arm explants, organotin compound or PCB contamination, muscle dedifferentiation occurs.

The presence of SLSs has not been clearly documented in sea stars, but this might be due to the fact that few if any studies have focused on the changes that take place within the muscle during arm regeneration. There is a brief description (abstract) of the dedifferentiation of myoepithelial cells of the water-vascular epithelium in the ambulacral feet of the sea star *Pisaster ochraceus* by Cavey and Marsden [53], but this was not followed by an in depth report. In another study of arm-tip regeneration in the sea star *Leptasterias hexactis*, Mladenov and colleagues [35] describe some of the events that occur at the level of the muscle system. It is interesting that they describe the occurrence of muscle histolysis in the area near the scar at 3 days post-amputation and of phagocytic cells intermixed with clumps of disorganized muscle at 7-d post amputation. These processes are reminiscent of what has been described in greater detail during SLSs formation and muscle dedifferentiation in holothurians and crinoids. Until recently, few studies addressed muscle regeneration in ophiuroids. However, a detailed study of arm regeneration in two brittle stars *Ophioderma longicaudum* and *Amphiura filiformis* have also shown that following arm autotomy there is a striking disorganization of the skeletal muscle and the presence of dedifferentiating myocytes [54].

Finally, dedifferentiating myocytes, characterized by the presence of SLS have also been documented in the sea urchin during test regeneration [38]. In this case, a hole in the sea urchin test was made with a scalpel and during the regenerative phase, the

mesothelial myocytes of the laminae adjacent to the wound site were highly disorganized with their myofilaments compacted into SLSs.

Thus, formation of SLS is an important feature of muscle cell dedifferentiation in echinoderms. Moreover the presence of SLS in the intact tissues of the animals shows the possibility of myocyte dedifferentiation in the course of normal life activities. This seems to reflect the process of normal growth and/or renewal (physiological regeneration) of muscle tissue in these animals.

However, presence of SLS in cytoplasm cannot be considered as an unambiguous indicator that the cell is a descendant of dedifferentiated myoepithelial or muscle cells. It was shown that some other cells located at the wounded area can phagocyte and utilize SLSs. First of all, these are peritoneocytes, which are in direct contact with the myoepithelial cells [48, 53, 55], then amoebocytes performing phagocytic functions in echinoderms [33, 47], and even glial cells of the nervous system [56].

Dedifferentiation might be due to the direct damage caused to some muscle cells by the transection or autotomy event. This is suggested by the large number of dedifferentiating cells that occur close to the damage site early in regeneration, as has been reported in the regenerating intestinal mesentery [47], mesothelium and water-vascular canals [43] and LMB [33] of holothurians and in the peripheral area of the stump muscles in the brittle star arm [54]. However, it is clear that dedifferentiation does not solely occur at the wound site, and is not only caused by direct damage on the muscle cells being impinged by the transection or wounding. First, cells at various distances from the damage site can be seen to dedifferentiate. In holothurians dedifferentiation occurs in regions far from the wound or from the rupture site, in both the LMB of the body wall after cutting and the mesentery muscle during intestinal regeneration. This dedifferentiation is usually observed in a gradient, where those regions close to the wound produce more SLSs early in the regenerative process, while regions farther from the wound produce SLSs later in the process [46, 57]. Second, the extent of de-differentiation extends temporally as well as spatially. Signs of cell dedifferentiation can sometimes be found in specimens well advanced in regeneration. For example, SLSs can be still seen, although in much lower densities, in holothurian muscle regenerating 3 weeks following transection [46] and in the brittle star arm, dedifferentiating myocytes are found in advanced regenerative phases that could extend over 5 weeks following arm autotomy [54]. On the other hand, the formation of SLS might not be solely due to regeneration events, but might well be part of a housekeeping process that serves to recycle the molecular machinery of cells that have undergone cell death or damage. This might explain the finding of SLSs in the normal tissues of echinoderms [19, 39-41].

The dedifferentiation process has been well studied at the electron and light microscopy level. As we have said above the process of destruction of contractile apparatus during dedifferentiation is different in myoepithelial cells and myocytes. Thus, we will consider separately the dedifferentiation of contractile cell of smooth and somatic musculature.

Dedifferentiation of Myoepithelial Cells

Following transection or evisceration, cellular destruction is observed in the damaged area. Some coelomic epithelial cells undergo apoptosis, but concurrently both peritoneocytes and myoepithelial cells begin to dedifferentiate. Careful examination of regeneration in different holothurian organs revealed that the cells can show different degrees of dedifferentiation.

Incomplete dedifferentiation can be best described by focusing on the regeneration of the respiratory trees in holothurians. These organs are highly branched outgrowths of cloaca wall and located inside coelom. In holothurians of the order Aspidochirotida they are removed together with intestine during evisceration and then regenerate again [21, 48]. Respiratory muscle regeneration occurs as a result of the reorganization of the cloacal coelomic epithelium that surrounds the wounded area. During dedifferentiation, bundles of intracellular filaments of peritoneal cells are fragmented (Fig. **3 (C)**). The cells loose connections to the basal lamina, flatten and begin migrating toward the wounded area. The intercellular junctions between the cells are retained. Unlike peritoneocytes, myoepithelial cells become isolated cells because desmosomes that fasten them to each other disappear. The cells also loose connections with the basal lamina. At the same time, the contractile apparatus of the cells begins to collapse. Myofilaments are fragmented and aggregated into SLS. The latter are accumulated within the cytoplasm, but later on, some are released to the outside. These SLS are phagocyted by peritoneocytes and amoebocytes.

Besides simplification of cellular structure, one of the signs of dedifferentiation is nuclear activation. In the non-injured animals, peritoneal and myoepithelial cells present irregular nuclei with condensed chromatin. However, during dedifferentiation, both cell types show large rounded euchromic nuclei, often with a large nucleolus. Thus, at the highest stage of dedifferentiation, the peritoneal and myoepithelial cells have a similar morphology and are distinguished only by their position in the epithelium. Dedifferentiated peritoneocytes form a continuous layer, under which the dedifferentiated myoepithelial cells remain. Their cytoplasm contains well-developed rough endoplasmic reticulum and many free ribosomes and polysomes. Dedifferentiated peritoneocytes proliferate actively, whereas no mitotically dividing dedifferentiated myoepithelial cell has been registered.

Complete dedifferentiation can be best observed during regeneration of the intestine or the aquapharyngeal complex. In these cases, the cells gradually dedifferentiate in the course of their migration toward the regenerating structure. The initial stages of this process proceed similarly to those in respiratory tree regeneration. Peritoneocytes and myoepithelial cells dedifferentiate, eliminating from their cytoplasm intracellular filaments and myofilaments, respectively. The latter case implies the formation of SLSs. In some cases de-differentiating myocytes with some remains of the contractile apparatus or SLS have been seen within the migrating cells in the regenerating crinoid arm explant [36], in the coelomic epithelium of regenerating intestine [58], and in the coelomic cavities of regenerating brittle star arms [54]. Simultaneously with the migration and dedifferentiation, they enter the mitotic cycle and begin dividing [59]. The cells of the coelomic epithelium of the regenerating structure show no morphological differences from each other and are connected to each other by junctions. Thus, they form the mesothelium and, therefore, the muscle layer appears to develop *de novo* at the expense of coelomic epithelial cells migrating from the body wall and mesentery [15, 43, 47, 58].

As there are no morphological markers available, the origin and further destiny of dedifferentiated cells making up such an epithelium are still uncertain. It is still not clear, whether they originate from only peritoneal cells or from both peritoneal and myoepithelial cells. As has been mentioned earlier, both the peritoneal and myoepithelial cells of coelomic epithelium share a histological origin. Thus, we can suppose that both cell types could dedifferentiate entirely and then differentiate in two directions (similar to what occurs during ontogenesis) giving rise to peritoneocytes and myoepithelial cells.

Dedifferentiation of Myocytes

The process of myocyte dedifferentiation has been studied in most detail during regeneration of LMB in the holothurians *E. fraudatrix* and *A. japonicus* [33, 55]. Following transection, cellular dedifferentiation and destruction is observed in the damaged area and some muscle cells undergo apoptosis (Fig. **5 (A-C)**). Some muscle cells undergo apoptosis. Two variations of this process have been documented; their incidence depends on the degree of damage to the muscle bundles and myocytes. If the damage is minor, myo-

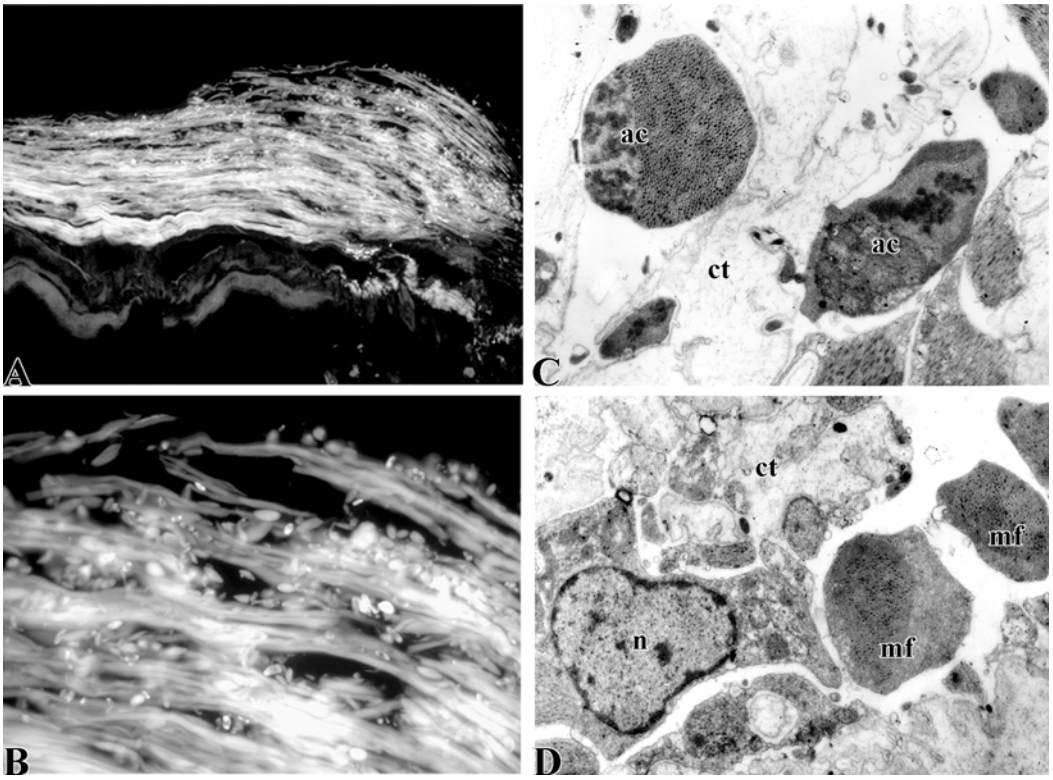

Fig. (5). Dedifferentiation of myocytes of somatic musculature. A. Large numbers of SLSs are found in the muscle stump 6-days following transection of longitudinal muscle band of *H. glaberrima*. B. Higher magnification shows the details of the SLSs among disorganized muscle fibers. C. Electron micrograph of apoptotic cells (ac) within in the connective tissue (ct) of the muscle stump 3-days after transection of longitudinal muscle band of *E. fraudatrix*. D. Electron micrograph of the myocyte with fragmented myofilament-containing cytoplasm (mf). Note activated nucleus (n) of the myocyte.

cyte dedifferentiation take place almost exactly as in myoepithelial cells of the mesothelium. SLS form at the periphery of the cell. First, a small SLSs appear, which then enlarge and finally occupy the bulk of the cell cytoplasm.

When major damages occur, there is no formation of SLSs. In these cases, the entire myofilament-containing cytoplasm is separated from the cell (Fig. **5 (D)**). Numerous microvesicles and plasmalemma invaginations appear in perinuclear zone. These vesicles fuse together to form a cytoplasmic membrane separating the nucleus with a small amount of cytoplasm from the myofilament-containing part of the cell. The latter then collapses forming several fragments that are taken up by amoebocytes.

During dedifferentiation, myocytes remain within the muscle bundle surrounded by the basal lamina. Activation of the nucleus occurs simultaneously with the destruction of the contractile apparatus. Chromatin is decondensed and a nucleolus appears. Organelles associated with protein synthesis develop within the cytoplasm, where we can see well-developed Golgi apparatus, cisterns of rough endoplasmic reticulum and numerous ribosomes and polysomes [55]. Later on, new bundles of myofilaments appear within the cells. The basal lamina of a muscle bundle is often retained and there are no signs of migration or proliferation, suggesting that the dedifferentiation of myocytes and their reverse myogenic specialization result in the restoration of the functions of the old muscle bundle rather than lead to the development of a new one.

In the crinoid arm it has been proposed that de-differentiated muscle cells become coelomocytes, however, the evidence for this transdifferentiation remains limited to light microscopical observations at various time points that suggest the transformation of cells into various lineages [36]. Dedifferentiated migrating cells might also form the blastemal cells although this has never been shown, and even if this is so, it is not clear what is their role or their final phenotype in the regenerating structure. Other dedifferentiated cells appear to remain within the coelomic epithelia. Some of these cells retain SLSs within their cytoplasm and remain connected to their neighbors in the coelomic epithelia with intercellular junctions. These cells have been proposed to migrate toward the regenerating structure as a continuous epithelial sheet rather than as individual cells [48, 58]. These dedifferentiated cells appear capable of undergoing mitotic divisions.

Do dedifferentiated contractile cells become the new myoepithelial cells or myocytes of the regenerated structure? There is not enough experimental evidence to answer this question. It is likely that some of the dedifferentiated myocytes are integrated into the migrating coelomic epithelium and this might give rise to new muscle cells. Particularly since it appears, as explained in the following section, that the new myocytes are formed from cells within the coelomic epithelium. The possibility that individual dedifferentiated cells that migrate within the connective tissue or coelomic cavities do become muscle is much harder to evaluate. There is no strong evidence that these cells actually reach the regenerating structure and no histological evidence whatsoever that these cells are capable of re-differentiating into or of integrating into the muscle layer or muscle bundles. In fact, Dubois and Ameye [37] doubt that this process occurs since it would imply that myocytes, which are surrounded by a basal lamina, would have to make this basal lamina *de novo*.

It is true that the temporal sequence, where de-differentiation precedes myogenesis, suggests some degree of dependence, where in order for myogenesis to occur, muscle de-differentiation needs to have occurred. Moreover, the formation of new muscle cells from the de-differentiated ones is possible, if one considers recent findings in other phyla. In the salamander *Ambyostoma mexicanum*

dedifferentiated blastemal cells in the regenerating limb were shown to retain a memory of their previous phenotype and dedifferentiated muscle cells were found to give rise solely to new muscle cells [60]. Nonetheless, other results suggest that in some cases muscle regeneration can occur in the absence of muscle dedifferentiation as is the case of the crinoid arm, where few signs of muscle de-differentiation are observed following amputation but a new arm is formed [61].

In summary, all echinoderms appear to have the capability of dedifferentiating their muscle cells. The mechanisms, involved in these processes, seem to be similar in all cases, and include elimination of contractile apparatus (the formation of SLS) and activation of the nucleus to prepare the cell toward proliferation or redifferentiation. An injury caused to the animal initiates dedifferentiation directly at the wounded area. However, later on cells of more remote areas can also be involved into this process. Obviously, destruction of tissues and dedifferentiation of numerous cells needs much energy and time, which decreases the rate of regeneration. In this connection, in the course of evolution the animals developed particular adaptations to minimize trauma caused by predators. One of such adaptation is autotomy. The mechanisms of autotomy appeared in crinoids in the late Paleozoic [62]. In crinoids no myocytes are present at the place of autotomy, therefore, if a part of an arm is removed no muscle is damaged [36]. This might be the reason for the absence of dedifferentiation during the arm regeneration in these animals following autotomy. However, this fact does not mean that the animals have no mechanisms of dedifferentiation. For example, as has been shown in the crinoid, when a more dramatic injury is produced, such as producing arm explants by the amputation of an arm section, then large rates of muscle dedifferentiation are observed along a gradient from the cut ends to the middle of the explant [36].

Myogenesis

All echinoderms have the capability to regenerate some or most organs or appendages. Since the regenerated structures are usually identical to the lost ones, including their muscular components, it has then been assumed that formation of new muscle cells or myogenesis occurs in all echinoderms. However, the cellular process by which myogenesis occurs have not always been well described or even studied. In fact, only in holothurians has the formation of the new muscle been well described. Initial studies by Dolmatov and colleagues [44, 45] show that in the regeneration of the somatic muscle of the body wall of *E. fraudatrix* and *A. japonicus*, the dedifferentiated coelomic epithelium at the lesion site forms deep furrows, which penetrate deeply into the underlying connective tissue; the submerged regions of the coelomic epithelium detach from the surface epithelium to close up and form tubular structures that eventually become new muscle bundles, whose cells develop myofilaments in their basal extensions and eventually become myocytes.

In visceral organs, where the muscle is part of the coelomic epithelium or mesothelium, the origin of the muscle appears to be the coelomic epithelium of the remaining organs. This was shown in the regenerating Cuvierian tubules of the holothurian *H. forskali* where Vandenspiegel and colleagues [63] showed that new myocytes originate from the mesothelial layer. In this system the cells also become myoepithelial as an initial step and then migrate into the connective tissue layer losing their apical attachments to other cells of the coelomic epithelium but remaining basally attached to the basal lamina. Similarly, the new muscle cells that appear in other regenerating viscera, such as the intestine and the respiratory trees in holothurians, also appear to originate from the coelomic epithelia, and ingress into the inside of the structure, differentiating into myocytes and forming the new muscle layers [15, 18, 48, 64]. A similar process gives rise to the mesenterial muscle layer following its dedifferentiation as the intestine regenerates [47].

A mesothelial origin of myocytes during regenerative phenomena is consistent with what has been proposed to be the embryological and evolutionary origin of myocytes as proposed by Rieger and Lombardi [9] and by Dolmatov's group [19, 31, 32, 65].

Visceral Musculature Regeneration

Information available to date suggests that the visceral muscle regenerates from the coelomic epithelial cells. This process, with few exceptions, occurs similarly in all organs [15, 18, 43, 48, 55, 63, 64]. Initially, as described above, dedifferentiated cells of the coelomic or water-vascular epithelia become flattened and migrate into the wound site. Cell dedifferentiation and migration are accompanied by mitotic divisions. Short bundles of intermediate filaments appear in the cytoplasm of the peritoneal cells. Myoepithelial cells start to repair their contractile apparatus (Fig. **6 (A)**). Small bundles of myofilaments appear in their basal region and processes. Later on the processes expand and connect to each other via desmosomes. After full dedifferentiation specializing peritoneal and myoepithelial cells remain attached by intercellular junctions on first steps of regeneration. Afterwards they lost connection and myoepithelial cells deep under peritoneocytes (Fig. **6 (B-I)**).

The visceral muscle of an organ often consists of several layers; the processes of myoepithelial cells are running in different directions, making up circular and longitudinal musculature. In this case muscle layers appear to arise differently in different organs. For example, during regeneration of Cuvierian tubules, longitudinal and circular muscles appear simultaneously at the 3rd stage [63]. During regeneration of respiratory trees, the circular musculature is first to be restored, while the longitudinal one appears 5-10 days later [48]. Some myoepithelial cells become submerged under the layer of circular muscles. Such cells form hemidesmosomal junctions with the basal lamina and there is progressive differentiation and an increase in the number of myofilaments. The development of longitudinal muscles is correlated with the formation of large bundles of nerve processes in the basiepithelial nerve plexus.

Muscle cells or cells in the process of forming the new muscle cells do not undergo cell division. In regenerating Cuvierian tubules, Vandenspiegel and colleagues [63] did not find labeled muscle cells after injecting tritiated thymidine, while other cellular types, including the undifferentiated cells and peritoneocytes were labeled. Similar results were obtained in the regenerating arm of crinoids, where animals were exposed to 2 hours of BrdU prior to sacrifice and most of the labeled cells were found within the blastema and overlying epithelium [66].

On the other hand, if BrdU pulse-chase experiments are done and enough time is allotted following BrdU injection for cells to undergo differentiation then at least some muscle cells do show BrdU incorporation. This was found in the regenerated intestine following 28 days after evisceration when BrdU was applied two weeks prior to sacrifice [18]. Labeled muscle cells were also found in the crinoid regenerating arm when animals were injected with BrdU, returned to the aquaria and allowed to complete the regenerative process [66]. In summary, smooth muscle cells appear to originate from coelomic epithelial cells that have the capacity to divide, but once muscle properties are acquired they loose the proliferation capacity.

Somatic Musculature Regeneration

The regeneration of somatic muscle has been best studied following transection of the body wall (which includes the LMB) in two holothurian species, *E. fraudatrix* and *A. japonicus* [33, 44, 45, 55]. Regeneration of LMB progresses as follows: One day after injury, the wound site begins filling with extracellular matrix. Simultaneously, the epithelization of the wound begins. Epidermal cells migrate across the surface of the connective tissue clot,

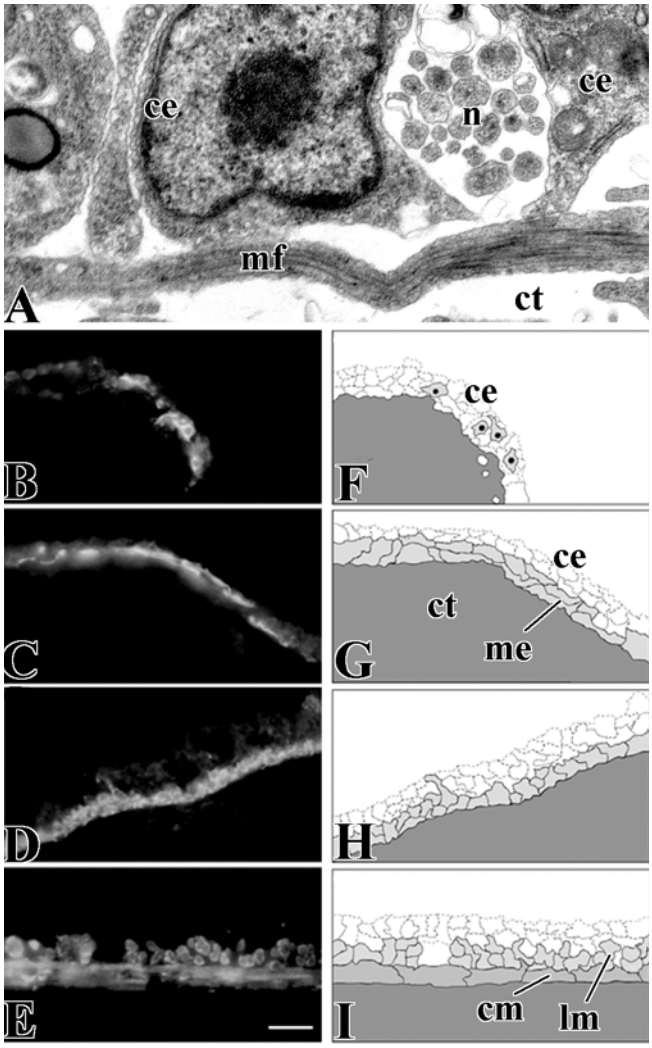

Fig. (6). Regeneration of visceral musculature. A. Electron micrograph of the differentiating myocyte situated under peritoneal cells (pc) in regenerating respiratory tree. Note bundle of nerve processes (n) and growing process of myocyte with bundle of myofilaments (mf). B-E. The process of muscle formation can be followed using a muscle-specific antibody in the regenerating intestine of *H. glaberrima*. Cells in the coelomic epithelium express the muscle epitope during the first week of regeneration (B, F). By the second week (C, G) myocytes (me) are found beneath the peritoneocytes (ce). By the third week (D, H) large numbers of myocytes are found although their orientation is not evident. Four weeks into regeneration (E, I) the two muscle layers, longitudinal (lm) and circular (cm) are evident.

forming a wound epidermis. By the 2-4th day, the wound is already entirely closed by the wound epidermis and connective tissue. Four days after the injury, some SLSs and myocyte fragments can be found within the extracellular matrix of LMB terminal areas. Destruction of muscle bundles continues up to 15-20th days, and occurs simultaneously with the process of restoration.

Regeneration of the muscle also begins around the 4th day following injury. The terminal areas of LMB are covered with flattened coelomic epithelium consisting of dedifferentiated peritoneal and myoepithelial cells. The amount of extracellular matrix beneath the epithelial cells increases. During the second week of regeneration new muscle bundles begin arising. Groups of coelomic epithelial cells sink into the connective tissue of the LMB anlagen (Fig. **7 (A)**). These cells loose their cilia and their centrioles move from the apical surface to the basal part of the cell. They then develop long processes directed into the underlying connective tissue. Intercellular junctions between the cells are retained, thus the process can be considered to be migration of groups of cells (epithelial morphogenesis). The differentiating cells contain nuclei with large nucleoli, and in their cytoplasm there are many free ribosomes and polysomes, numerous small vesicles, and scarce cisterns of rough endoplasmic reticulum. The first fine fibrillar material can be distinguished within the long processes directed into connective tissue and in the cytoplasm of already sunken myogenic cells. These are probably actin filaments. Then thick filaments begin arising in-between the thin ones. As the cells sink into the connective tissue, they produce basal lamina until eventually all muscle bundles become separated from connective tissue by a basal lamina.

Young muscle bundles represent spacious myocyte-lined cavities within the connective tissue. The myocytes are highly flattened and expanded along the longitudinal axis of the LMB; with very few myofilaments in their cytoplasm. There is no clear indication of what is contained in the internal area of the bundle as it appears empty in electronmicrographs. The earlier arising myocyte bundles are gradually sinking to the inside of the connective tissue anlage, being replaced by new groups of myogenic cells migrating down from the surface.

Neurons of basiepithelial nerve plexus of coelomic epithelium migrate into the muscle anlage together with epithelial cells. In their axons there are microtubules and neurofilaments, as well as numerous vesicles. Numerous processes of nerve cells were found also in the developing connective tissue.

The transformation of coelomic epithelial cells into myocytes occurs without mitotic divisions. The analysis of autoradiographic data suggested that thymidine was actively incorporated into the nuclei of epithelial cells, which is an indication of DNA synthesis [33, 67]. When the cells are sinking down into connective tissue of LMB anlage, the intensity of DNA synthesis decreases. No mitotic figures were observed as a new muscle was formed [33, 45].

Therefore, the proposed model for the formation of the regenerated musculature in the echinoderms begins with the coelomic epithelium. Some of the cells within this epithelium might originate from the dedifferentiated myoepithelial or muscle cells. The epithelium migrates as a sheet toward the regenerating structure and some of the cells can undergo mitosis, producing new cells. At the regeneration site, some of the coelomic epithelial cells ingress and as they detach from the overlying epithelium they also acquire the muscle phenotype. The final destination of these myogenic cells depend on whether the muscle layer is within the mesothelium, where the cells remain attached to the same basal lamina as the peritoneocytes, or whether the new myocytes completely detach from the epithelium and form muscle bundles surrounded by a basal lamina. There are some loose ends in this model that need to be studied. In particular the proposed outcome of undifferentiated cells or coelomocytes that might also originate from the dedifferentiated muscle cells. It has been proposed that some of these cells migrate into the regenerating structure and form part of the blastema. Now, whether they contribute to myogenesis and how this process might occur has not been shown.

Myocytes can also participate in the repair of muscle bundles [55]. As described above, myocytes don't form SLS after major damage. Their nuclei separate from the contractile apparatus and form myogenic cells. These cells then synthesize new bundles of myofilaments, repairing their contractile apparatus (Fig. **7 (B)**). There are no signs of migration or proliferation, suggesting that the dedifferentiation of myocytes and their reverse myogenic specialization result in the restoration of the functions of the old muscle

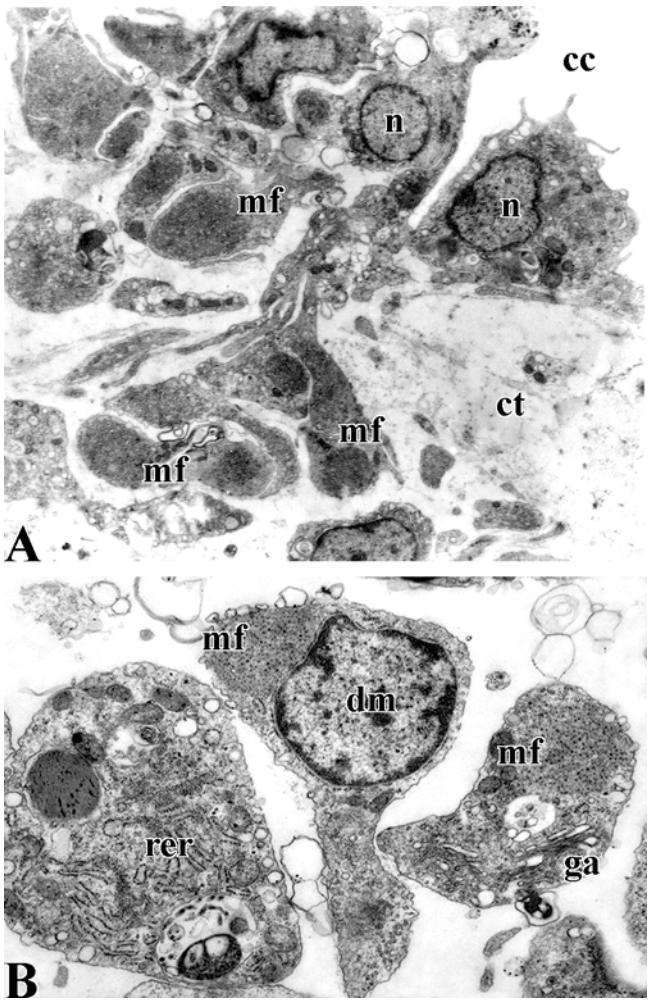

Fig. (7). Regeneration of somatic musculature. A. Electron micrograph of group of coelomic epithelial cells sinking into the connective tissue (ct) of the longitudinal muscle band of *E. fraudatrix*. Note cell nuclei (n) and long processes containing bundles of myofilaments (mf) adjacent to the coelomic cavity (cc). B. Electron micrograph of differentiating myocytes (dm). Their cytoplasm contains bundles of myofilaments (mf), and well-developed rough endoplasmic reticulum (rer) and Golgi apparatus (ga).

bundle rather than lead to the development of a new one. General schemes of the processes of muscle dedifferentiation and regeneration in echinoderms are represented on Fig. **8**.

As echinoderms and vertebrates are closely related taxa, it could be interesting to have a look, whether the myogenesis in these animals has anything in common. In vertebrates, starting from the fishes, the complexity of the structure and organization of contractile apparatus is much greater. They are characterized by multinuclear muscle fibers and development of a system of satellite cells. Thus, the myogenesis in these animals becomes much more complicated in terms of both ontogenesis and regeneration. However, certain evidence in favor of epithelial origin of vertebrate muscles is rather obviously pronounced in the course of embryogenesis [31]. Information is available that in mice somatic muscles of esophagus develop from smooth musculature [68, 69].

MOLECULAR ANALYSIS OF MUSCLE REGENERATION

While the cellular events associated with muscle regeneration in echinoderms have received some attention, little information is available on the molecular events that control the process.

The disappearance and re-expression of muscle-associated molecules, such as laminin and contractile proteins, have been used to analyze the process of dedifferentiation and myogenesis [18, 70]. Or otherwise, a 98-kDa cytoskeletal uncharacterized cytoskeletal protein that is expressed in the coelomic epithelia has been used to follow the transformation of the coelomic epithelial cells into muscle [45]. But these molecules have served more as markers of cellular phenotype rather than as contributors or modulators of the process itself.

Some general information on differential gene expression has recently been obtained for the complete process of intestinal regeneration [71], however, at present, it will be difficult to pinpoint which of these genes is specifically associated with muscle dedifferentiation or regeneration. Some hints might be obtained by focusing on genes known to be expressed by muscle cells. For example, various cytoskeletal genes show differential expression during regeneration; two actin genes are up-regulated while another actin gene and a myosin gene are down-regulated [71] suggesting that some of these genes might be associated with the initial dedifferentiation of muscle cells and the subsequent myogenesis. As has occurred with the histological-microscopy studies of organ regeneration that provided valuable information on the events of muscle regeneration, we expect these molecular analyses of organ regeneration to pave the way to more focused experiments where the molecular basis of the regeneration of different cellular phenotypes can be determined.

Pharmacological Studies of Muscle Regeneration

The effect of drugs or chemicals on echinoderm muscle regeneration is a chapter that still needs to be written. There is no in-depth study that might serve to address this topic. Experiments by Candia Carnevali and colleagues might provide some useful hints on the effect of some chemicals on muscle regeneration (Fig. **9**). They have focused on studying the effect of endocrine disrupter compounds (EDCs) on the regenerative capacity of crinoid arms [72-74]. In these experiments they have used both estrogenic contaminants such as polychlorinated biphenyl (PCB) as well as androgenic (triphenyltin-TPT, fenarimol-FEN) environmental contaminants [50, 73]. The results showed that the androgenic and estrogenic compounds increased recruitment and recycling of myocytes from the muscle bundles and massive migration of dedifferentiated cells in the coelomic fluids. Similar results were obtained with methyltestosterone. These effects were not strictly dose dependent, occurring mainly at medium and low doses but correlated with other effects observed on sea urchin and crinoid reproduction, in particular a reduced egg diameter. In additional experiments, two anti-androgenic compounds with somewhat different modes of action were studied; 1,1-dichloro-2,2-bis-p-chlorophenyl ethylene (p,p'-DDE) and cyproterone acetate (CPA) [74]. Although, upon short and long term exposure, both caused myocyte dedifferentiation at the level of the stump and also at distances remote to the amputation level, they showed differences on their effects to cell division and overall growth of the regenerate. DDE had some effect at 3 days decreasing cell division only at the highest dose but no overall effect on the size of the regenerate at 2 weeks. On the other hand CPA caused a dose dependent decrease in cell division at 1week and a decrease in the regenerate length at 2 week with all doses. Thus, it appears that while most EDCs accelerate the pace of muscle dedifferentiation, they have differential effects on the completion of the regeneration process be it by affecting cell division or the eventual differentiation of cells into new muscle.

Nonetheless these experiments, like many other *in vivo* experiments, present the problem of not knowing whether the effect is a direct effect on the muscle cells or an indirect effect at some of other level of regeneration control. Thus, it will remain to future investigators, when cultures of echinoderm cell types are available,

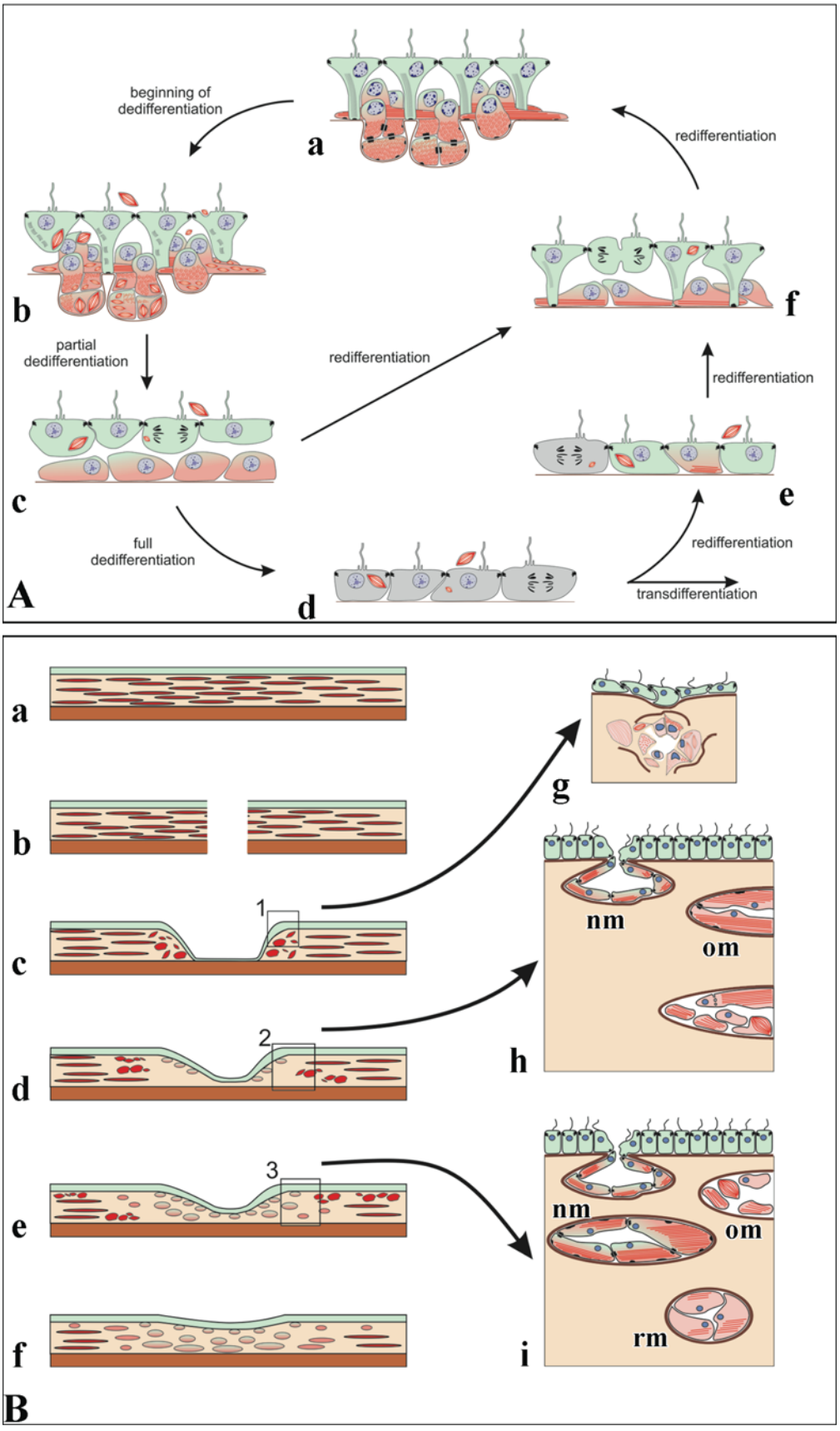

Fig. (8). Schemes of dedifferentiation and regeneration of muscle system in echinoderms. A. Transformation of the coelomic epithelium during regeneration. a. Organization of the coelomic epithelium. This epithelium is formed of ciliated peritoneal cells with bundles of intermediate filaments (green) and groups of myoepithelial cells (green-reddish) with myofilaments (red spots and lines). b. Dedifferentiation of the coelomic epithelial cells begins just after damaging.

Bundles of intermediate filaments are destroyed, peritoneal cells loose connection with basal lamina (brown). Spindle-like structures are formed in myoepithelial cells. Some of them are exocytosed into the coelomic cavity or endocytosed by peritoneal cells. c. Partial dedifferentiation of the coelomic epithelial cells. (As occurs during regeneration of respiratory trees in holothurians.) Dedifferentiated peritoneal cells have lost connections with basal lamina but remain attached to other by cell junctions. These cells can undergo mitosis. Dedifferentiated myoepithelial cells form a mesenchymal layer under the peritoneal cells. These cells do not contain myofilaments and do not divide. d. Full dedifferentiation of coelomic epithelium. (As occurs during gut regeneration in holothurians.) Peritoneal and myoepithelial cells intermingle to form a single epithelium on basal lamina. Cells of the epithelium divide mitotically. e. Beginning of redifferentiation. Some cells give rise to peritoneal cells and other - myoepithelial cells. Cytoplasm of the latter contains small bundles of myofilaments. f. Redifferentiation of the coelomic epithelium. Peritoneal cells form basal processes and attach to basal lamina by hemidesmosomes. Their cytoplasm contains small bundles of intermediate filaments. Myoepithelial cells are situated under peritoneal cells and develop long myofilament- containing processes.

B. Regeneration of longitudinal muscle band in holothurians. a. Undamaged muscle band composed of bundles of muscles cells (red) and covered by coelomic epithelium containing only peritoneal cells (green). Muscle band is situated on connective tissue layer (brown) of body wall. b. Muscle band following transection. c. Beginning of regeneration. Coelomic epithelium migrates to wound region and covers it. Damaged myocytes are destroyed. d. Formation of new muscle bundles (green-reddish ovals). Groups of peritoneal cells sink into connective tissue and form muscle bundle. Their cytoplasm contains myofilaments. Concurrently, destruction of several muscle bundles in the wound region continues. Myocytes dedifferentiate. They shed myofilament-containing cytoplasm and the cell transforms into a myoblast. e. Advanced stage of regeneration. Coelomic epithelium continues formatting new muscle bundles. Concurrently, groups of myoblasts begin to form new muscle bundle (reddish oval). f. Regenerated muscle band contains old bundles (red) and new bundles develop from coelomic epithelium (green-reddish ovals) so dedifferentiated myocytes (reddish ovals). g. Higher magnification of region 1 shows destruction of muscle bundles. h. Higher magnification of region 2 shows formation of new muscle bundle (nm) and old muscle bundles (om) with dedifferentiating myocytes. i. Higher magnification of region 3 shows formation of new muscle bundle (nm), old muscle bundles (om) with dedifferentiating myocytes, and muscle bundle regenerated from dedifferentiated myocytes (rm).

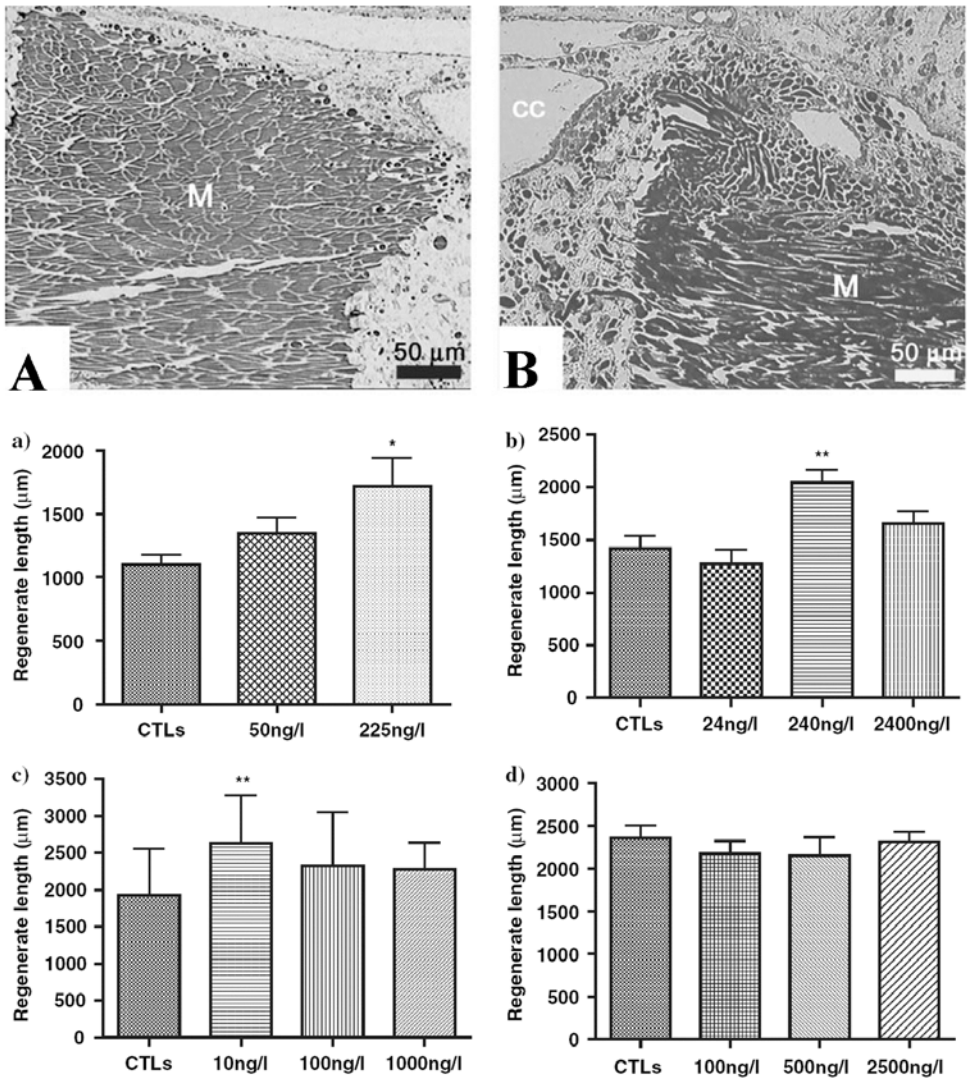

Fig. (9). Muscle regeneration in crinoids. A. Semi thin section of normal muscle (M) in crinoid arm. B. Dedifferentiating muscle in crinoid arm subjected to 2.5 µg/ml of the antiandrogenic drug DDE for 72 hrs. Muscle dedifferentiation is increased in the presence of various drugs (mainly endocrine disruptors). The drugs used were: (a) Triphenyltin (b) fenarimol (c) methyl-testosterone and (d) p, p'-DDE. Increases in muscle dedifferentiation cause a decrease in the length of the regenerating arm length as observed by the effect of various doses of the endocrine disruptor drugs (modified from Sugni *et al*. 2007, 2008).

or when specific cell types *in vivo* can be targeted to define the pathway of action of the chemicals.

CONCLUSIONS

We have now brought together the available information on muscle de-differentiation and regeneration in echinoderms. As is evident, these organisms present interesting model systems with a high potential for muscle regeneration studies. Their close phylogenetic relation to vertebrates and their high regenerative capacities makes them attractive models to determine what cellular and molecular processes are required for successful muscle regeneration to occur. The cellular process has been well described in a variety of species. However, there is an enormous need for studies at the molecular level in order to determine the molecules and genes that are responsible for the de-differentiation of muscle cells and for the myogenic potential of cell that become the new muscle in regenerating structures.

The potential of using echinoderms for many other biomedical applications remains largely unexploited. Initial experiments have been done focusing on effects of environmental contaminants. However, other areas of great importance such as the molecular basis of muscle cell de-differentiation and differentiation remain open to the use of echinoderm models. In view of the dramatic de-differentiation process that appears to occur in the muscle system of all echinoderm species studied, these animals might provide important clues to the de-differentiation process, a process that appears to be more restricted in other organisms. Similarly, the well-described cellular events by which echinoderms regenerate their muscle systems provide the basis to explore the molecular events directing muscle formation and differentiation. Thus, we expect that this review will serve to entice researchers into exploiting echinoderm model systems for in depth biomedical studies in muscle development and regeneration.

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ABBREVIATIONS

BrdU	=	5-Bromo-2-deoxyuridine
CPA	=	Cyproterone acetate
DDE	=	1,1-Dichloro-2,2-bis-p-chlorophenyl ethylene
EDC	=	Endocrine disrupter compound
FEN	=	Fenarimol
LMB	=	Longitudinal muscle band
PCB	=	Polychlorinated biphenyl
SLS	=	Spindle-like structure
TPT	=	Triphenyltin

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