Wound Healing in Jellyfish Striated Muscle Involves Rapid Switching between Two Modes of Cell Motility and a Change in the Source of Regulatory Calcium

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Small wounds (1.2 mm in diameter) made in the sheet of myoepithelial cells forming the “swimming” muscle of the jellyfish, *Polyorchis penicillatus*, were closed within 10 h by epithelial cells migrating centripetally to the wound center. Some 24 to 48 h later these cells redifferentiated into fully contractile muscle cells. Labeling with bromodeoxyuridine failed to reveal any cell proliferation during this process. Phenotype switching (within 1 h) from contractile muscle cells to migratory cells did not require synthesis of new protein as shown by treatment with 40 μM cycloheximide. Excitation–contraction coupling in undamaged muscle depended on entry of Ca\(^{2+}\) through voltage-gated ion channels, as shown by a block of contractility by 40 μM nitrendipine and also on calcium released from intracellular stores since caffeine (10 mM) caused a 25% reduction in contractile force. In contrast, migratory cells did not require a source of extracellular calcium since migration was unimpeded by low (1 mM) free Ca\(^{2+}\) or nitrendipine. Instead, modulatory calcium was derived from intracellular stores since caffeine (10 mM) and thapsigargin (10 μM) slowed migration. This lack of dependence on calcium influx in migratory cells was further confirmed by a dramatic down-regulation in voltage-gated inward current as shown by whole-cell patch recordings.

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Key Words: wound healing; striated muscles; cell motility; calcium; jellyfish.

INTRODUCTION

It has been known for over a century that jellyfish are capable of rapid wound healing without the formation of visible scar tissue, even when such a differentiated tissue as striated muscle is involved (Romanes, 1876, 1877, 1880). More recently, striated muscle cells from hydrozoan jellyfish have been shown to be capable of migration and trans-differentiation into neurons under specific conditions (Schmid et al., 1976; Schmid, 1992; Schmid and Reber-Müller, 1995). This led us to the supposition that scarless wound healing might occur if striated muscle cells were able to transform into migratory cells to cover the wound and then redifferentiate into contractile muscle cells. It also seemed likely that this kind of wound healing did not require cell proliferation from stem cells and only involved one cell type which is very different from both skeletal and cardiac muscle (Schaffer and Nanney, 1996; Witte and Barbul, 1997; Megeney et al., 1996; Schultz, 1996; Weber et al., 1996a). If phenotype switching proved to be the mechanism for wound healing in the jellyfish striated muscle layer then it would be interesting to examine the way calcium regulates these two forms of cell motility. We therefore developed a wounding procedure that allowed us to follow both the process of wound healing and the regulation of two forms of motility by calcium in the hydrozoan jellyfish, *Polyorchis penicillatus*.

The striated muscle sheets of the hydromedusa *Polyorchis* are analogous to the skeletal muscles of vertebrates since they act against a skeletal element, the mesoglea or jelly, and are responsible for swimming. Analogies can also be drawn to cardiac muscle of vertebrates as both the jellyfish bell and the heart are fluid pumps (Frye, 1987). Contraction of the “swimming” muscle of medusae is phasic, unlike all other muscles in this animal. Indeed swimming muscle is the only striated muscle known in...
this phylum, the Cnidaria (Hyman, 1940). The rapid onset of contraction was believed to be a consequence of the rapid influx of Ca\(^{2+}\) from the extracellular space via voltage-gated calcium channels (Spencer and Satterlie, 1981). In this study we wanted to see if this type of twitch motility also required contribution from intracellular stores of Ca\(^{2+}\), which could also act as local Ca\(^{2+}\) sinks. This proved to be the case. After muscle cells have dedifferentiated and lost their contractility to become migratory cells we were interested in knowing whether the source of calcium for regulation of lamellipodial locomotion had also changed. This was evaluated by examining the effects of a calcium channel blocker (nitrendipine) and of Ca\(^{2+}\) store modulators (caffeine and thapsigargin) on migration as well as the efficacy of Ca\(^{2+}\) entry through voltage-gated (Vg) ion channels using whole-cell patch recordings. We were able to establish that migrating cells down-regulated Vg Ca\(^{2+}\) channels and utilized intracellular Ca\(^{2+}\) for regulation of motility. Other requirements for both phenotype switching and migration were also determined, including protein synthesis and actin polymerization.

**MATERIALS AND METHODS**

Medusae of *P. penicillatus* were collected from Bamfield Inlet or Pachena Bay, near Bamfield, British Columbia, Canada, and held in aquaria with running seawater at 9–12°C.

**Field Stimulation of Muscle Strips**

Preliminary studies indicated that muscle strips from the subumbrella (internal bell lining) and velum had similar pharmacologies. Due to the thickness and elastic property of mesoglea in the bell region, recordings from muscle strips obtained from this region were extremely variable and it was difficult to measure contractile amplitude. Therefore, only muscle strips from vela were used in this study. The vela of medusae, anesthetized in 1:1 isotonic MgCl\(_2\) (0.33 M) and artificial seawater (ASW), were excised so as to provide continuous strips of maximal width. To avoid any contamination by nervous tissue, each velar strip was bisected lengthwise into two strips and only strips free from nerve-ring tissue were used in the study. The free ends of each velar strip were pinned to the Sylgard base of a 35-mm petri dish containing a pair of embedded Ag/AgCl\(_2\) stimulating electrodes connected to a Grass S44 stimulator. The velar strip ran between the two stimulating electrodes and around a small hook attached to an isometric force transducer (Kent Scientific Corp.). Tension on the strip was adjusted using a micro-manipulator so as to remove any slack in the preparation and to maximize tension excursions. The threshold for contraction was determined by increasing the stimulation voltage until a measurable contraction occurred. The frequency of stimulation was 0.2 Hz with each square pulse having a duration of 30 ms. The rate of perfusion was controlled by a peristaltic pump at 1.5 ml/min and the perfusate was removed by a vacuum pump. All perfusion solutions were kept at 12–14°C during experiments by running the perfusion tubing through an ice bucket. The transduced tension was recorded on a digital Dash-IV pen recorder (Astro-Med, Inc.). The amplitude of contractile tension for each condition (control, drug effect, and washed) was calculated by averaging 10 contractions from each preparation.

**Wounding Procedure**

A radial bisection of the bell of medusae exposed the subumbrellar striated muscle sheets and a Pasteur pipette was used to mark an area 1.2 mm in diameter in each quadrant for cell removal. A surgical blade was used to gently scrape away muscle cells within the marked area. As many as three wounds could be created in each jellyfish. Digital images of the wound-healing process were acquired through a JVC CCD video camera mounted on a dissecting microscope using SigmaScan image software (Jandel Scientific, Inc.). The speed of migration was calculated by measuring movement of the cell front at 3, 4, and 5 h after wounding. Jellyfish were immersed in ASW, pH 7.5, at 10–12°C for all experiments.

**Electron Microscopy and Laser Confocal Microscopy**

For transmission electron microscopy, jellyfish were fixed with 4% paraformaldehyde and 2% glutaraldehyde in phosphate-buffered saline (pH 7.5) and postfixed with 1% osmium tetroxide and processed as described previously (Spencer, 1979). For scanning electron microscopy, jellyfish were fixed with 0.01% osmium tetroxide in ASW, dehydrated, coated with gold, and examined using a JEOL scanning electron microscope. For laser scanning confocal microscopy, whole jellyfish were fixed with 4% paraformaldehyde in ASW and washed with PBS, and each quadrant with a wound in the center was excised and double stained with BODIPY FL phalloidin and propidium iodide (Molecular Probes, Inc.).

**Bromodeoxyuridine Labeling**

Immediately after wounding, whole jellyfish were incubated in 1 mM bromodeoxyuridine (BrdU) in ASW (Plickert and Kroicher, 1988) for 72 h and the BrdU solution was exchanged every 24 h. Following incubation specimens were fixed in 4% paraformaldehyde in ASW, acid treated, and then immunodetected following the manufacturer’s protocol (Cell Proliferation Detecting Kit, Boehringer Mannheim).

**Requirement of Protein Synthesis during Wound Healing**

To test whether initiation of cell migration requires protein synthesis whole jellyfish were treated with 40 μM cycloheximide 4 h before wounding. Jellyfish were bisected, wounded as described in the above section, and incubated in cycloheximide for another 6 h. At the end of the treatment, jellyfish were cut into quadrants with each wound at the center of each quadrant. Quadrants from the same jellyfish were used for both actin staining and the potassium depolarization experiments. The control for these experiments was ASW lacking cycloheximide.

To examine if functional recovery of contractility requires protein synthesis, jellyfish were wounded as described before and cycloheximide was added at 6 h after wounding. Jellyfish were incubated in cycloheximide for 24 h at 12°C and then cut into quadrants for actin staining and potassium depolarization experiments. In experiments in which cycloheximide and actinomycin D were removed after treatment, jellyfish were thoroughly washed in running seawater and kept in separated beakers with running seawater for a further 42 h. After this recovery period, jellyfish were processed as described above. A diagram of the experimental design is given in Fig. 7g.
Potassium Depolarization of Migrating Muscle Cells and the Healed Region

Quadrants of jellyfish with wounds in their centers were pinned to the Sylgard base of a 35-mm petri dish. Patch-clamp electrodes filled with 100 mM KCl were attached by Tygon tubing to a 1-ml plastic syringe. A micromanipulator was used to position the electrode just above the wounded region (within approx 200 μm) and a positive pressure applied to release a small amount of KCl locally. Slight negative pressure was applied before the electrode was advanced toward the tissue surface to prevent KCl leaking prematurely. Muscle contractions in response to the each KCl pulse could be easily observed under the dissecting microscope. As a control, ASW was ejected, which never caused muscle contraction.

Cell Dissociation and Electrophysiology

The experiments to examine dye coupling and electrophysiology in migrating cells were carried out on explants so as to reduce the thickness of the mesoglea to allow the use of an inverted microscope. Migrating cells were prepared by grafting a piece of jellyfish muscle, which was isolated by mechanically stripping muscles off mesoglea with a pair of sharp forceps without enzyme digestion, onto a mesoglea-coated petri dish and gently pressing the graft with a coverslip [Schmid and Bally, 1988]. Mesoglea was prepared with Ca2+/Mg2+-free seawater and then washed in distilled water [Freeman, 1981]. This sandwich was kept immersed in ASW at 12°C before recording. Recordings were made within 6–12 h after grafting when muscle cells migrated onto the mesoglea and formed a monolayer. Single-cell recordings were achieved by selectively removing unwanted cells. Dye coupling was evaluated by adding 1 mM Lucifer yellow to the recording electrode solution and iontophoresing it with repetitive hyperpolarizing current injection. Single cells used for intact muscle cell recordings were obtained by selectively removing the subumbrellar areas from the whole jellyfish and digesting with 1 mg/ml Pronase (Boehringer Mannheim) in ASW at 20 min. A high digestion temperature was chosen as it is premature. Muscle contractions in response to the each KCl pulse was kept immersed in ASW at 12°C with resistances of 2 MΩ.

Motility in Undamaged Muscle

The subumbrellar muscle sheets of the jellyfish P. penicillatus are formed by a layer of mononucleated, striated muscle cells, about 10 μm thick (Fig. 1a). These myoepithelial cells are the only cell type present in the muscle sheet, except for the extreme periphery where the muscle sheets are innervated by motor processes [Lin et al., 2000].
FIG. 1. Muscle contraction requires extracellular calcium. (a) TEM micrograph of striated muscle from the jellyfish *P. penicillatus*. M, myofibers; N, nucleus; Mi, mitochondria; Me, mesoglea. Scale bar, 1 μm. (b) Trace from force transducer of stimulated muscle contractions and the inhibitory effect of addition of 40 μM nitrendipine in ASW (closed arrow) and subsequent wash (open arrow). (c) The maximal contractile force generated with each contraction when treated with the drugs shown compared with the control in artificial seawater (ASW). The absence of contractions with nitrendipine and low-calcium seawater indicates a requirement for an external source of Ca²⁺. The amplitude of contractile tension for each condition (control, drug effect, and washed) was calculated by averaging 10 contractions from each preparation. Note that 0.2% DMSO served as a carrier control for nitrendipine. Significant differences are indicated by asterisks (ANOVA test followed by PLSD test, *P < 0.05*). *N* = 9 for each treatment group.
Myoepithelial cells are polarized into an apical, somal region and a basal region of muscle "feet" containing the contractile myofibrillar apparatus [Singla, 1978; Spencer, 1979; and Fig. 1a]. Muscle feet of neighboring cells interdigitate and are connected end-on by desmosomes. They attach to the underlying mesoglea (jelly) by hemidesmosomes and are aligned concentrically so that when contracted they reduce the internal diameter of the bell cavity to expel water out of the bell opening. Gap junctions are present between myoepithelial cells [Spencer, 1979] mostly in the "neck" joining the somata to the "feet" but also on the feet themselves [this study]. These junctions provide the electrical continuity required for myoid conduction [Spencer and Satterlie, 1981; Satterlie and Spencer, 1983]. Despite its morphological polarization, jellyfish swimming muscle has structural similarities to vertebrate cardiac muscle.
muscle, such as striations, gap junctions, and desmosomal connections. These similarities are presumably a result of convergent evolution to create tissues adapted for repetitive contraction of a fluid pump.

Swimming involves rhythmic, synchronous, and phasic contraction of all four muscle sheets making up the subumbrellar surface with a 1:1 relationship between motor action potentials and muscle contractions (Spencer, 1982). It is possible to mimic these phasic contractions using field stimulation of isolated muscle strips and recording the muscle tension produced by each contraction [Fig. 1c]. We were able to confirm previous studies [Spencer and Satterlie, 1981; Spencer, 1982; Satterlie and Spencer, 1983], which suggested that muscle contraction requires the presence of calcium in the surrounding medium. In this study we show that this calcium enters through voltage-gated calcium channels since nifedipine at 40 μM [L-type Ca²⁺ channel blocker] and low calcium [approx 1 μM] caused complete but reversible contraction block [Fig. 1c]. However, intracellular calcium stores are also likely to be involved in excitation contraction coupling as caffeine [10 mM] caused a 25% reduction in contractile force [Fig. 1c]. Once again much of this physiology is reminiscent of that found in vertebrate myocardium where contraction is rhythmic and excitation–contraction coupling involves influx of extracellular calcium through voltage-gated ion channels augmented to varying degrees by calcium-induced calcium release from intracellular stores [Bers, 1991].

Despite the large amount of information regarding regeneration and morphogenesis in cnidarians, particularly in Hydra [Hyman, 1940; Tardent, 1965; Gierer et al., 1972; Bode et al., 1988; Shimizu et al., 1993; Shimizu and Sugiyama, 1993], the cellular mechanism regulating the wound-healing process is still not clear [Campbell and Bibb, 1970; Bibb and Campbell, 1973; Campbell 1985].

Preliminary observations revealed that wound healing in swimming muscle of Polyorchis involved global phenotype transformations in the wound region. To examine this process we made circular wounds of about 1.2 mm in diameter in the center of a swimming muscle sheet by scraping off the muscle sheet from the mesoglea [Fig. 2]. We then allowed the animals to recover in artificial seawater, and over the next 96 h the healing process was monitored using several parallel techniques: light microscopy, SEM, and anti-actin labeling [Fig. 3].

Figure 3a illustrates an intact myoepithelial cell with two muscle feet (there can be as many as four feet), containing the contractile apparatus, projecting from the soma. Adjacent somata formed a homogeneous, hexagonally packed, epithelial pavement facing the seawater [Fig. 3b]. Interdigitalization of the muscle feet was sufficiently exact to form an uninterrupted monolayer of muscle fibers which could be visualized as parallel fiber arrays using phalloidin labeling of the actin filaments [Fig. 3c].

Within 1 h after wounding, undamaged striated muscle cells at the margin of the wound flattened and lost their polarization as muscle feet were withdrawn into the soma [Fig. 3d]. The neck, which connects the soma to the feet was lost as the cells began to flatten. Ruffled lamellipodial projections formed at the margin of cells that faced the wound [Fig. 3e]. These lamellipodia-bearing cells then migrated in concert across the vacant mesogleal surface toward the wound center. The centripetally migrating cell front was followed by several rows of submarginal cells whose lamellipodia were tucked under the row in front. Myofibrils lost their parallel alignment by shortening and condensing, eventually wrapping around the nucleus [Fig. 3f]. We were interested to know whether this condensed myofibrillar material had retained its contractility in migrating cells. We challenged the migrating cells with a

**FIG. 3.** Changes in cell morphology during the wound healing process in jellyfish striated muscle cells. [a] Drawing of a striated muscle cell from undamaged tissue. [b] SEM image of an undamaged muscle sheet indicates their polarized morphology with a superficial layer of somata apparent and a deeper layer of attached contractile feet being hidden. [c] Confocal image of undamaged muscle cells showing the ordered arrangement of striated myofibers stained by BODIPY-conjugated phalloidin (green) and nuclei stained with propidium iodide (red). [d] Drawing of a lamellipodia-bearing cell at the migratory front. [e] SEM image of cells at the migrating wound margin 1 h after wounding showing ruffled lamellipodia at the leading edge. [f] Confocal image taken 1 h after wounding showing that muscle feet were retracted at the wound margin. Myofibers lose their alignment and envelop the nuclei. [g] Drawing of an isolated migrating cell. [h and i] SEM and fluorescent images of isolated migrating cells [4 h after wounding] showing the loose arrangement of actin filaments in the ruffled lamellipodium and the nonfunctional myofibrillar apparatus wrapped around the nucleus. [j] Drawing of a flattened, migrating cell. [k] SEM image of flattened migrating cells as the wound approaches closure 6 h after wounding. [l] Migrating cells at the same stage as [k] showing the contractile apparatus bundled around the nuclei. [m, n, and o] Drawing and SEM and fluorescent images of cells just after wound closure (18 h after wounding) as they begin to redifferentiate by rounding up, thickening, and reorganizing the muscle feet. [p and q] Drawing and SEM image of a healed area 60 h after wounding showing that cells are no longer elevated. [r] Fluorescent image at the same stage as [q] showing that myofibers have reextended but they are not yet completely realigned. All pairs of images are of different cells or cell populations but they were fixed at the same time after wounding. Scale bars are 10 μm for all images.
depolarizing stimulus by addition of 100 mM KCl using a patch-clamp electrode [see Materials and Methods]. Such a stimulus failed to elicit contractions from migrating cells but did cause contractions of intact muscle cells.

Cells normally migrated as a continuous sheet, but occasionally some cells at the migrating edge broke free, revealing their individual morphology, which resembled migrating keratocytes [Figs. 3g and 3h]. These keratocyte-like cells contained radially arranged actin spikes [Theriot and Mitchison, 1991] at the lamellipodial margins in addition to the condensed actin surrounding the nucleus [Fig. 3i]. When the migrating front converged in the center of the denuded area, cell migration stopped, with the whole area becoming covered by flattened cells, some 3 to 4 μm thick [Figs. 3j and 3k]. At this stage there was maximal condensation of actin into bands around the nucleus [Fig. 3l]. After migrating cells met at the center of the wound, they began to repolarize with the somata elevating and separating from the muscular feet which were simultaneously extending [Figs. 3m–3o]. Finally, muscle feet became fully extended and the apical region of somata regained their squamous appearance, returning the tissue to its undamaged state [Figs. 3p–3r]. It took about 8 to 10 h for muscle cells to migrate and cover a denuded mesogleal surface 1.2 mm in diameter and another 24 to 48 h for the migrating cells to repolarize and recover contractility.

Many similar changes in cellular morphology have been seen during reepithelialization in vertebrates, which also involves cellular dedifferentiation, migration, and redifferentiation, though there is an additional process of proliferation [Stenn and DePalma, 1988]. At the border of the wound, cells are induced to migrate and express new integrins [Cavani et al., 1993]. Epithelial cells first retract tonofilaments and dissolve hemidesmosomes and desmosomes, which link them to basal lamina and each other, respectively. At the same time, they lose their cuboidal, polarized shape, flatten out, and form lamellipodia with actin filaments on the side facing the wound to provide the motor apparatus for migration [Gabbiani et al., 1978].

Since gap junctions are involved in myoid conduction in undamaged muscle [Satterlie and Spencer, 1983], we examined if gap junctions were maintained in migrating muscle sheets. We explanted small muscle strips onto mesoglea and allowed cells to migrate outward for 6 to 8 h [Fig. 4a] and then injected Lucifer yellow into a single cell [arrowed in Figs. 4a and 4b]. Migrating muscle cells appeared to maintain their gap junction connections as there was dye coupling of neighboring cells [Fig. 4b]. Transmission electron micrographs of migrating cells also revealed the presence of gap junctions [Fig. 4c], which suggested the involvement of gap junctions in coordinating migration. Migration as a sheet of cells has also been reported for reepithelialization in vertebrate wound healing [Stenn and DePalma, 1988], and gap junctions are retained between migrating epidermal keratinocytes and cornenal keratocytes [Gabbiani et al., 1978; Watsky, 1995a]. It is believed that maintenance of gap junctions is required for synchronizing locomotion of individual cells [Stenn and DePalma, 1988].

In most systems studied to date, wound healing involves the participation of myofibroblasts, which are the contractile elements used to bring the wounded margins together and reduce wound size [Gabbiani, 1998]. However, in this jellyfish, migrating muscle cells themselves are capable of exerting tonic traction forces during both migration and repositioning of muscle feet. Figure 2 shows that the force generated by migrating muscle cells created strain marks in undamaged areas. These strain marks ran parallel to the long axis of the muscle feet. Due to the pliability of the mesoglea [Schmid et al., 1976], the tonic forces generated during lamellipodial locomotion could help to close the wound by reducing the area that needs to be covered by migrating cells.

In many vertebrate systems, wound closure is achieved by both cell proliferation and the forces generated by myofibroblasts [Gabbiani, 1998]. However, in adult mammalian striated muscles [skeletal and cardiac] the majority of cells are terminally differentiated and are irreversibly withdrawn from the cell cycle [Zak, 1974; Nadal-Ginard, 1978], so they cannot undergo dedifferentiation, proliferation, and migration. Instead, several other cell types, including inflammatory cells, fibroblasts, and endothelial cells, are responsible for migration, proliferation, differentiation, and matrix remodeling [Schaffer and Nanney, 1996; Witte and Barbul, 1997]. In adult skeletal muscle, a small pool of quiescent, undifferentiated myogenic stem cells, the satellite cells, can proliferate, differentiate, and fuse into multinucleated myofibers to restore muscle function [Megeney et al., 1996; Schultz, 1996]. In cardiac tissue no such undifferentiated stem cell population exists and necrosis leads to a functional loss after healing [Weber et al., 1996b].

Although closure of the wound in jellyfish muscle appeared to be achieved by muscle cell migration and mesogleal retraction, we needed to determine if cell proliferation was also involved. Bromodeoxyuridine has been used to label mitotic cells in several cnidarians [Plickert and Kroiber, 1988]. During wound healing we treated whole jellyfish with 1 mM BrdU for 72 h, and no labeling was seen in the wounded muscle region of six treated jellyfish with 1 mM BrdU for 72 h, and no labeling was seen in the wounded muscle region of six treated jellyfish [Fig. 5]. In contrast, cell proliferation was detected at the bases of tentacles where no wound healing was occurring. This suggested that, unlike vertebrates [Lawrence, 1998], mitosis is not necessary for closure of small wounds; however, we have not examined whether cell proliferation is used to cover large wounds.

Initiation of migration and dedifferentiation by wounding might be a consequence of disruption of cell–cell junctional proteins, since this is known to evoke a cascade of cellular events [Clark and Brugge, 1995; Schwartz et al., 1995]. The precise signal that induces cells to become migratory is not known, although a change in the concentration of extracellular calcium is known to be associated with the beginning of migration during reepithelialization [Grzesiak and
Cell migration requires sophisticated regulation of the interaction between the cytoskeleton and the extracellular matrix, which is mediated by integrin receptors (Hynes, 1992; Huttenlocher et al., 1996). The precise nature of integrins in cnidarians is unknown, nevertheless an anti-human, H9252-integrin antibody shows cross-reactivity with neurons in the jellyfish Turritopsis nutricula (Piraino et al., 1996).

To determine whether transformation of contractile muscle cells to a migratory phenotype required synthesis of new proteins, we treated whole jellyfish with 40 μM cycloheximide 4 h before inflicting the wound. Cycloheximide did not significantly affect the ability of muscle cells to become migratory (Fig. 6e) nor the morphology of migratory cells (Figs. 7a and 7b). These data suggest that phenotype switching to a migratory state does not require the synthesis of new proteins. Transformation from contractile to migratory cells uses existing structures in the cells and presumably involves depolymerization and polymerization of cytoskeletal elements. We regard this transformation as dedifferentiation. However, if jellyfish were treated with cycloheximide (40 μM) or actinomycin D (10 μM, data not shown) 6 h after the wound was inflicted, which was approx 4 h before wound closure, redifferentiation did not take place. Migratory cells remained flat and muscle feet were not reextended after treatment for 24 h with either of the above drugs. Reorganization and alignment of sarcomeres did take place in controls when these drugs were not added (Figs. 7c and 7d). We did not use treatment times longer than 24 h since tissues deteriorated after 36 h. When cycloheximide (but not actinomycin D) was removed after 24 h treatment (approx 30 h after wounding) by washing in running seawater, cells redifferentiated to a fully polarized state, with realigned myofibers being seen some 72 h after wounding (Figs. 7e and 7f). Together these data suggest that redifferentiation does require protein synthesis. Since the onset of migration in jellyfish striated muscle cells does not require synthesis of new proteins it is likely that the receptors used for extracellular matrix attachment in intact muscle cells might also be used for cell migration.

**Requirements for the Migratory Phase**

The cytoskeleton plays a major role in cell migration, and in most cases, cell migration depends on actin-based, calcium-dependent, actin polymerization and depolymerization events [Mitchison and Cramer, 1996]. Therefore we developed an assay for migration rate during wound healing to evaluate whether locomotion in jellyfish migratory cells used similar machinery. Wounds of 1.2 mm diameter were made as for the previous section and the rate of wound closure was measured by photographing the position of the wound margin at 1-h intervals for up to 12 h after wounding (Fig. 6). From the previous experiments it seemed likely that cells were migrating across the wound using actin-based machinery since we had visualized actin in the lamellipodia using phalloidin labeling. Indeed, the actin-depolymerizing agent dihydrocytochalasin B (5 μg/ml) completely inhibited cell migration within 1 h, and prolonged treatment (3 h) caused muscle cells to round up and detach from the mesoglea [data not shown].

Since normal phasic contraction of jellyfish muscle during swimming partially depends on influx of extracellular calcium through voltage-gated calcium channels (Fig. 1 and Spencer and Satterlie, 1981; Spencer, 1982; Satterlie and...
compared to 1.2/H9262
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corrm in a separate series of experiments by addition of
cell migration on intracellular stores of calcium was
voltage-gated currents, particularly inward calcium cur-
cium for regulating locomotion, so it seemed probable that
migratory cells switched to an intracellular source of cal-
migrated and cover the wound area at the same
rate as under control conditions [Fig. 6e]. Clearly,
dihydropyridine-sensitive, voltage-gated calcium channels
were not required for cell migration to proceed. However,
voltage-gated calcium channels are known to control cal-
cium influx which in turn regulates migration in many
cells, such as neurons (Komuro and Rakic, 1992, 1996;
Schindelholz and Reber, 2000).
To establish whether intracellular calcium stores are
required for cell migration we examined the effect of 10
mM caffeine; the migration rate slowed to 0.2 /m/min,
compared to 1.2 /m/min in ASW [Fig. 6e]. The dependence
of cell migration on intracellular stores of calcium was
confirmed in a separate series of experiments by addition of
10 /m thapsigargin, a calcium ATPase inhibitor, which
empties calcium stores [Shmigol, 1995]. Thapsigargin
significantly reduced migration speed from 1.03 ± 0.09 to
0.46 ± 0.08 /m/min [mean ± SE, n = 12, P < 0.05, paired
t test]. This experiment also controlled for possible nonspe-
cific effects of caffeine [Butcher and Sutherland, 1962]. Thus
it is apparent that migration of dedifferentiated muscle cells
in jellyfish is dependent on modulation of the intracellular
calcium concentrations in various cytoplasmic compart-
ments. In migrating leukocytes it has been suggested that
the highest calcium concentrations lead to depolymeriza-
tion of actin filaments in the rear portion of a cell, whereas
a low calcium concentration at the front would favor the
polymerization of actin [Stossel, 1993].
Another cytoskeleton component known to be important
in regulating cell shape is microtubules. However, the
importance of microtubules in cell migration seems to be
cell-type specific. Mammalian endothelial cells require
microtubules for cell migration, whereas fish keratocytes
do not (Schliwa and Honer, 1993).
Migration does not appear to require protein synthesis
since treatment with 40 /m cycloheximide 4 h before
wounding did not affect the migration rate [Fig. 6e].

Electrophysiology and Contractility during Wound Healing

Experiments in the previous sections had suggested that
migratory cells switched to an intracellular source of cal-
cium for regulating locomotion, so it seemed probable that
voltage-gated currents, particularly inward calcium cur-
rents, might be down-regulated in migrating cells. We
measured this directly using whole-cell, patch-clamp re-
cordings to compare the density of voltage-gated ion chan-
nels between contractile and migrating cells. Figure 8a
shows that dissociated, contractile muscle cells possessed
both outward potassium currents [A-type and delayed rect-
tifier] and inward currents, whereas only the delayed recti-
fier current, albeit at a reduced amplitude, was present in
migrating cells from explants [Fig. 8b]. The predominant
inward current in intact striated myocytes is carried by
calcium ions, and channels passing this current resemble
T-type vertebrate channels as they are activated at low
voltages [Lin and Spencer, in preparation]. Therefore, it
appears that the dedifferentiation of contractile muscle
cells into migrating cells is accompanied by a down-
regulation of voltage-gated ion channels, especially those
responsible for inward currents, and this can explain the
insensitivity of migrating cells to nitrendipine, which do
not require an external source of calcium. The considerable
thickness of the mesoglea prevented us from directly moni-
toring changes in the population of voltage-gated ion chan-
nels during the wound-healing process. Nevertheless, we
were able to use contractility of muscle cells, in response to
a potassium depolarization stimulus, as an indirect way to
monitor the presence of a population of functionally com-
petent voltage-gated ion channels. Thus to examine
whether protein synthesis is required for restoration of
functionality after wound healing, we monitored muscle
contractility after treatment with cycloheximide and sub-
sequent recovery. As corroborative evidence we were able
to follow morphological markers of redifferentiation such
as realignment of the myofibrillar apparatus. As described
previously, jellyfish that had been allowed to recover for
42 h from cycloheximide treatment had reextented myofi-
bers [Figs. 7e and 7f]. We stimulated the healed area in these
jellyfish by local addition of 100 mM KCl and found that
this muscle was able to contract as did muscle from
jellyfish that had been wounded 30 h previously, but not
recovered with cycloheximide [Fig. 7g]. However, animals
treated with cycloheximide that was not removed failed to
contract when challenged by potassium-induced depolar-
ization 30 h after wounding [Fig. 7g]. Thus both morpho-
logical redifferentiation and excitability after wounding
require protein synthesis. However, whether the reappearance
of voltage-gated ion channels was prior to or after the
realignment of sarcomeres remains unclear. Nor do these
experiments determine whether the reappearance of ion
channels in the cell membrane represented de novo synthe-
sis of channels. The phenomenon of ion channel loss during
migration has been described for corneal keratocytes, which
also have an ectodermal origin [Watsky, 1995b].

CONCLUSIONS

The ability of striated muscle cells to behave like epithe-
lial cells during the wound-healing process is probably
related to their ectodermal origin, which is apparent during
FIG. 5. Wound healing without cell proliferation. (a) The wounded area in the muscle sheet of the subumbrella does not show any BrdU labeling 72 h after wounding. (b) In contrast the subtentacular region on the subumbrellar surface of the same jellyfish did show some cells labeled by BrdU antibodies. Red, propidium iodide; green, anti-BrdU. Scale bar, 5 μm.

FIG. 6. Effects of various pharmacological agents on rate of cell migration. (a–d) Time-lapse, phase-contrast images of cell migration in normal artificial seawater during the wound-healing process to show the method for measuring migration rate. Jellyfish were dissected and pinned down with cactus spines which also served as reference points for distance measurement. Arrows indicate the migrating front. (e) The effects of various pharmacological agents on cell migration. The rate of cell migration was estimated from the advance of the wound margin at measured time intervals. Note that 0.2% DMSO served as a carrier control for nitrendipine. Significant difference is indicated by the asterisk (ANOVA test followed by PLSD test, *P < 0.05). N = 9 for each treatment group.
FIG. 7. Effects of cycloheximide on arrangement of myofibers and muscle contractility during wound healing. Confocal images (a) to (f) are stained by BODIPY-conjugated phalloidin to show the arrangement of myofibers. (a) Migrating cells between the intact area and the migrating front 6 h after wounding in artificial seawater. (b) As for (a) except the jellyfish was treated with 40 μM cycloheximide 4 h before wounding. Note that there is no difference in the arrangement of myofibers between control and cycloheximide-treated muscle cells. The upper two bars in (g) show that a challenge with 100 mM KCl at 6 h after wounding did not produce any contraction in either control or cycloheximide-treated wounds. (c) Center of wounded area 30 h after wounding in seawater showing that muscle cells reextended and began to realign myofibers. (d) As for (c) except that the jellyfish was treated with 40 μM cycloheximide between 6 and 30 h after wounding. Note that myofibers were still wrapped around nuclei and cells remained flat, leaving large spaces between myofibers [as in Fig. 3i]. The middle two bars in (g) show that a challenge with 100 mM KCl at 30 h after wounding produced contractions in 5 of 9 preparations in the control and contractions in 0 of 9 cycloheximide-treated preparations. (e) Center of a wounded area 72 h after wounding in seawater showing realigned myofibers. (f) As for (e) except that the jellyfish was treated with 40 μM cycloheximide between 6 and 30 h after wounding and then allowed to recover in flowing seawater for a further 42 h. Note that there was no significant difference in the degree of alignment of myofibers between control and treated cells. The bottom two bars in (g) show that a challenge with 100 mM KCl at 72 h after wounding produced contractions in 9 of 9 preparations in the control and contractions in 8 of 9 cycloheximide-treated preparations which were allowed to recover. Scale bar, 50 μm. [g] Diagram to show the protocol for the experiments on the effects of cycloheximide on myofibrillar arrangement (differentiation) and contractility. Each bar shows the solution and duration of application. Jellyfish were wounded at time 0 h and the experiments commenced 4 h before this. Muscle cells at the migrating front (or center of wound after closure) were challenged with 100 mM KCl at 6, 30, or 72 h. The italicized alphabet label at the left of each bar indicates the corresponded phalloidin–BODIPY staining images above. The numbers at the right extremity of each bar show the ratio of preparations that showed a contraction in response to potassium depolarization to the number tested.

FIG. 8. Whole-cell currents recorded from normal muscle cells and migrating cells. (a) Whole-cell, voltage clamp recording from undamaged contractile muscle cells (averaged current traces from 15 cells) using bath and electrode solutions that support both inward and outward currents. (b) Similar recording from migrating cells (averaged current traces from 5 cells). The amplitudes of both inward and outward currents are significantly reduced in migrating cells compared with normal muscle cells, indicating that there is a down-regulation of ion channels prior to or during migration. Stimulus protocol was 20-ms test pulses incrementing in 10-mV steps from −70 to +90 mV from a holding potential of −80 mV.
budding from the polyp stage (Hyman, 1940; Spencer, 1974, 1975). This contrasts with triploblasts in which striated muscles have a mesodermal origin. Striated muscle cells appear only in the medusa stage of cnidarians, and under certain primary culture conditions these striated muscle cells can lose their striated pattern to become smooth (Schmid and Reber-Müller, 1995). Thus, jellyfish striated muscle cells can be regarded as a highly differentiated ectodermal epithelium. Molecular cloning experiments in the jellyfish Podocoryne carnea indicate that specific genes are activated during the development of medusae [Schuchert et al., 1993; Aerne et al., 1995] and presumably some of these are concerned with formation of the striated muscles used for swimming. The action potentials recorded from these differentiated muscle cells are reminiscent of those recorded from vertebrate cardiac muscle in that they have a long plateau phase of up to 170 ms duration [Spencer and Satterlie, 1981]. This action potential shape differs markedly from that of adjacent, simple, noncontractile, epithelial cells in the same monolayer (Spencer, 1978), where the plateau is lacking and the spike shape is very simple. Therefore we presume that the functional disappearance of the specific ion channels responsible for this characteristically square action potential, after wounding, represents a dedifferentiation event that may reflect an earlier developmental state of this tissue.

ACKNOWLEDGMENTS

This article is dedicated to Nikita Grigoriev, a scientist with a technical acumen that in our experience was unsurpassed. We thank the staff at Bamfield Marine Station for assistance in collecting jellyfish. Mr. R. Bhatnagar, Mr. G. Braybrook, and Mr. R. Mandryk provided technical support. N.G.G. received partial salary support from the Western Canadian Universities Marine Biological Society. We are grateful to Dr. S. D. Buckingham, Dr. J. P. Chang, Dr. W. J. Gallin, Dr. N. Syed, and Dr. C. J. Wong for reading the manuscript and providing valuable comments. This study was supported by a grant to A.N.S. from the Natural Sciences and Engineering Research Council, Canada.

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Received for publication March 30, 2000
Revised May 22, 2000
Accepted June 5, 2000