the observed tolerance to some drugs but also responses to internal pathological conditions and environmental influences, thus providing a potential tool for understanding the well-known cyclic nature of various psychopathologies.

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Thick and Thin Filaments in Postmitotic, Mononucleated Myoblasts

Abstract. *Addition of cytochalasin B to primary muscle cultures allows the physical separation of postmitotic myogenic cells from replicating presumptive myoblasts and replicating fibroblasts. Mononucleated, postmitotic myoblasts proceed without fusion to synthesize myosin and actin and to assemble these proteins into thick and thin filaments. Although sarcomeres oriented in tandem are not evident and Z, H, and I bands are atypical in these mononucleated myoblasts, the irregularly scattered clusters of myofilaments are assembled into remarkably normal interdigitating arrays. These scattered clusters of stacked thick and thin filaments permit the cell to contact spontaneously in the presence of cytochalasin B.*

It has been known for some time that multinucleated myotubes form by the fusion of mononucleated myogenic cells (*1–3*). Some investigators have argued that there is no obligatory coupling between fusion and the regulatory events associated with the initiation of contractile protein synthesis or the assembly of these proteins into striated myofibrils (*2–4*). They contend that the correlation in vitro between fusion and the initiation of contractile protein synthesis is more coincidental than causal and that, in the absence of fusion, mononucleated postmitotic myoblasts assemble thick and thin filaments (*3–7*).

This view has been challenged. Some workers (*8*) claim that neither transcription nor translation of myosin messenger RNA (mRNA) takes place in mononucleated myogenic cells, and that these events occur only in multinucleated myotubes. Others (*9*) argue that while myosin and creatine phosphokinase mRNA's are transcribed in mononucleated myogenic cells, these mRNA's are not translated until after the cells have fused to form myotubes [see also (*10*)].

In our experiments cytochalasin B (CB) was added to muscle cultures. This antibiotic, which does not block the synthesis of myosin or actin (*11–13*), blocks fusion and separates postmitotic myotubes and myoblasts from replicating myogenic and fibrogenic cells (*4, 14, 15*). Cultures were prepared from breast muscles of 10-day chick embryos and CB was added to a final concentration of 5 μg/ml (*12*). After 2 to 4 days in CB the cells were prepared for electron microscopy (EM) by fixation in glutaraldehyde.

Myogenic cultures and most "myogenic clones" consist of a heterogenous population of cells including myotubes, replicating "presumptive myoblasts" and their daughters, the postmitotic "myoblasts" (*2, 3, 6*), as well as replicating mononucleated cells which operationally are indistinguishable from fibroblasts (*16*). Contrary to some reports (*17*), neither we nor others (*18*) can reliably distinguish among replicating presumptive myoblasts, fibroblasts, and mesenchyme cells. In this report only postmitotic, mononucleated cells that can fuse and actually display thick and thin filaments are termed myoblasts.

Cytochalasin B has very different effects on postmitotic myotubes and myoblasts as compared to replicating cells. Forty-eight

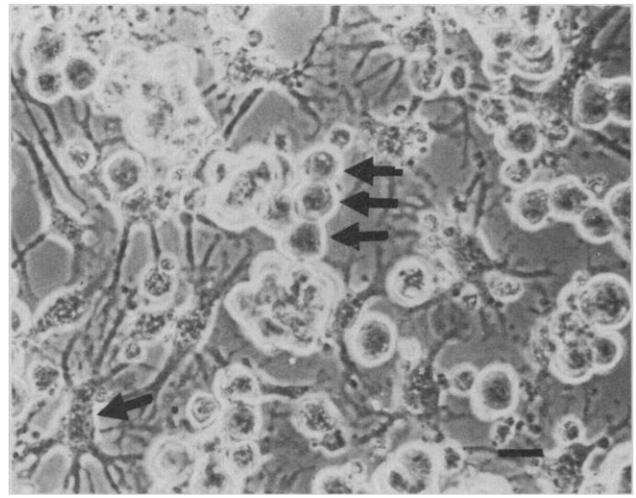

Fig. 1. Intermediate-power phase micrograph of a 5-day breast muscle culture exposed to CB for the last 48 hours. All of the myotubes have retracted from the substrate and will degenerate. The arborized cells adhere to the substrate and are characterized by their many spidery processes (single arrow). In another focal plane are clusters of spontaneously contracting, postmitotic, round, mononucleated myoblasts loosely attached to one another (three arrows). The arborized cells do not bind fluorescein-labeled antibody against myosin or tropomyosin, whereas the round cells bind both antibodies (*6, 14*). Scale bar, 20 μm.

hours after CB was added to the medium, all of the thousands of myotubes that had formed in 3-day-old cultures retracted from the substrate, became round, and floated off into the medium (*14, 15*). The remaining cells (Fig. 1) were of two classes: (i) arborized cells that adhered to the collagen substrate and (ii) round cells, either isolated or in clusters, that were readily dislodged from the substrate. Cytochalasin B inhibits cleavage of the midbody and so aborts cytokinesis (*11, 19*). Consequently, in addition to mononucleated cells, some cells in Fig. 1 are bi- and even oligonucleated. The proportions of these forms varied, depending on the frequency of cell division when CB was present.

Arborized cells have never been observed contracting. Of more than 20 arborized cells inspected by EM, not one exhibited interdigitating thick and thin filaments. Elsewhere we have shown that arborized cells (i) are largely replicating presumptive myoblasts and replicating fibroblasts, (ii) synthesize a different set of myosin light chains from those found in postmitotic myoblasts; and (iii) do not bind fluorescein-labeled antibodies against skeletal myosin or synthesize heavy chains that can be precipitated by such antibodies in agar immunodiffusion tests (*3, 6, 11, 20*). In all probability the myosin heavy and light chains synthesized by the arborized presumptive myoblasts and fibroblasts are the products of structural genes different from those active in myoblasts.

In contrast, most round cells contract spontaneously and bind fluorescein-labeled antibody against skeletal myosin. Figure 2 shows a section through a round, mononucleated myoblast; the three-dimensional arrangement of the myofilaments is atypical and is reminiscent of that induced by colcimide (*11, 21*). In mononucleated myoblasts I and H bands are rarely observed, whereas "dense bodies" often appear to be randomly distributed. Thin actin-like filaments approximately 60 angstroms in diameter often inserted into the dense bodies, although in other instances the dense bodies themselves appeared to consist of aligned filaments 30 to 40 angstroms in diameter. While sarcomeres are not arranged in tandem, the stacking of thick and thin filaments into functional, interdigitating arrays is impressive. Clearly this is an instance of a partially normal assembly of two supramolecular structures.

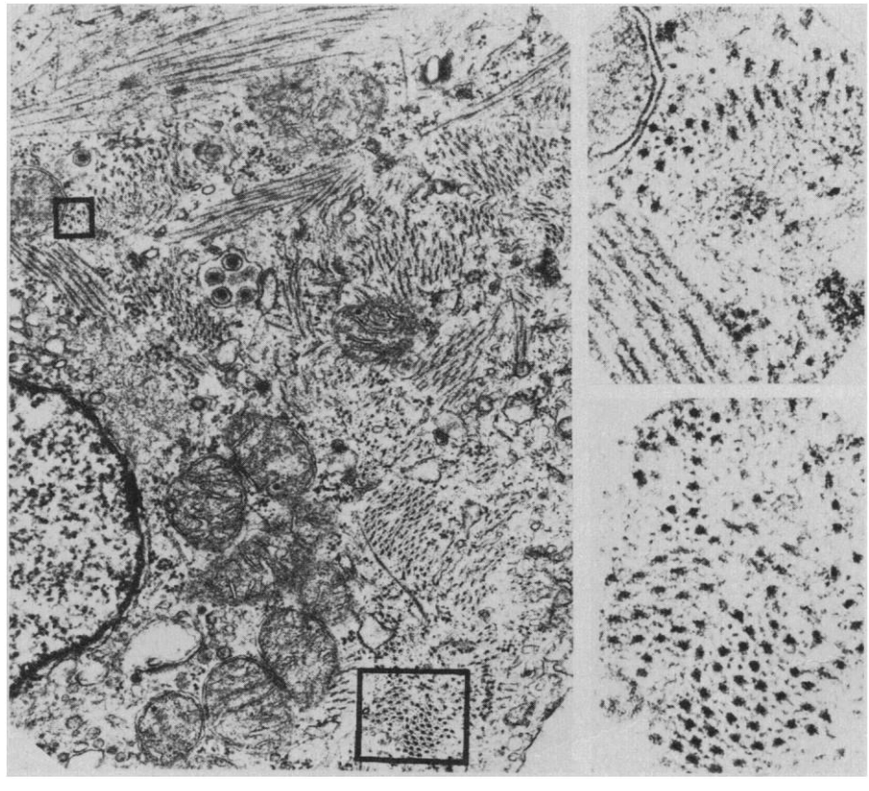

Fig. 2. Electron micrograph of a section through a postmitotic myoblast (left, × 8,000). The goodly number of irregularly scattered stacks of interdigitating thick and thin filaments demonstrates that fusion is not required for either the transcription or translation of myosin or actin mRNA's, or for the assembly of these proteins into filaments. The two areas shown enlarged at the right (× 25,000) illustrate the characteristic hexagonal array of thick and thin filaments. Note the poorly defined dense bodies toward the top of the micrograph.

The existence of these postmitotic, mononucleated myoblasts renders untenable claims (*22*) that mononucleated myogenic cells that fail to fuse remain in the cell cycle. Breast myogenic cells are not basically different from somite myogenic cells (*2, 4, 7*). Although fusion and the formation of multinucleated myotubes is a striking phenomenon, it does not perform the unique roles related to transcription or translation that some investigators (*8, 9*) have claimed. The stable postmitotic condition of the myoblasts also demonstrates that fusion does not induce cells to withdraw from the cell cycle (*22*); myoblasts are programmed to withdraw from the cell cycle as a precondition to fusion.

The failure of some investigators to detect myosin or myosin-like molecules in mononucleated cells is probably related to the reliability or sensitivity of their analytical procedures. With techniques used in muscle biochemistry, myosin and actin have been found in a variety of cells, including normal fibroblasts (*23*) and even sessile chondroblasts (*24*). Myosin heavy and light chains have been demonstrated (*20, 25*) in replicating presumptive myoblasts and myoblasts suppressed by 5-bromo-2′-deoxyuridine.

In summary, although fusion is an interesting marker for changes in the cell surface (*2, 3, 11*), it is not essential for either transcription or translation of the mRNA's for skeletal myosin or actin, nor for the polymerization and assembly of these proteins into functional, interdigitating filaments. Furthermore, these events occur in CB. Whether fusion actually alters the rate of myosin synthesis, the rate of accumulation per DNA per unit time, or the rate of turnover in myotubes compared to that in myoblasts has not been determined. But the central problem in myogenesis remains: How, during one quantal cell cycle, does a replicating presumptive myoblast, transcribing and translating genes for "cytoplasmic" myosin heavy and light chains, yield daughter postmitotic myoblasts that initiate the transcription and translation of the genes for the myofibrillar myosins (*2, 6, 11, 14*)?

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Tetanus Toxin: Direct Evidence for Retrograde Intraaxonal Transport

Abstract. *The neurotoxin tetanospasmin causes tetanus when it reaches the central nervous system. In this autoradiographic study, ^{125}I-labeled tetanospasmin was injected into the leg muscles of rodents, and the nerves supplying these muscles were crushed. The labeled toxin accumulated within axons on the distal side of the crush. This study provides direct evidence for retrograde axonal transport of a macromolecular toxin that acts at synapses in the central nervous system.*

In the time of Hippocrates, tetanus was known by the triad of wounding, lockjaw, and death (*1*); even now the disease continues to be a worldwide health problem of major proportions (*2*). Tetanus results when tetanospasmin, the protein neurotoxin produced in contaminated wounds by *Clostridium tetani*, reaches the central nervous system (CNS) (*2*). In the CNS, tetanospasmin interferes with inhibitory synaptic inputs on spinal motor neurons (*3*). The route by which the toxin reaches the CNS has been the subject of study for many years (*4*). Three pathways of entry have been proposed: intraaxonal transport from peripheral nerve endings to the CNS; passage through intraneural, extraaxonal tissue spaces; or vascular and lymphatic spread. In this study, autoradiography was used to localize [^{125}I]tetanospasmin in nerves after intramuscular and intraneural injection of toxin. The results provide direct evidence for retrograde intraaxonal transport of tetanospasmin.

Purified tetanus toxin was used in these studies; intramuscular injection of 0.1 μg of toxin (*5*) killed all mice at 24 hours. A portion of the toxin was iodinated by using chloramine T (*5*) followed by chromatography on a Sephadex (Pharmacia) G75 column equilibrated with 0.1*M* phosphate buffer (*p*H 7.0) with 0.1 percent bovine serum albumin. The final preparation of ^{125}I-labeled toxin had a specific activity of 3.19 μc/μg [7.027×10^6 disintegrations per minute (dpm) per microgram]. The labeled toxin retained toxicity in mice, producing tetanus followed by death 24 to 36 hours after intraperitoneal injection of 140 ng of toxin (40 μl containing 1×10^6 dpm). Before and after iodination, electrophoresis of the toxin on 7 percent polyacrylamide disc gels showed one major protein component (*5*).

This [^{125}I]tetanospasmin was used in a series of experiments designed to look for distal-proximal migration of labeled toxin within nerves. The strategy consisted of injecting labeled toxin into muscle or nerve, crushing the appropriate nerve proximally, and, after an interval, removing the nerves for autoradiographic studies (Table 1). After fixation, the nerve segments above and below the crush or injection were embedded in Epon, sectioned (1.5 μm), and processed for autoradiography (*6*). The use of a crush allowed collection of migrating material at a single site, so small amounts of radioactivity could be detected (*7*). The systems investigated are summarized in Table 1. We examined mouse and rat sciatic nerves after injection into the distal muscles of hind limb, and rat sciatic nerve segments proximal and distal to a direct intraneural microinjection (*8*).

In the experiments in which ^{125}I-labeled toxin was injected into rat or mouse

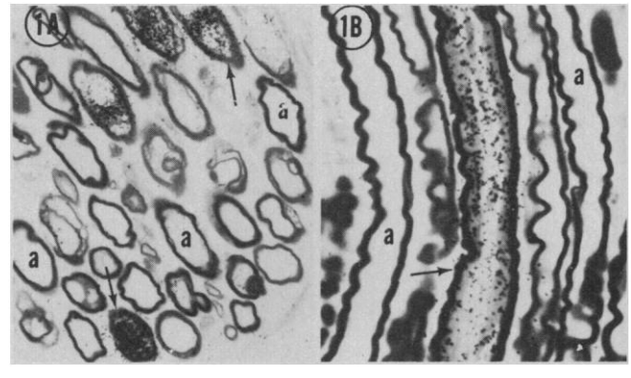

Fig. 1. Autoradiograms of sciatic nerves distal to crush. Sections cut at 1.5 μm are stained with toluidine blue. (A) Axons and myelin sheaths in cross section. The abnormal appearance of axons and myelin is typical of the alterations commonly present in proximity to a crush. Silver grains are present within four axons. Arrows point to two labeled axons. Three unlabeled axons are indicated by *a* (× 400). (B) Axons and myelin sheaths in longitudinal section. Silver grains are present within the axon in center of field. The label is heaviest over dark-stained material rather than over the clear central core. Electron microscopy showed that the basophilic material within the axon represents mitochondria, dense bodies, and accumulations of membranous and vesicular organelles. The central core contains neurofilaments. Unlabeled axons are indicated by *a* (× 900).