

From the Archive

Actin in locomotion

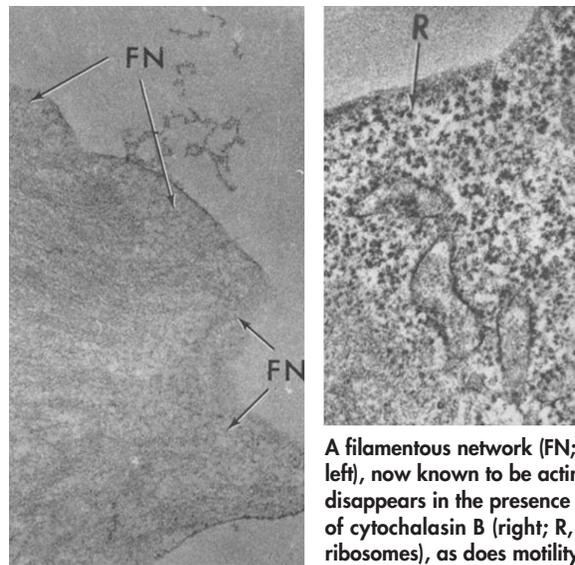
By 1970, several ultrastructural studies reported that different cell types contained thin filaments, 50 Å in diameter, which resembled the actin filaments seen in muscle cells. Similar filaments were also observed in the growing tips of axons, where they formed a network or mesh along the periphery. By analogy, most scientists assumed these actin-like filaments would also be involved in contraction and promote cellular movement. A series of papers published by Norman Wessells and his group at Stanford University (Yamada et al., 1970; Yamada et al., 1971; Spooner et al., 1971) provided the necessary correlative link: only when there was filament structure was there also motile function.

The studies were made possible by a new pharmacological tool. Cytochalasin B had been shown to inhibit motility (Carter, 1967), but without any real idea of mechanism. Schroeder (1969) then demonstrated that the drug also inhibited cytokinesis—a process that, like the action of muscle, was thought to be contractile—and he correlated this action with the disruption of the 50-Å filament networks in cells.

Wessells's group found a similar correlation between drug action and lack of filaments for cytochalasin B's inhibition of cell shape morphogenesis (Spooner and Wessells, 1970), and decided to see if the same held up when the drug was applied during neuron outgrowth and cell locomotion. "The lab was unusual in encouraging graduate students to wander off into unrelated projects," says Ken Yamada, then a student with Wessells and now at the NIH (Bethesda, MD). "So it was fascinating that everything fell together when we tested cytochalasin on the various systems."

First the group added the drug to neurons in culture, which caused the axons' growing tips, or growth cones, to round up. The drug disrupted the filamentous networks in the growth cones and halted axon elongation (Yamada et al., 1970;

Yamada et al., 1971). In a parallel study, the group described a network of microfilaments—similar in organization to the one found in growth cones—at the very leading edges of migratory glial cells. Cytochalasin B rapidly disrupted this network and halted cell migration (Spooner et al., 1971). When the drug was washed away in either system, the microfilament networks recovered and axon outgrowth or locomotion resumed.



A filamentous network (FN; left), now known to be actin, disappears in the presence of cytochalasin B (right; R, ribosomes), as does motility.

The very existence of the filament system was controversial. "It was quite peculiar to have such a meshwork associated with and forming the sole contents of protruding membranes," says Yamada. Coagulation after fixation was a concern, and one reviewer, recalls Yamada, "wondered if you would see the same if you fixed concentrated BSA." Still, the drug seemed to target the thin 50-Å filaments specifically, as microtubules and intermediate filaments remained intact. But little was known about its mode of action. "We were using the agent without knowing what it was affecting, other than what we could see by electron microscopy," says Yamada.

A year later, experiments by Spudich and Lin (1972) showed that cytochalasin B specifically binds to purified muscle actin, supporting the conclusion that the microfilaments required for cell movement were indeed actin-like proteins. Their identity was, however, not confirmed until later

studies demonstrated that the filaments could bind heavy meromyosin (Spooner et al., 1973) and again when actin antibodies became available (Spooner and Holladay, 1981). "In our original papers, we intentionally avoided using the word actin," says Yamada.

How actin might drive cell movement was even more obscure. Most of the speculation in the early papers was, by analogy with the known microfilament presence in muscle, centered on contractile possibilities. Contractile alignment of filaments might form microspikes, or contraction might pull the rearward cell contents forward, like an inchworm, to meet adhesions at the front. As yet there was no talk of pushing out the front of the cell with filament polymerization.

In their experiments, Spooner, Yamada, and Wessells also added the drug colchicine to cells to disassemble microtubules. This treatment did not affect growth cone elongation or glial cell migration. "Colchicine can collapse the axon, but the growth cone is still wriggling and trying to grow," explains Brian Spooner (Kansas State University), who was then a post-doc in Wessells's lab. "We now know that microtubules are involved in motility in all kinds of ways, but they are not *required* for cellular translocation. Our original conclusion has held up fairly well."

The thrill of finding part of the cell's motor was palpable. "It was a very exciting time," recalls Yamada. "Usually, 1 out of 10 experiments work; but at that time, 9 out of 10 would work." **JCB**

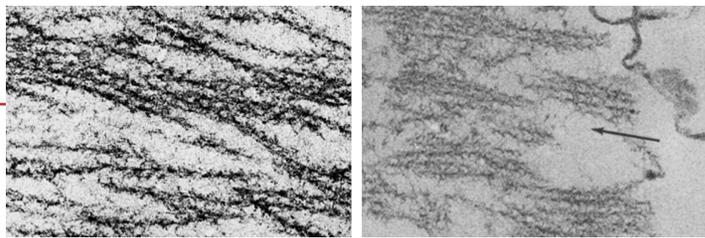
Carter, S.B. 1967. *Nature*. 213:261–264.
Schroeder, T.E. 1969. *Biol. Bull.* 137:413.
Spooner, B.S., and N.K. Wessells. 1970. *Proc. Natl. Acad. Sci. USA*. 66:360–364.
Spooner, B.S., et al. 1971. *J. Cell Biol.* 49:595–613.
Spooner, B.S., et al. 1973. *Tissue Cell*. 5:37–46.
Spudich, J.A., and S. Lin. 1972. *Proc. Natl. Acad. Sci. USA*. 69:442–446.
Yamada, K.M., et al. 1970. *Proc. Natl. Acad. Sci. USA*. 66:1206–1212.
Yamada, K.M., et al. 1971. *J. Cell Biol.* 49:614–635.

Actin in nonmuscle cells

In the late 1960s, Howard Holtzer's group at the University of Pennsylvania made the unexpected observation that virtually all eukaryotic cells assemble a variety of actin-based structures (Ishikawa et al., 1969). At that time, scientists thought that actin and myosin were restricted to muscle cells and ascribed contractile activity in other cells to a variety of molecules and structures. Holtzer's fluorescent antibodies to sarcomeric myosin decorated muscle but not any other cell type. "That's why," he says, "we were so surprised to find actin filaments in non-muscle cells."

Holtzer's work followed a classic study by Huxley (1963). Huxley had reported that in a cell-free system heavy meromyosin (HMM), a proteolytic fragment of myosin, could be incubated with polymerized, filamentous actin and form polarized arrowhead complexes that could be readily visualized in the EM. The orientation of the arrowhead complexes gave a readout of actin organization.

Holtzer and colleagues observed HMM-decorated filaments in every cell type examined, from skeletal and cardiac muscle cells to fibroblasts, chondroblasts, keratocytes, glia, and blood cells. Most decorated filaments in these different cell types localized to stress fibers. HMM-decorated filaments were also seen at the cleavage furrow of metaphase cells and at the core of the microvilli of intestinal



Decoration of actin filaments is similar in muscle (left) and epithelial (right) cells.

and tracheal cells. In an early review of this work, Holtzer et al. (1972) suggested that there might be more than one type of actin and that each might be associated with a variety of actin-binding proteins in different cell types.

By the mid- to late-1970s, numerous studies using fluorescent antibodies to nonsarcomeric actin (Lazarides and Weber, 1974) and nonsarcomeric myosin (Adelstein et al., 1971), as well as fluorescent phalloidin for visualizing filamentous actin, confirmed the presence of both actin and myosin in most cells. Different cell types were later shown to contain distinct isoforms of both contractile proteins. In plant cells, actin filaments capable of binding HMM were shown to be involved in cytoplasmic streaming and moving organelles (Palevitz et al., 1974); Allen (1974) made similar observations for animal cells. **JCB**

Adelstein, R.S., et al. 1971. *Proc. Natl. Acad. Sci. USA.* 68:2703–2707.

Allen, N.S. 1974. *J. Cell Biol.* 63:270–287.

Holtzer, H., et al. 1972. *Curr. Top. Dev. Biol.* 7:229–256.

Huxley, H.E. 1963. *J. Mol. Biol.* 77:281–308.

Ishikawa, H., et al. 1968. *J. Cell Biol.* 38:538–555.

Ishikawa, H., et al. 1969. *J. Cell Biol.* 43:312–328.

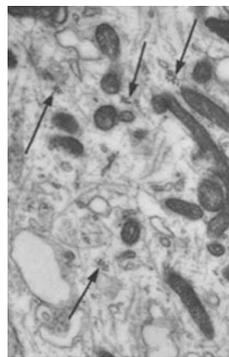
Lazarides, E., and K. Weber. 1974. *Proc. Natl. Acad. Sci. USA.* 71:2268–2272.

Palevitz, B.A., et al. 1974. *Proc. Natl. Acad. Sci. USA.* 71:363–366.

Growth cones make proteins, too

Axons branch out from neurons as they respond to chemical cues in their extracellular environment. Until recently, many scientists did not believe that elongating axons could synthesize proteins locally. But in the past three years, this view has been largely overturned. One of the first clues that protein synthesis might occur in the growing tips of axons—the growth cones—came from morphological studies conducted over 30 years ago by Virginia Tennyson.

In the mid-1960s, several electron microscopy studies of neurons had been published, but few of them focused on the growth cone. Tennyson, then a researcher at Columbia University, decided to examine the axons of fetal rabbit dorsal root neuroblasts at 11–12 days, a time in development when many growth cones are present. "I remember, I wanted to study growth



Ribosomes (arrows) turned up in axons.

cones," recalls Tennyson. "I certainly was not expecting to see any ribosomes."

Tennyson observed clusters of particles along the length of an entire axon and in several growth cones. The particles were 150–250 Å in diameter and morphologically identical to ribosomes (Tennyson, 1970). "The presence of ribosomes in the early embryonic axons suggests that protein synthesis may continue in these segments at a considerable distance from the perikaryon [neuron cell body]," Tennyson wrote in her 1970 paper. "Of course I had no evidence of protein synthesis at that time, so I did not want to make too much of that observation," she says. Shortly after Tennyson's study, further ultrastructural analyses confirmed the presence of polyribosomes in growth cones of cultured neurons (Yamada et al., 1971; Bunge, 1973).

Since then, several studies have

documented ribosomes, mRNA, translational initiation proteins, and protein synthesis in axons and growth cones. Douglas Campbell and Christine Holt (University of Cambridge) demonstrated that molecules that guide the growth of axons rapidly trigger protein synthesis in isolated retinal growth cones (Campbell and Holt, 2001). Inhibition of protein synthesis by translation blockers abolishes the response of these growth cones to guidance molecules. This and other studies (Brittis et al., 2002; Zheng et al., 2001) showed that, at least in vitro, fast, local synthesis of proteins not only occurs but is necessary for guiding axon growth in response to external cues. "In retrospect," muses Holt, "it is surprising how remarkable everyone thought this was." **JCB**

Brittis, P.A., et al. 2002. *Cell.* 110:223–235.
Bunge, M.B. 1973. *J. Cell Biol.* 56:713–735.
Campbell, D.S., and C.E. Holt. 2001. *Neuron.* 32:1013–1026.
Tennyson, V.M. 1970. *J. Cell Biol.* 44:62–79.
Yamada, K.M., et al. 1971. *J. Cell Biol.* 49:614–635.
Zheng, J.Q., et al. 2001. *J. Neurosci.* 21:9291–9303.