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, kerocytes, 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 cells (Holtzer, 1972; Huxley, 1963; Ishikawa et al., 1968; Ishikawa et al., 1969). 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 myosin (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.

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Growth cones make proteins, too

Axons branch out from neurons as they respond to chemical cues in their extra-cellular environment. Until recently, many scientists did not believe that elongating axons could synthesize protein 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 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.”

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