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Actin Localization and Function in Higher Plants

Review Article

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1. Introduction

Eukaryotes from all four kingdoms contain cytoskeletal actin and associated proteins. Functions for actin are well-defined in animals and include cell contraction, intracellular movement, cell morphology determination, cell locomotion, and cytokinesis (ALLEN and ALLEN 1978, SCHLIWA, 1986). In addition, it is now well-established that actin-myosin interaction provides the motive force for cytoplasmic streaming in some lower plants and fungi (for reviews, see ALLEN and ALLEN, 1978, KAMIYA 1981, WILLIAMSON 1980). Evidence for the existence of vascular plant actin and myosin has been accumulating in recent years (reviewed by JACKSON, 1982), although their role in the cell has not been unequivocally defined. This review summarizes current knowledge of actin and associated proteins in plants, considers higher plant actomyosin function, and suggests areas deserving future attention.

2. Lower Plant Actomyosin

Although the remainder of this overview will focus on the occurrence of actin in higher plant cells, a brief look at Characean algae is useful. More is known about actin and its role in cytoplasmic motility in *Characeae* than for any other plants. Light microscopic examination of the giant internode cells of these algae reveals rapid endoplasmic streaming that requires the integrity of cortical filamentous cables (NAGAI and REBHUN, 1966, NAGAI and HAYAMA, 1979 b). Ultrastructurally, these cables consist of 50–100 microfilaments with a 5–7 nm diameter, a double helical structure, and a 36–38 nm repeat (NAGAI and REBHUN, 1966, NAGAI and HAYAMA 1979 a, b, PALEVITZ and HEPLER, 1975). Characean microfilaments reversibly bind mammalian heavy meromyosin (HMM) or S-1 myosin fragments, resulting in arrowhead patterns. All microfilaments in a bundle show uniform polarity, with arrowheads pointing opposite the direction of cytoplasmic flow (KERSEY *et al.* 1976, KERSEY and WESSELS 1976, PALEVITZ and HEPLER 1975, WILLIAMSON 1974). Fluorescence microscopy confirms the presence of actin microfilaments at the interface between stationary ectoplasm and mobile endoplasm which, as suggested by numerous earlier studies (reviewed in KAMIYA 1981), is the site of motive force generation (NOTHNAGEL *et al.* 1981, WILLIAMSON and TOH, 1979, WILLIAMSON *et al.* 1986).

Proteolytic activity in cytoplasmic extracts of plant cells, particularly those possessing large vacuoles, constitutes a problem for biochemical approaches to the study of plant cytoskeletal proteins. It is perhaps for this reason that knowledge about the biochemical properties of plant cytoskeletal proteins is still scarce, at least when compared to animal cells. Cytoplasmic extracts from the Characean alga, *Nitella,* contain an actin-like protein with electrophoretic mobility, solubility properties, and ultrastructure comparable to mammalian actins (ALLEN and ALLEN 1978). A 55.000 MW component copurifies with the 46.000 MW actin from these extracts and may be involved in microfilament bundling (ALLEN and ALLEN 1978, NAGAI and HAYAMA 1979 a, b). Early attempts to identify algal myosin from fractionated extracts elucidated no

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200,000 MW protein in the supernatant, but a Mg^{++}-ATPase in the particulate fraction and a 260,000 MW minor band were noted (ALLEN and ALLEN 1978). Using different extraction procedures, KATO and TONOMURA (1977) described a putative *Nitella* myosin heavy chain of >200 kD MW, which formed bipolar filaments of 0.2 μm length at low ionic strength: they also observed a Ca^{++} sensitive Mg^{++}-ATPase that was activated by skeletal muscle F-actin. However, these are preliminary and as yet unconfirmed observations, and should be viewed with caution. Endoplasmic organelles frequently show irregularily shaped rod- or horn-like protuberances, and 20–30 nm globular bodies often attached to fine filaments of <4 nm diameter. These structures are believed to be membrane-bound myosin, but direct proof is lacking (NAGAI and HAYAMA 1979 a, b).

The value of Characean algae for the study of actin-dependent processes in plants lies in the fact that reactivatable *in vitro* systems can be obtained with relative ease, and that both they and the intact cells themselves are amenable to a wide variety of experimental manipulations. These include electrical and mechanical stimulaton of cells (KAMITSUBO 1980), vacuolar perfusion (ALLEN and ALLEN 1978, WILLIAMSON and TOH 1979, WILLIAMSON 1980), compression, centrifugation, ligation, and mechanical damage (KAMITSUBO 1980, NAGAI and KAMIYA 1977, CHEN and KAMIYA 1975) light microbeaming (KAMITSUBO 1980), and chemical stimulation and inhibition (ALLEN and ALLEN 1978, CHEN and KAMIYA 1975, NAGAI and KAMIYA 1977, NOTHNAGEL *et al.* 1981, WILLIAMSON and TOH 1979). Cell models and *in vitro* cytoplasmic streaming systems were developed using demembranated endoplasmic droplets which continue to show chloroplast rotation and other motility phenomena (HIGASHI-FUJIME 1980). Molecular analysis of *in vitro* motility led to the discovery that the actin cables of mechanically dissected algal cells are capable of supporting the movement of organelles (WILLIAMSON and TOH, 1979, SHIMMEN and TAZAWA 1982), myosin-coated beads (SCHEETZ and SPUDICH 1983, SHIMMEN and YANO 1984), and membranous organelles of *Acanthamoeba* by myosin-I (ADAMS and POLLARD 1986). These studies have provided a current model of Characean cytoplasmic streaming that envisions the motive force for rotational streaming to be generated at the interface between the moving endoplasm and the stationary layer of actin cables attached to cortical chloroplasts. Myosin associated with endoplasmic organelles or soluble myosin is proposed to create a shear force that drives the endoplasm around the cell in a direction determined by actin microfilament orientation. However, the actual distribution of myosin in Characean cells is not known, and the molecular details of this model still need to be elucidated.

3. Higher Plant Actomyosin

3.1. Methods of Higher Plant Actin Identification

Studies on Characean algae set the precedent that allowed researchers to identify actin in vascular plants. The methods used include electron, light, and fluorescence microscopy, inhibitor studies, biochemical characterization, and gene cloning and sequencing.

Early light microscopic studies correlated 0.1–0.2 μm cytoplasmic fibers with active streaming in the transvacuolar strands of higher plants (KAMIYA 1959, O'BRIAN and THIMANN 1966, PARTHASARATHY and MUHLETHALER 1972). In other cells, cytoplasmic fibrils were frequently found to be oriented parallel to the main axis and to the direction of flow in elongated cells (FRANKE *et al.* 1972, O'BRIAN and MCCULLY 1970). Cinematography (MASCARENHAS and LAFOUNTAIN 1972), videotaping (SEAGULL and HEATH 1980 b, PARTHASARATHY 1985), differential interference contrast microscopy (O'BRIAN and MCCULLY 1970, PESACRETA and PARTHASARATHY 1984), and computer image enhancement (PARTHASARATHY 1985) have since improved the visualization of fibrils in regions of active streaming and have expanded our knowledge of their disposition. By analogy with the situation in Characean algae (NAGAI and REBHUN 1966), it was suspected that these fibers were composed, at least in part, of actin filaments. However, confirmation of this supposition has met with difficulty.

The earliest evidence for the existence of actin in higher plant cells came from extruded *Amaryllis* cytoplasm which contained 6 nm filaments that specifically bound HMM with a 35 nm periodicity and released it in the presence of ATP (CONDELLIS 1974). Similar results were obtained with glycerinated preparations from *Haemanthus* endosperm cells (FORER and JACKSON 1975, 1976). Fixation and localization of actin microfilaments *in situ* by conventional electron microscopic techniques has proven cumbersome. Osmium tetroxide treatment, routinely used in sample preparation, destroys actin microfilaments under many conditions (MAUPIN-SZAMIER and POLLARD 1978, SMALL 1981, SEAGULL and HEATH 1979). To increase plant microfilament preservation by preferentially stabilizing actin filaments, several approaches have been used, with varying success: HMM binding (CONDEELIS 1974,

Table 1. *Actin in higher plants*

Plant	Tissue	Technique	Function	References
Gymnosperms:				
Chamaecyparis	roots	LM/EM/FM	CS	70, 71
Pseudotsuga	roots	LM/FM	CS	70
Angiosperms:				
Monocotyledons:				
Allium	bulb, scale epidermis	EM/FM	CS/SP	67
	cotyledon GMCs	LM/INH	CS/OM/CK	25, 60, 61, 63, 64
	root tips	FM	CS/OM/CK	6, 65
Alstroenemia	pollen tubes	EM/FM	CS/SP	67, 69
Amaryllis	pollen tubes, protoplasts	LM/EM	CS	7
Avena	coleoptile	LM/INH/EM/FM	CS/SP	3, 4, 57, 67, 68, 96, 103
Chamaedorea	stem and root:			
	procambium and vascular tissue	EM	CS	66
Clivia	endosperm	FM	SP/KK/CK	80, 81, 82, 83
	pollen tubes	EM/INH	CS/TG	18
Cocos	stem and root:			
	procambium and vascular tissue	EM	CS	66
Egeria	leaf epidermis	EM/FM	CS (2°)/SP	67
	leaves	LM/FM/INH/EM	CS (2°)/OM	110
Elodea	leaf epidermis	EM/FM	CS (2°)/SP	67
	leaves	EM/FM/LM/INH	CS (2°)/OM	11, 110
Endymion	pollen	FM	CS	28
Haemanthus	endosperm	EM/FM/LM/INH	CS/SP/KK/CK	13, 14, 16, 17, 80, 81, 82, 83
Hordeum	coleoptile epidermis	LM/EM/FM/INH	CS/SP/Gravi	67, 68, 104
	pollen	FM	CS	28
Hydrilla	leaf epidermis	EM/FM	CS (2°)	67
	leaves	EM/FM/LM/INH	CS (2°)/OM	110
Lilium	pollen grains and tubes	LM/EM/FM/INH	CS/SP/TG	18, 27, 67, 69, 74, 76
Limnobium	root hairs	EM/FM	CS/SP	67
Narcissus	pollen	FM	CS	28
Phleum	root tips	EM	—	98
Prestoea	root: procambium and vasc. tissue	EM	CS	66
Tradescantia	pollen grains and tubes	LM/FM/INH/EM	CS/TG	43, 46, 74
	stamen hairs	LM/EM/FM/INH	CS/SP	10, 43, 67, 98
	male meiocytes	EM	—	26
Triticum	coleoptile epidermis	EM/FM	CS/SP	67
	wheat germ	BC/EM	—	32
	leaf trichomes	EM	—	98
Vallisneria	leaf epidermis	LM/EM/INH	CS (2°)	112
	leaf mesophyll	LM/INH	CS (2°)/OM	33, 95
Zea	genomic library	Mol. Biol.	—	89
	coleoptiles	LM/EM/FM/INH	CS/Gravi	4, 77, 104
Dicotyledons:				
Cucumis	fruit thin-walled parenchyma	EM/FM	SP	67
Cucurbita	fruit thin-walled parenchyma	EM/FM	CS/SP	67
	stem: 1′ & 2′ vasc. tissue, cambium	EM	CS	66
Fraxinus	stem: active cambium	EM	CS	19
Glycine	seedlings	BC/EM	—	49, 50
	hypocotyls	Mol. Biol.	—	30, 31, 55, 88, 89
Helloborus	pollen	FM	CS	28
Heracleum	petiolar hairs	LM	CS	58
Hibiscus	callus protoplasts	LZR/INH/LM	CS/SP	20
Impatiens	pollen tubes	EM/FM	CS/SP	67, 69
Lepidium	root hairs, root tips	LM/INH/EM/FM	CS/TG/OM/Gravi	22, 23, 27, 102
Lycopersicon	fruit parenchyma and endocarp	BC/EM	CS	99, 100
	stem, petiolar hairs, fruit thin-walled parenchyma	EM/FM	CS/SP	67

Table 1 (continued)

Plant	Tissue	Technique	Function	References
Medicago	suspension culture cells	FM	CW/CS	87
Nicotiana	pith callus	EM/FM	CS/SP	67
	pollen	FM	TG	74
	mesophyll callus protoplasts	LZR/INH/LM	CS/SP	20
	stem: 1′ & 2′ vasc. tissue, cambium	EM	CS	66
Petunia	pollen grains and tubes	EM/FM	SC/SP/TG	67, 69, 74
Phaseolus	root tips	BC/EM	—	34
Pisum	internode cells	EM/LM/INH	CS	4
Raphanus	root hairs	LM/EM/INH	CS/TG/CW	28, 84, 85, 86
Salix	stem: cambium, 2′ vascular tissue	EM	CS	66
Trifolium	seedlings	BC/EM	—	50

KEY:

Techniques:		Function:	
EM	Electron microscopy	CS	Cytoplasmic streaming
LM	Light microscopy	SP	Cell shape determination
FM	Fluorescence microscopy	TG	Tip growth
INH	Inhibitor studies	OM	Organelle movement
BC	Biochemistry	KK	Karyokinesis
Mol. Biol.	Gene cloning and sequencing	CK	Cytokinesis
LZR	Laser ablation	Gravi	Graviperception
		CW	Cell wall deposition

FORER and JACKSON 1976, 1979 a), tannic acid treatment (SEAGULL and HEATH 1979), tissue glycerination (FORER and JACKSON 1979 a), and freeze-substitution techniques (HEPLER and LANCELLE 1986, LANCELLE *et al.* 1986, TIWARI *et al.* 1984). Electron microscopy using these techniques reveals 5–7 nm microfilaments with the morphological characteristics of F-actin (*e.g.* double-helices with a 34–40 nm periodicity) (SEAGULL and HEATH 1979). Mammalian HMM or S-1 fragments will reversibly decorate these microfilaments (SEAGULL and HEATH 1979, YAMAGUCHI and NAGAI 1981).

Recently, fluorescence microscopy, although not having the resolution of electron microscopy, has become increasingly popular for visualizing three-dimensional cytoskeletal arrays in plant cells. Fluorochrome-tagged phallotoxins [fungal metabolites known to bind evolutionarily diverse F-actins (WULF *et al.* 1979, PARTHASARATHY 1985, PESACRETA *et al.* 1982)], and indirect immunofluorescence with antibodies against actin have added to our knowledge of actin arrays in plant tissues (see Table 1). The distribution patterns revealed with these techniques will be discussed in more detail in the next section.

Despite the progress that has been made in recent years, one needs to ask how reliable are these visualization procedures, and where are their limitations. For example, HMM decoration, although capable of unequivocally identifying actin filaments and revealing their subcellular distribution, nevertheless requires a preparatory step, such as glycerination or permeabilization, that may cause rearrangements of actin filaments, and may even induce the polymerization of previously unpolymerized actin. Glycerination also permeabilizes the tonoplast and hence can lead to proteolysis and degradation from the release of vacuolar components. Related problems arise with the use of antibodies or fluorescent phallotoxins for light microscopic visualization of F-actin. Therefore, fixation/extraction protocols required for fluorescent visualization may cause partial destruction, rearrangements, selective extraction, or preferential stabilization of certain cellular components. One has to ask, for example, whether it is only the most stable actin that is visualized, or the actin that is associated with other, more resistant, cellular structures. It is disturbing that, with a few notable exceptions, staining patterns obtained with fluorescent phalloidin are rather diffuse, although one would expect a distinct fibrillar staining based on what is known about phalloidin's interaction with F-actin. In many cases, the techniques used, and the results obtained with fluorescent molecular probes are taken at face-value and are often not evaluated critically enough.

Actin-like proteins have been biochemically identified

and partially characterized in *Phaseolus* root tips (JACKSON and DOYLE 1977), wheat germ (ILKER *et al.* 1979), *Glycine* seedlings (METCALF *et al.* 1980, 1984), and *Lycopersicon* endocarp (VAHEY and SCORDILIS 1980, VAHEY *et al.* 1982). Based on electrophoretic mobility, solubility and immunological and electron microscopic criteria, these proteins show homology to cytoplasmic and muscle actins. All four of the aforementioned plant tissues contain a protein of about 45 kD that comigrates with mammalian actins, while *Phaseolus* (JACKSON and DOYLE 1977) and *Triticum* (ILKER *et al.* 1979) also contain a slower migrating polypeptide. A 58.000 MW protein from *Triticum* copurifies with the 45 kD actin and has properties similar to, and comigrates with, a sea urchin actin binding protein (ILKER *et al.* 1979). Immunologically, these 45 and 58 kD proteins crossreact in Ouchterlony immunodiffusion assays with antibodies against turkey gizzard actin (ILKER *et al.* 1979). Actins from crude cytoplasmic extracts of *Glycine* and *Trifolium,* or from partially purified *Lycopersicon* extract, form 6–7 nm wide filaments at >50 mM ionic strength and reversibly bind HMM or rabbit S-1 fragments in an arrowhead pattern with a periodicity of 28–37 nm (METCALF *et al.* 1984, VAHEY and SCORDILIS 1980, VAHEY *et al.* 1982). *Lycopersicon* actin, which constitutes approximately 6% of extracted proteins, is capable of activating skeletal or tomato myosin Mg^{++}-ATPase (VAHEY *et al.* 1982).

More recently, molecular cloning techniques have aided in the study of the plant cytoskeleton in general, and of plant actin in particular. Actin genes have been cloned from *Glycine max* and *Zea mays* using a *Dictyostelium* actin sequence as a probe (NAGAO *et al.* 1981, SHAH *et al.* 1982, 1983). Soybean contains six or more genes in a multigene family of actin-related sequences (HIGHTOWER and MEAGHER 1985), while maize has at least two actin genes (SHAH *et al.* 1983). Sequences from one maize and three soybean actin genes suggest plant actin genes evolved from a common ancestral gene, with a unique sequence of the N-terminal 9 amino acids and three introns (SHAH *et al.* 1982, 1983, HIGHTOWER and MEAGHER 1986). The deduced amino acid sequence of plant actins shows homology to animal cytoplasmic and muscle actins, yet there is extensive coding sequence heterogeneity among soybean actin genes and between soybean and maize genes (SHAH *et al.* 1983, HIGHTOWER and MEAGHER 1986). The three soybean actin gene sequences differ by 6–9% replacement substitutions (nucleotide changes resulting in amino acid substitutions). Maize and soybean have 8–10% replacement substitutions, while plant actin genes have diverged 11–15% from their animal counterparts. On the other hand, typical animal-animal replacement substitutions only range from 3–7% (SHAH *et al.* 1983, HIGHTOWER and MEAGHER 1986, VANDEKERCKHOVE and WEBER 1978). The six genes of the soybean actin multigene family fall into three pairs of sequence homology by northern and dot-blot analyses, with one pair highly expressed in seedlings and mature plant organs, constituting $>80\%$ of all actin mRNAs (HIGHTOWER and MEAGHER 1985, 1986). It is not known whether actin genes are differentially expressed in plant development or whether different isotypes perform unique cellular functions.

3.2. Actin Microfilament Arrays

Plant microfilaments, like microtubules, are organized into distinct arrays that change in distribution during the cell cycle. Several types of interphase microfilament arrays have been reported. Most common are the large cytoplasmic microfilament bundles oriented parallel to the long axis of the cell or the direction of streaming, as in transvacuolar cytoplasmic strands (CLAYTON and LLOYD 1985, PARTHASARATHY and MUHLETALER 1972, PARTHASARATHY *et al.* 1985, PERDUE and PARTHASARATHY 1985, SEAGULL and HEATH 1979, 1980 b, SEAGULL *et al.* 1986, TAKAGI and NAGAI 1983, WHITE and SACK 1986). In addition, other cell types, such as *Haemanthus* and *Clivia* endosperm cells, exhibit a loose cytoplasmic meshwork of microfilaments that intermingles with the interphase microtubule array (SCHMIT and LAMBERT 1985 a, b, 1986 b, SCHMIT *et al.* 1986 a). Recently, a perinuclear microfilament "basket" was described in alfalfa suspension culture cells (SEAGULL *et al.* 1986), *Equisetum* root tip cells (DERKSEN *et al.* 1986), and *Hordeum* and *Zea* coleoptile cells (WHITE and SACK 1986). In these same cell types, as well as in *Raphanus* root hairs and other cells, fine cortical arrays of microfilaments were observed (FRANKE *et al.* 1972, PARTHASARATHY 1985, SEAGULL and HEATH 1979, 1980 b, TAKAGI and NAGAI 1983, WHITE and SACK 1986, YAMAGUCHI and NAGAI 1981, DERKSEN *et al.* 1986). These cortical microfilament arrays are mostly oriented parallel to the cell's long axis and lie in close apposition to the plasmalemma. Individual microfilaments are sometimes observed in close proximity to, or even parallel to cortical microtubules which are believed to be important in cell wall deposition (SEAGULL and HEATH 1979, 1980 b). Finally, pollen grain of a number of angiosperms contain numerous fusiform, phalloidin-binding bodies ($\sim 2.5 \times 15$ µm) that possibly constitute an actin storage form. These bodies are dispersed into fine fibrils localized near the germination

Table 2. *Actin associated proteins in higher plants*

Plant	Tissue	Protein	Technique	Function	References
Allium	root tips	myosin	FM/BC	CK	65
		troponin T	FM	—	44
Egeria	leaves	myosin	BC/EM	CS	59
Lycopersicon	endocarp	myosin	BC/EM	CS	99, 100

aperture and in the pollen tube during pollen activation (HESLOP-HARRISON *et al.* 1986).

The distribution of microfilaments during mitosis has long been a controversial topic, and although several microfilament configurations have been described, their functional relevance in mitosis is disputed (for review, see FORER 1978, MCINTOSH 1979, PETZELT 1979, PICKETT-HEAPS *et al.* 1982). During prophase in *Haemanthus* and *Clivia* endosperm cells, the cytoplasmic microfilament array gradually redistributes to the cortex (SCHMIT and LAMBERT 1985 a, b, 1986 b, SCHMIT et al., 1986 a). As the mitotic spindle forms in prometaphase and elongates in anaphase, it tends to occupy the cell volume, forcing a rearrangement of the cortical actin network which forms an envelope around the spindle, being stretched until many microfilaments eventually lie parallel to spindle microtubules (SCHMIT and LAMBERT *1985 a, b, 1986 b,* SCHMIT *et al.* 1986 a). These findings may help explain early observations on glycerinated *Haemanthus* endosperm mitotic cells which reportedly contain microfilaments between the chromosomes and poles. Most of these microfilaments are oriented parallel to the microtubules and those associated with kinetochore fibers show common polarity (FORER and JACKSON 1975, 1976, 1979 a, b). A localization of microfilaments near spindle fibers has also been observed by fluorescence microscopy in mitotic spindles from alfalfa suspension culture cells (SEAGULL *et al.* 1986), but not in nonglycerinated *Haemanthus* spindles (SCHMIT and LAMBERT 1985 a) or in *Allium* and *Equisetum* spindles (CLAYTON and LLOYD 1985, DERKSEN *et al.* 1986). Late in karyokinesis increased numbers of microfilaments are observed by fluorescence microscopy in the equatorial division plane. This is the earliest sign of a cytokinetic apparatus (CLAYTON and LLOYD 1985, SCHMIT and LAMBERT 1985 a, b, 1986 b, SCHMIT *et al.* 1986 a).

Cytokinesis in higher plant cells is effected by an organelle known as the phragmoplast which is composed of parallel arrays of microtubules. These presumably guide vesicles to the division plane where they fuse to form a cell plate separating the daughter cells. Fluorescence microscopy indicates the presence of a diffuse actin network codistributing with phragmoplast microtubules in *Allium* root cells (CLAYTON and LLOYD 1985, PARKE *et al.* 1986), *Equisetum* root tip cells (DERKSEN *et al.* 1986), and alfalfa suspension culture cells (SEAGULL *et al.* 1986). A more distinct microfilament pattern has been observed in phragmoplasts of *Haemanthus* and *Clivia* endosperm cells. In these cells the actin has a definite fibrillar appearance, closely associated with, and parallel to, microtubules (SCHMIT and LAMBERT 1985 a, b, 1986 b, SCHMIT *et al.* 1986 a). When the nuclear envelope reassembles in telophase, the interphase microfilament arrays also reform (SEAGULL *et al.* 1986).

Of the four major microtubule structures found in higher plant cells (cortical arrays, preprophase band, mitotic spindle, and phragmoplast), only the preprophase band so far has not been found to have associated microfilaments (CLAYTON and LLOYD 1985, PARKE *et al.* 1986).

3.3. Actin-associated Proteins

Whereas the presence of actin in higher plant cells is now well-established, much less is known about the disposition of actin-associated proteins involved in force generation, structural interactions, and actin filament dynamics. Little information is available in part due to the difficulty of applying to plant cells what are routine biochemical approaches for animal cells. Myosin-like proteins have been reported in *Allium* root tips (PARKE *et al.* 1986), *Egeria* leaf extracts (OHSUKA and INOUE 1979), and *Lycopersicon* (tomato) endocarp (VAHEY and SCORDILIS 1980, VAHEY *et al.* 1982) (see Table 2). In some respects these myosins show a similarity to skeletal muscle myosin. High ionic strength extracts of *Egeria* and *Lycopersicon* contain a K^+-EDTA ATPase activity, which is inhibited by Mg^{++} in tomato (VAHEY and SCORDILIS 1980, VAHEY *et al.* 1982) but not *Egeria* (OHSUKA and INOUE 1979). *Egeria* myosin also shows no p-nitrophenyl phosphatase activity (OHSUKA and INOUE 1979). At low ionic strength

and in the presence of Ca^{++}, tomato myosin contains a Mg^{++}-ATPase that is activated 19-fold by tomato F-actin and 10-fold by rabbit skeletal muscle actin (VAHEY *et al.* 1982). Both of these myosins can be induced to form 0.5–1.0 μm bipolar filaments in a low ionic strength medium (OHSUKA and INOUE 1979, VAHEY and SCORDILIS 1980). Also, *Egeria* myosin can reversibly bind to muscle F-actin with the 40 nm periodicity of projections characteristic of animal myosins (OHSUKA and INOUE 1979). *Allium* root tips contain a protein of 200,000 MW that reacts in Western blots with an antibody against 3 T 3 cell myosin (PARKE *et al.* 1986). This protein comigrates on gels with mammalian myosin heavy chains (PARKE *et al.* 1986) and is similar in size to the ~200 kD heavy chain of *Egeria* (OHSUKA and INOUE 1979). Tomato myosin weighs only 100 kD and is believed to be a proteolytic fragment of the actual heavy chain; the authors find no polypeptides >100 kD in polyacrylamide gels (VAHEY *et al.* 1982). Tomato myosin light chains are less well characterized but two, with molecular weights of 16 and 14 kD, have been reported (VAHEY *et al.* 1982).

There are no published reports on myosin genes or mRNA's in higher plants. Likewise, ultrastructural studies of myosin are few. Using an antibody against animal myosin, indirect immunofluorescent localization of myosin in *Allium* root tip cells has been achieved (PARKE *et al.* 1986). In interphase, diffuse cytoplasmic staining and aggregates of label were observed, none of which co-localized with actin microfilament arrays. No labeling of preprophase bands or spindles was noted, but a diffuse cytoplasmic staining prevailed in these stages. the cytokinetic phragmoplast is the only structure in which the myosin antibody shows costaining with rhodamine phalloidin-labeled F-actin.

Besides myosin, the only higher plant actin-associated protein on which there is some information is a troponin T-like protein, identified in *Allium* root tip cells by immunofluorescent staining with a monoclonal antibody against striated muscle troponin T (LIM *et al.* 1986). Again, the staining pattern does not strictly correspond to actin arrays. This epitope, resembling troponin T, was found associated with interphase cortical microtubules, preprophase band microtubules, mitotic spindle fibers and pole complexes, and with phragmoplast microtubules. Much work is still to be done to identify other actin associated proteins in plants, as there is copious evidence for their occurrence in other kingdoms (for reviews, see KORN 1978, SCHLIWA 1986, STOSSEL *et al.* 1985).

3.4. Actomyosin Function

An involvement of actin in certain plant cellular processes is based on two lines of evidence. The first is localization in cell domains concerned with the execution of motile processes, such as mitotic spindles, phragmoplasts, cell cortex, etc. ... However, conclusions based solely on this principle of "guilt by association" need to be viewed with caution. The second line of evidence entails the use of pharmacological agents thought to specifically disrupt actin-based processes. Of these inhibitors, the cytochalasins are the most widely used microfilament toxins. These fungal metabolites are thought to disrupt F-actin (MACLEAN-FLETCHER and POLLARD 1980, SCHLIWA 1982), but in a number of systems, especially plants, no such destruction occurs even at concentrations of 1–100 μg/ml (FORER *et al.* 1972, THOMAS 1978). In fact, cytochalasins are reported to have variable and complex effects on plant cells and it is very difficult to judge whether this is due to differential effects on specific microfilament populations, or nonspecific disruption of other structures and processes (SEAGULL and HEATH 1980 b, WESSELS *et al.* 1971, POPE *et al.* 1979). Phallotoxins are another set of fungal products which reportedly interfere with actin microfilament function by binding to, and stabilizing, F-actin (WIELAND 1977). In many instances the concentrations of these compounds required for disruption of cellular functions are so high that one has to worry about the specificity of the effect observed. On the other hand, it is plausible that the effective intracellular concentrations are much lower since the cell wall may act as a permeability barrier. However, unless these uncertainties are resolved, studies based on inhibitor experiments alone are often difficult to interpret.

Table 1 lists reports of actin occurrence in higher vascular plant tissues, the techniques used for identification, and some proposed microfilament functions. Cytoplasmic streaming occurs in all of these tissues with only a few exceptions (see PARTHASARATHY *et al.* 1985). By extrapolation from the well-documented involvement of actomyosin in Characean algal motility and other lines of indirect evidence, *e.g.*, presence of myosin, orientation of microfilaments parallel to the direction of streaming and its reversible disruption by cytochalasins and phalloidin (BRADLEY 1973, CANDE *et al.* 1973, FORDE and STEER 1976, FRANKE *et al.* 1972, HAHNE and HOFFMAN 1984, HERTH *et al.* 1972, ISHIGAMI and NAGAI 1980, MASCARENHAS and LAFOUNTAIN 1972, PALEVITZ 1980, PARTHASARATHY *et al.* 1985,

PARTHASARATHY 1985, PERDUE and PARTHASARATHY 1985, SEAGULL and HEATH 1980 b, WESSELS *et al.* 1971, WITZMUN and PARTHASARATHY 1985), it seems plausible to suggest a role for actin in cytoplasmic streaming of higher plant cells as well (see JACKSON 1982 for a review).

Another process in which microfilaments are thought to play a role is tip growth of pollen tubes (FRANKE *et al.* 1972, HERTH *et al.* 1972, MASCARENHAS and LAFOUNTAIN 1972, PIERSON *et al.* 1986) and root hairs (HERTH *et al.* 1972). Both processes are disrupted by cytochalasins. This inhibitory action is likely due to a disturbance of the vectorial control of vesicular wall material transport which may be mediated by axial microfilaments.

Organelle movement is a third cytoplasmic event believed to require the presence of microfilaments. Light-induced chloroplast migration (2° streaming) in *Vallisneria, Egeria, Elodea,* and *Hydrilla* has been demonstrated to be dependent on a peripheral microfilament network by cytochalasin-induced disruption of movement, structural evidence, and physiological and mechanical manipulations (ISHIGAMI and NAGAI 1980, PARTHASARATHY *et al.* 1985, TAKAGI and NAGAI 1983, WITZMUN and PARTHASARATHY 1985, YAMAGUCHI and NAGAI 1981). *Allium* guard mother cells (GMC) require microfilaments for nuclear migration following division, as shown by specific disruption with cytochalasin and phalloidin (PALEVITZ 1980).

Many authors posit a role for cytoplasmic microfilaments in cell shape determination (O'BRIEN and THIMANN 1966, PARTHASARATHY *et al.* 1985, PARTHASARATHY 1985, SCHMIT and LAMBERT 1985 a, b, SEAGULL and HEATH 1980 a, b). In plant cells, the cell wall, a semirigid cellulosic matrix, defines the boundaries to be filled by turgid protoplasts. Whereas cortical microtubules are generally believed to play an important role in cell shape determination by controlling the orientation of cellulose microfibril deposition, the involvement of actin is less well understood (HEPLER and PALEVITZ 1974, PALEVITZ and HEPLER 1975, HEATH and SEAGULL 1982). A few reports suggest that cell wall deposition might be mediated by both microtubules and microfilaments (reviewed by HEATH and SEAGULL 1982). Cytochalasin B treatment of *Raphanus* root hairs did not result in a disruption of cellulose microfibril deposition or cortical microtubule arrays but rather led to an overall increase in microtubule lengths and in the number of microtubule-microtubule associations (SEAGULL and HEATH 1980 a). *Allium* guard mother cells treated with cytochalasin B or phalloidin also do not show specific disruption of microtubule arrays, atypical wall thickenings, or cellulose microfibril orientation (PALEVITZ and HEPLER 1976, PALEVITZ 1980). The observation that normal wall morphogenesis can occur despite the inhibition of microfilament function seemingly refutes the claims of microfilament-microtubule interactions in cell wall formation and cell shape determination. In a unique study using *Hibiscus* and *Nicotiana* protoplasts, either laser microsurgery or cytochalasin treatment led to the reversible disruption of large cytoplasmic strands, a cessation of streaming, and the formation of isodiametric protoplasts (HAHNE and HOFFMAN 1984). These observations are suggestive of a role for cytoplasmic microfilaments in protoplast shape determination, but whether processes of protoplast shape determination influence intact plant cell morphogenesis is poorly understood.

Karyokinesis has also been suggested to be a process requiring microfilament-microtubule interactions. Kinetochore fiber-associated microfilaments could, theoretically, drive chromosome-to-pole movement (FORER 1978, MCINTOSH 1979). This is controversial since cytochalasin treatment or antimyosin antibody injections, in general, do not disrupt chromosome movement (MCINTOSH 1979). Cytochalasin B does not inhibit chromosome movement in *Haemanthus* spindles (SCHMIT and LAMBERT 1985 b). The spindle-associated cortical microfilaments reported in *Haemanthus* endosperm might instead control the lateral movement of interzone microtubules in anaphase observed in these cells; alternatively, they might be involved in vesicle transport on the spindle surface (SCHMIT and LAMBERT 1985 a, SCHMIT *et al.* 1986 a). This cortical framework might also maintain spindle integrity and cell shape during mitosis (SCHMIT and LAMBERT 1985 b, 1986 b, SCHMIT *et al.* 1986 a). Treatment of *Allium* guard mother cells with cytochalasins or phalloidin does not disrupt spindle formation or spindle structure, nor does it interfere with chromosome movement (PALEVITZ 1980), which therefore suggests that microtubule-microfilament interactions are not important in these processes. In contrast to these observations, spindle reorientation in anaphase and telophase of *Allium* guard mother cells is sensitive to microfilament, microtubule, and metabolic inhibitors (HEPLER 1981, PALEVITZ and HEPLER 1974 b, 1976, PALEVITZ 1980).

Numerous reports of actin and myosin localization led to speculation on their role in phragmoplast and cell plate formation and function (CLAYTON and LLOYD 1985, PARKE *et al.* 1986, SCHMIT and LAMBERT 1985 a, b, 1986 b, SCHMIT *et al.* 1986 a). Cell plate orientation

in *Allium* guard mother cells has been shown to be cytochalasin sensitive, but attempts to visualize significant numbers of microfilaments by electron microscopy so far have been unsuccessful (HEPLER 1981, PALEVITZ and HEPLER 1974 b, 1976, PALEVITZ 1980). In contrast to the disruption of cell plate orientation, cytochalasin or phalloidin treatment does not disrupt phragmoplast microtubule organization, or the transport of vesicles to the division plane for cell plate formation (PALEVITZ and HEPLER 1974 b, PALEVITZ 1980). These results do not rule out the possibility that phragmoplast actin plays a role in the centrifugal expansion of this organelle or in vesicle fusion at the cell plate.

Finally, a cytoskeletal role in graviperception has been suggested based on an apparent structural interaction between amyloplasts and microfilaments in the statocytes of *Zea* and *Hordeum* coleoptiles (WHITE and SACK 1986). Light microscopic observation of these cells suggests that cytoplasmic streaming may contribute to the dynamics of amyloplast sedimentation (SACK and LEOPOLD 1985). Other reports suggest that actin microfilaments are important in generating a polar organization of cellular organelles, specifically the rough endoplasmic reticulum, in presumptive statocytes of cress (*Lepidium*) roots (HENSEL 1985, WENDT and SIEVERS 1986). This polar organization is thought to be crucial to the perception of gravity by these cells, and can be disrupted by cytochalasin treatment (WENDT and SIEVERS 1986, HENSEL 1985). Localization of microfilaments with rhodamine-phalloidin did not reveal any specific interaction with the rough endoplasmic reticulum, but instead displays weak or diffuse cortical staining and perpendicular cytoplasmic bundles (HENSEL 1986).

4. Outlook

This account of higher plant actin and associated proteins makes it apparent that much work remains to be done to understand the role these cytoskeletal proteins play in plant cellular processes. To date, most researchers have relied upon "function by correlation", *i.e.*, actin is localized in motile structures therefore it plays a role in driving the motility. This is a necessary, yet insufficient, step in understanding actin's involvement in cellular motile processes. It is important to complement these observations with physiological and mechanical manipulations, as was done in Characean algae. Similarly, inhibitor studies with, for example, cytochalasins, can provide valuable first insights but are of limited value because of their reported nonspecific effects (WESSELS *et al.* 1971, POPE *et al.* 1979). Phallotoxins, which apparently also disrupt actin-mediated events even when applied to intact cells, should be used to complement experiments employing cytochalasins. The ability to microinject plant cells has been demonstrated (CROSSWAY *et al.* 1986), and injection of antibodies against actin, myosin, and other cytoskeletal proteins will provide inhibitory molecules of higher specificity than pharmacological agents. More effort should be directed towards the development of *in vitro* systems since they bridge traditional biochemistry and *in vivo* observations. Reactivatable lysed cells have proven invaluable tools in the study of animal cell motility, and there is reason to believe that this approach will be similarly informative for plant cell motility, once the horrendous problems posed by the presence of vacuoles, and the design of cell milieu-compatible buffers, can be overcome. Other approaches that should prove fruitful in defining the role of actomyosin in the cell cycle are analyses of mutants and genomic manipulation by molecular genetic techniques. Finally, a search for actin-associated proteins in general, and proteins that mediate microtubule-microfilament interactions in particular seems desirable, as these interactions will prove crucial for understanding higher plant cytoskeletal function. The study of the cytoskeleton of higher plant cells is just beginning. However, one can be satisfied with the progress that has been made to date, and can look forward to significant contributions in the near future.

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Note Added in Proof:

Rhodamine-phalloidine localization of F-actin in the preprophase band of *Allium* root tip cells (PALEWITZ, BA (1987) J Cell Biol. 104: 1515–1519) and *Daucus* suspension culture cells (TRAAS JA *et al.* (1987) J Cell Biol 105: 387–395) has recently been reported in the literature.

References

1. ADAMS RJ, POLLARD TD (1986) Propulsion of organelles isolated from *Acanthamoeba* along actin filaments by myosin-I. Nature 322: 754–756
2. ALLEN NS, ALLEN RD (1978) Cytoplasmic streaming in green plants. Ann Rev Biophys Bioeng 7: 497–526
3. BRADLEY MO (1973) Microfilaments and cytoplasmic streaming: Inhibition of streaming with cytochalasin. J Cell Sci 12: 327–343

4. Cande WZ, Goldsmith MHM, Ray PM (1973) Polar auxin transport and auxin-induced elongation in the absence of cytoplasmic streaming. Planta 111: 279–296
5. Chen JWC, Kamiya N (1975) Localization of myosin in the internodal cell of *Nitella* as suggested by differential treatment with N-ethylmaleimide. Cell Struct Funct 1: 1–9
6. Clayton L, Lloyd CW (1985) Actin organization during the cell cycle in meristematic plant cells. Exp Cell Res 156: 231–238
7. Condeelis JS (1974) The identification of F-actin in the pollen tube and protoplast of *Amaryllis belladonna*. Exp Cell Res 88: 435–439
8. Crossway A, Hauptli H, Houck CM, Irvine JM, Oakes JV, Perani LA (1986) Micromanipulation techniques in plant biotechnology. Biotechniques 4: 320–333
9. Derksen J, Traas JA, Oostendorp T (1986) Distribution of actin filaments in differentiating cells of *Equisetum hymale* root tips. Plant Science 43: 77–81
10. Doree M, Picard A (1980) Release of Ca^{++} from intracellular pools stops cytoplasmic streaming in *Tradescantia* staminal hairs. Experientia 36: 1291–1292
11. Forde J, Steer MW (1976) Cytoplasmic streaming in *Elodea*. Can J Bot 54: 2688–2694
12. Forer A, Emmersen J, Behnke O (1972) Cytochalasin B: does it affect actin-like filaments. Science 175: 774–776
13. — Jackson WT (1975) Actin in the higher plant *Haemanthus katherinae* Baker. Cytobiol 10: 217–226
14. — — (1976) Actin filaments in the endosperm mitotic spindles in a higher plant. *Haemanthus katherinae* Baker. Cytobiol 12: 199–214
15. — (1978) Chromosome movements during cell-division: Possible involvement of actin filaments. In: Heath IB (ed) Nuclear division in the fungi. Academic Press Inc, NY, pp 21–88
16. — Jackson WT (1979 a) Actin in the spindles of *Haemanthus katherinae* endosperm. I. General results using various glycerination methods. J Cell Sci 37: 323–347
17. — — (1979 b) Actin in the spindles of *Haemanthus katherinae* endosperm. II. Distribution of actin in chromosomal spindle fibres, determined by analysis of serial sections. J Cell Sci 37: 349–371
18. Franke WW, Herth W, VanDerWoude WJ, Morre DJ (1972) Tubular and filamentous structures in pollen tubes: Possible involvement as guide elements in protoplasmic streaming and vectorial migration of secretory vesicles. Planta 105: 317–341
19. Goosen-de Roo L, Burggraaf PD, Libbenga KR (1983) Microfilament bundles associated with tubular endoplasmic reticulum in fusiform cells in the active cambial zone of *Fraxinus excelsior* L. Protoplasma 116: 201–208
20. Hahne G, Hoffman F (1984) The effect of laser microsurgery on cytoplasmic strands and cytoplasmic streaming in isolated plant protoplasts. Eur J Cell Biol 33: 175–179
21. Heath IB, Seagull RW (1982) Oriented cellulose fibrils and the cytoskeleton: a critical comparison of models. In: Lloyd CW (ed) The cytoskeleton in plant growth and development. Academic Press, London, pp 163–187
22. Hensel W (1985) Visualization of the membrane bound cytoskeleton and coated pits of plant cells by means of dry cleaving. Protoplasma 119: 212–218
23. — (1986) Demonstration of microfilaments in statocytes of cress roots. Naturwissenschaften 73: 510–511
24. Hepler PK, Palevitz BA (1974) Microtubules and microfilaments. Ann Rev Plant Physiol 25: 309–362
25. — (1981) Morphogenesis of tracheary elements and guard cells. In: Kiermayer O (ed) Cytomorphogenesis in plants vol 8. Cell Biol Monographs. Springer Inc, Wien, pp 327–347
26. — Lancelle SA (1986) Cytoskeletal details in freeze-substituted meiotic cells of *Tradescantia blossfeldiana*. J Cell Biol 103: 555 a
27. Herth W, Franke WW, Vanderwoude WJ (1972) Cytochalasin stops tip growth in plants. Naturwissenschaften 59: 38–39
28. Heslop-Harrison J, Heslop-Harrison Y, Cresti M, Tiezzi A, Ciampolini F (1986) Actin during pollen germination. J Cell Sci 86: 1–8
29. Higashi-Fujime S (1980) Active movement *in vitro* of bundles of microfilaments isolated from *Nitella* cells. J Cell Biol 87: 569–578
30. Hightower RC, Meagher RB (1985) Divergence and differential expression of soybean actin genes. EMBO J 4: 1–8
31. — — (1986) The molecular evolution of actin. Genetics 114: 315–332
32. Ilker RA, Breidenbach RW, Murphy TM (1979) Partial purification of actin from wheat germ. Phytochemistry 18: 1781–1783
33. Ishigami M, Nagai R (1980) Motile apparatus in *Vallisneria* leaf cells. II. Effects of Cytochalasin B and lead acetate on the rate and direction of streaming. Cell Struct Funct 5: 13–20
34. Jackson WT, Doyle BG (1977) Characterization of actin from root tips of *Phaseolus vulgaris*. J Cell Biol 75: 268 a
35. — (1982) Actomyosin. In: Lloyd CW (ed) The cytoskeleton in plant growth and development. Academic Press, London, pp 3–29
36. Kamitsubo E (1980) Cytoplasmic streaming in characean cells: role of subcortical fibrils. Can J Bot 58: 760–765
37. Kamiya N (1952) Protoplast streaming. Protoplasmatologia 8: 3 a
38. — (1981) Physical and chemical basis of cytoplasmic streaming. Ann Rev Plant Physiol 32: 205–236
39. Kato T, Tonomura Y (1977) Identification of myosin in *Nitella flexilis*. J Biochem 82: 777–782
40. Kersey YM, Hepler PK, Palevitz BA, Wessels NK (1976) Polarity of actin filaments in Characean algae. PNAS 73: 165–167
41. — Wessels NK (1976) Localization of actin filaments in internodal cells of characean algae. A scanning and transmission electron microscope study. J Cell Biol 88: 264–275
42. Korn ED (1978) Biochemistry of actomyosin-dependent cell motility. PNAS 75: 588–599
43. Lancelle SA, Callaham DA, Hepler PK (1986) A method for rapid freeze fixation of plant cells. Protoplasma 131: 153–165
44. Lim SS, Hering GE, Borisy GG (1986) Widespread occurrence of anti-troponin T crossreactive components in non-muscle cells. J Cell Sci 85: 1–19
45. MacLean-Fletcher S, Pollard TD (1980) Mechanism of action of cytochalasin B on actin. Cell 20: 329–341
46. Mascarenhas JP, Lafountain J (1972) Protoplasmic streaming, cytochalasin B, and the growth of the pollen tube. Tissue Cell 4: 11–14
47. Maupin-Szamier P, Pollard TD (1978) Actin filament destruction by osmium tetroxide. J Cell Biol 77: 837–852

48. McIntosh JR (1979) Cell Division. In: Roberts K, Hyams JS (eds) Microtubules. Academic Press, London, pp 381–441
49. Metcalf TN, Szabo LJ, Schubert KR, Wang JL (1980) Immunochemical identification of an actin-like protein from soybean seedlings. Nature 285: 171–172
50. — — — — (1984) Ultrastructural and immunocytochemical analyses of the distribution of microfilaments in seedlings and plants of *Glycine max*. Protoplasma 120: 91–99
51. Nagai R, Rebhun LI (1966) Cytoplasmic microfilaments in streaming *Nitella* cells. J Ultrastruct Res 14: 571–589
52. — Kamiya N (1977) Differential treatment of *Chara* cells with cytochalasin B with special reference to its effect on cytoplasmic streaming. Exp Cell Res 108: 231–237
53. — Hayama T (1979 a) Ultrastructural aspects of cytoplasmic streaming in Characean cells. In: Hatano S, Ishikawa H, Sato H (eds) Cell motility: molecules and organization. University Park Press, Baltimore, pp 321–337
54. — — (1979 b) Ultrastructure of the endoplasmic factor responsible for cytoplasmic streaming in *Chara* internodal cells. J Cell Sci 36: 121–136
55. Nagao RT, Shah DM, Eckenrode VK, Meagher RB (1981) Multigene family of actin-related sequences isolated from soybean genomic library. DNA 2: 1–9
56. Nothnagel EA, Barak LS, Sanger JW, Webb WW (1981) Fluorescence studies on modes of cytochalasin B and phallotoxin action on cytoplasmic streaming in *Chara*. J Cell Biol 88: 364–372
57. O'Brien TP, Thimann KV (1966) Intracellular fibers in oat coleoptile cells and their possible significance in cytoplasmic streaming. PNAS 56: 888–894
58. — McCully ME (1970) Cytoplasmic fibres associated with streaming and saltatory-particle movement in *Heracleum mantegazzianum*. Planta 94: 91–94
59. Ohsuka K, Inoue A (1979) Identification of myosin in a flowering plant *Egeria densa*. J Biochem 85: 375–378
60. Palevitz BA, Hepler PK (1974a) The control of the plane of division during stomatal differentiation in *Allium*. I. Spindle reorientation. Chromosoma 46: 297–326
61. Palevitz BA, Hepler PK (1974b) The control of the plane of division during stomatal differentiation in *Allium*. II. Drug studies. Chromosoma 46: 327–344
62. Palevitz BA, Hepler PK (1975) Identification of actin *in situ* at the ectoplasm-endoplasm interface of *Nitella*. J Cell Biol 65: 29–38
63. Palevitz BA, Hepler PK (1976) Cellulose microfibril orientation and cell shaping in developing guard cells of *Allium*: The role of microtubules and ion accumulation. Planta 132: 71–93
64. — (1980) Comparative effects of phalloidin and cytochalasin B on motility and morphogenesis in *Allium*. Can J Bot 58: 773–785
65. Parke J, Miller C, Anderton BH (1986) Higher plant myosin heavy-chain identified using a monoclonal antibody. Eur J Cell Biol 41: 9–13
66. Parthasarathy MV, Muhlethaler K (1972) Cytoplasmic microfilaments in plant cells. J Ultrastruct Res 38: 46–62
67. — Perdue TD, Witzmun A, Alvernaz J (1985) Actin network as a normal component of the cytoskeleton in many vascular plant cells. Am J Bot 72: 1318–1323
68. — (1985) F-actin architecture in coleoptile epidermal cells. Eur J Cell Biol 39: 1–12
69. Perdue TD, Parthasarathy MV (1985) In situ localization of F-actin in pollen tubes. Eur J Cell Biol 39: 13–20
70. Pesacreta TC, Carley WW, Webb WW, Parthasarathy MV (1982) F-actin in conifer roots. PNAS 79: 2898–2901
71. — Parthasarathy MV (1984) Microfilament bundles in the roots of a conifer, *Chamaecyparis obtusa*. Protoplasma 121: 54–64
72. Petzelt C (1979) Biochemistry of the mitotic spindle. Int Rev Cytol 60: 53–92
73. Pickett-Heaps JD, Tippit DH, Porter KR (1982) Rethinking mitosis. Cell 29: 729–744
74. Pierson ES, Derksen J, Traas JA (1986) Organization of microfilaments and microtubules in pollen tubes grown *in vitro* or *in vivo* in various angiosperms. Eur J Cell Biol 41: 14–18
75. Pope DG, Thorpe JR, Al-Azzawi MJ, Hall JL (1979) The effect of cytochalasin B on the rate of growth and ultrastructure of wheat coleoptiles and maize roots. Planta 144: 373–383
76. Reiss HD, Herth W (1979) Calcium ionophore A 23187 affects localized wall secretion in the tip region of pollen tubes of *Lilium longiflorum*. Planta 145: 225–232
77. Sack FD, Leopold AC (1985) Cytoplasmic streaming affects gravity-induced amyloplast sedimentation in maize coleoptiles. Planta 164: 56–62
78. Schliwa M (1982) Action of cytochalasin D on cytoskeletal networks. J Cell Biol 92: 79–91
79. — (1986) The cytoskeleton, an introductory survey. Springer, Wien New York, 328 pp
80. Schmit AC, Lambert AM (1985 a) F-actin dynamics is associated to microtubule function during cytokinesis in higher plants. A revised concept. In: De Brabander M, DeMey J (eds) Microtubules and microtubule inhibitors. Elsevier Science Publishers B.V., Amsterdam, pp 243–252
81. — — (1985 b) F-actin distibution during the cell cycle of higher plant endosperm cells. J Cell Biol 101: 38 a
82. — Vantard M, Lambert AM (1986 a) Microtubule and F-actin rearrangement during the initiation of mitosis in acentriolar higher plant cells. In: Ishikawa H, Hatano S, Sato H (eds) Cell motility: mechanism and regulation. Alan R Liss Inc, New York, pp 415–433
83. — Lambert AM (1986 b) F-actin as an active component of the plant cell cortex during mitosis. J Cell Biol 103: 555 a
84. Seagull RW, Heath IB (1979) The effects of tannic acid on the *in vivo* preservation of microfilaments. Eur J Cell Biol 20: 184–188
85. — — (1980 a) The organization of cortical microtubule arrays in the radish root hair. Protoplasma 103: 205–229
86. — — (1980 b) The differential effects of cytochalasin B on microfilament populations and cytoplasmic streaming. Protoplasma 103: 231–240
87. Seagull R, Falconer M, Weerdenburg C (1986) Microfilaments: Dynamic arrays in higher plant cells. J Cell Biol 103: 110 a
88. Shah DM, Hightower RC, Meagher RB (1982) Complete nucleotide sequence of a soybean actin gene. PNAS 79: 1022–1026
89. — — — (1983) Genes encoding actin in higher plants are highly conserved but the coding sequences are not. J Mol Appl Gen 2: 111–126
90. Sheetz MP, Spudich JA (1983) Movement of myosin-coated fluorescent beads on actin cables *in vitro*. Nature 305: 31–35

91. Shimmen T, Tazawa M (1982) Reconstitution of cytoplasmic streaming in *Characeae*. Protoplasma 113: 127–131
92. — Yano M (1984) Active sliding movement of latex beads coated with skeletal muscle myosin on *Chara* actin bundles. Protoplasma 121: 132–137
93. Small JV (1981) Organization of actin in the leading edge of cultured cells. J Cell Biol 91: 695–705
94. Stossel TP, Chapponier C, Ezzel RM, Haartwig JH, Janmey PA, Kwiatkowski DJ, Lind SE, Smith DB, Southwick FS, Yin HL, Zaner KS (1985) Non-muscle actin binding proteins. Ann Rev Cell Biol 1: 353–402
95. Takagi R, Nagai R (1983) Regulation of cytoplasmic streaming in *Vallisneria* mesophyll cells. J Cell Sci 62: 385–405
96. Thomas DDESS, Dunn DM, Seagull RW (1977) Rapid cytoplasmic responses of oat coleoptiles to cytochalasin B, auxin, and colchicine. Can J Bot 55: 1797–1800
97. — (1978) Cytochalasin effects in plants and eukaryotic microbial systems. In: Tannenbaum SW (ed) Cytochalasins-Biochemical and Cell Biomedical Aspects. Elsevier/North-Holland Biomedical Press, pp 258–275
98. Tiwari SC, Wick SM, Williamson RE, Gunning BES (1984) Cytoskeleton and integration of cellular function in cells of higher plants. J Cell Biol 99: 63s–69s
99. Vahey M, Scordilis SP (1980) Contractile proteins from tomato. Can J Bot 58: 797–801
100. — Titus M, Trautwein R, Scordilis S (1982) Tomato actin and myosin: Contractile proteins from a higher land plant. Cell Motility 2: 131–147
101. Vandekerckhove J, Weber K (1978) Actin amino acid sequences. Comparison of actins from calf thymus, bovine brain, and SV-40 transformed 3T3 cells with rabbit skeletal muscle actin. Eur J Biochem 90: 451–462
102. Wendt M, Sievers A (1986) Restitution of polarity in statocytes from centrifuged roots. Plant Cell Env 9: 17–23
103. Wessels NK, Spooner BS, Ash JF, Bradley MO, Luduena MA, Taylor EL, Wrenn JT, Yamada KM (1971) Microfilaments in cellular and developmental processes. Science 171: 135–143
104. White RG, Sack FD (1986) Actin microfilaments in the presumptive statocytes of grass coleoptiles. J Cell Biol 103: 110a
105. Wieland T (1977) Modifications of actins by phallotoxins. Naturwissenschaften 64: 303–309
106. Williamson RE (1974) Actin in the alga, *Chara corallina*. Nature 248: 801–802
107. — Toh BH (1979) Motile models of plant cells and the immunofluorescent localization of actin in a motile *Chara* cell model. In: Hatano S, Ishikawa H, Sato H (eds) Cell motility: molecules and organization. University Park Press, Baltimore, pp 339–346
108. — (1980) Actin in motile and other processes in plant cells. Can J Bot 58: 766–772
109. — Perkin JL, McCurdy DW (1986) Production and use of monoclonal antibodies to study the cytoskeleton and other components of the cortical cytoplasm of *Chara*. Eur J Cell Biol 41: 1–8
110. Witzmun A, Parthasarathy MV (1985) Role of actin in chloroplast clustering and banding in leaves of *Egeria, Elodea,* and *Hydrilla*. Eur J Cell Biol 39: 21–26
111. Wulf E, Deboben A, Bautz FA, Faulstich H, Wieland T (1979) Fluorescent phallotoxin, a tool for the visualization of cellular actin. PNAS 76: 4498–4502
112. Yamaguchi Y, Nagai R (1981) Motile apparatus in *Vallisneria* leaf cells. I. Organization of microfilaments. J Cell Sci 48: 183–205