

Studies on *Saprolegnia ferax* suggest the general importance of the cytoplasm in determining hyphal morphology¹

Susan G. W. Kaminskyj²

I. Brent Heath

Department of Biology, York University, 4700 Keele Street, North York, Ontario, Canada, M3J 1P3

Abstract: Tip growth of hyphae is the dominant growth form of the vast majority of fungi, and of their morphological allies, the oomycetes. Tip growth has been considered to result from the balance between the extensibility properties of the apical cell wall and the pressure generated by turgor. This model places regulation of tip growth in the extracellular domain, outside of the direct influence of the normal intracellular regulatory systems, which is at odds with the extraordinary level of regulatory precision needed to produce the typically even-diameter tube characteristic of hyphae. An alternative model, wherein regulation of tip extensibility resides in a part of the cytoskeleton linked to the plasma membrane, places control of hyphal morphology in the cytoplasm. This review focuses on the diverse processes of tip growth as they relate to the latter model, drawing heavily on our results from of the oomycete, *Saprolegnia ferax*. The concept of tensegrity is suggested as a possible integrator of these processes.

Key words: actin, cytoskeleton, cytoplasmic migration, integrin, *Saprolegnia ferax*, spectrin, tensegrity, tip growth

INTRODUCTION

Tip growth is polarized extension of walled cells, producing a tubular cell morphology, examples of which are found in all kingdoms of walled eukaryotes. Fungi and oomycetes have tip growing hyphae. Higher plants have specialized tip-growing cell types, root hairs and pollen tubes. Lower plant cells such as the protonemata and caulonemata of ferns and mosses grow in

this way, as do algal rhizoids. Non-walled nerve cells (Heidemann, 1990) have similar mature forms also, as do some prokaryotes (Prosser, 1990) although these latter will not be discussed.

Despite their taxonomic diversity, tip-growing cells share an important characteristic: they are not motile, suggesting that tip growth is a consistent mechanical/bioengineering solution for exploring the environment through directed growth. For this strategy to be successful, these cells must be able to detect changes in the physical and chemical nature of their surroundings and respond by altering their growth direction or by differentiating. However, the polyphyletic distribution of tip-growing cells makes it likely that the details of their tip growth mechanisms will vary.

Tip growth has been studied for over a century, particularly in heterotrophic tip-growing organisms, fungi and oomycetes, because they have a strong impact on human life, both beneficial and otherwise. These organisms secrete diverse substances, some of which are the foundation of major industries, while others are essential for nutrient recycling. However, these organisms can also cause decay and disease. Tip growth regulation is an important topic if we are to exploit or control these organisms in more sophisticated ways because, as Gooday and Gow (1990) rightly observed, "For a fungal hypha, life is at the tip." In this paper we focus primarily on recent advances relating to morphological aspects of tip growth in the hyphal oomycete, *Saprolegnia ferax* (Gruith.) Thuret, where a number of concepts and aspects of tip growth have been developed and investigated. Our intent is to introduce some of these ideas, and discuss them in the context of our current understanding of tip growth regulation, rather than to provide an encyclopedic overview of the topic. For this reason, much of the original literature is referred to only via appropriate reviews.

CYTOPLASM

General appearance and behavior of cytoplasm in growing tips.—Hyphae extend at their tips, which contain organelle-rich cytoplasm. In contrast, the subapical regions (the majority of hyphal colonies) are typically highly vacuolate with a thin layer of mostly peripheral cytoplasm. Both regions have a full range of organelle

Accepted for publication September 6, 1995.

¹ Dedicated to Melvin Fuller in the year of his official retirement, in recognition and appreciation of his long record of contributions to our understanding of fungal cell biology.

² Author for correspondence; current address: Department of Biological Sciences, 333 Hansen Life Sciences Research Building, Purdue University, West Lafayette, Indiana 47907-1392. E-mail: skamin@bilbo.bio.purdue.edu.

•
•
•

•
•

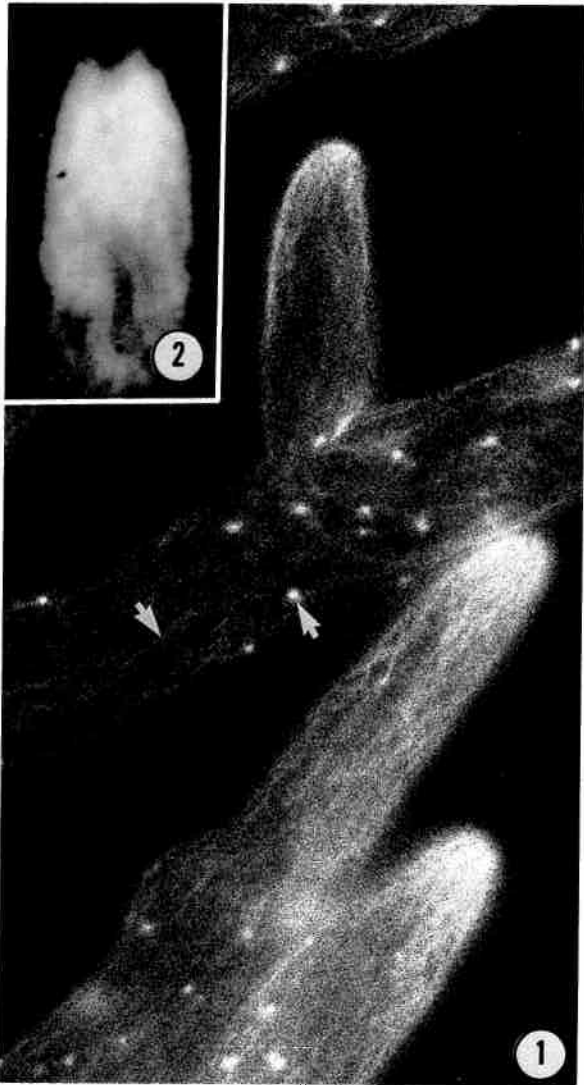
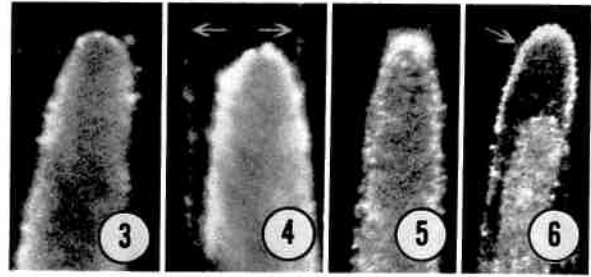


FIG. 1. Formaldehyde-fixed *Saprolegnia* hyphae showing rhodamine phalloidin stained peripheral filamentous actin arrays in apical and subapical regions, which are closely associated with the plasma membrane. The tips have a dense population of fine longitudinal fibrils which merge with the sub-apical populations of coarser fibrils and plaques (arrows). This field of view is unusual in that the periphery of both apical and subapical hyphal regions are in focus. $\times 2,000$. From Heath, 1987.

FIG. 2. A median optical section of the apex of a living, growing *Saprolegnia* hypha stained with rhodamine-labelled phalloidin, showing the filamentous actin which permeates the central cytoplasm and is concentrated just behind the tip. The peripheral arrays shown in FIG. 1 do not stain under these conditions. The characteristic cleft in the extreme tip behaves much like a Spitzenkörper in changing lateral position before a corresponding change of growth direction. The staining intensity of this central actin is much lower than the peripheral arrays in FIG. 1; this image was captured with an image intensifier and is reproduced on a different intensity scale. $\times 1,900$. From Jackson and Heath, 1993b.



FIGS. 3–6. Median optical confocal immunofluorescence micrographs of formaldehyde-fixed hyphal tips of *Saprolegnia*, using primary antibodies directed against spectrin (FIGS. 3 and 4) and β_1 -integrin cytoplasmic domain (FIGS. 5 and 6). In all cases, the stain is primarily concentrated in the periphery of the hyphae in the form of plaques. In FIGS. 4 and 6, the cytoplasm separated from the apical cell wall (indicated by arrows) during processing, showing that the spectrin-staining plaques mostly retracted with the cytoplasm (FIG. 4), whereas the integrin-staining plaques primarily remained attached to the cell wall (FIG. 6). FIGURES 3 and 4 $\times 2,200$, FIG. 5 $\times 1,600$, FIG. 6 $\times 1,050$.

types and subapical regions can generate new growing tips. The relative abundance of organelles and vacuoles varies along the hyphal length and, since this distribution is maintained as the colony enlarges, the organelle-rich apical cytoplasm appears to stay with the hyphal tip as it grows. Therefore, tip growth seems to be an energetically economical process, in that hyphae synthesize mostly wall and membranes as they grow, but relatively little organelle-rich cytoplasm.

Cytoplasm is permeated by a protein meshwork, the cytoskeleton, which generates and/or regulates structural and force-generating cytoplasmic functions. In hyphae, predominant filamentous components of the cytoskeleton are microtubules and filamentous actin (Heath, 1990, 1994), whose organization and functions are determined by microtubule-associated (Olmsted, 1986, 1991) and actin-binding proteins (Stossel et al., 1985; Hartwig and Kwiatkowski, 1991; Vanderkerckhove and Vancompernelle, 1992), respectively.

The apices of tip-growing cells contain abundant cytoskeletal arrays, including actin (Heath, 1990; FIGS. 1, 2). *Saprolegnia* hyphae also contain a spectrin homologue (FIGS. 3, 4) and at least one protein, an integrin homologue (FIGS. 5, 6), which are likely to play a role in linking the cytoskeleton to the cell membrane and wall (Kaminskyj and Heath, 1995). *Aspergillus nidulans* hyphae also have an integrin homologue with a similar cellular distribution (Kaminskyj and Hamer, 1995). The presence of spectrin homologues in fungal hyphae has not yet been investigated. Likely arrangements of these proteins are summarized for fungal and oomycete hyphae in Fig. 7.

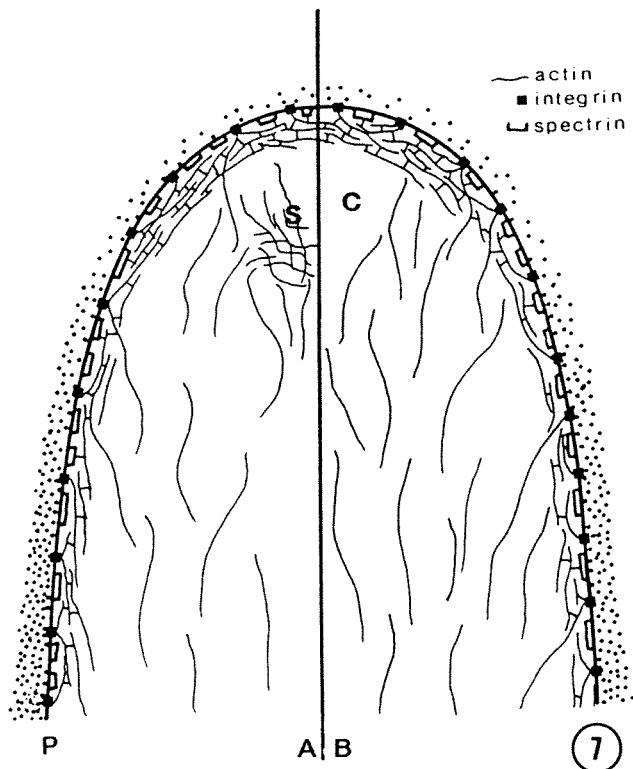


FIG. 7. Diagrammatic interpretation of the construction of a growing hyphal tip of a Spitzenkörper-containing fungal species (A) and an oomycete (B). The degree of cross linking, and thus strength, in the cell wall is indicated by the intensity of stippling outside the plasma membrane (P). The plasma membrane is shown as being strengthened by filamentous actin and spectrin linked to its inner surface and to the integrin, linked to the cell wall. The strength of the membrane-associated skeleton is indicated by the abundance of filaments and the number of cross links. The cytoplasm is permeated by filamentous actin, which is concentrated in the Spitzenkörper (S), but which is depleted in the cleft (C).

Saprolegnia hyphae have distinct peripheral (FIG. 1) and central (FIG. 2) actin populations, based on rhodamine phalloidin staining affinity in living *vs* fixed cells (Jackson and Heath, 1990b). In contrast, rhodamine phalloidin staining patterns in living *Uromyces* hyphae are similar to those in fixed specimens (Corrêa and Hoch, 1993). In *Saprolegnia*, the peripheral arrays appear to be closely associated with the cell membrane, and are considerably more abundant and variable in appearance than the central arrays. Both populations are more concentrated at the tips of growing hyphae, and both are predominantly arranged parallel to the hyphal axis (Jackson and Heath, 1990b). Therefore in *Saprolegnia* the variability in cytoplasmic organelle abundance between apical and subapical regions, and peripheral and central zones, is complemented by their being associated with distinguishable actin arrays.

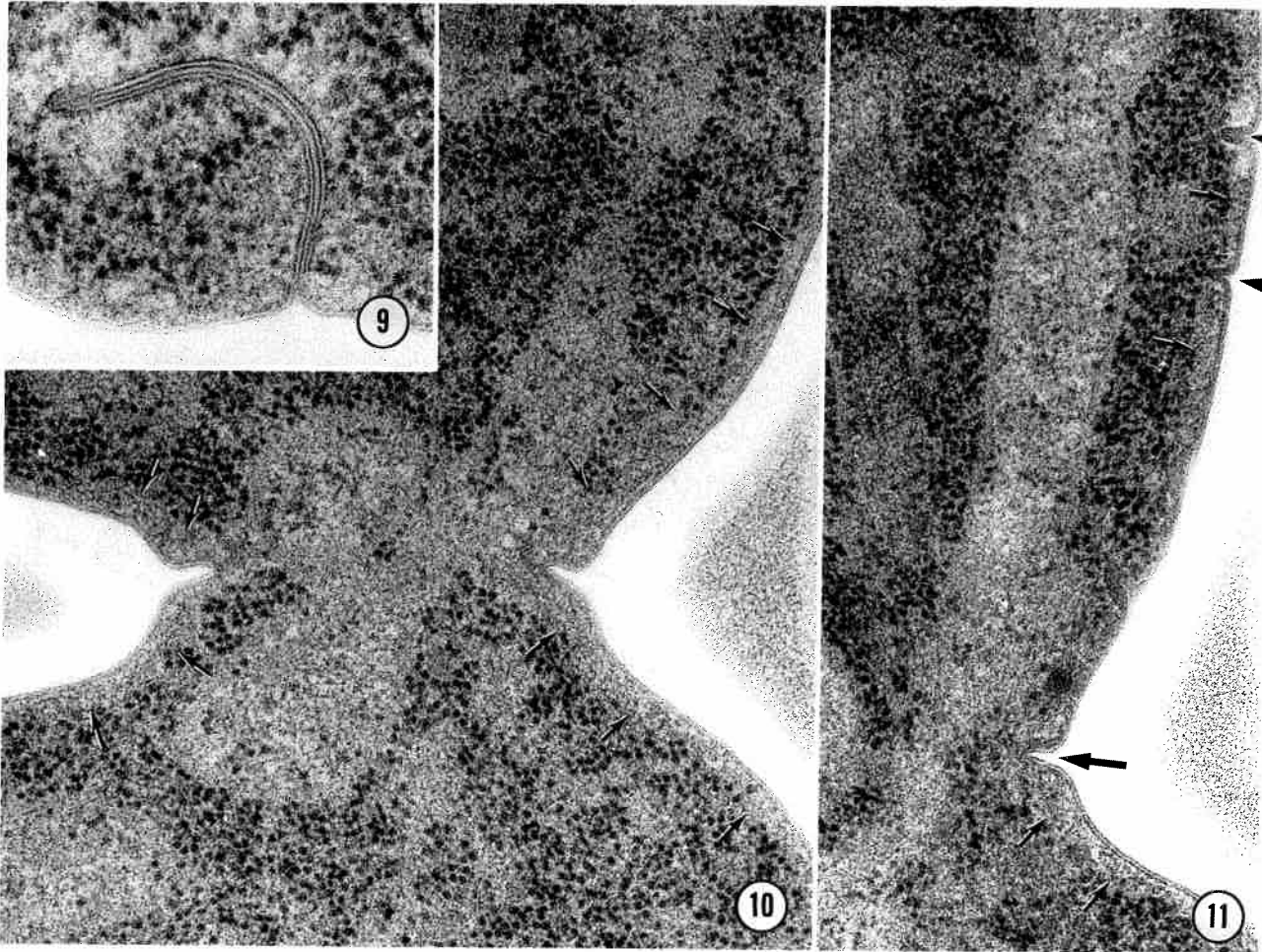
In *Saprolegnia* hyphae the peripheral arrays form



FIG. 8. Portion of the peripheral cytoplasm in the subapical region of a freeze substituted hypha of *Saprolegnia*. A patch of poorly preserved fibrillar material (indicated by arrows) adjacent to the plasma membrane (p), from which ribosomes are excluded, is comparable in size to the subapical actin plaques in FIG. 2. $\times 66,000$.

an apical cap of fine fibrils, which intergrades subapically to coarser fibrils and cables with interspersed plaques (FIG. 1) (Heath, 1990), the details of which are preparation dependent (Kaminskyj and Heath, 1994). Although the function of these subapical plaques remains elusive, they can be immunofluorescently localized in freeze-substituted hyphae (Kaminskyj and Heath, 1994). Plaque-sized accumulations of as yet uncharacterized fibrillar material can be found adjacent to the cell membrane in subapical regions of *Saprolegnia* hyphae using freeze-substitution electron microscopy (FIG. 8).

The central actin arrays in *Saprolegnia* hyphae are diffuse, and vary in concentration but not dramatically in pattern, although in growing hyphae they are more fibrillar in appearance (Jackson and Heath, 1993c). Jackson and Heath (1990b) suggest that differences in appearance and staining affinity of the central and



FIGS. 9-11. Portions of freeze substituted budding cells of *Saccharomyces cerevisiae* showing the peripheral zone of fibrillar material adjacent to the plasma membrane (arrows). This zone extends across the neck of the bud region (FIG. 10) and envelopes plasma membrane invaginations in the neck and elsewhere (arrows in FIG. 11). These have recently been shown in chemically fixed specimens to be foci for actin accumulations (Mulholland et al., 1994). These invaginations can be very large (FIG. 9), and their concentration in growing regions of cells suggests their importance in growth. Their demonstration in freeze substituted material suggests that they are not an artefact of chemical fixation. FIGURES 9, 10 $\times 68,000$, FIG. 11 $\times 105,600$.

the various patterns of peripheral actin in *Saprolegnia* hyphae may relate to their actin-binding protein complements, implying that the actin in peripheral and central zones has different functions.

Saprolegnia actin patterns cannot be generalized to all tip-growing cells, since fungi have apical actin spots instead of obvious fibrillar arrays (Heath, 1990). *Uromyces* (Hoch and Staples, 1983; Corrêa and Hoch, 1993) and *Saccharomyces* (Adams and Pringle, 1984) actin arrays bind phalloidin, suggesting that their apical spots contain actin filaments. Actin has been immunolocalized in fungi using electron microscopy. In *Magnaporthe* (Bourett and Howard, 1991) and *Sclerotium* (Roberson, 1992) hyphae, actin is found throughout the cytoplasm, and localizes to filosomes, fibril-coated vesicles of unknown function. However, filosomes may not be coincident with all actin spots

and, while there are intriguing similarities (cf. FIG. 8 in Hoch and Staples, 1983), their distributions are somewhat different. *Saccharomyces* actin has been immunolocalized to apical cell membrane invaginations in expanding regions, and along subapical cables, using electron microscopy of chemically-fixed cells (Mulholland et al., 1994), consistent with light microscopic patterns (Adams and Pringle, 1984). These invaginations can also be preserved using freeze-substitution electron microscopy (FIGS. 9-11). Since cells with either fibrillar or spot-like apical actin arrays can grow by tip growth, these very different actin patterns may have some functions in common.

The subapical regions of *Saprolegnia* hyphae usually have a thin layer of peripheral cytoplasm, which may be relatively stable given the continuity of peripheral actin patterns (Heath, 1990), and which may be de-

posited there as the cell extends, analogous to the wall. The central cytoplasm appears instead to be migrating to keep up with the apex. Along with this seeming difference in migration behavior during growth, organelles in *Saprolegnia* have characteristic radial distributions in the apical cytoplasm (Heath and Kaminskyj, 1989), so that even at the tip the central and peripheral zones have somewhat different organelle complements. This suggests that central and peripheral cytoplasm may also have different functions.

Apex-directed cytoplasmic migration.—Organelle-rich cytoplasm appears to stay at hyphal tips as they extend. The force for this migration may be intrinsic to the apical cytoplasm, or may be imposed by the subapical regions. The latter model implies that apical cytoplasm might be pushed forward by the subapical vacuolate regions, but this is unlikely. Pushing would require an internal pressure gradient, which has not been detected by direct pressure probing (Money, 1990).

In many hyphae, large subapical vacuoles form from smaller ones in the near-apical cytoplasm, so there is a gradation from organelle- to vacuole-rich cytoplasm (e.g. Heath and Heath, 1979), although this is not obvious in all tip-growing cells, e.g. pollen tubes (Pierson and Cresti, 1992) and algal rhizoids (Kiss and Staehelin, 1993). A pressure gradient remains unlikely in the latter systems since membranes probably lack the strength to sustain a pressure differential. Therefore it seems likely that the apical cytoplasm migrates actively to maintain its position at the tip as it extends, and indeed, hyphae have been described as tube-dwelling amoebae (Reinhardt, 1892; Heath, 1990).

Amoeboid cells use actin-myosin based force generating systems, particularly for extension and/or locomotion. Both actin (Heath, 1990) and myosin (Watts et al., 1985; van Tuinen et al., 1986; Heslop-Harrison and Heslop-Harrison, 1989; Tang et al., 1989; Johnston et al., 1991; Kaminskyj and Heath, 1994b; McGoldrick et al., 1995) have been reported in tip-growing cells, consistent with an amoeboid model of cytoplasmic migration. As with amoeboid cells, specialized actin patterns are associated with extending regions of hyphae (Heath, 1990). Furthermore, treatments which damage these actin arrays interfere with extension (Grove and Sweigard, 1980; Jackson and Heath, 1990a, 1993b; Levina et al., 1994).

Actin-myosin based motility systems, such as amoeboid cells and muscle, are calcium regulated, as are tip-growing cells (Picton and Steer, 1982). Tip growing cells have apex-high calcium gradients (Jackson and Heath, 1993a; Pierson et al., 1994) which are essential for extension (Garrill et al., 1993; Pierson et al., 1994). In *Basidiobolus* and *Saprolegnia* hyphae, local (McKerracher and Heath, 1986; Jackson and Heath, 1992)

and general (Kaminskyj et al., 1992b) treatments that disturb ion homeostasis induce cytoplasmic contractions. Similar contractions can be induced by the calcium-selective ionophore, A23187 (Kaminskyj et al., 1992b). In all these cases, contraction characteristics can be influenced by changes in exogenous calcium levels. These contractions are predominantly parallel to the hyphal axis, i.e. aligned with the actin arrays in *Saprolegnia*. Apex-directed cytoplasmic migration may be supplemented by consistent polarity of actin filaments in the cytoplasm, but this has not been examined. Cytoplasmic contractions can significantly reduce hyphal diameter in *Saprolegnia* (Kaminskyj et al., 1992b), implying that the longitudinal cytoskeletal arrays may be interconnected laterally.

Cytoplasmic microtubules appear to be important in organelle positioning and cytoplasmic cohesion, but they are not essential for tip extension (Heath, 1990, 1994). Co-distributed microtubules and organelles, particularly nuclei and mitochondria, have been widely reported (Heath, 1994). Microtubules may be short (McKerracher and Heath, 1986, 1987; Heath and Kaminskyj, 1989) or extended (McKerracher and Heath, 1987; Meyer et al., 1988; Roberson and Fuller, 1988; Kaminskyj et al., 1989; Raudaskoski et al., 1991). Short microtubules are unlikely to be tracks for organelle motility because they are often shorter than the organelles with which they associate (Heath and Heath, 1978; McKerracher and Heath, 1986). However, the alignment of short microtubules in *Saprolegnia* after tubulin immunofluorescence (Kaminskyj and Heath, 1994) suggests that apparently separate (in freeze-substituted electron microscopy; Heath and Kaminskyj, 1989) microtubules may be functionally interconnected, possibly through linkages with actin by diverse filament binding proteins (Heath, 1994).

Tubulin immunofluorescent arrays are appropriately arranged to mediate nucleus spacing (Kaminskyj et al., 1994), and in several species disruption of microtubules by microbeam ablation (McKerracher and Heath, 1986) or antimicrotubule drugs (Heath, 1982; Kaminskyj et al., 1989) affects nucleus positioning. In fungi with extended microtubules, microtubule-based motor protein homologues have been identified, and are associated with organelle motility (Meluh and Rose, 1989; Enos and Morris, 1990; Eshel et al., 1993; Xiang et al., 1994).

During induced, general cytoplasmic contractions in *Saprolegnia*, the central cytoplasm moves faster, further and longer than the peripheral layer (Kaminskyj et al., 1992b). This is consistent with the inferred pattern of cytoplasmic migration during normal growth, where the central cytoplasm appears to migrate with respect to the peripheral layer, suggesting that there is a shear zone between them. The peripheral cyto-

plasm seems to be relatively immobile, and is likely to be anchored to the cell wall to provide a stable base against which force can be exerted.

Cytoplasmic anchoring.—Amoeboid cells have regulated anchorage to their extracellular matrix, through membrane-spanning proteins such as integrins, which mediate attachment and signalling (Hynes, 1992; Sastri and Horwitz, 1993; Williams et al., 1994). If the apical cytoplasm in *Saprolegnia* hyphae is migrating actively, using a mechanism analogous to the apical contraction model of amoeboid motion (Allen, 1961), wherein attachments to the substrate underlie directed cell movement, then hyphal cytoplasm should require attachments to its substrate, the cell wall, in order to pull itself forward.

Walled and amoeboid cells have analogous protein components (Marcantonio and Hynes, 1988; Quatrano et al., 1991; Sanders et al., 1991; Wagner et al., 1992; Hostetter et al., 1993; Zhu et al., 1993) and functional similarities (Schindler et al., 1989; Wayne et al., 1992) in their cytoskeleton-extracellular matrix interactions, suggesting that specific cytoplasm-cell wall interactions are required for normal cellular functions (Kropf, 1992; Sanders and Lord, 1992; Goodner and Quatrano, 1993; Wayne et al., 1992; Wyatt and Carpita, 1993; Hostetter, 1994). *Saprolegnia* (FIGS. 5, 6) and *Aspergillus* (Kaminskyj and Hamer, 1995) have immunoreactive integrin homologues, whose peripheral distribution and behavior suggest they are important for anchoring the cytoplasm to the cell wall (Kaminskyj and Heath, 1995).

The *Saprolegnia* integrin homologue is more abundant near hyphal tips than subapically, although it is found in both regions (Kaminskyj and Heath, 1995). Differential cytoskeleton-wall anchoring which is stronger at the tips could contribute to polarized cytoplasmic migration. Apical and subapical cytoplasmic anchoring may also be required for organelle motility, as has been proposed for nucleus migration in several systems (Aist and Berns, 1981; McKerracher and Heath, 1987; Kaminskyj et al., 1989; Aist and Bayles, 1991). This is consistent with the longitudinal distribution of the integrin homologue in *Saprolegnia* and *Aspergillus*. As well, since transverse cytoplasmic contractions that affect hyphal diameter in *Saprolegnia* (Kaminskyj et al., 1992b) do not cause cytoplasm-wall separation, the force of these contractions may be transmitted to the wall by linkages between wall and cytoplasm.

Amoeboid cells can move with respect to their extracellular matrices. In contrast, *Saprolegnia* hyphae appear to retain a stable peripheral cytoplasm, discussed earlier, past which the central cytoplasm seems to migrate. This suggests that the cell membrane and

wall of these hyphae might not move with respect to each other except, for example, during zoosporegenesis (Heath and Harold, 1992). Cytoskeleton-integrin homologue interactions in *Saprolegnia* hyphae may be organized differently from the potentially reversible focal contacts of amoeboid cells.

HYPHAL MORPHOLOGY

Roles of the hyphal wall.—The hyphal wall likely has several functions, of which the most obvious is structural support for the cell. In subapical regions, the mature hyphal form is enclosed by a wall which is fully crosslinked (Wessels, 1990; Gooday, 1994) and largely inextensible (Robertson and Rizvi, 1968). The wall is also the 'skin' of the cell and, as with our skin, it is where environmental stimuli are first intercepted. Some of these must traverse the wall before encountering membrane-based receptors that can interact with the cytoplasm (e.g. Madden et al., 1991; Zhou et al., 1991). Cell wall components sometimes appear to attach hyphae to their surroundings (e.g. Epstein et al., 1985, 1987; Terhune and Hoch, 1993; Braun and Howard, 1994a, b) which is important for recognition of physical features (Terhune and Hoch, 1993). The wall may also have a regulatory role defined by its chemical constituents, likely not including structural components.

Rust fungus urediospore infection structures have a succession of habitats: germlings grow on external plant surfaces, infection hyphae grow intercellularly, and haustoria penetrate plant cell walls (Littlefield and Heath, 1979). These stages each have distinct wall composition (Kaminskyj and Heath, 1983; Freytag and Mendgen, 1991). Similarly, the aerial hyphae of *Schizophyllum* fruiting structures have hydrophobic wall protein components not seen in submerged, vegetative mycelium (Wessels, 1993). In amoeboid cells, cytoskeletal interactions with different substrate compositions are mediated by different integrins (Hynes, 1992), which is also expected for hyphal cytoplasm interacting with walls of different composition. Consistent with this, fungal cell differentiation is accompanied by laying down a new inner wall layer (e.g. Littlefield and Heath, 1979).

Wall deposition.—Hyphae grow at their tips, where the cytoplasm produces and simultaneously occupies a membrane-wall tube. The tip expands at the apex as deposition and growth continue, and the final mature form is attained subapically. Tip-growing cells have even profiles, so their form appears to develop in a regulated way. As well, different species and life cycle stages are distinguishable (e.g. FIG. 1 in McKerracher

and Heath, 1987), so their morphology regulation systems appear to be precisely controlled.

Wall deposition seems to be coordinated with tip extension because, typically, the wall is evenly thick. Normally, the wall is smoothly lined with membrane, again implying depositional coordination. A mechanism for recycling excess cell membrane and wall material has not been described in hyphae (Heath, 1990), and certain growth disturbances are characterized by excess material (e.g. lomasomes) being deposited inside an otherwise smooth wall (Heath, 1987; Heath and Greenwood, 1970).

Localized wall deposition involves several processes which must be individually controlled and coordinated. The cell wall is composed of a matrix of proteins and glucans crosslinked to fibrils, which are composed of chitin or cellulose (Gooday and Gow, 1990; Wessels, 1990; Gooday, 1994). These wall-building materials appear to be synthesized in the subapical cytoplasm (e.g. Heath et al., 1985) and delivered to the tip in wall-forming vesicles (FIG. 12, 13), where the majority of exocytosis occurs (Fèvre and Rougier, 1982). Heath and Kaminskyj (1989) suggest that, in *Saprolegnia* hyphae, apex-directed wall vesicle transport may depend on the peripheral actin arrays, since wall vesicle patterns and subapical actin cables are similar. The timing of vesicle fusion must be controlled also, so that it does not occur precociously between exocytic vesicles within the cytoplasm (e.g. Heath, 1987), although this has not been studied systematically. Interestingly, wall-forming vesicles in *Saprolegnia* (FIGS. 12, 13) and *Aspergillus* (FIGS. 14, 15) hyphal tips have sparse, fibrillar coats (Heath et al., 1985; Heath and Kaminskyj, 1989), which may be involved in controlling fusion (Heath, 1995).

In fungi there appear to be two types of wall-forming vesicles, those which contain matrix materials and others which contain fibril synthesizing enzymes (Bartnicki-Garcia, 1990), although this may not be so in *Saprolegnia* (Heath and Kaminskyj, 1989). Fibrils are produced by enzyme synthetic complexes which are probably integral proteins in the wall vesicle membrane (Bartnicki-Garcia, 1990). Matrix material is likely to be in the wall vesicle lumen, corresponding to the localization of secreted materials in other cells.

Hyphae also produce a variety of materials such as extracellular enzymes (Gooday and Gow, 1990) which need not directly involved in wall building. Vesicles apparently destined for exocytosis can have variable appearance (Bartnicki-Garcia, 1990) and content (e.g. Hill and Mullins, 1980a, b). Deposition of an even, membrane-lined wall is likely to be regulated by controlling the proportions of different types of secreted materials. An appropriate sorting mechanism(s) has not yet been described in tip growing cells, however,

in animal systems constitutive and regulated secretion are separately controlled.

The site of wall deposition in the hyphal apex correlates with the direction that the hypha will grow. Bartnicki-Garcia (1990) proposed that wall-forming vesicles are marshalled through a vesicle supply centre, from which they would be directed to the cell membrane. Treating *Gilbertella* (Grove and Sweigard, 1980) and *Saprolegnia* (Jackson and Heath, 1990a) hyphae with the actin-selective poison, cytochalasin, induced tip swelling, suggesting that actin might have a role in this process.

The Spitzenkörper is an aggregation of vesicles in the tips of growing fungal hyphae (Grove and Bracker, 1970), whose position is coincident with the theoretical position of the vesicle supply centre (Bartnicki-Garcia, 1990), and the lateral position of the Spitzenkörper correlates with growth direction in living hyphae (Grove and Bracker, 1970). In fungi, the Spitzenkörper is associated with actin (Bourett and Howard, 1991) and microtubules (Robertson and Vargas, 1994; FIGS. 16, 17) and suggesting that its position is likely to be controlled by the cytoskeleton. *Saprolegnia*, which lacks a Spitzenkörper, has an apical cleft in its central actin arrays (FIG. 2) whose relationship to growth direction suggests an analogous function (Jackson and Heath, 1993c).

Control of wall plasticity.—The newly-deposited apical wall is plastic, i.e. deformable, and this correlates with the region of the hypha that is extending and expanding to full width. However, wall plasticity is limited in duration, since subapically the wall is largely inextensible. If a tip stops growing for more than ~40 s, its form is fixed (Robertson and Rizvi, 1968). New growth from tips whose growth was interrupted is associated with near-apical branching, a pattern distinct from the subapical branching pattern of undisturbed hyphae (Robertson and Rizvi, 1968) but which is probably similar mechanistically.

One model for regulating wall plasticity proposes that the wall is plasticized by "softeners" in the wall-forming vesicles, so that the relative frequency of vesicle insertion determines wall deformability (Bartnicki-Garcia, 1990). This model describes what appears to happen at developing branch sites, where the mature wall must be softened for extension to begin (Mullins, 1973), although these enzymes also seem to be secreted by established tips (Gooday, 1994).

Another model, which may apply to extending tips, proposes that the wall initially is a semi-liquid matrix, which is rigidified as extension continues (Wessels, 1990). Plasticity of the apical wall is suggested to be reduced by progressive crosslinking between matrix components and by crosslinking the matrix with fibrils

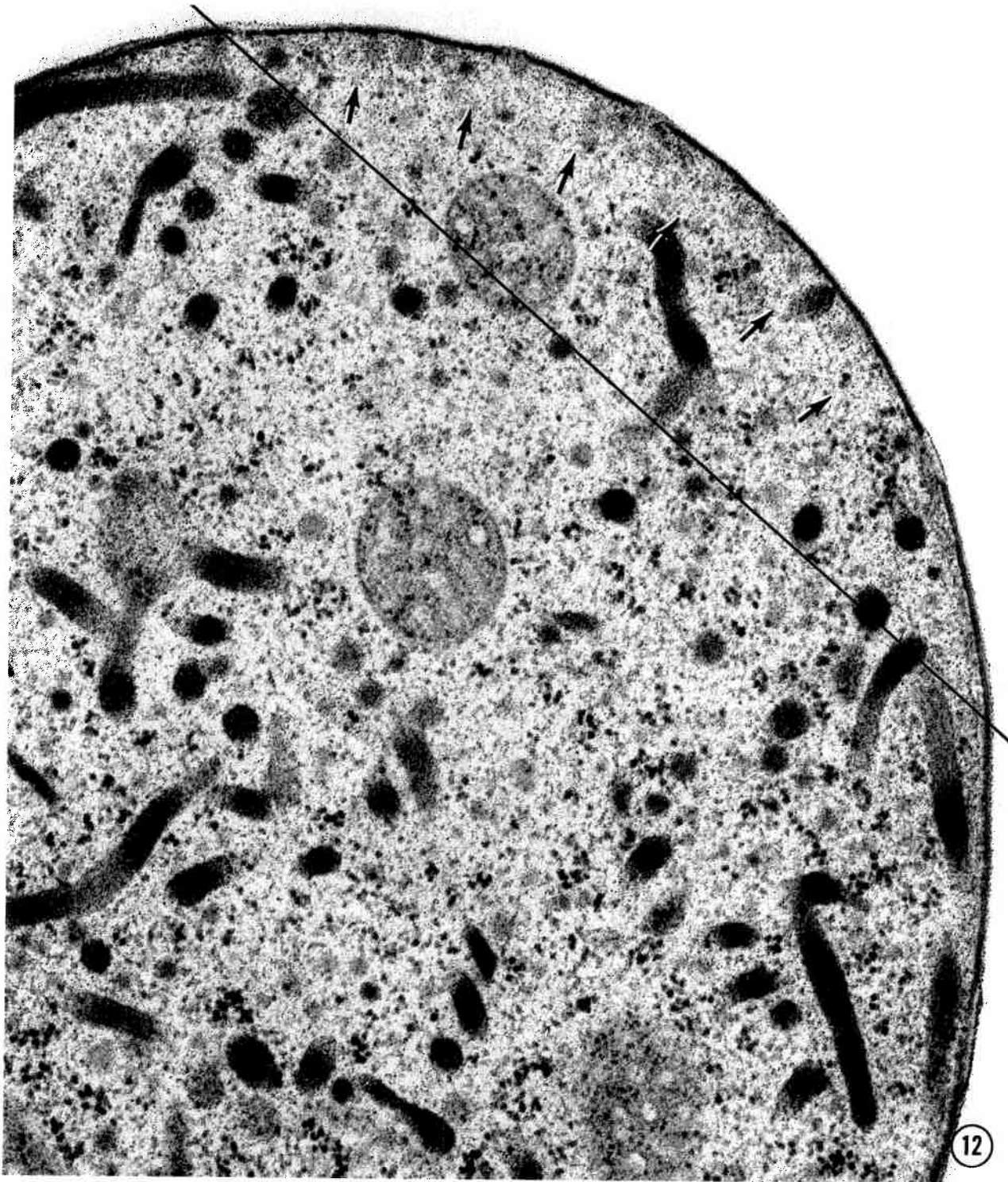


FIG. 12. Portion of a median longitudinal section of a freeze substituted hyphal tip of *Saprolegnia*, showing the characteristically elongated, darkly stained wall vesicles, some of which are embedded in a peripheral zone of fine fibrillar material which lies adjacent to the plasma membrane. This diffuse material typically excludes cytoplasmic structures such as ribosomes. The interface between the peripheral zone and the rest of the cytoplasm is indicated by arrows. The line shows the approximate level of the transverse section shown in FIG. 13. $\times 68,000$.

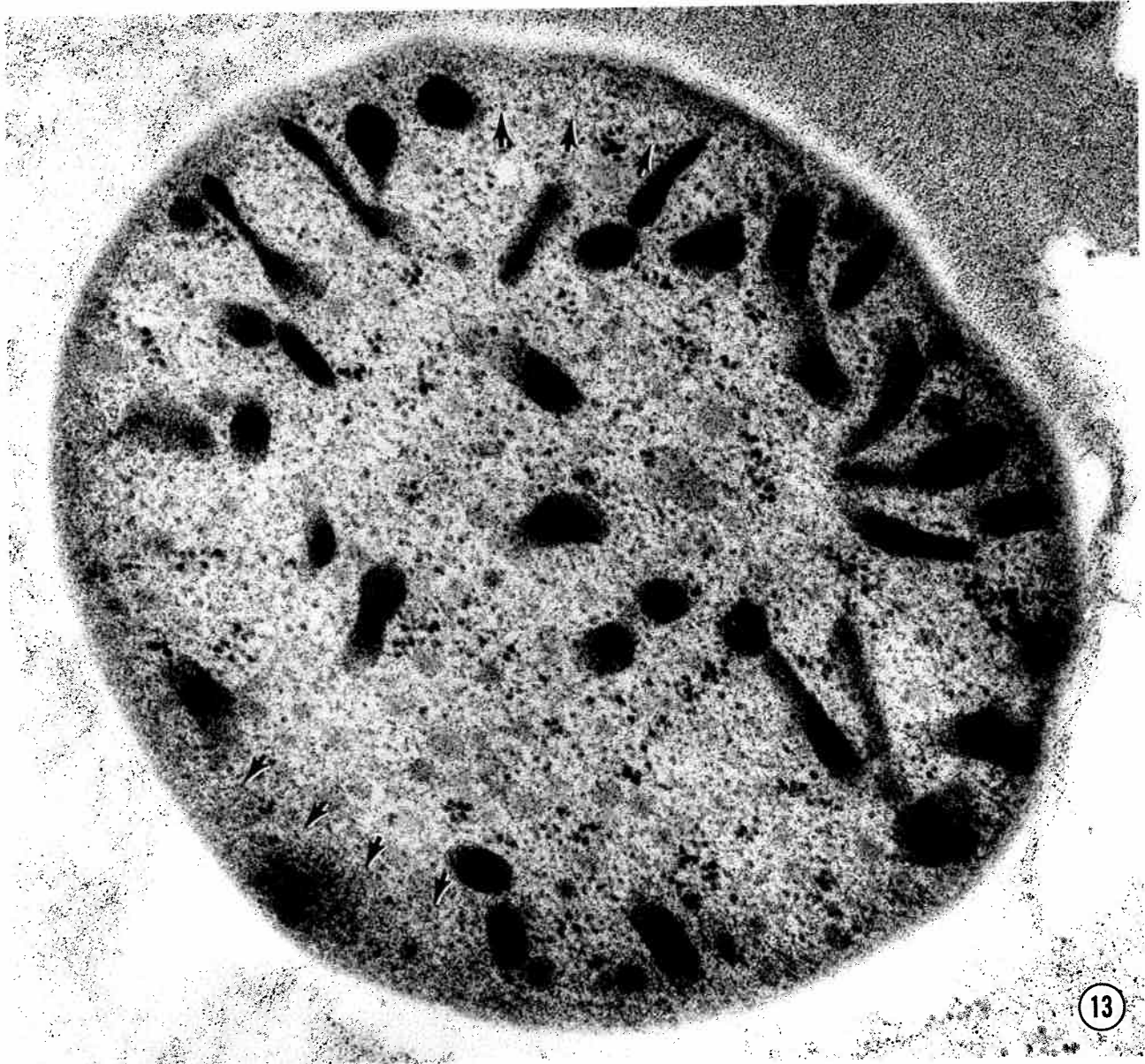


FIG. 13. Transverse section through the tip of a freeze substituted hypha of *Saprolegnia*, $\sim 0.6 \mu\text{m}$ behind the absolute tip, as indicated by the line on FIG. 12. Here, the plasma membrane is sectioned obliquely, and so is not sharp, but the peripheral fibrillar zone is obvious (inner boundary indicated by arrows). Many wall vesicles have their outer ends embedded in this peripheral zone. $\times 68,000$.

produced by enzyme synthesizing complexes. These complexes are integral in the cell membrane and become active in the nascent wall (Bartnicki-Garcia, 1990; Wessels, 1990). Subapically, these processes generate a somewhat flexible, but largely inextensible fabric. This model is supported by detailed biochemical studies of wall composition in *Schizophyllum* hyphae (Wessels, 1990). This model also proposes that 'bulk flow' of material from the inner to outer wall surface during wall deposition can transport materials like enzymes through the wall (Wessels, 1993). These materials are

often considerably larger than the pore size of the mature wall, and their secretion is difficult to explain otherwise.

In the hyphal expansion zone, between deposition of the apical plastic wall and its subapical maturation, forces acting in this region mold the developing hypha into its mature morphology. Factors describing hyphal morphology include diameter, straightness and branching pattern, and a dynamic component, extension rate. Branching has been reviewed elsewhere (Harold, 1990) and will not be discussed here.

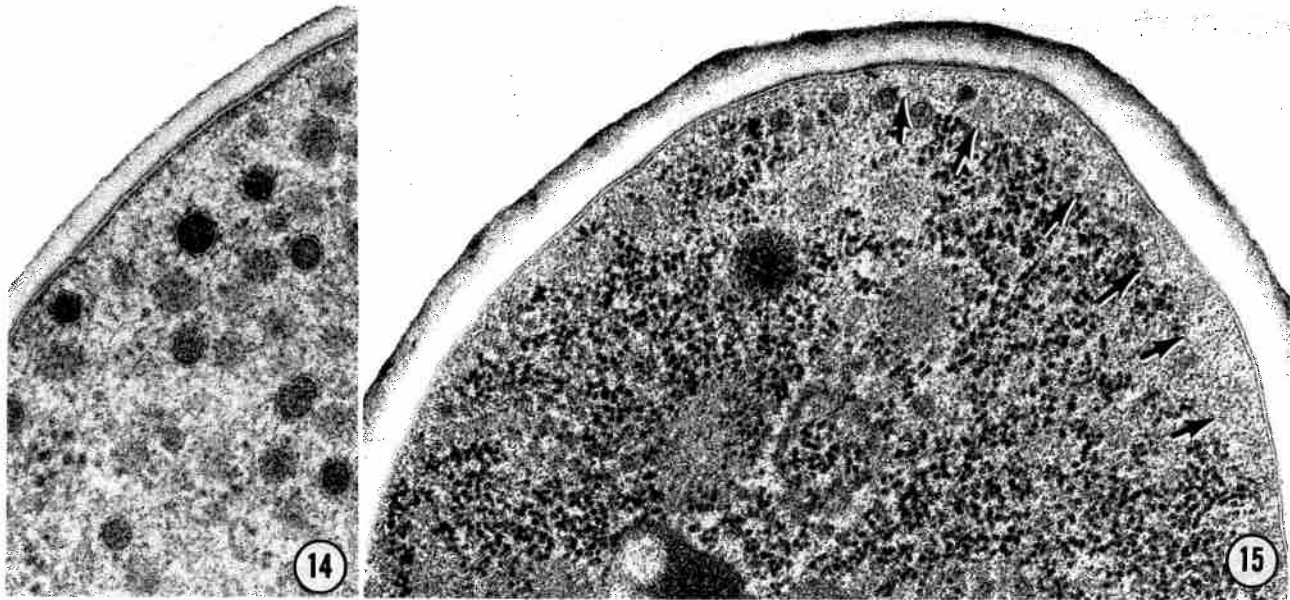


FIG. 14. Portion of a freeze substituted hyphal tip of *Aspergillus flavus* showing the fibrillar matrix which is adjacent to the plasma membrane, in which the wall vesicles are enmeshed. $\times 79,200$. From Kurtz et al., 1994.

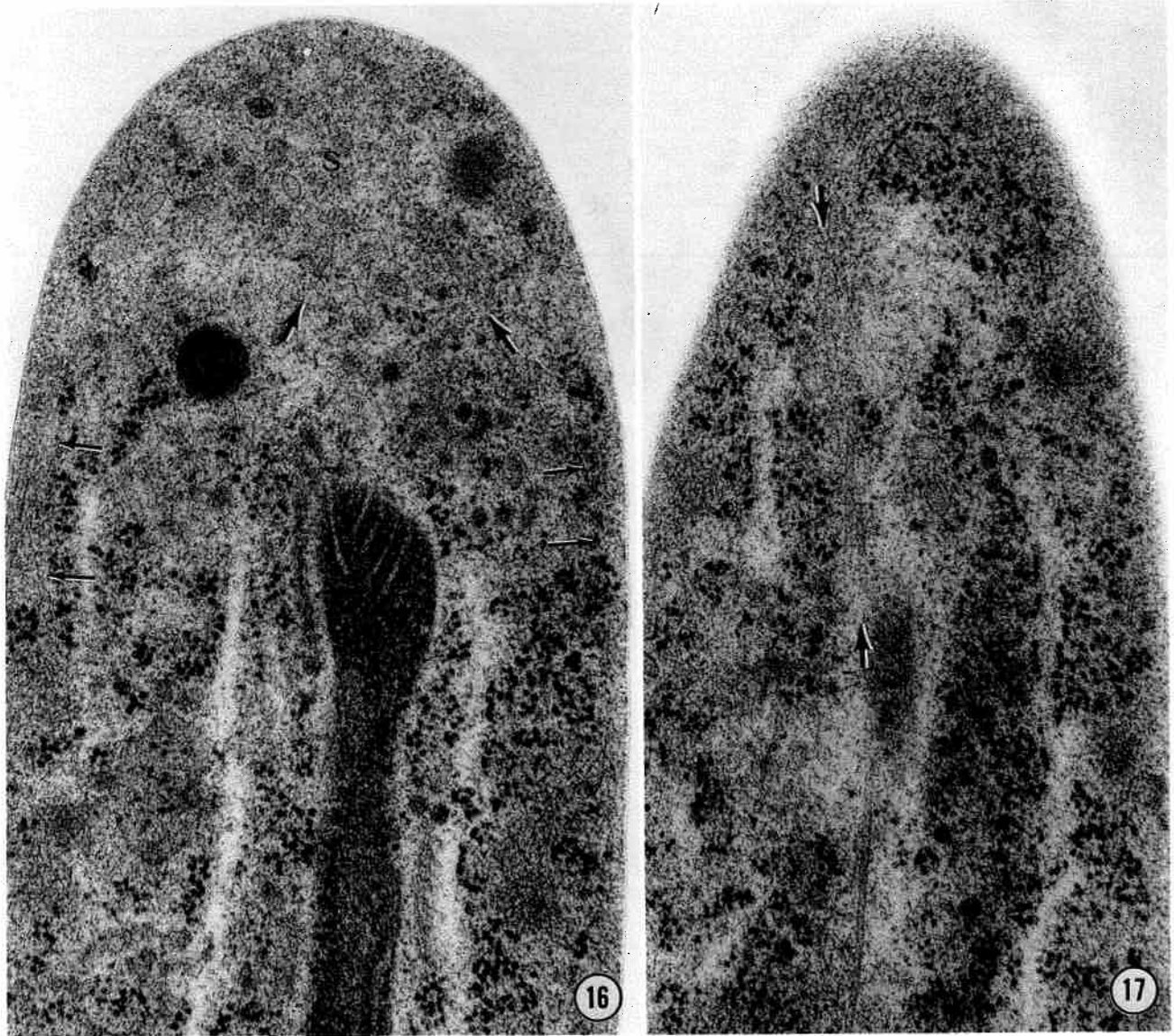
FIG. 15. Portion of the apex of a freeze substituted hypha of *Aspergillus flavus*, whose growth had been severely inhibited by a lipopeptide (Kurtz et al., 1994) prior to fixation. The reduced growth rate correlates with fewer wall vesicles, but makes the peripheral zone of fibrillar material adjacent to the plasma membrane more prominent (arrows). $\times 51,000$.

Hyphal extension rate and control of hyphal morphology.— The membrane lining the wall tube allows the cytoplasm to have a different ionic composition from its surroundings. Because the cytoplasm of walled cells typically contains more solute than the environment, these cells develop an hydrostatic pressure called turgor. The force of turgor is on the cell membrane, which is supported by the cell cortex. In subapical regions this force is borne largely by the mature wall (Robertson and Rizvi, 1968). However, in extending regions, where the wall is plastic, there may be internal reinforcement from actin arrays (Grove and Sweigard, 1980; Jackson and Heath, 1990a). Walled cells can use turgor to provide force for substrate penetration (Money, 1995), and to support aerial structures, which can be important for spore dispersal. Turgor is regulated by membrane permeability (e.g. Clipson and Jennings, 1990) and metabolic processes (e.g. Luard, 1982).

Since the hyphal tip is deformable, turgor has been proposed to determine both tip extension rate and hyphal morphology (Saunders and Trinci, 1979; Koch, 1982; Wessels, 1988). However, these models are not well supported by experimental evidence. The few studies on turgor-growth rate correlations in fungi have given variable results, and similarly contradictory data have been found in the relation between medium concentration and growth rate (reviewed in Kaminskyj et al., 1992a).

The relation between turgor and growth rate has been studied intensively in oomycetes, whose colonies are coenocytic, forming single pressure compartments uncomplicated by the possibility that septal pores might plug. Oomycetes grow rapidly, so that rate variations can be measured accurately even over short intervals, and oomycete hyphae are large enough that turgor pressures can be measured directly by probing (Money, 1990; Money and Harold, 1992, 1993), and can also be estimated for a colony (Money, 1990; Kaminskyj et al., 1992a) as is done for narrow hyphae. In these studies, only some growth rate variation could be ascribed to turgor level. When growth rate was reduced by increasing medium concentration, turgor levels fell to a plateau wherein there was a two-fold growth rate variation without a corresponding turgor variation. When *Saprolegnia* growth rates were reduced by half with a potassium channel blocker, turgor was unaffected (Kaminskyj et al., 1992a).

Growth rates are also highly variable under conditions where turgor is likely to be constant (Kaminskyj et al., 1992a; Lopez-Franco et al., 1994, 1995), and there are regulated differences in growth rate between hyphae and their branches in most species. Typically, hyphal branching is subapical (Harold, 1990), but hyphal density at the colony margin stays approximately constant as the colony grows. For this to happen, the branches must grow faster than hyphae at the colony margin. Taken together these results suggest that tur-



FIGS. 16, 17. Median (FIG. 16) and peripheral (FIG. 17) longitudinal sections from a freeze substituted hyphal tip of *Aspergillus nidulans*. The wall vesicles are enmeshed in a diffuse fibrillar matrix which occupies much of the tip, excludes ribosomes, and extends subapically as a peripheral zone adjacent to the plasma membrane (small arrows in FIG. 16). This peripheral zone is more extensively seen in FIG. 17, where its inner boundary is indicated by the dashed line. The fibrils are especially clear where they are aligned predominantly parallel to the microtubules (arrows). In the median section, cytoplasmic microtubules (large arrows in FIG. 16) appear to be associated with an aggregation of vesicles, the Spitzkörper (s). Both $\times 68,000$.

gor does not regulate growth rate under many conditions. Growing *Saprolegnia* hyphae have peripheral caps of actin whose length correlates with growth rate (Jackson and Heath, 1990a), particularly when turgor level is not growth rate-related (Kaminskyj et al., 1992), suggesting that cytoskeletal plasticity may be a relevant factor. Indeed, for hyphae growing with extremely low turgor, the cytoskeleton may provide the force for tip extension (Heath, 1995; FIG. 18) comparable to amoeboid locomotion.

Turgor level and wall strength must be correlated,

since turgor is balanced by tension in the wall. If a cell is growing when turgor is known to be low, as for oomycete hyphae growing in concentrated media (Kaminskyj et al., 1992a; Money and Harold, 1992, 1993), then the apical wall (and subtending actin arrays) must be soft in order to be deformed by a low internal pressure. Conversely, if the wall is known to be soft, as is expected for an *Aspergillus* mutant with chitin-poor walls (Katz and Rosenberger, 1970), then turgor must be low to maintain cellular integrity. In both cases hyphal morphology was normal, so forces other

than turgor, probably involving the cytoskeleton, are likely to be regulating factors.

The cytoplasm's role in determining hyphal morphology.— Hyphal morphology appears to be regulated by forces within the cytoplasm, particularly in cases as above where the wall is likely to be very soft. Similarities between amoeboid and tip growing systems, coupled with the observation that actin is found in the protruding regions of amoeboid cells, led Picton and Steer (1982) to propose that apical actin arrays should be found in the extending regions of tip growing cells. They reasoned that the apical wall alone might lack the mechanical strength to withstand turgor, and suggested that actin would be well suited for a supportive role, since both its mechanical properties and the tip growth process are calcium-regulated. This model has since been supported by finding actin enriched in the apices of tip growing cells (FIGS. 1, 2; Heath, 1990, 1994).

Picton and Steer (1982) proposed that these apical actin arrays should have the appearance of fine fibrils, since actin polymers are filamentous. Consistent with this prediction, in *Saprolegnia* a filamentous actin cap extends from the apex to the base of the expanding region of growing hyphae (Jackson and Heath, 1990a). In fungi, actin immunolocalizes to filamentous structures (Bourett and Howard, 1991; Roberson, 1992; Mulholland et al., 1994), even when the details are not obvious with light microscopy. Consistent with a supportive role for the apical actin arrays, osmotically shocked *Saprolegnia* hyphae did not burst at the apex (Jackson and Heath, 1990a), which would be expected if the tip were supported by the wall alone. The burst point was subapical, in the expansion zone, suggesting that the tip is supported by additional, presumably cytoskeletal, factors. A similar pattern of subapical bursting after osmotic shock has been reported for *Neurospora* (Robertson and Rizvi, 1968). Treating *Gilbertella* (Grove and Bracker, 1980) and *Saprolegnia* (Jackson and Heath, 1990a) hyphae with cytochalasin induced localized apical swelling, as if the tip wall were unable to maintain the hyphal profile without internal reinforcement.

If peripheral, and particularly apical, actin arrays are ubiquitous in tip-growing cells, why are they not routinely identified using electron microscopy? The reasons likely include the lower absolute levels of actin in walled cells, which are supported by turgor pressure and need not employ a robust cytoskeleton to maintain their shape. These arrays may also be less ordered than those in muscle or microvilli. Nevertheless, careful inspection of peripheral hyphal regions (FIGS. 8-17) reveals a narrow zone of fibrillar material containing relatively few organelles. These fibrils may indicate

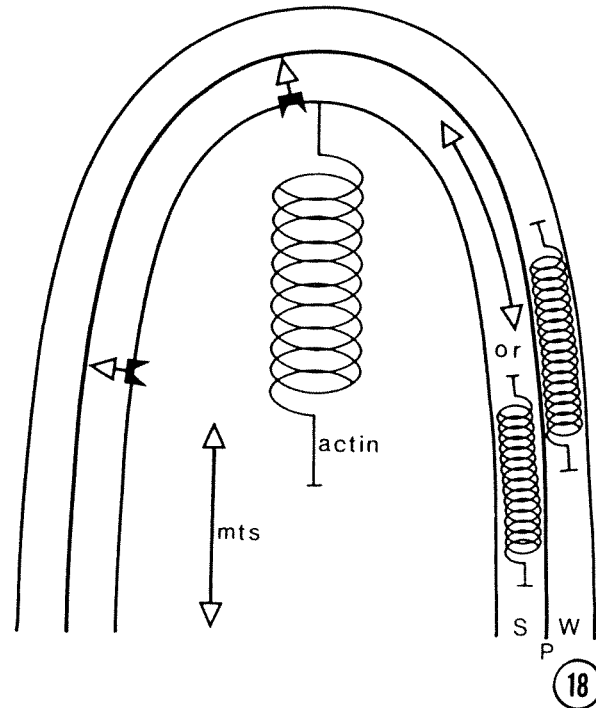


FIG. 18. Summary of the forces postulated in the integrity concept of hyphal tip growth. Springs indicate tension bearing elements (i.e. pulling on the ends of the springs) and the two-headed arrows indicate compression-resistant elements (i.e. pushing in the direction of the arrows). Internally, microtubules (mts) and turgor pressure (tailed arrows) are compression resistant, while the filamentous actin is tension bearing. Externally, the wall (W) is also under tension. The plasma membrane (P) associated skeleton (S) is shown as either compression or tension bearing, depending on the presence or absence of the major intracellular compression resistant force, turgor pressure.

poorly preserved or poorly contrasted actin filaments of a cortical actin cytoskeleton. Actin has not yet been immunolocalized in these regions using freeze-substitution electron microscopy (Bourett and Howard, 1991; Roberson, 1992), but its immunoreactivity is affected by diverse factors (Kaminskyj and Heath, 1994; Mulholland et al., 1994) so negative results are not compelling.

Apical actin arrays are ideally placed for regulating tip deformability from within the cell, and actin is probably also involved in regulating wall vesicle transport and localized wall deposition, as discussed earlier. Furthermore, cytoskeletal control of tip deformation is likely to be subtly, rapidly, and locally responsive to environmental and physiological factors. For actin to be involved in regulating tip deformability, it should be attached to the cell membrane and cell wall, either directly or through intermediate proteins, such as integrin homologues, discussed above.

The amount of wall support to the cell membrane probably depends on wall plasticity, and so the apical region may also require a membrane-reinforcing skeleton (Goodman et al., 1988; Bennett, 1990; Bloch and Pumplin, 1992; Heath, 1995). *Saprolegnia* hyphae have a spectrin homologue which is enriched in hyphal tips (FIGS. 5, 6; Kaminskyj and Heath, 1995), where it may be a membrane-reinforcement in extending regions. Bloch and Pumplin (1992) show that the spectrin tetramers are not always the extended molecules typically seen in spread erythrocyte membrane preparations, but rather are compactly folded 'concertinas'. A transition from more- to less-folded spectrin may occur in the expanding apex, consistent with the longitudinal immunofluorescence distribution of spectrin in *Saprolegnia* hyphae (Kaminskyj and Heath, 1995).

For actin arrays to be involved in regulating tip deformability, they should be interlinked, so that they could not slide freely past each other. *Aspergillus* hyphae have a single-headed myosin which localizes to hyphal tips (McGoldrick et al., 1995) where it might interlink actin filaments according to a functional model for single-headed myosins (Adams and Pollard, 1989). In *Dictyostelium* amoebae, a single-headed myosin is found at the leading edge of pseudopodia, where it is associated with force-requiring activities (Fukui et al., 1989; Baines et al., 1992; Jung et al., 1993) consistent with a recent model for amoeboid crawling (Stossel, 1993). *Saprolegnia* also has a filamin homologue, identified by immuno-blotting and fluorescence (Bachewich, Gupta, Kaminskyj, unpublished), which could regulate actin array extensibility, as well as contribute to cytoplasmic cohesion.

Hyphal growth responses to environmental stimuli.—To this point, we have considered the existence and discussed the possible roles for actin-cell membrane-wall linkages in regulating hyphal growth and morphology. Tip growth appears to be a strategy employed by non-motile organisms to explore their environment through directed growth, requiring that they be able to sense their surroundings through their walls. Environmental responses may be shown by a change in growth direction, suggesting that the mechanisms which are involved in straight growth can be modified for steering. Environmental responses can also trigger differentiation, as has been characterized in mating interactions (e.g. Madden et al. 1991) and pathogenesis (e.g. Hoch et al., 1987).

The environmental responsiveness of tip growth has been refined by the biotrophic rust fungi, whose urediospore germlings are an invasion system, seeking stomata in the early stages of a quest to parasitize a host plant. The oriented growth of rust fungus germlings does not depend on resource detection, since it is directed by physical features of the host surface

(Wynn, 1976). Germling adherence is important for stimulus detection (Terhune and Hoch, 1993), and adherence seems to require extracellular glycoproteins (Epstein et al., 1985, 1987), some of which have been partially characterized (Moloshok et al., 1994). Perceiving these external stimuli through the wall appears to involve the near-apical region of the germ-tube tip on the contact surface (Corrêa and Hoch, 1995; Kwon and Hoch, 1991), for which the sensor may be stretch-activated ion channels (Zhou et al., 1991).

Growth responses vary with species and environmental stimulus. As an example, *Saprolegnia* hyphae change from straight to helical growth in response to the agar substitute, gellan gum (Kaminskyj and Heath, 1992). These tips are responding to diffusible substances plus the physical barrier of the gel. Although the identity of the factor(s), and the mechanisms involved in their detection and subsequent events are not yet determined, they must be detected through the hyphal wall (Kaminskyj and Heath, 1992). It is possible that these factors are detected by an integrin-mediated system interacting with the cytoskeleton (Williams et al., 1994), which consistent with an integrin homologue being enriched at hyphal tips (Kaminskyj and Heath, 1995).

Unexpectedly, the gellan response differs for two classes of morphologically indistinguishable tips, those of established hyphae and their branches. This is a novel indication of functional differences between apparently similar, physically interconnected, tips. However, this difference in growth form response is consistent with the regulated difference in growth rates between hyphae and their subapical branches, discussed earlier.

INTEGRATION

Tensegrity.—Cytoplasmic structure and function in amoeboid cells has been proposed to depend on interactions between compression-resistant and tension-bearing elements (Ingber, 1993; Ingber et al., 1993), essentially a system composed of struts linked by elastic filaments. Structures of this type, including cytoplasm, maintain their shape because they are under tension, giving rise to the name, tensegrity systems. Tension in this sense implies cytoskeletal elasticity, distinct from the physiological meaning of 'negative turgor'. In cytoplasm, the compression-resistant and tension-bearing elements correspond to microtubules and actin filaments, respectively. Stabilized regions between cytoskeleton-to-extracellular matrix linkages are also compression-resistant regions. Hyphal cytoplasm has similar cytoskeletal elements (FIG. 18; Heath, 1990, 1994) which appear to be linked to an extracellular matrix, the cell wall (Kaminskyj and Heath, 1995), that

is relatively incompressible because it is supported by turgor.

In neurons, cytoskeletal tension appears to regulate and promote axonal extension, and experimental manipulation to reduce tension inhibits extension (Heidemann and Buxbaum, 1990). Hyphal cytoplasm seems to have cytoskeletal tension also, because localized ablation of microtubules (McKerracher and Heath, 1986) or actin (Jackson and Heath, 1992) induces contraction away from the irradiation site. General loss of cytoskeletal tension within the hypha, as caused by injury or hypertonic stress, also inhibits growth.

Heidemann and Buxbaum (1990) proposed that the cytoskeletal tension might be a "second messenger" for growth regulation in neurons, and perhaps tension in the peripheral actin cytoskeleton of hyphal tips has an analogous role. In many situations, turgor appears to be unrelated to growth rate, but it is possible that changes in turgor level/cytoskeletal tension are significant. However, as with other systems and second messengers, hyphae can apparently adapt to and grow with different levels of turgor (Kaminskyj et al., 1992a; Money and Harold, 1992, 1993).

A tensegrity-based cytoplasmic model also predicts that the presence and nature of cytoskeleton-extracellular matrix linkages will have a profound effect on cell shape and function (Ingber, 1993; Ingber et al., 1993), which is supported by recent evidence of integrin-mediated mechanotransduction of external stimuli (Wang et al., 1993; He and Grinnell, 1994). The hyphal cytoskeleton and wall may form a similarly interactive system, because treatments which selectively affect actin (Jackson and Heath, 1990a; Levina et al., 1994), or the wall (Jackson and Heath, 1990a) affect growth in *Saprolegnia* hyphae.

Steering.—Hyphal steering responses to environmental factors that cause a change in growth direction, likely involve lateral displacement of the Spitzenkörper in fungi or the apical actin cleft in oomycetes. Since generation of hyphal morphology seems to be regulated by cytoplasmic factors, the same must be true of changes in growth direction. If deformability of the apical actin arrays regulates hyphal morphology, then differential deformability on opposite sides of the tip may contribute to steering.

Actin and actin-membrane-wall attachment proteins are enriched at hyphal apices. Another polarized feature of tip growing systems is an apex-high calcium gradient, which is necessary for growth (Jackson and Heath, 1993a; Garrill et al., 1993; Pierson et al., 1994), and which is generated by a tip-high distribution of stretch-activated ion channels in the cell membrane. Apical actin arrays and stretch-activated channels in *Saprolegnia* hyphal tips appear to interact (e.g. Levina et al., 1994), most likely contributing to the polariza-

tion of the multiple processes involved in tip extension. In unperturbed conditions at least, the integrin and spectrin gradients in *Saprolegnia* hyphae (Kaminskyj and Heath, 1995) correlate with that of stretch-activated calcium channels (Garrill et al., 1992; Levina et al., 1994), suggesting that they may be interrelated. This has also been proposed for gravity sensing in algae (Staves et al., 1992; Wayne et al., 1992), and to regulate environmental responses of plant cells (Pickard, 1994).

In animal systems, spectrins are proposed to isolate and specialize membrane domains by restricting diffusion of integral membrane proteins (Edidin, 1992). By analogy, spectrins may play a similar role in contributing to the specialized distribution of enriched proteins in the hyphal tip. Furthermore, in other systems, there are spectrin-integrin (Goodman et al., 1988), and integrin-ion channel associations, which are implicated in growth regulation (Schwartz et al., 1991). The specific mechanisms which control steering are yet to be explored, although there is evidence that external ion gradients are sufficient to polarize growth in oomycetes (Harold and Harold, 1986; Hyde and Heath, 1995).

CONCLUSION

This review has examined structural and functional features of *Saprolegnia* hyphae and other tip growing systems, in the context of the many roles of the cytoplasm in the tip growth process. Cytoplasm is specialized in hyphal tips compared to subapical regions which form the majority of mycelial colonies, and it differs in peripheral and central zones of the hyphae. The central cytoplasm appears to migrate actively to keep up with the extending tip, and to synthesize and deposit non-migrating components of the cell. Thus, the cytoplasm seems to be fundamental to the generation of hyphal morphology as it molds the hypha and steers its growth. However, although we now appear to have identified major components required for tip growth, our understanding of the interactions between them is rudimentary. This brings us full circle, because "the complex interweaving of interactive events", which has fascinated people for a hundred years, has now opened onto a new level of molecular description and dissection. In this we are at a new beginning.

ACKNOWLEDGMENTS

The authors' original work was supported by grants and scholarships from the Natural Sciences and Engineering Research Council of Canada, and scholarships from the Ontario Ministry of Colleges and Universities, all of which are gratefully acknowledged.

REFERENCES

- Adams, A. E. M., and J. R. Pringle. 1984. Relationship of actin and tubulin distribution to bud growth in wild-type and morphogenetic mutant *Saccharomyces cerevisiae*. *J. Cell Biol.* 98: 934-945.
- Adams, R. J., and T. D. Pollard. 1989. Binding of myosin I to membrane lipids. *Nature* 340: 565-568.
- Aist, J. R., and C. J. Bayles. 1991. Ultrastructural basis of mitosis in the fungus *Nectria hematococca* (sexual stage of *Fusarium solani*). I. Asters. *Protoplasma* 161: 111-122.
- , and M. W. Berns. (1981). Mechanics of chromosome separation during mitosis in *Fusarium* (*Fungi imperfecti*): new evidence from ultrastructural and laser microbeam experiments. *J. Cell Biol.* 91: 446-458.
- Allen, R. D. 1961. Amoeboid movement. Pp. 135-216. In: *The Cell. Volume II. Cells and their component parts*. Academic Press, New York and London.
- Baines, I. C., H. Brzeska, and E. D. Korn. 1992. Differential localization of *Acanthamoeba* myosin I isoforms. *J. Cell Biol.* 119: 1193-1203.
- Bartnicki-Garcia, S. 1990. Role of vesicles in apical growth and a new mathematical model of hyphal morphogenesis. Pp. 211-232. In: *Tip Growth in Plant and Fungal Cells*. Ed., I. B. Heath. Academic Press, Toronto and New York.
- Bennett, V. 1990. Spectrin: a structural mediator between diverse plasma membrane proteins and the cytoplasm. *Curr. Opin. Cell Biol.* 2: 51-56.
- Bloch, R. J., and D. W. Pumphlin. 1992. A model of spectrin as a concertina in the erythrocyte membrane skeleton. *Trends Cell Biol.* 2: 186-189.
- Bourett, T. M., and R. J. Howard. 1991. Ultrastructural immunolocalization of actin in a fungus. *Protoplasma* 163: 199-202.
- Braun, E. J., and R. J. Howard. 1994a. Adhesion of fungal spores and germlings to host plant surfaces. *Protoplasma* 181: 202-212.
- , and ———. 1994b. Adhesion of *Cochliobolus heterosporus* conidia and germlings to leaves and artificial surfaces. *Exp. Mycol.* 18: 211-220.
- Clipson, N. J. W., and D. H. Jennings. 1990. The role of potassium and sodium in generation of osmotic potential of the marine fungus *Dendryphiella salina*. *Mycol. Res.* 94: 1017-1022.
- Corrêa Jr., A., and H. C. Hoch. 1993. Microinjection of urediospore germlings of *Uromyces appendiculatus*. *Exp. Mycol.* 17: 253-273.
- , and ———. 1995. Identification of thigmoresponsive loci for cell differentiation in *Uromyces* germlings. *Protoplasma* 186: 34-40.
- Edidin, M. 1992. Patches, posts and fences: proteins and plasma membrane domains. *Trends Cell Biol.* 2: 376-380.
- Enos, A. P., and N. R. Morris. 1990. Mutation of a gene that encodes a kinesin-like protein blocks nuclear division in *A. nidulans*. *Cell* 60: 1019-1027.
- Epstein, L., L. Laccetti, R. C. Staples, and H. C. Hoch. 1987. Cell-substratum adhesive protein involved in surface contact responses of the bean rust fungus. *Physiol. Mol. Plant Pathol.* 30: 373-388.
- , ———, ———, ———, and W. A. Hoose. 1985. Extracellular proteins associated with induction of differentiation in bean rust urediospore germlings. *Phytopathology* 75: 1073-1076.
- Eshel, D., A. Urrestarazu, S. Vissers, J. C. Jauniaux, J. C. Van Vliet-Reedijk, R. J. Planta, and I. R. Gibbons. 1993. Cytoplasmic dynein is required for normal nuclear segregation in yeast. *Proc. Natl. Acad. Sci. U.S.A.* 90: 11172-11176.
- Fèvre, M., and M. Rougier. 1982. Autoradiographic study of hyphal cell wall synthesis of *Saprolegnia*. *Arch. Microbiol.* 131: 212-215.
- Freytag, S., and K. Mendgen. 1991. Surface carbohydrates and cell wall structure of *in vitro*-induced urediospore infection structures of *Uromyces viciae-fabae* before and after treatments with enzymes and alkali. *Protoplasma* 161: 94-103.
- Fukui, Y., T. J. Lynch, H. Brzeska, and E. D. Korn. 1989. Myosin I is located at the leading edges of locomoting *Dictyostelium* amoebae. *Nature* 341: 328-331.
- Garrill, A., S. L. Jackson, R. R. Lew, and I. B. Heath. 1993. Ion channel activity and tip growth: tip-localized stretch-activated channels generate an essential Ca²⁺ gradient in the oomycete *Saprolegnia ferax*. *Eur. J. Cell Biol.* 60: 358-365.
- , R. R. Lew, and I. B. Heath. 1992. Stretch-activated Ca²⁺ and Ca²⁺-activated K⁺ channels in the hyphal tip plasma membrane of the oomycete *Saprolegnia ferax*. *J. Cell Sci.* 101: 721-730.
- Gooday, G.W. 1994. Cell Walls. Pp. 43-62. In: *The Growing Fungus* Eds., N. A. R. Gow, and G. M. Gadd. Chapman and Hall, London.
- , and N. A. R. Gow. 1990. Enzymology of tip growth in fungi. Pp. 31-58. In: *Tip Growth in Plant and Fungal Cells*. Ed., I. B. Heath. Academic Press, Toronto and New York.
- Goodman, S. R., K. E. Krebs, C. F. Whitfield, B. M. Riederer, and I. S. Zagon. 1988. Spectrin and related molecules. *CRC Critical Rev. Biochem.* 23: 171-234.
- Goodner, B., and R. S. Quatrano. 1993. *Fucus* embryogenesis: a model to study the establishment of polarity. *The Plant Cell* 5: 1471-1481.
- Grove, S. N., and C. E. Bracker. 1970. Protoplasmic organization of hyphal tips among fungi: vesicles and Spitzenkörper. *J. Bacteriol.* 104: 989-1009.
- , and J. A. Sweigard. 1980. Cytochalasin A inhibits spore germination and hyphal tip growth in *Gilbertella persicaria*. *Exp. Mycol.* 4: 239-250.
- Harold, F. M. 1990. To shape a cell: an inquiry into the causes of morphogenesis of microorganisms. *Microbiol. Rev.* 54: 381-431.
- Harold, R. L., and F. M. Harold. 1986. Ionophores and cytochalasins modulate branching in *Achlya bisexualis*. *J. Gen. Microbiol.* 132: 213-219.
- Hartwig, J. H., and D. J. Kwiatkowski. 1991. Actin-binding proteins. *Curr. Opin. Cell Biol.* 3: 87-97.
- He, Y., and F. Grinnell. 1994. Stress relaxation of fibroblasts activates a cyclic AMP signalling pathway. *J. Cell Biol.* 126: 457-464.
- Heath, I. B. 1982. The effect of nocodazole on the growth and ultrastructure of the fungus *Saprolegnia ferax*. *Ev-*

- idence against a simple mode of action. Pp. 275–311. In: *Microtubules in Microorganisms*. Eds., P. Cappuccinelli, and N. R. Morris. Marcel Dekker, New York and Basel.
- . 1987. Preservation of a labile cortical array of actin filaments in growing hyphal tips of the fungus *Saprolegnia ferax*. *Eur. J. Cell Biol.* 44: 10–16.
- . 1990. The roles of actin in tip growth of fungi. *Int. Rev. Cytol.* 123: 95–127.
- . 1994. The cytoskeleton. Pp. 99–134. In: *The Growing Fungus*. Eds., N. A. R. Gow, and G. M. Gadd. Chapman and Hall, London.
- . 1995. Integration and regulation of hyphal tip growth. *Can. J. Bot.* 73: 5131–5139.
- , and Greenwood, A. D. 1970. The structure and formation of lomasomes. *J. gen. Microbiol.* 62: 129–137.
- , and R.L. Harold. 1992. Actin has multiple roles in the formation and architecture of zoospores of the oomycetes, *Saprolegnia ferax* and *Achlya bisexualis*. *J. Cell Sci.* 102: 611–627.
- , and M. C. Heath. 1978. Microtubules and organelle movements in the rust fungus *Uromyces phaseoli* var. *vignae*. *Cytobiologie* 16: 393–411.
- , and ———. 1979. Structural studies of the development of infection structures of cowpea rust, *Uromyces phaseoli* var. *vignae*. II. Vacuoles. *Can. J. Bot.* 57: 1830–1837.
- , and S. G. W. Kaminskyj. 1989. The organization of tip-growth-related organelles and microtubules revealed by quantitative analysis of freeze-substituted oomycete hyphae. *J. Cell Sci.* 93: 41–52.
- , K. Rethoret, A. L. Arsenaault, and F. P. Ottensmeyer. 1985. Improved preservation of the form and contents of wall vesicles and the Golgi apparatus in freeze substituted hyphae of *Saprolegnia*. *Protoplasma* 128: 81–93.
- Heidemann, S. R. 1990. Neuronal tip growth. Pp. 285–316. In: *Tip Growth in Plant and Fungal Cells*. Ed., I. B. Heath. Academic Press, Toronto and New York.
- , and R. E. Buxbaum. 1990. Tension as regulator and integrator of axonal growth. *Cell Motil. Cytoskel.* 17: 6–10.
- Heslop-Harrison, J., and Y. Heslop-Harrison. 1989. Myosin associated with the surfaces of organelles, vegetative nuclei and generative cells in angiosperm pollen grains and tubes. *J. Cell Sci.* 94: 319–325.
- Hill, T. W., and J. T. Mullins. 1980a. Hyphal tip growth in *Achlya*. I. Cytoplasmic organization. *Can. J. Microbiol.* 26: 1132–1140.
- , and ———. 1980b. Hyphal tip growth in *Achlya*. II. Subcellular localization of cellulase and associated enzymes. *Can. J. Microbiol.* 26: 1141–1146.
- Hoch, H. C., and R. C. Staples. 1983. Visualization of actin *in situ* by rhodamine-conjugated phalloin in the fungus *Uromyces phaseoli*. *Eur. J. Cell Biol.* 32: 52–58.
- , ———, B. Whitehead, J. Comeau, and E. D. Wolf. 1987. Signalling for growth orientation and cell differentiation by surface topography in *Uromyces*. *Science* 235: 1659–1661.
- Hostetter, M. K. 1994. Interactions of *Candida albicans* with eukaryotic cells. *Amer. Soc. Microbiol. News* 60: 370–374.
- , E. J. Michael, and C. M. Bendel. 1993. Complement receptors in *Candida albicans* and other yeasts: structure, function and role in pathogenesis. Pp. 281–289. In: *Dimorphic Fungi in Biology and Medicine*. Eds., H. Vanden Bossche, F. C. Odds and D. Kerridge. Plenum Press, New York and London.
- Hyde, G. J., and I. B. Heath. 1995. Ca²⁺-dependent polarization of axis establishment in the tip-growing organism, *Saprolegnia ferax*, by gradients of the ionophore A23187. *Eur. J. Cell Biol.* 67: 356–362.
- Hynes, R. O. 1992. Integrins: versatility, modulation, and signaling in cell adhesion. *Cell* 69: 11–25.
- Ingber, D. E. 1993. Cellular tensegrity: defining new rules of biological design that govern the cytoskeleton. *J. Cell Sci.* 104: 613–627.
- , S. Karp, G. Plopper, L. Hansen, and D. Mooney. 1993. Mechanochemical transduction across extracellular matrix and through the cytoskeleton. Pp. 61–78. In: *Physical forces and the mammalian cell*. Ed., J.A. Franngos. Academic Press, Toronto and New York.
- Jackson, S. L., and I. B. Heath. 1990a. Evidence that actin reinforces the extensible hyphal apex of the oomycete *Saprolegnia ferax*. *Protoplasma* 157: 144–153.
- , and ———. 1990b. Visualization of actin arrays in growing hyphae of the fungus *Saprolegnia ferax*. *Protoplasma* 154: 66–70.
- , and ———. 1992. UV microirradiations elicit Ca²⁺ dependent apex-directed cytoplasmic contractions in hyphae. *Protoplasma* 170: 46–52.
- , and ———. 1993a. Roles of calcium ions in hyphal tip growth. *Microbiol. Rev.* 57: 367–382.
- , and ———. 1993b. UV microirradiation implicates F-actin in reinforcing growing hyphal tips. *Protoplasma* 175: 67–74.
- , and ———. 1993c. The dynamic behaviour of cytoplasmic F-actin in growing hyphae. *Protoplasma* 173: 23–34.
- Johnston, G. C., J. A. Prendergast, and R. A. Singer. 1991. The *Saccharomyces cerevisiae* MYO2 gene encodes an essential myosin for vectorial transport of vesicles. *J. Cell Biol.* 113: 539–551.
- Jung, G., Y. Fukui, B. Martin, and J. A. Hammer. 1993. Sequence, expression pattern, intracellular localization pattern and targeted disruption of the *Dictyostelium* myosin ID heavy chain isoform. *J. Biol. Chem.* 268: 14981–14990.
- Kaminskyj, S. G. W., A. Garrill, and I. B. Heath. 1992a. The relation between turgor and tip growth in *Saprolegnia ferax*: turgor is necessary but not sufficient to explain apical extension rates. *Exp. Mycol.* 16: 64–75.
- , and Hamer, J. E. 1995. Why search for integrin homologs in fungi? *Fung. Genetics News.* 42A: 91.
- , and Heath, I. B. 1992. Age-dependent differential responses of *Saprolegnia* hyphal tips to a helical growth-inducing factor in the agar substitute, gellan. *Exp. Mycol.* 16: 230–239.
- , and ———. 1994. A comparison of techniques for localizing actin and tubulin in hyphae of *Saprolegnia ferax*. *J. Histochem. Cytochem.* 42: 523–530.

- , and ———. 1995. Integrin and spectrin homologues, and cytoplasm-wall adhesion in tip growth. *J. Cell Sci.* 108: 849–856.
- , and M. C. Heath. 1983. Histological responses of infection structures and intercellular mycelium of *Uromyces phaseoli* var. *typica* and *U. phaseoli* var. *vignae* to the HNO₂-MBTH-FeCl₃ and the IKI-H₂SO₄ tests. *Physiol. Plant Pathol.* 22: 173–179.
- , S. L. Jackson, and I. B. Heath. 1992b. Fixation induces differential polarized translocations of organelles in hyphae of *Saprolegnia ferax*. *J. Microsc.* 167: 153–168.
- , K. S. Yoon, and I. B. Heath. 1989. Cytoskeletal interactions with post-mitotic migrating nuclei in the oyster mushroom fungus, *Pleurotus ostreatus*: evidence against a force-generating role for astral microtubules. *J. Cell Sci.* 94: 663–674.
- Katz, D., and R. F. Rosenberger. 1970. A mutation in *Aspergillus nidulans* producing hyphal walls which lack chitin. *Biochim. Biophys. Acta* 208: 452–460.
- Kiss, J. Z., and L. A. Staehelin. 1993. Structural polarity in the *Chara* rhizoid: a reevaluation. *Amer. J. Bot.* 80: 273–282.
- Koch, A. L. 1982. The shape of the hyphal tips of fungi. *J. Gen. Microbiol.* 128: 947–951.
- Kropf, D. L. 1992. Establishment and expression of cellular polarity in fucoid zygotes. *Microbiol. Rev.* 56: 316–339.
- Kurtz, M. B., I. B. Heath, J. Marrinan, S. Dreikorn, J. Onishi and C. Douglas. 1994. Morphological effects of lipopeptides against *Aspergillus fumigatus* correlate with activities against (1,3)- β -D-glucanase. *Antimicrobial Agents and Chemotherapy* 38: 1480–1489.
- Kwon, Y. H., and H. C. Hoch. 1991. Temporal and spatial dynamics of appressorium formation in *Uromyces appendiculatus*. *Exp. Mycol.* 15: 116–131.
- Lovina, N. N., R. R. Lew, and I. B. Heath. (1994). Cytoskeletal regulation of ion channel distribution in the tip growing organism *Saprolegnia ferax*. *J. Cell Sci.* 107: 127–134.
- Littlefield, L. J., and M. C. Heath. 1979. *Ultrastructure of Rust Fungi*. Academic Press, Toronto and New York.
- Lopez-Franco, R., S. Bartnicki-Garcia, and C. E. Bracker. 1994. Pulsed growth of fungal hyphal tips. *Proc. Natl. Acad. Sci. U.S.A.* 91: 12228–12232.
- , R. J. Howard, and C. E. Bracker. 1995. Satellite Spitzenkörper in growing hyphal tips. *Protoplasma* 188: 85–103.
- Luard, E. J. 1982. Growth and accumulation of solutes by *Phytophthora cinnamomi* and other lower fungi in response to changes in external osmotic potential. *J. Gen. Microbiol.* 128: 2583–2590.
- Madden, K., C. Costigan, and M. Snyder. 1991. Cell polarity and morphogenesis in *Saccharomyces cerevisiae*. *Trends Cell Biol.* 1: 22–29.
- Marcantonio, E. E., and R. O. Hynes. 1988. Antibodies to the conserved cytoplasmic domain of the integrin β_1 subunit react with proteins in vertebrates, invertebrates and fungi. *J. Cell Biol.* 106: 1765–1772.
- McGoldrick, C. A., Gruver, C. and May, G. S. 1995. *myoA* of *Aspergillus nidulans* encodes an essential myosin I required for secretion and polarized growth. *J. Cell Biol.* 106: 1765–1772.
- McKerracher, L. J., and I. B. Heath. 1986. Fungal nuclear behaviour analyzed by ultraviolet microbeam irradiation. *Cell Motil. Cytoskel.* 6: 35–47.
- , and ———. (1987). Cytoplasmic migration and intracellular organelle movements during tip growth of fungal hyphae. *Exp. Mycol.* 11: 79–100.
- Meluh, P. B., and M. D. Rose. 1990. *KAR3*, a kinesin-related gene required for yeast nuclear fusion. *Cell* 60, 1029–1041.
- Meyer, S. L. F., S. G. W. Kaminskyj, and I. B. Heath. 1988. Nuclear migration in a *nud* mutant of *Aspergillus nidulans* is inhibited in the presence of a quantitatively normal population of cytoplasmic microtubules. *J. Cell Biol.* 106: 773–778.
- Moloshok, T. D., B. T. Terhune, J. S. Lamboy, and H. C. Hoch. 1994. Fractionation of extracellular matrix components from urediospore germlings of *Uromyces*. *Mycologia* 86: 787–794.
- Money, N. P. 1990. Measurement of hyphal turgor. *Exp. Mycol.* 14: 416–425.
- . 1995. Turgor pressure and the mechanics of fungal penetration. *Can. J. Bot.* 73: S96–S102.
- , and F. M. Harold. 1992. Extension growth of the water mold *Achlya*: interplay of turgor and wall strength. *Proc. Natl. Acad. Sci. U.S.A.* 89: 4245–4249.
- , and ———. 1993. Two water molds can grow without measurable turgor pressure. *Planta* 190, 426–430.
- Mulholland, J., D. Preuss, A. Moon, A. Wong, D. Drubin, and D. Botstein. 1994. Ultrastructure of the yeast actin cytoskeleton and its association with the plasma membrane. *J. Cell Biol.* 125: 381–391.
- Mullins, J. T. 1973. Lateral branch formation and cellulase production in the water molds. *Mycologia* 65: 1007–1014.
- Olmsted, J. B. 1986. Microtubule-associated proteins. *Ann. Rev. Cell Biol.* 2: 421–57.
- . 1991. Non-motor microtubule-associated proteins. *Curr. Opin. Cell Biol.* 3: 52–58.
- Picton, J. M., and M. W. Steer. 1982. A model for the mechanism of tip extension in pollen tubes. *J. theor. Biol.* 98: 15–20.
- Pickard, B. G. 1994. Contemplating the plasmalemma control centre model. *Protoplasma* 182: 1–9.
- Pierson, E.S., and M. Cresti. 1992. Cytoskeleton and cytoplasmic organization of pollen and pollen tubes. *Int. Rev. Cytol.* 140: 73–125.
- , Miller, D. D., Callaham, D. A., Shipley, A. M., Rivers, B. A., Cresti, M. and Hepler, P. K. 1994. Pollen tube growth is coupled to the extracellular calcium flux and the intracellular calcium gradient: effect of BAPTA-type buffers and hypotonic media. *The Plant Cell* 6: 1815–1828.
- Prosser, J. I. 1990. Comparison of tip growth in prokaryotic and eukaryotic filamentous organisms. Pp. 233–259. In: *Tip Growth in Plant and Fungal Cells*. Ed., I. B. Heath. Academic Press, Toronto and New York.

- Quatrano, R. S., L. Brian, J. Aldridge, and T. Schultz. 1991. Polar axis fixation in *Fucus* zygotes: components of the cytoskeleton and extracellular matrix. *Devel. Suppl.* 1: 11-16.
- Raudaskoski, M., I. Rupes, and S. Timonen. 1991. Immunofluorescence microscopy of the cytoskeleton in filamentous fungi after quick-freezing and low-temperature fixation. *Exp. Mycol.* 15: 167-173.
- Reinhardt, M. O. (1892). Das Wachstum der Pilzhyphen. *Jahrb. wiss. Botanik* 23: 479-566.
- Roberson, R. W. 1992. The actin cytoskeleton in hyphal cells of *Sclerotium rolfisii*. *Mycologia* 84, 41-51.
- , and M. S. Fuller. 1988. Ultrastructural aspects of the hyphal tip of *Sclerotium rolfisii* preserved by freeze substitution. *Protoplasma* 146: 143-149.
- , and M. M. Vargas. 1994. The tubulin cytoskeleton and its sites of nucleation in hyphal tips of *Allomyces macrogynus*. *Protoplasma* 182: 19-31.
- Robertson, N. F., and S. R. H. Rizvi. 1968. Some observations on the water-relations of the hyphae of *Neurospora crassa*. *Ann. Bot.* 32: 279-291.
- Sanders, L. C., and E. M. Lord. 1992. A dynamic role for the stylar matrix in pollen tube extension. *Int. Rev. Cytol.* 140: 297-316.
- , C. S. Wang, L. L. Walling, and E. M. Lord. 1991. A homolog of the substrate adhesion molecule vitronectin occurs in four species of flowering plants. *The Plant Cell* 3: 629-635.
- Sastry, S. K., and A. F. Horwitz. 1993. Integrin cytoplasmic domains: mediators of cytoskeletal linkages and extra- and intracellular initiated transmembrane signalling. *Curr. Opin. Cell Biol.* 5: 819-831.
- Saunders, P. T., and A. P. J. Trinci. 1979. Determination of tip shape in fungal hyphae. *J. Gen. Microbiol.* 110: 469-473.
- Schindler, M., S. Meiners, and D. A. Cheresh. 1989. RGD-dependent linkage between plant cell wall and plasma membrane: consequences for growth. *J. Cell Biol.* 108: 1955-1965.
- Schwartz, M. A., C. Lechene, and D. E. Ingber. 1991. Insoluble fibronectin activates the Na^+/H^+ antiporter by clustering and immobilizing integrin $\alpha_5\beta_1$, independent of cell shape. *Proc. Natl. Acad. Sci. U.S.A.* 88: 7849-7853.
- Staves, M. P., R. Wayne, and A. C. Leopold. 1992. Hydrostatic pressure mimics gravitational pressure in characean cells. *Protoplasma* 168: 141-152.
- Stossel, T. P. 1993. On the crawling of animal cells. *Science* 260, 1086-1094.
- , C. Chaponnier, R. M. Ezzell, J. H. Hartwig, P. A. Janmey, D. J. Kwiatowski, S. E. Lind, D. B. Smith, F. S. Southwick, H. L. Yin, and K. S. Zaner. 1985. Non-muscle actin-binding proteins. *Ann. Rev. Cell Biol.* 1: 353-402.
- Tang, X., P. K. Hepler, and S. P. Scordilis. 1989. Immunocytochemical and immunocytochemical identification of a myosin heavy chain polypeptide in *Nicotiana* pollen tubes. *J. Cell Sci.* 92: 569-574.
- Terhune, B. T., and H. C. Hoch. 1993. Substrate hydrophobicity and adhesion of *Uromyces* urediospores and germlings. *Exp. Mycol.* 17: 241-252.
- van Tuinen, D., R. Ortega Perez, and G. Turian. 1986. A search for myosin in elongating hyphae of *Neurospora crassa*. *Bot. Helv.* 96: 299-302.
- Vanderkerckhove, J., and K. Vancompernelle. 1992. Structural relationships of actin-binding proteins. *Curr. Opin. Cell Biol.* 4: 36-42.
- Wagner, V. T., L. Brian, and R. S. Quatrano. 1992. Role of a vitronectin-like molecule in embryo adhesion of the brown alga, *Fucus*. *Proc. Natl. Acad. Sci. U.S.A.* 90: 3644-3648.
- Wang, N., J. P. Butler, and D. E. Ingber. 1993. Mechanotransduction across the cell surface and through the cytoskeleton. *Science* 260, 1124-1127.
- Watts, F. Z., D. M. Miller, and E. Orr. (1985). Identification of myosin heavy chain in *Saccharomyces cerevisiae*. *Nature* 316: 83-85.
- Wayne, R., M. P. Staves, and A. C. Leopold. 1992. The contribution of the extracellular matrix to gravisensing in characean cells. *J. Cell Sci.* 101: 611-623.
- Wessels, J. G. H. 1988. A steady-state model for apical wall growth in fungi. *Acta Bot. Neerl.* 37: 3-16.
- . 1990. Role of cell wall architecture in fungal tip growth generation. Pp. 1-29 In: *Tip Growth in Plant and Fungal Cells*. Ed., I. B. Heath. Academic Press, Toronto and New York.
- . (1993). Wall growth, protein excretion and morphogenesis in fungi. (Tansley Review No. 45) *New Phytol.* 23: 397-413.
- Williams, M. J., P. E. Hughes, T. E. O'Toole, and M. H. Ginsberg. 1994. The inner world of cell adhesion: integrin cytoplasmic domains. *Trends Cell Biol.* 4: 109-112.
- Wyatt, S. E., and N. C. Carpita. 1993. The plant cytoskeleton-cell-wall continuum. *Trends Cell Biol.* 3: 413-417.
- Wynn, W. K. 1976. Appressorium formation over stomates by the bean rust fungus: response to a surface-contact stimulus. *Phytopathology* 66: 136-146.
- Xiang, X., S. M. Beckwith, and N. R. Morris. 1994. Cytoplasmic dynein is involved in nuclear migration in *Aspergillus nidulans*. *Proc. Natl. Acad. Sci. U.S.A.* 91: 2100-2104.
- Zhou, X.-L., M. A. Stumpf, H. C. Hoch, and C. Kung. 1991. A mechanosensitive channel in whole cells and in membrane patches of the fungus *Uromyces*. *Science* 253: 1415-1417.
- Zhu, J.-K., J. Shi, U. Singh, S. E. Wyatt, R. A. Bressan, P. M. Hasegawa, and N. C. Carpita. 1993. Enrichment of vitronectin- and fibronectin-like proteins in NaCl-adapted plant cells and evidence for their involvement in plasma membrane-cell wall adhesion. *The Plant Journal* 3: 637-646.

