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A steady-state model for apical wall growth in fungi

J. G. H. WESSELS

Department of Plant Physiology, Biological Centre, University of Groningen, 9751, NN Haren, The Netherlands

INTRODUCTION

The presence of cell walls makes plants immobile but fungi (defined as a separate kingdom, Whittaker 1969) have evolved as organisms which, despite the presence of cell walls, have a certain degree of mobility due to apical growth. The mycelial colony, consisting of a system of branched hyphae, may thus grow over and through substrates. Consequently, the actively growing part of the fungal mycelium constantly moves away from its original position while colonizing dead substrata (saprotrophs) or living organisms (biothrophs) as in parasitic and symbiotic associations.

These activities are particularly prominent in relation to plants, as evidenced by the fact that fungi are the main decomposers of the lignocellulosic plant cell wall (Crawford 1981), they are also the main plant pathogens (Dickinson & Lucas 1982) and they form mycorrhizae with nearly all land plants (Harley & Smith 1983). Although the association of fungi with healthy animals (including man) is limited, their colonizing ability is now becoming a problem in immuno-compromised patients (Chandler 1986).

This paper is concerned with the mechanisms responsible for the continuous extension of the wall at the apex of growing fungal hyphae. It should be noted, however, that some cells in plants show intrusive growth by apical extension, e.g. root hairs, pollen tubes, and rhizoids (Schnepf 1986). It is only in fungi that a view of apical wall extension can be formulated based on the knowledge of the emerging wall structure. It is possible, however, that the principles involved may ultimately prove to be similar in apically growing cells of both plants and fungi although details would be different because of differences in the chemistry of wall polymers.

GENERAL PERSPECTIVE

Many of the basic observations on apical wall growth were made in the nineteenth century and various hypotheses then put forward to explain apical growth still survive. Most notable is a publication from 1892 by Reinhardt in which he described experiments with the wide hyphae of *Peziza* species showing that disturbances of growth were first manifested at the apices of hyphae. Many of these observations were later repeated and

Key-words: apical growth, β-glucan, chitin, fungal cell wall, fungal hypha, *Schizophyllum commune*, wall growth.

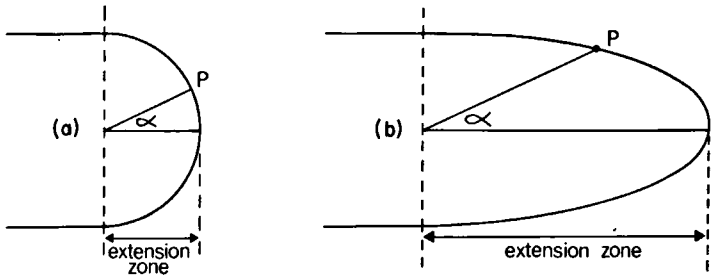

Fig. 1. The rate of expansion of any point at the apex is proportional to the cosine of the angle α when the shape of the tip is hemispherical (a), or to the co-tangent of the angle α when the shape of the tip is half ellipsoid (b).

extended by Robertson (1958 1965). A change in the shape of the apex from more or less hemispherical to half-ellipsoids of revolution with increasing growth rate (Fig. 1), as noted by Trinci & Saunders (1977), was also observed by Reinhardt. In addition, he noted that the origin of curvature of hyphae was at the apex and together these observations provided the evidence that hyphal extension occurs at the apex. Direct observations by Reinhardt (1892) on the displacement of particles on the surface during apical growth could only be made with root hairs of *Lepidium sativum*. Similar experiments with a fungal system (Castle 1958) confirmed the validity of his conclusion that fungal hyphae extend at their apices.

With regard to the mechanisms involved in apical wall growth, Reinhardt (1892) discussed the then prevailing theory of leading botanists who regarded enlargement of the wall area as a process in which an elastic or plastic wall expands due to turgor pressure while new wall material is added by apposition or intussusception. However, he considered such a theory inadequate because it would require an increase in mechanical strength of the wall from the very apex to the base of the extension zone. As he puts it, such an increase in strength could be achieved by a proportional increase in wall thickness or by a change in the quality of the molecules that make up the wall. He found no evidence for a change in wall thickness. Later investigators (Girbardt 1969, Grove & Bracker 1970, Trinci & Collinge 1975) also found that wall thickness remains uniform in the extension zone. A change in the quality of the wall molecules—which is precisely the kind of change which is suggested by recent work from our laboratory (see below)—was considered unlikely by Reinhardt. Thus one would expect that an increase in hydrostatic pressure, as caused by flooding with water, would result in swelling or bursting at the extreme tip. Instead he observed that bursting occurred just under the apex where the cylindrical form is attained and where the circumferential stress in the wall increases. Subapical swelling and bursting was also observed by later investigators (Robertson 1958, 1965, Bartnicki-Garcia & Lippman 1972, see Fig. 2). Reinhardt concluded that the wall must have uniform strength over the whole apex and does not grow by plastic expansion. He proposed intussusception of wall material maximally at the extreme tip and declining to zero at the base of the extension zone. The fact that relief of turgor pressure, by applying solutions of low osmotic potential, resulted in cessation of growth at the apex was interpreted as being due to detachment of cytoplasm from the apical wall, thus interrupting the organized delivery of new wall material by the cytoplasm.

Disregarding the observations made by Reinhardt (1892), and probably inspired by considerations of D'Arcy Thompson (1917, see Bonner 1961) on the origin of cellular form, mathematical models have been put forward to describe apical morphogenesis.

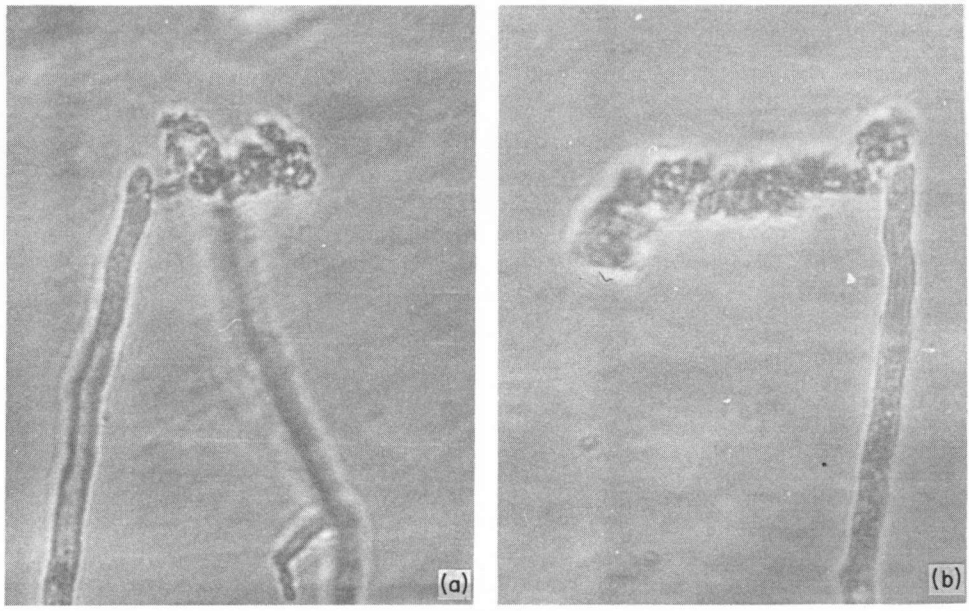

Fig. 2. Explosive apical bursting of agar-grown hyphae of *Schizophyllum commune* after flooding with 0·5% acetic acid, following the procedure of Park & Robinson (1966). The cytoplasm is extruded through a hole located at the base of the apical dome (a) or the whole apical dome is blown off (b). (Courtesy of Dr J.H. Sietsma.)

They all rely on the concept that extension at the hyphal apex is due to the presence of a gradient in the plasticity of the wall such that there is a decrease in the tendency of the wall to yield to turgor pressure from the extreme tip downwards (de Wolff & Houwink 1954, De Riva Ricci & Kendrick 1972, Green 1974, Trinci & Saunders 1977, Saunders & Trinci 1979, Koch 1982). In all these models expansion at any point on the apical dome is determined by its position, according to a mathematical function which depends on the shape of the apex (Fig. 1). To put these models through a test it would seem necessary to determine the plastic and elastic properties of the wall at various points over the growing apex; obviously such measurements are difficult to make.

If Reinhardt's theory is to be dismissed, another explanation must be found for the tendency of hyphal tips to swell and burst at the base of the apical dome instead of the extreme apex when subjected to high turgor pressure. It is unfortunate that none of those who have advanced a theory for a plastic wall expanding under turgor pressure has cared to explain subapical bursting. Possibly the recently discovered cytoskeletal elements in the hyphal apex (for review see McKerracher & Heath 1987) protect the newly formed delicate wall from becoming subject to high hydrostatic pressure (Wessels 1986). This has also been proposed for the apical wall in extending pollen tubes (Picton & Steer 1982). Protection of the apical wall against high internal pressure by the underlying structured cytoplasm may explain why the wall does not always burst at its weakest point, i.e. at the very apex, when turgor is suddenly increased.

At the same time, recognition of the presence of cytoskeletal elements at the apex would question the validity of mathematical models assuming expansion under turgor pressure. Such models may be wrong in assuming that turgor pressure, probably generated subapically in vacuoles, is uniformly present over the whole apical wall area. New models

taking into account the presence of cytoskeletal elements might assign a role to the cytoplasm in shaping the hyphal apex (as already advocated by Reinhardt) while retaining the concept of a deformable wall expanding under the influence of hydrostatic pressure generated in subapical hyphal parts.

If there is a gradient in the plasticity of the wall, maximal at the very apex and declining towards the base of the apical extension zone, then how is this gradient generated? Two possibilities were considered in the nineteenth century. Either the wall is originally plastic and expands until it becomes rigid or the wall is synthesized as a rigid entity and cannot expand until it becomes plastic. The first concept was generally accepted in the nineteenth century; controversies mainly concerned whether wall addition was by intussusception (Nägeli) or apposition (Strasburger) (cit: Reinhardt 1892). This concept of rigidification of the wall after its formation was implicit in formulations by Robertson (1958, 1965) to explain his observations on living hyphae. Work on wall biogenesis in *Schizophyllum commune* (to be discussed below) has given this concept a molecular and experimental basis (Wessels & Sietsma 1981b, Wessels *et al.* 1893) and has been named the 'steady-state model of apical wall growth' (Wessels 1986). The alternative concept that the wall must be continuously loosened by lytic enzymes in order to expand was expressed a century ago by Marchall-Ward (1888) and has more recently been favoured by Bartnicki-Garcia (1973) and Gooday (1978). Although direct evidence for this concept, which presumes a 'delicate balance between wall synthesis and wall lysis' (Bartnicki-Garcia 1973), is missing, it is rather uncritically cited as a fact in many research papers and textbooks. I have critically examined the evidence and have come to the conclusion that, to date, the evidence for this concept is very thin indeed (Wessels 1984, 1986). Lysins play no role in the preferred steady-state model of apical wall growth. However, this model does not disprove a role for lytic enzymes. For the moment it seems sufficient to explain the structural and experimental data. If hard evidence for a role of lysins in apical wall growth were to arise, e.g. through genetic studies, this would necessitate modification of the model.

STRUCTURE OF FUNGAL WALLS

At first sight fungal walls contain a bewildering number of polymers and constituent monomers (for reviews see Bartnicki-Garcia 1968, Wessels & Sietsma 1981a, Bartnicki-Garcia & Lippman 1982, Wessels 1986). However, when only the alkali-insoluble components are considered a much more simple picture emerges. The alkali-insoluble wall portions of ascomycetes and basidiomycetes mainly consist of (1→4)-β-D-glucosaminoglycans, [poly-*N*-acetylglucosamine (chitin) and partially or wholly deacetylated derivatives (e.g. chitosan)] and (1→3)-β-D/(1→6)-β-D-glucan. There is evidence that this alkali-insoluble portion of the wall is solely responsible for hyphal morphogenesis (Sietsma & Wessels 1988) and for the sake of the present discussion the alkali-soluble components of the wall will therefore be largely ignored.

In order to reveal the molecular architecture of the hyphal wall, electronmicroscope observations, combined with the use of more or less specific enzymic or chemical extractions, have been made on the walls of a variety of fungi (lit. cit. in Wessels & Sietsma 1981a). On the basis of such observations, Hunsley & Burnett (1970) have modelled the wall as a co-axially layered structure. As Burnett (1979) has pointed out, it should be understood that the co-axially arranged regions are not supposed to be discrete but grade into each other. Wessels & Sietsma (1981a), however, noted that the techniques used can

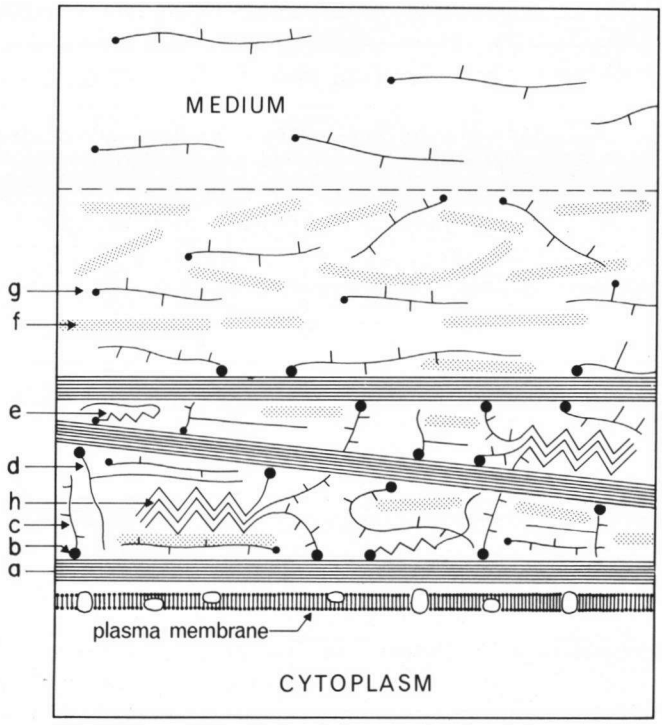

Fig. 3. Model of the mature hyphal wall of *Schizophyllum commune*. Partially crystallized chitin chains (a) are hydrogen-bonded to chitin chains which carry covalently linked β-glucan chains. The coupling fragment (b) contains amino acids with a high proportion of lysine. The β-glucan chains are (1→3)-β linked and carry single (1→6)-β-linked glucose branches (c) or longer (1→6)-β-linked glucan branches (d) or alternatively, (1→3)-β and (1→6)-β linked glucan branches (e). Some unsubstituted or sparsely branched (1→3)-β glucan segments may form triple helices (h) which add to the strength of the glucan network. Crystalline (1→3)-α-glucan fibrils (alkali-soluble s-glucan) (f) occur throughout the wall and accumulate at the outer surface as a layer. Free water-soluble (1→3)-β-glucan chains with single (1→6)-β-linked glucose branches (g) are also present in the wall and may be excreted into the medium. (Adapted from Wessels & Sietsma 1981b.)

easily lead to misinterpretations of wall structure. They considered most published studies to be in agreement with a model of the wall in which the various wall components are more closely associated with each other to form essentially one layer, with some components accumulating at the outside apparently forming extra layers. This simple model only applies to vegetative hyphae and not to the walls of specialized structures, e.g. spores and aerial hyphae, where genuine outer layers may be present.

Figure 3 depicts a model of the hyphal wall of the basidiomycete *Schizopyllum commune* integrating the results of a number of chemical, enzymic and ultrastructural analyses (Wessels *et al.* 1972, Sietsma & Wessels 1977, 1979, van der Valk *et al.* 1977). In this case a water-soluble gel-like (1→3)-β-/(1→6)-β-glucan and a water-insoluble but alkali-soluble (1→3)-α-glucan (s-glucan) accumulate at the outside of a layer which contains an alkali-insoluble glucosaminoglycan–glucan complex. In this complex the glucan chains are (1→3)-β-linked with (1→6)-β-linked branches attached. In some of the chains, branches consist of just one glucose residue and these chains thus resemble the gel-like glucan accumulating on the outside of the hyphae. Other (1→3)-β-linked chains carry longer (1→6)-β-linked glucan branches. Both types of branched glucans are thought to be attached to (1→4)-β-linked glucosaminoglycan chains through their reducing ends via

Table 1. Solubilization of non-aminoglycans by depolymerization of aminoglycans*

	Nitrous acid treatment		Chitinase treatment	
	Aminoglycan degraded	Non-aminoglycan released	Aminoglycan degraded	Non-aminoglycan released
S. commune	—(100)†	10 (95·4)†	98	88
A. bisporus				
Vegetative mycelium	41	38	67	48
Fruit-body stipe	19	41	94	56
M. mucedo	79	100	—	—

*In *S. commune* and *A. bisporus* figures refer to the percentages of aminoglycan and glucan in the alkali-insoluble wall fraction becoming soluble in water and alkali after the treatments. In *M. mucedo* the figures refer to the percentages of aminoglycan and glycuronan in the whole wall which were soluble in water after the treatment. Nitrous acid treatments were done before exposure of the walls to alkali. Chitinase treatments were done on alkali-insoluble wall fractions.

†Nitrous acid treatment after heating the wall fraction in 10 M NaOH to deacetylate chitin.

amino acids, particularly lysine. Probably these substituted glucosaminoglycan chains are partially hydrogen-bonded to microfibrillar chitin consisting of hydrogen-bonded unsubstituted chains of poly-*N*-acetylglucosamine. The presence of hydrogen-bonded triple helices among the glucan chains was inferred from the weak hydroglucan reflections seen in X-ray analysis of the chitin–glucan complex. Treatment of the complex with hot dilute acid breaks the linkages between glucosaminoglycan and glucan and hydrolyses (1→6)-β-linked glucan chains thus leading to sharp X-ray reflections of hydroglucans and chitin.

The most important evidence for postulating linkage between glucan and glucosaminoglycan chains is that the glucan chains become soluble in water or alkali after specific depolymerization of (acetyl)glucosamine-containing polymers (Stagg & Feather 1973, Sietsma & Wessels 1979). Such depolymerizations (employing nitrous acid to break bonds in the glucosaminoglycans where non-acetylated glucosamine residues occur (Datema *et al.* 1977b) and chitinase to break bonds in homopolymer stretches of *N*-acetylglucosamine) have been shown to solubilize all the alkali-insoluble β-glucans from the wall of a number of basidiomycetes and ascomycetes (Sietsma & Wessels 1981; Mol *et al.* 1988). Recently we found that even the very small amount of glucosaminoglycan in the wall of *Saccharomyces cerevisiae* must be held responsible for keeping the β-glucan in the wall in an alkali-insoluble form (Mol & Wessels 1987).

Table 1 shows data for two basidiomycetes, *Schizophyllum commune* (Sietsma & Wessels 1979) and *Agaricus bisporus* (Mol & Wessels unpublished). In the first species nearly all the glucosaminoglycan is acetylated and nitrous acid has little effect unless the glucosaminoglycan is first deacetylated. Chitinase (from *Serratia marcescens*), on the other hand, is very effective in degrading this acetylated glucosaminoglycan (chitin) and solubilizing the β-glucan. In *Agaricus bisporus*, however, a large number of glucosamine residues in the glucosaminoglycan occur in a deacetylated form because the polymer is extensively degraded by nitrous acid releasing β-glucan into solution. Consequently, the glucan is less effectively released by chitinase but a nitrous acid treatment, followed by a chitinase treatment, effectively brings all the β-glucan into solution (Mol & Wessels 1988). Table 1 also shows data derived from a study on the wall of *Mucor mucedo* (Datema *et al.* 1977b). In this zygomycete an even larger fraction of the glucosamine residues of the

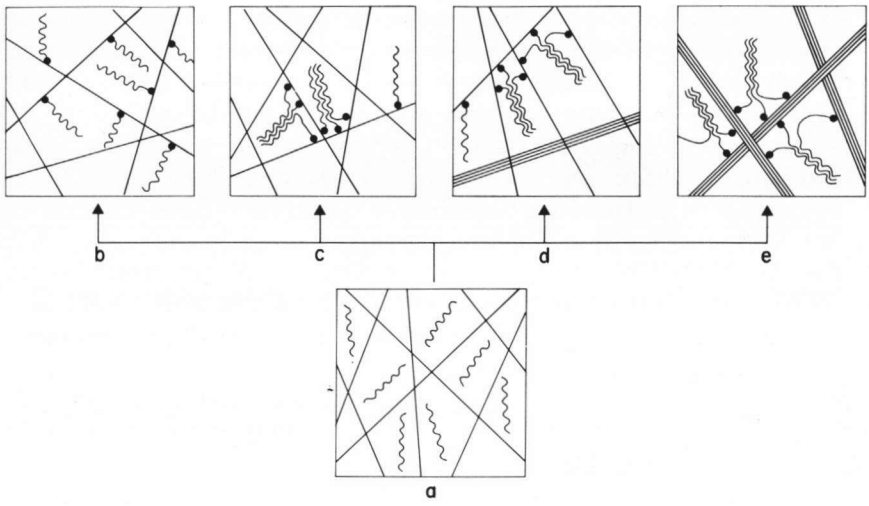

Fig. 4. Possible interactions between glucosaminoglycans (——) and (1→3)-β-glucan (~) involving hydrogen bonding between heterologous chains.

glucosaminoglycans is deacetylated resulting in a poly-cation. Depolymerization of this glucosaminoglycan releases an anionic heteroglucuronan containing fucose, mannose, galactose and glucuronic acid (5:1:1:6 on a molecular basis). Since in this case the non-aminoglycan can also be extracted by strong salt solutions and partly by alkali, the linkage between the two polymers is probably ionic and not covalent, as surmised in the ascomycetes and basidiomycetes.

The linkage of the β-glucan chains to glucosaminoglycan chains in the walls of ascocomycetes and basidiomycetes not only leads to insolubilization of the glucan chains but, in combination with hydrogen-bonding between homologous chains, may also result in a highly cross-linked complex as shown in Fig. 3. This may, however, represent an extreme case.

Figure 4 schematically depicts some wall structures which may actually arise from the interactions between glucosaminoglycan and (1→3)-β-glucan chains. Included are possible interactions between homologous chains involving hydrogen bonds leading to the formation of chitin microfibrils in the case of acetylated glucosaminoglycans and triple helices in the case of (1→3)-β-glucans (Jelsma & Kreger 1975). Figure 4e thus shows a situation in which all the chains are involved in hydrogen bonding while the outer chains of the chitin microfibrils covalently bind the glucan chains. In fact, all the structures depicted (Fig. 4a–e) may actually be present in the wall and contribute to its mechanical properties.

BIOSYNTHESIS AND ASSEMBLY OF THE WALL

Most evidence (Vermeulen *et al.* 1979 and references cited therein) indicates that chitin chains are made at the outer surface of the plasma membrane probably by an integral membrane protein, chitin synthase, that accepts the precursor uridine-diphosphate-*N*-acetylglucosamine at the cytoplasmic side of the membrane. Chitosomes (Bracker *et al.* 1976) are minivesicles which probably act as conveyers for inactive chitin synthase *en route*

to the plasma membrane, although the discoverers of these chitosomes still entertain the possibility that chitosomes themselves synthesize chitin after their extrusion through the plasma membrane into the domain of the wall (Bartnicki-Garcia & Bracker 1984, Ruiz-Herrera 1984). In *Saccharomyces cerevisiae* evidence for vectorial synthesis of chitin by plasma membranes has come from Cabib's laboratory (Cabib's *et al.* 1984) but complications have arisen after the gene for this chitin synthase had been cloned, inactivated *in vitro* by an insertion, and used to replace the wild-type allele by transformation (Bulawa *et al.* 1986). The gene replacement abolished the appearance of the chitin synthase studied thus far *in vitro* but did not affect synthesis of chitin *in vivo*, apparently catalysed by another enzyme (Orlean 1987). However, autoradiography after labelling *S. commune in vivo* with tritiated-*N*-acetylglucosamine has shown that akali-insoluble glucosaminoglycan is indeed synthesized just outside the plasma membrane (van der Valk & Wessels 1977). In this case the ultimate product was mainly poly-*N*-acetylglucosamine (chitin), but in other cases the product may be subject to enzymic deacetylation immediately after synthesis (Davis & Bartnicki-Garcia 1984).

Numerous studies have demonstrated the synthesis of an alkali-soluble (1→3)-β-D-glucan from uridine-diphosphate-glucose by membranous preparations from fungi (lit. cit: in Sonnenberg *et al.* 1985; Szaniszlo *et al.* 1985) but only in *S. cerevisiae* has the glucan synthase rigorously been shown to be (partially) associated with the plasma membrane (Shematek *et al.* 1980).

Since the polymerization of the *N*-acetylglucosamine residues at the outer plasma membrane surface immediately results in a water-insoluble and alkali-insoluble product (Wessels *et al.* 1983), it follows that after synthesis of the individual glucosaminoglycan and β-glucan chains (indicated in Fig. 4a) the processes that lead to cross-linked wall structures (indicated in Fig. 4b–e) must all take place within the domain of the wall. Because the nature of the coupling fragment between the glucosaminoglycan and glucan chains is insufficiently known, and may differ among fungi, the mechanism of coupling remains elusive. In addition, because of the polymeric nature of the two reacting components it is unlikely that the coupling itself is mediated by an enzyme. Rather we envisage a scheme in which amino acids such as lysine or phenolic residues are attached to aminosugars within the glucosaminoglycans and/or to the reducing ends of β-glucans. Coupling might then occur via radicals produced by oxidases in the wall. As an analogy we may recall the cross-linking between lysine residues in collagen as occurring in the extracellular matrix of animals (Hay 1981) or the cross-linking of tryptophane residues in extensin as occurring in cell walls of vascular plants (Wilson & Fry 1986). Alternatively, lysine residues attached to aminosugars within the glucosaminoglycans may directly and non-enzymically interact with the reducing end of glucan chains forming a Schiff base and Amadori products (Monnier *et al.* 1984). Also direct interactions of the reducing ends of glucan chains with amino-groups on the glucosaminoglycans cannot be excluded.

Irrespective of the type of cross-links between the glucosaminoglycans and β-glucans, post-synthetic transitions within the wall, such as depicted in Fig. 4, may have important biological consequences. For instance, it can be envisaged that the highly hydrated precursor wall structure shown in Fig. 4a has visco-elastic properties and can be deformed; whereas the cross-linked composite structures shown in Fig. 4c–e display various degrees of rigidity.

The evidence collected with *S. commune* to support such a two-step process of synthesis and assembly of wall components with an accompanying change in mechanical properties can be summarized as follows.

1. Water-soluble and alkali-soluble (1→3)-β-glucans are the precursor molecules for the alkali-insoluble β-glucans in the wall. This was shown by pulse-chase of radioactivity both with regenerating protoplasts (Sonnenberg *et al.* 1982) and with growing hyphae (Wessels *et al.* 1983).

2. In the presence of polyoxin D, protoplasts can form a wall made of (1→3)-α-glucan without chitin and alkali-insoluble glucan (de Vries & Wessels 1975, van der Valk & Wessels 1976). Although the soluble (1→3)-β-glucan precursor molecules are normally formed they can then not be linked to an alkali-insoluble glucosaminoglycan and thus remain soluble (Sonñenberg *et al.* 1982). Similarly, Elorza *et al.* (1987) have shown that nikkomycin, another specific inhibitor of chitin synthase, inhibits the conversion of an alkali-soluble glucan into an alkali-insoluble glucan on regenerating protoplasts of *Candida albicans.*

3. Autoradiography shows that alkali-insoluble glucosaminoglycans are maximally synthesized at the extreme hyphal apex decreasing in subapical direction. A similar gradient is found for water-soluble β-glucan but very little alkali-insoluble glucan is present at the extreme apex. While displaced in a subapical direction during extension in the absence of label, the water-soluble glucans become alkali-insoluble. If extension does not occur during the chase, the water-soluble glucan can be seen to become insoluble over the whole apex (Wessels *et al.* 1983).

4. Electron microscopy of shadowed preparations combined with autoradiography (Vermeulen & Wessels 1984) has shown that the alkali-insoluble glucosaminoglycans at the growing hyphal apex are non-fibrillar and very susceptible to chitinase and hot dilute mineral acid. This is taken as evidence that at the growing apex the glucosaminoglycans have not yet crystallized and are still available for linkage to glucan chains. Hyphae that have ceased to grow not only have alkali-insoluble glucan but also microfibrillar chitin in their apical walls. Recently, it was shown that also *in vitro* a time gap exists between synthesis and crystallization of the chitin chains (Vermeulen & Wessels 1986).

5. High shearing forces, as generated by the passage of hyphae through an X-press, disrupt growing tips and remove the pulse-labelled glucosaminoglycan and glucan. However, after a chase of a few minutes these labelled polymers, present subapically in growing hyphae or apically in hyphae not growing after the chase, are not removed by these shearing forces (Wessels *et al.* 1983). This is the only direct evidence we have to show that the mixture of individual glucosaminoglycan and glucan chains present at the tip mechanically differs from the cross-linked complex in the matured wall.

MECHANISM OF APICAL WALL GROWTH

The evidence summarized above serves as the basis for the steady-state growth model as shows in Fig. 5. In the extension zone, individual glucosaminoglycan and (1→3)-β-glucan chains are deposited into the wall by apposition. They are probably polymerized at the membrane–wall interface. Per unit area maximal deposition occurs at the extreme apex and then declines towards the base of the extention zone in accordance with autoradiographic estimates of incorporation of labelled precursors (Gooday 1971, Wessels *et al.* 1983). The glucosaminoglycan and β-glucan chains extruded into the wall are supposed to be highly hydrated and to form a visco-elastic wall that expands under the internal pressure of the cytoplasm. As a visco-elastic wall segment is stretched and displaced in subapical direction by growth, it also moves to a more external side of the wall because appositional addition of visco-elastic wall material on the inside continues so that uniform

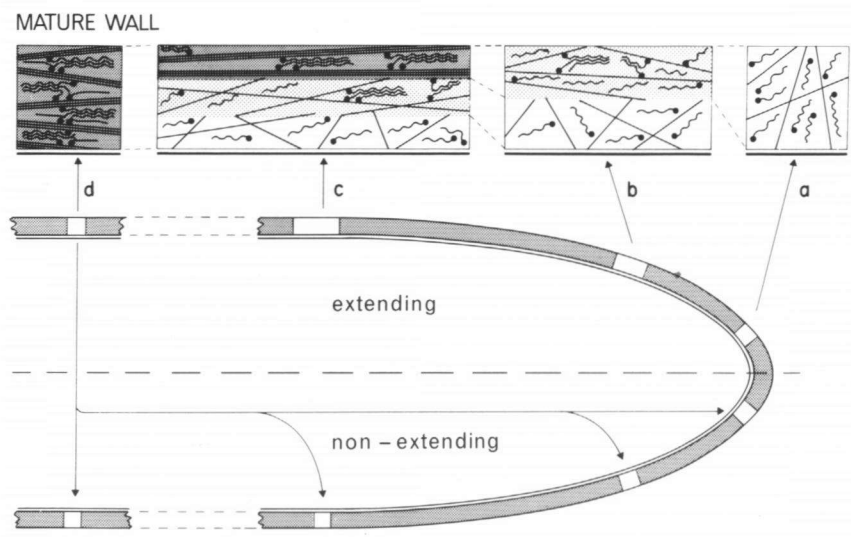

Fig. 5. Steady-state model of apical wall growth. Glucosaminoglycan (——) and (1→3)-β-glucan (~) interact while being displaced in a subapical direction. (1→6)-β-linked glucose residues also appear subapically. When extension ceases the interactions occur over the whole apex.

wall thickness is maintained (Green 1974). At the same time the subapically and externally displaced wall segment undergoes rigidification because of covalent bonding between heterologous chains and hydrogen bonding between homologous chains. At point c in Fig. 5 these processes have advanced to such an extent that the wall no longer yields to the internal pressure and the maximal diameter of the hyphae is attained. However, the interactions between the polymers continue (Fig. 5d) and probably lead to further rigidification, as deduced from continued insolubilization of β-glucan chains (Wessels *et al.* 1983). Also shown in Fig. 5d are (1→6)-β-linked glucan chains or glucose residues appearing as branches on the (1→3)-β-linked glucan chains. Such (1→6)-β linkages are initially very scarce in the insoluble glucan but rapidly appear when the wall matures, eventually outnumbering the (1→3)-β-linkages (Sietsma *et al.* 1985). It is also possible that they are part of a mixed-linkage glucan that is secondarily hooked up to the glucosaminoglycan chains. It should also be stressed that the final wall structure probably deviates from the highly regular structure depicted in Fig. 5d. In fact, the alkali-insoluble complex shows very little crystallinity in X-ray diffraction and may actually be made up of various structures as depicted in Fig. 4. Also not shown are the water-soluble (1→3)-β-glucan chains with single (1→6)-β-linked glucose residues attached which freely occur in the wall and the alkali-soluble and partly crystalline (1→3)-α-glucan (s-glucan) which accumulates at the outer surfaces of the wall.

The model described above is a steady-state model because continued extension depends on the continuous extrusion of the visco-elastic wall material which is subsequently hardened by the cross-linking process in the wall. The rate of extrusion of wall polymers into the wall and the rate of cross-linking (rigidification) are supposed to be independent. Consequently, when growth ceases cross-linking and stiffening will occur over the whole apex (Fig. 5). The experimental evidence does indeed show that the wall over the apex then assumes the same structure as in subapical parts, including the appearance of (1→6)-β-linked glucose residues and chitin microfibrils (Vermeulen & Wessels 1984, Sietsma *et al.* 1985).

With respect to observations with living hyphae, the steady-state model of wall growth explains why hyphal tips do not lyse when growth ceases because no lytic enzymes are implicated. Instead the model predicts rigidification of the tip wall which would explain why extension becomes irreversibly blocked when growth is interrupted for a short time (Reinhardt 1892, Robertson 1958). In addition, a positive relationship between the rate of hyphal growth and the length and width of the extension zone (Reinhardt 1892; Steele & Trinci 1975) can be envisaged. Interference with the cross-linking process could produce a variety of deformations of hyphal tips. Endogenously produced substances that interfere with the homologous or heterologous interactions between polymers in the wall may give rise to bulbous cells such as in blastic conidia (Cole 1986). In symbiotic associations such substances, when produced by host tissue, may be responsible for the formation of various fungal infection structures. Experimentally, the generation of bulbous tips can be induced by substances such as congo red (Pancaldi *et al.* 1984, Vermeulen & Wessels, unpublished) which interferes with the crystallization of chitin chains (Vermeulen & Wessels 1986). For branch formation, i.e. the generation of an extending tip from a matured wall segment, lysins would still be required to initiate the process. However, such lysine could be quite specific enzymes that, for example, break the cross-links between glucosaminoglycan and glucan chains.

In essence, the scheme suggested above may be applicable to basidiomycetes and ascomycetes in general but details may differ. It is tempting to speculate that in the zygomycetes the role of the chitin–glucan linkages is played by the ionic linkages between partially deacetylated chitin and glucuronans. Similar to the solubilization of β-glucans by depolymerization of chitin, specific depolymerization of the partially deacetylated chitin chains in zygomycetes causes the solubilization of glucuronans (Datema *et al.* 1977a, Table 1). Deacetylation of chitin seems to occur in the wall shortly after synthesis of the chitin chains and before crystallization has occurred (Davis & Bartnicki-Garcia 1984). Assuming that these processes occur at the apex, the generation of the cationic groups by the deacetylase at the apex could lead to cross-linking of the glucosaminoglycan chains by acidic glucuronans. These in turn may be hydrogen-bonded among themselves. Thus the process of deacetylation of chitin at the apex of a zygomycete hypha could also lead to a significant change in the visco-elastic properties of the wall.

CONCLUSIONS

Growth of the wall at the hyphal apex requires that the wall in this region has plastic properties which contrast with the requirement of rigidity elsewhere in the hypha. A widely held view involves the participation of wall-lytic enzymes to plasticize the wall at the apex and to allow new wall material to be inserted. As an alternative, a steady-state model is discussed based on observations in the author's laboratory. In essence this model holds that the assemblage of polymers synthesized at the apex is inherently plastic. However, this assemblage develops rigidity by interactions, in the wall, between the various individual polymers present while the assemblage is stretched and moves in a subapical direction during elongation. This model seems to fit many of the observations made on living hyphae.

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