Hyphal Wall Growth in *Neurospora crassa* and *Geotrichum candidum*

By A. P. J. TRINCI AND ANNETTE J. COLLINGE

Microbiology Department, Queen Elizabeth College, University of London, London W8 7AH

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**SUMMARY**

Growth of the walls of hyphae of *Neurospora crassa* and *Geotrichum candidum* was studied using longitudinal and serial transverse sectioning methods. Rigidification of the hyphal wall below the extension zone did not appear to involve the gross formation of a secondary wall since the transition from extensible to non-extensible wall was not associated with an increase in thickness. However, behind the extension zone the walls of leading hyphae of *N. crassa* increased in thickness until eventually they attained a thickness which was up to five times that of the tip wall. A hypothesis of hyphal wall growth is proposed.

**INTRODUCTION**

Hyphal growth is polarized, i.e. extension is confined to the hyphal tip (Trinci & Banbury, 1967). The length of the apical extension zone of a hypha may be determined by measuring the length of the tapered region of its tip (Trinci & Halford, 1975). The tips of fungal hyphae contain vesicles (Grove & Bracker, 1970) which are assumed to carry wall precursors and/or the enzymes required for the insertion of these precursors into the existing wall (Bartnicki-Garcia, 1973). The vesicles are also thought to supply the membrane which enables the plasmalemma at the tip to expand rapidly in surface area. It has been estimated that about 38000 vesicles fuse with a tip of a *Neurospora crassa* hypha (wild-type) during 1 min of extension growth at 25°C (Collinge & Trinci, 1974). The vesicles and other precursors required to maintain the high apical extension rate observed in fungi are probably generated throughout the peripheral growth zone (Trinci, 1971) of the hypha (Collinge & Trinci, 1974). It is not known if increase in wall thickness behind the tip (i.e. secondary wall growth) involves this vesicular system (Collinge & Trinci, 1974).

Robertson (1959) has proposed that hyphal extension involves a balance between the insertion of new wall material at the hyphal tip and rigidification of the newly formed wall at the base of the extension zone. The rigidification process may involve the addition of non-extensible, secondary wall material to the extensible primary wall and/or the formation of cross-linkage between the existing wall polymers of the primary walls. Robertson (1968) favours the hypothesis that deposition of secondary wall material is involved in the rigidification process.

This study was undertaken to determine if there was an increase in wall thickness at the hyphal tip in the region of transition from extensible to non-extensible wall. Studies were made on hyphae of undifferentiated mycelia (mycelia formed during the early exponential stage of growth on solid medium; Steele & Trinci, 1975) and the ‘leading’ hyphae at the margin of ‘mature’ colonies.
METHODS

Organisms and media. The spreading colonial mutants of Neurospora crassa (spco-I and -12) and the colonial temperature-sensitive mutant (cot-3) were supplied by the Fungal Genetics Stock Center, Humboldt State College Foundation, Arcata, California, U.S.A.; cot-3 has wild-type characteristics at 25 °C. The wild-type strain of N. crassa (sYR-I~A) (inos + C, D, E) was kindly supplied by Professor E. L. Tatum. Geotrichum candidum (Queen Elizabeth College strain FI, formerly called G. lactis) was also used in this investigation. The N. crassa strains were grown on Vogel's minimal medium (Vogel, 1956) with 1% (w/v) sucrose as the carbon source; this medium was prepared as described previously (Trinci, 1973). Geotrichum candidum was grown on DM medium (Trinci, 1971).

Undifferentiated hyphae (Steele & Trinci, 1975) of N. crassa cot-3 and G. candidum were grown on the surface of solid media at 25 °C; the hyphae were fixed in 4% (v/v) glutaraldehyde at 25 °C (Trinci & Collinge, 1973) at 27 and 16 h after inoculation respectively. The total hyphal length of the mycelia did not exceed more than about 5 mm. 'Mature' colonies were grown at 25 °C on solid media and the 'leading' hyphae at the margin of the colony were fixed whilst in the linear phase of growth. Procedures employed for electron microscopy have been described previously (Trinci & Collinge, 1973; Collinge & Trinci, 1974). Measurements of wall thickness were made upon photographs of transverse hyphal sections; the hyphae had a more-or-less circular outline in these sections. The sections were usually silver or gold in colour and thus varied in thickness from about 60 to about 150 nm. The values for the thickness of the wall at various distances from the hyphal tip (Figs. 1, 6 and 7) are always the means of five measurements made around the hyphal circumference.

RESULTS

Wall thickness of 'leading' hyphae at the margin of 'mature' colonies of Neurospora crassa

Figures 1, 3 and 4 show the wall thickness and diameter of 'leading' hyphae at the margin of a 'mature' colony of N. crassa cot-3. The hypha in Fig. 1 had an extension zone of about 28 μm but there was no appreciable change in wall thickness over the apical 56 μm of the hypha. The wall thickness of 'leading' hyphae of the wild-type N. crassa (sYR-17-3A) also did not vary over the terminal 50 μm of the hypha. In longitudinal sections the wall over the apical dome of the hypha was sometimes thicker than the wall of the basal part of the extension zone. The 'leading' hyphae of wild-type and spreading colonial mutants (spco) of N. crassa had tip walls of about the same thickness (Table 1).

The walls of 'leading' hyphae of N. crassa cot-3 showed an appreciable increase in thickness about 500 μm from the margin of the colony (Fig. 1) and finally attained a thickness (about 270 nm) which was more than five times that of the tip wall.

Wall thickness of undifferentiated mycelia of Neurospora crassa cot-3 and Geotrichum candidum

Figures 2 and 6 show the change in wall thickness and diameter of hyphae of undifferentiated mycelia of N. crassa cot-3 as observed in serial transverse sections (Fig. 6b) and a median longitudinal section (Fig. 6a). These hyphae were narrower and had shorter extension zones (about 2 μm) than the 'leading' hyphae at the margin of 'mature' colonies (about 28 μm). There was no significant change in wall thickness over the terminal 10 μm of the hyphae but between about 10 to about 200 μm from the tip there was a gradual increase in thickness until the wall attained a more-or-less constant thickness of 120 to 140 nm. The
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Fig. 1. Wall thickness (●) and diameter (○) of 'leading' hyphae at the margin of 'mature' colonies of *N. crassa* cot-3 at 25 °C. Measurements from 0 to 50 μm were made on a median longitudinal section of a single hypha. Measurements from 500 μm to 30 mm from the margin of the colony were made on different 'leading' hyphae.

![Graph showing wall thickness and diameter of hyphae](image)

**Table 1. Thickness of the tip walls of 'leading' hyphae of *N. crassa* at the margin of 'mature' colonies and hyphae of undifferentiated mycelia**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Type of hypha</th>
<th>Mean wall thickness* (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SYR-17-3A (wild-type)</td>
<td>'Leading'</td>
<td>45</td>
</tr>
<tr>
<td><em>spc-1</em></td>
<td>'Leading'</td>
<td>50</td>
</tr>
<tr>
<td><em>spc-12</em></td>
<td>'Leading'</td>
<td>49</td>
</tr>
<tr>
<td><em>cot-3</em></td>
<td>'Leading'</td>
<td>50</td>
</tr>
<tr>
<td><em>cot-3</em></td>
<td>Undifferentiated</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>0-5 μm from tip</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>5-10 μm from tip</td>
<td>51</td>
</tr>
</tbody>
</table>

* Mean of at least 15 measurements.

tip walls of immature hyphae of *N. crassa* were slightly thicker than those of the 'leading' hyphae of the same strain (Table 1). In regions distal from the tip, the walls of hyphae of undifferentiated mycelia of *N. crassa* were made up of an electron-dense inner layer and an electron-transparent outer layer (Fig. 2d); 200 to 1500 μm from the tip, where the wall thickness was approximately constant (Fig. 6), the inner layer had a mean thickness of 51 ± 10 nm and the outer layer of 87 ± 12 nm. The vesicles at the tip of immature hyphae of *N. crassa* had a mean diameter of 62 ± 13 nm and were thus smaller than the vesicles (mean diameter 134 ± 26 nm) at the tips of the 'leading' hyphae of this same strain (Collinge & Trinci, 1974).

Figures 5 and 7 show the wall thickness, diameter and vesicle concentration of hyphae of an undifferentiated mycelium of *G. candidum*. Like *N. crassa*, these hyphae had extension zones of about 2 μm. The concentration of vesicles in the cytoplasm only increased in the regions where the diameters of the hyphae narrowed, i.e. in the extension zones. The vesicles of *G. candidum* had a mean diameter of 76 ± 20 nm. Longitudinal sections showed that the vesicles were not elongated in the direction of the long axis of the hypha.

The thickness of the hyphal wall of *G. candidum* remained constant (about 70 nm) over the terminal 300 μm of the hypha (Figs. 5 and 7). Other sections showed that the wall of this particular hypha had not increased in thickness at distances of up to 1.4 μm from the tip. The
apparent increase in wall thickness (Fig. 7) over the hyphal dome (the apical 1 μm) can almost certainly be accounted for in terms of the curvature of the wall at the tip. Throughout the terminal 300 μm of the hypha the wall was made up of two layers (Fig. 5) of more or less equal thickness: an electron-transparent inner layer and an electron-dense outer layer.

**DISCUSSION**

The results obtained with *N. crassa* and *G. candidum* suggest that rigidification of the wall below the extension zone does not involve the formation of a substantial secondary wall. Published electron micrographs of longitudinal sections of hyphae of other species also indicate that there is no appreciable change in wall thickness in areas at the base and immediately below the extension zone (Grove & Bracker, 1970). The apparent increase in wall
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Fig. 6. Wall thickness (●), diameter (○) and vesicle concentration (■) of undifferentiated hyphae (27 h culture) of *N. crassa* cot-3 at 25 °C. (a) Measurements made on a median longitudinal section of a single hypha; (b) measurements made on serial transverse sections of a single hypha.

Fig. 7. Wall thickness (●), diameter (○) and vesicle concentration (■) of an undifferentiated (16 h culture) hypha of *G. candidum*. Measurements made on serial transverse sections of a single hypha.

thickness which was sometimes observed over the apical domes of hyphae cut longitudinally was probably caused by the non-median nature of these particular sections (see McClure, Park & Robinson, 1968). Rigidification of the wall below the extension zone may involve the addition of small amounts of substances to the wall which do not increase its thickness appreciably, and/or the formation of cross-linkages between existing wall polymers. It is possible that the primary wall only remains extensible whilst vesicles (containing lytic enzymes?) continue to fuse with it. Rigidification may thus occur at the base of the extension zone simply because vesicles are no longer fusing with the wall at this point.

In *N. crassa* (Figs. 1 and 6), but not apparently in *G. candidum* (Fig. 7), there is an increase in wall thickness behind the hyphal tip due to secondary wall growth. D. Hunsley (personal communication) also observed changes in wall thickness in *N. crassa* similar to those we
Fig. 8. Tentative model of hyphal wall growth based mainly upon studies with N. crassa. Zone A: tapered extension zone with a primary wall, containing microfibrils, which becomes progressively less extensible with distance from the hyphal apex. Wall thickness maintained approximately constant and extensible by the fusion with it of vesicles containing wall precursors and/or lytic and synthetic enzymes. Hyphal extension dependent upon the rate of supply of vesicles. Zone B: region of constant hyphal diameter. The wall is inextensible and of approximately the same thickness as in zone A. Rigidification of the wall may involve the formation of cross-linkages between existing wall polymers or the addition of small quantities of new wall material. It is possible that vesicles do not normally fuse with this region of the hyphal wall. Zone C: region of secondary wall formation. The wall increases in thickness with distance from the tip, eventually becoming a 'mature' wall of approximately constant thickness. Zone D: under conditions of carbon starvation (Zonneveld, 1972) some of the wall polymers may be degraded to provide substrates for endogenous metabolism.

The observation that the inner layer of the 'mature' hyphal wall of N. crassa (present results, and Hunsley & Burnett, 1970) has approximately the same thickness (about 50 nm) as the primary tip wall (Table 1) suggests that the former may be continuous with the latter (Fig. 8).

The chitin content of the inner layer of the 'mature' hyphal wall of N. crassa (Hunsley & Burnett, 1970) provides further evidence in support of the above hypothesis, since the tips of hyphae of N. crassa and other fungi are covered by a microfibrillar mesh of chitin (Hunsley & Burnett, 1970). The chemistry of the secondary wall probably differs both qualitatively and quantitatively from that of the primary wall (Hunsley & Burnett, 1970). Support for these contentions and the hypothesis presented in Fig. 8 is provided by the observations that walls at the tips of fungal hyphae differ from those below the tip both antigenically (Fultz & Sussman, 1966) and in their reaction to the fluorescent brightener, Calcofluor (Gull & Trinci, 1974). The glycoprotein reticulum of the walls of hyphae of N. crassa appears to develop progressively behind the tip as a superficial wall layer (Hunsley & Burnett, 1970).

Thus our results and those of Hunsley & Burnett (1970) and Hunsley (1973) suggest that hyphal walls of N. crassa and Phytophthora parasitica increase in thickness largely or wholly by secondary wall layers being deposited upon the existing primary wall (Fig. 8). The possibility that secondary wall material may sometimes (e.g. during unbalanced growth and differentiation processes such as spore formation) be deposited on the cytoplasmic side of the primary wall is not excluded.

As observed by Robertson (1965), 'The key to the fungal hypha lies in the apex.' Certainly, the crucial events involved in hyphal morphogenesis probably occur in the apical and immediate sub-apical areas rather than in regions of secondary wall formation far removed from the tip. Thus it is unlikely that comparison of the gross chemistry of 'mature' hyphal walls (e.g. De Terra & Tatum, 1963) will reveal the subtle chemical changes at the hyphal tip which are responsible for a particular type of morphogenetic change.
We thank Dr D. Hunsley for helpful discussion, and the Royal Society and the Science Research Council for financial support.

REFERENCES


