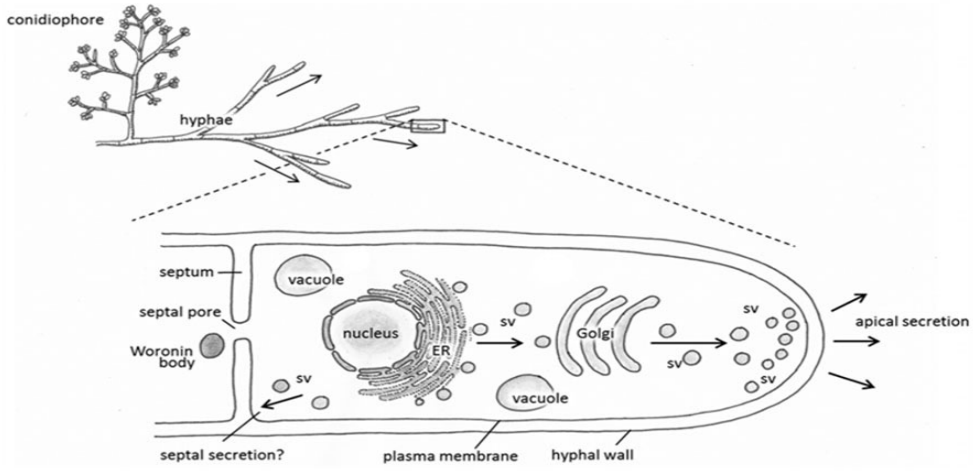
Apical hyphal growth



The fungal cell wall is a dynamic structure that protects the cell from changes in osmotic pressure and other environmental stresses, while allowing the fungal cell to interact with its environment. The structure and biosynthesis of a fungal cell wall is unique to the fungi, and is therefore an excellent target for the development of anti-fungal drugs.

the cell wall synthesis in apical hyphal growth. It appears that the hyphal apex is best viewed as a highly polarized system of exocytosis. Wall materials, extracellular enzymes, and probably other substances are excreted at the growing end of a tubular cell. The most obvious cellular features that accompany this polarized system are

(1) the unidirectional flow of vesicles in the cytoplasm fusing with the plasma membrane at the apex,

(2) the gradients in wall synthesis at the apex, and

(3) the cytoplasmic gradients in ion distribution that are maintained at the apex. New microscopic techniques reveal a cytoskeletal organization of the cytoplasm at the apex, which may be crucial to its polarized activity.

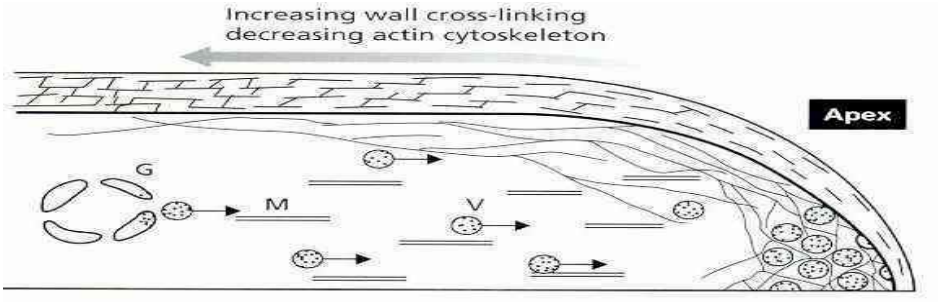
Growth of the wall at the hyphal apex requires that the wall in this region has plastic properties, which contrast with the requirement of rigidity elsewhere in the hypha. A widely held view involves the participation of wall-lytic enzymes in plasticizing the wall at the apex and in allowing new wall material to be inserted. A critical evaluation of the evidence presented to support this view makes this hypothesis less attractive. As an alternative a steady-state model is discussed based on recent observations in the author's laboratory. 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 and among the various individual polymers present while the wall segment moves in subapical directions during elongation. This model seems to fit many of the original observations made on living hyphae.

Ultrastructural studies have shown that many organelles within the growing hyphal tip are distributed in steep gradients, as would be expected of a cell growing in a polarized mode . This is visible even with the light microscope by careful observation of an unstained hypha using phase-contrast optics, and more so with the aid of simple staining techniques .The cytoplasm of the extreme apex is occupied almost exclusively by secretory vesicles and microvesicles
In the higher fungi (Asco- and Basidiomycota), the former are arranged as a spherical shell around the latter, and the entire formation is called the SpitzenkoŐrper or 'apical body' .
The SpitzenkoŐrper may be seen in growing hyphae even with the light microscope.
Hyphae of the Oomycota and some lower Eumycota (notably the Zygomycota) do not contain a recognizable SpitzenkoŐrper, and the vesicles are instead distributed more loosely in the apical dome. Hyphal growth can be simulated by means of computer models based on the assumption that the emission of secretory vesicles is coordinated by a 'vesicle supply centre', regarded as the mathematical equivalent of the SpitzenkoŐrper in higher fungi.

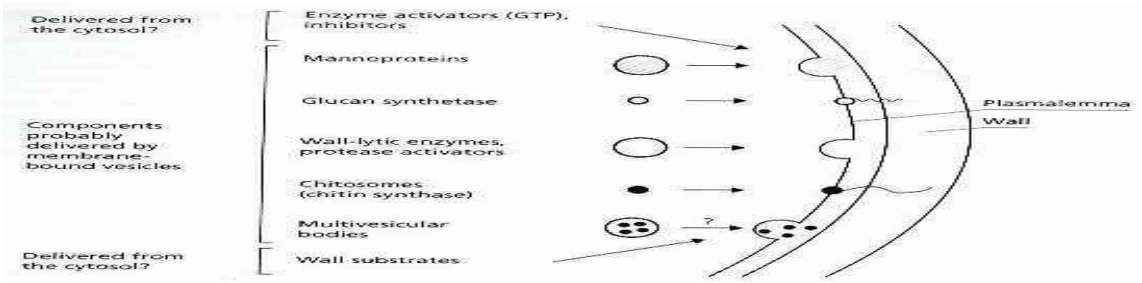
Physiology of the growing hyphae

Hyphae grow at their tips. During tip growth, cell walls are extended by the external assembly and polymerization of cell wall components, and the internal production of new cell membrane. The spitzenkörper is an intracellular organelle associated with tip growth. It is composed of an aggregation of membrane-bound vesicles containing cell wall components. The spitzenkörper is part of the endomembrane system of fungi, holding and releasing vesicles it receives from the Golgi apparatus. These vesicles travel to the cell membrane via the cytoskeleton and release their contents outside the cell by the process of exocytosis, where it can then be transported to where it is needed. Vesicle membranes contribute to growth of the cell membrane while their contents form new cell wall. The spitzenkörper moves along the apex of the hyphal strand and generates apical growth and branching; the apical growth rate of the hyphal strand parallels and is regulated by the movement of the spitzenkörper.

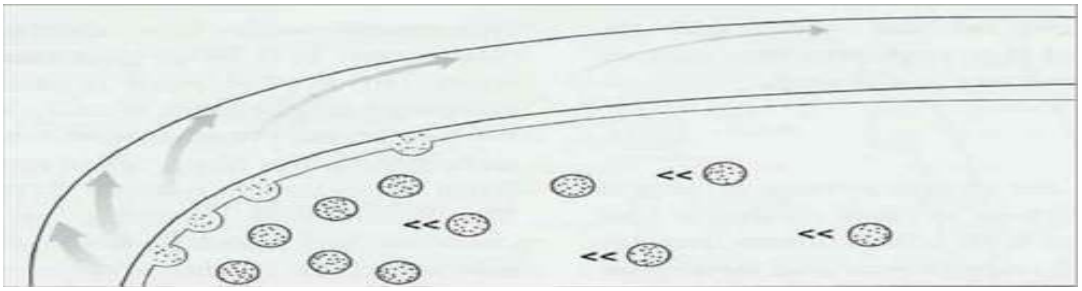
As a hypha extends, septa may be formed behind the growing tip to partition each hypha into individual cells. Hyphae can branch through the bifurcation of a growing tip, or by the emergence of a new tip from an established hypha.



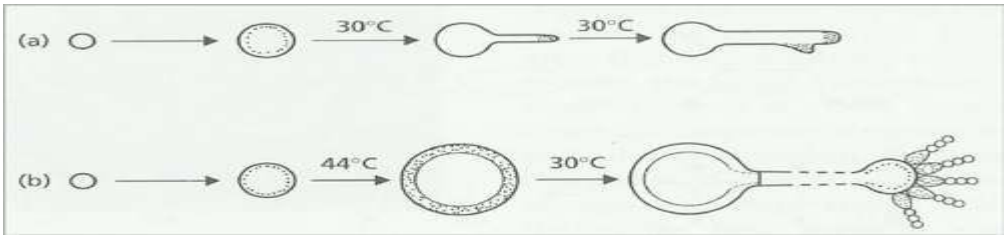
Diagrammatic representation of the possible organisation of wall growth at the hyphal apex. Only half of the hypha is shown. Vesicles (V) derived from the endoplasmic reticulum and Golgi body (G) are transported to the apex, probably by microtubule (M) -associated motor proteins. The vesicles could then be directed to the plasma membrane, perhaps by actin-associated motor proteins. The newly-formed wall at the extreme hyphal tip is thin and has few cross-linkages, but becomes increasingly cross-linked further back. In contrast, the actin cytoskeleton is highly developed at the extreme tipand might help to provide structural support, compensating for the lack of wall cross-linking at the tip. The concentration of actin progressively decreases behind the tip



illustrates some of the components of wall synthesis at the hyphal tip. Vesicles are thought to deliver the main wall-synthetic enzymes (chitin synthase and glucan synthase) to the tip, where they lodge in the plasma membrane as integral membrane proteins. Mannoproteins and other glycoproteins are transported in vesicles from the Endoplasmic reticulum - Golgi secretory system (because the glycosylation of proteins occurs only in the Golgi). Multivesicular bodies, whose functions are still unclear, may be carried as vesicular cargoes along microtubules. Enzyme activators and inhibitors also are thought to be involved in the orchestration of tip growth, but the substrates for wall synthesis arrive from metabolic reactions in the cytosol.

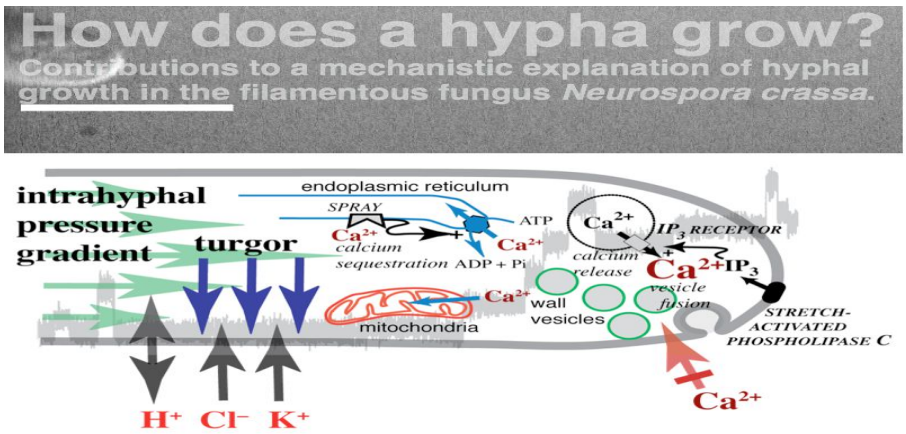


Representation of the steady state model of hyphal tip growth, in which the wall is envisaged as being visco-elastic. New wall polymers synthesised at the extreme tip are suggested to flow outwards and backwards as new components are continually added at the tip. The decreasing thickness of the arrows behind the tip signifies progressively reduced flow as the polymers become cross-linked.



Stages in germination of spores of *Aspergillus niger*. **(a)** In normal conditions (e.g. 30°C) the spore swells and incorporates new wall material over the whole of the cell surface (shown by stippling), then a germ-tube emerges and all new wall incorporation is localised to the hyphal tip. **(b)** At 44°C the spore continues to swell and incorporates wall material in a non-polar manner, producing a giant cell with a thick wall. If the temperature is lowered to 30°C this cell produces an outgrowth, which immediately differentiates to produce a spore-bearing head.

NOTE:



Fungal Tip Growth Characterization of cytoplasmic Ca^{2+} has identified at least two mechanisms associated with the process of hyphal growth.

One involves Ca^{2+} influx at the growing tip (*Saprolegnia*). The other involves internal generation of a tip-high Ca^{2+} gradient (*Neurospora*). In both cases, a tip-high Ca^{2+} gradient is required for hyphal growth. Our most recent model of Ca^{2+} regulation and ion transport during *Neurospora* hyphal growth is shown. We are now exploring the role of turgor in hyphal tip growth. Movie of *Neurospora crassa* tip growth .

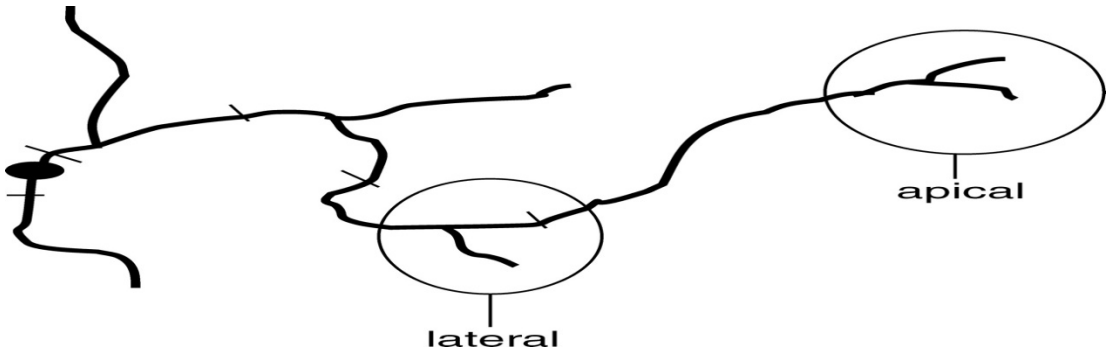
At the growing tip, the major transport system may function to maintain the tip-high Ca^{2+} gradient. In *Neurospora crassa*, inositol-1,4,5-trisphosphate ($InsP_3$), which is probably produced by a stretch-activated phospholipase , mediates the release of Ca^{2+} from internal stores at the tip, and then Ca^{2+} causes fusion of vesicles at the expanding tip. The Ca^{2+} is sequestered behind the tip by the endoplasmic reticulum (by the action of a Ca^{2+}-ATPase pump) and the mitochondria. SPRAY is a putative regulator of Ca^{2+} sequestration .

Behind the tip (within the mycelial network), ion and nutrient uptake are driven by the activity of the plasma membrane H^+-ATPase. Some of the known (and assumed, such as Cl^-) H^+ transport partners are shown, including amino acids, nitrate (NO_3^-), phosphate ($H_2PO_4^-$) and glucose. The intra-hyphal pressure gradient causes cytoplasmic movement into the tip

Branching of fungal hyphae:

Apical branching of fungal hyphae:

The emergence of a branch from the hyphal tip is referred to as apical branching. This pattern of branching has been observed in a large number of fungi . In many of these fungi, apical branching presumably occurs in response to the abnormal accumulation of exocytic vesicles at the hyphal tip. This could conceivably be triggered by perturbations that slow extension of hyphal tips without interrupting the flow of exocytic vesicles through the cytoplasm. Because the supply of vesicles exceeds their capacity to be incorporated into the existing tip, they accumulate leading to the formation of a new tip Although numerous mutations that cause increased apical branching (also referred to as dichotomous branching or tip splitting) have been described in fungi such as *A. nidulans*, *A. niger* and *N. crassa* ,the fact that it occurs in wild type isolates suggests that it is not merely an abnormal pattern . Furthermore, there is limited evidence that apical branching shares common control mechanisms with the more prevalent branching pattern, lateral branching . Instead, it seems likely that apical branching is a general response that enables continued growth under conditions that compromise organization of hyphal tips and thereby disrupts apical dominance.



Lateral branching.

Lateral branching would only occur when a potential site is far enough removed from the tip so as to escape the effects of these factors. Accordingly, the nature of these factors and their mode-of-action is of great interest (see below).

There appears to be two broad patterns of lateral branching; branches associated with septa, and random branching. In the former pattern, new branches emerge adjacent to septa , and it seems likely that some component(s) of the septum provides a spatial cue that specifies the position of the branch. Several fungi exhibit this pattern , including members of the Saccharomycotina (*A. gossypii*, *Geotrichum candidum*), as well as zygomycetes (*Basidiobolus ranarum*) and basidiomycetes (*Coprinus* species). Note that in *A. gossypii*, lateral branching predominates during the early stages of growth that precede hyphal maturation and the switch to apical branching . In most cases of lateral branching, the branch emerges just behind the septum, which would be expected if the septum were serving as a barrier that impeded the tip-bound flow of exocytic vesicles and thus led to their local accumulation. However, the analysis of the *A. gossypii bud3* mutant suggests that this interpretation may be too simple. In this

mutant, delocalization of actin rings at septation sites results in the accumulation of aberrant chitin aggregates instead of normal septa . Nevertheless, branches still emerge from these sites. It is tempting to speculate that a component involved in an early step in septum formation .