

Chapter 2

Tropic Orientation Responses of Pathogenic Fungi

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Abstract Cellular orientation allows growth, differentiation and behaviour to respond to vectorial cues generated in the environment and in relation to cells of the same organisms or different organisms that exist in proximity to one another. In the case of fungal pathogens, the orientation of hyphae may allow the fungus to detect a host and to make strategic penetrations at points of weakness on the host surface. Within a host, tropic orientation may facilitate colonisation, ramification and dispersal within the host tissues. To achieve this, cells have to be able to coordinate their cell cycles, growth and expansion of their margins with directional growth responses. In this chapter, we review the tropic orientation responses of fungi and, with an emphasis on fungal pathogenesis, discuss and speculate on the underlying molecular mechanisms that regulate cellular tropisms. Examples are taken across the fungal kingdom, including from work on saprophytes, plant and animal pathogens, to construct a working model that speculates how a wide range of tropisms may be controlled by a more-or-less common tropic mechanism that regulates the orientation of the hyphal tip.

2.1 Introduction

Pathogenic fungi have developed efficient growth strategies that allow them to penetrate and infiltrate through host tissue. A common mechanism in this process is the formation of elongating structures, such as hyphal filaments, penetration pegs, shmoos and rhizoids, which translocate fungal growth within the host or environment, for example, through leaf cuticles or between epidermal cell layers. Each host offers its own unique environment, so fungi have evolved hard-wired host-specific sensing and response mechanisms (tropisms) that regulate their vectorial

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growth. The study of tropic responses *in vitro* has demonstrated how subtle the interplay between environmental cues and fungal response can be and potentially involves the integration of mechanical, chemical and electrical signals. The challenge now is to develop a holistic understanding of the process during infection *in vivo*, with a view to destabilising the relationship between host environment and pathogen growth response. In this chapter, we review the tropic, or pre-programmed, growth responses of fungal pathogens which are involved in disease progression in plants and humans.

Thigmotropism is the movement or orientation of an organism or cell in relation to the topography, shape and physical properties of the underlying substrate on which it is growing. In both the macrobiotic and microscopic worlds, there are many well-recognised examples of thigmotropism. Roots of plants grow around stones and obstacles or along the crevices of rocks, ivy infiltrates the wall facing of houses and grows along drain pipes and seaweeds penetrate suitable holdfasts on the sea floor to maintain their position in the tide. At the cellular level, pollen tubes enter the stigma and head straight through the style to locate and fertilise the plant ovaries, and nerve cells execute complex orientation responses according to the tissues through which they are growing and generate complex neural networks that somehow work together to generate the bewildering complexity of the brain. The hyphae of endophytes trace the perimeters of the plant cells they associate with (Fig. 2.1d), and mating gametes of fungi grow directly towards each other and, with exquisite precision, fuse precisely at their tips to facilitate karyogamy. All these orientation responses require growth to be orchestrated and directed.

The best evidence for thigmotropism facilitating fungal pathogenesis comes from work on a range of plant pathogenic species. On the outer surfaces of plants, some fungi trace the junctions between plant cortical cells, while others grow across the junctions at right angles (Fig. 2.1a, b). Good examples of the former behaviour come from fungi growing on dicotyledenous plants where the cells are arranged as a mosaic, while examples of growth perpendicular to plant epithelial cells are exhibited by some monocot pathogens (Fig. 2.1). In both cases, this behaviour seems to be adapted to facilitate the searching out of guard cells, which are often the natural infection site targets. In monocots, the guard cells are usually in staggered rows; therefore, crossing the cells at right angles will maximise the chance of a guard cell encounter. If the guard cells are scattered between a mosaic of cells, a more effective strategy is to follow the peripheries of the cortical cells. In some fungi, the guard cell lip may then trigger thigmo-differentiation of the appressorium (Fig. 2.1c) (Allen et al. 1991; Collins and Read 1997; Read et al. 1992) (see later).

Human fungal pathogens also exhibit hypha thigmotropism (Fig. 2.2) (Gow 1993, 2004; Gow et al. 1994; Perera et al. 1997), although it has not yet been established whether this is a *bone fide* virulence attribute. It has been shown that non-thigmotropic mutants (described below) are less good at causing tissue damage (Brand et al. 2008), but such mutations are normally pleiotropic and this tropism phenotype cannot, therefore, be uniquely attributed to alterations in the tropic behaviour of the fungus (Davies et al. 1999). However, we can speculate that the ability to grow between the cells of a tissue or within the strata of a cornified

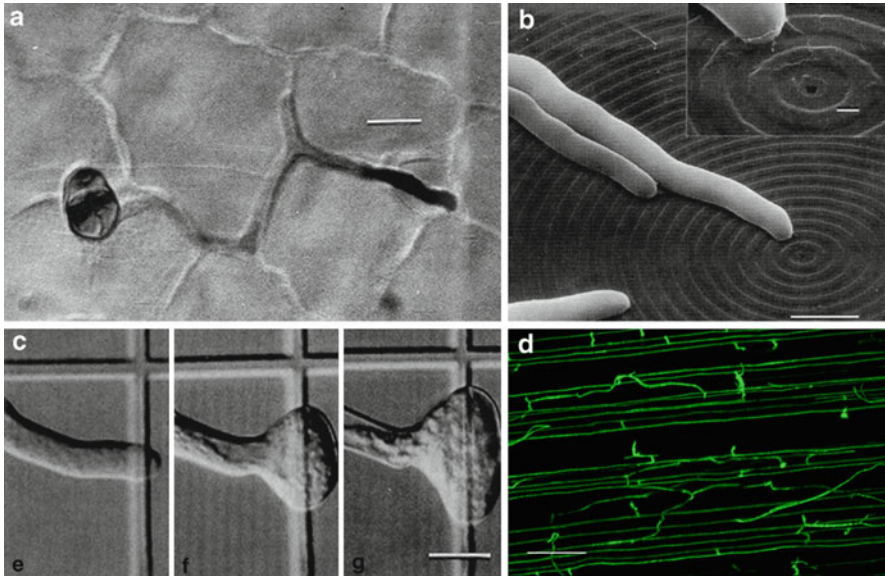


Fig. 2.1 Thigmotropism and thigmo-differentiation of plant-associated fungi. Fungi that penetrate the host leaf via stomata have evolved host-specific hyphal growth strategies to locate and recognise stomatal guard cells. (a) A hypha of the fungus *Cymodothea trifolii* follows the depressions surrounding adjoining epidermal cells of the host plant *Trifolium repens* (white clover) in order to locate a stoma, on which an appressorium is formed (Roderick 1993) (Bar = 10 μm). (b) Hyphae of *Uromyces appendiculatus* (bean rust fungus) cross cortical cells of the leaf surface at right angles to maximise the likelihood of locating a stoma, which are arranged in staggered rows in its host. This tropism appears to be elicited entirely through mechanical contact sensing because it can be replicated by growth on an inert microfabricated surface, resulting in the targeting of the bullseye of a surface consisting of concentric circles (Hoch et al. 1993, C IEEE). (c) Thigmo-differentiation – the formation of appressoria by *U. appendiculatus* grown on a polystyrene substrate can be triggered on contact with 0.5- μm -high ridges that precisely mimic the height of guard cells of the host plant (Kwon and Hoch 1990) (Bar = 11.8 μm). (d) Hyphae of *Epichloë* endophytes, here shown tagged with fluorescent green protein, extend along the longitudinal axis of the host leaf. Hyphal extension precisely matches that of the leaf. It is thought that, through their tight association with the intercellular space, hyphae are subjected to mechanical stretching as the host cells expand, and this stress is mitigated by the onset of intercalary growth (Christensen et al. 2008) (Bar = 100 μm) (image courtesy of C. Voisey)

epithelium may confer an advantage on such pathogens (Perera et al. 1997; Hutton et al. 1978; Kumamoto and Vines 2005). It is also clear that *Candida albicans* hyphae sometimes directly enter human cells, sometimes move between them and sometimes induce their own phagocytosis. Therefore, thigmotropism should be regarded as an adjunct penetration mechanism, and not the only one. It is also quite clear that non-pathogenic fungi also exhibit strong thigmotropic responses. It may be that similar advantages are conferred if hyphae of a saprophyte or symbiont can sense the dead or living cells or tissues on which they are living (Kumamoto 2008; Brand and Gow 2009; Christensen et al. 2008).

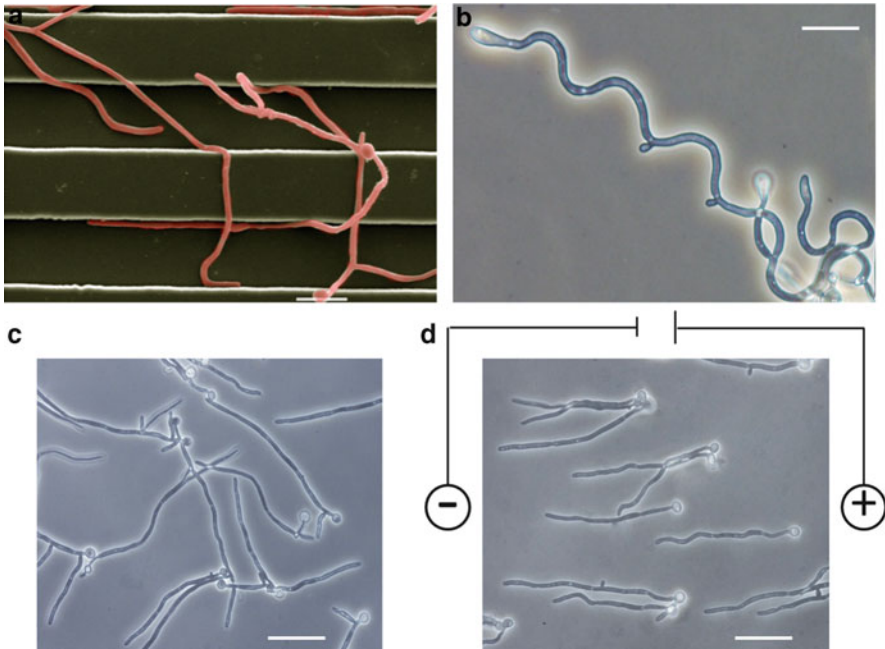


Fig. 2.2 Tropic growth of the human fungal pathogen *Candida albicans*. (a) Hyphae adhered to a microfabricated quartz slide (etched with ridges of $3.25\ \mu\text{m}$) follow the contours of the substrate by re-orienting their tip growth ($\text{Bar} = 15\ \mu\text{m}$) (image courtesy A Brand and K Mackenzie). (b) When *C. albicans* is grown on a semi-solid surface with poor nutrients, hyphae form regular sinusoidal waves, with a strong tendency of septa to form at apices of the curves (Brand et al. 2008) ($\text{Bar} = 12\ \mu\text{m}$) (image courtesy A Brand and K Lee). (c) The random direction of growth by hyphae germinated after adhesion to a glass slide can be strikingly overridden by the application of a DC electric field prior to germination (d), which causes growth to be cathode oriented (Crombie et al. 1990; Brand et al. 2007) ($\text{Bars} = 25\ \mu\text{m}$) (images courtesy A Brand)

2.2 Mechanism of Growth and Tropic Orientation

Considering how fundamental cellular orientation is in nature, there is really rather little information available about how this is achieved and regulated. The mechanism is assumed to be part of the machinery that is involved in cell growth and extension. But is there in addition a subset of proteins and processes that operate in parallel to those controlling growth? To create a metaphor, is there a separate molecular steering wheel that is separately regulated and distinct from the engine that drives the cell tip forward during growth? In this chapter, we consider this question in the context of thigmotropic orientations and in turn other tropic mechanisms that are exhibited by fungal hyphae. Fungi are excellent models to address such fundamental issues. Their hyphae often grow rapidly and their trajectories can be readily mapped and measured. They respond to a wide range of environmental cues by reorienting growth and can often be manipulated

physiologically and genetically, and mutants that have altered growth and orientation behaviours can be studied.

Thigmotropism can be broken down into a number of component steps that can be considered separately or as part of an integral mechanism. To respond to the undulation of a surface, the surface must first be sensed directly. This requires intimate contact to be made with the surface, and so adhesion mechanisms are a component of the information chain that results in tropic growth. Once the surface is bound and sensed, the vectorial information that is defined by the surface contours must be translated into a signal that articulates with the cell biological processes that bring about polarised apical growth. This implies that orientation mechanisms involve a very large number of proteins with signalling, scaffolding and mechanical properties so that cell polarity establishment and maintenance are regulated in the context of ambient environmental cues. The cellular apparatus for tip growth in fungi is in itself an enormous field involving the microfilament and microtubular cytoskeleton and their motor proteins, the secretory pathway that provides vesicles for membrane expansion at the tip, enzymes to catalyse apical cell wall growth, and regulatory proteins such as those found in the polarisome, Arp2/3 and exocyst protein complexes (Virag and Harris 2006; Steinberg 2007; Sudbery and Court 2007; Machesky and Gould 1999; Lipschutz and Mostov 2007). In filamentous fungi, the apex has an assemblage of vesicles within a structure called the Spitzenkörper, whose dynamic properties and position are critical in defining the growth axis of the cell. In yeast, the growth axis is determined by cell-cycle regulated cortical markers that form adjacent to septin rings. They are regulated by a complex genetic circuit involving *BUD* and other genes that encode a developmental programme that determines where the site of outgrowth will occur (Casamayor and Snyder 2002; Fischer et al. 2008). The mechanism of cell orientation is, therefore, governed in part by endogenous cues, such as the cortical proteins, whilst remaining responsive to exogenous ones that have the potential to override these endogenous cues.

Therefore, the biology of cell tropic behaviour is a highly integrative field comprising aspects of cell biology, cytoskeletal function, secretion, polarity, the cell cycle and cell wall growth. A number of general reviews on the aspects of these various component fields have been suggested, but here we will focus on the studies that deal most specifically and directly with hyphal orientation and responses to topography in the context of fungal pathogenesis.

2.2.1 Requirement for Initial Adhesion

For the fungal spore or cell, the naked host surface, such as the leaf of a plant or epithelial mucosa, is an unstable and even hostile environment. On plants, vibration and the shearing effect of abrasion, water flow and raindrop splash are immediate threats to successful colonisation. In the mammalian host, mechanical abrasion, the sloughing off of keratinised surface epithelial cells, blood shear flow and attack by

components of the innate immune system threaten survival of the fungus. The fungal strategy is, therefore, to transfer as quickly as possible from the hostile host surface into a safer environment. This involves differentiation to a morphology specialised for penetration of the underlying host tissue (leaf and root cuticles, or endothelial cell layers), intercalation between keratinised cells or establishment of a protective biofilm layer on mucosal or plastic surfaces. The primary function of the infective fungal particle is, therefore, to remain adhered to the host long enough for this process to take place.

Surprisingly, little is known about the molecules that mediate the initial adhesion of fungi to the host, although some general concepts are well understood. Dispersive forces and non-specific interactions such as electrostatic or hydrophobic attraction provide avidity rather than affinity, and it is likely that they play a greater role in the infection of plants than mammals. Although topographically variable, plant leaves offer a relatively uniform surface of epicuticular waxes, and plant pathogens are thought to adhere via surface hydrophobic rodlets. These amphiphilic molecules not only act during spore dissemination and initial adhesion, but are also subsequently shed onto the leaf surface to mediate adhesion of the developing appressorium (Wösten et al. 1994; Talbot et al. 1993). In the complex mammalian environment, heterogeneity in surface molecules appears to be the key to adhesion success in *C. albicans*. Unlike *A. fumigatus*, which primarily infects the lung, *C. albicans* adheres to multiple host sites, including hydrophobic in-dwelling medical plastic devices, and to itself through flocculation in biofilms. We and others have shown that *C. albicans* yeast consistently binds more avidly to collagen IV, found in the kidney and the epithelial and endothelial basal lamina, than to the general extracellular matrix protein collagen I, suggesting a degree of binding specificity by the *C. albicans* yeast cell surface (Yan et al. 1998). Initial adhesion of yeast cells to extracellular matrix proteins was proposed to be mediated by as-yet unidentified promiscuous fungal receptors in a calcium-sensitive manner, where charge interactions are important (Klotz et al. 1993). Charge is strongly influenced by pH, which differs dramatically by host body site, ranging from pH 1–4 in the gut, to pH 4.2 in the vaginal mucosa and pH 7.4 in the mouth. *C. albicans* expresses a family of 8 *ALS* (Agglutinin-Like Sequence) genes that encode proteins with hypervariable N-termini. Together they present a range of physico-chemical properties that are involved in non-specific adhesion – hydrophobicity, electrostatic charge and hydrogen-bonding interactions. Some *ALS* genes are expressed specifically in hyphae, e.g. *ALS3*, and so are not involved in the initial adhesion of yeast cells, but when expressed heterologously in *Saccharomyces cerevisiae*, distinct adhesion profiles were identified for individual Als proteins, suggesting that this gene family contributes significantly to the predicted overall heterogeneity of surface adhesive molecules in *C. albicans* (Hoyer and Hecht 2001; Sheppard et al. 2004; Hoyer et al. 2008; Liu and Filler 2011). In addition, the amyloid-like properties of the Als proteins could allow them to increase binding avidity through the formation of adhesive plaques (Alsteens et al. 2010). Experiments using Als5 showed the plaques were initiated through the mechanical stretching of individual surface molecules. Stretching of the protein fibril unfolds the N-terminal β -sheet

domains, exposing hydrophobic patches that recruit by-stander Als5 molecules to form self-propagating adhesive patches. The process is independent of cell viability, but it is conceivable that such dramatic surface changes in living cells could initiate a signal to the cell interior to indicate that adhesion had occurred.

2.2.2 Initial Adhesion and Mechanosensing

Cell attachment to a surface triggers a “differentiate to survive” response in microorganisms. The energetic cost of this transition is high, so a requirement for the integration of multiple signals ensures that this irreversible step is taken only when conditions are favourable. For example, in the bacterium *Vibrio parahaemolyticus*, adhesion is sensed by the steric hindrance to rotation of the polar flagellum by the proximity of the surface, but the switch to swarming growth also requires a nutrient signal (i.e. a lack of iron) (Gode-Potratz et al. 2011). Surface hardness is a key inducer of differentiation in many plant pathogens and even transient contact induces *Magnaporthe grisea* conidia to produce appressoria (Liu et al. 2007). How do fungi sense contact through rigid cell walls? Unlike mammalian cells, there is no evidence in fungi that the sensing of adhesion is receptor mediated via *trans*-wall fungal molecular signalling. Instead, sensing is probably mediated by perturbation of the cell wall and the consequences of this on the stretch of the underlying cell membrane. The cell wall counters a high internal turgor pressure (0.17–0.24 MPa) in the mammalian pathogen, *Pythium insidiosum* (Ravishankar et al. 2001), but it is not known whether the weight of the spore leads to wall deformation. By extrapolation from studies carried out with plant cells, tangential shear forces generated within the wall–plasma membrane–cytoskeleton linkages may be sensed by the adhered spore when it is jostled relative to the attachment surface. Shear forces are sensed in plant cells by such a mechanism and result in an immediate increase in cytosolic Ca^{2+} via plasma membrane Ca^{2+} channels (Pickard 1992; Ding and Pickard 1993). Hechtian strands, thin linkages composed of plasma membrane, actin and integrin-like peptides, have been observed at attachment points at the internal face of the cell wall in plasmolysed plant cells and the hyphal oomycete, *Saprolegnia ferax*, and it has been suggested that mechanosensing Ca^{2+} channels are clustered at these focal points to co-ordinate the response to perturbations in cell shape (Lang et al. 2004; Volgger et al. 2010; Jaffe et al. 2002; Kaminskyj and Heath 1995). To our knowledge, Hechtian strands have not been visualised in fungi, but two proteins have been identified in *C. albicans* that are predicted to be involved in conveying information from the wall to the membrane. Dfi1 is a small transmembrane protein that extends into the cell wall, where it is cross-linked to the primary cell wall structural polymer, β -glucan. Deletion of Dfi1 renders the cell sensitive to cell wall stresses such as treatment with Congo Red (Zucchi et al. 2010). *C. albicans* also contains an orthologue of Wsc1, the cell wall integrity sensor first identified in *S. cerevisiae* (Verna et al. 1997). The distal portion of ScWsc1 protrudes from the membrane into the cell wall, and molecular

engineering experiments have shown that it displays the properties of a nanospring (Dupres et al. 2009). CaWsc1 lies upstream in an orthologous cell wall integrity signalling pathway which is activated through the phosphorylation of the kinase CaMkc1 when cells are in contact with a semi-solid medium or plastic (Kumamoto 2005). Thus, adhesion seems to be sensed as cell wall perturbation, but cell responses are likely to also require integration of this signal with others from the external environment.

2.2.3 Initial Adhesion and Physico-Chemical Interactions

The requirement for additional signals for spore differentiation has been primarily established during the study of plant pathogens, but the characteristics of the inductive surfaces identified are only understood at the macro level. Many plant pathogens require a hard, hydrophobic surface and limited nutrients to activate differentiation (Warwar and Dickman 1996; Apoga et al. 2004; Shaw et al. 2006). Even though inert hard surfaces such as plastic can induce differentiation in *Uromyces appendiculatus* and *M. grisea*, the precise interplay between the fungus and an inert surface may nevertheless involve complex physico-chemical signals that operate at the nano-environmental level. Differentiation of these spores requires that the cells come into contact with moisture. At the cell surface, the “unstirred” aqueous diffusion boundary layer can extend from the cell surface by tens of micrometres. Within this zone, fungal surface molecules and effluxed ions are likely to create a localised chemical signature that could itself subsequently feedback to mediate cell behaviour, as is observed in mammalian cells (Smith et al. 2010). At the molecular level, some infective fungal particles are pre-coated with contact-activated molecular signals. Spores of the plant pathogens *Uromyces viclaid-fabae* and *Blumeria graminis* carry esterases, cutinases and lipases on their surfaces. On contact with the host plant, the enzymes from *U. viclaid-fabae* form an adhesive pad within seconds, and in both species, released enzyme activity generates specific breakdown products from the long-chain waxes of the host leaf cuticle. The chemical signal generated subsequently activates differentiation (Deising et al. 1992; Feng et al. 2009). Even inert hydrophobic surfaces could generate a chemical signal. It has been proposed that contact with a hydrophobic surface allows the diffusion of a surface-borne inhibitor, pyriculol, away from conidiospores of *M. grisea*, thereby relieving inhibition of differentiation (Hegde and Kolatukudy 1997). This strategy of priming the spore in a host-specific manner, therefore, serves three functions – surface adhesion, host recognition, and cell differentiation activation. A similar system may be employed by the human pathogen *C. albicans*, which features cell surface-bound proteases that operate at a variety of pH optima, giving the fungus a potential means to identify which human surface it has become adhered to by interpreting the peptide signature that is generated (Schild et al. 2011).

2.2.4 Requirement for Polarised Growth

The infective particles, spores and yeasts of most “professional” pathogenic fungi generate specialised polarised morphologies such as hyphae, germ tubes, appressorial penetration pegs and branched haustoria, which are designed to achieve directional mobility and to penetrate the host interior. In *U. appendiculatus*, *U. maydis*, *M. griseae* and oomycetes such as *P. infestans*, a polarised germ tube of approximately 20 μm emerges from the spore prior to the formation of the dome-like appressorium. In *C. albicans*, hyphae evaginate from the infecting yeast cell. Regulated directional growth is apparent in these morphologies. In hyphae and germ tubes, initial emergence is observed to be planar, while haustoria and the appressorial penetration peg form at an interface with the plant, indicating that surface sensing elicits a pre-programmed directional growth response in these pathogens.

A feature of differentiation in *C. albicans* is a change in the profile of surface adhesion molecules. The complement of wall adhesins changes to include, for example, *Hyphal Wall Protein 1* (Hwp1), which is a substrate for host transglutaminases (Staab et al. 1999). Where the requirement during initial adhesion is to remain attached until cell differentiation could occur, successful host penetration demands an adhesive force that enables the fungus to apply sufficient pressure to penetrate and infiltrate host tissue. Adhesion is just one part of the equation. In invasive hyphae, hydrolytic enzymes produced at the tip, and even the shape of the tip itself, aid direct penetration of the host cell membrane, thereby lowering the critical level of adhesive force required to anchor the hypha. In appressoria, penetration is achieved by generating a turgor pressure of 8 MPa (80 bar) to power the penetration peg through the leaf cuticle (Howard et al. 1991). The pressure required to penetrate the host must be adequately countered by the adhesive force so that the appressorium does not merely push itself away from the leaf. In *M. griseae*, the adhesive force has been estimated at a minimum of 500 J/m^2 , i.e. in the value region for sticky tape rather than superglue (Goriely and Tabor 2006; Gent and Kaang 1986). Treatments that can tip the balance between host-specific fungal avidity and the mechanical resistance offered by the host might be an effective method to reduce infection, particularly by plant pathogens.

2.2.5 Directionality in Polarity Establishment

Directional growth requires the establishment of a single growth site to which vesicles and new wall material are delivered. In *S. cerevisiae* and *C. albicans* yeast, polarity is established and maintained by the small GTPase Cdc42 module. This module is essential in these two fungi and is highly conserved in all eukaryotes, but can be substituted by other related small GTPases in some plant pathogens (Harris and Momany 2004). The site at which Cdc42 is activated is

determined in *S. cerevisiae* by cortical landmark proteins that are pre-anchored within the plasma membrane adjacent to previous bud sites (Casamayor and Snyder 2002). In *C. albicans* hyphae, Cdc42-GFP localises to the hyphal apex and immediate sub-apical region, but its activation is limited to the positioning of its guanine exchange factor (GEF), Cdc24, which in turn is localised by another small GTPase, Rsr1/Bud1 (Fig. 2.3a). Thus, it is the positioning of Rsr1 that determines the site at which polarised growth is established. Deletion of Rsr1 or its GTPase-activating protein Bud2 causes erratic wandering of the polarisome complex, which maintains

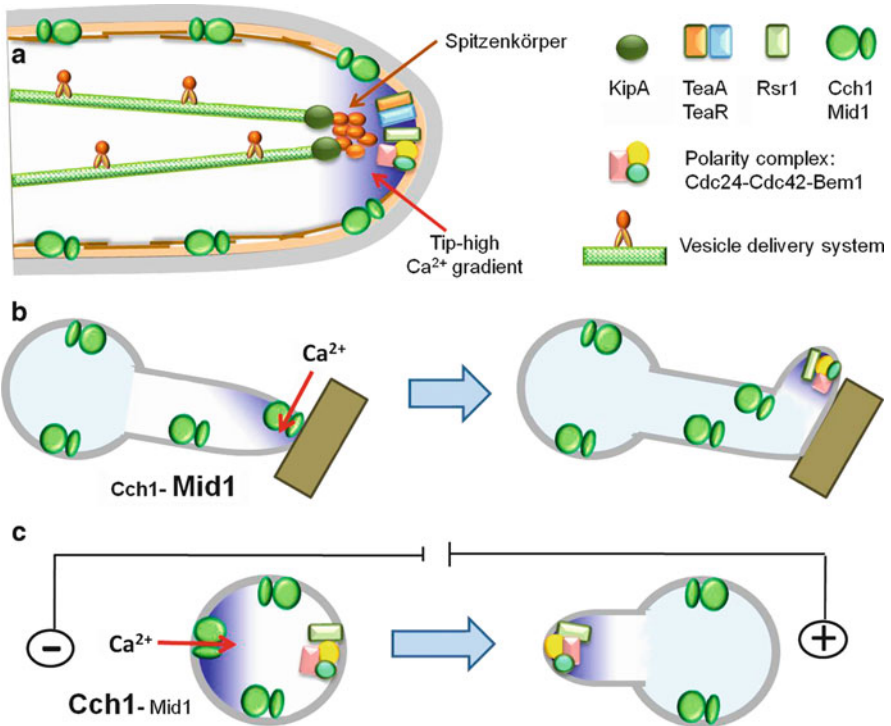


Fig. 2.3 Model for tip growth and re-orientation of hyphae. (a) Fungal hyphae share conserved molecular complexes that drive polarised growth. These include a vesicle delivery system, a vesicle supply centre (Spitzenkörper), plasma membrane calcium channels and a tip-high calcium gradient. A system of cell-end markers and small GTPases marks the site of growth and directs delivery of the necessary materials (Brand and Gow 2009). (b) Model for thigmotropic response in *C. albicans*. Hyphal tips that contact an obstacle undergo wall and plasma membrane deformation and stress. This is sensed by the stretch-activated calcium channel regulator Mid1, causing localised calcium influx via Cch1. The asymmetry of the calcium gradient results in repositioning of the active cell polarity Cdc24–Cdc42–Bem1 complex and the establishment of a new growth axis. (c) Model for the galvanotropic response in *C. albicans*. In an applied electric field, the anodal face of the yeast cell becomes hyperpolarised, while the cathodal face becomes depolarised. Membrane depolarisation activates the L-type voltage-gated calcium channel Cch1, which permits entry of calcium at the cathodal side of the cell. The presence of a localised calcium gradient overrides the signal generated by other internal polarity markers, and hyphae subsequently emerge and grow towards the cathode

polarised growth, leading to hyphal evagination at random sites on the cell surface (Hausauer et al. 2005). In *C. albicans*, the site of hyphal emergence from the mother yeast cell is reported as 50% random (lateral) and 50% determined by the position of the previous bud site (Herrero et al. 1999), which allows environmental cues to play a role in hyphal directionality. The early positional markers and molecular organisers of cell polarity have been studied in depth in *S. cerevisiae* and are generally conserved in *C. albicans* but not well characterised in filamentous fungi. However, directionality of germ tube emergence is particularly important in fungal conidia and appressoria that are heavily melanized, where wall strength is an impedence for the emergence of new growth. Hyphal germination in *Podospora anserina*, a pathogen that does not form an appressorium, occurs through a specific pore in the melanized ascospore (Lambou et al. 2008). Similarly, the penetration peg emerges from appressoria within an area defined by a heavily melanized ring that surrounds a polysaccharide bilayer (Wolkow et al. 1983; Bourett and Howard 1992). The sites of these emergence zones are probably laid down during wall biosynthesis prior to melanisation. How this spatial regulation is achieved in *P. anserina* ascospores is unknown, but in *de novo* appressoria its position is likely to be determined by detection of the host surface. Yeast-like cortical landmark proteins (BUDs) are either lacking or are poorly conserved in these fungi, and alternative mechanisms that mark the site of polarity establishment remain to be identified. The Cdc42 module, essential in *C. albicans* for organising the cytoskeleton and polarised vesicle transport, is not essential for growth in *M. grisea*, *P. marneffei*, *U. maydis* or *W. dermatitidis* (Bassilana et al. 2003; Zheng et al. 2006; Boyce et al. 2001; Ye and Szanislo 2000). In *M. grisea*, *Colletotrichum lindemuthianum* and *Botrytis cinerea*, a role for a tetraspannin-like protein has been suggested in the establishment of polarised outgrowth. According to a model proposed by Harris and Momany, positional signals in filamentous fungi could be conveyed by membrane receptors that recognise the host plant (Harris and Momany 2004). Tetraspannins generally function to cluster membrane proteins, and mutants (*pls1*⁻) were observed to have “abortive and mislocalised” penetration pegs, suggesting that Pls1 may be important for the organisation of positional signalling complexes or cell-end markers (Clergeot et al. 2001; Gourgues et al. 2004; Veneault-Fourrey et al. 2006). In *C. lagenarium*, deletion of the orthologue of the *Schizosaccharomyces pombe* cell-end marker, Tea1, resulted in abnormal appressoria that could not penetrate cellulose, but the mutant phenotype was rescued by growth on the host plant or in the presence of calcium ions. Thus, the all-important emergence of a penetration peg into the host plant provides a clear example of a contact sensing-regulated directional growth response.

2.3 Thigmotropic Sensing

The host specificity of fungal thigmotropic responses has best been characterised in the rust fungus *U. appendiculatus*, which undergoes thigmo-differentiation on contact with the stomatal ridge of the host leaf (Hoch et al. 1987). The response

ensures that the appressorium forms at the correct host penetration site and could be elicited *in vitro* by ridges in an inert plastic surface (Fig. 2.1c). It was concluded that the fungus could detect the precise height of the inductive host feature (5 μm) and that the process was purely mechanical. The mechanisms whereby hyphae sense perturbations in the substratum are as yet unknown, but there is some evidence that sensing could occur through mechanosensing (MS) Ca^{2+} -permeable channels. Such channels have been identified in *U. appendiculatus* and the hyphae of *C. albicans*, which also displays thigmotropic turning *in vitro* (Zhou et al. 1991; Watts et al. 1998). Similar to *U. appendiculatus*, *C. albicans* hyphae (2 μm diameter) respond to low ridges (0.8 μm high) in the substratum, which cause them to re-orient and grow along the ridge. An orthologue of the *S. cerevisiae* Ca^{2+} -permeable MS channel, Mid1, has been identified in *C. albicans*. Its deletion or that of the large L-type voltage-gated channel it putatively regulates, CaCch1; another putative Ca^{2+} channel, CaFig1; or the Crz1 transcription factor that regulates expression of Cch1 reduces *C. albicans* hypha re-orientation by approximately 50% (Brand et al. 2007; Karababa et al. 2006). Thigmotropism was also attenuated in the presence of a Ca^{2+} chelator, gadolinium or verapamil, blockers of stretch-activated and L-type channels, respectively, but not on deletion of CaYvc1, which releases Ca^{2+} from intracellular stores (Brand, unpublished). Abnormally high intracellular Ca^{2+} also abolishes sinusoidal growth, another contact-dependent response of wild-type hyphae in *C. albicans* (Fig. 2.1b). Hyphae and branches of the *pmr1* Δ mutant, which cannot pump Ca^{2+} into the Golgi, grew as remarkably straight rods instead of developing regular oscillating waves on low-nutrient, high-concentration agar (Bates et al. 2005; Brand et al. 2009). Thus, normal Ca^{2+} flux and signalling are involved in hyphal tip directionality in *C. albicans*. The current model for contact sensing in hyphae is, therefore, one whereby wall deformation causes perturbation of the membrane, which is sensed by stretch-activated Ca^{2+} channels. Localised Ca^{2+} influx could then act as a signal to influence the polarity machinery, but the pathway between the two has not been characterised (Fig. 2.3c) (Brand et al. 2007).

Nevertheless, by compromising the function of components of the Cdc42 polarity complex, several of which are essential in *C. albicans*, some interesting cell polarity and tropism phenotypes have emerged. On deletion of the cell polarity Ras-like GTPase, CaRsr1/Bud1 or its GAP, Bud2, polarised growth was established and maintained, but the hyphal trajectory became increasingly erratic and the polarisome (visualised using Spa2-YFP) moved randomly within the tip (Hausauer et al. 2005). Rsr1 GTP-GDP cycling is, therefore, required to anchor the polarisome stably within the apex, and without this linkage, hyphae were completely unable to respond to any of the known external tropism cues. The mutants were also attenuated in their ability to penetrate and damage cells in a model of oral epithelial infection, suggesting that normal tip directionality could be important for tissue invasion (Brand et al. 2008). Conversely, in mutants where the loss of GTPase activity was in the Rho-like GTPase Cdc42, the thigmotropic response was reduced, although hyphal trajectories, which meandered slightly as normal, were maintained. This suggests that GTP-GDP cycling may be required to

“unlock” the position of the Cdc42 module so that it can relocate within the apex in response to external cues (Brand, unpublished).

In obligately filamentous fungi, microtubules play a prominent role alongside actin cables in vesicle transport and polarity maintenance. Correct association of microtubules with the hyphal apex is mediated by a cell-end marker, a cortical receptor and a kinesin (TeaA, TeaR and KipA in *A. nidulans*, respectively) (reviewed by Fischer et al. 2008) (Fig. 2.3a). The involvement of the cell marker system in the control of tip orientation is evidenced by the zig-zag trajectories of the deletion mutants. How the balance of power is regulated or co-ordinated between the microtubule- and actin-based polarity systems in hypha tip directionality is not known.

Work by Bowen et al. suggests that the point of contact between the obstacle and the growth zone in the hyphal tip influences the directional response. Experiments where hyphae were grown on ridges with shallow inclines led to the conclusion that the apical growth zone in hyphal tips, estimated to describe an arc of approximately 60°, was insensitive to touch because cross-linking between the immature cell wall, plasma membrane and cytoskeleton was incomplete (Bowen et al. 2007). One could speculate that the zone of tip sensitivity might, therefore, lie sub-apically to the growth zone but forward of the zone of endocytosis where polarity effectors are internalised for recycling to the tip. This concept could explain the observations of *C. albicans* thigmotropism in vitro. When *C. albicans* hyphae are tightly adhered to the substratum, tips re-orient on contact with ridges of 0.8 µm, less than half the diameter of a hypha (2 µm). Moreover, tip re-orientation increases with reducing ridge height (Brand et al. 2007). A refinement of this experiment with a wider range of ridge heights could help to determine the region of sensitivity in the hyphal tip. In contrast to tightly adhered cells, in in vitro assays, the undulating growth of *C. albicans* hyphae seen in infection models allows the tip to approach host cells orthogonally. Instead of deflecting on contact with the host cell membrane, the hyphal growth trajectory is maintained, resulting in host cell penetration (Dalle et al. 2010) and supporting the view that the hyphal growth zone is insensitive to touch.

2.4 Modulation of the Thigmotropic Response

In light of in vitro investigations, numerous and complex factors that could influence a pathogen’s ability to sense and respond to contact have been identified. Nutrient availability, temperature and other environmental factors affect hyphal diameter, growth trajectory, turgor pressure, tip shape, cell wall elasticity and the expression of surface adhesins (Brand et al. 2009; Bowen et al. 2007, Ravishankar et al. 2001; Money and Harold 1992; Bastidas et al. 2009). The thigmotropic sensitivity of *Aspergillus niger* increased in low nutrient conditions due to a change in hyphal tip shape, which became more closely apposed to the substratum (Bowen et al. 2007). In *C. albicans*, low nutrient availability and surface hardness caused wavy and undulating growth, away from contact and the confines of the substratum

(Brand et al. 2009, V. Veses, personal communication). In *P. insidiosum*, a temperature increase from 24 to 37°C caused hyphae to increase their diameter and exert a greater force on skin, but although the hyphal diameter remained the same, when the cells were grown in serum, the effect of temperature was reversed (Ravishankar et al. 2001). Thus, pathogenic behaviour and success are the products of genetic interactions with a number of environmental variables. The role of thigmotropism in fungal infections within the human host will, therefore, be difficult to examine, but the different orientation responses observed in *C. albicans* hyphae could reflect niche-dependent tip regulation. Re-orientation in response to small obstacles might be important in biofilm formation or during superficial mucosal infection, where hyphae intercalate between keratinised cell layers. In contrast, in blood stream infections, tissue penetration and escape from internalisation by macrophages require that hyphae do not deflect on contact.

2.5 Lessons from Other Tropic Responses

2.5.1 Galvanotropism

Galvanotropism is the directional growth response of cells to an imposed D.C. electrical field. A wide range of cell types respond tropically or tactically to exogenous electrical fields, and most eukaryotic cells and tissue generate electrical fields (Gow 1987, 1989, 1994; Bowling et al. 1986). Fungi grow directionally in electrical fields in the order of $>2\text{--}5$ V/cm, but zoospores of oomycetes swim towards the positive (anode) or negative (cathode) poles of much weaker fields. In the case of the latter, this is of interest since plant roots and other tissue generate sufficiently large electrical fields to orient the trajectory of the swimming cells to specific sites on the plant surface (Miller and Gow 1989; Morris et al. 1992; van West et al. 2002). In the case of fungal hyphae, the galvanotropism response is not sufficiently sensitive to believe that they respond to the relatively weak electrical fields generated by plant or animal tissues which they may inhabit.

There are several possible consequences of cells experiencing an external electrical field. The field may influence the behaviour, activity or mobility of *trans*-membrane proteins (Gow et al. 1984; Gow 1989). At the cathode-facing end of a cell, the internally negative membrane potential will become depolarised, while at the anode end of the cell, the membrane will be hyperpolarised. Membrane depolarisation can lead to triggering of action potentials by voltage-sensing proteins and channels (Gow 1987, 2004). Imposed electrical fields can also lead to membrane electrophoresis or electro-osmosis – the counter-flow of water due to the displacement of counter-ions around the charged groups of proteins within the field.

The application of electric fields is an useful experimental system for inducing marked and uniform hyphal alignments. Such experiments can assess what environmental conditions influence the ability of a hypha to respond to an imposed

tropic stimulus and to test whether a specific mutation influences the galvanotropic response (Crombie et al. 1990; McGillivray and Gow 1986, 1987). In an electric field (10 V/cm), germ tube emergence of *C. albicans* hyphae is 70–80% cathodal and can be further increased by the presence of extracellular Ca^{2+} in a dose-dependent manner (Crombie et al. 1990; Brand et al. 2007). The model generated from these experiments postulates that the cathodal face of the yeast cell is depolarised by the electric field, activating the voltage-gated calcium channel CaCch1. The resulting calcium influx overrides the existing positional information within the cell and indicates a new growth site (Fig. 2.3c). Such experiments have demonstrated that certain aspects of the regulation of the galvanotropic response are shared with those required for thigmotropism. For example, both tropic responses are attenuated in media of very low calcium ion concentration (Lever et al. 1994; Brand et al. 2007) and in both cases, Rsr1, a Ras-like GTPase component of the bud-site selection mechanism, is required for tropic orientation (Brand et al. 2008). These findings suggest that the underlying mechanism that is responsible for tropic orientation contains elements that are shared and are common for a wide range of tropic responses. However, it is interesting to note that deletion of the gene encoding the voltage-sensitive calcium ion channel Cch1 strongly attenuated galvanotropism, but had less of an effect on thigmotropism. Reciprocally, the membrane stretch sensor Mid1, which is thought to modulate the properties of Cch1, is critical for thigmotropism, but not for galvanotropism (Brand et al. 2007). These experiments all point to the importance of calcium regulation in the control of tropic orientation responses.

It has also been shown that the galvanotropic response of *Neurospora crassa* is strongly dependent on the pH of the medium – and indeed the response has an isoelectric point where there is no galvanotropism (Lever et al. 1994). This suggests that the pH may affect the mobility of key polarity-determining proteins embedded in the cell membrane, which in turn can be moved laterally in an electrical field, resulting in turn in directionality of hypha growth. Putting these phenomena together, it can be speculated that electrical fields induce tropic responses by influencing both the distribution of and ionic fluxes through calcium ion pumps and calcium channels in the cell membrane. Many aspects of cellular physiology resulting in tip growth are calcium dependent. For example, actin polymerisation and depolymerisation are both Ca^{2+} dependent. Galvanotropic mechanisms often implicate actin organisation as the ultimate target of the changes induced by electrical field exposure (Gow 1994). However, it should also be pointed out that electrical fields can also result in the orientation of cell division plane of bacteria (Rajnicek et al. 1994), and although bacteria are now known to have some elements that are orthologues of eukaryotic cytoskeletal proteins, they do not have actin-dependent cell growth. A second actin-dependent cell biological process is that of vesicle fusion with the cell membrane. It is, therefore, feasible that these orientation mechanisms induce gradients and asymmetries in calcium ions that result in preferential vesicle fusion events and the spatial organisation of actin assembly and disassembly – and hence, directed cell extension and tropic growth.

2.5.2 *Aerotropism and Chemotropism to Nutrients and Pheromones*

Mycelia of filamentous fungi form well-spaced, usually non-overlapping hyphae, and individual hyphae often exhibit obvious negative autotropisms (Aoki et al. 1998). The environmental cues that explain these tropic responses have not been established – but they have been hypothesised to be due to either positive aerotropism towards regions of high oxygen tension or a form of negative chemotropism away from secreted fungal staling products. However, the evidence for *bone fide* chemotropism to soluble nutrients such as glucose and amino acids in most classes of fungi is not strong (Gooday 1975; Jansson et al. 1988). It is quite easy to use point sources of solutes at the ends of filled capillary tubes to reveal chemotaxis of bacteria, oomycete zoospores and chemotropic tropic alignments of zygomycete rhizoids, such as for the fungus *Allomyces macrognus* or *Blastocladiella emersonii* (Youatt et al. 1988). In contrast, chemotropism of ascomycete and basidiomycete hyphae has been very difficult to demonstrate (Gooday 1975). The notable exception to this is the very strong and noticeable tropic response seen to sex pheromones of gametes of all the major classes of fungi (Gooday and Adams 1993). Sometimes such encounters occur in complex environments with competing environmental tropic cues. It is possible that the formation of biofilms stabilises such matrices and facilitates chemotropic orientations (Daniels et al. 2006). It is not known as yet if such sexual orientations are also subject to the tropic regulation mechanisms underpinning thigmotropic and galvanotropic orientation.

2.6 Models and Speculations

The studies summarised above suggest a number of common elements and themes emerging from studies of fungal tropic growth responses. Calcium ion flux across the apical cell membrane of the hypha mediated by Mid1 and Cch1 influences a number of tropic responses including thigmotropism and galvanotropism. Proteins that regulate calcium transport, such as Fig. 1 in *C. albicans*, are also implicated in the growth of mating shmoos (Yang et al. 2011), which undergo chemotropism in response to sex pheromone. Elements within the RAC/Rho GTPase signalling pathways such as Rsr1/Bud1 and Cdc42 that influence bud-site selection in yeast are also clearly involved in tropic growth, as are components of the actin-based and microtubule-based cytoskeleton (e.g. Tea1). Connecting these observations is the possibility that standing gradients of calcium ion gradients in the apical dome of the hypha could modulate the activities of components of the cortical polarity establishment and maintenance apparatus, which in turn regulates the site of vesicle secretion and cytoskeleton function. Both these latter processes are strongly influenced by Ca^{2+} , and some of the upstream signalling proteins contain calcium-binding motifs, such as EF hands, and it remains to be tested whether these

domains are required for the integrity of the tropism signalling mechanism to be maintained. This model has certain attractions in so far as it would account for the ability of hyphal cells to respond to both exogenous (nutrients, pheromones, obstacles and electric fields) and endogenous signals (cortical markers for budding, germ tube evagination sites and septal junctions). What seems clear from the analysis of some of the mutants listed above is that orientation mechanisms can be dissociated from core growth mechanisms. Therefore, it seems that hyphae do have a cellular steering wheel and a range of sensors that enable them to explore, respond to and exploit their environments and to refine strategies for pathogenesis efficiently.

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References

- Allen EA, Hoch HC, Stavely JR, Steadman JR (1991) Uniformity among races of *Uromyces appendiculatus* in response to topographical signalling for appressorium formation. *Phytopathology* 81:883–887
- Alsteens D, Garcia MC, Lipke PN, Dufresne YF (2010) Force-induced formation and propagation of adhesion nanodomains in living fungal cells. *Proc Natl Acad Sci USA* 107:20744–20749
- Aoki A, Ito-Kuwa S, Nakamura K, Vidotta V, Takeo K (1998) Oxygen as a possible tropic factor in hyphal growth of *Candida albicans*. *Mycoscience* 39:231–238
- Apoga D, Barnard J, Craighead HG, Hoch HC (2004) Quantification of substratum contact required for initiation of *Colletotrichum graminicola* appressoria. *Fungal Genet Biol* 41:1–12
- Bassilana M, Blyth J, Arkowitz RA (2003) Cdc24, the GDP-GTP exchange factor for Cdc42, is required for invasive hyphal growth of *Candida albicans*. *Eukaryot Cell* 2:9–18
- Bastidas RJ, Heitman J, Cardenas ME (2009) The protein kinase Tor1 regulates adhesin gene expression in *Candida albicans*. *PLoS Pathog* 5:e1000294
- Bates S, MacCallum DM, Bertram G, Munro CA, Hughes HB, Buurman ET, Brown AJP, Odds FC, Gow NAR (2005) *Candida albicans* Pmr1p, a secretory pathway P-type $\text{Ca}^{2+}/\text{Mn}^{2+}$ -ATPase, is required for glycosylation and virulence. *J Biol Chem* 280:23408–23415
- Bourett TM, Howard RJ (1992) Actin in penetration pegs of the fungal rice blast pathogen, *Magnaporthe grisea*. *Protoplasma* 168:20–26
- Bowen AD, Davidson FA, Keatch R, Gadd GM (2007) Induction of contour sensing in *Aspergillus niger* by stress and its relevance to fungal growth mechanics and hyphal tip structure. *Fungal Genet Biol* 44:484–491
- Bowling DFJ, Edwards MC, Gow NA, Bowling DFJ, Edwards MC, Gow NAR (1986) Electrical currents at the leaf surface of *Commelina communis* and their relationships to stomatal activity. *J Exp Bot* 179:876–882
- Boyce KJ, Hynes MJ, Adrianopoulos A (2001) The CDC42 homolog of the dimorphic fungus *Penicillium marneffeii* is required for correct cell polarization during growth but not development. *J Bacteriol* 183:3447–3457
- Brand A, Gow NAR (2009) Mechanisms of hypha orientation of fungi. *Curr Opin Microbiol* 12:1–8
- Brand A, Shanks S, Duncan VMS, Yang M, Mackenzie K, Gow NAR (2007) Hyphal orientation of *Candida albicans* is regulated by a calcium-dependent mechanism. *Curr Biol* 17:347–352

- Brand A, Vacharaksa A, Bendel C, Norton J, Haynes P, Henry-Stanley M, Wells C, Ross K, Gow NAR, Gale CA (2008) An internal polarity landmark is important for externally induced hyphal behaviours in *Candida albicans*. *Eukaryot Cell* 7:712–720
- Brand A, Lee K, Veses B, Gow NAR (2009) Calcium homeostasis is required for contact-dependent helical and sinusoidal tip growth in *Candida albicans* hyphae. *Mol Microbiol* 71:1155–1164
- Casamayor A, Snyder M (2002) Bud-site selection and cell polarity in budding yeast. *Curr Opin Microbiol* 5:179–186
- Christensen MJ, Bennett RJ, Ansari HA, Koga H, Johnson RD, Bryan GT, Simposon WR, Koolarard JP, Nickless EM, Voisey CR (2008) Epichloe endophytes grow by intercalary hyphal extension in elongating grass leaves. *Fungal Genet Biol* 45:84–93
- Clergeot PH, Gourgues M, Cots J, Laurans F, Latorse MP, Pepin R, Tharreau D, Notteghem JL, Lebrun MH (2001) PLS1, a gene encoding a tetraspanin-like protein, is required for penetration of rice leaf by the fungal pathogen *Magnaporthe grisea*. *Proc Natl Acad Sci USA* 98:6963–6968
- Collins TJ, Read ND (1997) Appressorium induction by topographical signals from six cereal rusts. *Physiol Mol Plant Pathol* 51:169–179
- Crombie T, Gow NAR, Gooday GW (1990) Influence of applied electrical fields on yeast and hyphal growth of *Candida albicans*. *J Gen Microbiol* 136:311–317
- Dalle F, Wächtler B, L'Ollivier C, Holland G, Bannert N, Wilson D, Labruère C, Bonnin A, Hube B (2010) Cellular interactions of *Candida albicans* with human oral epithelial cells and enterocytes. *Cell Microbiol* 12:248–271
- Daniels KJ, Srikantha T, Lockhart SR, Pujol C, Soll DR (2006) Opaque cells signal white cells to form biofilms in *Candida albicans*. *EMBO J* 25:2240–2252
- Davies JM, Stacey AJ, Gilligan CA (1999) *Candida albicans* hyphal invasion: thigmotropism or chemotropism? *FEMS Microbiol Lett* 171:245–249
- Deising H, Nicholson RL, Haug M, Howard RJ, Mendgen K (1992) Adhesion pad formation and the involvement of cutinase and esterases in the attachment of uredospores to the host cuticle. *Plant Cell* 4:1101–1111
- Ding JP, Pickard BG (1993) Mechanosensory calcium-selective cation channels in epidermal cells. *Plant J* 3:83–110
- Dupres V, Alsteens D, Wilk S, Hansen B, Heinisch JJ, Dufrene YF (2009) The yeast Wsc1 cell surface sensor behaves like a nanospring in vivo. *Nat Chem Biol* 5:857–862
- Feng J, Wang F, Liu G, Greenshields D, Shen W, Kaminskyj S, Hughes GR, Peng Y, Selvaraj G, Zou J, Wei Y (2009) Analysis of a *Blumeria graminis*-secreted lipase reveals the importance of host epicuticular wax components for fungal adhesion and development. *Mol Plant Microbe Interact* 22:1601–1610
- Fischer R, Zekert N, Takeshita N (2008) Polarized growth in fungi – interplay between the cytoskeleton, positional markers and membrane domains. *Mol Microbiol* 68:813–826
- Gent AN, Kaang S (1986) Pull-off forces for adhesive tapes. *J Appl Polym Sci* 32:4689–4700
- Gode-Potratz CJ, Kustusch RJ, Breheny PJ, Weiss DS, McCarter LL (2011) Surface sensing in *Vibrio parahaemolyticus* triggers a programme of gene expression that promotes colonization and virulence. *Mol Microbiol* 79:240–263
- Gooday GW (1975) Chemotaxis and chemotropism in fungi and algae. In: Carlile MJ (ed) *Primitive sensory and communication systems*. Academic, London, pp 155–204
- Gooday GW, Adams DJ (1993) Sex hormones and fungi. *Adv Microb Physiol* 34:69–145
- Goriely A, Tabor M (2006) Estimates of biomechanical forces in *Magnaporthe grisea*. *Mycol Res* 110:755–759
- Gourgues M, Brunet-Simon A, Lebrun MH, Levis C (2004) The tetraspanin BcPls1 is required for appressorium-mediated penetration of *Botrytis cinerea* into host plant leaves. *Mol Microbiol* 51:619–629

- Gow NAR (1987) Polarity and branching induced by electrical fields. In: Poole RK, Trinci APJ (eds) Spatial organisation in eukaryotic microbes. Special publications of the society for general microbiology, vol 23. IRL Press, Oxford, pp 25–41
- Gow NAR (1989) The circulating ionic currents of microorganisms. *Adv Microb Physiol* 30:89–123
- Gow NAR (1993) Non-chemical signals used for host location and invasion by fungal pathogens. *Trends Microbiol* 1:45–50
- Gow NAR (1994) Growth and guidance of the hyphal apex. *Microbiology* 140:3193–3205, Fleming Lecture
- Gow NAR (2004) New angles in mycology: studies in directional growth and directional motility. *Mycol Res* 108:5–13
- Gow NAR, Kropf DL, Harold FM (1984) Growing hyphae of *Achlya bisexualis* generate a longitudinal pH gradient in the surrounding medium. *J Gen Microbiol* 130:2967–2974
- Gow NAR, Perera THS, Sherwood-Higham J, Gooday GW, Gregory DW, Marshall D (1994) Investigation of touch-sensitive responses by hyphae of the human pathogenic fungus *Candida albicans*. *Scanning Microsc* 8:705–710
- Harris SD, Momany M (2004) Polarity in filamentous fungi: moving beyond the yeast paradigm. *Fungal Genet Biol* 41:391–400
- Hausauer DL, Gerami-Nejad M, Kistler-Anderson C, Gale CA (2005) Hyphal guidance and invasive growth in *Candida albicans* require the Ras-Like GTPase Rsr1p and its GTPase-activating protein Bud2p. *Eukaryot Cell* 4:1273–1286
- Hedge Y, Kollatukudy PE (1997) Cuticular waxes relieve self-inhibition of germination and appressorium formation by conidia of *Magnaporthe grisea*. *Physiol Mol Plant Pathol* 51:75–84
- Herrero AB, Lopez MC, Fernandez-Lago L, Dominguez A (1999) *Candida albicans* and *Yarrowia lipolytica* as alternative models for analysing budding patterns and germ tube formation in dimorphic fungi. *Microbiology* 145:2727–2737
- Hoch HC, Bojko RJ, Comeau GL, Allen EA (1993) Integrating microfabrication and biology. *Circuits and Devices* 9:16–22
- Hoch H, Staples R, Whitehead B, Comeau J, Wolf E (1987) Signalling for growth orientation and cell differentiation by surface topography in *Uromyces*. *Science* 235:1659–1662
- Howard RJ, Ferrari MA, Roach DH, Money NP (1991) Penetration of hard substrates by a fungus employing enormous turgor pressures. *Proc Acad Sci USA* 88:11281–11284
- Hoyer LL, Hecht JE (2001) The ALS5 gene of *Candida albicans* and analysis of the Als5p N-terminal domain. *Yeast* 18:49–60
- Hoyer LL, Green CB, Oh SH, Zhao X (2008) Discovering the secrets of the *Candida albicans* agglutinin-like sequence (ALS) gene family – a sticky pursuit. *Med Mycol* 46:1–15
- Hutton RD, Kerbs S, Yee K (1978) Scanning electron microscopy of experimental *Trichophyton mentagrophytes* infections in guinea pig skin. *Infect Immun* 21:247–253
- Jaffe MJ, Leopold AC, Staples RC (2002) Thigmo responses in plants and fungi. *Am J Bot* 89:375–382
- Jansson H-B, Johansson T, Nordbring-Herts B, Tunlid A, Odham G (1988) Chemotropic growth of germ tubes of *Cochliobolus sativus* to barley roots or root exudates. *Trans Br Mycol Soc* 90:647–650
- Kaminskyj SG, Heath IB (1995) Integrin and spectrin homologues, and cytoplasm-wall adhesion in tip growth. *J Cell Sci* 108:849–856
- Karababa M, Valentino E, Pardini G, Coste AT, Bille J, Sanglard D (2006) CRZ1, a target of the calcineurin pathway in *Candida albicans*. *Mol Microbiol* 59:1429–1451
- Klotz SA, Rutten MJ, Smith RL, Babcock SR, Cunningham MD (1993) Adherence of *Candida albicans* to immobilized extracellular matrix proteins is mediated by calcium-dependent surface glycoproteins. *Microb Pathog* 14:133–147
- Kumamoto CA (2005) A contact-activated kinase signals *Candida albicans* invasive growth and biofilm development. *Proc Natl Acad Sci USA* 102:5576–5581

- Kumamoto CA (2008) Molecular mechanisms of mechanosensing and their roles in fungal contact sensing. *Nat Rev Microbiol* 6:667–673
- Kumamoto CA, Vences MD (2005) Alternative *Candida albicans* lifestyles: growth on surfaces. *Annu Rev Microbiol* 59:113–133
- Kwon YH, Hoch HC (1990) Temporal and spatial dynamics of appressorium formation in *Uromyces appendiculatus*. *Exp Mycol* 15:116–131
- Lambou K, Malagnac F, Barbisan C, Tharreau D, Lebrun MH, Silar P (2008) The crucial role of the Pls1 tetraspanin during ascospore germination in *Podospora anserina* provides an example of the convergent evolution of morphogenetic processes in fungal plant pathogens and saprobes. *Eukaryot Cell* 7:1809–1818
- Lang I, Barton DA, Overall RL (2004) Membrane – wall attachments in plasmolysed plant cells. *Protoplasma* 224:231–243
- Lever M, Robertson B, Buchan ADB, Gooday GW, Gow NAR (1994) pH and Ca²⁺ dependent galvanotropism of filamentous fungi: implications and mechanisms. *Mycol Res* 98:301–306
- Lipschutz J, Mostov K (2007) Exocytosis: the many masters of the exocyst. *Curr Biol* 1:212–214
- Liu Y, Filler SG (2011) *Candida albicans* Als3, a multifunctional adhesin and invasin. *Eukaryot Cell* 10:168–173
- Liu H, Suresh A, Willard FS, Siderovski DP, Lu S, Naqvi NI (2007) Rgs1 regulates multiple G α subunits in *Magnaporthe* pathogenesis, asexual growth and thigmotropism. *EMBO J* 26:690–700
- Machesky LM, Gould KL (1999) The Arp2/3 complex: a multifunctional actin organizer. *Curr Opin Cell Biol* 11:117–121
- McGillivray AM, Gow NAR (1986) Applied electrical fields polarize the growth of mycelial fungi. *J Gen Microbiol* 132:2515–2525
- McGillivray AM, Gow NAR (1987) The transhyphal electrical current of *Neurospora crassa* is carried principally by protons. *J Gen Microbiol* 133:2875–2881
- Miller AL, Gow NAR (1989) Correlation between profile of ion-current circulation and root development. *Physiol Plant* 75:102–108
- Money NP, Harold FM (1992) Extension growth of the water mold *Achlya*: interplay of turgor and wall strength. *Proc Natl Acad Sci USA* 89:4245–4249
- Morris BM, Reid B, Gow NAR (1992) Electrotaxis of zoospores of *Phytophthora palmivora* at physiologically relevant field strengths. *Plant Cell Environ* 15:645–653
- Perera THS, Gregory DW, Marshall D, Gow NAR (1997) Contact sensing in hyphae of dermatophytic and saprophytic fungi. *J Med Vet Mycol* 35:289–294
- Pickard BG (1992) Wall to membrane linkers, stretch activated channels, and the detection of tension, voltage, temperature, auxin, and pH. *ASGSB Bull* 6:31
- Rajnicek AM, McCaig CD, Gow NAR (1994) Electric fields induce curved growth of *Enterobacter cloacae*, *Escherichia coli* and *Bacillus subtilis* cells: implications for mechanisms of galvanotropism and bacterial growth. *J Bacteriol* 176:702–713
- Ravishankar JP, Davis CM, Davis DJ, MacDonald E, Makselan SD, Millward L, Money NP (2001) Mechanics of solid tissue invasion by the mammalian pathogen *Pythium insidiosum*. *Fungal Genet Biol* 34:167–175
- Read ND, Kellock LK, Knight H, Trewavas AJ (1992) Contact sensing during infection by fungal pathogens. In: Callow JA, Green JR (eds) *Perspectives in plant cell recognition*, vol 48. Cambridge University Press, Cambridge, pp 137–172
- Roderick HW (1993) The infection of white clover (*Trifolium repens*) by conidia of *Cymadothea trifolii*. *Mycol Res* 97:227–232
- Schild L, Heyken A, de Groot PWJ, Hiller E, Mock M, de Koster C, Horn U, Rupp S, Hube B (2011) Proteolytic cleavage of covalently linked cell wall proteins by *Candida albicans* Sap9 and Sap10. *Eukaryot Cell* 10:98–109
- Shaw BD, Carroll GC, Hoch HC (2006) Generality of the prerequisite of conidium attachment to a hydrophobic substratum as a signal for germination among *Phyllosticta* species. *Mycologia* 98:186–194

- Sheppard DC, Yeaman MR, Welch WH, Phan QT, Fu Y, Ibrahim AS, Filler SG, Zhang M, Waring AJ, Edwards JE Jr (2004) Functional and structural diversity in the Als protein family of *Candida albicans*. *J Biol Chem* 279:30480–30489
- Smith PJS, Collis LP, Messerli MA (2010) Windows to cell function and dysfunction: signatures written in the boundary layers. *Bioessays* 32:514–523
- Staab JF, Bradway SD, Fidel PL, Sundstrom P (1999) Adhesive and mammalian transglutaminase substrate properties of *Candida albicans* Hwp1. *Science* 283:1535–1538
- Steinberg G (2007) Hyphal growth: a tale of motors, lipids, and the Spitzenkörper. *Eukaryot Cell* 6:351–360
- Sudbery P, Court H (2007) Polarised growth in fungi. In: Howard RJ, Gow NAR (eds) *The Mycota VIII*, 2nd edn. Springer, Berlin, pp 137–166
- Talbot NJ, Ebbole DJ, Hamer JE (1993) Identification and characterization of *MPG1*, a gene involved in pathogenicity from the rice blast fungus *Magnaporthe grisea*. *Plant Cell* 5:1575–1590
- van West P, Morris BM, Reid B, Appiah AA, Osborne MC, Campbell TA, Shepherd SJ, Gow NAR (2002) Plant pathogens use electric fields to target plant roots. *Mol Plant Microbe Interact* 15:790–798
- Veneault-Fourrey C, Lambou K, Lebrun MH (2006) Fungal Pls1 tetraspanins as key factors of penetration into host plants: a role in re-establishing polarized growth in the appressorium? *FEMS Microbiol Lett* 256:179–184
- Verna J, Lodder A, Lee K, Vagts A, Ballester R (1997) A family of genes required for maintenance of cell wall integrity and for the stress response in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* 94:13804–13809
- Virag A, Harris SD (2006) The Spitzenkörper: a molecular perspective. *Mycol Res* 110:4–13
- Volgger M, Lang I, Ove-ika M, Lichtscheidl I (2010) Plasmolysis and cell wall deposition in wheat root hairs under osmotic stress. *Protoplasma* 243:51–62
- Warwar V, Dickman M (1996) Effects of calcium and calmodulin on spore germination and appressorium development in *Colletotrichum trifolii*. *Appl Microbiol Environ* 62:74–79
- Watts H, Very A-A, Perera THS, Davies J, Gow NAR (1998) Thigmotropism and stretch-activated channels in the pathogenic fungus *Candida albicans*. *Microbiology* 144:689–695
- Wolkow PM, Sisler HD, Vigil EL (1983) Effect of inhibitors of melanin biosynthesis on structure and function of appressoria of *Colletotrichum lindemuthianum*. *Physiol Plant Pathol* 23:55–71
- Wösten HA, Schuren FH, Wessels JG (1994) Interfacial assembly of a hydrophobin into an amphipathic protein membrane mediates fungal attachment to hydrophobic surfaces. *EMBO J* 13:5848–5854
- Yan S, Rodrigues RG, Cahn-Hidalgo D, Walsh TJ, Roberts DD (1998) Hemoglobin induces binding of several extracellular matrix proteins to *Candida albicans*. *J Biol Chem* 273:5638–5644
- Yang M, Brand A, Srikantha T, Daniels K, Soll DR, Gow NAR (2011) Fig1 facilitates calcium influx and localises to membranes destined to undergo fusion during mating in *Candida albicans*. *Eukaryot Cell* 10:435–444
- Ye X, Szaniszló PJ (2000) Expression of a constitutively active Cdc42 homologue promotes development of sclerotial bodies but represses hyphal growth in the zoopathogenic fungus *Wangiella (Exophiala) dermatitidis*. *J Bacteriol* 182:4941–4950
- Youatt J, Gow NAR, Gooday GW (1988) Bioelectric and biosynthetic aspects of cell polarity in *Allomyces macrogynus*. *Protoplasma* 146:118–126
- Zheng W, Zhao Z, Chen J, Liu W, Ke H, Zhou J, Lu G, Darvill AG, Albersheim P, Wu S, Wang Z (2006) A Cdc42 ortholog is required for penetration and virulence of *Magnaporthe grisea*. *Fungal Genet Biol* 46:450–460
- Zhou XL, Stumpf MA, Hoch HC, Kung C (1991) A mechanosensitive channel in whole cells and in membrane patches of the fungus *Uromyces*. *Science* 253:1415–1417
- Zucchi PC, Davis TR, Kumamoto CA (2010) A *Candida albicans* cell wall-linked protein promotes invasive filamentation into semi-solid medium. *Mol Microbiol* 76:733–748



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