Review

Reproduction without sex: conidiation in the filamentous fungus *Trichoderma*Johanna M. Steyaert,¹ Richard J. Weld,² Artemio Mendoza-Mendoza¹ and Alison Stewart¹

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Trichoderma spp. have served as models for asexual reproduction in filamentous fungi for over 50 years. Physical stimuli, such as light exposure and mechanical injury to the mycelium, trigger conidiation; however, conidiogenesis itself is a holistic response determined by the cell's metabolic state, as influenced by the environment and endogenous biological rhythms. Key environmental parameters are the carbon and nitrogen status and the C:N ratio, the ambient pH and the level of calcium ions. Recent advances in our understanding of the molecular biology of this fungus have revealed a conserved mechanism of environmental perception through the White Collar orthologues BLR-1 and BLR-2. Also implicated in the molecular regulation are the PacC pathways and the conidial regulator VELVET. Signal transduction cascades which link environmental signals to physiological outputs have also been revealed.

Introduction

Fungi reproduce both sexually and asexually, producing a vast array of structures which have evolved over time to suit habitat and in some cases host. These structures are of great economic importance to society. Approximately 48 % of the world's food crop yield is lost due to plant diseases, of which the majority are caused by fungi (Agrios, 2005). For most fungal diseases, the primary sources of inoculum are sexual and/or asexual spores. As well as economic losses, fungi can have positive economic benefits for agriculture, such as biocontrol of plant diseases. Numerous fungi have been successfully developed as biocontrol agents (BCAs) of plant diseases and the majority of these are sold as spore preparations (Chernin & Chet, 2002). The global fungal BCA market is dominated by species of the ubiquitous ascomycete *Trichoderma* (Harman *et al.*, 2004). In general, commercial preparations of *Trichoderma* spp. for biological control consist of bulk-produced conidia (asexual spores), whereas good biocontrol activity in the environment relies upon the fungus remaining vegetative, and thus antagonistically active. The ideal *Trichoderma* BCA produces ample conidia in a cost-effective manner during production and maintains long periods of vigorous vegetative growth during usage. Understanding the factors that control this morphogenic switch from mycelia to conidia is integral to biocontrol research. Over 50 years of studies on conidiation in the genus have established *Trichoderma* as a model for asexual reproduction in fungi. In this review, we will present what is known about the physiological responses of *Trichoderma* to the environmental cues that induce conidiation, and then provide

insights into the molecular basis of these responses, including an examination of the signal transduction pathways which link environmental signals to physiological outputs.

Environmental cues which influence *Trichoderma* conidiation

The transition from fungal mycelium to spore is determined by the interplay of environmental cues, whereby one factor alone is not necessarily sufficient to evoke change; rather, it is the combination of factors which determines the outcome. Known cues influencing conidiation in *Trichoderma* include light, endogenous rhythms, C:N status, the ambient pH of the medium, extracellular calcium, physical injury to the mycelium and the presence of fungal-derived volatile organic compounds.

Light

Exposure to light is an important component of commercial production of *Trichoderma* conidia; however, surprisingly, there is little evidence of optimization of this morphogenic cue. A typical light regime during production is alternating cycles of 12 h light/12 h dark, which is based on the assumption that this reflects the natural environment; however, in temperate climate zones this is only 'natural' for above-ground fungi at the equinoxes. Conidiogenesis proceeds in a series of five stages following a discreet burst of light (Fig. 1) (Betina & Zajacová, 1978a, b; Gressel & Galun, 1967; Horwitz *et al.*, 1990). Though reported to occur within 24 h of light exposure, conidial maturation has been observed to take up to 5 days

Fig. 1. Conidiogenesis in *Trichoderma* following perception of different stimuli. (a) Appearance of vertical hyphae, (b) hyphal branching, (c) development of phialides, (d) appearance of hyaline conidia, (e) maturation of conidial pigmentation.

depending on the species and media composition (Steyaert *et al.*, 2010b, c). Physiological and molecular responses to light by species of *Trichoderma* have recently been reviewed (Schmoll *et al.*, 2010).

In constant light, conidiation occurs continuously across the fungal colony, whereas under alternating light–dark conditions, concentric rings of conidial formation can be seen in cultures, and this is a characteristic feature of all *Trichoderma* spp. Gutter (1957) put forward the theory of competency, whereby only cells of a specific age are responsive to light, as an explanation for the production of conidiation rings. Using *Trichoderma viride* as a model system, Gutter (1957) demonstrated that a minimal hyphal age of approximately 10 h is necessary for photoconidiation (light-induced conidiation), and that dark-grown mycelium older than 20 h is no longer responsive to the light stimulus. It was assumed that under constant light conditions, all cells become competent when they pass through the responsive metabolic phase during development and growth.

Studies on nucleic acid synthesis during photoinduction have linked hyphal cell competency to the metabolic rate of the cell rather than to hyphal age per se. Using labelled precursors, Gressel & Galun (1967) tested for increased nucleic acid synthesis associated with photoconidiation. At 5 h post light induction, there was a massive increase in nucleic acid synthesis in the conidial ring area when compared with the dark-grown controls, and this increased until 8 h. The addition of RNA synthesis inhibitors at 3, 5 and 7 h inhibited conidiation, but had little or no effect on growth. RNA synthesis inhibitors added after 7 h had no effect on conidiation. Protein synthesis inhibitors have also been shown to inhibit conidiation (Betina & Zajacová, 1978b). Together, these results clearly demonstrate that *de novo* transcription and translation are required during photoconidiation from at least 3 h and up to 7 h post light exposure. The authors concluded that the ability to conidiate in response to light is related to the metabolic rate of the hyphal cell rather than to the size of the colony

or the hyphal cell age. This conclusion supports the findings of Galun (1971), who demonstrated that competency in *T. viride* decreases from the colony margin inward.

More recently, it has been suggested that competency to photoconidiate is dependent on the metabolic state of the hyphal cell, rather than metabolic rate and age (Steyaert *et al.*, 2010b, c). Conidiation in response to a single light burst in *Trichoderma* spp. has been reported to be restricted to what was the colony perimeter at the time of light exposure in *T. viride* and *Trichoderma atroviride*, which supports the theory that the youngest, most metabolically active cells are competent (Betina & Farkaš, 1998; Casas-Flores *et al.*, 2004; Gutter, 1957). In contrast, Steyaert *et al.* (2010c) have observed a single ring at the perimeter, a disk of conidia, either encompassing the whole light-exposed colony or constrained to the centre, or no conidiation at all, in response to light, and this is dependent on the media composition and specific isolate used (Steyaert *et al.*, 2010b, c) (Fig. 2).

Induction of conidiation in response to light in *Trichoderma* spp. falls within the ‘blue-light effects’ in fungi. The most photoresponsive wavelengths (peaks) are within the near-UV

Fig. 2. Morphological patterns of photoconidiation of *T. atroviride*. PDB, potato dextrose broth.

(320–380 nm) and blue (380–500 nm) spectra (Betina & Farkaš, 1998; Gressel & Hartmann, 1968; Kumagai & Oda, 1969). A correlation exists between light intensity and exposure period, which determines the level of conidiation. This correlation is in accordance with the Bunsen–Roscoe reciprocity law of 1863, which states that light exposures of different durations and intensities which produce the same total energy dose will produce the same level of response (Gressel *et al.*, 1975; Gutter, 1957; Horwitz *et al.*, 1990). A light intensity of 25 % for 60 s duration resulted in the same conidial rate as 12.5 % intensity for 120 s in *T. viride* (Galun, 1971). Horwitz *et al.* (1990) validated the Bunsen–Roscoe reciprocity law in the nanosecond to minutes range in *T. atroviride* [the *Trichoderma harzianum* isolate used in that study has since been reidentified as *T. atroviride* (Kindermann *et al.*, 1998)]. In addition, they demonstrated no difference in the kinetics of the photoreponse at 3 °C compared with 26 °C, which suggests that no enzymic processes are involved. Their results suggest that the inductive event is a simple first-order photobleaching reaction. Photobleaching reactions can also occur in the absence of oxygen. Gressel *et al.* (1975) demonstrated that if oxygen is briefly removed from *T. viride* cultures and a pulse of light given, then conidiation will begin when cultures are transferred back to the air, whereas Gutter (1957) demonstrated that light-induced conidiation is inhibited in hyphal cells which develop in the absence of oxygen. Together, these studies show a clear differentiation between the initial photoreactions and the development of conidiation and suggest that oxidative processes are a requirement for the developmental step. This is supported by the observation that cultures which are exposed to light at low temperatures, then kept in the cold for an extended period of time, will conidiate upon return to normal incubation conditions (Gressel *et al.*, 1975).

Biological rhythms

In response to cyclical changes in light, temperature and other environmental influences, organisms have adapted to anticipate daily changes through the evolution of circadian rhythms (Lakin-Thomas & Brody, 2004). These rhythms have been well characterized in *Neurospora crassa*, which produces multiple bands of conidia in the dark in a circadian fashion following a single exposure to blue and/or UVA light (Dunlap *et al.*, 2007). Similar to *N. crassa*, *Trichoderma* spp. also conidiate in the dark following light exposure; however, this response is generally considered non-circadian and strictly light-inducible (Betina & Zajacová, 1978a; Schrüfer & Lysek, 1990). More recently, an endogenous, possibly circadian, conidiation rhythm has been described in *Trichoderma pleuroticola* (Steyaert *et al.*, 2010a). In dark-grown cultures of *T. pleuroticola*, multiple rings of conidia are produced at intervals of approximately 24 h, and although light does not induce the rings, a single light exposure at 48 h constrains and intensifies the associated band. Deitzer *et al.* (1988) observed an endogenous rhythm in light sensitivity in *T. atroviride*, as

measured by photo-induced conidiation, but no rhythmic conidiation was observed in the dark, and conidiation in rings was light-inducible only. These studies suggest differences in conidiation-associated endogenous rhythms between *T. pleuroticola* and *T. atroviride*; however, it is important to note that rhythmic conidiation is not observed under all conditions in *T. pleuroticola* and is likely dependent on the media composition (Steyaert *et al.*, 2010a, b, c). It is possible that rhythmic dark conidiation may also occur in other *Trichoderma* spp., including *T. atroviride*, but the appropriate conditions for this to be observed have yet to be identified.

Carbon and nitrogen status

Commercial production of conidia typically relies on manipulation of nutrients and substrates to promote conidiation, which has led to much research into the optimal growth conditions for *in vitro* conidiation in many species of *Trichoderma*. From the literature, it is clear that the carbon and nitrogen status and the C:N ratio, in addition to the ambient pH, are the main nutritional factors influencing conidiation in *Trichoderma* (Aube & Gagnon, 1969; Bastos, 2001; Brian & Hemming, 1950; Friedl *et al.*, 2008; Gao *et al.*, 2007; Jackson *et al.*, 1991; Kredics *et al.*, 2004; Lewis & Papavizas, 1983; Monga, 2001; Steyaert *et al.*, 2010b, c).

Despite a wealth of studies into the nutritional factors that govern conidiation, no single set of optimal parameters has been assigned to a particular species. Species-specific parameters may exist; however, inadequate taxonomic identification of *Trichoderma* isolates has made it difficult to interpret the studies in the context of true species. Few early studies of the influence of nutrition on conidiation in *Trichoderma* state that the isolates were identified by molecular methods; therefore, it is possible the identifications were incorrect. In older studies pre-dating Bissett (1991a, b, c) and, in particular, Rifai (1969), species identification was based on species-groups, and individual species within a group were not recognized. Further, studies in which molecular identifications are offered have focused on one isolate per species. The question of whether species-specific optimal conditions for conidiation exist remains largely unanswered.

The presence/absence of light within an experiment and the influence that this may have on the results is seldom considered (Schmoll *et al.*, 2005); however, it is becoming clear from the literature that there is an interactive effect between light and nutritional influences on conidiation. The source of carbon has been suggested to be the primary determinant for both conidiation in the dark and light-induced conidiation in *T. viride* and *T. atroviride* (Chovanec *et al.*, 2001; Friedl *et al.*, 2008). Chovanec *et al.* (2001) observed conidia in *T. viride* cultures grown on 30 out of 32 carbon sources, including polysaccharides, amino acids and alcohols. Conidiation rates varied depending on the carbon source, and the level of variation in dark-grown

cultures was comparable with that observed in response to a single light exposure. In contrast, conidiation is not observed in *T. atroviride* when amino acids or alcohols are the sole carbon source, and although light-induced conidiation correlates with conidiation in the dark, light appears to inhibit conidiation on D-arabinose and D-gluconic acid (Fiedl *et al.*, 2008). The *T. viride* study employed a single light exposure to dark-grown cultures to evaluate light-induced conidiation, whereas alternating light/dark conditions were used to evaluate the influence of light on conidiation in *T. atroviride*. The differential responses between these two isolates may, therefore, be a consequence of experimental design, or could simply reflect species (isolate)-specific parameters.

The nitrogen status of the environment has been demonstrated to cross-regulate photoconidiation (Ellison *et al.*, 1981; Steyaert *et al.*, 2010c). In the presence of preferred (primary) nitrogen sources, organisms repress expression of genes required for the utilization of secondary sources and this is referred to as nitrogen catabolite repression (NCR). Under nitrogen deprivation, or when primary sources are low and secondary sources are high, derepression occurs. Out of 40 *T. viride* soil isolates, half sporulated at the periphery and did not produce concentric rings when grown on potato dextrose agar (PDA) under natural light, whereas repetition of the same experiments on richer media resulted in concentric circles (Ellison *et al.*, 1981). By supplementing PDA with single nutrients present in the richer media, these authors demonstrated that addition of primary forms of nitrogen can convert a peripheral sporulator to a concentric one and concluded that the uptake of primary nitrogen in a cell must be sufficient to allow simultaneous growth and conidiation. In contrast, Schröder & Lysek (1990) were unable to convert conidiation phenotypes in their *T. viride* isolates through increasing available nitrogen. It should be noted that no reliable identification of the *T. viride* isolates was provided in either study, and therefore it is possible that they were investigating different species. Indeed, Lieckfeldt *et al.* (1999) later revised this species and separated it into *T. viride* and *Trichoderma asperellum*.

Primary sources of nitrogen strongly promote photoconidiation in *T. asperellum*, *T. atroviride* and *T. pleuroticola*, but not *Trichoderma hamatum* or *Trichoderma virens*, suggesting the interactive effect to be species (isolate)-specific (Steyaert *et al.*, 2010c). When high amounts of glutamine or urea are present in the medium as the sole nitrogen source, *T. asperellum* produces a disk of conidia in response to a single light exposure, whereas a photoconidiation ring is produced from cultures grown in the presence of KNO_3 as the nitrogen source (Steyaert *et al.*, 2010c). Amino- and ammonium-based primary nitrogen sources are routinely used to induce nitrogen catabolite repression in filamentous fungi, and growth on KNO_3 or other nitrates strongly induces nitrogen derepression (Marzluf, 1997; ter Schure *et al.*, 2000). Further, when incremental amounts of glutamine are added to the PDA to

invoke nitrogen-repressing conditions, the conidial pattern changes from a ring to a disk. In the absence of sufficient nitrogen, fungal cells can assimilate nitrates by reduction to ammonia, which is then converted to glutamate or glutamine; thus, primary nitrogen metabolism can occur when nitrates are the sole nitrogen source (Marzluf, 1997; Wiame *et al.*, 1985). Hyphal cells store and translocate amino acids in a system which allows for the regulation of developmental genes by controlling the intracellular nitrogen status (Olsson, 1999; Watkinson, 1999). Under conditions of nitrogen repression in *T. asperellum*, both primary nitrogen scavenged from the environment and intracellular reserves generated through the reduction of nitrates can be translocated to the colony perimeter, thus maintaining NCR in the hyphal front, which in turn promotes photoconidiation in a ring.

The relative ratio between carbon and nitrogen has been shown to have a strong influence on conidiation and growth. Aube & Gagnon (1969) observed that conidiation decreases with increasing nitrogen levels, and Jackson *et al.* (1991) found that mycelial biomass increases significantly as the nitrogen levels decrease. This concept was investigated further by Gao *et al.* (2007), who demonstrated an interactive effect on conidiation in *T. viride* between the level of carbon and the C:N ratio. Alone or in combination with different amounts of nitrogen, 6 g l⁻¹ carbon (sucrose) produced the highest quantity of conidia. The rate of conidiation did not vary significantly as the C:N (soy peptone) ratio rose from 10:1 to 80:1; however, at 160:1 the rate of conidiation more than doubled compared with previous values. At 6 and 8 g carbon l⁻¹, mycelial growth was highest at a C:N ratio of 10:1, whereas at 12 g carbon l⁻¹, the optimum C:N ratio rose to 40:1. Higher amounts of nitrogen favoured mycelial growth, whereas nitrogen limitation favoured conidiation. In direct contrast, Steyaert *et al.* (2010c) observed that high amounts of primary nitrogen promote conidiation in *T. asperellum*, *T. atroviride* and *T. pleuroticola*. The differing observations may relate to the use of glucose as the main carbon source (Steyaert *et al.*, 2010c) compared with sucrose (Gao *et al.*, 2007), or could simply reflect species-specific differences.

Ambient pH

The initial pH of the medium has also been demonstrated to have an effect on conidiation, and unlike the C:N ratio, pH levels which favour conidiation have been shown to favour mycelial growth as well (Aube & Gagnon, 1969; Bastos, 2001; Brian & Hemming, 1950; Lewis & Papavizas, 1983; Steyaert *et al.*, 2010b). The pH optima vary among isolates from pH 4.0 to 6.8. While the initial pH of the medium has some influence on conidiation and growth, the *Trichoderma* culture itself alters the pH of the medium (Lewis & Papavizas, 1983; Steyaert *et al.*, 2010b, c). Changes in the medium pH are dependent on the nitrogen sources: with primary nitrogen, the pH decreases, whereas

alkalinization occurs when the medium is supplemented with secondary nitrogen or when primary nitrogen is limiting. In *T. atroviride*, *T. hamatum* and *T. pleuroticola*, photoconidiation on pH-buffered PDA has been shown to be strictly low pH-dependent, with maximum response values at pH 3.6, 4.0 and 4.4, respectively (Steyaert *et al.*, 2010b). One possible explanation for the disparity between buffered and unbuffered experiments is that conidiation may be dependent on intracellular acidification, which in turn is dependent on the ambient pH and the buffering state of the medium and influenced by light exposure. Low ambient pH of the growth medium has been demonstrated to result in intracellular acidification in *Aspergillus niger* and *Saccharomyces cerevisiae* (Caspani *et al.*, 1985; Gradisnik-Grapulic & Legisa, 1997). In *T. viride*, intracellular acidification occurs when hyphae are exposed to light (Gresík *et al.*, 1991); however, it is not known whether this response is constrained in a highly buffered environment. It is possible that on pH-buffered media the low ambient pH stimulates intracellular acidification and that light-induced acidification is constrained, whereas on the unbuffered medium light stimulates intracellular acidification, which brings the internal pH to below the threshold for photoconidiation. Based on the work of Steyaert *et al.* (2010b), the internal pH threshold would be around pH 4.0; however, this, as with many aspects of conidiation, is likely to be species-specific and further studies are required.

Calcium signalling

In eukaryotes, Ca^{2+} serves as a universal secondary messenger whose intracellular concentration is tightly regulated. Multiple Ca^{2+} protein targets have been identified in eukaryotes; these include calcium-calmodulin, protein kinases and calcineurin-like proteins. In fungi, calcium plays important roles during differentiation (Krystofová *et al.*, 1995; Roncal *et al.*, 1993; Silverman-Gavrila & Lew, 2003). In *T. viride*, extracellular Ca^{2+} induces conidiation in submerged cultures after 48 h of incubation (Simkovic *et al.*, 2008). The mechanism by which calcium induces conidiation in *T. viride* is not known; however, calcium has been demonstrated to induce conidiation independently of the nutritional state and light conditions, suggesting an alternative pathway for conidial formation in *Trichoderma* (Simkovic *et al.*, 2008).

Mycelial injury

Physical injury to the mycelium has been demonstrated to induce conidiation in multiple species of *Trichoderma* (Casas-Flores *et al.*, 2004; Steyaert *et al.*, 2010b, c). This was inadvertently discovered during a mutational study, in which key genes involved in mediating blue-light responses were knocked out in *T. atroviride* (Casas-Flores *et al.*, 2004). These mutants were unable to respond to light, but conidiated wherever the mutant cultures were injured by a scalpel during growth. The authors further investigated this

mechanism of induction in the wild-type and again observed conidiation in response to injury. Cultures were grown in total darkness and injured with a scalpel under safe red light.

Similar to photoconidiation, both the nitrogen status and the ambient pH of the environment have been demonstrated to have an interactive effect on mycelial injury-induced conidiation (Steyaert *et al.*, 2010b, c). Primary nitrogen promotes injury-induced conidiation in *T. asperellum*, *T. atroviride* and *T. pleuroticola*, and in *T. atroviride* and *T. hamatum*, injury-induced conidiation is strictly low pH-dependent on buffered media.

The exact mechanism by which injury to the mycelium stimulates conidiation in *Trichoderma* is not known; however, studies in other fungi strongly suggest the involvement of oxidative stress. Oxidative stress has been suggested to be involved in the differentiation of several fungi, including *Aspergillus nidulans*, *N. crassa*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Sclerotinia minor*, and in *Sclerotium rolfsii*, sclerotial biogenesis in response to mycelial injury occurs as a result of oxidative stress (Aguirre, *et al.*, 2005; Georgiou *et al.*, 2006; Papapostolou & Georgiou, 2010).

Volatile organic compounds (VOCs)

Microbial VOCs are the intermediate or end products of various microbial metabolic pathways and belong to a diversity of structural classes, such as alkenes, ketones, alcohols, esters, lactones, furanes and terpenes. Microorganisms produce VOCs either inadvertently as a result of normal metabolism or deliberately in order to synchronize intra-population responses, such as sporulation, or to impose an effect, such as competitor growth inhibition, vector attraction and host growth promotion, upon other organisms (Insam & Seewald, 2010; Lin & Phelan, 1992; Schöller *et al.*, 2002; Wheatley *et al.*, 1997).

As with many fungi, a diverse array of VOCs has been detected from cultures of *Trichoderma* (Fiedler *et al.*, 2001; Stoppacher *et al.*, 2010; Wheatley *et al.*, 1997). These compounds include the eight-carbon VOCs 1-octen-3-ol and its analogues, which are the end products of fatty acid metabolism (Schnürer *et al.*, 1999). These C8 compounds produced by *Trichoderma* and other fungi have been shown to stimulate conidiation in *Trichoderma* and likely provide a signalling system for synchronization of conidiation (Nemcovic *et al.*, 2008). The production of VOCs can itself be influenced by the exogenous presence of other secondary metabolites. For example, the production of *Trichoderma* 1-octen-3-ol is increased in the presence of the mycotoxin fusaric acid produced by *Fusarium moniliforme* (Stoppacher *et al.*, 2010), which gives some insight into the extent of inter-community communication in fungi.

The mechanism by which VOCs stimulate conidiation in *Trichoderma* is not known. The addition of 1-octen-3-ol to

Penicillium paneum conidia results in a slight permeabilization of the plasma membrane and significantly alters protein expression (Chitarra *et al.*, 2005). Changes in membrane permeability are known to accompany conidiation in *Trichoderma* but are not themselves sufficient to induce conidiation (Nemcovic *et al.*, 2008). It has been suggested, but not tested, that the specificity of the cell response to particular C8 VOCs implies the presence of specific receptors on the plasma membrane that could transduce the VOC signal into the conidiation regulation pathways (Nemcovic *et al.*, 2008).

Genetic regulation of conidiation

BLR-1 and BLR-2

Blue-light-induced conidiation in *T. atroviride* is mediated by the Blue Light Regulators BLR-1 and BLR-2 (Casas-Flores *et al.*, 2004; Castellanos *et al.*, 2010). *blr-1* and *blr-2* are orthologous to the well-characterized zinc finger transcription factors *wc-1* and *wc-2*. Both BLR and WC proteins contain conserved Per-ARNT-Sim (PAS) domains, which have been shown to be involved in signal transduction through monitoring of the energy state of the cell, in protein–protein interactions and in the sensing of environmental signals (Möglich *et al.*, 2009; Taylor & Zhulin 1999). In *N. crassa*, WC-1 and WC-2 (White Collar 1 and White Collar 2) mediate all known light responses (Ballario *et al.*, 1996; Cheng *et al.*, 2003; Degli-Innocenti & Russo, 1984; Froehlich *et al.*, 2002; Harding & Turner, 1981; He *et al.*, 2002; Herrera-Estrella & Horwitz, 2007; Linden & Macino, 1997). However, in *T. atroviride*, there appear to be both BLR-dependent and -independent blue-light perception pathways (Berrocal-Tito *et al.*, 2000; Casas-Flores *et al.*, 2006; Rocha-Ramirez *et al.*, 2002; Schmoll *et al.*, 2010). Loss of either *blr-1* or *blr-2* in *T. atroviride* results in mutants unable to conidiate in response to light but able to conidiate in response to starvation and mycelial injury (Casas-Flores *et al.*, 2004). Loss-of-function *wc-1* and *wc-2* mutants are similarly deficient in photoconidiation, yet conidiate in response to nutrient deprivation (Ballario *et al.*, 1996; Linden & Macino, 1997). These studies demonstrate a clear separation between light perception and conidiation, and suggest that the different signalling pathways that stimulate conidiation merge at a step prior to the development of conidia. In addition to photoconidiation, BLR-1 and BLR-2 have been shown to play a role in mycelial growth and glucose sensing of *T. atroviride*, and this is both light-dependent and -independent (Casas-Flores *et al.*, 2004, 2006).

In *T. atroviride* dark-grown cultures, sudden glucose deprivation triggers a conidial ring at the perimeter of the colony, whereas this conidiation response is impaired in $\Delta blr-1$ and $\Delta blr-2$ mutants, suggesting a light-independent role for BLR-1/BLR-2 in carbon deprivation-induced conidiation (Casas-Flores *et al.*, 2006; Friedl *et al.*, 2008). Photoconidiation is also carbon source-dependent, since carbon sources that do not allow carbon deprivation-induced

conidiation in the dark also do not allow photoconidiation (Friedl *et al.*, 2008). It is possible that BLR binds to one or more ligands, responding to the availability of specific carbon sources, or that BLR responds to the intracellular balance between $\text{NAD}^+/\text{NADP}^+$ and NADH/NADPH (the redox state) (Casas-Flores *et al.*, 2006). If the BLR complex can detect the redox state, the effect of different carbon sources on photoconidiation could be explained by different carbon sources altering the redox state of the cells (Friedl *et al.*, 2008).

More than 40 genes have been shown to be regulated by BLR-1/BLR-2, possibly including post-transcriptional autoregulation of BLR-2 (Esquivel-Naranjo & Herrera-Estrella, 2007; Mikus *et al.*, 2009; Rosales-Saavedra *et al.*, 2006). In contrast to WC-1 and WC-2, negative regulation of gene expression by BLR-1/BLR-2 has also been demonstrated (Rosales-Saavedra *et al.*, 2006). *Phr1*, a DNA photolyase gene, has been demonstrated to be regulated in response to light in a BLR-dependent manner (Berrocal-Tito *et al.*, 1999, 2000, 2007; Rosales-Saavedra *et al.*, 2006). DNA photolyases are flavoproteins that repair UV damage, and it is postulated that their induction in response to blue light is in preparation for more damaging rays. Interestingly, *phr1* transcripts can be detected across the whole colony following a light exposure, suggesting that all cells are sensitive to the light, whereas conidiation occurs only in a ring at the edge of the colony in these cultures, suggesting that light perception and conidial development are separate functions. This is further supported by the fact that atropine inhibits photoconidiation but does not block *phr1* transcription in response to light (Casas-Flores *et al.*, 2006).

Many of the *T. atroviride* BLR-1/BLR-2-regulated, blue-light-inducible genes are downregulated in constant blue light, a phenomenon known as photoadaptation. In *N. crassa*, VIVID, a small PAS/LOV domain protein, acts as the blue-light photoreceptor for photoadaptive responses (Schwerdtfeger & Linden, 2001, 2003). In addition to transient expression, VIVID has been demonstrated to be involved in regulating the 2 h refractory period before a second light exposure is effective in *N. crassa* (Schwerdtfeger & Linden, 2001). Refractory periods between which light doses are effective have also been demonstrated in *T. atroviride* (Deitzer *et al.*, 1988; Gressel & Galun, 1967). An orthologue of VIVID, designated ENVOY, has been found in *Hypocrea jecorina* (anamorph *Trichoderma reesei*) and shown to regulate photoadaptation of BLR-1/BLR-2-regulated, blue-light-inducible genes (Castellanos *et al.*, 2010; Schmoll *et al.*, 2005, 2010; Schuster *et al.*, 2007).

Analysis of the promoter regions of *phr1* and other rapid blue-light-inducible genes in *Trichoderma* spp. has revealed the presence of conserved light-responsive elements (LREs), which supports a role for these in light regulation. In *N. crassa*, it has been demonstrated that the light-activated WC-1/WC-2 complex interacts with the promoters of early light-inducible genes at the consensus LRE sequence GATNC—CGATN (He & Liu, 2005). Rosales-Saavedra *et al.* (2006) found LREs

in most of the light-responsive genes from their microarray study, and at least one perfect consensus sequence was found in the promoters of all the BLR-dependent genes. These analyses support a role for this element in light-associated regulation and suggest that most of the light-responsive genes are under the control of BLR-1/BLR-2.

Regulation of circadian rhythms

In *N. crassa*, the WC-1 and WC-2 proteins together with FRQ (Frequency) are the key regulators of circadian photoconidiation, where WC-1 and WC-2 regulate conidiation and FRQ acts as the oscillator (Lakin-Thomas & Brody, 2004; Loros & Dunlap, 2001). In *N. crassa*, light input to the circadian system is mediated by binding of the WC-1/WC-2 complex to the *frq* promoter at the consensus LREs (Froehlich *et al.*, 2002; He & Liu, 2005). Endogenous rhythms associated with conidiation have been identified in some strains of *T. atroviride* and *T. pleuroticola* (Deitzer *et al.*, 1988; Steyaert *et al.*, 2010a). In *T. atroviride*, a rhythmic sensitivity to light has been observed, and in *T. pleuroticola*, conidiation has been observed to occur in a 24 h rhythm (Deitzer *et al.*, 1988; Steyaert *et al.*, 2010a). The expression of the clock-controlled gene *gpd* was investigated in *T. atroviride* and a *blr-2* mutant in a free-running rhythm assay, which is the standard analysis for the circadian model *N. crassa* (Dunlap & Loros, 2006; Steyaert, 2007). Over a 4 day period, the expression levels were observed to oscillate in the wild-type and this appeared to follow an unusually long 48 h rhythm. No rhythm was observed in *gpd* expression in the *blr-2* mutant, suggesting BLR-1/BLR-2 involvement in rhythmicity; however, the wild-type expression pattern could not be repeated in subsequent experiments (J. M. Steyaert and L. L. Loguercio, unpublished data). The promoter sequences of the putative *frq* genes from *T. atroviride*, *T. reesei* and *T. virens* have been analysed for the presence of LREs; however, none has been identified (Steyaert *et al.*, 2010a). It is not known whether BLR-1/BLR-2 and FRQ are involved in circadian responses in *Trichoderma* spp.

Nitrogen regulation of photoconidiation

In *N. crassa*, many WC-1/WC-2 blue-light-inducible genes have been shown to be induced by nitrogen starvation independently of WC-1/WC-2, which suggests these genes to be under the influence of nitrogen catabolite repression (Sokolovsky *et al.*, 1992). It is not known whether BLR-regulated genes are also under this control; however, sudden nitrogen deprivation has been shown to stimulate conidiation in *T. atroviride* independently of BLR-1/BLR-2, and primary sources of nitrogen strongly promote photoconidiation in *T. asperellum*, *T. atroviride* and *T. pleuroticola* (Casas-Flores *et al.*, 2006; Steyaert *et al.*, 2010c).

pH regulation: Pac1 (PacC)

Transcriptional regulation of gene expression by the ambient pH is mediated in the filamentous fungi by the

zinc finger protein transcription factor PacC (Peñalva & Arst, 2002, 2004). Genes under the control of PacC are preferentially expressed under either acid or alkaline growth conditions, depending on the particular gene. As the ambient pH is lowered or raised, the level of gene expression increases or decreases uniformly. Active alteration of the ambient pH by the fungus is considered a mechanism by which fungi exploit the PacC regulation of various secreted proteins, permeases and enzymes involved in the synthesis of exported metabolites such as toxins and antibiotics (Peñalva & Arst, 2002; Prusky & Yakoby, 2003). Mutational studies have implicated Pac1 (PacC) regulation of conidiation in the *T. harzianum* isolate, CECT 2413 (Moreno-Mateos *et al.*, 2007). The majority of experiments reported by Moreno-Mateos *et al.* (2007) were carried out using pH-buffered and unbuffered minimal medium with 2 % glucose and 5 g ammonium sulphate l⁻¹ (18.9 mM nitrogen). No major differences in phenotype associated with the buffering state were reported in that work, whereas Steyaert *et al.* (2010b) found that conidiation was directly affected by both the ambient pH and the buffering state of the medium. On buffered PDA, no conidiation occurred above pH 3.6 in *T. hamatum*, above pH 4.0 in *T. atroviride* and above pH 4.4 in *T. pleuroticola*, whereas on unbuffered medium it occurred at all pH values tested (2.8–5.2). Further, alkalization of the media occurred for both *T. hamatum* and *T. atroviride*, raising the ambient pH well above the cut-off value for buffered PDA. These responses appear to be more complex than the proposed pH-regulated control mediated through Pac1 (PacC).

Global regulation by the VELVET protein

The VELVET protein is a global regulator of morphogenesis and secondary metabolism in filamentous fungi (Calvo, 2008). First identified in 1965, it has since been shown to positively regulate sexual reproduction and both positively and negatively regulate asexual reproduction in a number of filamentous fungi (Calvo, 2008). Despite a high level of structural conservation among filamentous ascomycetes, *velvet* orthologues have divergent roles depending on the fungal species. In *N. crassa*, *A. nidulans* and *Penicillium chrysogenum*, deletion of *veA* orthologues leads to an increase in light-independent conidial formation, whereas deletion in *Aspergillus parasiticus* and *Aspergillus fumigatus* results in a general decrease in conidiation, which is dependent on the nutritional composition of the medium (Bayram *et al.*, 2008; Calvo *et al.*, 2004; Hoff *et al.*, 2010; Krappmann *et al.*, 2005; Mooney & Yager, 1990). A reduction in conidiation has also been observed in *Aspergillus flavus* and *Fusarium fujikuroi* *veA* orthologue deletion mutants (Amaike & Keller, 2009; Wiemann *et al.*, 2010).

The *Trichoderma* *veA* orthologue (*vel1*) has recently been isolated and analysed through mutation in *T. virens* (Mukherjee & Kenerley, 2010). Similar to *F. fujikuroi*, deletion of *vel1* in *T. virens* results in a total loss of

conidiation on solid medium. In contrast, incubation of *vel1* deletion mutants in submerged cultures results in a massive increase in chlamydospore production, whereas in *F. fujikuroi* there is no difference in phenotype between solid and liquid medium. These results suggest that in *T. virens*, VeA acts as negative regulator of chlamydospore production in liquid media (Mukherjee & Kenerley, 2010; Wiemann *et al.*, 2010). Interestingly, the authors also observed loss of gliotoxin production, mycoparasitic activity and plant disease biocontrol capability, demonstrating a link between morphogenesis and biocontrol in *Trichoderma*.

Stage-specific marker genes

Molecular-based studies in *Trichoderma* have revealed a number of potential marker genes for conidiation. The gene corresponding to an upregulated 48 kDa (62 kDa on SDS-PAGE) species has been isolated and designated *cmp1* for 'conidial multidomain protein' (Baum & Horwitz, 1991; Puyesky *et al.*, 1997). The deduced amino acid sequence shows similarity to developmentally regulated cell surface proteins of animal cells and labelling experiments locate the protein to the plasmalemma; therefore, Cmp1 is considered to be a cell surface protein. *cmp1* expression was first detected 12 h post induction, when conidia begin to appear, and peaked at 16–24 h when conidial development was at a maximum. Expression of this gene is considered specific to conidiophores and conidia, and has been extended to other *Trichoderma* strains (our unpublished results). Multiple potential markers for conidiation have been identified in *T. atroviride* and include *Spo14* (encodes phospholipase D), *Spo75* (meiosis-specific protein, required for spore formation), *StuI* (encodes a cell pattern formation-associated protein) and *Wet1* (developmental regulatory protein), and the hydrophobin genes *srh1* (*hfb2*), *hfb-2c*, *hfb-6a* and *hfb-6b* (Mikus *et al.*, 2009; Muñoz *et al.*, 1997; Seidl *et al.*, 2009).

Trichoderma as a molecular model for conidiation

Orthologues of key conidiation genes from the filamentous fungal models *N. crassa* and *A. nidulans* are present within the *Trichoderma* genome and have been demonstrated to have roles in conidiation. This genetic conservation highlights the commonality among filamentous fungi and further validates the use of *Trichoderma* as a molecular model for conidiation studies. Three *Trichoderma* genomes are now publicly available (<http://genome.jgi-psf.org/>), representing taxonomically diverse species in which conidiation is studied: *T. reesei*, *T. atroviride* and *T. virens*. Combining genome resources with modern sequencing technologies will enable the identification of vast numbers of conidiation genes from within these species, and those highly conserved among the three *Trichoderma* genomes will likely be represented in other filamentous fungi. Conversely, those conidiation genes exhibiting less conservation may be more representative of species-specific adaptations to the environment and are of equal interest.

It should be noted that as a model for conidiation, unless otherwise stated, all early studies in *Trichoderma* were conducted on wild-type isolates, whereas the *N. crassa* and *A. nidulans* early studies were inadvertently performed in strains carrying *band* and *velvet* mutations, respectively (Loros & Dunlap, 2001; Adams *et al.*, 1998).

Signal transduction cascades

Organisms respond to environmental cues through a complex regulatory network of signal transduction pathways, which interact with each other in a tightly regulated fashion dependent on extra- and intracellular conditions. Both cAMP and MAP kinase signalling cascades play central roles in morphological and physiological changes associated with both external and internal cues (Lee *et al.*, 2003; Xu, 2000). Research into the biochemistry of photoconidiation and the discovery of *blr-1* and *blr-2* have revealed links between conidiation induction pathways and multiple signalling cascades within the hyphal cell. This has enabled the formation of preliminary models of the networks associated with photoconidiation and has given insight into the signalling pathways that lead from environmental cues at the cell surface to the molecular changes which initiate conidiation.

Rapid biochemical changes associated with conidiation

Multiple biochemical changes have been shown to occur within *T. viride* hyphal cells in response to light. These biochemical responses include changes in the membrane potential, intracellular acidification, a transient rise in ATP production, a biphasic rise in cAMP and an increase in the rate of oxygen consumption (Gresik *et al.*, 1988, 1991; Nemcovic & Farkas, 1998; Sulova *et al.*, 1990). It has been suggested that these biochemical responses are part of the same physiological response; however, the exact order of events is uncertain. Gresik *et al.* (1991) put forward the following hypothesis regarding the biochemical changes associated with exposure to light. In *T. viride*, light triggers the opening of K⁺ channels in the cellular membrane, which results in an efflux of K⁺ ions into the outer membrane space, causing hyperpolarization. Light also stimulates an oxidative burst in the cell, and H⁺ ions are released from the light-stimulated mitochondria into the cytoplasm to compensate for the charge loss. This proton release results in intracellular acidification, which depolarizes the cellular membrane. The H⁺ gradient established across the mitochondrial membrane drives ATP production. Both increased ATP production and intracellular acidification have been shown to activate adenylate cyclase, which suggests a mechanism by which light stimulates cAMP production. Sulova *et al.* (1990) postulated that the energy released from the initial photoreactions drives the oxidative burst, and further that this is likely flavin-mediated. It is not known whether BLR-1 participates in these reactions. More recently it has been postulated that a BLR-1/BLR-2-independent blue-light photoreceptor

is involved in the activation of adenylate cyclase (Casas-Flores *et al.*, 2006).

cAMP regulation of conidiation

cAMP acts as a secondary messenger for morphogenic signals in both prokaryotes and eukaryotes, and in species of *Trichoderma* it has been shown to influence conidiation. cAMP levels have been shown to rise transiently during photoconidiation in *T. viride*, and photoconidiation is significantly increased when *T. viride* is grown in the presence of an inhibitor of cAMP degradation (Gresík *et al.*, 1988; Sulova & Farkas, 1991). In addition, exogenous cAMP or its derivatives stimulate conidiation in both light and dark cultures of *T. viride* and *T. atroviride* (Casas-Flores *et al.*, 2006; Friedl *et al.*, 2008; Gresík *et al.*, 1988; Nemcovic & Farkas, 1998; Sulova & Farkas, 1991). In prokaryotes, cAMP acts as a starvation signal, with intracellular levels low when glucose levels are high. The effect of exogenous cAMP on *T. viride* has been shown to be more pronounced when cultures are grown on glucose-rich medium, which suggests that cAMP may similarly act as a starvation signal (Nemcovic & Farkas, 1998). This is supported by recent molecular studies in *T. virens* on the adenylate cyclase gene *tac1*. Adenylate cyclase catalyses the formation of cAMP from ATP, and is therefore essential for cAMP production (Lee *et al.*, 2003). Knockout mutants of *tac1* in *T. virens* are unable to conidiate in the dark, which clearly demonstrates that starvation induction in the absence of light is cAMP-dependent (Mukherjee *et al.*, 2007). Interestingly, conidiation in the light still occurs in $\Delta tac1$ mutants, which suggests that while photoconidiation is promoted by cAMP, it is not dependent on it. To elucidate the role of carbon source and cAMP in conidiation, a systematic analysis was performed in *T. atroviride* using 95 different carbon sources in the presence or absence of cAMP (Friedl *et al.*, 2008). This analysis showed that while some carbon sources induce conidiation in the presence of cAMP, others repress the differentiation programme.

Protein phosphorylation studies support a role for cAMP in photoconidiation. Phosphorylation of proteins is a common mechanism of post-translational regulation and is due to the action of protein kinases. Both cAMP-dependent and Ca^{2+} /calmodulin-activated protein kinases have been described in fungi. Gresík *et al.* (1989) demonstrated phosphorylation of at least two protein species in cell-free extracts of illuminated mycelium and showed that the illumination effects could be largely substituted by addition of cAMP. Addition of Ca^{2+} inhibited phosphorylation of all proteins. These results suggest that phosphorylation is due to the action of cAMP-dependent protein kinases.

Cross regulation: BLR-1/BLR-2 and protein kinase A (PKA)

Combined molecular and biochemical studies have revealed regulatory interactions between the cAMP signalling pathway and blue-light responses in *T. atroviride*. Sudden carbon deprivation induces a ring of conidiation in *T. atroviride*

wild-type but not in $\Delta blr-1$ and $\Delta blr-2$, which demonstrates that this response requires an active BLR pathway (Casas-Flores *et al.*, 2006). When mutant cultures were transferred to medium without glucose and containing cAMP, the wild-type phenotype was restored, which suggests that cAMP is involved in the BLR-associated carbon response. Further work has also shown that photoconidiation is carbon source-dependent and that dependence can be altered by the addition of cAMP (Friedl *et al.*, 2008).

The physiological effects of cAMP in fungi are mediated through PKA, a cAMP-dependent protein kinase (Lee *et al.*, 2003). cAMP binds to the PKA regulatory subunit, which releases the catalytic subunit, thus activating its kinase activity. In *T. atroviride*, a rise in PKA activity levels was detected within 5 min of light exposure, and this was independent of BLR-1/BLR-2, which suggests that PKA activity is induced via a novel blue-light pathway (Casas-Flores *et al.*, 2006). Those authors further investigated the role of PKA through mutations in the PKA regulatory subunit gene *pkr-1*. PKA activity was constitutively high in *pkr-1* antisense mutants and conidiation was not inducible in response to light or glucose deprivation. Some conidiation was observed in *pkr-1* antisense mutants when transferred to nil glucose and cAMP; however, this conidiation was not in a ring but at the centre of the colony. The authors postulated that the cAMP-induced conidiation is, in part, independent of PKA activity. In mutants where *pkr-1* was overexpressed, PKA activity was very low and cultures hypersporulated under all test conditions, particularly in response to light and cAMP. Though no conidiation occurred in the *pkr-1* antisense mutants, induction of BLR-regulated genes in response to light was still observed. In contrast, no induction of BLR-regulated genes in response to light was detected in the *pkr-1* overexpression mutants. These results clearly demonstrate cross-regulation of the BLR pathway by the cAMP-PKA pathway. In addition, the results suggest that high PKA activity is essential for the transcription of rapid BLR-inducible genes, whereas low PKA activity is required for conidial development. The authors postulate that this cross-regulation is due to phosphorylation of BLR-1 by PKA.

Following a dose of light, WC-1 becomes hyperphosphorylated, and this transient phosphorylation is essential for induction of rapid blue-light-inducible genes (Schwerdtfeger & Linden, 2003). In dark-grown cells and light-adapted cells, protein kinase C (PKC) is thought to phosphorylate WC-1, thus modulating its activity (Franchi *et al.*, 2005). Casas-Flores *et al.* (2006) suggested that PKA may be involved in the phosphorylation of BLR-1 following a light burst, thus stimulating transcription. Phosphorylation sites for both PKC and PKA are present in both WC-1 and BLR-1, which supports their assertion.

Heterotrimeric G proteins and MAP kinase cascades

In heterotrimeric G protein signalling, the binding of a ligand to a cell receptor results in a conformational change

and release of the cytoplasmic G protein (made up of α , β and γ subunits) from the receptor, exchange of GDP for GTP on the $G\alpha$ subunit and dissociation of that subunit from its $\beta\gamma$ partners, allowing both units to regulate downstream effectors. G protein-mediated signals are propagated through two signalling branches in fungi: cAMP-dependent protein kinase (PKA) or mitogen-activated protein kinase (MAPK). To date, four G protein-coupled receptors have been identified in *T. atroviride*, one of which has been shown to be essential for conidiation (Brunner *et al.*, 2008).

G proteins are an integral part of cell signalling, and in fungi have been shown to be involved in sporulation, secondary metabolite production and vegetative incompatibility (Lee *et al.*, 2003). In *Trichoderma*, G proteins have been shown to negatively regulate conidiation. Loss of the G protein α -subunit gene (*tga1*) in *T. atroviride* results in intense conidiation. Conversely, conidiation is inhibited in *tga1* overexpression mutants (Rocha-Ramirez *et al.*, 2002). Loss of *tga3* also results in hyperconidiation in *T. atroviride* and conidiation in the dark (Zeilinger *et al.*, 2005). The

steady-state levels of cAMP are reduced in *tga1* and *tga3* loss-of-function mutants, which is expected; however, addition of cAMP to the *tga3* mutant does not restore the wild-type phenotype. This result suggests that Tga3-mediated effects on conidiation are via a cAMP-independent pathway. It is also possible, as suggested by Casas-Flores *et al.* (2006), that exogenous cAMP acts differently, through a cAMP receptor. In contrast, knockout mutations of *tgaA* (orthologue of *tga1*) and *tgaB* in *T. virens* do not differ from the wild-type in conidial phenotype (Mukherjee *et al.*, 2004), and knockout mutants of *gna1*, a *tga1* orthologue, in *T. reesei* are reduced or delayed in conidiation (Seibel *et al.*, 2009). Recently, Schmoll *et al.* (2009) demonstrated that the *T. reesei* Tga3 orthologue (GNA3) regulates cellulase expression in response to light and is itself induced by light exposure. It is not known whether GNA3 in *T. reesei* regulates conidiation by a mechanism similar to that of Tga3 in *T. atroviride*.

MAPK signalling cascades have been shown to regulate a variety of responses in the cell associated with growth, proliferation and virulence (Schaeffer & Weber, 1999; Xu,

Fig. 3. Model for conidiation signal transduction pathways in a hypothetical *Trichoderma* cell. The different colours indicate the *Trichoderma* species used in this model: blue, *T. atroviride*; yellow, *T. virens*; red, *T. reesei*; magenta, *T. atroviride* and *T. virens*; green, *T. atroviride* and *T. reesei*; brown, *T. viride*; white, postulated but no experimental data available. HR, hypothetical receptor; R, regulatory subunit of PKA; C, catalytic subunit of PKA; BLR-I and BLR-2, Blue Light Regulators 1 and 2, respectively. The arrow and line colours correspond to the *Trichoderma* species indicated above. Arrowheads indicate positive regulation and bars negative regulation. Solid lines indicate data supported and dashed lines hypothetical regulation.

2000). The *T. virens* MAPK homologue belonging to the YERK1 class has been described by two independent groups (*tvk1*, Mendoza-Mendoza *et al.*, 2003; *tmkA*, Mukherjee *et al.*, 2003). Loss of the MAPK gene *tmkA* from *T. virens* (IMI304061) results in hyperconidiation in the light and dark (Mukherjee *et al.*, 2003). However, the effect of this deletion is much less drastic than those observed in the G α -protein mutants (Mukherjee *et al.*, 2003, 2004; Rocha-Ramirez *et al.*, 2002; Schmoll *et al.*, 2009; Zeilinger *et al.*, 2005). In *T. virens* (Gv28.9), deletion of *tvk1* results in a reduction in the production of conidia in solid medium and less intense conidial pigmentation compared with the wild-type (Mendoza-Mendoza *et al.*, 2003). The deletion mutants of *tvk1* conidiate profusely in liquid medium and this is correlated with an increase in the production of hydrophobins (Mendoza-Mendoza *et al.*, 2003, 2007). These data suggest a novel mechanism of regulation by TVK1: positive regulation of conidiation in solid medium and negative regulation in liquid medium. The *T. virens tmk1* (*tvk1*) studies utilized different light and nutritional conditions during incubation; therefore, it is not known whether their respective phenotypes are isolate-specific or an artefact of the experimental design.

Concluding remarks

Trichoderma predominantly reproduces in a relatively simple fashion through asexual reproduction; however, the combination of factors that signals reproduction and the molecular basis of this response are extremely complex. Any researcher who has studied conidiation in controlled laboratory conditions will revel in the extent to which the process is still unpredictable and even a little mysterious. Conidiation is a precise response to an imprecise multitude of permutations of factors such as light, injury, carbon and nitrogen nutrition, ambient pH, environmental calcium, circadian rhythms and the internal metabolism of the fungus (Fig. 3). Further, the ability, or competency, of an individual *Trichoderma* hyphal cell to undergo conidiogenesis in response to light and injury is determined primarily by the nutritional environment rather than the nature of the physical stimuli. Conidiation is fundamental to *Trichoderma* survival and so the molecular basis of conidiation might be expected to be conserved throughout the genus; however, some disparity exists between species. Conidial responses within the same nutritional background have been shown to vary between species. It may be that the genes themselves function in the same manner but that species-specific metabolic adaptations to the environment alter the response thresholds and hence the conidial response, masking the similarity in regulation. In earlier reviews, one or two species of *Trichoderma* have been presented as model(s) for conidiation in *Trichoderma*. It is clear from the literature that this is inadequate and that greater comparative studies with multiple isolates from multiple species are urgently required to establish a complete model for conidiation in this genus.

Understanding species-specific differences in metabolic adaptations to the environment should assist biocontrol design and implementation. Knowledge of the appropriate conditions for maximal yields of viable spores would likely reduce production costs. Knowledge of survivability and vigour within a complex environment could enable targeting of biocontrol strains to the soil or foliar condition appropriate for their species. It may also be possible to create designer BCAs which incorporate desired traits through protoplast fusion or genetic modification.

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