

Emergence of self-organised oscillatory domains in fungal mycelia

M. Tlalka, D.P. Bebber, P.R. Darrah, S.C. Watkinson, M.D. Fricker *

Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, UK

Received 22 December 2006; accepted 22 February 2007

Available online 13 March 2007

Abstract

Fungi play a central role in the nutrient cycles of boreal and temperate forests. In these biomes, the saprotrophic wood-decay fungi are the only organisms that can completely decompose woody plant litter. In particular, cord-forming basidiomycete fungi form extensive mycelial networks that scavenge scarce mineral nutrients and translocate them over long distances to exploit new food resources. Despite the importance of resource allocation, there is limited information on nutrient dynamics in these networks, particularly for nitrogen, as there is no suitable radioisotope available. We have mapped N-translocation using photon-counting scintillation imaging of the non-metabolised amino acid analogue, ^{14}C -aminoisobutyrate. We describe a number of novel phenomena, including rapid, preferential N-resource allocation to C-rich sinks, induction of simultaneous bi-directional N-transport, abrupt switching between different pre-existing transport routes, and emergence of locally synchronised, oscillatory phase domains. It is possible that such self-organised oscillatory behaviour is a mechanism to achieve global co-ordination in the mycelium.

© 2007 Elsevier Inc. All rights reserved.

Keywords: *Phanerochaete velutina*; Phase domain; Bi-directional amino acid transport; Fungal network; Metabolic oscillation; Self-organisation

1. Introduction

Fungi play a central role in the nutrient cycles of boreal and temperate forests where most of the Earth's terrestrial carbon is sequestered (Malhi et al., 1999; Lindahl et al., 2002). In these biomes, saprotrophic basidiomycete wood-decay fungi are the only organisms that can completely decompose woody plant litter to provide a source of carbon (Boddy, 1999). They form extensive foraging mycelial networks (Rayner et al., 1994) that persist for decades or centuries (Smith et al., 1992; Ferguson et al., 2003). These networks are adept at scavenging scarce mineral nutrients, including anthropogenic pollutant nitrogen (Currie, 1999; Currie et al., 2004), and act as the main repositories of soil nitrogen for which plants and microbes compete (Lindahl et al., 2002; Watkinson et al., 2006). Network-enabled long-distance translocation of nutrients both across the forest floor (Wells et al., 1990, 1995, 1998) and

vertically between soil horizons (Frey et al., 2000, 2003) allows rapid colonisation and exploitation of new food resources (Boddy, 1999).

Despite the importance of basidiomycete mycelial systems, remarkably little is known about the development, anatomy and physiology of long-distance transport pathways within them, or about how resource allocation through the network is regulated (Jennings, 1987; Cairney, 2005). Stable isotope labelling and destructive harvesting has been extensively used to determine fluxes of carbon (^{13}C) and nitrogen (^{15}N) over a range of scales (Lindahl et al., 2002; He et al., 2003; Read and Perez-Moreno, 2003; Simard and Durall, 2004; Govindarajulu et al., 2005). In contrast, detailed analysis of nutrient fluxes in individual mycelia has used non-invasive mapping of radioisotopes, typically ^{14}C and ^{32}P . The emerging picture for C and P dynamics is complex, with evidence from microcosms for highly responsive shifts in nutrient allocation depending on the size and quality of resource units, the sequence of their encounter and the presence of other competing organisms (Boddy, 1999). In general, substan-

* Corresponding author. Fax: +44 0 1865 275074.

E-mail address: mark.fricker@plants.ox.ac.uk (M.D. Fricker).

tial levels of isotope are taken up, but with varying amounts retained at the loading site (Clipson et al., 1987; Olsson and Gray, 1998). Net allocation of the remainder through the network is a complex function of multiple competing source-sink relationships (Wells et al., 1995, 1998, 1999). Movement can be bi-directional (Granlund et al., 1985; Olsson and Gray, 1998; Lindahl et al., 2001; Nielsen et al., 2002), with features similar to a circulatory system (Wells et al., 1998). However, absolute rates of translocation vary over at least two orders of magnitude, from 1.8 mm h⁻¹ (Olsson and Gray, 1998) to 200 mm h⁻¹ (Brownlee and Jennings, 1982), depending on the degree that specific transport pathways, such as cords and rhizomorphs, have developed. The mechanism(s) driving movement are also unclear, with evidence for diffusion, mass flow and cytoplasmic streaming in different systems over different time and length scales (Jennings, 1987; Olsson and Jennings, 1991; Olsson and Gray, 1998; Cairney, 2005; Darrah et al., 2006). Thus, whilst indeterminate growth and flexible resource allocation is of great benefit in exploiting a patchy resource environment (Boddy, 1999; Ettema and Wardle, 2002; Watkinson et al., 2006), co-ordination of these activities poses a considerable challenge within an interconnected, but locally responsive, network (Rayner et al., 1994).

In comparison to C and P, even less information is available for nitrogen (N) dynamics as there is no suitable radioisotope tracer. However, some progress has been made using 2-amino[1-¹⁴C]isobutyrate (¹⁴C-AIB), a methylated analogue of alanine, as a marker for the soluble amino acid pool. AIB¹ is taken up by amino acid transporters (Ogilvie-Villa et al., 1981; Lilly et al., 1990) and accumulates without being metabolised or incorporated in protein (Kim and Roon, 1982). Although AIB competitively inhibits amino acid uptake, it can be used a subtoxic (micromolar) concentrations as a marker for long-distance amino acid transport. It shows rapid uptake and bi-directional transport in cords (Watkinson, 1984), as well as movement through specific transport pathways in undifferentiated mycelia on agar (Olsson and Gray, 1998). Recently we have shown using photon-counting scintillation imaging (PCSI) that there is a pulsatile component to AIB transport, with evidence for differential behaviour between the assimilatory and foraging mycelia (Tlalka et al., 2002, 2003). However, at this stage there is no information on how N-dynamics alter in response to addition of new resources, particularly in corded systems.

In this paper, we consider the effects of adding C-rich, N-poor resources on the dynamics of N-distribution, in microcosms designed to mimic the sporadic capture of wood fragments during mycelial foraging or stochastic input from the forest canopy. Using PCSI (Tlalka et al., 2002, 2003) we describe a number of novel phenomena,

including rapid, preferential N-resource allocation to C-rich sinks, induction of simultaneous bi-directional transport, and abrupt switching between different pre-existing transport routes. Furthermore, analysis of the pulsatile transport component shows that as the colony forms, it self-organises into well demarcated domains that are identifiable by differences in the phase relationship of the pulses. This suggests that self-organised synchronous behaviour can emerge within the seemingly simple network architecture, and that this behaviour may be a manifestation of global co-ordination and organisation in the mycelium.

2. Materials and methods

2.1. Fungal material

Cultures of *Phanerochaete velutina*, from an isolate collected by Prof. L. Boddy (University of Cardiff), were maintained on 2% malt agar (2% Oxoid malt extract, 2% Oxoid No. 3 agar) at 22 ± 1 °C in darkness in a temperature-controlled incubator (Gallenkamp, England) as previously described (Tlalka et al., 2002, 2003).

2.2. Experimental microcosms

Microcosms were prepared as in (Tlalka et al., 2002, 2003) using either a Lite Plus intensifying screen or a Bio-Max TranScreen LE intensifying screen (Sigma, Poole, UK) in the presence or absence of additional cellulose bait(s) (13 or 26 mm disc, Grade AA filter paper, Whatman, Maidstone, England) according to figure legends.

2.3. Visualisation of ¹⁴C-AIB transport using photon-counting scintillation imaging

¹⁴C-AIB was imaged using a high-resolution, photon-counting camera system (HRPCS-3, Photek Inc., St. Leonards on Sea, UK) as described previously (Tlalka et al., 2003). For most experiments, 20 µl (37 kBq) of a 0.9 mM solution of 2-amino[1-¹⁴C]isobutyric acid, ¹⁴C-AIB, specific activity 2.11 GBq mmol⁻¹ (Amersham, UK) in 2% v/v EtOH was applied to either the inoculum or to a bait. In 4-baited experiments, the amount of ¹⁴C-AIB was increased to 50–100 µl. The chambers were sealed and imaged in a temperature-controlled room at 19–22 °C, monitored with “Diligence”™ data loggers (Comark Ltd., UK). Temperatures for individual experiments were kept to ±0.5 °C. Images were integrated over 60 min and experiments lasted up to 400 h. In some experiments, different regions of the colony were harvested at the end of the experiment and the total amount of ¹⁴C-AIB measured using liquid scintillation counting. One-microliter samples were diluted with 4 ml OptiPhase “Hisafe” 3 (Fisher, UK), vortexed, left for 24 h and counted using a Beckman LS1801 β-spectrometer (Beckman Instruments Inc., USA).

¹ Abbreviations: AIB, aminoisobutyrate; DFT, discrete Fourier transform; HSB, hue, saturation brightness colour space; PCSI, photon-counting scintillation imaging; RGB, red, green, blue colour space.

2.4. Construction of phase, amplitude and trend images

To visualise differences in the phase of the oscillatory component across the colony, an image processing suite written in MatLab (The Mathworks, Inc., Natick, MA, USA) was used to apply Fourier analysis (Tlalka et al., 2002, 2003) on a pixel-by-pixel basis according to Fricker et al. (2007). In brief, the raw data for each pixel was smoothed, de-trended and filtered with a Hanning window to provide a time-series suitable for Fourier analysis. The discrete Fourier transform (DFT) was calculated on a pixel-by-pixel basis to give arrays of phase and magnitude of the oscillation at each Fourier frequency. The dominant Fourier frequency was determined for each pixel and the corresponding phase and magnitude images were extracted at this frequency. Pseudo-colour maps of each Fourier variable were constructed with the parameter of interest, such as phase, coded by colour. The angular mean and angular deviation of the phase for specific regions such as the inoculum, bait, connecting cord and mycelium from different experiments were calculated according to Jammalamadaka and SenGupta (2001).

2.5. Movie and image presentation

Movie files in *avi* format were output directly from the IFS32 Imaging Software (Photek Inc.) or assembled from individual images using MatLab (The Mathworks Inc., Natick, MA, USA). Web movies were formatted with QuickTime Pro v6.5 (Apple Computer Inc.).

3. Results

3.1. Transport to recently colonised baits is exclusively acropetal

To set up a minimal system to investigate directional N-transport, growing mycelia of *Phanerochaete velutina* were established from a discrete inoculum on an otherwise inert environment (a scintillation screen). Growth over the first 48–96 h was symmetrical with a uniform marginal growth zone of fine hyphae. ^{14}C -AIB loaded at the inoculum at the start of the growth period was distributed relatively evenly throughout the colony (Fig. 1A). Around 96–128 h, growth became concentrated on a restricted number of foci at the margin and by 192–240 h these were supported by the development of cords from the inoculum. The transition to asymmetric growth was associated with the accumulation of high levels of ^{14}C -AIB at the growing margin (Fig. 1A, see also Supplementary Movie 1A).

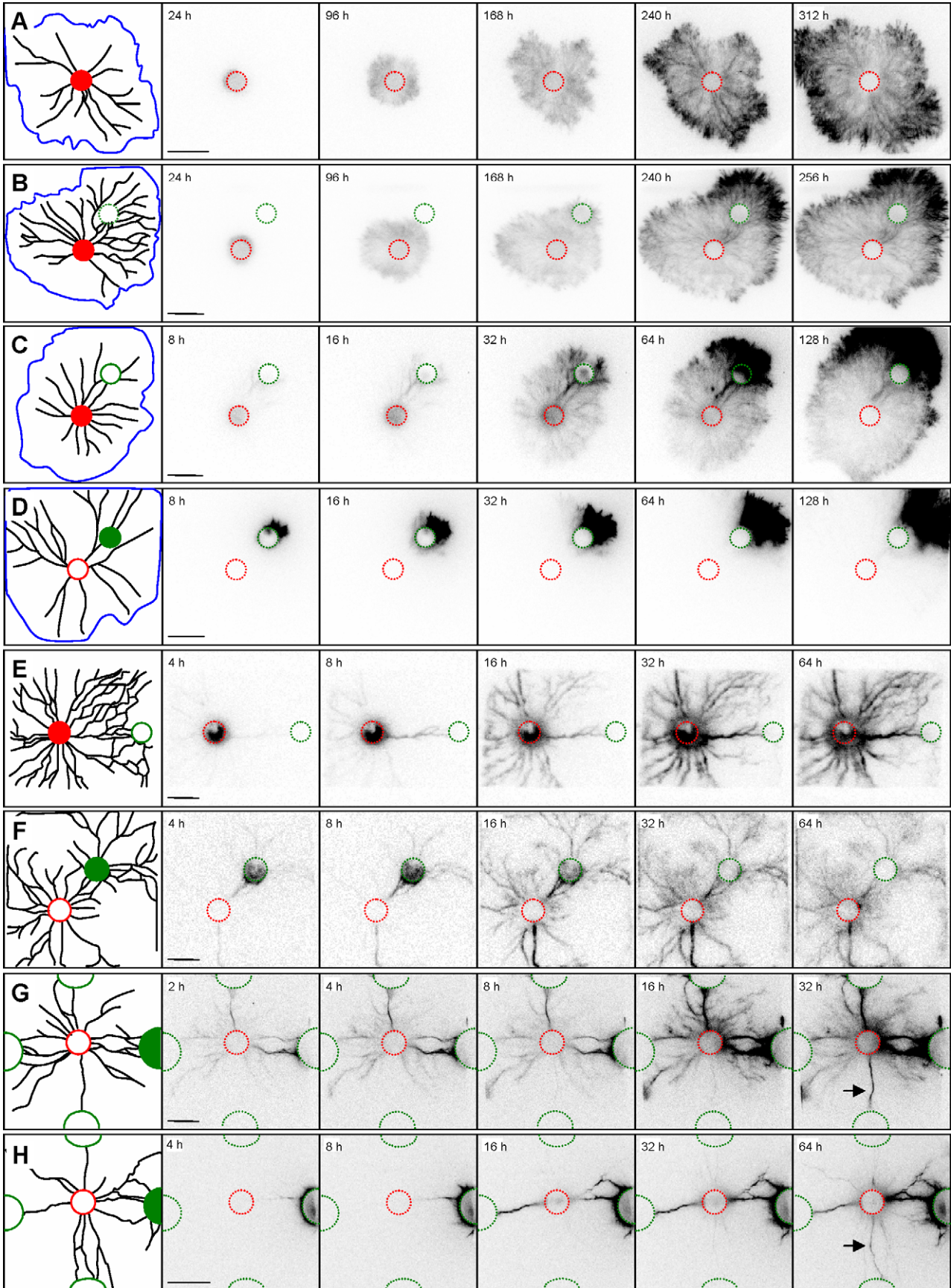
To mimic encounter with a C-rich resource, a single cellulose disc was added at the colony margin after 144–192 h. ^{14}C -AIB loaded at the inoculum at the start of the growth period preferentially accumulated in the growing mycelium subtended by the bait (Fig. 1B). The local growth rate also increased in response to the bait, often with a commensu-

rate decrease in growth of more distal regions of the colony, leading to a bulge in the colony outline (Fig. 1B, see also Supplementary Movie 1B). In these experiments, ^{14}C -AIB localization reflected the cumulative effects of its distribution over the whole growth period. To probe the instantaneous direction of N movement, ^{14}C -AIB was added to either the inoculum (Fig. 1C) or to the bait (Fig. 1D) of previously unlabelled, juvenile baited microcosms. ^{14}C -AIB was rapidly taken up from either location, but in both cases transport was highly polarised towards the colony margin, with no retrograde movement to the inoculum (see also Supplementary Movie 1C&D). Thus at this early stage of development, only unidirectional acropetal transport was observed.

3.2. Both basipetal and acropetal transport occurs in more mature corded systems

At some point it was expected that the system would develop the capacity to redistribute nutrients both acropetally and basipetally. Preliminary observations suggested that as the juvenile colonies became more corded, typically after a further 3–4 d growth, ^{14}C -AIB could be transported basipetally to the inoculum from the loaded bait, and thereafter through the rest of the colony (see also Supplementary Movie 1D). To test whether bi-directional transport was a common feature of corded systems, ^{14}C -AIB transport was mapped in mature 5-week-old baited colonies, when the relatively fine, diffuse mycelium associated with the marginal growth zone of younger colonies had resolved into a network of cords (Fig. 1E and F). In contrast to juvenile systems, ^{14}C -AIB loaded at either the inoculum (Fig. 1E) or the bait (Fig. 1F) was distributed throughout the whole system (see also Supplementary Movie 1E&F). Typically, the cord interconnecting the bait and inoculum was labelled most rapidly and to the highest level. In some cases, ^{14}C -AIB was also distributed throughout the rest of the mycelium (Fig. 1E and F). In other cases there was evidence of marked preferential transport routes and restricted allocation to a sub-set of the whole colony (data not shown). Importantly, and in contrast to recently colonized baits, ^{14}C -AIB was clearly seen to move basipetally from the resource to the inoculum as well as acropetally to the colony margin adjacent to the resource (Fig. 1F). Thus, system-wide translocation developed from the uniquely polarized transport seen in younger mycelia capturing a fresh carbon source.

It was not possible to test simultaneous bi-directional transport between a single bait and the inoculum using ^{14}C -AIB in an analogous manner to experiments using differential detection of ^{31}P and ^{32}P (Lindahl et al., 2001). However, it was possible to examine whether flow could occur from both bait to inoculum and then from inoculum to other baits in the same mycelium. Thus, more complex systems with four baits around the inoculum were established for 6–7 weeks and ^{14}C -AIB added to one of the baits. Transport was detected with a minimal lag period of 2–4 h,



similar or faster than that for loading at the inoculum. The ^{14}C -AIB moved rapidly from the loaded bait to the inoculum and, via the inoculum, to one or more of the other connected baits (Fig. 1G and H, see also Supplementary Movie 1G&H). Thus, these corded systems are capable of both acropetal and basipetal movement in the same colony at the same time.

3.3. N-distribution shows discrete route opening events

The ability to continuously image N-dynamics also revealed further unexpected levels of sophistication in routing of N through the network. ^{14}C -AIB distribution from the inoculum to the unloaded baits was not necessarily simultaneous. In several cases there was a considerable delay before detectable signal appeared in one of the connecting cords (Fig. 1G and H, see also Supplementary Movie 1G&H). Once transport was initiated, the cord filled within 1 h and to a similar overall level to that observed for the other cords that conducted immediately. These transitions had the appearance of a discrete switch in behaviour opening up a pre-existing transport route, rather than a gradual transition from a low to a high conductance state.

3.4. Substantial levels of N are allocated to new resources added to mature systems

To mimic the effect of deposition of plant litter on to mycelial systems on the forest floor, a fresh cellulose disc was added to the established, 4-baited networks. New cords formed to connect the fresh bait to the inoculum and to the adjacent carbon sources and amino acid was routed towards it within 24 h (Fig. 2A and B), with the earliest detectable response within about 6 h. ^{14}C -AIB, measured by scintillation counting after destructive harvesting, was distributed to the inoculum, mycelium, existing baits and new resource, but the levels were very variable between experiments. For example, there was a 5-fold difference in the absolute amount mobilised from the loading site and a 3- to 5-fold difference in allocation between the three notionally equivalent existing baits (Table 1). This may reflect the normal variability in the colony development and number of cords connecting each bait to the inoculum. Nevertheless, in 3 out of 4 experiments, a substantial fraction of the total ^{14}C -AIB transported was allocated to the fresh carbon source.

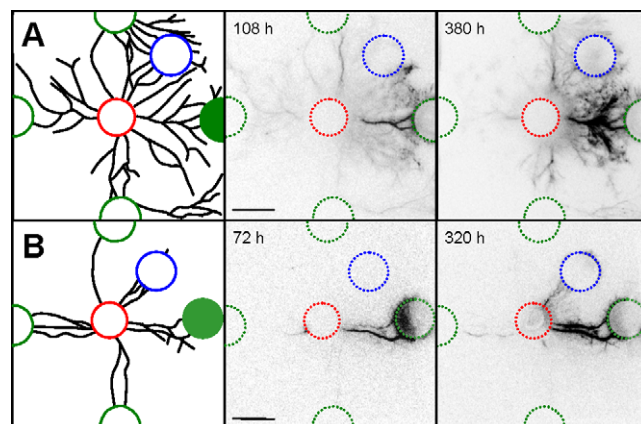


Fig. 2. N-allocation following addition of a new cellulose resource. Images of ^{14}C -AIB movement in colonies of *P. velutina* grown from a central inoculum across a scintillation screen in the absence or presence of four additional cellulose baits and imaged using a photon-counting camera. The first column shows schematic outline of the colony and the major cords, with the position of the inoculum in red and the baits in green. Solid colour represents the loading site of ^{14}C -AIB. Fifty microliters ^{14}C -AIB was added after 28 d (A) or 33 d (B). The time points are given in hours after loading. At 120 h after loading, a new cellulose bait was added at the position indicated in blue. This triggered development of new cords and directed transport of ^{14}C -AIB. Images show two examples from four experiments. Scale bars = 25 mm.

Table 1
Distribution of ^{14}C -AIB in mycelial networks of *P. velutina*

% ^{14}C -AIB transported	Inoculum	Mycelium	Bait 2	Bait 3	Bait 4	Extra bait
11.7	23.9	39.6	0.5	0.2	0.1	35.7
36.0	12.7	4.2	26.8	14.8	9.4	32.1
37.0	11.9	39.5	11.5	5.0	2.2	30.5
61.4	7.7	4.8	49.6	19.8	11.4	6.6

Levels of ^{14}C -AIB were determined by liquid scintillation counting in specific regions of mycelial systems with 4 cellulose baits 200–260 h after addition of an extra bait. Overall recoveries of ^{14}C -AIB were $95.7 \pm 2.8\%$. Values are shown for four separate experiments as there was considerable biological variation between each replicate in the amount translocated and distributed between individual baits.

3.5. Emergence of synchronised oscillatory phase domains

As in our previous work (Tlalka et al., 2002, 2003), all the mycelial systems here displayed regular pulses in amino acid transport, with a period of between 14 and 18 h. Thus, in addition to imaging the pattern of ^{14}C -AIB distribution, we also examined how the phase of

Fig. 1. N-dynamics during mycelial network formation. Time-series of ^{14}C -AIB movement in colonies of *Phanerochaete velutina* grown from a central inoculum across a scintillation screen in the absence (A) or presence of one (B–F) or four (G and H) additional cellulose baits and imaged using a photon-counting camera. The first column shows schematic outline of the colony and the major cords with the position of the inoculum in red and the bait(s) in green. Solid colour represents the loading site of ^{14}C -AIB. ^{14}C -AIB was added at the start of growth (A and B), or to colonies grown for 8 d (C and D), 47 d (E), 35 d (F) or 31 d (G and H). The time points are given in hours after loading ^{14}C -AIB. Arrows in (G and H) highlight a cord that appears to switch on rapidly after transport throughout the rest of the colony has been initiated. Images are representative examples of $n = 8$ (A); 13 (B); 4 (C and D); 6 (E); 5 (F–H). All scale bars = 25 mm.

the pulsatile component was affected during development of corded systems and in response to bait addition. We extended our previous Fourier analysis techniques (Tlalka et al., 2002, 2003) to spatially map the frequency, phase and magnitude of the pulsatile component over the whole colony on a pixel-by-pixel basis (Fricker et al., 2007). In these maps, the Fourier variable of inter-

est was expressed as colour for each pixel. To avoid ambiguity in the phase and amplitude images, only regions with the same dominant Fourier frequency were colour-coded, the remainder are represented in greyscale (Fricker et al., 2007).

In juvenile mycelial systems with no bait addition, the mycelium under the inoculum and the mycelium growing over the screen formed distinct oscillatory domains with the same frequency, but that were 150° out-of-phase with each other, corresponding to a lag of about 6 h (Fig. 3A and G). There was no change in the frequency of the pulses with the introduction of a new C-resource (Fig. 3B), but in 7 out of 12 colonies a distinct phase domain became established at the bait that differed from the remainder of the mycelium and the inoculum (Fig. 3B and H). In most of these cases, the phase difference in the bait was slightly (~ 3 –5 h) ahead of the inoculum (Fig. 3H). However, in one case the bait was observed to lag by up to 5 h behind (Fig. 3C). Thus, although well demarcated phase domains were commonly observed in these baited colonies, their precise phase relationship was not fixed.

In more established colonies, distinct phase domains comprising the inoculum, bait and rest of the mycelium, were observed irrespective of whether ^{14}C -AIB was loaded at the inoculum (Fig. 3D and I) or bait (Fig. 3E, F and J). In individual experiments, the inter-connecting cord appeared to have a slightly different phase from the rest of the mycelium (Fig. 3E and F). However, the overall variation in phase of the different regions between experiments obscured any trend for the cord, with considerable overlap for the average phase vector for these regions (Fig. 3I and J).

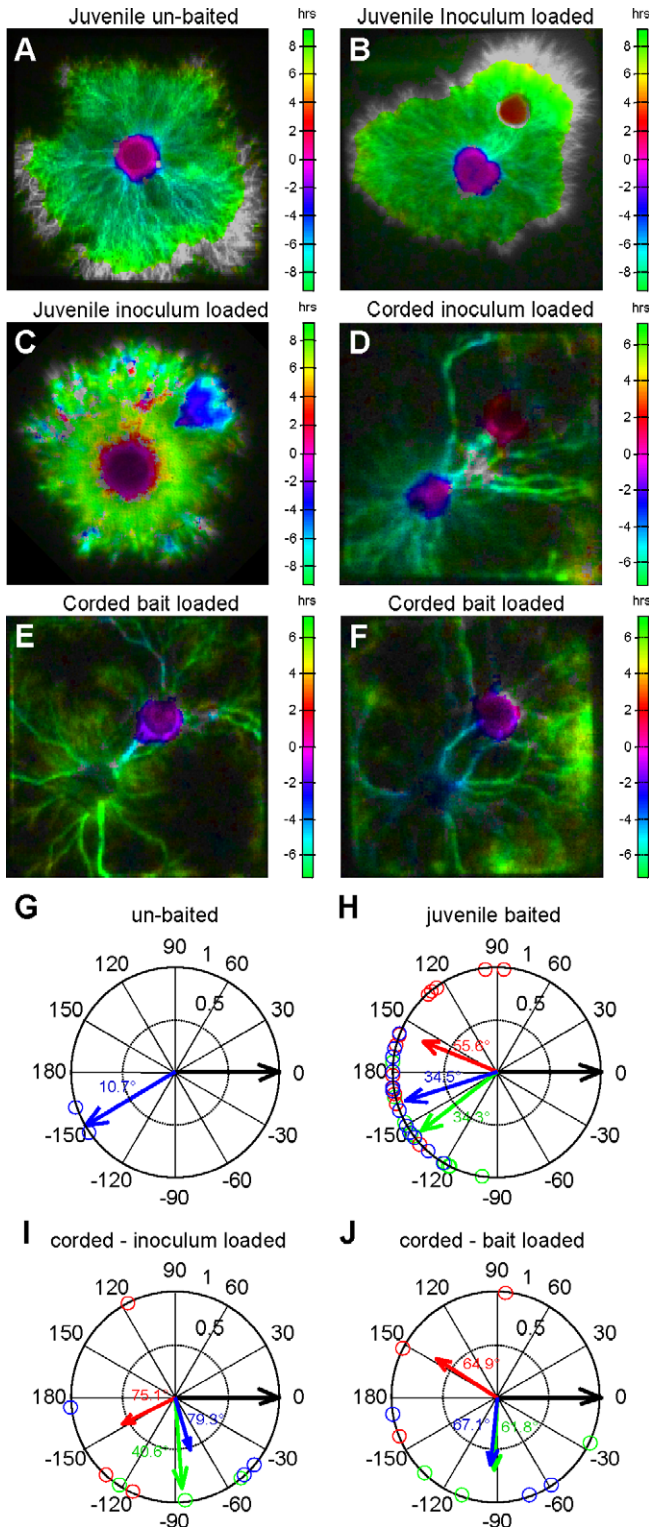


Fig. 3. Establishment of oscillatory phase domains. Pseudo-colour coded images showing the relative phase of the oscillatory component of ^{14}C -AIB transport determined pixel-by-pixel using Fourier analysis (see Fricker et al., 2007). The colour represents the phase difference in hours from the oscillation recorded at the loading site. Only regions with the same frequency are colour-coded. Regions with a different frequency or where the Fourier analysis was not possible, such as the growing colony margin, are coded in greyscale. The period of the oscillation ranged from 18.3 h (A and B) to 14.2 h (C–F), depending on the growth temperature with a Q_{10} of around 2. (A) A control colony grown for 240 h showing distinct phase domains in the inoculum and foraging mycelium with ~ 7 h phase difference. (B) Development of an additional phase domain in the bait of a juvenile colony. The bait lags the inoculum by ~ 3.5 h. (C) One example in which the bait still formed a distinct domain but was ~ 5 h ahead of the inoculum. (D) Phase map of a 35-d-old colony following application of ^{14}C -AIB to the inoculum. (E and F) Phase maps of 35-d-old colonies following loading at the bait with one (E) or two (F) connecting cords from the inoculum. (G–J) Phase maps showing phase in degrees relative to the inoculum (black) for the mycelium (blue) and, when present, bait (red) and interconnecting cord (green). Individual experiments are shown as open circles. The angular mean for each domain is shown by vectors whose magnitude provides a measure of dispersion. The angular deviation in degrees is given next to the vector.

4. Discussion

4.1. Simple mycelial systems show sophisticated N-dynamics

Continuous scintillation imaging and Fourier mapping has allowed detailed characterisation of the complexity of N-dynamics in mycelial systems. The results presented indicate that N-allocation, as reported by a non-metabolised amino acid analogue, is considerably more dynamic and responsive than previously suspected, and a number of novel phenomena occur, including rapid, preferential N-resource allocation to C-rich sinks, induction of bi-directional transport and abrupt switching between different pre-existing transport routes. The speed of some of the responses observed, such as switching of transport routes or detection of the new resource, are close to the current temporal resolution of PCSI, with changes detectable within 1–2 and 6 h, respectively. Such rapid responses are perhaps not unexpected in an environment with strong competition for ephemeral resources.

In juvenile colonies, transport was unidirectional towards the growing margin, consistent with the predicted direction of mass flow from the inoculum needed to support growth across the otherwise inert scintillation screen. However, mass flow would not be sufficient to supply nutrients all the way to the extending hyphal tips so within the peripheral growth zone, nutrient transport may be augmented by cytoplasmic streaming, vesicular transport (Cairney, 1992, 2005) and/or diffusion through the extensive interconnected reticulate vacuole system (Darrah et al., 2006; Bebber et al., 2007). The effect of a cellulose bait was to preferentially focus N-transport and further growth to the baited sector of the colony, at the expense of transport to other regions. As the colony developed further, the fine mycelial system resolved into a corded network (Bebber et al., 2007). However, once a network of cords was established, a transition occurred to bi-directional translocation. In several of the systems studied, baits were connected to the inoculum by a single cord. Given that each system seemed to be capable of moving ^{14}C -AIB in either direction along such cords, we infer that either a bi-directional transport system was constitutively present in every cord or the direction of transport switched between acropetal and basipetal movement within the same cord (Cairney, 1992; Bebber et al., 2007). Although PCSI has sufficient spatial resolution to see transport in individual cords, we have not yet devised a methodology to measure bi-directional transport directly with a single radioisotope. Bi-directional translocation has been demonstrated previously in double labelling experiments with related fungi using ^{32}P and ^{33}P (Lindahl et al., 2001), or inferred in ectomycorrhizal mycelium from double labelling with ^{13}C and ^{14}C (Simard et al., 1997). However, the spatial resolution in these investigations was not sufficient to assign transport to a specific transport route.

In the 4-baited networks, there appeared to be a capacity to switch rapidly between one route and another. There is evidence from other systems for transport along a sub-set of all possible routes at any one time (Brownlee and Jennings, 1982; Olsson and Gray, 1998), but these studies were not able to identify switching between different transport routes. If route-switching is a common phenomenon, it provides evidence of much more sophisticated orchestration of transport dynamics than hitherto suspected. As yet, however, we do not have a mechanistic explanation of how such routing may be controlled.

4.2. Foraging hyphae rapidly sense a new carbon supply

As might be expected in environments with strong competition for resources, both juvenile and corded networks were very responsive to the addition of new C-resources. Fresh cellulose added to a pre-existing mycelium was colonized within hours. Subsequently, connecting cords developed and amino acid was translocated into the fresh carbon source. The mechanism leading to detection of the cellulose resource is not known. As with other fungal species, it is likely that the response is not triggered directly by the cellulose and hemi-cellulose polymers, but by soluble sugars already present or possibly derived from breakdown by low levels of constitutive extracellular cell wall degrading enzymes (CWDE, Aro et al., 2005; Chavez et al., 2006). Whatever the method of initial induction of CWDE, their subsequent action generates a range of soluble mono- and disaccharides that further stimulate expression of various CWDE through transcriptional activators such as XlnR, ACEI and II, and Hap complexes (Aro et al., 2005; Chavez et al., 2006). CWDE expression is also strongly repressed by high levels of fermentable sugars, in a process termed carbon catabolite repression (CCR), to prevent excess production of costly enzymes when glucose is no longer limiting. Although CCR has not been fully elucidated in saprotrophic basidiomycetes, it is probably mediated by Cre-proteins activated by one or more of a number of functionally overlapping external and internal sugar sensing pathways that are highly conserved across eukaryotes (Rolland et al., 2006; Santangelo, 2006; Bahn et al., 2007). Thus in yeast potential extracellular sensors include high and low affinity non-translocating hexose transporters (Hxts) and a G-protein coupled receptor (GPCR) system leading to production of cAMP and activation of PKA. Sensing of internal sugar levels may be mediated by a monomeric G-protein, Ras/cAMP/PKA pathway and/or hexose kinase (Hxk2) that antagonises the Snf1/Mig1 pathway needed for de-repression (Rolland et al., 2001, 2002; Santangelo, 2006). A number of homologues to components of these pathways have been identified in filamentous fungi, particularly *Neurospora* and *Aspergillus* (Flipphi et al., 2003; Li and Borkovich, 2006; Palmer and Horton, 2006; Divon and Fluhr, 2007), but even a partial pathway awaits characterisation in a cord-forming basidiomycete.

4.3. Early responses to new C-resources include preferential N-translocation and increased growth

Mass balance analysis by destructive sampling and scintillation counting of ^{14}C -AIB suggested nitrogen, as amino acid, was preferentially allocated to fresh baits in comparison with existing partly utilised cellulose. At present we cannot determine whether the differential N-allocation observed is in response to a preferential demand specifically for extra N, needed to fully exploit the C-rich resource, or a reflection of greater overall metabolic activity during colonisation and exploitation of the newly added bait. Following the asymmetric distribution, high internal levels of amino acids are normally sensed through the TOR (target or rapamycin) pathway that activates GATA transcription factors leading to increased transcription, particularly of ribosomal components, needed for rapid growth (Cooper, 2002; Inoki et al., 2005). Conversely, under N-starvation, inactivation of TOR reduces ribosome biogenesis, but leads to an increase in expression of stress genes, nitrogen catabolite-repressed (NCR) genes and induction of autophagy (Rohde et al., 2001; Cooper, 2002; Rohde and Cardenas, 2003; Inoki et al., 2005). Such regulation by N would be consistent with the increased colony expansion observed around the new resource and the cessation of growth, or even autophagy, in distal parts of the mycelium. However, there are a number of caveats in this interpretation. First we do not know whether the N-translocated is located in the cytoplasm, vacuole or other storage compartment, so high levels of radiotracer may not correspond to high cytoplasmic N availability. Second, the spatial separation of nutrient sources in these microcosms has the potential to generate conflicting internal and external signals. Thus, whilst high levels of internal translocated amino acids achieved would be expected to repress NCR genes through the TOR pathway, the very low levels of external N available in the cellulose resource might be expected to activate externally facing transduction pathways, such as the Ssy1-Ptr3-Ssy5 (SPS) non-translocating amino acid permease complex (Bahn et al., 2007) that alleviate NCR. Third, there is the possibility that the increases in radiolabel observed are a consequence of using a non-metabolised tracer that accumulates as it cannot be processed further and no longer accurately reflects the dynamics of the soluble amino acid pool.

4.4. Changes in ^{14}C -AIB distribution are rapid and co-ordinated across the colony

Although from the discussion above it is clear that much more work is required to understand the signalling processes involved in spatial N-redistribution, we infer from the speed and magnitude of the shifts in AIB that the system can adapt its metabolism rapidly and manages to coordinate resource allocation throughout the whole colony in response to local demands. At present we can only monitor changes in the soluble amino acid pool with radiola-

belled AIB. However, it may be possible to fully characterise C and N metabolism using the type of spatially resolved metabolomics and gene expression studies pioneered by Shachar-Hill and co-workers for mycorrhizal systems (Govindarajulu et al., 2005, Tlalka et al., unpub.).

Such fine control of N-distribution may be an adaptive response with ecological relevance in a patchy resource environment (Frey et al., 2000, 2003; Ettema and Wardle, 2002). If borne out in soil-based microcosms, these observations would also suggest that C, N and P fluxes show different allocation dynamics and are subject to different controls (Wells et al., 1990, 1995, 1998). Responsive, network-enabled N-acquisition by these fungi and targeted allocation to woody resources are likely to contribute substantially to net accumulation of N by the forest floor (Currie, 1999; Currie et al., 2004) and also restriction of subsequent N transfer to plants (Lindhahl et al., 2002). Both these factors have been highlighted as critical components needed to rationalise predictive models of C and N cycling against field data (Currie et al., 2004) and may explain the capacity of the forest floor for responsive absorption of pollutant nitrogen (Lindhahl et al., 2002).

4.5. Physiological oscillations reveal the emergence of self-organised functional domains

With competition for limited, ephemeral resources, mycelial networks must remain highly responsive, continuously balancing resource allocation between maintenance, recycling, exploration or exploitation. How such conflicting activities are integrated into co-ordinated colony-wide responses is not clear (Rayner et al., 1994). At this stage we do not know what significance to attribute to the emergence of oscillatory phase domains in terms of global signalling within the colony, but the co-ordinated activity implies there is some benefit for the system to switch between different states in a synchronized manner. There are many circadian and ultradian rhythms in fungi that are thought to allow distinct biochemical processes to proceed under more optimal conditions (Lloyd and Murray, 2006a, 2006b; Tu and McKnight, 2006; Wijnen and Young, 2006). It is likely that as experimental methods improve (see for example Klevecz et al., 2004; Tu et al., 2005; Murray et al., 2007), oscillations and temporal compartmentalization will increasingly emerge as an effective strategy used by many organisms to improve metabolic efficiency (Wijnen and Young, 2006).

In this case, it is unlikely that pulsing in AIB transport is tightly linked to a canonical circadian oscillator (Bell-Pedersen et al., 2005; Dunlap and Loros, 2005; Liu and Bell-Pedersen, 2006) as the period differs markedly from 24 h and is not temperature-compensated (Tlalka et al., 2003). On this basis it is more plausible to propose that pulsing arises from a metabolic oscillator, uncoupled from any genetic pacemaker (Kippert, 2001; Lloyd and Murray, 2005; Lloyd, 2006). Recently, however, the conceptual separation between circadian-linked oscillators and metabolic

oscillators has become less distinct. For example, mutants in the key *frq* and *wc* clock genes in *Neurospora* have revealed a number of underlying *frq*-less metabolic oscillators (FLOs, Iwasaki and Dunlap, 2000; Lakin-Thomas, 2006; Wijnen and Young, 2006). These mutants may sporulate rhythmically in the absence of external cues, or when subjected to temperature cycles (Dunlap and Loros, 2004; Dunlap et al., 2004; Lakin-Thomas and Brody, 2004; Lakin-Thomas, 2006). Unlike the intact *frq*–*wc* system, temperature compensation is often poor, typical circadian entrainment is lost, and the oscillation period can differ widely from circadian. A current model of general fungal rhythmicity envisages a system of FLO's, which may be co-ordinated by a central circadian system when this is present, but which can run on their own when it is not (Dunlap et al., 2004). One FLO of particular interest in this context occurs in nitrate reductase in *Neurospora* (Christensen et al., 2004). This FLO controls an approximately diurnal cycle in nitrate assimilation activity in *frq*- and *wc*-null mutants, which is not temperature-compensated, and may be mediated by a feedback loop in nitrate assimilation, where nitrate uptake periodically feeds back to reduce the level of assimilating activity (Dunlap et al., 2004).

We have previously developed a kinetic model of nutrient storage and loading into a transport pathway that could underlie amino acid transport pulsation and explain phase differences between the inoculum and foraging mycelium in the absence of an added resource (Tlalka et al., 2003). To generate oscillatory behaviour, the model required sequential vacuolar accumulation and release of amino acids, which were triggered at fixed concentration thresholds, coupled to translocation away from the inoculum. In this paper, addition of the new resource causes a switch in the oscillation for a localised region of mycelium to establish a new, synchronised domain. As there is no change in the external N-status of the resource, it is not possible to generate this type of behaviour in the existing model without introducing additional regulatory processes (data not shown). Furthermore, we have preliminary evidence that similar oscillations can be observed with non-metabolised glucose analogues (Fricker et al., 2007; Bebber et al., unpublished), that would argue against a mechanism dependent on specific kinetic properties of amino acid transporters. Thus at this stage the clearly demarcated phase domains correlate well with different predicted functional activities in the mycelium, but do not lend themselves to a simple mechanistic model.

Synchronisation throughout the interconnected network of hyphal tubes within each domain may be more easily explained. There is an extensive literature documenting the emergent properties of coupled oscillatory systems (see for example Strogatz, 2001; Lloyd and Murray, 2005; Aon et al., 2007) and it is likely that synchronised oscillatory behaviour arises spontaneously from weak coupling through metabolic intermediates (Lloyd and Murray, 2005; Lloyd, 2006; Aon et al., 2007; Murray et al., 2007).

Such coupled oscillatory behaviour may underpin co-ordination of metabolic and physiological activity in basidiomycete fungi, as with other simple microbial systems (Gerisch, 1987; Dormann et al., 2002; Lloyd and Murray, 2005, 2006a; Ueda, 2005; Lloyd, 2006). This may represent a general principle used by self-organising biological systems to improve metabolic efficiency (Lloyd and Murray, 2005; Tu and McKnight, 2006), achieve global co-ordination without centralised control, and in spatial networks, such as mycelial fungi and acellular slime moulds, provide a mechanism to solve complex routing problems (Nakagaki et al., 2000, 2004; Tero et al., 2006).

Acknowledgments

We gratefully acknowledge financial support from the NERC (GR3/12946 & NER/A/S/2002/882), BBSRC (43/P19284) and from the Dunstan Bequest (University of Oxford). We thank Prof. J.A. Langdale for many helpful comments on the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fgb.2007.02.013.

References

- Aon, M.A., Cortassa, S., Lemar, K.M., Hayes, A.J., Lloyd, D., 2007. Single and cell population respiratory oscillations in yeast: a 2-photon scanning laser microscopy study. *FEBS Lett.* 581, 8–14.
- Aro, N., Pakula, T., Penttila, M., 2005. Transcriptional regulation of plant cell wall degradation by filamentous fungi. *FEMS Microbiol. Rev.* 29, 719–739.
- Bahn, Y.S., Xue, C.Y., Idnurm, A., Rutherford, J.C., Heitman, J., Cardenas, M.E., 2007. Sensing the environment: lessons from fungi. *Nat. Rev. Microbiol.* 5, 57–69.
- Bebber, D.P., Tlalka, M., Hynes, J., Darrach, P.R., Ashford, A., Watkinson, S.C., Boddy, L., Fricker, M.D., 2007. Imaging complex nutrient dynamics in mycelial networks. In: *Fungi in the Environment*. Cambridge University Press.
- Bell-Pedersen, D., Cassone, V.M., Earnest, D.J., Golden, S.S., Hardin, P.E., Thomas, T.L., Zoran, M.J., 2005. Circadian rhythms from multiple oscillators: lessons from diverse organisms. *Nat. Rev. Genet.* 6, 544–556.
- Boddy, L., 1999. Saprotrophic cord-forming fungi: meeting the challenge of heterogeneous environments. *Mycologia* 91, 13–32.
- Brownlee, C., Jennings, D.H., 1982. Long-distance translocation in *Serpula lacrimans*—velocity estimates and the continuous monitoring of induced perturbations. *Trans. Brit. Mycol. Soc.* 79, 143–148.
- Cairney, J.W.G., 1992. Translocation of solutes in ectomycorrhizal and saprotrophic rhizomorphs. *Mycol. Res.* 96, 135–141.
- Cairney, J.W.G., 2005. Basidiomycete mycelia in forest soils: dimensions, dynamics and roles in nutrient distribution. *Mycol. Res.* 109, 7–20.
- Chavez, R., Bull, P., Eyzaguirre, J., 2006. The xylanolytic enzyme system from the genus *Penicillium*. *J. Biotechnol.* 123, 413–433.
- Christensen, M.K., Falkeid, G., Loros, J.J., Dunlap, J.C., Lillo, C., Ruoff, P., 2004. A nitrate-induced *frq*-less oscillator in *Neurospora crassa*. *J. Biol. Rhythms* 19, 280–286.
- Clipson, N.J.W., Cairney, J.W.G., Jennings, D.H., 1987. The physiology of basidiomycete linear organs .1. Phosphate-uptake by cords and mycelium in the laboratory and the field. *New Phytol.* 105, 449–457.

- Cooper, T.G., 2002. Transmitting the signal of excess nitrogen in *Saccharomyces cerevisiae* from the Tor proteins to the GATA factors: connecting the dots. *FEMS Microbiol. Rev.* 26, 223–238.
- Currie, W.S., 1999. The responsive C and N biogeochemistry of the temperate forest floor. *Trends Ecol. Evol.* 14, 316–320.
- Currie, W.S., Nadelhoffer, K.J., Aber, J.D., 2004. Redistributions of N¹⁵ highlight turnover and replenishment of mineral soil organic N as a long-term control on forest C balance. *Forest Ecol. Manag.* 196, 109–127.
- Darrah, P.R., Tlalka, M., Ashford, A., Watkinson, S.C., Fricker, M.D., 2006. The vacuole system is a significant intracellular pathway for longitudinal solute transport in basidiomycete fungi. *Eukaryot. Cell* 5, 1111–1125.
- Divon, H.H., Fluhr, R., 2007. Nutrition acquisition strategies during fungal infection of plants. *FEMS Microbiol. Lett.* 266, 65–74.
- Dormann, D., Vasiev, B., Weijer, C.J., 2002. Becoming multicellular by aggregation; the morphogenesis of the social amoebae *Dictyostelium discoideum*. *J. Biol. Phys.* 28, 765–780.
- Dunlap, J.C., Loros, J.J., 2004. The *Neurospora* circadian system. *J. Biol. Rhythms* 19, 414–424.
- Dunlap, J.C., Loros, J.J., 2005. Analysis of circadian rhythms in *Neurospora*: overview of assays and genetic and molecular biological manipulation. In: *Circadian Rhythms*. pp 3–22.
- Dunlap, J.C., Loros, J.J., Lee, K., Froehlich, A., Colot, H., Shi, M., Pregelero, A., 2004. The genetics and molecular biology of circadian rhythms. In: Brambl, R., Marzluf, G.A. (Eds.), *The Mycota III. Biochemistry and Molecular Biology*. Springer-Verlag, Berlin, pp. 209–229.
- Ettema, C.H., Wardle, D.A., 2002. Spatial soil ecology. *Trends Ecol. Evol.* 17, 177–183.
- Ferguson, B.A., Dreisbach, T.A., Parks, C.G., Filip, G.M., Schmitt, C.L., 2003. Coarse-scale population structure of pathogenic *Armillaria* species in a mixed-conifer forest in the Blue Mountains of northeast Oregon. *Can. J. Forest Res.* 33, 612–623.
- Flipphi, M., van de Vondervoort, P.J.I., Ruijter, G.J.G., Visser, J., Arst, H.N., Felenbok, A., 2003. Onset of carbon catabolite repression in *Aspergillus nidulans*—parallel involvement of hexokinase and glucokinase in sugar signaling. *J. Biol. Chem.* 278, 11849–11857.
- Frey, S.D., Elliott, E.T., Paustian, K., Peterson, G.A., 2000. Fungal translocation as a mechanism for soil nitrogen inputs to surface residue decomposition in a no-tillage agroecosystem. *Soil Biol. Biochem.* 32, 689–698.
- Frey, S.D., Six, J., Elliott, E.T., 2003. Reciprocal transfer of carbon and nitrogen by decomposer fungi at the soil-litter interface. *Soil Biol. Biochem.* 35, 1001–1004.
- Fricker, M.D., Tlalka, M., Beber, D.P., Tagaki, S., Watkinson, S.C., Darrah, P.R., 2007. Fourier-based spatial mapping of oscillatory phenomena in fungi. *Fungal Genet. Biol.*, in press. doi:10.1016/j.fgb.2007.02.012.
- Gerisch, G., 1987. Cyclic-AMP and other signals controlling cell-development and differentiation in *Dictyostelium*. *Annu. Rev. Biochem.* 56, 853–879.
- Govindarajulu, M., Pfeffer, P.E., Jin, H.R., Abubaker, J., Douds, D.D., Allen, J.W., Bucking, H., Lammers, P.J., Shachar-Hill, Y., 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. *Nature* 435, 819–823.
- Granlund, H.I., Jennings, D.H., Thompson, W., 1985. Translocation of solutes along rhizomorphs of *Armillaria mellea*. *Trans. Brit. Mycol. Soc.* 84, 111–119.
- He, X.H., Critchley, C., Bledsoe, C., 2003. Nitrogen transfer within and between plants through common mycorrhizal networks (CMNs). *Crit. Rev. Plant Sci.* 22, 531–567.
- Inoki, K., Ouyang, H., Li, Y., Guan, K.L., 2005. Signaling by target of rapamycin proteins in cell growth control. *Microbiol. Mol. Biol. Rev.* 69, 79, +.
- Iwasaki, H., Dunlap, J.C., 2000. Microbial circadian oscillatory systems in *Neurospora* and *Synechococcus*: models for cellular clocks. *Curr. Opin. Microbiol.* 3, 189–196.
- Jammalamadaka, S.R., SenGupta, A., 2001. *Topics in Circular Statistics*. World Scientific, Publishing, London, 336 p.
- Jennings, D.H., 1987. Translocation of solutes in fungi. *Biol. Rev.* 62, 215–243.
- Kim, K.W., Roon, R.J., 1982. Transport and metabolic effects of alpha-aminoisobutyric-acid in *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta* 719, 356–362.
- Kippert, F., 2001. Cellular signalling and the complexity of biological timing: insights from the ultradian clock of *Schizosaccharomyces pombe*. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 356, 1725–1733.
- Klevecz, R.R., Bolen, J., Forrest, G., Murray, D.B., 2004. A genome-wide oscillation in transcription gates DNA replication and cell cycle. *Proc. Natl. Acad. Sci. USA* 101, 1200–1205.
- Lakin-Thomas, P.L., 2006. New models for circadian systems in microorganisms. *FEMS Microbiol. Lett.* 259, 1–6.
- Lakin-Thomas, P.L., Brody, S., 2004. Circadian rhythms in microorganisms: New complexities. *Annu. Rev. Microbiol.* 58, 489–519.
- Li, L., Borkovich, K.A., 2006. GPR-4 Is a predicted G-protein-coupled receptor required for carbon source-dependent asexual growth and development in *Neurospora crassa*. *Eukaryot. Cell* 5, 1287–1300.
- Lilly, W.W., Higgins, S.M., Wallweber, G.J., 1990. Uptake and translocation of 2-aminoisobutyric acid by *Schizophyllum commune*. *Exp. Mycol.* 14, 169–177.
- Lindahl, B., Finlay, R., Olsson, S., 2001. Simultaneous, bidirectional translocation of ³²P and ³³P between wood blocks connected by mycelial cords of *Hypholoma fasciculare*. *New Phytol.* 150, 189–194.
- Lindahl, B.O., Taylor, A.F.S., Finlay, R.D., 2002. Defining nutritional constraints on carbon cycling in boreal forests—towards a less ‘phytcentric’ perspective. *Plant Soil* 242, 123–135.
- Liu, Y., Bell-Pedersen, D., 2006. Circadian rhythms in *Neurospora crassa* and other filamentous fungi. *Eukaryot. Cell* 5, 1184–1193.
- Lloyd, D., 2006. Ultradian rhythms and clocks in plants and yeast. *Biol. Rhythm Res.* 37, 281–296.
- Lloyd, D., Murray, D., 2006a. An ultradian clock in yeast: metronome for intracellular coherence. *FEBS J.* 273, 5–6.
- Lloyd, D., Murray, D.B., 2005. Ultradian metronome: timekeeper for orchestration of cellular coherence. *Trends Biochem. Sci.* 30, 373–377.
- Lloyd, D., Murray, D.B., 2006b. The temporal architecture of eukaryotic growth. *FEBS Lett.* 580, 2830–2835.
- Malhi, Y., Baldocchi, D.D., Jarvis, P.G., 1999. The carbon balance of tropical, temperate and boreal forests. *Plant Cell Environ.* 22, 715–740.
- Murray, D.B., Beckmann, M., Kitano, H., 2007. Regulation of yeast oscillatory dynamics. *PNAS* 104, 2241–2246.
- Nakagaki, T., Yamada, H., Hara, M., 2004. Smart network solutions in an amoeboid organism. *Biophys. Chem.* 107, 1–5.
- Nakagaki, T., Yamada, H., Toth, A., 2000. Maze-solving by an amoeboid organism. *Nature* 407, 470.
- Nielsen, J.S., Jøner, E.J., Declerck, S., Olsson, S., Jakobsen, I., 2002. Phospho-imaging as a tool for visualization and noninvasive measurement of P transport dynamics in arbuscular mycorrhizas. *New Phytol.* 154, 809–819.
- Ogilvie-Villa, S., Debusk, R.M., Debusk, A.G., 1981. Characterization of 2-aminoisobutyric acid transport in *Neurospora Crassa* - a general amino-acid permease-specific substrate. *J. Bacteriol.* 147, 944–948.
- Olsson, S., Gray, S.N., 1998. Patterns and dynamics of ³²P-phosphate and labelled 2-aminoisobutyric acid (¹⁴C-AIB) translocation in intact basidiomycete mycelia. *FEMS Microbiol. Ecol.* 26, 109–120.
- Olsson, S., Jennings, D.H., 1991. Evidence for diffusion being the mechanism of translocation in the hyphae of 3 molds. *Exp. Mycol.* 15, 302–309.
- Palmer, G.E., Horton, J.S., 2006. Mushrooms by magic: making connections between signal transduction and fruiting body development in the basidiomycete fungus *Schizophyllum commune*. *FEMS Microbiol. Lett.* 262, 1–8.
- Rayner, A.D.M., Griffith, G.S., Ainsworth, A.M., 1994. Mycelial interconnectedness. In: Gow, N.A.R., Gadd, G.M. (Eds.), *The Growing Fungus*. Chapman and Hall, London, pp. 21–40.

- Read, D.J., Perez-Moreno, J., 2003. Mycorrhizas and nutrient cycling in ecosystems - a journey towards relevance? *New Phytol.* 157, 475–492.
- Rohde, J., Heitman, J., Cardenas, M.E., 2001. The TOR kinases link nutrient sensing to cell growth. *J. Biol. Chem.* 276, 9583–9586.
- Rohde, J.R., Cardenas, M.E., 2003. Nutrient signaling through TOR kinases controls gene expression and cellular differentiation in fungi. In: *Tor-Target of Rapamycin*. pp 53–72.
- Rolland, F., Baena-Gonzalez, E., Sheen, J., 2006. Sugar sensing and signalling in plants: conserved and novel mechanisms. *Annu. Rev. Plant Biol.* 57, 675–709.
- Rolland, F., Winderickx, J., Thevelein, J.M., 2001. Glucose-sensing mechanisms in eukaryotic cells. *Trends Biochem. Sci.* 26, 310–317.
- Rolland, F., Winderickx, J., Thevelein, J.M., 2002. Glucose-sensing and -signalling mechanisms in yeast. *FEMS Yeast Research* 2, 183–201.
- Santangelo, G.M., 2006. Glucose signaling in *Saccharomyces cerevisiae*. *Microbiology and Molecular Biology Reviews* 70, 253, +.
- Simard, S.W., Durall, D.M., 2004. Mycorrhizal networks: a review of their extent, function, and importance. *Can. J. Bot.* 82, 1140–1165.
- Simard, S.W., Perry, D.A., Jones, M.D., Myrold, D.D., Durall, D.M., Molina, R., 1997. Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature* 388, 579–582.
- Smith, M.L., Bruhn, J.N., Anderson, J.B., 1992. The fungus *Armillaria Bulbosa* is among the largest and oldest living organisms. *Nature* 356, 428–431.
- Strogatz, S.H., 2001. Exploring complex networks. *Nature* 410, 268–276.
- Tero, A., Kobayashi, R., Nakagaki, T., 2006. *Physarum* solver: a biologically inspired method of road-network navigation. *Physica A* 363, 115–119.
- Tlalka, M., Hensman, D., Darrah, P.R., Watkinson, S.C., Fricker, M.D., 2003. Noncircadian oscillations in amino acid transport have complementary profiles in assimilatory and foraging hyphae of *Phanerochaete velutina*. *New Phytol.* 158, 325–335.
- Tlalka, M., Watkinson, S.C., Darrah, P.R., Fricker, M.D., 2002. Continuous imaging of amino-acid translocation in intact mycelia of *Phanerochaete velutina* reveals rapid, pulsatile fluxes. *New Phytol.* 153, 173–184.
- Tu, B.P., Kudlicki, A., Rowicka, M., McKnight, S.L., 2005. Logic of the yeast metabolic cycle: temporal compartmentalization of cellular processes. *Science* 310, 1152–1158.
- Tu, B.P., McKnight, S.L., 2006. Opinion—metabolic cycles as an underlying basis of biological oscillations. *Nat. Rev. Mol. Cell Biol.* 7, 696–701.
- Ueda, T., 2005. An intelligent slime mold: a self-organizing system of cell shape and information. In: Armbruster, D., Kaneko, K., Mikhailov, A.S. (Eds.), *Networks of Interacting Machines: Production Organisation in Complex Industrial Systems and Biological Cells*. World Scientific, Singapore, pp. 1–35.
- Watkinson, S.C., 1984. Inhibition of growth and development of *Serpula lacrimans* by the non-metabolized amino-acid analog alpha-aminoisobutyric-acid. *FEMS Microbiol. Lett.* 24, 247–250.
- Watkinson, S.C., Bebbler, D., Darrah, P.R., Fricker, M.D., Tlalka, M., Boddy, L., 2006. The role of wood decay fungi in the carbon and nitrogen dynamics of the forest floor. In: Gadd, G.M. (Ed.), *Fungi in Biogeochemical Cycles*. Cambridge University Press, Cambridge, pp. 151–181.
- Wells, J.M., Boddy, L., Evans, R., 1995. Carbon translocation in mycelial cord systems of *Phanerochaete velutina* (Dc, Pers) Parmasto. *New Phytol.* 129, 467–476.
- Wells, J.M., Harris, M.J., Boddy, L., 1998. Temporary phosphorus partitioning in mycelial systems of the cord-forming basidiomycete *Phanerochaete velutina*. *New Phytol.* 140, 283–293.
- Wells, J.M., Harris, M.J., Boddy, L., 1999. Dynamics of mycelial growth and phosphorus partitioning in developing mycelial cord systems of *Phanerochaete velutina*: dependence on carbon availability. *New Phytol.* 142, 325–334.
- Wells, J.M., Hughes, C., Boddy, L., 1990. The fate of soil-derived phosphorus in mycelial cord systems of *Phanerochaete velutina* and *Phallus impudicus*. *New Phytol.* 114, 595–606.
- Wijnen, H., Young, M.W., 2006. Interplay of circadian clocks and metabolic rhythms. *Annu. Rev. Genet.* 40, 409–448.