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MiniReview

Conidial anastomosis tubes in filamentous fungi

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Abstract

Conidial anastomosis tubes (CATs) can be recognized in 73 species of filamentous fungi covering 21 genera, and develop in culture and in host-pathogen systems. They have been shown to be morphologically and physiologically distinct from germ tubes in *Colletotrichum* and *Neurospora*, and under separate genetic control in *Neurospora*. CATs are short, thin, usually unbranched and arise from conidia or germ tubes. Their formation is conidium-density dependent, and CATs grow towards each other. MAP kinase mutants of *Neurospora* are blocked in CAT induction. Nuclei pass through fused CATs and are potential agents of gene exchange between individuals of the same and different species. CAT fusion may also serve to improve the chances of colony establishment.
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Keywords: Hyphal fusion; Conidia; Gene transfer; Incompatibility; *Colletotrichum*; *Neurospora*

1. Introduction

The conidium is the main type of asexual spore produced by fungi, and is particularly characteristic of the Ascomycota and Basidiomycota. It is also the defining cell type of the mitosporic fungi (previously called Fungi Imperfecti or Deuteromycota) which lack a recognizable sexual stage, although the majority of this group has been found to belong to the Ascomycota [1].

Under appropriate conditions, a conidium germinates to form a tip-growing germ tube that extends and successively branches to establish the fungal colony. A colony can arise from a single spore but it has been long appreciated that conidia and conidial germlings in close vicinity to each other commonly undergo fusion to produce an interconnected network of germlings (Figs. 1–3). In 1863, Tulasne and Tulasne [2] first reported fusions between conidia and conidial germlings in several species (Fig. 1). Since then we have found descriptions of this phenomenon in 21 genera and 73 species (Table 1). However, what has not been appreciated until recently is that this process of fusion between conidia and/or conidial germlings involves the formation and interaction of specialized hyphae, called *conidial anastomosis tubes* (CATs) [3,4].

The aims of this minireview are to: (1) describe the defining characteristics of CATs; (2) review recent insights that have been gained into their cell biology; (3) discuss their possible roles; and (4) define key questions which need to be addressed about their biology.

2. Characteristics of CATs

CATs were first described as being morphologically and physiologically distinct from conidial germ tubes

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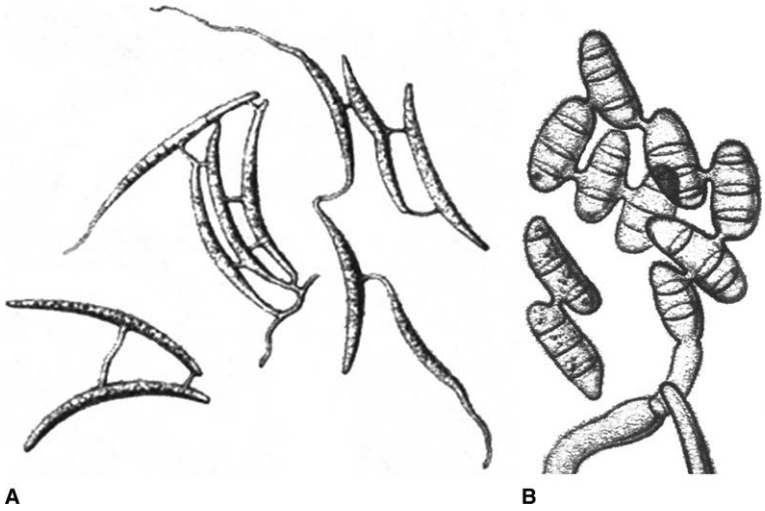

Fig. 1. Two drawings from the earliest description of fusions between conidia and conidial germlings. (A) *Cryptospora auta*; (B) *Hypomyces rosellus.* (Reproduced from [2].)

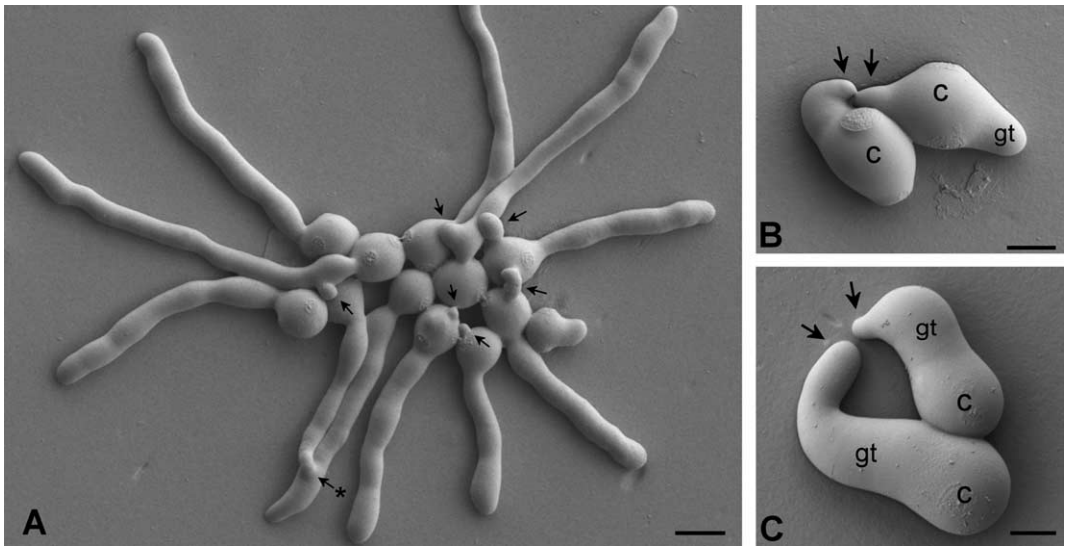

Fig. 2. CAT fusion between conidia and conidial germlings in *Neurospora crassa* imaged by low-temperature scanning electron microscopy. (A) Conidial germlings interconnected by CATs (arrows). Note that the wider germlings are growing away from each other. Most CATs are formed directly from conidia but some also form from germ tubes (asterisk). Bar = 10 μm. (B, C) CATs (arrows) emerging directly from conidia and homing towards each other. Bar = 5 μm.

in the plant pathogen, *Colletotrichum lindemuthianum* [3]. Subsequently, CATs were characterized in *Neurospora crassa* in which they were also shown to be under separate genetic control from germ tubes [4]. These studies identified the following features which distinguish CATs from germ tubes (Figs. 2 and 3): (1) CATs are thinner, shorter and exhibit determinate growth; (2) CATs are usually unbranched; (3) CAT induction is dependent on conidial density; (4) CATs home towards each other whilst germ tubes avoid each other; and (5) CATs are under separate genetic control.

In *C. lindemuthianum*, CATs have been shown to emerge from conidia: (1) within the asexual spore-producing structure, the acervulus [3]; (2) in vitro after removal from acervuli (Fig. 3A) [3]; (3) on the surface of the leaf (Fig. 3B) [5]; and (4) in anthracnose lesions on bean pods [3]. However, only conidia removed from acervuli form germ tubes because germination self-inhibitors are produced in these spore-producing structures [6,7]. In *N. crassa*, both CATs and germ tubes emerge from conidia during a similar time period in culture (Fig. 2) [4]. CAT frequency can vary: in *Colletotrichum,* 10% of conidia can participate in CAT fusions within the acervulus [3]; in *Neurospora,* up to 50% of the conidia can participate in CAT fusions during germination in vitro [4].

Neurospora crassa produces three types of conidia: macroconidia, microconidia and arthroconidia [8]. All

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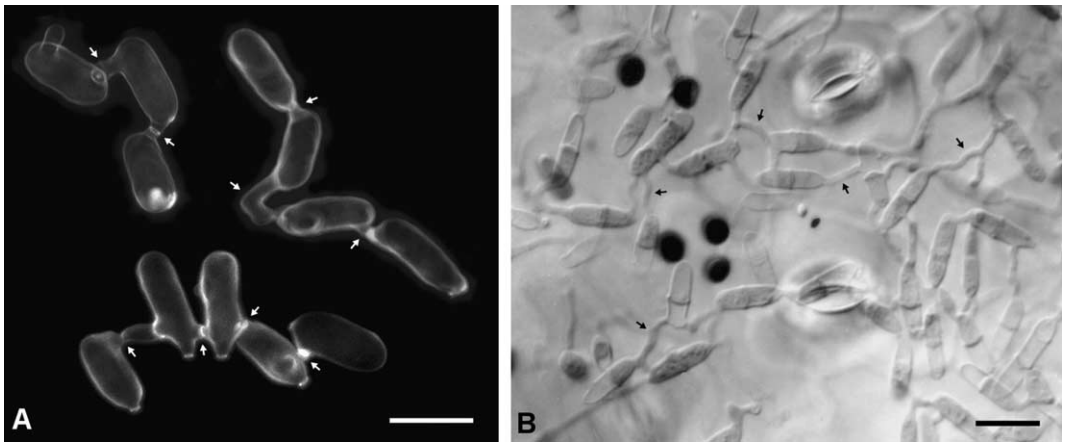

Fig. 3. Chains of conidia of *Colletotrichum* spp. fused together by CATs. (A) Confocal microscopy of fused conidia of *C. lindemuthianum* isolated from an acervulus and stained with Calcofluor White M2R. Arrows indicate fused CATs. Bar = 15 μm. (B) Light microscopy of fused conidia of *Colletotrichum* sp. on the surface of a cowpea leaf (from [5] with permission). Bar = 20 μm.

Table 1
Phylogenetic distribution of CATs[a] in the Ascomycota and mitosporic fungi

Genus	Number of species	References
Arthrobotrys	2	[44]
Aspergillus	20	[28]
Botrytis	3	[18,39]
Colletotrichum	6	[3,5,45–47]
Cryptospora	1	[2]
Dissconium	2	[30]
Fusarium	4	[18,48]
Gloeosporium	1	[46]
Hypocrea	1	[2,12]
Hypomyces	1	[2]
Leptosphaeria	1	[29]
Monascus	1	[28]
Monilia	1	[29]
Neurospora	3	[4,18]
Nectria	2	[2,49]
Penicillium	17	[25,28]
Pleospora	1	[2]
Sclerotinia	2	[18]
Sordaria	2	[11]
Venturia	1	[14]
Verticillium	1	[50]

[a] CATs have been simply defined here as hyphae connecting conidia or conidial germ tubes.

conidial types produce CATs and a CAT from one conidial type will fuse with a CAT of another [4]. Two types of CATs are found in *N. crassa*. The first type is the most common and emerges directly from the conidium (Fig. 2A and B) while the second type develops from the germ tube (Fig. 2A and C). The latter CATs are morphologically different from many of the fusion hyphae which form in subperipheral regions of mature colonies; mycelial fusion hyphae are usually wider and often dichotomously branched [9]. However, it is not known whether the short, unbranched fusion hyphae (or pegs), which are also common in the mature colony [9,10], are different from CATs arising from germ tubes [4].

Organelles (e.g. nuclei) have been shown to pass through fused CATs (Fig. 4) [3,4]. In addition, microtubules have been found to extend through CATs from both conidial germlings which have fused, and as a result become intermixed [4].

By definition, CATs arise from conidia but other spore types also produce CAT-like structures. For example, ascospores and urediospores of the Ascomycota and Basidiomycota, respectively, develop specialized hyphae or branches from their germ tubes which home towards each other and fuse [11–13].

3. Cell biology of CATs

The cell biology of CATs can be divided into three phases: (a) CAT induction; (b) CAT homing; and (c) CAT fusion (Fig. 5A).

3.1. CAT induction

Evidence for an extracellular CAT inducer has been obtained in *N. crassa* and *Venturia inaequalis* because CAT induction is dependent on conidium density [4,14]. CAT induction in these cases thus seems to involve a form of quorum sensing (i.e. a mechanism in which cells monitor their population density by releasing signal molecules into their environment). The CAT inducer in *N. crassa* was shown not to be cyclic AMP (cAMP) because a mutant lacking cAMP formed CATs [4].

The CAT inducer signal in *N. crassa* seems to activate a mitogen-activated protein (MAP) kinase cascade which has orthologues in the MAP kinase pathway involved in pheromone signalling in *Saccharomyces*

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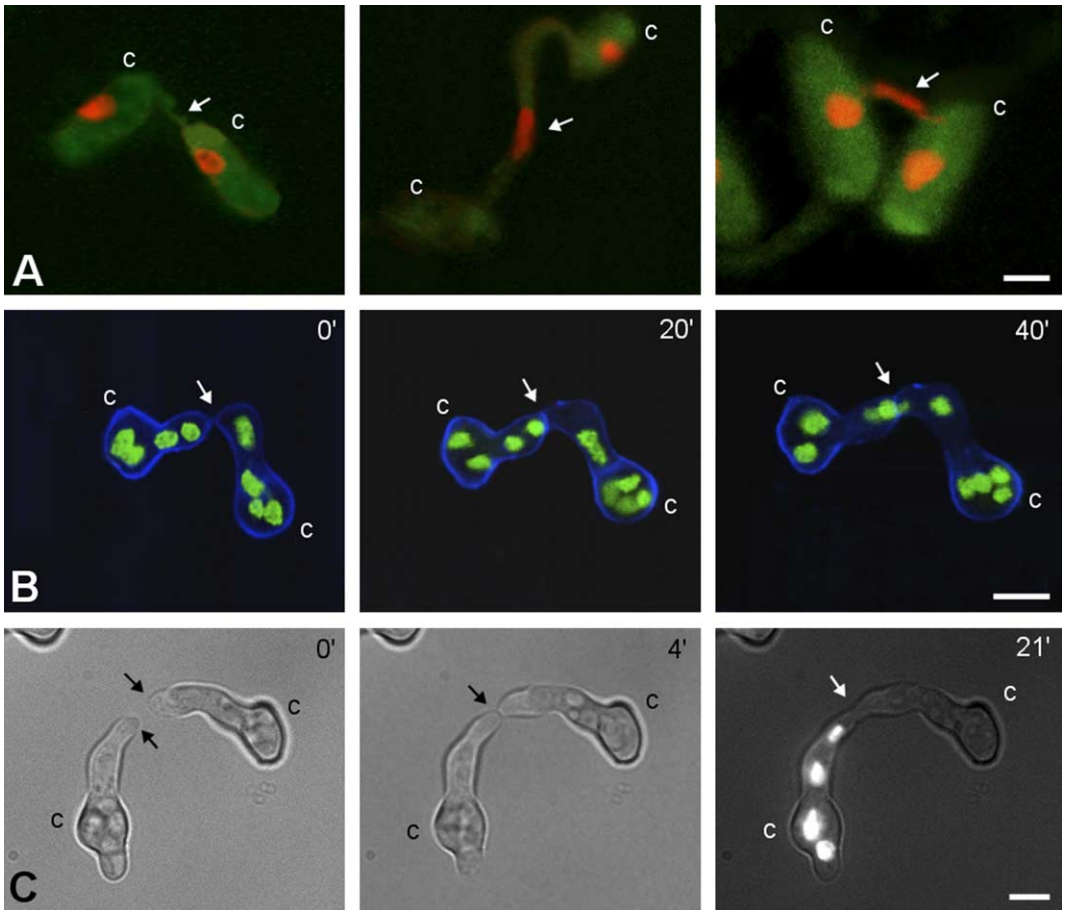

Fig. 4. Nuclear behaviour during CAT homing and fusion. (A) *Colletotrichum lindemuthianum*. Left image: fused CATs with each conidium containing one nucleus. Centre image: nucleus (arrow) from one conidium migrating through fused CATs (from [3] with permission). Right image: nucleus (arrow) migrating through fused CATs from one of the conidia; this nucleus must have arisen by division of the initially single nucleus found in a conidium. Conidial germlings fixed, stained with propidium iodide (red) and FITC-phalloidin (green) and imaged by confocal microscopy. (B) Time course showing two CATs of *Neurospora crassa* of the same mating type (*mat a*) which have homed towards and made contact with each other (0 min), fused (20 min) and through which nuclei are migrating (40 min). The arrows indicate the point of CAT contact and fusion. Confocal microscopy of germlings labelled with H1-GFP (nuclei shown in green) and Calcofluor White M2R (cell walls shown in blue). (C) Time course showing two CATs of *N. crassa* of different mating types homing towards each other (0 min, the arrows indicate the tips of the two CATs), having made contact (4 min) and fused (21 min). The right-hand germling is a *mat a* strain labelled with H1-GFP targeted to nuclei. The first two images in the sequence have been imaged with brightfield optics; the last image is a confocal image showing fluorescent nuclei which has been superimposed on the brightfield image. Bars = 5 μm.

cerevisiae [4,15]. Two mutants blocked in CAT induction are mutated in genes encoding a MAP kinase kinase kinase (NRC-1) and a MAP kinase (MAK-2) (Fig. 5). Furthermore, deletion of the orthologue of the yeast gene *ste12,* which encodes the transcription factor that is the downstream target of the pheromone response MAP kinase pathway, is also a hyphal fusion mutant with a similar phenotype to *nrc-1* and *mak-2* mutants [16]. It is not clear which receptor or other upstream components activate the MAP kinase pathway. Possible candidates include G-protein coupled receptors or a two-component signalling system [16].

Another mutant in *N. crassa* unable to form CATs is *ham-2* [4]. HAM-2 is a putative transmembrane protein [17], but its role in CAT induction is not known [4] (Fig. 5).

3.2. CAT homing

To our knowledge, Köhler in 1930, first showed CATs growing towards each other and proposed that "these growth reactions were due to substances secreted by the fungal hyphae" [18]. The CAT chemoattractant remains unidentified. A novel assay to analyse CAT homing, involving the use of optical tweezer micromanipulation, has been developed [4]. This technique allows an individual conidium or conidial germling to be optically trapped and moved relative to another conidium or germling. When CATs of the wild type which were growing towards each other were moved apart their tips subsequently grew back towards each other indicating that the CAT tips were both the sites of chemoattractant secretion and reception [4].

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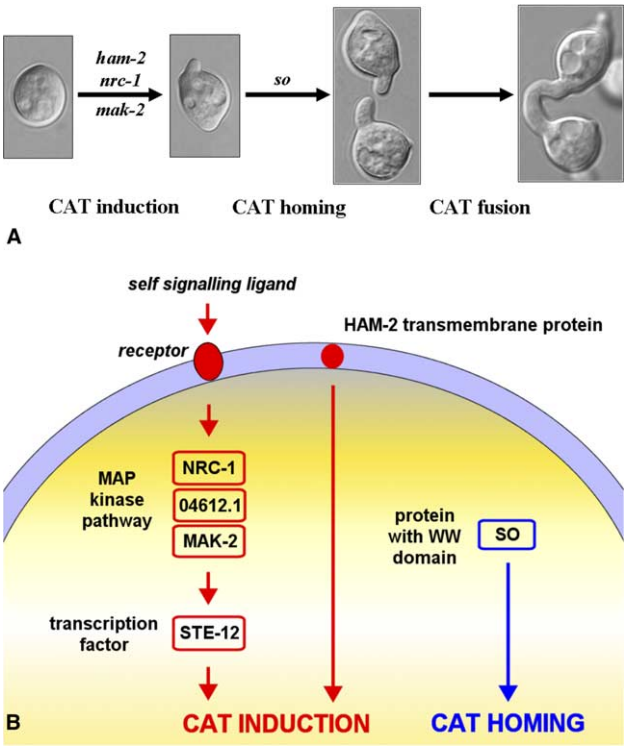

Fig. 5. CAT induction, homing and fusion in *Neurospora crassa*, and signalling which occur during CAT induction and signalling. (A) Mutants blocked in CAT induction and homing. (B) Model of the signalling pathways involved in CAT induction and homing. 04612.1 is the *N. crassa* NCU number (http://www.broad.mit.edu/annotation/fungi/neurospora/) for the predicted orthologue of *STE7* in *Saccharomyces cerevisiae*.

The extracellular chemoattractant in *N. crassa* is not cAMP since a mutant lacking cAMP undergoes normal homing, as assessed with the optical tweezer assay [4]. It is likely that the chemoattractant is a peptide because this would provide more species specificity than a molecule such as cAMP (see Section 4.2). Peptide sex pheromones that orient hyphal or yeast cell growth towards other hyphae or yeasts cells are well documented in the sexual stages of a number of fungal species [19–21]. However, in contrast to the non-self interactions involved in the sexual phase, CAT formation and homing are commonly responses to extracellular signals between the same genotype (i.e. they involve self-signalling ligands).

A *N. crassa* mutant in the *so* gene still forms CATs, but they are unable to home towards or fuse with other CATs of the *so* mutant or wild type (Fig. 5A). The *so* mutation seems to confer a defect in the biochemical machinery involved in the synthesis and/or secretion of the chemoattractant and in the signalling apparatus involved in the perception and/or transduction of the chemoattractant signal. Nevertheless, this mutant undergoes normal trichogyne homing towards, and fusion with, conidia during the sexual phase, indicating that the mechanism of homing between CATs during the vegetative phase is different [22].

3.3. CAT fusion

Following contact, the tips of two CATs adhere, a fusion pore forms between them and cytoplasmic and organelle mixing occurs. Adhesion and fusion pore formation presumably involve the secretion of extracellular adhesives and extracellular cell wall-degrading enzymes, respectively. Similar processes have been described during fusion between mycelial fusion hyphae [9,23]. However, one difference between these processes is that the organelle fluxes between fused conidial germlings are typically several orders of magnitude slower than those between fused mycelial fusion hyphae (Fig. 4B and C).

In *Colletotrichum*, conidia are initially uninucleate but after 15 days of acervulus (asexual fruitbody) development, and coincident with the onset of anastomoses, an increasing fraction of conidial nuclei undergo division, migration and fragmentation. Sometimes this is accompanied by nuclear loss from one of the conidia (Fig. 4A) [3].

4. CAT homing and fusion between different conidial genotypes

Self-fusions here are fusions that occur between conidia and/or conidial germlings of the same genotype (i.e. the same strain). Non-self fusions are conversely fusions between different strains (and thus different genotypes).

4.1. Non-self fusions in the same species

Heterokaryosis resulting from hyphal fusion, causes an incompatible response, and ultimately cell death, if the hyphae which fuse contain nuclei with genetically different heterokaryon incompatibility genes, such as *het* genes in *N. crassa* [24]. Evidence has also been obtained for pre-fusion recognition by vegetatively incompatible strains [4]. Strains of *N. crassa* which are of opposite mating type are vegetatively incompatible because they contain the different *mat A-1* and *mat a-1* *het* genes. When combined, the CATs of strains of opposite mating type homed towards each other. Although these CATs made contact with each other, it was found that there was a 50% reduction in complete CAT fusion that resulted in cytoplasmic continuity being achieved between the interacting CATs [4]. The mechanistic basis of this inhibition is unknown.

Cytological and genetic evidence that heterokaryons can form via CAT fusions without an incompatible response have been obtained in *Penicillium notatum* [25] and *C. lindemuthianum* [3,26]. Furthermore, in *N. crassa*, the vegetative incompatible response following CAT fusion between vegetatively incompatible strains is significantly delayed compared with that following the fusion

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of mycelial fusion hyphae in the mature colony [Roca, M.G. unpubl; Jacobson, D.J. Pers. Comm.]. These studies raise the question of how the incompatible responses in these cases might have been suppressed (see Section 6.3).

Heterokaryosis can also result in parasexuality, a term devised by Pontecorvo [29] from studies on *Aspergillus nidulans* to account for the creation of diploids and subsequent mitotic crossing over and haploidization by the loss of individual chromosomes. Heterokaryons were produced in these experiments by growing pairs of complementary auxotrophic mutants together on minimal medium. It has been assumed that the actual mechanism for generating these heterokaryons is through anastomoses between mycelial fusion hyphae [27], but it is possible that CAT fusion may also be important in mediating this process.

4.2. Inter-specific fusions

Homing between CATs of different species was first analysed by Köhler [18] who found that CATs homed towards each other in some species combinations. In no case did he obtain clear evidence of CAT fusion. Ishitani and Sakaguchi [28] later showed that *A. oryzae* was able to undergo CAT fusion with *Monoascus* and a large number of other *Aspergillus* and *Penicillium* species although the progeny of these fusions were not analysed.

Different species of *Colletotrichum* were combined using morphological and genetic markers (spore colour, shape and size, cultural characteristics, and a PCR marker for the hygromycin-resistance gene) to detect hybrid progeny [26]. High-density co-inoculation was made with conidia from a hygromycin resistant strain of *C. lindemuthianum* and a hygromycin-sensitive strain of *C. gossypii*. The hybrids exhibited morphological and genetic properties of both parents when grown on hygromycin-selective medium. When propagated via monosporic culture through eight monthly passages, hybrid characters were maintained, sometimes even in the absence of selection, although patchy sectoring of spore colour was observed. One hybrid grown on different bean cultivars was found to be one of the most pathogenic strains of *Colletotrichum* infecting beans so far reported [26]. These data strongly suggest that the colonies contained recombinant nuclei that were of mixed parentage and that the genetic composition was at least semi-stable.

5. Possible roles of CATs

We suggest two explanations for CAT formation. They are not mutually exclusive and there is some evidence supporting each of them.

5.1. Improving the chances of colony establishment

There is some evidence that CAT fusion is promoted by nutrient starvation. A number of studies have shown that the frequency of germling fusion can be increased by diluting the growth medium or by germinating conidia in water [18,29,30]. These observations suggest that CATs may improve the chances of colony establishment by allowing heterogeneously distributed nutrients or water within the environment to be shared between different germlings. We have found that conidia joined by CATs can germinate faster than single conidia [3].

5.2. Gene exchange

It is not uncommon for sexual forms of filamentous fungi to be rare or non-existent in nature [31]. Previously unexplained recombination that occurs during vegetative growth with the absence of a stable diploid form may be explained by a process mediated by CAT fusion. The recombinants could involve either or both nuclear and extranuclear genomes.

A detailed genetic analysis of two different vegetatively incompatible biotypes of *C. gloeosporioides* infecting *Stylosanthes* spp. was made [32–34]. A mechanism that generated chromosome variation and gene transfer between these normally incompatible genotypes was suggested [35]. In these studies there was no corresponding cytological work. However, we have suggested that CAT fusions could have allowed the introgression of genetic material (horizontal gene transfer), a mechanism for the acquisition of supernumerary chromosomes, and also an explanation for the origin of genetic diversity in species with rare or no sexual reproduction [26].

In natural populations, mitochondria and prions can have an influence on virulence and vegetative incompatibility resulting from hyphal fusion [36,37]. In *N. crassa*, the transfer of extranuclear genes between vegetatively incompatible strains is even possible without using selection markers (e.g. complementary auxotrophic mutants or hygromycin resistance) to promote and maintain unstable heterokaryons [38].

6. Some important questions

6.1. Is CAT fusion a model for vegetative hyphal fusion?

CAT fusion during colony establishment provides a much simpler and more readily manipulated experimental system than does vegetative hyphal fusion in subperipheral regions of the older colony. How CAT fusion will provide a model for vegetative hyphal fusion remains to be determined. So far, all hyphal fusion mutants that have been analysed are blocked in both mycelial hyphal fusion and CAT fusion [4,22]. However,

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morphologically there are significant differences between CATs and many mycelial fusion hyphae (see Section 2) and downstream events following non-self fusions between CATs may be also different (see Section 6.3).

6.2. What signalling pathways are involved in CAT induction, homing and fusion?

The simplicity of the CAT fusion system makes it easy to score for mutants blocked in CAT induction, homing or fusion (Fig. 5A). With the increasing availability of gene knockout mutants from the *Neurospora* genome project (http://www.dartmouth.edu/~neurosporagenome/), it should be possible to identify quickly which signalling pathways are involved in these different processes. One of the major challenges will be to discover how the mechanism of self-signalling operates between conidia and between CATs of the same genotype. Virtually all previous studies on signalling during cell fusion in fungi have involved studies on the interaction between cells of different genotypes producing different pheromones (e.g. [19,21]). Identification of the self-signalling ligand(s) involved in CAT induction and homing should be very revealing in this respect.

6.3. How might vegetative incompatibility be avoided following CAT fusion?

For gene exchange to be a significant outcome of CAT fusion, vegetative incompatibility barriers must be overcome. It is known that this can be achieved in two ways. First, vegetative incompatibility may not fully function during the early stages of colony establishment allowing the heterokaryons resulting from CAT fusions to be tolerated (Fig. 4C) [4,15,26,39]. Vegetative incompatibility is suppressed, for example, when nuclei of opposite mating type share the same cytoplasm in trichogynes, ascogonia or ascogenous hyphae during sexual reproduction [40]. Second, vegetative incompatibility may be overcome by the loss of functional *het* genes through mutation or chromosome rearrangements [32,34,41]. An analysis of *het* gene expression and genetic composition during the early stages of colony establishment following CAT fusion will be very important to determine which of these possibilities may be occurring.

6.4. What is the fate of nuclei following CAT non-self fusions?

If vegetative incompatibility is overcome following CAT non-self fusions [25,26], it will be important to determine the fate of the individual nuclei in the heterokaryons formed. Questions to be addressed include the following. How long during the early stages of colony establishment is the incompatible response suppressed? Do the non-self nuclei become randomly mixed in the young microcolony or do they form genetic mosaics with genetically similar nuclei grouping together? Do any nuclei fuse and form diploids or aneuploids? What is the stability of such hybrid nuclei? What is the genetic nature of any long-term products from such fusions?

The events immediately post-fusion can be studied using live-cell imaging techniques involving the specific fluorescent labelling of genetically different nuclei sharing the same cytoplasm. Once the colony has developed, quantitative measurement of nuclear DNA content, quantitative PCR analyses and sequencing of a range of markers from single spores, and in situ hybridization to identify specific marker genes in individual nuclei could also be revealing [42,43].

7. Final comments

CAT fusions seem to be a very common phenomenon within fungi but we know very little about their biology or importance. Although we have suggested that CAT fusion may be important for increasing the chances of colony establishment and/or for gene transfer, these possible roles need to be rigorously analysed experimentally both in culture and in nature. A multidisciplinary approach combining molecular, genetic, and cell biological techniques together with population genetics approaches will be important to achieve this.

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