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Mini-Review

Carbonic anhydrases in fungi

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Carbonic anhydrases (CAs) are metalloenzymes that catalyse the interconversion of carbon dioxide and bicarbonate with high efficiency. This reaction is fundamental to biological processes such as respiration, photosynthesis, pH homeostasis, CO_2 transport and electrolyte secretion. CAs are distributed among all three domains of life, and are currently divided into five evolutionarily unrelated classes (α, β, γ, δ and ζ). Fungal CAs have only recently been identified and characterized in detail. While *Saccharomyces cerevisiae* and *Candida albicans* each have only one β-CA, multiple copies of β-CA- and α-CA-encoding genes are found in other fungi. Recent work demonstrates that CAs play an important role in the CO_2-sensing system of fungal pathogens and in the regulation of sexual development. This review focuses on CA functions in *S. cerevisiae*, the fungal pathogens *C. albicans* and *Cryptococcus neoformans*, and the filamentous ascomycete *Sordaria macrospora*.

Introduction

Carbon dioxide (CO_2) is a small molecule that is among the most important gases for all living organisms. It is a waste product of respiration, but it is also a nutrient, a sensing factor and a major component of the carbon cycle. Despite its ubiquitous importance, CO_2 comprises only 0.033 % of the atmospheric gases, although it is found at higher levels in several tissues. Plants, algae and cyanobacteria are able to fix CO_2 during photosynthesis, while animals and micro-organisms produce and return it to the atmosphere.

In nature, the concentration of CO_2 is balanced by an interconversion to bicarbonate (HCO_3^-) through the spontaneous reaction $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$. Bicarbonate is also an important biological substrate, and since the average amount of HCO_3^- required by organisms is much greater than the amount produced spontaneously from CO_2, bicarbonate production requires finely tuned regulation (Jones, 2008). For this reason, a highly diverse family of enzymes has evolved that are able to accelerate the interconversion reaction by up to 10 000-fold (Wistrand, 1981). Members of this protein family, called carbonic anhydrases (CAs), are able to catalyse the reversible hydration of CO_2 to give HCO_3^- and a proton (Supuran, 2008). The production of H^+ results in a pH decrease, so CAs are also involved in pH homeostasis. In addition, CAs are involved in biosynthesis or detoxification pathways that use HCO_3^- as a co-factor or as a co-substrate, such as fatty-acid or arginine biosynthesis, the cAMP pathway and cyanate degradation (Aguilera *et al.*, 2005b; Anderson *et al.*, 1990; Bahn & Mühlschlegel, 2006).

CAs have evolved in all three domains of life and are currently divided into five, evolutionarily unrelated classes (α, β, γ, δ and ζ) that independently arose from different precursors during convergent evolution. The best-studied group is the α-class of mammals, prokaryotes, plants and fungi. The β-class has been identified in plants, bacteria and fungi but not mammals, while γ-CAs, which have strikingly different sequence features, are predominantly found in archaea (Supuran, 2008; Tripp *et al.*, 2001). The β-class can be further subdivided into three subclasses. The plant-like β-CAs and the cab-like β-CAs, named after the carbonic anhydrase β (CAB) from the archaeon *Methanobacterium thermoautotrophicum*, were first described by Kimber & Pai (2000). The third subclass of β-CAs, which is designated the ε-class, was identified in the chemolithoautotrophic bacterium *Halothiobacillus neapolitanus* (Sawaya *et al.*, 2006). Recently, two more CA classes, the δ- and ζ-classes, have been described. So far, these have been found only in marine diatoms (Xu *et al.*, 2008). Although the primary structures of the various classes of CAs are strikingly different, the metal- (in most cases a zinc atom) coordinating sites are remarkably similar at the structural level. The number of subunits also varies greatly among the different CA classes. Nonetheless, all CA enzymes catalyse the same chemical reaction (Supuran, 2008; Tripp *et al.*, 2001).

At least 16 α-CAs or carbonic-anhydrase-related proteins (CARPs) are encoded in mammals (Supuran, 2008, and references therein). These isoenzymes are tissue or organ specific in their expression and localize to different subcellular compartments. The cytosolic isozymes CA I and CA II were first characterized in human and bovine blood cells, where they play a significant role in gas exchange, while the distribution of CA III is restricted to the cytosol of skeletal muscles and adipocytes. The remaining cytosolic CA VII and the CARPs CA VIII, X

and XI were demonstrated to be located in the central nervous system and CA XIII was detected in the kidney, liver and brain. Mitochondrial CAs are exclusively localized in the liver (CA VA) or distributed in heart and skeletal muscles, kidney, pancreas, spinal cord and gastrointestinal tract (CA VB). Five isoforms are associated with the membrane of cells in the kidney (CA IV, CA XIV and CA XV), heart muscles (CA IV), brain (CA IV and CA XIV), pancreas (CA IV), liver (CA XIV) and eye (CA XII). CA IX is a transmembrane enzyme with significant scientific relevance, because of its clinical application as a tumour marker. CA VI is a secreted protein, present in salivary and mammary glands (Supuran, 2008). Phylogenetic analysis has revealed that all human CA isoenzymes clearly originated from a common ancestor.

Since CAs are a ubiquitous group of enzymes, the conservation of their physiological roles among organisms is worth investigating, because it suggests a complex evolutionary history. Genome sequencing projects have identified CAs in all organisms analysed. To the best of our knowledge, only *Symbiobacterium thermophilum*, a syntrophic bacterium that effectively grows on CO_2 generated by other bacteria, has lost its CA genes (Nishida *et al.*, 2009).

Fungi possess α- and β-CAs

Fungi react to different stimuli, such as temperature and nutrients. Gases can also serve as signalling molecules (Bahn *et al.*, 2007). A prominent example of a gas-driven process is the switching of *Candida albicans* between yeast and filamentous growth in response to varying CO_2 levels. This process was demonstrated to be controlled by a fungal adenylyl cyclase and a β-CA (Klengel *et al.*, 2005).

All known fungal CAs belong either to the α- or to the β-class (Elleuche & Pöggeler, 2009a). To date, members of the other three groups have not been identified in fungal genomes. Fungal CA-encoding genes, like the CA multigene families in mammals, algae and plants, have diversified extensively during fungal evolution. The genomes of most filamentous ascomycetes contain three isoforms of β-class CAs and at least one α-class CA, whereas only β-class CAs have been identified in hemiascomycetous yeasts (Elleuche & Pöggeler, 2009a). Within the basidiomycetes, only *Cryptococcus neoformans* encodes two β-CAs, while the *Coprinopsis cinerea*, *Laccaria bicolor*, *Malassezia globosa* and *Ustilago maydis* genomes all contain single β-CA genes (Elleuche & Pöggeler, 2009a). Interestingly, eight genes encoding putative α-CAs can be identified in the genome of *L. bicolor* (S. Pöggeler, unpublished).

β-CAs have been functionally characterized in only a few fungal species (Table 1). The *NCE103* gene encodes a plant-like β-CA in the hemiascomycetous yeasts *Candida albicans*, *Candida glabrata* and *Saccharomyces cerevisiae*, whereas two closely related isozymes, Can1 and Can2, have been described in the basidiomycete *Cryptococcus neoformans* (Bahn *et al.*, 2005; Götz *et al.*, 1999; Innocenti *et al.*, 2009; Klengel *et al.*, 2005). Interestingly, an additional putative cab-like β-CA has been identified in the pathogenic yeast *C. albicans* (XP_715817) and in *Pichia stipitis* (XP_001383682.1) (Elleuche & Pöggeler, 2009a). Recently, a multigene family of β-CAs was shown to influence the sexual development of the filamentous ascomycete *Sordaria macrospora* (Elleuche & Pöggeler, 2009b). CA activity from secreted CA isoforms has also been detected in various *Penicillium* isolates, and this activity is believed to be involved in limestone dissolution (Li *et al.*, 2009).

The CA Nce103 of *Saccharomyces cerevisiae*

The first fungal CA was discovered in 1996, in the yeast *S. cerevisiae*. Initially, it was identified as a component of a non-classical protein export pathway and designated Nce103 (Cleves *et al.*, 1996). Subsequent sequence alignments of the predicted amino acid residues assigned Nce103 to the β-class of CAs (Götz *et al.*, 1999). A deletion mutant of *NCE103* is unable to grow under ambient air conditions (0.033 % CO_2). Since the lethality of the *nce103*Δ mutation can be complemented by high CO_2 concentration (5 % CO_2), the phenotype was defined as high-CO_2-requiring (HCR). However, the defect of the *nce103*Δ mutant can be complemented by heterologous expression of CAs from plants [β-CA from *Medicago sativa*, tobacco chloroplast β-CA (Götz *et al.*, 1999; Slaymaker *et al.*, 2002)], mammals [human α-CAII (Clark *et al.*,

Table 1. Functionally characterized β-CAs in fungi

Species	Protein	Reference
Candida albicans	Nce103	Klengel *et al.* (2005)
Candida glabrata	Nce103	Innocenti *et al.* (2009)
Cryptococcus neoformans	Can1	Bahn *et al.* (2005)
	Can2	Bahn *et al.* (2005)
Saccharomyces cerevisiae	Nce103	Cleves *et al.*(1996); Götz *et al.* (1999)
Sordaria macrospora	CAS1	Elleuche & Pöggeler (2009b)
	CAS2	Elleuche & Pöggeler (2009b)
	CAS3	Elleuche & Pöggeler (2009b)

2004)], bacteria [β-CA *can* from *Escherichia coli* (Cronk *et al.*, 2001)] or *C. neoformans* [β-CA Can1 and Can2 (Bahn *et al.*, 2005)], indicating that non-specific CA activity is sufficient to ensure an efficient supply of HCO_3^- for yeast cells.

In addition, it has been shown that *NCE103* is transcriptionally upregulated under ambient air conditions (0.033 % CO_2), and the detectable CA activity corresponds to an increase in *NCE103* mRNA accumulation under inducing, low-CO_2, conditions (Amoroso *et al.*, 2005). High levels of CO_2 result in the downregulation of *NCE103* transcript (Aguilera *et al.*, 2005a). In agreement with these results, no CA activity is detectable in cells grown at high (5 %) CO_2 levels (Amoroso *et al.*, 2005).

The HCR phenotype of the *S. cerevisiae nce103*Δ mutant indicates the importance of Nce103 in CO_2/HCO_3^- homeostasis and suggests that *S. cerevisiae* CA may participate in supplying intracellular HCO_3^- for anaplerosis or for the regulation of the intracellular pH (Amoroso *et al.*, 2005). Aguilera *et al.* (2005b) identified several HCO_3^--dependent carboxylation reactions that are impaired in the *nce103*Δ mutant strain. To test the nutritional requirements of the *nce103*Δ mutant under non-CO_2-enriched conditions, the mutant was complemented by adding L-aspartate, L-arginine, uracil or fatty acids to the medium. The results demonstrated that the main physiological role of the *S. cerevisiae* CA is the production of bicarbonate for HCO_3^--dependent metabolic carboxylation reactions catalysed by pyruvate carboxylase, acetyl-CoA carboxylase and carbamoyl-phosphate synthetase under low CO_2 concentrations (Aguilera *et al.*, 2005b).

CAs of the fungal pathogens *Candida albicans* and *Cryptococcus neoformans*

The role of CAs in development and virulence has been investigated in the pathogenic fungi *Candida albicans* and *Cryptococcus neoformans* (Bahn & Mühlschlegel, 2006; Mogensen & Mühlschlegel, 2008), which are both exposed to drastic environmental changes during their infectious life cycles. In its natural habitat, *C. neoformans* grows as a saprophyte at 0.033 % CO_2, but when it infects the human lung, it must adapt to levels as high as 5 % CO_2. Similarly, *C. albicans* can survive low levels of CO_2 such as on the skin, but it is also able to infect the bloodstream in immunocompromised patients, where the CO_2 concentration is ~5 %. Elevated CO_2 concentrations promote a prominent switch from yeast to hyphal growth in the hemiascomycetous yeast *C. albicans* and the induction of capsule biosynthesis in the basidiomycete (Bahn *et al.*, 2005; Klengel *et al.*, 2005).

The CA-encoding genes *CAN1* and *CAN2* of *C. neoformans*, and *NCE103* of *C. albicans*, have been characterized. All these genes encode β-class CAs and are able to rescue CA deletion strains of *E. coli* or *S. cerevisiae* (Bahn *et al.*, 2005; Mogensen *et al.*, 2006). The crystal structure of *C. neoformans* Can2 revealed that the enzyme carries a unique N-terminal extension that can interact with the active site entrance of the dimer, so the N-terminus was hypothesized to be an internal regulator or an interaction site for a different protein (Schlicker *et al.*, 2009). Deletion analysis of the CA-encoding genes in *C. neoformans* and *C. albicans* revealed that *CAN2* and *NCE103* are essential for survival under ambient air, but dispensable for *in vivo* proliferation and virulence at the high CO_2 levels found in the host (Bahn *et al.*, 2005; Klengel *et al.*, 2005). In *C. neoformans*, the *in vitro* growth defects under ambient air were largely attributable to defective fatty acid synthesis. The *C. neoformans CAN2* mutant had physiological impairments in addition to the virulence defect. The mating ability of *C. neoformans* wild-type is inhibited under high-CO_2 conditions, but the *can2*Δ strain had lost this regulation and initiated mating independently of the CO_2 level. This showed that HCO_3^-, as the conversion product of CO_2, is the key molecule for mating inhibition. Furthermore, the transcription of pheromone genes no longer decreases at elevated CO_2 conditions in the *can2*Δ strain (Bahn *et al.*, 2005).

Interestingly, CAs of both these fungal pathogens are involved in CO_2 sensing and virulence. In both fungi, adenylyl cyclase (AC) was shown to transmit the CO_2 signal in a CA-dependent manner (Bahn *et al.*, 2005; Klengel *et al.*, 2005; Mogensen *et al.*, 2006). Like the mammalian and bacterial soluble ACs (Chen *et al.*, 2000), the catalytic domains of *C. albicans* and *C. neoformans* ACs were shown to be activated by bicarbonate *in vitro* (Klengel *et al.*, 2005; Mogensen *et al.*, 2006).

Sordaria macrospora encodes four carbonic anhydrases

The filamentous ascomycete *S. macrospora* is a close relative of the well-known red bread mould *Neurospora crassa*, and is used as a model system for investigating processes of sexual development (Kück *et al.*, 2009; Nowrousian *et al.*, 2004; Pöggeler *et al.*, 2006). The role of HCO_3^- metabolism during sexual reproduction has been investigated by characterizing three β-CA genes (Elleuche & Pöggeler, 2009b). A total of four CA-encoding genes, *cas1*, *cas2*, *cas3* and *cas4*, have been identified in the *S. macrospora* genome. CAS1 and CAS2 are closely related proteins that belong to the plant-like subgroup of β-CAs, while *cas3* encodes a cab-type β-CA and *cas4* an α-CA. Based on the similarity of *cas1* and *cas2*, these genes are proposed to be the result of an ancient gene duplication event (Elleuche & Pöggeler, 2009a). In contrast to the widely distributed plant-like β-CAs in fungi, cab-type β-CA genes seem to be restricted to the ascomycete phylum. Filamentous ascomycetes of the order Hypocreales exhibit multiple cab-like β-CAs, but only a single gene has been identified in members of the Eurotiales, Sordariales and Pleosporales, and in some hemiascomycetous yeast species (Elleuche & Pöggeler, 2009a).

In mammals, plants and algae, different isoforms of CAs are targeted to different tissues and organelles (Fabre *et al.*, 2007; Supuran, 2008; Ynalvez *et al.*, 2008), so it is likely that fungal CAs are also unequally distributed within the fungal cell. The first evidence for an intracellular CA comes from yeast, where CA activity was measured only inside the living cell, indicating that *Saccharomyces cerevisiae* does not encode a secreted CA isoform (Amoroso *et al.*, 2005). In *Sordaria macrospora*, β-CAs are targeted to different subcellular compartments. CAS1 and CAS3 are cytoplasmic CAs, while CAS2 is specifically translocated into mitochondria, and the α-CA CAS4 is predicted to be secreted (Elleuche & Pöggeler, 2009b).

A detailed survey of CAs in the genomes of mycelial fungi demonstrated that nearly all filamentous ascomycetes investigated so far encode a single mitochondrial plant-like β-CA. Interestingly, this specific isozyme is not homologous when comparing members of the orders Sordariales and Eurotiales, indicating that the translocation of CA into mitochondria has evoloved at least twice in the fungi (Elleuche & Pöggeler, 2009a).

CAS1 and CAS2 are involved in *Sordaria macrospora* fruiting body development

A comprehensive molecular investigation of the *S. macrospora* β-CAs revealed that the plant-like isozymes CAS1 and CAS2 are involved in the bicarbonate-dependent regulation of fruiting body formation and maturation (Fig. 1). Surprisingly, the deletion of a single *cas* gene does not cause lethality, as it does in yeasts and several prokaryotes (Götz *et al.*, 1999; Klengel *et al.*, 2005; Kusian *et al.*, 2002). From analysis of the phenotypes of Δ*cas1*, Δ*cas2* and Δ*cas3* deletion strains, *cas2* appears to encode the major CA in *S. macrospora*, since only the Δ*cas2* mutant is drastically impaired in vegetative growth. This

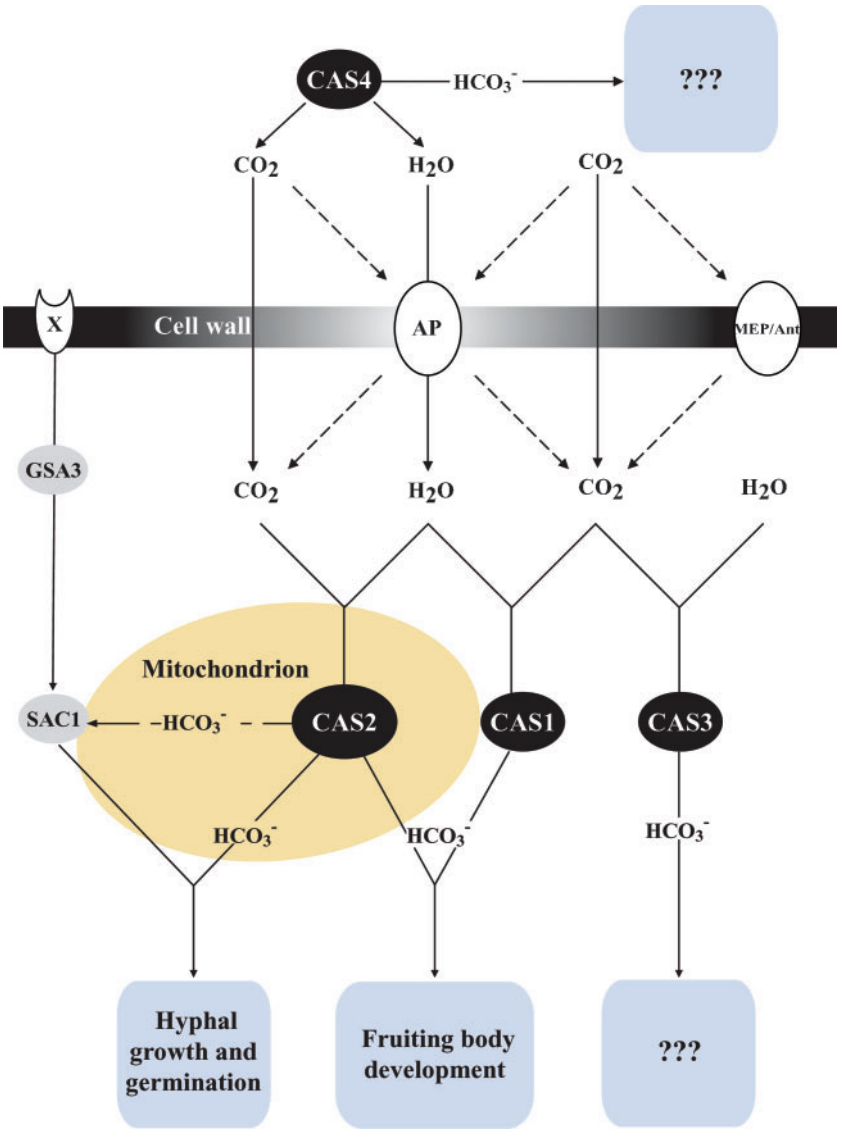

Fig. 1. Hypothetical model of bicarbonate regulation in the filamentous fungus *Sordaria macrospora*. CO_2 is transported into the fungal cell by diffusion or putative transport systems (AP, MEP/Ant; according to Bahn & Mühlschlegel, 2006). The figure depicts the localization and influence of various processes of α- and β-CAs and the adenylyl cyclase (AC) pathway in *S. macrospora*. The α-CA CAS4 is predicted to be secreted from the fungal cell, whereas CAS1 and CAS3 are cytoplasmically located and CAS2 is translocated into the mitochondria. The plant-like β-CAs CAS1 and CAS2 play a role in fruiting body development and CAS2 is additionally involved in the regulation of hyphal growth and germination. The latter processes might be connected to the well-known G-protein/AC pathway (GSA3/SAC1). The functional roles of cab-like β-CA CAS3 and α-CAS4 are so far unknown. X, unknown receptor; AP, aquaporin; MEP/Ant, putative ammonium permease/transporter. Dashed lines indicate hypothetical signal cascades that have yet to be proven for *S. macrospora*.

defect can be functionally complemented non-specifically by high CO_2 concentrations, or by overexpression of *cas1* and *cas3*. A partial complementation of the growth defect was also achieved by providing a mixture of palmitic, stearic and myristic acid, indicating that HCO_3^- produced by CAS2 is involved in the biosynthesis of fatty acids. In addition to the vegetative growth impairment, the germination rate of the Δ*cas2* mutant is drastically reduced compared to the wild-type, Δ*cas1* or Δ*cas3* strains. Because this defect cannot be restored by the addition of a non-specific bicarbonate source such as elevated CO_2 concentration, or by overexpression of *cas1* or *cas3*, *S. macrospora* is hypothesized to require a well-defined amount of CA activity within the mitochondria to assure efficient ascospore germination (Elleuche & Pöggeler, 2009b). Ascospores have a depressed respiration, so their low intracellular levels of HCO_3^- must be increased to catalyse the bicarbonate-dependent carboxylation reactions required for germination. Young germlings of the *S. macrospora* Δ*cas2* mutant are drastically impaired in growth rate and exhibit thin, highly vacuolated hyphae. In contrast to CAS2, CAS1 and CAS3 appear to provide a low level of HCO_3^- that is sufficient only for the basal metabolic reactions of growing hyphae. Finally, the production of a mycelium from germinated Δ*cas2* spores is drastically delayed. However, when the mycelium of these colonies is consecutively transferred to fresh agar plates, the production of perithecia increases. Thus, after prolonged growth, adequate amounts of CO_2/HCO_3^- that are sufficient for fruiting body development accumulate in the hyphae (Elleuche & Pöggeler, 2009b).

Comparable observations regarding the influence of CAs on developmental processes have been described in the higher plant *Arabidopsis thaliana*. Similar to *S. macrospora*, *A. thaliana* encodes multiple CA isoenzymes that are localized to the cytoplasm, plasma membrane and mitochondria, as well as to chloroplasts (Fabre *et al.*, 2007; Parisi *et al.*, 2004). The major CA of *A. thaliana* was shown to be the chloroplast-localized *Atβ*CA1 (Ferreira *et al.*, 2008). Deletion and RNA_i suppression of *Atβ*CA1 result in the loss of $\geqslant 70\,\%$ of total CA activity, indicating the dominant role of this specific isoform. Most of the germlings derived from *Atβ*CA1 mutants produce no true leaves, have a reduced root growth rate and show growth arrest after several days in ambient air conditions. All impairments can be fully restored by increased CO_2 concentration in the air. However, some mutant germlings grow to maturity and are not morphologically distinguishable from the wild-type, even though their CA activity is approximately 30 % of wild-type levels (Ferreira *et al.*, 2008). The specific plastid CA activity of *A. thaliana* is thought to be required to mobilize energy reserves during early developmental stages, although during later stages, the activity of other CAs is sufficient for development (Ferreira *et al.*, 2008). Similarly, in *S. macrospora*, the activity of the mitochondrial CAS2 enzyme seems to be especially important for the supply of HCO_3^- during early stages of development, but during later stages, CAS1, CAS3 and CAS4 can produce sufficient bicarbonate. The interconnection and dependence of *S. macrospora β*-CAs is clearly visible in a Δ*cas1/2* double-deletion strain. This mutant displays an even more obvious delay in fruiting body development than the Δ*cas2* single mutant and is completely impaired in the production of mature ascospores (Elleuche & Pöggeler, 2009b). Interestingly, deletion of the *S. macrospora* adenylyl cyclase gene *sac1* causes a phenotype comparable to the Δ*cas2* mutant (Kamerewerd *et al.*, 2008). The Δ*sac1* deletion strain is impaired in vegetative growth and ascospore germination as well as in the production of fruiting bodies. While growth defects and fruiting body development are restored by the addition of cAMP to the medium, the germination defect cannot be complemented by the supply of cAMP. Furthermore, SAC1 was shown to be genetically linked to G-protein signalling (Kamerewerd *et al.*, 2008).

By analogy to the adenylyl cyclases of *Candida albicans* and *Crytococcus neoformans*, SAC1 of *S. macrospora* might also be activated by bicarbonate. Because CAS2 and SAC1 seem to regulate the same processes in *S. macrospora* development, the mitochondrial CA CAS2 might provide the bicarbonate for the regulation of SAC1 activity. Interestingly, *in silico* analysis predicts a mitochondrial localization of SAC1 (Fig. 1) (authors' unpublished results).

The *Sordaria macrospora* cyanase is a bicarbonate-dependent enzyme

The CYN1 cyanase of *S. macrospora* is also regulated by bicarbonate. Cyanases catalyse the HCO_3^--dependent degradation of toxic cyanate in a reaction that produces CO_2 and NH_3 (Anderson *et al.*, 1990). In heterotrophic bacteria and cyanobacteria, cyanases are encoded together with a CA in a cyanate-inducible operon (Guilloton *et al.*, 1993). In *E. coli*, the CynT CA of the cyanase operon is important for the specific supply of HCO_3^- for the cyanase CynS. Toxic cyanate kills *cynT* deletion mutants, even though a second CA is encoded in *E. coli* by the *can* gene. Under ambient air, the deletion of *can* is lethal. Interestingly, the lethal phenotype of a *can* deletion mutant can be complemented by the addition of cyanate, because this leads to the induction of the *cyn* operon under ambient air conditions. These results indicate that the functions of *cynS* and *cynT* are strongly interconnected (Guilloton *et al.*, 1993). The genomes of filamentous ascomycetes, but not those of hemiascomycetous yeasts, also contain a single cyanase gene, although it is not clustered with a CA gene (Elleuche & Pöggeler, 2008; Guilloton *et al.*, 2002). Functional characterization of the *cyn1* product after heterologous expression in *E. coli* revealed that CYN1 catalyses the reaction of cyanate with bicarbonate to give NH_3 and CO_2. Transcription of the *S. macrospora cyn1* gene is upregulated by the addition of cyanate to the medium, and depressed when arginine is the sole nitrogen source.

A Δ*cyn1* knockout mutant of *S. macrospora* is completely devoid of cyanase activity and shows an increased sensitivity to exogenously supplied cyanate in an arginine-depleted medium and defects in ascospore germination, but no other obvious morphological phenotypes (Elleuche & Pöggeler, 2008). Thus, detoxification of exogenous cyanate does not seem to be the predominant function of the *S. macrospora* cyanase. From *E. coli* studies, cyanate appears to inhibit carbamoyl-phosphate synthetase (CPS), an enzyme responsible for the conversion of glutamine, bicarbonate and ATP to the arginine precursor carbamoyl phosphate (Anderson *et al.*, 1973). In addition, CPS is specifically inhibited by cyanate (Guilloton & Karst, 1987). Therefore, cyanase has been proposed to be involved in the regulation of arginine biosynthesis (Elleuche & Pöggeler, 2008; Guilloton *et al.*, 2002). Future analysis will focus on the functional connection between fungal CAs and cyanases, and their impact on arginine biosynthesis.

Conclusion

Recent work demonstrates that CAs have a far more extensive and fundamental role in fungal biology than previously recognized. Fungal CAs are essential for growth in ambient air because they provide bicarbonate for HCO_3^--dependent metabolic carboxylation reactions, such as those catalysed by pyruvate carboxylase, acetyl-CoA carboxylase and carbamoyl-phosphate synthetase. They are involved in CO_2 sensing by producing bicarbonate for the activation of adenylyl cyclase. Moreover, CAs are required to establish sexual reproduction in basidiomycetes and filamentous ascomycetes. Multiple isoenzymes of filamentous ascomycetes have been found in the cytoplasm and in mitochondria, and secreted into the medium. However, in most cases very little is known about the exact physiological role for each of these isoforms. Genome sequencing projects have identified multiple CAs in several fungal species. The future challenge for fungal biologists will be to determine the biological functions of these enzymes.

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