The metabolism of 4-aminobutyrate (GABA) in fungi

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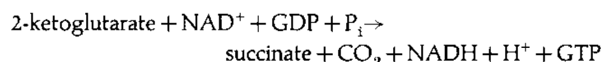
Information on the genetics and metabolism of 4-aminobutyrate (GABA) in yeasts and fungi is reviewed. In spite of ubiquitous occurrence, there is limited information on its function and biological role. Most fungi utilize GABA both as a carbon and a nitrogen source. Fungal endogenous GABA largely originates from the decarboxylation of L-glutamate and is associated with sporulation/spore metabolism. Whatever its source, GABA is catabolized to succinate via succinicsemialdehyde. Taken together these steps define a potential bypass outside the classical tricarboxylic acid cycle. Evidence for the existence of such a functional bypass in fungi is reviewed. The role of GABA and its metabolism in various facets of fungal biology is gradually emerging.

4-Aminobutyrate (GABA) is an ubiquitous nonprotein amino acid. Presence of this amino acid was first demonstrated in plant tissues (Hulme & Arthington, 1950) and subsequently in rat brain (Awapara *et al.*, 1950; Roberts & Frankel, 1950). Later, it was detected in many parts of vertebrates/nonvertebrates and also from various plant components like root nodules, germinating seeds and leaves. GABA was found in some bacteria, especially in their germinating spores. To date, the role of GABA in most organisms is not fully understood. As an exception, the well known function of GABA is that of a negative neurotransmitter in the central and peripheral nervous system of vertebrates and certain non-vertebrates (Tillakratne, Mediana-Kauwe & Gibson, 1995). It functions by binding the GABA (A/B) receptor thereby increasing the membrane conductance to chloride ions; this results in the hyperpolarization of membranes.

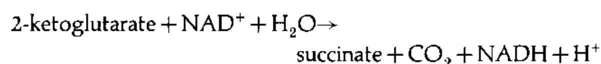
GABA is an intermediate involved in bypassing certain key steps of the TCA cycle (Balazs *et al.*, 1970). In this variant of the TCA cycle, 2-ketoglutarate is aminated to form L-glutamate rather than being oxidatively decarboxylated to succinate. The glutamate thus formed is decarboxylated to GABA, by glutamate decarboxylase (GAD, EC 4.1.1.15). The GABA is then transaminated to succinicsemialdehyde (SSA) by 4-aminobutyrate transaminase (GABAT, EC 2.6.1.19), and finally, SSA is irreversibly oxidized to succinate by succinicsemialdehyde dehydrogenase (SSADH, EC 1.2.1.16). This sequence of reactions circumvents the critical 2-ketoglutarate dehydrogenase step of the TCA cycle and allows the flow of carbon from 2-ketoglutarate to succinate outside the classical TCA cycle (Fig. 1). The reaction stoichiometry for the two alternative pathways for the flow of 2-ketoglutarate carbon is given below:

(*a*) in the classical TCA cycle,



$$\text{2-ketoglutarate} + NAD^+ + GDP + P_i \rightarrow \text{succinate} + CO_2 + NADH + H^+ + GTP$$

(*b*) via the GABA bypass,



$$\text{2-ketoglutarate} + NAD^+ + H_2O \rightarrow \text{succinate} + CO_2 + NADH + H^+$$

Clearly the GABA bypass is an energetically less efficient route than the direct oxidation of 2-ketoglutarate to succinate by the TCA cycle. It is, therefore, difficult to visualize the preferential operation of this shunt pathway under normal metabolic states.

In an *in vitro* study using brain tissue, the flux through the GABA bypass was estimated to be 8–10% of that via the TCA cycle (Balazs *et al.*, 1970). Such a GABA bypass is also postulated/evidenced in a few plants including potato tubers (Satya Narayan & Nair, 1990). Although the role of GABA and its metabolism in plants is not clearly understood, it has been correlated to environmental stress. Glutamate appears to be catabolized via GABA during imbibition but was metabolized through 2-ketoglutarate during the later phase of seed germination (Vandewalle & Olsson, 1983; Shelp *et al.*, 1995). These observations suggest possible roles for GABA during germination in plants. The postulation of a GABA bypass in bacteria is simply based on the demonstration of all the relevant enzymes (GAD, GABAT & SSADH) (Jakoby, 1962). Although a clear-cut role (other than that as C or N source) for GABA in bacteria is not evident, a possible involvement in *Bacillus megaterium* spore germination was postulated (Foerster & Foerster, 1973); GAD activity enhanced dramatically during germination leading to an elevated GABA pool, which was subsequently metabolized to succinate.

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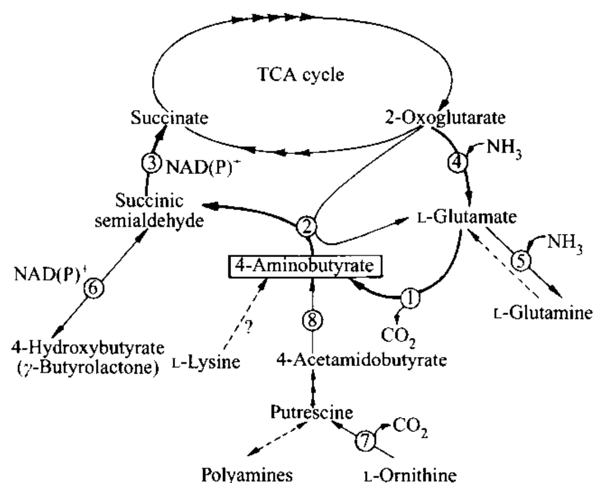

Fig. 1. A summary of pathways of GABA metabolism in fungi. The GABA bypass is represented by bold arrows. Enzymic step: 1. Glutamate decarboxylase; 2. GABA transaminase; 3. Succinic-semialdehyde dehydrogenase; 4. Glutamate dehydrogenase; 5. Glutamine synthetase; 6. Succinicsemialdehyde reductase; 7. Ornithine decarboxylase; 8. 4-Acetamidobutyrate deacetylase. Steps that have variations or are hypothetical are represented by broken arrows.

OCCURRENCE OF GABA IN FUNGI

The occurrence of GABA is not limited to animals, higher plants and bacteria but is also found in many moulds and yeasts. This nonprotein amino acid was isolated from acid-treated yeast extracts (Reed, 1950). It was later shown to be present in the free state, to the extent of 1–2% by dry weight, in untreated yeast. While investigating the amino acid composition of red yeast, *Rhodotorula glutinis*, GABA was again detected (Krishnaswamy & Giri, 1953). An elevated GABA pool was observed in the early phase of *Neurospora crassa* spore germination (Schmit & Brody, 1975). Other filamentous fungi like *Aspergillus nidulans* and *Aspergillus niger* are also known to contain GABA. An interesting and unusual accumulation of GABA in *A. niger* during acidogenesis is documented (Kubicek, Hampel & Rohr, 1979). The GABA levels increased in parallel with citric acid accumulation, while under normal conditions it was virtually undetectable.

FUNGAL GABA METABOLISM AND ENZYMOLOGY

Fungal GABA metabolism involves the formation of GABA, mostly from L-glutamate, and the catabolism of GABA to succinate via SSA (Fig. 1). Individual enzymes from a few fungi have been purified and characterized with respect to their regulation at enzyme and/or genetic levels. All the three enzymes of GABA bypass were detected in *Saccharomyces cerevisiae* and *Torulopsis utilis* (Pietruszko & Fowden, 1961). These yeasts, alternatively, could reduce SSA to 4-hydroxybutyrate by elaborating a SSA reductase. Such a reduction is well known in mammals (Rating *et al.*, 1984).

The presence of all the relevant enzymes of the GABA shunt in *A. niger* was detected recently (Fig. 2; Punekar *et al.*, 1995). Externally supplied GABA was catabolized in this filamentous fungus by specific induction of GABAT and SSADH activities. GAD, the other enzyme, was associated only with conidiating *A. niger* mycelia. The enzymes of the glutamate decarboxylation loop exist in *Coprinus cinereus* and it appears to be the normal route of TCA metabolism in this organism (Moore & Ewaze, 1976; Ewaze, Moore & Stewart, 1978; Moore, 1984). In such situations NAD^+-glutamate dehydrogenase is effectively a normal component of the TCA cycle of *Coprinus* (Moore, 1984). Within 2 h of the exposure of *Agaricus bisporus* fruit body material to L-[U-^{14}C]glutamate, the label appeared in GABA, succinate and malate (Piquemal, Latche & Baldy, 1972). In addition, the enzymes of the glutamate decarboxylation loop occur in *Agaricus* spores (Rast, Stauble & Zobrist, 1976). These are the best reports yet in fungal systems, where all the enzymes of GABA bypass have been demonstrated *in vitro*. However, there is a need for examples that conclusively and directly demonstrate a flux through this shunt in fungi.

GABA is a metabolic intermediate (as depicted in Fig. 1) in *Candida boidinii* cells grown on spermidine, putrescine and 4-acetamidobutyrate (Haywood & Large, 1986; Large, 1986, 1992; Large & Robertson, 1988). Correspondingly, the GABAT activity in this yeast was elevated 20–40 fold. Other enzymes of the proposed route of polyamine breakdown are non-coordinately induced or derepressed during growth on spermidine or its putative breakdown intermediate(s) (Gillyon *et al.*, 1987; Large & Robertson, 1988). There is some genetic evidence for a possible minor route of ornithine catabolism through GABA in *A. nidulans* as well (Arst, 1977). A strong induction of GABAT by L-lysine is reported in the lysine-adapted cells of *Candida guilliermondii*. This observation suggests a new hypothetical route of L-lysine catabolism, where GABA may be an intermediate metabolite (Der Garabedian, 1986; Large, 1986; Der Garabedian & Vermeersch, 1989). Alternatively, it may reflect the activity of a separate 5-aminovalerate aminotransferase, which is probably induced in lysine breakdown (Der Garabedian, 1986).

Whatever the biosynthetic origin of GABA, its catabolism in fungi results in the formation of succinate via SSA (Fig. 1).

Decarboxylation of L-glutamate

The first enzyme of GABA bypass metabolism is GAD, glutamate decarboxylase – which generates GABA. A few salient properties of this catalyst include: an acidic pH optimum (between 4·0 and 6·0 pH), a Michaelis constant for L-glutamate in the lower millimolar range, exclusively cytosolic location, and finally, an absolute requirement for pyridoxal phosphate as a cofactor. An early report on the yeast enzyme appeared in 1953 (Krishnaswamy & Giri, 1953, 1956). The *R. glutinis* enzyme was partially purified and characterized. A potent inhibition by hydroxylamine is reversed by the addition of large excess of pyridoxal phosphate. To date, the *N. crassa* enzyme is the best studied fungal GAD; it was purified to homogeneity from the conidia. The enzyme is a monomer of around 30–33 kDa and exhibits a K_D for pyridoxal phosphate of 40 nM. From a preliminary account (Baldy, 1975), the *A. bisporus* GAD appears to be analogous to the *N. crassa* enzyme. The GAD activity could only be demonstrated in conidiating *A. niger* mycelia and not in the vegetative cells. This enzyme was undetectable in mycelia from the early stages of citric acid fermentation (acidogenesis),

but was markedly elevated during later time points (Fig. 2, Punekar *et al.*, 1995). The reported accumulation of GABA during acidogenesis (Kubicek *et al.*, 1979) is nicely mirrored by our data on increased GAD levels.

Formation of succinicsemialdehyde from GABA

It was recognized quite early that the nitrogen from GABA becomes available to the mould (like *Aspergillus fumigatus*) chiefly by a transamination reaction with 2-ketoglutarate (Roberts, Ayengar & Posner, 1953). The broad distribution of ω-amino acid transaminases (including GABAT) in a variety of micro-organisms was investigated by Yonaha *et al.* (1983). Evidence for a distinct ω-amino acid metabolism in yeasts and filamentous fungi has emerged from these studies. The yeast species like *Saccharomyces*, *Hansenula* and *Candida* develop a specific GABAT when grown on GABA but not on β-alanine. On the contrary, GABAT activity was detected on GABA as well as on β-alanine, in many moulds such as *Rhizopus*, *Aspergillus*, *Penicillium* and *Neurospora* species. The presence of a relatively specific GABAT activity in the cell extracts of yeasts (and not in those of moulds!) is thus apparent (Yonaha *et al.*, 1983). The ability of filamentous fungi to elaborate multiple ω-amino acid transaminases (see below, Molecular genetics of fungal GABA metabolism) could explain this broad substrate specificity.

A GABA-inducible GABAT was purified and characterized from *Candida* species (Der Garabedian, Lotti & Vermeersch, 1986). It is a homodimer (with monomers of 55 kDa). The salient catalytic properties of this pyridoxal phosphate enzyme are: an alkaline pH optimum (around pH 8·0); GABA and higher ω-amino acids as excellent amino donors while β-alanine is hardly used; the K_m for GABA and 2-ketoglutarate in the lower millimolar range (1·0–3·0 mM); a reasonable inhibition by short chain fatty acids such as propionate and butyrate. The GABAT activity from *A. bisporus* carpophore extracts was resolved on a DEAE-cellulose matrix (Baldy, 1976). It resembles the yeast enzyme (Pietruszko & Fowden, 1961; Der Garabedian *et al.*, 1986) in terms of its pH optimum, pyridoxal phosphate requirement etc.; the enzyme is specific for 2-ketoglutarate but not for GABA. Although aspergilli elaborate GABAT activity (activities?) to assimilate GABA, β-alanine and 6-aminocaproic acid (Arst, 1978), these have not been characterized in any detail. From the GABA grown *A. niger* mycelia, however, two distinct GABAT fractions were resolved by DEAE-Sephacel chromatography (unpublished observations). Preliminary studies suggest that they differ in their specificity towards ω-amino acids.

Oxidation of succinicsemialdehyde to succinate

The dehydrogenation of SSA to form succinate concludes the catabolism of GABA and commits the carbon skeleton into the TCA cycle. The enzyme catalysing this step, succinic-semialdehyde dehydrogenase (SSADH) from fungi, has received relatively little attention. In most instances, simply SSADH activity was monitored from crude cell extracts (Pietruszko & Fowden, 1961; Baldy, 1977; Vissers *et al.*, 1989).

A few properties of the *A. bisporus* and the yeast enzymes have been recorded. The kinetics, substrate and cofactor ($NAD(P)^+$) specificities along with the differential heat inactivation data indicate the possibility of two distinct SSADH activities, in both these organisms (Pietruszko & Fowden, 1961; Baldy, 1977). Our laboratory is exploring the molecular and kinetic characterization of this enzyme from *A. niger* mycelia (Kumar & Punekar, 1994). In summary, the salient features shared by these SSADHs are: (i) their mitochondrial location, (ii) high specificity for their aldehyde substrate (K_m for SSA in the lower μM range) while being relatively less specific for their nicotinamide cofactors, (iii) extreme lability with an absolute thiol requirement for activity, (iv) an alkaline pH optimum around pH 9·0, and (v) a characteristic substrate inhibition by SSA (the *A. bisporus* enzyme being a possible exception, Baldy, 1977).

None of the SSADHs studied so far catalyses the reverse reaction (i.e. the reduction of succinate to SSA) to any appreciable extent (Pietruszko & Fowden, 1961; Rating *et al.*, 1984; Satya Narayan & Nair, 1989). However, the *T. utilis* extracts were shown to reduce SSA to 4-hydroxybutyric acid (Pietruszko & Fowden, 1961).

MOLECULAR GENETICS OF FUNGAL GABA METABOLISM

The reported genetic analysis of GABA catabolism is mostly confined to the two fungi, *S. cerevisiae* and *A. nidulans*. On the other hand, molecular cloning and characterization of the crucial biosynthetic gene for GAD are limited to the *N. crassa* system. In this case Schmit and coworkers have established an excellent correlation between conidiation/conidial germination events with a functional GAD protein (see below). The various genes, gene products and their functions relevant to fungal GABA metabolism are summarized in Table 1.

Saccharomyces cerevisiae

The catabolic route for GABA in *S. cerevisiae* is established on a firm genetic basis (Ramos *et al.*, 1985). The two structural genes involved are *UGA1* (for GABAT) and *UGA2* (for SSADH). Together they are responsible for the conversion of GABA to succinate. Both these enzymes are inducible by GABA and their expression is under the control of the *UGA3*

Table 1. Fungal GABA metabolism: genes and their assigned functions*

Organism/gene	Function
Saccharomyces cerevisiae	
UGA1	GABA transaminase
UGA2	SSADH
UGA4	GABA permease
UGA3, *UGA35(DURL)*	Positive regulators
UGA43	Negative regulator
Neurospora crassa	
gad	Glutamate decarboxylase
Aspergillus nidulans	
*gat*A	GABA transaminase
*gat*B	GABA transaminase?
*ssu*A	SSADH
*int*A	Positive regulator
*gab*A	GABA permease
*lam*A	Lactamase

* Summarized from references: Arst, 1976, 1977, 1978; Arst *et al.*, 1978; Bailey *et al.*, 1979, 1980; Richardson *et al.*, 1989; Vissers *et al.*, 1989, 1990; Hao & Schmit, 1993.

gene product. The yeast *UGA1* gene was sequenced and the predicted amino acid sequence indicates a high degree of homology with three L-ornithine transaminases (André & Jauniaux, 1990).

The uptake and accumulation of GABA in *S. cerevisiae* is mediated by three transport systems. These include the general amino acid permease, a proline permease and a specific GABA permease (encoded by *UGA4* gene). Of these, the GABA permease is induced in the presence of GABA but also requires the *UGA3* gene product. Taken together, the pathway of GABA catabolism behaves as a regulon (under *UGA3* control) of three unlinked genes *UGA1*, *UGA2* and *UGA4*. Additional regulatory mutations provide evidence for the existence of both positive and negative regulatory elements which control the final expression of the *UGA4* gene (Vissers *et al.*, 1989). Three classes of mutants with a constitutive GABA-specific permease have been isolated: (i) a recessive mutation in the *UGA43* gene behaving like a *trans*-acting negative regulator for *UGA4* expression, (ii) the semi-dominant mutation (*uga11*) closely linked to *UGA4* gene product, and (iii) the *uga81* mutation, closely linked to the *UGA3* gene, making the whole UGA regulon constitutive. On the other hand, recessive mutations at the *uga35* locus lead to non-inducibility of the UGA regulon. Thus, the *UGA35* gene product behaves like a second *trans*-acting positive regulator, in addition to *UGA3* (Vissers *et al.*, 1989).

The *UGA35* gene is identical to the *DURL* gene that was previously identified as a positive regulatory factor for the urea catabolic route (Vissers *et al.*, 1990). It is worth noting that GABA and urea catabolic pathways are expressed under specific conditions and do not share any enzymic step or metabolite. The *UGA43* gene is yet another regulator (but acting negatively) that is common to both these pathways.

Aspergillus nidulans

The genetic analysis of GABA uptake/utilization by *A. nidulans* has been studied in some detail. Over the years, Arst and coworkers have elegantly characterized a large number of mutants related to this system (Arst, 1977, Kinghorn & Pateman 1977; Arst, Bailey & Penfold, 1980). They include mutations in many structural and regulatory genes.

The *int*A mutation in *A. nidulans* prevents the utilization of GABA, β-alanine and 5-aminovalerate as nitrogen sources and GABA as a carbon source (Arst, 1976). The expression of *gab*A (structural gene for GABA permease) is under the control of (i) *int*A gene for the induction by GABA, (ii) ammonium repression mediated by *are*A, and probably (iii) carbon catabolite repression as well (Bailey, Penfold & Arst, 1979). The regulatory mutations *gab*I-1, *gab*I-2 and *gab*I-3 (selected on GABA as the nitrogen source), result in an increased *gab*A expression and are *cis*-dominant in their effects. The *int*A gene also controls the expression of acetamidase and a lactamase (for utilizing lactam; Arst, Penfold & Bailey, 1978). The acid phosphatase specified by the *pac*C-5 is probably involved in GABA uptake by influencing the permease; mutation in *pac*C-5 leads to considerable reduction in GABA uptake and growth (Arst *et al.*, 1980).

The *gat*A gene, encoding a GABA transaminase of *A. nidulans*, is under the positive control of *int*A. Yet another gene (*gat*B) influences the GABAT levels and is implicated in GABA utilization (Bailey, Arst & Penfold, 1980). The exact role of *gat*B remains to be further elucidated. Partial or complete loss of *gat*A, *gat*B, and *ssu*A (the SSADH locus) leads to an accumulation of ω-amino acids. The *gat*A is not closely linked to any other genes involved in the ω-amino acid metabolism or related pathways.

The *A. nidulans gat*A gene was successfully cloned from a cosmid library and the DNA sequence of this 2·6 Kb genomic fragment is available (Richardson, Hurley & Hynes, 1989). An open reading frame of 1497 nucleotides is interrupted by three putative introns and predicts a 55 kDa polypeptide. A Northern analysis has confirmed that (i) *gat*A mRNA levels are under *int*A control and (ii) *gat*A is not strongly regulated by *are*A-mediated nitrogen metabolite repression. More recently, this cloned *gat*A was exploited, via heterologous hybridization, to isolate a putative GABAT gene (*ugat*A) from *Ustilago maydis* (Straffon, Hynes & Davis, 1996). This basidiomycete gene shows considerable homology to GABAT genes from the ascomycetes *A. nidulans* and *S. cerevisiae*. Interestingly, the *ugat*A promoter did not function in *A. nidulans*. Unlike the phenotype of a *gat*A$^-$ strain of *A. nidulans* or a *uga1*$^-$ strain of *S. cerevisiae*, disruption of *ugat*A gene did not hinder the growth of *U. maydis* on GABA as the sole nitrogen source. However, a significant loss of growth on β-alanine and a decrease in GABAT levels were observed. The *ugat*A product seems to participate primarily in the β-alanine metabolism while an alternative GABA metabolic route is predicted. This is clearly in line with the observed ability of many fungi to elaborate multiple ω-amino acid transaminases (discussed above).

GABA METABOLISM – FUNCTIONAL CONTEXTS

From the often demonstrated parsimony in cellular metabolism, it follows that GABA metabolism could possibly be recruited to fulfil other important role/s as well. The following valid connections, largely based on metabolic studies, are of relevance to fungal biology. However, applications of recombinant DNA techniques, being developed rapidly for these systems, are sure to contribute greatly in our understanding of fungal GABA metabolism.

Catabolism of nitrogen compounds

Various fungi like *S. cerevisiae* (Ramos *et al.*, 1985; Vissers *et al.*, 1989, 1990) and *A. nidulans* (Arst, 1978; Yonaha *et al.*, 1983) can utilize GABA as a sole nitrogen source. For this purpose, GABA specifically induces the relevant enzymes and a GABA permease. GABA catabolic machinery (such as GABAT and SSADH) may also be recruited to degrade a number of related compounds. For example, fungal GABAT may participate in the utilization of caprolactam, β-alanine, and other ω-amino acids (Yonaha *et al.*, 1983; Straffon *et al.*, 1996). At least in *C. boidinii*, the catabolic route for polyamines culminates in GABA and its eventual breakdown (Haywood & Large, 1986; Large, 1986, 1992; Large & Robertson, 1988).

The GABAT from *A. nidulans* acts on alanine, GABA and 6-aminohexanoate. More interestingly, it can functionally

substitute an enzyme in the L-ornithine biosynthetic route; the conversion of N-acetyl-L-glutamic-4-semialdehyde to N-acetyl-L-ornithine (Arst, 1978). The same enzyme also provides an alternate route of β-alanine synthesis from malonic semialdehyde. These two cases provide us examples of a possible biosynthetic role for GABAT.

Conidiation and germination of conidia

The L-glutamate pool constitutes the predominant nitrogen store (up to 2·5 % of conidial dry weight) of *N. crassa* conidia. This is possibly true for most other fungal conidia as well. During the early phase of conidial germination this glutamate is mobilized to L-aspartate (Schmit & Brody, 1975; Loo, 1976; Christensen & Schmit, 1980). The significance of this glutamate pool and its metabolism to GABA was evidenced by using a double mutant strain of *N. crassa*. This *en(am)-2;am* mutant was a glutamate auxotroph and had lower NADP$^+$-glutamate dehydrogenase levels. The greatly delayed germ-tube formation in this mutant responded to glutamate supplementation of the medium (Guignard & Brody, 1983).

Elegant work of Schmit and coworkers has established GAD as the first cloned, conidiation stage-specific marker enzyme (Schmit & Brody, 1975; Hao & Schmit, 1991, 1993). The GAD activity could not be detected in mycelia. Instead, high levels of this enzyme were found in the mature conidia. The GAD levels decline in the early phase of conidial germination; loss of this activity and the rate of glutamate catabolism are well correlated (Christensen & Schmit, 1980). This GAD activity does not reappear until the next round of conidial germination. Interestingly, in spite of their simultaneous presence in the conidia, GAD does not decarboxylate glutamate until the conidial germination begins.

The GAD structural gene from *N. crassa* was cloned and expressed in *Escherichia coli* (Hao & Schmit, 1993). The GAD protein is restricted to mature *N. crassa* conidia while the corresponding mRNA could be detected only in the conidiating mycelia. There are indications that the GAD mRNA levels are controlled by differential regulation of mRNA synthesis, turnover, or both. These and other observations are consistent with the interpretation that the GAD polypeptide is synthesized in the mycelia but is then packaged into conidia. Such detailed investigation on other GABA metabolic enzymes and their relationship to conidial metabolism are lacking. Similar data for other fungal systems are yet to be collected.

We have monitored GAD levels in *A. niger* mycelia. This enzyme activity was markedly elevated during acidogenic growth (Fig. 2). The reported accumulation of GABA (Kubicek *et al.*, 1979) is compatible with this profile of GAD activity. Further, *A. niger* mycelia do not conidiate normally during acidogenesis. It therefore remains to be seen whether the acidogenic state represents a unique case of altered development or differentiation.

Special metabolic states and the GABA shunt

The context within which various components of GABA-bypass exist, in the asexual developmental cycle of fungi, is summarized in Fig. 3. These metabolic parameters are aligned along the stages of fungal life-cycle for the sake of clarity and to highlight the possibility of a functional GABA shunt, if any. This schematic representation is based on the enzyme surveys and metabolite measurements alluded to above. A short temporal overlap of relevant enzymes and metabolites during the early phase of conidiospore germination is apparent. This is when the GABA shunt could possibly operate as a normal route of TCA metabolism. One reasonable approach to confirm this would be to use the noninvasive ^{13}C NMR technique to study GABA metabolism *in vivo*. A direct measurement of flux through the glutamate decarboxylation

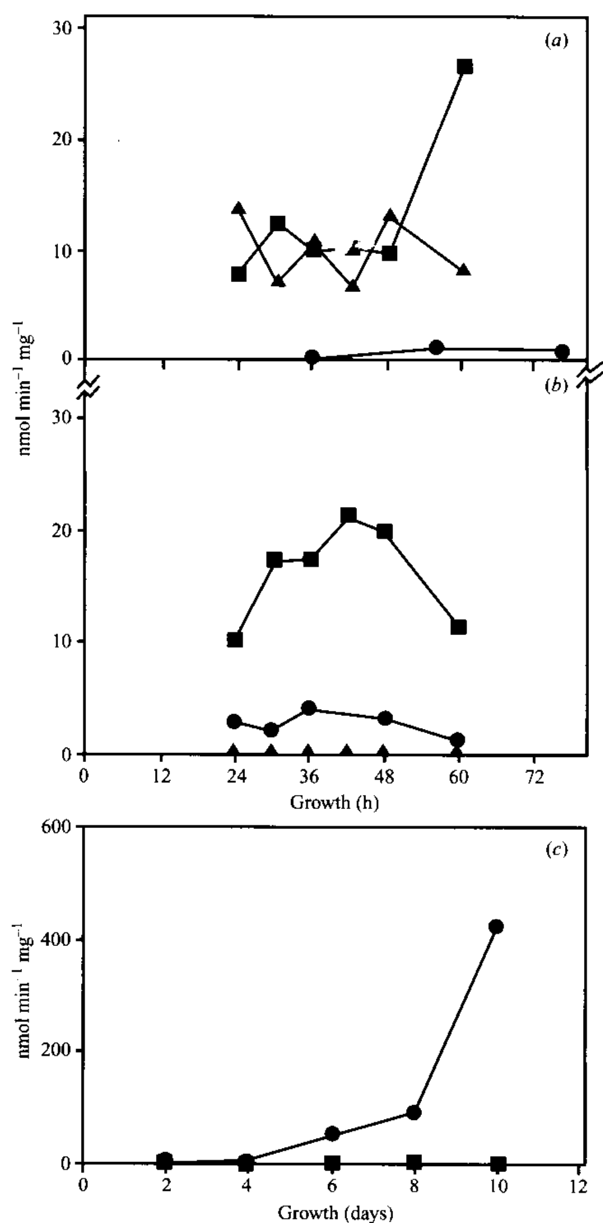

Fig. 2. The *Aspergillus niger* GABA metabolism: enzyme activities as a function of growth. Fungal surface cultures were grown in (i) minimal medium with L-glutamate (*a*) or ammonium nitrate (*b*) as nitrogen source and (ii) acidogenic medium (*c*). The data are from Kumar & Punekar (1994) and Kumar & Punekar (unpublished). The enzymes in the crude extracts were assayed according to Satya Narayan & Nair (1990). ●, GDC; ■, GABAT; ▲, SSADH.

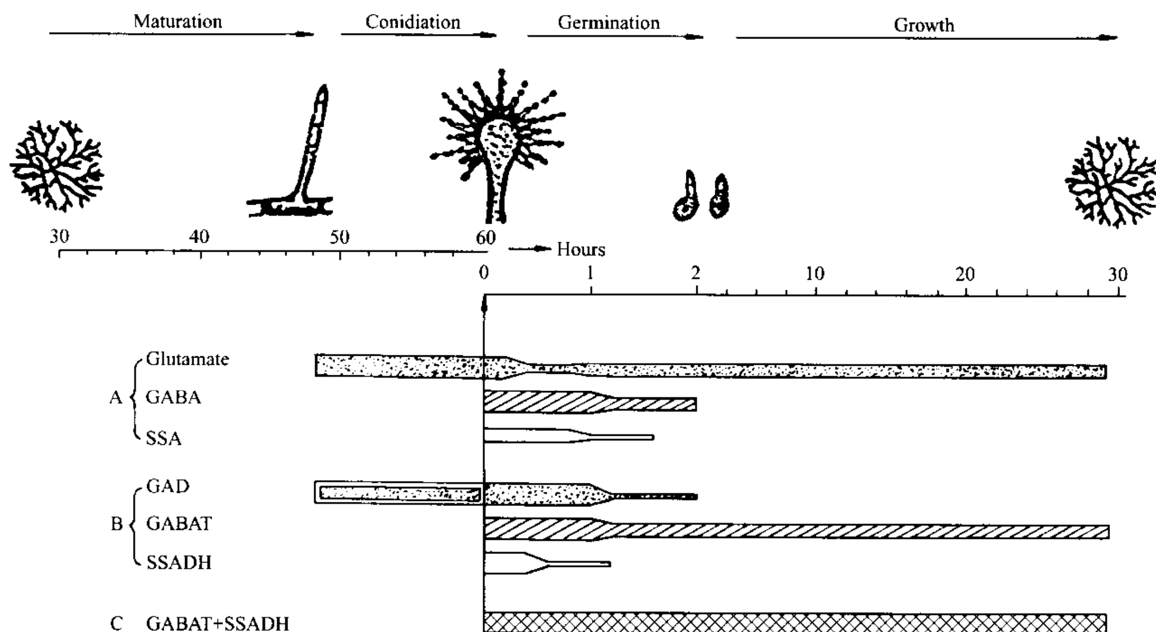

Fig. 3. Metabolites and enzymes of GABA metabolism along the asexual developmental cycle of fungi: a schematic temporal representation. The cartoons indicate different stages in the life-cycle of a representative filamentous fungus. The thickness of the horizontal bars relates to changes in enzyme/metabolite levels. Zero corresponds to the beginning of spore germination and GAD is functional only in the germinating spores. GABAT and SSADH are induced in GABA grown mycelia (shown in the figure as C).

loop is also feasible through careful radiotracer studies (e.g. Balazs *et al.*, 1970). Since the apparatus exists for a functional GABA shunt in *A. niger*, such an approach is being pursued in our laboratory.

The maturation of the cap tissue of *C. cinereus* is accompanied by a specific pattern of changes in the metabolism. Of these, amplified flux through the TCA cycle and the urea cycle are significant. The accumulation of amino compounds and urea is a candidate for osmoregulation in the cap tissue of this organism (Ewaze, Moore & Stewart, 1978; Moore, 1984). The activities of 2-ketoglutarate dehydrogenase and isocitrate lyase were either very low or could not be detected in the fruit bodies (Moore & Ewaze, 1976). A conventional TCA cycle/glyoxylate bypass may therefore not be operative. As activities of the enzymes of the glutamate decarboxylation loop were amplified, it is believed to be the normal route of TCA metabolism in this tissue. Similar lines of evidence support the existence of a functional GABA bypass pathway in the fruit body (Piquemal *et al.*, 1972) and dormant spores (Rast *et al.*, 1976) of *A. bisporus*.

CONCLUSIONS

Fungi possess GABA and GABA metabolism as an important component of their metabolic repository. An ubiquitous occurrence of GABA in them clearly supports this view. From the literature reviewed, it appears that GABA metabolic machinery is interfaced with some of the major facets of fungal cell cycle. These include nitrogen and energy metabolism, sporulation, differentiation, and development. A definitive picture of the importance of GABA in these processes is emerging. Some advance has been made towards the biochemical and enzymological aspects, but a continued research effort is needed. The applications of molecular genetics and genetic engineering of GABA pathway(s) in a few fungi is underway. It is anticipated that such an approach will illuminate and more clearly define the place of GABA in the fungal physiology and metabolism.

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