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A third gene affecting GABA transaminase levels in *Aspergillans nidulus*

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SUMMARY

Mutations in the *gat*B gene as well as mutations in the putative structural gene *gat*A and the positive acting regulatory gene *int*A can affect γ-amino-*n*-butyrate (GABA) transaminase (EC 2.6.1.19) levels in the ascomycete fungus *Aspergillus nidulans*. Partial or complete loss of function mutations in *gat*A, *gat*B and *ssu*A, which specifies succinic semialdehyde dehydrogenase, can lead to accumulation of ω-amino acids resulting in pseudo-constitutivity and elevated expression of (retained) activities under *int*A control. These regulatory effects underlie selective methods for *gat*B⁻, *ssu*A⁻ and leaky *gatA*⁻ mutations. However, all three *gat*B⁻ alleles which have been selected lead only to partial loss of GABA transaminase activity as judged by both *in vivo* and *in vitro* criteria. It has not been established whether the leakiness of these three *gat*B⁻ mutations is an allele-specific or a locus-specific effect and whether or not the GABA transaminase present in *gat*B⁻ strains differs from the wild type enzyme. Thus the rôle of the *gat*B product remains to be elucidated. The *gat*B gene is not closely linked to any other gene involved in ω-amino metabolism or related pathways.

1. INTRODUCTION

The γ-amino-*n*-butyrate (GABA) transaminase (4-amino-*n*-butyrate: 2-oxoglutarate aminotransferase, EC 2.6.1.19) of the ascomycete fungus *Aspergillus nidulans* is principally involved in the catabolism of GABA, β-alanine and other ω-amino acids (see Fig. 1). However, the enzyme can fulfil two other physiological rôles: (1) It can substitute for the transaminase which normally catalyses the conversion of N-acetyl-L-glutamic γ-semialdehyde to N^{α}-acetyl-L-ornithine for the biosynthesis of L-ornithine (and thence polyamines) and L-arginine (Arst, 1976,

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1977). (2) It can provide an alternative pathway of β-alanine synthesis, presumably by catalysing the transamination of malonic semialdehyde, for the biosynthesis of coenzyme A (Arst, 1978).

The regulation of GABA transaminase activity is particularly interesting. Previous work has identified two genes where mutation can affect GABA transaminase levels, the putative structural gene *gat*A and the positive acting regulatory gene *int*A (Arst, 1976, Arst & Bailey, 1977). The *int*A gene resembles an integrator gene on the Britten & Davidson (1969) model for regulation of gene expression. GABA transaminase is one of at least four activities specified by genes under *int*A control. The other three activities are a GABA permease specified by *gab*A (Arst, 1976; Bailey, Penfold & Arst, 1979), acetamidase specified by *amd*S (Arst,1976; Hynes, 1978; Arst, Penfold & Bailey, 1978), and an activity necessary

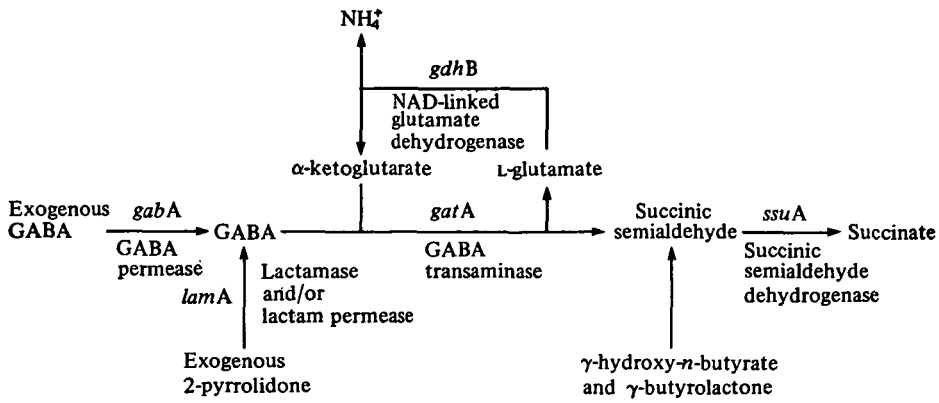

Fig. 1. Catabolic pathway for GABA and related compounds in *Aspergillus nidulans* (Arst, 1976; Arst *et al.* 1978).

for lactam utilisation – probably a lactamase – specified by *lam*A (Arst *et al.* 1978). β-alanine, GABA, and other ω-amino acids act as co-inducers of these four activities. It follows that mutations leading to intracellular accumulation of ω-amino acids may have consequences for the regulation of activities under *int*A control. In this paper we show that mutations in the *gat*B gene leading to partial loss of GABA transaminase result in pseudo-constitutivity and elevated expression of (retained) activities under *int*A control. These regulatory effects underlie selective techniques for *gat*B$^-$ mutations, as well as *ssu*A$^-$ (leading to loss of succinic semialdehyde dehydrogenase) and leaky *gat*A$^-$ mutations, which also result in ω-amino acid accumulation.

The main selective technique described in this paper makes use of *are*A^r mutations, which lead to loss of a positive acting regulatory product necessary for the expression of genes subject to nitrogen metabolite (or ammonium) repression (Arst & Cove, 1973; Rand & Arst, 1977). Reversion of strains carrying *are*A^r mutations has been an extremely fruitful method for obtaining mutations having direct or indirect regulatory effects. One of the reasons for the power of *are*A^r

reversion as a selective technique is the degree to which individual revertants can be characterised in the original *are*A^{r} background. Firstly, suppression of the *are*A^{r} phenotype can be monitored on a large number of nitrogen sources. Secondly, effects on carbon source utilisation can be screened because, although *are*A^{r} mutations prevent utilisation of nitrogen sources other than annonium in the presence of carbon catabolite repressing carbon sources such as D-glucose, they do not affect utilisation of any compound as sole carbon source (including those compounds able to serve as nitrogen sources) (Arst & Cove, 1973; Bailey & Arst, 1975; Arst & Bailey, 1977).

Previous work has shown that regulatory mutations which increase expression of the *gab*A gene can suppress *are*A^{r} mutations for utilization of GABA as a nitrogen source (Arst, 1976; Bailey *et al.* 1979). These regulatory mutations include *cre*A^{d} mutations leading to carbon catabolite derepression (Arst & Bailey, 1977; Arst, Bailey & Penfold, 1980), *gab*I mutations, which are tightly linked to and control in *cis* the expression of *gab*A (Bailey *et al.* 1979), and *int*A^{c} mutations leading to constitutive expression of *gab*A as well as the other activities under *int*A control (Arst, 1976). *gat*B^{-} mutations, described in this paper, also suppress *are*A^{r} mutations for GABA utilization.

2. MATERIALS AND METHODS

(i) *Strains, genetical techniques, and growth tests*

A list of *A. nidulans* mutations studied in this paper is given in Table 1. Other markers carried by strains are in general use (Clutterbuck, 1974; Clutterbuck & Cove, in the press). *gat*A-20 and -21 were isolated as partial suppressors of the arginine or ornithine auxotrophy *orn*B-7 in a strain of genotype *panto*B-100 (D-pantothenate requiring) *fw*A-1 (fawn conidial colour) *orn*B-7 after ultraviolet mutagenesis (D. C. Currie, P. F. Searle and H. N. Arst, Jr, unpublished results). The selection medium was a standard minimal medium (Cove, 1966) containing 1% (w/v) D-glucose as carbon source and 10 mM-ammonium (as the (+)-tartrate) as nitrogen source and supplemented with (final concentrations) 40 nM-biotin and 15 μM-D-pantothenate (as the calcium salt), and plates were incubated at 37 °C. Allelism of *gat*A-20 and -21 to *gat*A-2 was confirmed by lack of complementation in diploids and tight ($< 0{\cdot}1$ cm) meiotic linkage. *int*A^{-}-504 was selected as a partial reversion of the *int*A^{-}-101 mutation (leading to loss of *int*A function and non-inducibility of activities under *int*A control) in a strain of genotype *bi*A-1 (biotin requiring) *int*A^{-}-101 *gab*I-3 (leading to elevated expression of the GABA permease when in *cis*) after ultraviolet mutagenesis. The selection medium was standard glucose-minimal medium (Cove, 1966) containing 5 mM GABA as nitrogen source and supplemented with biotin, and the selection temperature was 37 °C. *int*A^{-}-504 differs from *int*A^{-}101 in that it does not reduce utilization of GABA, β-alanine, δ-amino-*n*-valerate or acetamide but it remains almost as stringent as *int*A^{-}-101 in its prevention of 2-pyrrolidone utilization as nitrogen or carbon source. Upon outcrossing to an *int*A^{+} strain, no progeny having the *int*A^{-}-101

phenotype were recovered and it is therefore likely that *int*A⁻-504 is a partial intracistronic reversion of *int*A⁻101. The selection of *gat*A-3, *gat*B-100, -200 and -201, and *ssu*A-1 is described in the Results section. In each case the glucose-minimal medium described by Cove (1966) was used with supplementation

Table 1. *Phenotypes of mutations central to this work*

Mutation	Relevant phenotype	Reference
*amd*S-17	Loss of acetamidase activity	Hynes, 1979
*are*A^{r}-2	Leads to an inability to utilize nitrogen sources other than ammonium	Arst & Cove, 1973
*are*A^{r}-600	Leaky *are*A^{r} allele, but strongly reduces 2-pyrrolidone utilization	Rand, 1978; this work
*are*A-102	Derepressed synthesis of some ammonium repressible enzymes and permeases	Arst & Cove, 1973; Hynes, 1975
*cre*A^{d}-1	Derepressed for carbon catabolite repressible activities	Bailey & Arst, 1975; Arst & Bailey, 1977
*gab*A-2	Reduced GABA permease activity	Arst, 1976; Bailey *et al.* 1979
*gab*I-1, 2, 3	*cis*-acting regulatory mutations affecting expression of GABA permease	Bailey *et al.*, 1979
*gat*A-1, 2	Inability to utilize ω-amino acids due to loss of GABA transaminase	Arst, 1976; Penfold, 1979
*gat*A-3	Slightly leaky *gat*A⁻ allele	This work
*gat*A-20, 21	*gat*A⁻ alleles able to suppress the *orn*B-7 auxotrophy	This work
*gat*B-100, 200, 201	Reduced GABA transaminase activity	This work
*int*A^{c}-2	Constitutive synthesis of acetamidase, GABA permease, GABA transaminase, and lactamase	Arst, 1976; Arst *et al.* 1978; Bailey *et al.* 1979; this work
*int*A⁻-101	Uninducible synthesis of acetamidase, GABA permease, GABA transaminase, and lactamase	Arst, 1976; Arst *et al.* 1978; Bailey *et al.* 1979; this work
*int*A⁻-504	Leaky *int*A⁻ allele, probably uninducible only for lactamase	This work
*lam*A-5	Unable to utilize 2-pyrrolidone, probably due to loss of lactamase activity	Arst *et al.* 1978
*orn*B-7	Auxotrophy for L-arginine or L-ornithine, probably due to loss of *N*-acetyl-L-glutamic γ-semialdehyde transaminase	Clutterbuck, 1974; Clutterbuck & Cove, in the press; Arst, 1976, 1977
*panto*C-3	Blocked in the biosynthesis of β-alanine and hence D-pantothenate and coenzyme A	Arst, 1978
*ssu*A-1	Probable loss of succinic semialdehyde dehydrogenase activity	Arst, 1976; this work

with (final concentrations) 40 nM-biotin, 30 μM-*p*-aminobenzoate, and 15 μM-D-pantothenate (as the calcium salt), where appropriate. Criteria used to establish allelism have been lack of complementation in diploids with, and tight meiotic linkage to, standard alleles.

Genetical techniques were modified after Pontecorvo, Roper, Hemmons Macdonald & Bufton (1953), McCully & Forbes (1965) and Clutterbuck (1974). Growth testing of *A. nidulans* has been described previously (Arst & Cove, 1969, 1973).

Growth tests were carried out by incubating for 2–3 days at 37 °C on standard minimal medium (Cove, 1966) containing appropriate supplements. Unless otherwise specified, 1 % (w/v) D-glucose served as carbon source. When utilization of carbon sources other than glucose was tested, 10 mM-ammonium chloride was used as nitrogen source.

(ii) *Enzyme assays*

Procedures for growth and extraction of mycelia, assay of GABA transaminase (EC 2.6.1.19) and acetamidase (EC 3.5.1.4), and determination of soluble protein in extracts were those given by Arst *et al.* (1978), with the modification that strains carrying mutant *are*A alleles were grown in media also containing 2 mM-ammonium (as the (+)-tartrate), which does not appreciably affect enzyme levels. Specific activities of acetamidase are expressed as nmoles ammonium formed per mg soluble protein in extract per minute.

3. RESULTS

(i) *The selection of gatB mutations*

As described in the Introduction, constitutive *int*A^{c} mutations suppress *are*A^{r} mutations for GABA utilization. It might be predicted that mutations leading to accumulation of co-inducers (ω-amino acids or metabolically related compounds) able to interact with the *int*A^{+} product to elicit *gab*A expression would also suppress the *are*A^{r} phenotype on GABA.

A collection of ultraviolet-induced revertants selected as able to utilize 5 mM-GABA as nitrogen source in a strain of genotype *pab*aA-1 (*p*-aminobenzoate requiring) *are*A^{r}-2 was kindly made available to us by Miss D. J. Gorton and Mr R. A. Peel. This collection of mutants enabled the identification of several further classes of mutations able to suppress the *are*A^{r} phenotype on GABA but recognizably different from previously characterized suppressors (see Introduction) by their diminution of the ability to utilize GABA and its lactam 2-pyrrolidone as carbon sources (see Fig. 1). It will be remembered that *are*A^{r} mutations do not affect the utilization of these compounds as sole carbon sources, so identification of the new supressor mutations was easily made in the *are*A^{r}-2 background.

One of the new suppressor mutations was shown to be a leaky *gat*A^{-} allele, designated *gat*A-3 after linkage and complementation tests. *gat*A-3 leads to considerable, but not total, loss of GABA transaminase (*vide infra*). Apparently it allows accumulation of enough GABA (and/or other endogenous ω-amino acids) intracellularly for sufficient *gab*A induction to suppress the *are*A^{r} mutation yet retains residual GABA transaminase activity to allow GABA to fulfil the nitrogen requirement. *gat*A-3 is recessive in diploids. Its phenotype is a convincing demonstration that GABA permease, not GABA transaminase, is the activity which limits GABA utilization by *are*A^{r} strains.

Another suppressor mutation, designated *ssu*A-1, defines a further class by resulting in inability to utilize 50 mM-γ-hydroxy-*n*-butyrate (sodium salt) and

γ-butyrolactone as well as GABA and 2-pyrrolidone as carbon sources. This indicates that it probably leads to loss of succinic semialdehyde dehydrogenase (see Fig. 1). *ssu*A-1 strains would thus accumulate succinic semialdehyde which, given the reversability of the GABA transaminase reaction, would result in GABA accumulation with consequent *gab*A induction. *ssu*A-1 is also recessive in diploids. GABA and 2-pyrrolidone are inhibitory to *ssu*A$^-$ strains. This might be due to accumulation of GABA which is toxic (Arst, 1976; Arst *et al.* 1978, 1980; Bailey *et al.* 1979) or to succinic semialdehyde toxicity or to both.

A third new suppressor mutation, also recessive, apparently lowers GABA transaminase levels because it reduces utilization of both 50mM GABA and 2-pyrrolidone as carbon sources (albeit much less drastically than *gat*A-3) without affecting utilization of γ-hydroxy-*n*-butyrate or γ-butyrolactone. Genetic analysis showed that this mutation, designated *gat*B-200, identifies a previously unidentified locus because it recombines freely with both *int*A and *gat*A mutations. Like *gab*I mutations (Bailey *et al.* 1979), *gat*A-3 and *ssu*A-1, *gat*B-200 suppresses *are*A^r-2 uniquely for GABA utilization. They are thus unlike truly constitutive *int*A^c mutations which can suppress *are*A^r mutations additionally for utilisation of acetamide and, to a lesser extent, 2-pyrrolidone as nitrogen sources (Arst & Cove, 1973; Arst, 1976; Arst *et al.* 1978, 1980).

Nevertheless the methods of selection of two other *gat*B alleles might indicate an effect on 2-pyrrolidone utilisation. *are*A^r-600 is an extremely leaky *are*A^r allele which has little or no effect on the utilization of most nitrogen sources, including GABA, but does strongly reduce 2-pyrrolidone utilization (Rand, 1978). Even on 2-pyrrolidone, however, the *are*A^r-600 phenotype is less extreme than that of non-leaky *are*A^r alleles such as *are*A^r-2. Dr K. N. Rand gave us some ultraviolet-induced revertants of a strain of genotype *paba*A-1 *are*A^r-600 selected as able to utilize 10 mM 2-pyrrolidone as nitrogen source. Amongst these revertants, we identified strains carrying *int*A^c mutations, leaky *gat*A$^-$ mutations, and a mutation, which, after linkage and complementation analysis, was designated *gat*B-100. The ability of *gat*B$^-$ and leaky *gat*A$^-$ mutations to suppress *are*A^r-600 on 2-pyrrolidone probably indicates that GABA accumulation can enhance *lam*A expression.

A different selection method for a *gat*B$^-$ mutation involved use of a leaky *int*A$^-$ allele. Complete loss of *int*A function mutations such as *int*A$^-$-101 are not suppressed for 2-pyrrolidone or GABA utilization by standard *gat*A$^-$, *ssu*A$^-$ or *gat*B$^-$ alleles (Arst, 1976; Arst *et al.* 1978 and unpublished results). This is hardly surprising because any regulatory effects of accumulation of GABA or other endogenous ω-amino acids must be mediated by a functional *int*A product. Moreover, 2-pyrrolidone is not itself an inducer of activities under *int*A control but must be converted to GABA in order to induce (Arst *et al.* 1978). The phenotype of the intA$^-$-504 allele is probably best explained as resulting from an *int*A product which, although functional, has reduced affinity for GABA. It does not significantly affect utilization of GABA or β-alanine as nitrogen sources but does drastically reduce 2-pyrrolidone utilization. 100 μM-β-alanine, although too dilute to contribute noticeably to nitrogen nutrition, considerably enhances growth of *int*A$^-$-504

strains on 5 mM-2-pyrrolidone as nitrogen source, presumably by inducing *int*A-mediated expression of *lam*A and *gat*A. The growth of *int*A$^-$-504 strains on 5 mM-GABA as nitrogen source indicates that relatively high concentrations of GABA achieve sufficient induction in *int*A$^-$-504 strains whilst exogenous 5 mM-2-pyrrolidone cannot maintain sufficiently high internal levels of GABA to induce appreciably. It is also possible that the active form of the *int*A$^-$-504 product elicits more efficiently *gab*A expression, required for GABA but not 2-pyrrolidone utilization, than *lam*A expression, required for 2-pyrrolidone but not GABA utilization.

After N-methyl-N′-nitro-N-nitrosoguanidine mutagenesis (Alderson & Hartley, 1969) of a strain of genotype *y*A-2 (yellow conidial colour) *int*A$^-$-504 *mol*A-33 (molybdate resistant) *panto*B-100 (D-pantothenate requiring), revertants able to utilize 5 mM-2-pyrrolidone as nitrogen source were selected. A common class of revertants, upon outcrossing to a wild type strain, yielded *int*A$^+$ recombinants resembling *gat*B-200 strains in phenotype. One of these mutations was analysed genetically, found to be tightly linked to *gat*B-200, and designated *gat*B-201. The ability of *gat*B$^-$ mutations to suppress *int*A$^-$-504 for utilization of 2-pyrrolidone as nitrogen source was confirmed upon construction of *gat*B-200 *int*A$^-$-504 double mutants. Another *int*A$^-$-504 suppressor selected in the same experiment was shown to be an *ssu*A$^-$ mutation. There were probably also leaky *gat*A$^-$ mutations responsible for the phenotypes of some revertants, but this was not confirmed. However, *gat*A-3 *int*A$^-$-504 double mutants were constructed and found to utilize 2-pyrrolidone as nitrogen source considerably better than *int*A$^-$-504 single mutants. Apparently GABA accumulation resulting from *gat*B$^-$, *ssu*A or leaky *gat*A$^-$ mutations can overcome the induction defect resulting from *int*A$^-$-504.

(ii) *The phenotype of gatB$^-$ mutations*

*gat*B-100, -200, and -201 have approximately the same phenotype. They reduce utilization of 5 mM-β-alanine, GABA, δ-amino-*n*-valerate and 2-pyrrolidone as nitrogen sources but much more drastically reduce utilization of 50 mM-GABA and 2-pyrrolidone as carbon sources. Their reduced utilization of GABA and 2-pyrrolidone is in part a consequence of the toxicity of accumulated GABA (Arst, 1976; Arst *et al.* 1978, 1980; Bailey *et al.* 1979) because 50 mM-GABA or 2-pyrrolidone reduces growth in the presence of alternative carbon sources such as 1 % (v/v) ethanol.

*gat*B$^-$ mutations enhance slightly, but less than *gat*A$^-$ or *int*A^c mutations (Arst, 1976; Hynes, 1978), utilization of 10 mM-acetamide and acrylamide as nitrogen sources. This suggests an effect on expression of the acetamidase encoded by *amd*S.

The fact that *gat*B$^-$ mutations (and leaky *gat*A$^-$ mutations such as *gat*A-3) enhance utilization of 5 mM-2-piperidone (δ-valerolactam) as nitrogen source suggests an effect on *lam*A expression also. 2-piperidone, like acrylamide, is a nitrogen source for certain *int*A^c mutants but not for wild type, presumably because it does not result in sufficient induction (Arst *et al.* 1978). 100 μM-β-

alanine considerably enhances utilization of 5 mM-2-piperidone and 10 mM-acrylamide as nitrogen sources by wild type strains without itself serving appreciably as a nitrogen source.

The ability of *gat*B$^-$ mutations to suppress *are*A^r mutations for GABA utilization (see Section (i)) indicates an effect on *gab*A expression. In this respect *gat*B$^-$ mutations resemble *int*A^c (Arst, 1976) and *gab*I (Bailey *et al.* 1979) mutations. Suppression of *are*A^r-2 by *gat*B-200 for GABA utilization is dependent on a functional *gab*A allele because *gat*B-200 *gab*A-2 *are*A^r-2 triple mutants are unable to use GABA as a nitrogen source.

GABA transaminase activity can also be monitored *in vivo* using *orn*B$^-$ strains, which lack the arginine biosynthetic enzyme responsible for the conversion of *N*-acetyl-L-glutamic γ-semialdehyde to N^{α}-acetyl-L-ornithine (Arst, 1976, 1977; Arst *et al.* 1978). This reaction can apparently also be catalysed by GABA transaminase, and co-inducers such as β-alanine and GABA can supplement *orn*B$^-$ auxotrophies. *int*A^c mutations can suppress *orn*B$^-$ auxotrophies in double mutants whereas non-leaky *int*A$^-$ and *gat*A$^-$ mutations prevent suppression of *orn*B$^-$ auxotrophies by *int*A^c mutations in triple mutants. *gat*A-3 behaves similarly to non-leaky *gat*A$^-$ mutations in this respect. Both *gat*A-3 *orn*B-7 double mutants and *int*A^c-2 *gat*A-3 *orn*B-7 triple mutants have a requirement for L-arginine or L-ornithine, which cannot be replaced by β-alanine or GABA. Nevertheless, certain leaky *gat*A$^-$ mutations can partially suppress *orn*B$^-$ auxotrophies because two, designated *gat*A-20 and -21, were obtained by reverting an *orn*B-7 strain to partial prototrophy (H. H. Arst, Jr, D. C. Currie & P. F. Searle, unpublished results. Details are given in Materials and Methods section). The ability of leaky *gat*A$^-$ mutations to suppress *orn*B$^-$ mutations would seem to depend upon their reducing activity towards (leading to the accumulation of) endogenous co-inducers for GABA transaminase such as β-alanine whilst retaining considerable activity towards *N*-acetyl-L-glutamic γ-semialdehyde. Reversion of *orn*B$^-$ strains to prototrophy should be a method for obtaining mutant GABA transaminases with altered substrate specificity. The heterogeneous behaviour of different leaky *gat*A$^-$ mutations (i.e. *gat*A-3 v. *gat*A-20 and -21) in an *orn*B$^-$ background is preliminary evidence that the putative structural gene *gat*A codes for the substrate binding site of the enzyme.

Neither *gat*B-200 nor *ssu*A-1 suppresses *orn*B-7 in corresponding double mutants nor do they affect the ability of β-alanine or GABA to supplement *orn*B-7. *int*A^c-2 *gat*B-200 *orn*B-7 and *int*A^c-2 *ssu*A-1 *orn*B-7 triple mutants are, like *int*A^c-2 *orn*B-7 double mutants, prototrophic.

Another *in vivo* method for monitoring GABA transaminase is afforded by the *panto*C-3 mutation, blocking biosynthesis of β-alanine and hence D-pantothenate and coenzyme A (Arst, 1978). GABA transaminase can provide an alternative pathway of β-alanine synthesis, presumably by catalysing the transamination of malonic semialdehyde. Thus *int*A^c mutations can suppress the *panto*C-3 auxotrophy and co-inducers of GABA transaminase such as DL-β-aminoisobutyrate can supplement it in strains carrying functional *int*A and *gat*A alleles (Arst, 1978). The

sensitivity of this *in vivo* test is less than that using *orn*B⁻ strains because *panto*C-3 is itself somewhat leaky. However, in *gat*B-200 *panto*C-3 double mutants there is no suppression of the *panto*C-3 auxotrophy, and supplementation responses are identical to those of *panto*C-3 single mutants.

It was of interest to determine whether the *gat*B product interacts with either the *int*A or the *gat*A product by constructing a series of double mutant strains. The reduced utilization of 2-pyrrolidone, GABA and other ω-amino acids resulting from *gat*B-200 is not suppressed by *int*A^c-2 in *int*A^c-2 *gat*B-200 double mutants. The non-leaky *int*A⁻ mutation *int*A⁻101 and the non-leaky *gat*A⁻ mutations *gat*A-1 and *gat*A-2 are completely epistatic to *gat*B-200 in double mutants. With leaky *gat*A mutations the effects of *gat*B-200 are additive: *gat*B-200 *gat*A-3, *gat*B-200 *gat*A-20 and *gat*B-200 *gat*A-21 double mutants are all more extreme in phenotype than any of the corresponding single mutants. There is thus no evidence for interactions although the possibility cannot be ruled out.

(iii) *GABA transaminase levels*

Data in Table 2 show that whereas *gat*A⁻, *int*A⁻ and *int*A^c mutations all drastically affect GABA transaminase levels, *gat*B-200 has a rather modest effect which is only apparent under strongly inducing conditions. This suggests that the reduced GABA and 2-pyrrolidone utilization due to *gat*B⁻ mutations owes more to the toxicity of non-metabolized GABA (Arst, 1976; Arst *et al.*, 1978, 1980; Bailey *et al.* 1979) than to carbon or nitrogen insufficiency. Another example where substrate toxicity makes the *in vivo* phenotype a sensitive indicator of reductions in enzyme levels is given by Rand & Arst (1977).

Table 2. *Relative activities of GABA transaminase in strains of various genotypes*

Relevant genotype	GABA transminase relative activity with co-inducer (at 5 mM)		
	None	β-alanine	GABA
wild type	14	100	47
*gat*A-2	5	0	tox
*gat*A-3	9	9	tox
*int*A⁻-101	12	13	n.t.
*int*A^c-2	142	134	n.t.
*int*A^c-2 *gat*A-2	2	0	n.t.
*gat*B-200	17	64	49
*are*A^r-2	22	110	n.t.
*are*A-102	38	tox	124
*int*A^c-2 *are*A^r-2	49	92	n.t.

Relative activities are expressed as percentages of the specific activity of the wild type strain induced with β-alanine. tox = toxicity of β-alanine or GABA to strains of this genotype precludes testing. n.t. = not tested. The *gat*A-2, *gat*A-3, *int*A⁻-101, *intA*^{c}-2, *int*A^c-2 *gat*A-2, *gat*B-200, and *int*A^c-2 *are*A^r-2 strains all carry the *p*-aminobenzoate auxotrophy *paba*A-1. The wild type, *are*A^r-2, and *are*A-102 strains carry the biotin auxotrophy *bi*A-1. The *are*A^r-2 and *are*A-102 strains also carry *fw*A-1 (fawn conidial colour).

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The *are*A^{r}-2 mutation appears to have little effect on GABA transaminase levels although comparison of data for the *int*A^{c}-2 and *int*A^{c}-2 *are*A^{r}-2 strains would suggest that the *are*A product is involved in control of GABA transaminase. There is also apparently an effect of the *are*A-102 mutation, which leads to derepressed expression of certain ammonium-repressible activities (Hynes & Pateman, 1970; Arst & Cove, 1973; Hynes, 1975). However, this effect might be a consequence of enhanced GABA uptake resulting in increased induction (Penfold, 1979). *are*A-102 leads to enhanced utilization of a number of α-amino acids (Hynes, 1973*a*) which in at least two cases is associated with increased uptake (Hynes, 1973*b*; Arst, 1977). β-alanine is extremely toxic to *are*A-102 strains, probably because of increased uptake (Penfold, 1979), precluding its use as a co-inducer. The uptake of β-alanine and α-amino acids must involve at least one common step or component because a class of mutation results in inability to utilize a large number of α-amino acids along with β-alanine and δ-amino-*n*-valerate (but excluding GABA) and to resistance to a number of toxic amino acid analogues (H. N. Arst Jr, unpublished results). *are*A-102 would appear to enhance expression of this common step or component because it also enhances utilization of 5 mM-δ-amino-*n*-valerate as a nitrogen source. Moreover, *are*A-102 *gat*A-2 double mutants are even more subject to the toxicity of 5 mM-β-alanine (in the presence of 1% (v/v) ethanol as carbon source and 10 mM-sodium nitrate as nitrogen source) than *are*A-102 or *gat*A-2 single mutants and more subject to the toxicity of 5 mM-δ-amino-*n*-valerate (in the presence of 1% ethanol as carbon source and 10 mM-sodium nitrate as nitrogen source) than *gat*A-2 single mutants.

A further point to be noted from Table 2 is that although, by other criteria, *gat*B-200 leads to accumulation of ω-amino acids, this accumulation appears to be insufficient for constitutive expression of the level of GABA transaminase which it retains. This might indicate that GABA transaminase induction requires higher levels of co-inducer than GABA permease induction (as indicated by *gat*B^{-} suppression of *are*A^{r} mutations) or acetamidase induction (*vide infra*). In contrast, *int*A^{c}-2 clearly leads to constitutive expression of GABA transaminase, provided a functional *gat*A allele is carried. *int*A^{-}-101 is predictably non-inducible. GABA permease activity has not been measured in a *gat*B^{-} strain, but it has been shown to be expressed constitutively in a *gat*A-2 strain (Penfold, 1979).

(iv) *Acetamidase levels*

Data in Table 3 show that *gat*B-200 considerably elevates the uninduced level of acetamidase. This partial constitutivity for acetamidase is an indirect but convincing demonstration that the reduction in GABA transaminase levels is sufficient for ω-amino acids to accumulate. It is responsible for the enhanced acetamide and acrylamide utilization by *gat*B^{-} strains. Partial constitutivity due to ω-amino acid accumulation is also apparent in the *ssu*A-1 and *gat*A-2 strains. Hynes (1978) reported partially constitutive synthesis of acetamidase in a *gat*A-312 strain. Indeed, elevated expression of acetamidase was crucial to the selection of both *gat*A-312 (Hynes, 1978) and *gat*A-2 (Arst, 1976). The uninduced levels of acetami-

dase shown in Table 3 correlate well with phenotypes on acetamide- and acrylamide-containing media: *ssu*A-1 has no effect whilst enhancement of utilization increases in the order *gat*B-200 < *gat*A-2 < *int*A^{c}-2. Decreased β-alanine catabolism apparently also elevates β-alanine-induced acetamidase levels in the *gat*B-200 strain and more markedly in the *gat*A-2 strain, although the *int*A^{c}-2 strain exhibits even greater superinducibility. The greater acetamidase levels associated with *int*A^{c}-2 apparently correlate not only with its effects on acetamide and acrylamide utilization by *are*A^{+} strains but also with the fact that it alone of the mutations listed in Table 3 is able to suppress *are*A^{r} mutations for acetamide utilization.

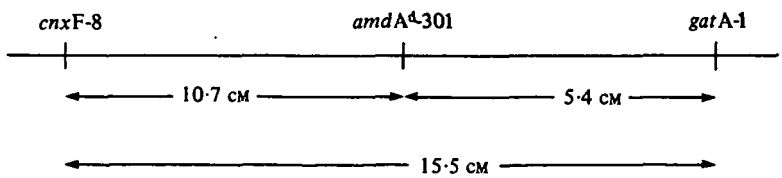
Table 3. *Specific activities of acetamidase in strains of various genotypes*

Relevant genotype	Acetamidase specific activity with co-inducer (5 mM)	
	None	β-alanine
wild type	63	226
*gat*A-2	208	374
*int*A^{c}-2	274	437
*int*A^{-}-101	36	60
*ssu*A-1	112	174
*gat*B-200	174	278

All strains carry the *p*-aminobenzoate auxotrophy *paba*A-1 except the *int*A^{c}-2 and *int*A^{-}101 strains, which carry the biotin auxotrophy *bi*A-1. The *ssu*A-1 and *gat*B-200 strains also carry *fw*A-1 (fawn conidial colour).

(v) *Map positions of genes involved in catabolism of GABA and related compounds*

The locations of *gat*B, *ssu*A and *int*A in linkage group II have been reported previously (Arst, 1977; Arst, Rand & Bailey, 1979). All three genes are located on the right arm of the linkage group, but they recombine freely and are separated by a number of unrelated genes. *gat*A is located in linkage group VII (Arst, 1976) and a three point cross has established the following linkage relationship (based on 355 progeny analysed):



*lam*A-5 is tightly linked (< 1 cM) to *fpa*D-43 in linkage group VIII, but its position in relation to other markers has not been determined. The positions of *pac*C, where mutations lead to pleiotropic loss of acid phosphatase activity and GABA permease (Arst *et al.* 1980), and *gab*A in linkage group VI have been reported previously (Bailey *et al.* 1979). They show only loose linkage and are separated by a number of unrelated genes. The other gene whose expression is known to be under *int*A control, *amd*S, is located in linkage group III, where its position

relative to other markers has been determined (Gunatilleke, Arst & Scazzocchio, 1975; Hynes, 1979). (Standard linkage maps of *A. nidulans* are given by Clutterbuck (1974) and Clutterbuck & Cove (in the press)). There is thus no clustering of genes involved in catabolism of GABA and related compounds or of genes under *int*A control.

4. DISCUSSION

Clearly, mutations in *gat*B as well as those in *gat*A and *int*A can affect GABA transaminase levels. The complex phenotype of *gat*B$^-$ mutants can best be explained in terms of a direct effect on GABA transaminase activity which leads to an intracellular accumulation of ω-amino acids. The ω-amino acids act as co-inducers in the *int*A$^-$ regulatory system with consequent effects on acetamidase, GABA permease and lactamase activities.

A crucial consideration is whether the three *gat*B$^-$ alleles selected in this work lead to complete loss of *gat*B function or whether the selective methods used might require some retention of *gat*B function, as, for example, the selective methods used to obtain leaky *gat*A$^-$ alleles do. Clearly, mutations leading to complete loss of GABA transaminase activity will not be selected using the methods described in this paper. An equally important question is whether or not the GABA transaminase present in *gat*B$^-$ strains is structurally identical to that present in wild type strains. Comparisons of thermolability and kinetic parameters might establish whether *gat*B codes for a structural component of GABA transaminase. Unfortunately the only assay procedure which seems satisfactory for GABA transaminase in *A. nidulans* (Arst *et al.* 1978; Penfold, 1979) is cumbersome and ill-suited to kinetic studies.

Present data are compatible with *gat*B coding for a structural component of GABA transaminase, for a co-factor required for maximal activity, or for some element involved in the regulation of *gat*A expression. A further question is whether the *gat*B gene is a fifth gene under *int*A control. However, an answer to this must await clarification of the rôle of the *gat*B product.

Note added in proof. Hynes (*Journal of Bacteriology* **142**, 400–406 (1980)) has recently questioned the necessity for use of the term integrator gene to describe *int*A. We believe this terminology to be helpful because it describes an aspect of the rôle of *int*A which is unique amongst *characterized* positive acting regulatory genes in prokaryotes and lower eukaryotes. In a particular context (i.e. the presence of ω-amino acids), the *int*A product integrates the expression of one structural gene (i.e. *amd*S) with that of several others (i.e. *gab*A, gatA and *lam*A) whereas in other contexts (i.e. the presence of acetate (or precursors) or benzoate (Arst, 1976; Hynes, 1978)) that structural gene can be expressed independently. This type of regulatory circuit whereby synthesis of a structural gene product can be induced through the action of any one out of two or more positive acting regulatory genes enables a structural gene to be expressed in as many contexts as there are regulatory genes. Thus the genome need contain only a single copy of the

structural gene rather than as many copies as there are contexts in which it is expressed. We believe that no other *characterized* regulatory gene shares this aspect with *int*A. For example, although *are*A is a positive acting regulatory gene which is similarly not metabolic pathway-specific, it does not integrate the expression of structural genes under its control. It could not possibly do so because it mediates repression rather than induction. Indeed, *are*A can be viewed as the antithesis of an integrator gene because it integrates, if anything, the non-expression of many structural genes in the context of nitrogen-rich growth conditions. Nevertheless, other integrator genes will probably be found (Arst, 1976; Rand, 1978). It is also possible that, upon further characterization, some of the positive acting regulatory genes mediating induction now considered to be pathway-specific will turn out to be integrator genes. In addition to references given in the text of this paper, more information about *int*A is given by Arst in *Genetics as a Tool in Microbiology* (eds. S. W. Glover and D. Hopwood), Society for General Microbiology Symposium 31, Cambridge University Press, 1981 (in the press).

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