Gene, 37 (1985) 207–214
Elsevier

GENE 1363

Transformation of *Aspergillus niger* using the *argB* gene of *Aspergillus nidulans*

(Recombinant DNA; integration; shuttle plasmid; λ phage; cosmid)

Frank P. Buxton, David I. Gwynne and R. Wayne Davies

Allelix Incorporated, 6850 Goreway Drive, Mississauga, Ontario, L4V1P1 (Canada) Tel. (416)6770831

(Received April 15th, 1985)
(Accepted May 6th, 1985)

SUMMARY

A mutant of *Aspergillus niger* defective in ornithine transcarbamylase function was transformed with plasmids carrying a functional copy of the *argB* gene of *Aspergillus nidulans* after treatment of spheroplasts in the presence of polyethylene glycol and calcium ions. The plasmid pDG3 gave stable transformants at a frequency of 4 per μg of input DNA. Southern blot analysis of DNA from transformants showed that pDG3 DNA had integrated into the *A. niger* chromosomes at a variety of locations. The transformants were phenotypically stable for many mitotic divisions. This procedure may potentially be used to insert any gene into the genome of *A. niger*. A cosmid shuttle vector, pDG1, for cloning in *Aspergillus* was also constructed.

INTRODUCTION

A. niger is of considerable economic importance, being used for the production of industrial enzymes and other metabolites such as organic acids. Genetical work on the available strains of this organism has been prevented by the failure of sexual crosses. An essential first step towards developing the molecular genetics for *A. niger* is the establishment of a method of transforming *A. niger* with exogenous DNA. Once transformation is possible, the well developed genetics and molecular biology of the related fungus *A. nidulans* is available and should allow the study of many *A. niger* genes and their regulation. Comparative studies of the details of gene regulation and other important processes in the two species will add considerably to our understanding of the molecular biology of filamentous fungi. In addition, successful transformation opens the way to the expression of foreign genes coding for desirable products in a system for which a great deal of industrial fermentation experience exists.

Methods of transformation of fungal spheroplasts with cloned genes, in the presence of PEG and Ca^{2+} ions have been developed in yeast (Hinnen et al., 1978), *Neurospora crassa* (Case et al., 1979; Buxton et al., 1984) and *A. nidulans* (Ballance et al., 1983; Tilburn et al., 1983). As *A. niger* and *A. nidulans* are regarded by taxonomists as being related, we chose to start with the transformation system as used for *A. nidulans* and to use cloned genes from *A. nidulans* to see if they could complement *A. niger* mutants.

We report here the successful complementation of a mutation in the *A. niger* gene coding for OTC by expression of the *A. nidulans argB* gene, which forms the basis of a useable transformation system for *A. niger*. This is a key step towards the exploitation of this important industrial organism as a host for the synthesis of peptides and proteins from other species.

Abbreviations: Km, kanamycin; PEG, polyethyleneglycol; OTC, ornithine transcarbamylase; ^{R}, resistance; SSC, 0.15 M NaCl, 0.015 M Na_3·citrate pH 7–8; Tn, transposon; UV, ultraviolet.

MATERIALS AND METHODS

(a) Strains

A. niger ATCC46951 was used as our standard wild type. Media for growing *A. niger* were as described by Cove (1966). All plasmids were grown in *Escherichia coli* HB101. Plasmid pBB116 (Berse et al., 1983), containing the *argB* gene of *A. nidulans*, was obtained from Dr. P. Węgleński.

(b) Recombinant DNA procedures

All enzymes were obtained from International Biotechnologies Incorporated and were used according to the manufacturers' instructions. Plasmids were prepared by the alkaline lysis procedure of Ish-Horowitz et al. (1981). Rapid DNA preparations from *A. niger* were as described by Yelton et al. (1984) or *A. nidulans*. Southern transfers, hybridisations and nick translations were as described in Buxton et al. (1983). DNA for dot blots was denatured in 9 vols. of 0.1 M NaOH for 10 min and neutralised with a further 1.5 vol. of 0.7 M Tris · HCl, 0.3 M NaCl and 0.02 M EDTA pH 6.8. After application to nitrocellulose using a dot blot apparatus (Bethesda Research Laboratories) the filters were treated as for Southern transfers.

(c) Transformation of *A. niger*

Mycelium was grown from an inoculum of 10^5 spores/ml in 500 ml of complete medium + 0.02% arginine + 10^{-5}% biotin in a 2-liter conical flask at 30°C with shaking at 250 rev./min. The mycelium was harvested through Whatman No.54 filter paper, washed with sterile water and sucked dry. The mycelium was added to 50 ml of filter-sterilised 1.2 M $MgSO_4$, 10 mM potassium phosphate pH 5.8, to which was added 20 mg of Novozym234 (Novo Enzyme Industries), 0.1 ml of β-glucuronidase (= 15000 units, Sigma) and 3 mg of bovine serum albumin for each gram of mycelium. Digestion was allowed to proceed at 37°C with gentle shaking for 50–70 min, and spheroplast production was checked periodically by light microscopy. Water (50 ml) was added and the spheroplasts were separated from the undigested fragments by filtering through 30-μm nylon mesh and harvested by centrifuging at 2500 × *g* for 5 min. The spheroplasts were washed by resuspending and centrifuging twice in 10 ml of 0.6 M KCl. The number of spheroplasts was determined using a hemacytometer and they were resuspended at a final concentration of 10^8/ml in 1.2 M sorbitol 10 mM Tris · HCl, 10 mM $CaCl_2$ pH 7.5. 0.4-ml aliquots were placed in plastic tubes to which DNA was added and incubated at room temperature for 25 min. In single experiments as much as 80 μg of DNA in 40 μl of 10 mM Tris · HCl, 1 mM EDTA can be used. An 0.4 ml aliquot of 60% PEG4000, 10 mM Tris · HCl and 10 mM $CaCl_2$ pH 7.5 was added to each tube with gentle but thorough mixing. Two further aliquots of this PEG solution were added similarly, the first being of 0.4 ml and the second of 1.6 ml. This was followed by a 20-min incubation at room temperature. The transformed spheroplasts were then added to appropriately supplemented minimal media containing 1% agar with or without 0.6 M KCl at 46°C. The suspension was poured immediately onto corresponding plates to form an overlay. After 3–5 days at 37°C transformants could be seen.

RESULTS

The key components of a useful transformation system are a cloned selectable gene and a recipient in which expression of the marker can be selected. Until dominant selectable markers become available in *Aspergillus*, it is necessary to have a recipient strain carrying a complementable mutation that has a low reversion frequency, and a corresponding cloned gene. Defects in amino acid biosynthetic pathways usually results in very clear phenotypes so that even poor complementation is easy to detect. Of the available *A. nidulans* genes, we chose to work with the *argB* gene which codes for OTC. As this gene was cloned by complementation in yeast (Berse et al., 1984), it probably contains no introns, since other *Aspergillus* genes containing introns could not be expressed in yeast (Penttila et al., 1984); the absence of introns removes one possible barrier to heterologous gene expression. The *argB* gene is also likely to have codon usage compatible with most fungal translation systems.

(a) Isolation of *A. niger* arginine-requiring mutants

Spores with auxotrophic mutations do not ger-

TABLE I

Analysis of the mutant phenotypes from a filtration enrichment experiment

Mutagenesis and filtration enrichment of *A. niger* was based on the method developed for *Ophiostoma multiannulatum* (Fries, 1947). Vegetative spores were harvested, in 0.01% Tween-80, from plates that had been inoculated with about 1000 spores and incubated for 1 week at 37°C on complete medium, filtered through 30-μm nylon mesh and washed 3 times by centrifuging and resuspending in the same solution. These spores can be stored in 0.01% Tween-80 for several weeks at 4°C without noticeable loss of viability. Spores were mutagenised by placing 10 ml of a 10^7/ml suspension, in 0.01% Tween-80, in a 9-cm petri dish and exposing them to UV light from a Chromatovue Transilluminator TM36 (Ultra-violet Products Inc.) for 10 min at 2 cm distance with constant stirring (in the absence of a lid). After 1 h in the dark the spores were suspended at 10^6/ml in minimal medium and incubated at 30°C with shaking at 200 rev./min. At 3-h intervals the germinated colonies were removed by filtering the suspension through 30-μm nylon mesh. The ungerminated spores were incubated until no further germination was visible by microscopic examination (typically 30 h). The spores were then plated on complete medium plus 0.05% sodium deoxycholate to limit the size of colonies (Mackintosh et al., 1963). The resulting colonies were tested for growth requirements on supplemented minimal media.

Supplement required	Frequency[a] (%)
Adenine	22.2
Arginine	9.0
Biotin	1.2
Choline chloride	0.6
Cysteine or methionine	12.6
Histidine	1.2
Leucine	4.8
Lysine	6.6
Methionine	10.2
Nicotinamide	1.2
Nitrogen, reduced	2.4
p-Aminobenzoic acid	4.8
Pyridoxine	4.2
Serine	0.6
Thiamine	1.8
Unclassified[b]	16.6

[a] The frequency at which each mutant phenotype was found is expressed as a percentage.

[b] Mutants having more than one requirement or not supplemented by any of the defined media tested are unclassified.

minate in minimal medium, and are therefore easily separated by filtration from germinated prototrophic spores, which put out hyphae. Wild-type *A. niger* conidia were mutagenised and the survivors filtration enriched in minimal media for auxotrophs as described in the footnote to Table I. In a typical experiment 10^8 spores were mutagenised, of which 0.5% survived as determined by growth on complete medium. Of these 5×10^5 viable spores 99.7% were removed during the filtration enrichment to give 1500 colonies on complete medium of which 26% were auxotrophs. These mutants were tested for their growth requirements on supplemented minimal media and the types of auxotrophs obtained are summarised in Table I. To selectively isolate arginine auxotrophs, filtration-enriched spores were plated directly onto arginine-supplemented minimal media. In this experiment auxotrophs other than those that require arginine will not grow.

The arginine auxotrophs were analysed in more detail by testing their growth on minimal medium supplemented with ornithine, citrulline or arginine (Table II). Mutants lacking OTC should grow on minimal plus citrulline or arginine but not on ornithine. Several of these were obtained, one of which, *argB*52 (350.52), was used for further experiments as it showed good growth on arginine-supplemented medium, no growth on minimal medium, and reverts spontaneously at a frequency of less than 1 in 10^8.

(b) Characteristics of plasmid vectors

Two plasmids were constructed that contained the *argB* gene of *A. nidulans* for this and further work. Plasmid pDG1, which contains the *cos* site of bacteriophage λ and hence may be used as a cosmid cloning vector, was constructed by inserting the 5.8-kb *Eco*RI-*Bam*HI fragment, containing the *argB*$^+$ gene of *A. nidulans*, from pBB116 (Berse et al., 1983) into pHC79 (Hohn et al., 1980), as outlined in Fig. 1. This plasmid can be used for cosmid cloning, having unique *Bam*HI and *Eco*RI sites and selectable markers in *E. coli* and *Aspergillus*.

As the Km^R gene of Tn*5* can be expressed in both *E. coli* and yeast (Jimenez et al., 1980) we also constructed a plasmid, pDG3, that contains both *argB* of *A. nidulans* and the Km^R gene of Tn*5* in order to test for the expression of Km^R in *A. niger*. To do this pNE02 was first constructed by cloning the 1.4-kb *Hin*dIII-*Sal*I fragment of Tn*5* (Berg et al., 1983), containing the Km^R gene, into pUC12 (Vieira et al., 1982) and then the 3.4-kb *Xba*I fragment from pDG1 was subcloned into the unique *Xba*I site of pNE02

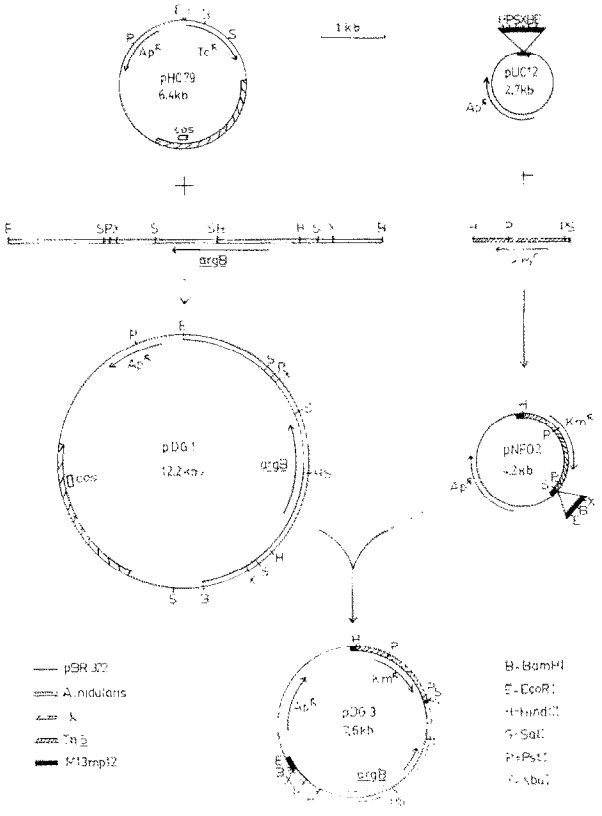

Fig. 1. Plasmids used in this work. For details of their construction see RESULTS, section **b**.

to give pDG3 (Fig. 1). This plasmid has markers selectable in yeast, *E. coli* and *Aspergillus* and has unique *Eco*RI and *Bam*HI sites for cloning. The Km^R determinant in this construction is expressed in yeast and *E. coli* but not in *Aspergillus*.

(d) Stability of ArgB$^+$ transformants

We transformed a strain (350.52) carrying the mutant *argB*52 with either pDG1 or pDG3 as described in MATERIALS AND METHODS, section **c**. The regeneration frequency of spheroplasts, as determined by comparing the number of spheroplasts counted microscopically with the number of colonies on complete medium containing 0.6 M KCl, was 40–90%. Contamination with nonosmotically sensitive cells was less than 1%. On the selective plates there were a few large and up to 100 times as many small colonies. On subculturing, the small colonies did not continue growing, whereas the large ones did. Small colonies were not obtained in the absence of added DNA or when the spheroplasts were treated with pUC12. Similar observations have been made with other fungal species (Hinnen et al., 1978; Case 1982; Tilburn et al., 1983; Yelton et al., 1984) and the small colonies are thought to be derived from spheroplasts that have failed to integrate the DNA that they have taken up. Transient expression of the *arg*B gene on the unintegrated plasmid may allow some growth to take place, but the plasmid is rapidly lost in the absence of integration (Buxton et al., 1984) and the colony stops growing. Scoring only the large colonies as real transformants, plasmid pDG1 gave 0.6 transformants per μg while pDG3 gave as many as 4 transformants per μg.

To demonstrate unequivocally that transformation had been achieved (rather than some DNA-mediated reversion event), DNA was prepared from

TABLE II

Growth characteristics of arginine auxotrophs

Mutation	Missing enzyme	Growth[a] on minimal media plus				Frequency[b] of *arg* mutants
		Nothing	Ornithine	Citrulline	Arginine	
wild type		+	+	+	+	–
argA	carbamoyl phosphate synthetase	–	+	+	+	24
argB	ornithine transcarbamoylase	–	–	+	+	35
argC	arginosuccinate synthetase	–	–	–	+	41
argD	arginosuccinase	–	–	–	+	

[a] Growth was scored after 3 days at 37°C on minimal medium (Cove, 1966) plus various supplements at 10 mM. +, good growth; –, no growth.

[b] The frequency at which the different types of arginine mutants are observed in a group of arginine auxotrophs obtained from a filtration enrichment experiment (see Table I). Note that *argC* and *argD* are not distinguishable on these media.

a number of independent colonies and dot blots made and probed with pDG1. 15 out of 16 colonies tested did contain sequences homologous to pDG1; the DNA of the host strain, 350.52, shows no homology to pDG1, pDG3 and pBR322 as determined by hybridisation to DNA immobilised on nitrocellulose filters when washed in 0.1 × SSC at 65 °C. To test if unselected parts of the plasmid were integrated, transformants obtained with pDG3 were probed with the 1.4-kb *Hin*dIII-*Sal*I Km^R gene fragment, and all eight tested were hybridised.

(d) Stability of $ArgB^+$ transformants

The mitotic stability of the transformed phenotype was assayed by growing transformants from a central inoculum on a petri dish containing complete medium plus 10 mM arginine. After 5 days spores were removed from the edge of the plate, diluted appropriately and plated on the same medium. The resulting single colonies were replicated and scored for auxotrophy. Of 400 colonies obtained from eight transformants none were auxotrophs, as one would expect for a chromosomally integrated gene but not for an autonomously replicating plasmid by analogy with the behavior of such plasmids in yeast (Stinchcomb et al., 1979).

(e) Analysis of the integration events in some transformants

To show whether stable transformation is mediated by autonomous replication or by integration into the genome of *A. niger*, it was necessary to analyse in more detail the fate of the transforming DNA. DNA was prepared from a number of transformants, run on an agarose gel and probed with pBR322. All the hybridisation was to the smear of randomly sheared DNA at the top of the gel (Fig. 2), indicating that the detectable incoming plasmid DNA had either integrated into the chromosomal DNA or was present as large concatamers.

Plasmid pDG3 is not cut by *Xho*I and is cut once by *Eco*RI in the sequence which is not homologous to pBR322. If recombination occurred between the chromosome and the plasmid sequences not derived from pBR322, the transformants should have one *Eco*RI fragment that is detectable by hybridisation to pBR322 (Fig. 3a). On the other hand if recombination occurred between the chromosome and the plasmid sequences derived from pBR322, the transformants should have two *Eco*RI fragments that are detectable by hybridisation to pBR322 (Fig. 3b). If transformants have direct tandem inserts of the plasmid at the same site, then their *Eco*RI-digested DNA should have a strong band hybridising to pBR322 at 6.8-kb monomer length of pDG3, and either one or two flanking fragments depending on the position of the recombination event in the plasmid (Fig. 3c). The multiple inserts could be produced by insertion of a single copy followed by further successive homologous rounds of recombination into the integrated plasmid, or by integration of a pre-existing multimer into the chromosome, this multimer having been generated in *E. coli* or *A. niger*.

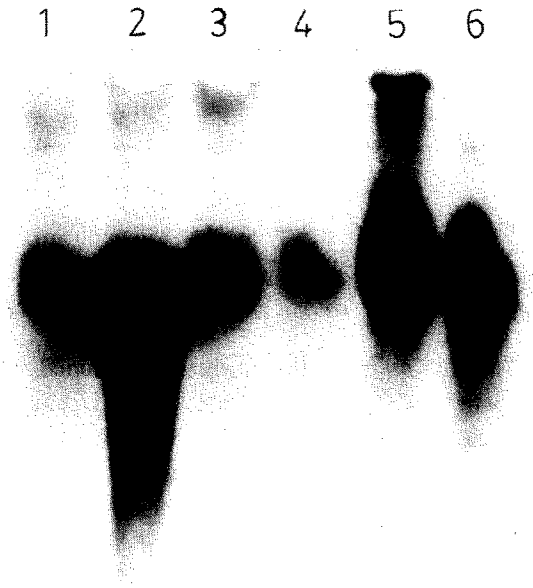

Fig. 2. Southern transfers of undigested DNA from *A. niger* transformants, probed with pBR322. The lane numbers correspond to transformant numbers. The hybridisation conditions were as described in MATERIALS AND METHODS, section **b**. Filters were washed at 65 °C in 0.1 × SSC.

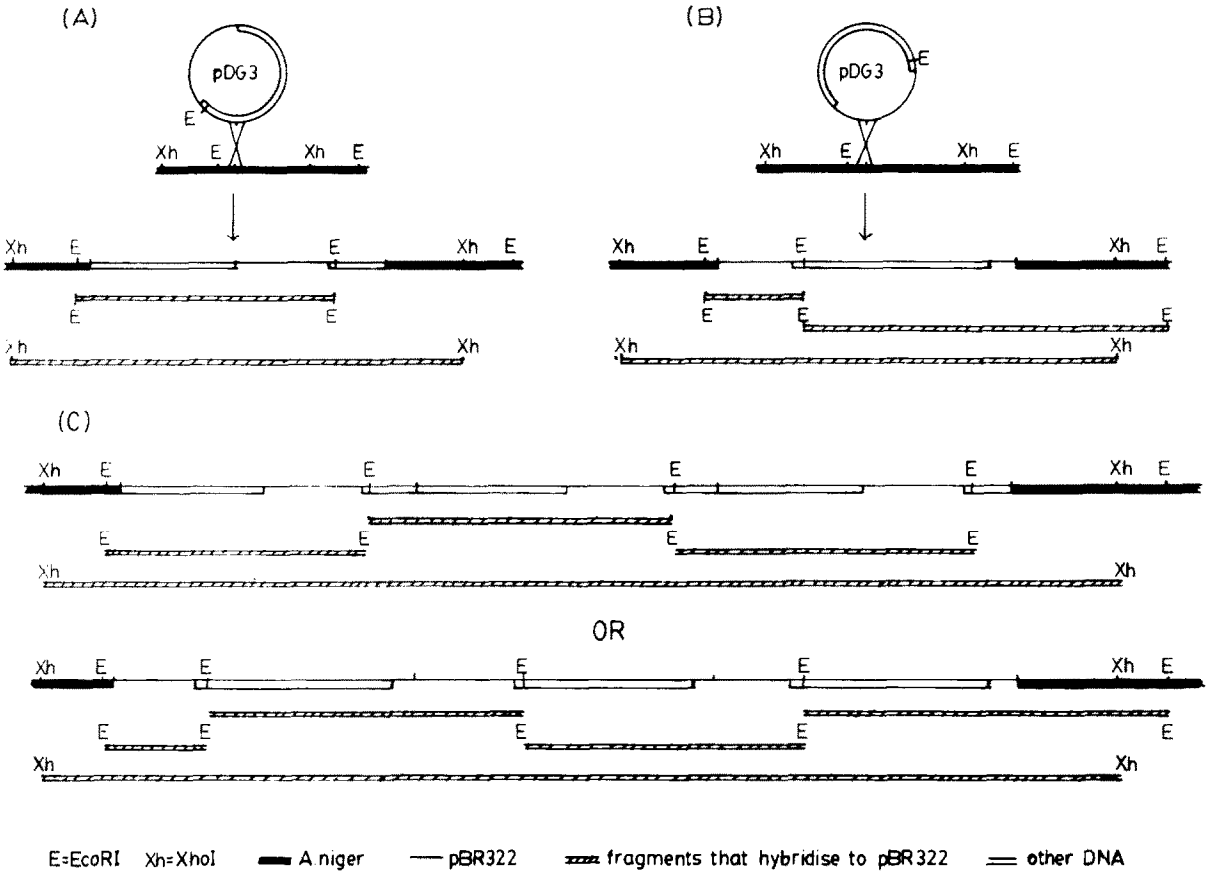

Fig. 3. Diagram of the integration events that may have occurred in various transformants. The restriction fragments that would hybridise to pBR322 are also indicated. For further details see RESULTS, section e.

The DNA from transformants obtained with pDG3 were cut with either *Eco*RI or *Xho*I, run on an agarose gel and probed with pBR322 (Fig. 4). As can be seen from Fig. 4, transformants 1 and 2 have two major *Eco*RI fragments that hybridise to pBR322 as expected for a single insert that recombined into the chromosome via recombination within the pBR322 sequences. One can also see some minor bands that have probably arisen from rearrangements of the inserted DNA. The pattern of hybridisation observed for transformant 4 (Fig. 4), when digested with *Eco*RI, consists of a strong 6.8-kb band and two weaker higher M_r bands. This can be explained as a tandem integration of a number of copies of pDG3 at the same site. The two very faint smaller bands are again probably the result of rearrangements. Other more complex patterns were also obtained, e.g., transformants 3 and 5 (Fig. 4). As the size of the hybridising *Xho*I fragment and the sum of the hybridising *Eco*RI fragments are variable between the different transformants with single inserts, the site of integration in the genome must be different in different transformants.

DISCUSSION

For the first time, transformation of *A. niger* and stable insertion of foreign DNA into the genome of *A. niger* has been demonstrated. The frequency at which transformants were obtained compares well with that obtained for non-autonomous vectors in yeast (Hinnen et al., 1978), *N. crassa* (Case et al., 1978; Buxton et al., 1984) or *A. nidulans* (Ballance et al., 1983; Tilburn et al., 1983; Yelton et al., 1984). This system is adequate for the insertion of any cloned sequence into the genome of *A. niger*, allowing foreign genes to be introduced and maintained stably in this organism.

In the transformants the vector DNA was integrated into the genomic DNA, and no evidence could be obtained for the existence of free plasmid. The transformed phenotype was stable for many

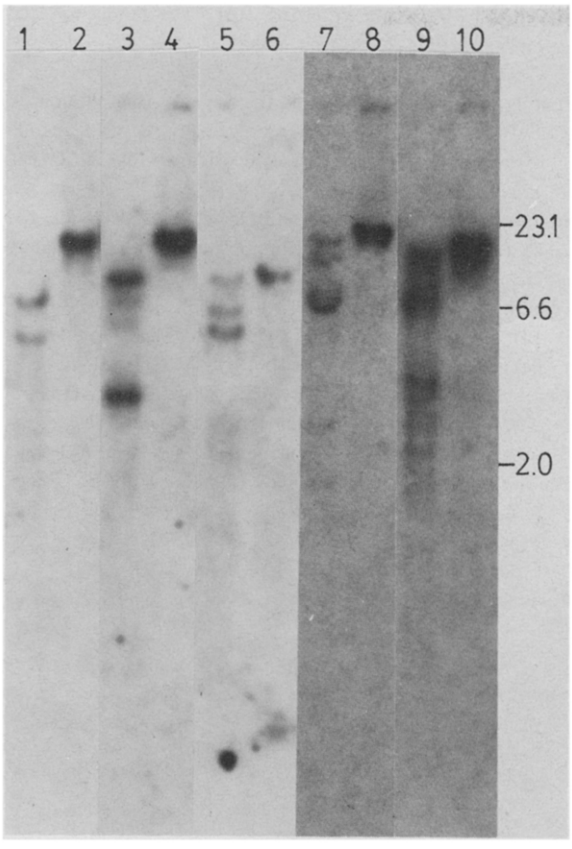

Fig. 4. Southern transfers of DNA from *A. niger* transformants digested with *Eco*RI (odd numbered lanes) or *Xho*I (even numbered lanes) and probed with pBR322. Lanes 1 and 2, transformant 1; lanes 3 and 4, transformant 2; lanes 5 and 6, transformant 3; lanes 7 and 8, transformant 4; lanes 9 and 10, transformant 5. Hybridisations and washing conditions were as given in Fig. 2.

generations as expected for the chromosomal location of the introduced gene. However, the occurrence of minor bands in the Southern transfers probed with pBR322 indicates that some rearrangement of the inserted DNA is occurring, although the *argB*$^+$ phenotype is not lost. Genetic analysis (by diploidisation) in *A. niger* is not yet developed enough to allow us to map the sites of integration reliably. It is clear from RESULTS, section **e** that the plasmid DNA is integrating into different sites in the genome. This differs from the results of Yelton et al. (1984) with *A. nidulans*, where 13 out of 15 *trpC*$^+$ transformants had integrated the plasmid into the *trpC* gene. Ballance et al. (1983) showed, however, that little homology is required for integration in *A. nidulans*, and in the absence of homology the transforming DNA is integrated at numerous sites in the genome (Ballance et al., 1985). Our results would agree with this if the *argB* genes of *A. niger* and *A. nidulans* are only weakly homologous. It seems that in these species recombination between sequences with extensive homology is the preferred event, but recombination between sequences having very little homology is a significantly frequent mode of integration.

The system described here is clearly a prototype. The frequency of transformation should be susceptible to considerable improvement, as it has been in *A. nidulans*, by modifications in the procedure for making protoplasts, by changing the recipient strain and by plasmid rearrangements or the incorporation of particular DNA fragments (Ballance et al., 1985). We are also incorporating a cloned *A. niger* gene which should be better expressed than the *A. nidulans* gene and thus perhaps increase the number of surviving transformants, and a dominant selectable marker expressed from a controllable promoter which will allow transformation of non-mutant recipient strains. As unselected parts of the vector were also incorporated into the genome along with the selected marker, we are able to insert almost any piece of DNA into the genome of *A. niger* by coupling it to the *argB* gene, or another selectable marker, and maintain the inserted DNA fragment stably for many generations.

REFERENCES

Ballance, D.J., Buxton, F.P. and Turner, G.: Transformation of *Aspergillus nidulans* by the orotidine 5′-phosphate decarboxylase gene of *Neurospora crassa*. Biochem. Biophys. Res. Commun. 112 (1983) 284–289.

Ballance, D.J. and Turner, G.: Development of a high frequency transforming vector for *Aspergillus nidulans*. Gene (1985) in press.

Berg, D.E. and Berg, C.M.: The prokaryotic transponsable element Tn5. Biotechnology 1 (1983) 417–435.

Berse, B., Dmochowska, A., Skrzypek, M., Węgleński, P., Bates, M.A. and Weiss, R.L.: Cloning and characterization of the ornithine carbamoyltransferase gene from *Aspergillus nidulans*. Gene 25 (1983) 109–117.

Buxton, F.P. and Radford, A.: Cloning of the structural gene for orotidine 5′-monophosphate carboxylase of *Neurospora crassa* by expression in *Escherichia coli*. Mol. Gen. Genet. 190 (1983) 403–405.

Buxton, F.P. and Radford, A.: The transformation of mycelial spheroplasts of *Neurospora crassa* and the attempted isolation of an autonomous replicator. Mol. Gen. Genet. 196 (1984) 339–344.

Case, M.E., Schweizer, M., Kushner, S.R. and Giles, N.H.: Efficient transformation of *Neurospora crassa* by utilising hybrid plasmid DNA. Proc. Natl. Acad. Sci. USA 76 (1979) 5259–5263.

Case, M.E.: Transformation of *Neurospora crassa* utilizing recombinant plasmid DNA, in Hollaender, A., DeMoss, R.D., Kaplan, S., Konisky, J., Savage, D. and Wolfe, R.S. (Eds.), Genetic Engineering of Microorganisms for Chemicals. Plenum, New York, 1982, pp. 87–100.

Cove, D.J.: The induction and repression of nitrate reductase in the fungus *Aspergillus nidulans*. Biochim. Biophys. Acta 113 (1966) 51–56.

Fries, N.: Experiments with different methods of isolating physiological mutations of filamentous fungi. Nature 159 (1947) 199.

Hinnen, A., Hicks, J.B. and Fink, J.R.: Transformation of yeast. Proc. Natl. Acad. Sci. USA 75 (1978) 1929–1933.

Hohn, B. and Collins, J.: A small cosmid for efficient cloning of large DNA fragments. Gene 11 (1980) 291–296.

Ish-Horowitz, D. and Burke, J.F.: Rapid and efficient cosmid cloning. Nucl. Acids Res. 9 (1981) 2989–2998.

Jimenez, A. and Davies, J.: Expression of a transposable antibiotic resistance element in *Saccharomyces*. Nature 287 (1980) 869–871.

Lusky, M. and Botchan, M.: Inhibition of SV40 replication in simian cells by specific plasmid pBR322 DNA sequences. Nature 293 (1981) 79–81.

Mackintosh, M.E. and Pritchard, R.H.: The production and replica plating of microcolonies of *Aspergillus nidulans*. Genet. Res. Camb. 4 (1963) 320–322.

Penttila, M.E., Nevalainen, K.M.H., Raynal, A. and Knowles, J.K.C.: Cloning of *Aspergillus niger* genes in yeast. Expression of the gene coding *Aspergillus* β-glucosidase. Mol. Gen. Genet. 194 (1984) 494–499.

Stinchcomb, D.T., Struhl, K. and Davis, R.W.: Isolation and characterisation of a yeast chromosomal replicator. Nature 282 (1979) 3943.

Tillburn, J., Scazzocchio, C., Taylor, G.G., Zabicky-Zissman, J.H., Lockington, R.A. and Davies, R.W.: Transformation by integration in *Aspergillus nidulans*. Gene 26 (1983) 205–221.

Vieira, J. and Messing, J.: The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene 19 (1982) 259–268.

Yelton, M.M., Hamer, J.E. and Timberlake, W.E.: Transformation of *Aspergillus nidulans* by using a *trpC* plasmid. Proc. Natl. Acad. Sci. USA 81 (1984) 1370–1374.

Communicated by A.J. Podhajska.