Vol. 112, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 15, 1983 Pages 284-289

TRANSFORMATION OF *ASPERGILLUS NIDULANS* BY THE OROTIDINE-5'-PHOSPHATE DECARBOXYLASE GENE OF *NEUROSPORA CRASSA*

*D. J. Ballance, **F. P. Buxton and *G. Turner

*Department of Microbiology, The Medical School, University Walk, Bristol BS8 1TD and **Department of Genetics, University of Leeds, Leeds LS2 9JT, England

Received February 16, 1983

SUMMARY Relief of an auxotrophic requirement for uridine in *Aspergillus nidulans* strain G191 has been achieved by transformation with a segment of *Neurospora crassa* DNA containing the corresponding gene coding for orotidine-5'-phosphate decarboxylase. The mitotic stability of such transformants suggests that the DNA has integrated into the genome. Southern hybridisation analysis of DNA isolated from transformants revealed the presence of pBR322 sequences which have integrated into the host genome along with the *N. crassa* DNA.

There are many examples in the literature of expression of genes in non-homologous host systems [1]. Most extensively studied has been the expression of eukaryotic genes in *Escherichia coli* [2, 3]. Transformation of *E. coli* with randomly cloned fragments of DNA has enabled the isolation of eukaryotic genes by direct [4] and indirect [5, 6] methods. Among the lower eukaryotes, structural genes from *Saccharomyces cerevisiae* [4, 7, 8], *Neurospora crassa* [9, 10] and *Aspergillus nidulans* [11] have been isolated by their ability to complement the corresponding auxotrophs of *E. coli*. Expression of foreign genes in lower eukaryotes has been largely restricted to yeast, e.g. the prokaryotic aminoglycoside phosphotransferase-3'(I) [12] and chloramphenicol acetylase [13] genes. There are very few reports of complementation of enzyme lesions in lower eukaryotes by foreign eukaryotic DNA [14]. Indeed, it has been shown that the yeast genes *trp1*, *his3*, *ura3* and *leu2* do not express in *N. crassa* when introduced on a selectable plasmid [15].

In common with *S. cerevisiae* and *N. crassa*, the genetics of *A. nidulans* has been well characterised [16]. Efficient transformation systems have been developed for both *S. cerevisiae* [17, 18] and *N. crassa* [19, 20] which have permitted the extension of earlier classical genetic studies to investigations at the molecular level. To date, it has not been possible to transform *A. nidulans*. Clearly the development of

0006-291X/83 $1.50

such a system in this species would be invaluable in the elucidation of the molecular genetics of *Aspergillus* sp.

Here we report the transformation of an *Aspergillus nidulans* pyrimidine auxotroph to prototrophy by the orotidine-5'-phosphate decarboxylase (EC 4.1.1.23, *pyr4*) gene segment isolated from *Neurospora crassa* [21].

MATERIALS AND METHODS

The following strains were used in this work:

E. coli: FB1009, a *pyrF* strain harbouring pFB6, an 8.4 kilobase plasmid carrying the *pyr4* gene from *Neurospora crassa* (Fig. 1) [21].

A. nidulans: G191 *pyrG89, pabaA1; mauA2; fwA1*
R153 *wA3; pyroA4*.

High molecular weight *A. nidulans* DNA was prepared by the method of Ullrich *et al.* [22] omitting the sucrose gradient step. Plasmid DNA was prepared essentially as described by Cornelis *et al.* [23].

The method used for transformation of *A. nidulans* was as follows: Overnight cellophane cultures [24] of G191 on complete medium [25] plus uridine (10mM) were incubated in a solution of Novozym 234 (5mg/ml in 0.6M KCl, Novo Enzyme Products) for 90 minutes at 30°C. The protoplast suspension was then filtered through sintered glass (porosity 2) and washed twice with 0.6M KCl. The protoplasts were resuspended in 1ml 0.6M KCl, 50mM $CaCl_2$ and divided into 200μl aliquots. DNA dissolved in 15-20μl of Tris-EDTA buffer and 50μl 25% polyethylene glycol 6000, 50mM $CaCl_2$, 10mM Tris-HCl pH 7.5 were added and the solution maintained on ice for 20-30 minutes. After this time, 2.5ml of the polyethylene glycol solution was added and the mixture left for a further 15 minutes at room temperature. The protoplasts were then centrifuged and resuspended in 500μl 0.6M KCl, diluted and 100μl aliquots added to 5-10ml molten regeneration agar (minimal medium [25] + 0.9% agar, 0.6M KCl) and poured. Transformant colonies began to appear after 2-3 days incubation at 37°C.

Transfer of DNA to nitrocellulose filters (BRL Inc.) was as described by Southern [26]. EcoRI-digested pBR322 [27] was end-labelled with 10μCi [α ^{32}P]dA TP (New England Nuclear) in the presence of the Klenow fragment of DNA polymerase I (BRL Ltd.) and dTTP. Hybridisation was carried out at 60°C in the presence of formamide (40%).

All transformation experiments were performed in accordance with Good Microbiological Practice as defined by the UK Genetic Manipulation Advisory Group.

RESULTS

Co-precipitation of pFB6, which carries the orotidine-5'-phosphate decarboxylase gene from *Neurospora crassa*, and Ca^{2+}-treated *Aspergillus nidulans* strain G191 protoplasts with polyethylene glycol resulted in the appearance of uridine-independent colonies on regeneration in selective medium. Southern hybridisation [26] using DNA isolated from such a clone revealed the presence of pBR322 sequences not normally present in strain G191 (pFB6 contains the large BamHI-EcoRI fragment of pBR322, Fig. 1) indicating that a transformation event had occurred. The frequency of such transformation was 5-10 transformants from

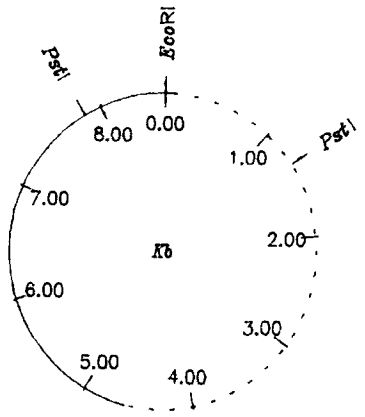

Figure 1. Plasmid pFB6. *N. crassa* sequences are shown as a dotted line. The solid line represents the large BamHI-EcoRI fragment of pBR322 plus the *Serratia marcescens trp* promoter/operator sequence. The *pyr4* gene is situated clockwise of the *N. crassa* PstI site. For full details see reference 21.

5×10^5 potential colony-forming units and 15μg of DNA. This is comparable with the frequency of transformation by integration in yeast and *Neurospora*.

Transformation was also attempted using EcoRI-generated fragments of G191 or R153 DNA ligated with linearised pFB6. This would provide homologous DNA for recombination at various sites in the genome - the degree of homology between the *pyr4* and *pyrG* gene segments is not known. There was no discernible difference in the frequency of transformation with the ligation mix or pFB6 on its own.

Transformants were mitotically stable under non-selective conditions (831/831 and 369/369 colonies from two transformants uridine-independent after growth on complete medium supplemented with uridine) indicating that the transforming DNA had probably integrated into the genome. Progeny from crosses with strain R153 included an unexpectedly high number of Pyr^- colonies. This, together with an observed reduction in ascospore viability on the medium used (down to 5-10%), seems to indicate a degree of instability through meiosis. This phenomenon is still under investigation.

Figure 2 shows the pattern of hybridisation of pBR322 to EcoRI and PstI digests of DNA prepared from two types of transformants; one resulting from transformation with pFB6 alone and one with ligated pFB6 and R153 DNA. The strong bands in lanes 7-9 can be explained by integration of the DNA via homologous recombination involving the *N. crassa* DNA and the *pyrG* region of the *A. nidulans* genome. The

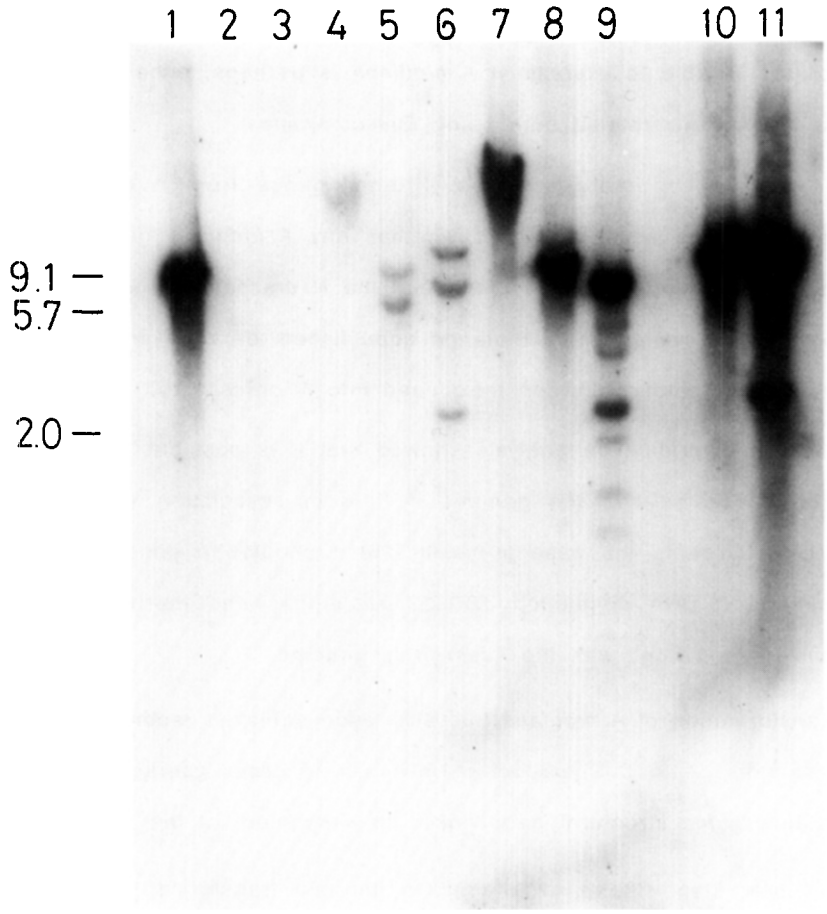

Figure 2. Autoradiograph showing hybridisation of ^{32}P-labelled pBR322 to *A. nidulans* transformant DNA. DNA from two transformants (5-10μg) was digested with EcoRI or PstI for 4hrs at 37°C, electrophoresed through 1% agarose then transferred to nitrocellulose paper. The DNA was then hybridised to end-labelled pBR322. Lanes 1 and 10 are EcoRI-digested, and lane 11 PstI-digested, pFB6; lane 2 is uncut DNA from G191 (untransformed); lane 3 is a λ size-marker; lanes 4-6 are uncut, EcoRI-, and PstI-digested DNA from a pFB6 transformant; lanes 7-9 are uncut, EcoRI-, and PstI-digested DNA from a pFB6/R153 ligation mix transformant. All samples were run on the same gel and the film exposed for 24hrs. Further exposure did not reveal any additional bands. The λ-derived scale is in kilobases.

presence of fainter bands indicate that the situation is rather more complicated, presumably due to the presence of R153 DNA in the ligation.

The simplest explanation for the pattern seen in lanes 4-6 is that a dimer of pFB6 has integrated via recombination, with the break point within the *pyr4* gene segment.

DISCUSSION

Until now it has not been possible to transform *Aspergillus nidulans* with exogenous DNA. This paper reports the introduction of a gene from *Neurospora crassa*

into *A. nidulans* protoplasts and the subsequent relief of auxotrophy. That a gene from *N. crassa* is able to express in *A. nidulans* is, perhaps, to be expected since the organisms are closely related, both being *Euascomycetes*.

It would be of interest to see if other genes from *N. crassa* are able to transform *A. nidulans* auxotrophs and whether any *A. nidulans* genes can transform *N. crassa*. Interestingly, we have found that the *N. crassa pyr4* gene is expressed in *Saccharomyces cerevisiae* (D. J. Ballance, unpublished data) even though the yeast *ura3* gene is non-functional when introduced into *N. crassa* [15].

Southern hybridisation analysis showed that it is possible for non-homologous DNA to be integrated into the genome if it is in association with an homologous segment. Thus, as is the case in yeast [28], it should be possible to transform a second time with DNA containing pBR322 sequences and thereby introduce genes which have no homology with the *Aspergillus* genome.

Transformation of *A. nidulans* has also been achieved recently by Tilburn *et al.* in the laboratory of Dr. C. Scazzocchio, University of Essex (personal communication) using an *Aspergillus* structural gene linked to a segment of the ribosomal repeat.

Our next step will be to attempt to improve transformation efficiency by the construction of vectors able to replicate autonomously in *A. nidulans* as has been done with yeast [18] and recently also in *N. crassa* [20]. This would also facilitate the reisolation of cloned genes.

A considerable body of knowledge about regulatory systems already exists for *A. nidulans* as a result of classical genetic analysis, e. g. that of nitrogen metabolism [29], and these systems, and others such as sporulation and development [30], should now be amenable to study at the molecular level.

In addition, it seems likely that experience gained in *A. nidulans* transformation will facilitate the extension of this technique to the industrially important *Aspergillus niger*. The consequent development of methods for genetic manipulation should permit improvement in performance of industrial strains.

ACKNOWLEDGEMENTS

This work was supported by an SERC grant to G. T. and an SERC studentship to D. J. B.

REFERENCES

1. Old, R. W. and Primrose, S. B. (1981) Studies in Microbiology, Vol. 2: Principles of Gene Manipulation. An Introduction to Genetic Engineering, 2nd edition, pp. 104-120, Blackwell Scientific, Oxford, England.
2. Struhl, K, Cameron, J. R. and Davis, R. W. (1976) Proc. Natl. Acad. Sci. USA 73, 1471-1475.
3. Crabeel, M., Messenguy, F., Lacroute, F. and Glansdorff, N. (1981) Proc. Natl. Acad. Sci. USA 78, 5026-5030.
4. Ratzkin, B. and Carbon, J. (1977) Proc. Natl. Acad. Sci. USA 74, 487-491.
5. Grunstein, M. and Hogness, D. S. (1975) Proc. Natl. Acad. Sci. USA 72, 3961-3965.
6. Broome, S. and Gilbert, W. (1978) Proc. Natl. Acad. Sci. USA 75, 2746-2749.
7. Struhl, K. and Davis, R. W. (1977) Proc. Natl. Acad. Sci. USA 74, 5255-5259.
8. Clarke, L. and Carbon, J. (1978) J. Mol. Biol. 120, 517-532.
9. Vapnek, D., Hautala, J. A., Jacobson, J. W., Giles, N. H. and Kushner, S. R. (1977) Proc. Natl. Acad. Sci. USA 74, 3508-3512.
10. Keesey, J. K. and DeMoss, J. A. (1982) J. Bacteriol. 152, 954-958.
11. Kinghorn, J. R. and Hawkins, A. R. (1982) Mol. Gen. Genet. 186, 145-152.
12. Jimenez, A. and Davies, J. (1980) Nature (London) 287, 869-871.
13. Cohen, J. D., Eccleshall, T. R., Needleman, R. B., Federoff, H., Buchferer, B. A. and Marmur, J. (1980) Proc. Natl. Acad. Sci. USA 77, 1078-1082.
14. Beach, D. H. and Nurse, P. (1981) Nature (London) 290, 140-142.
15. Case, M. E. (1982) In; Basic Life Sciences. Vol. 19: Genetic Engineering of Microorganisms for Chemicals (Hollaender, A., DeMoss, R. D., Koninsky, J., Savage, D. and Wolfe, R. S., eds.), pp. 87-100, Plenum Press, New York.
16. Smith, J. E. and Pateman, J. A., eds. (1977) The British Mycology Society Symposium Series No. 1: Genetics and Physiology of *Aspergillus*, Academic Press, London.
17. Hinnen, A., Hicks, J. B. and Fink, G. R. (1978) Proc. Natl. Acad. Sci. USA 75, 1929-1933.
18. Beggs, J. D. (1978) Nature (London) 275, 104-109.
19. Case, M. E., Schweizer, M., Kushner, S. R. and Giles, N. H. (1979) Proc. Natl. Acad. Sci. USA 76, 5259-5363.
20. Stohl, L. L. and Lambowitz, A. M. (1983) Proc. Natl. Acad. Sci. USA In the press.
21. Buxton, F. P. and Radford, A. (1983) Mol. Gen. Genet. In the press.
22. Ullrich, R. C., Kohorn, B. D. and Specht, C. A. (1980) Chromosoma 81, 371-378.
23. Cornelis, G., Bennet, P. M. and Grinsted, J. (1976) J. Bacteriol 127, 1058-1062.
24. Ferenczy, L., Kevei, F. and Szegedi, M. (1975) Experientia 31, 50-52.
25. Rowlands, R. T. and Turner, G. (1973) Mol. Gen. Genet. 126, 201-216.
26. Southern, E. M. (1975) J. Mol. Biol. 98, 503-517.
27. Bollvar, F., Rodriguez, R. L., Greene, P. J., Betlach, M. C., Heyneker, H. L. and Boyer, H. W. (1977) Gene 2, 95-113.
28. Hicks, J. B., Hinnen, A. and Fink, G. R. (1979) Cold Spring Harbor Symp. Quant. Biol. 43, 1305-1313.
29. Cove, D. J. (1979) Biol. Rev. Cambridge. Philos. Soc. 54, 291-237.
30. Zimmerman, C. R., Orr, W. C., Leclerc, R. F., Barnard, E. C. and Timberlake, W. E. (1980) Cell 21, 709-715.