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Effect of the *bncA* gene on the instability of *Aspergillus nidulans*

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Summary

The presence of a gene designated *bncA* which produces binucleate and trinucleate conidia in *A. nidulans* alters the instability of disomics, diploids, and strains with chromosome duplication. In disomics, the gene *bncA* increases instability. In duplicate and diploid strains, the *bncA* gene reduces instability by acting as a partial stabilizer. In the strain with chromosome duplication, the *bncA* gene produces increased percentages of bi- and trinucleate conidia, a fact that may be interpreted to be due to the larger conidial volume of this strain or to the combined effect of *bncA* and of the strain, which normally already exhibits a small amount of binucleate conidia.

1. Introduction

Haploid strains of the filamentous fungus *Aspergillus nidulans* are mitotically stable. Diploids, disomics and strains with duplicate segments, however are unstable. This instability is verified by the presence of sectors after inoculation and growth of colonies. In diploid strains, sectors are due to well known causes such as haploidization and mitotic recombination (Pontecorvo *et al.* 1953). In this case, several agents including *p*-fluorophenylalanine, benlate, chloroneb and others can increase instability (Morpurgo, 1961; Van Arkel, 1963; Hastie, 1970; Kappas *et al.* 1973; Kappas & Georgopoulos, 1974; Bignani *et al.* 1974; Azevedo *et al.* 1977). Balanced lethal systems (Azevedo & Roper, 1967) and certain genes such as *rec* and *pop* can alter the frequency of sectors produced by diploid colonies (Parag, 1977). Disomic strains of $n+1$ type present a restricted growth and produce vigorous haploid sectors due to the loss of the excess chromosome (Upshall, 1971; Kafer & Upshall, 1973). In this case instability can be reduced by a balanced lethal system similar to that used in diploids (Pizzirani, 1977; Azevedo *et al.* 1983). Strains carrying duplicate segments are also unstable; one such strain which has been studied in depth produces different types of sectors, mainly improved ones, due to the loss of duplicate segments and deteriorated sectors (Nga & Roper, 1968; Azevedo & Roper, 1970). Instability in this strain can be altered by certain physical agents or drugs added to the culture medium. Some of them increase instability (Palmer & Roper, 1970; Cooke *et al.* 1970; Rosato & Azevedo, 1978; Menezes, 1974; Azevedo *et al.* 1977; Majerfield & Roper, 1978), whereas others decrease it (Azevedo *et al.* 1977; Bonatelli Jr. & Azevedo, 1977; Parag & Roper, 1975; Lieber, 1976; Niffinegger-Souza, 1979). It has also been shown that a gene designated *stf-1* acts as a stabilizer of the duplication strain (Azevedo, 1975).

All the studies on the instability of diploid, disomic and duplication strains have been carried out on strains having uninucleate conidia, which is the normal condition for *A. nidulans* (Yuill, 1950). An unusual *A nidulans* strain which exhibited a high frequency of conidia with two or three nuclei in addition to the normal uninucleate conidia was detected by Pizzirani in 1977. This trait is due to a single gene designated *bncA* and located in linkage group IV of the species (Pizzirani-Kleiner & Azevedo, 1986). To study the effect of this gene on unstable strains, the present study was carried out using *A. nidulans* diploid and disomic strains as well as a strain bearing a chromosome duplication.

2. Materials and methods

(i) *Strains and culture media*

The following strains, originally obtained from Glasgow stocks, were utilized: Strain A (Nga & Roper, 1968), which carries a duplication of linkage group I translocated to linkage group II (Fig. 1); haploid strains *biA1*; *methG1* (designated by H) and MSE (McCully & Forbes, 1965); strain bnc that originated from MSE by spontaneous mutation and carrying the gene *bncA*, which produces conidia with more than one nucleus

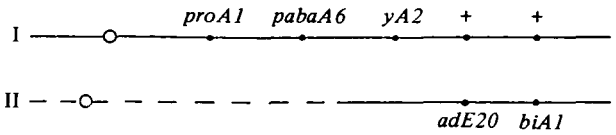

Fig. 1. Duplication strain A. Linkage groups I and II are shown by unbroken and broken lines respectively. Centromeres are designated by open circles. Genetic markers are explained in the text.

(Pizzirani, 1977; Pizzirani-Kleiner & Azevedo, 1986). The mutants used were: *adE20*, *biA1*, *methG1*, *nicB8*, *pabaA6*, *proA1*, *pyroA4*, *riboB2* and *sB3* (requirement for adenine, biotin, methionine, nicotinic acid, *p*-aminobenzoic acid, proline, pyridoxine, riboflavin, and thiosulfate, respectively); *facA303*, *galA1* (inability to grow on acetate and galactose as carbon sources, respectively); *suA1adE*20 (suppressor of *adE20*); *wA3* and *yA2* (white and yellow conidia, respectively), and *bncA* (formation of conidia with more than one nucleus). The location of the genes employed has been given by Clutterbuck (1974) and Pizzirani-Kleiner (1981).

Minimal medium (MM) was Czapeck Dox with 1% glucose, and complete medium (CM) was MM enriched with peptone, hydrolyzed casein, yeast extract, vitamins and yeast nucleic acid (Pontecorvo *et al.* 1953). MM containing vitamins and amino acids added separately, as well as MM containing galactose as carbon source and MM containing ammonium acetate (Apirion, 1962), were used for strain selection after meiotic crosses. CM with 0·08% sodium deoxycholate added (Mackintosh & Pritchard, 1963) was used to reduce colony size and to facilitate the visualization of aneuploids.

(ii) *Derivation of disomic strains*

Disomics were obtained spontaneously from MSE and bnc strains by plating conidia onto CM with sodium deoxycholate added and by observing colony growth. Conidia from colonies showing abnormal morphology or growth were plated onto CM. Microcolonies were transferred once again to CM and, after growth, disomic colonies were selected and classified for the various linkage groups on the basis of their typical morphology (Käfer & Upshall, 1973).

(iii) *Transfer of gene* bncA *to strains A and H and derivation of diploids having the* bncA *gene*

Gene *bncA* was transferred from strain bnc to strains A and H by meiotic crossing (Pontecorvo *et al.* 1953). The proportion of binucleate conidia of segregant colonies was determined by the HCl-Giemsa technique (Robinow & Caten, 1969). The presence of duplicate segments in the selected segregants and in segregants obtained from the A × bnc cross was ascertained by inoculating each segregant into the center of plates containing CM and by determining whether typical sectors of a strain with the chromosome duplication were present. Thus, the resulting strains Abnc and Hbnc were used in the present study. Both are genotypically identical to strains A and H except for the presence of the introduced *bncA* gene; these strains, with and without gene *bncA*, were utilized to form diploids with MSE by the technique of Roper (1952).

(iv) *Evaluation of instability*

Disomic strains with uninucleate conidia (originating from MSE) and disomic strains with uni-, bi- and trinucleate conidia (originating from strain bnc), for the same linkage groups, were investigated for instability by plating conidia from the disomic center onto CM and counting the resulting disomic and haploid colonies. For the strains with duplicate segments, the effect of gene *bncA* was evaluated in strain Abnc in relation to strain A. Conidia from the two strains were inoculated separately into the center of plates containing CM and in cubated at different temperatures. Plates incubated at 28° and 37 °C were maintained in the incubator for seven days, and plates incubated at 44 °C were maintained for 10 days because of their slower growth. After incubation, yellow, green, deteriorated and heterokaryotic sectors were counted for each treatment as previously described (Azevedo, 1975). Similarly, the instability of the diploids obtained was assessed by counting the number of visible sectors produced after inoculation and incubation by the same procedure as used for the duplicate strains. Two diploids heterozygous for gene *bncA* were utilized: Abnc//MSE and Hbnc//MSE. The diploids A//MSE and H//MSE were used as controls. All four diploid strains were also inoculated on CM plates and visible sectors were scored for haploid or diploid state based on conidial size. For strains A//MSE and Abnc//MSE only white sectors were considered. From H//MSE and Hbnc//MSE, white and yellow sectors were used.

3. Results

(i) *Derivation and instability of disomic strains*

A total of 5985 normal colonies and seven unstable, supposedly aneuploid colonies were obtained from strain bnc after plating on CM containing sodium deoxycholate, giving an aneuploid frequency of 0·117%. Of these, six were classified as disomic on the basis of typical morphology (Upshall, 1971; Käfer & Upshall, 1973), with two of them being disomic for chromosome IV and four for chromosome III. One strain could not be identified in terms of the extra chromosome by morphology. Among the disomics obtained, two were chosen for instability studies, one disomic for linkage group IV, designated MSEbncDS IV and one disomic for linkage group III designated

Table 1. *Frequency of haploid colonies originating from disomic colonies of bncA⁻ and bncA⁺ strains*

Disomic	Total no. of colonies	Number and percentage of haploid colonies
MSEbnc Ds IV	1382	205 (14·83)
MSE Ds IV	1116	31 (2·78)
MSEbnc Ds III	1151	175 (15·20)
MSE Ds III	1106	43 (3·89)

Table 2. *Percentage of uni-, bi- and trinucleate conidia in strains Hbnc, Abnc and bnc*[a]

Strain	Conidia %			Total no. of conidia	χ^{2b} (2 D.F.)
	Uninucleate	Binucleate	Trinucleate		
Hbnc	76·5	23·2	0·4	561	2·3 (N.S.)
Abnc	60·5	36·4	3·1	539	33·6 (Significant at 0·1% level)
bnc	74·8	24·1	1·0	787	

[a] Strains H, MSE and A produce respectively 100, 100 and 99·7% of uninucleate conidia.
[b] χ^2 values for the comparisons between type of conidia for each strain with strain bnc.

MSEbncDs III. These two disomics produce uni-, bi-and trinucleate conidia in the same proportions as strain bnc. For strain MSE, 5765 normal and eight supposedly aneuploid colonies were obtained from a total of 5773 colonies. Of these, five were identified as disomics, whose morphological pattern permitted the identification of three as disomic for linkage group III, one for linkage group IV and the last one for linkage group VI. Thus aneuploid frequency was 0·138%. The disomic for linkage group IV and one disomic for linkage group III, designated MSE Ds IV and MSE Ds III, respectively, were selected for studies in terms of their instability.

The instability of the selected disomics was investigated on the basis of the haploid colonies resulting from disomic conidia. Table 1 shows that the disomics carrying gene *bncA* exhibited a higher frequency of haploid colonies and therefore were more unstable than disomics from strains that formed only uninucleate conidia.

(ii) *Expression of gene* bncA *in a normal strain and in a strain carrying a chromosome duplication*

The numbers of uni-, bi- and trinucleate conidia in strains bnc, Hbnc and Abnc, expressed as percentages, are shown in Table 2. The types of conidia formed by strain Hbnc did not differ statistically from those formed by strain bnc, whereas significant differences were detected for strain Abnc.

(iii) *Effect of gene* bncA *on the instability of the duplication strain and of the diploids*

Duplication strain A gives rise to green and yellow sectors by spontaneous loss of the duplicated chromosome segments as well as to deteriorated morphologic sectors (Nga & Roper, 1968; Azevedo & Roper, 1970).

The effect of gene *bncA* on the instability of the chromosome duplication strain was assessed by comparing the number of sectors produced by strain Abnc and by strain A (Table 3). The table also shows the number and frequency of the various types of sectors produced at 28°, 37° and 44 °C by the two strains. When the total numbers of sectors produced or the mean sectors per plate are compared, it can be seen that the instability of Abnc was reduced at all three temperatures in relation to the instability of strain A.

Diploid instability was also evaluated by counting the number of spontaneous sectors produced by diploids resulting from combining the strain carrying the *bncA* gene and strain MSE. Diploids resulting from the same strains but without the bncA gene were used as controls. Table 4 shows the results obtained in terms of number and frequency of the different types of sectors produced at different temperatures for the diploids Abnc//MSE and A//MSE, and Table 5 shows the results for the diploids Hbnc//MSE and H//MSE. The data in these tables show that the presence of gene *bncA* also reduces the instability of diploids in relation to that of diploids that do not carry this gene. The relative percentage of haploid and dip-

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Table 3. *Number of sectors formed by strain Abnc and strain A at different temperatures*[a]

	Temperature					
	28 °C		37 °C		44 °C	
Sectors	Abnc	A	Abnc	A	Abnc	A
Yellow	68	106	87	180	122	434
Green	1	19	3	37	6	41
Deteriorated	4	3	8	6	6	17
Heterokaryotic	3	0	6	8	1	3
Total	76	128	104	231	135	495
Mean sectors/colony	0·95	1·60	1·30	2·89	1·69	6·19

[a] 80 colonies per strain.

Table 4. *Number of sectors obtained from diploids Abnc//MSE and A//MSE at different temperatures*[a]

	Temperature					
	28 °C		37 °C		44 °C	
Sectors	Abnc//MSE	A//MSE	Abnc//MSE	A//MSE	Abnc//MSE	A//MSE
Yellow	382	494	433	689	197	556
White	14	22	58	70	24	60
Green	0	0	0	0	5	0
Deteriorated	2	0	0	1	0	0
Total	398	516	491	760	226	616
Mean sectors/colony	9·95	12·90	12·28	19·00	5·65	15·40

[a] 40 colonies per strain.

Table 5. *Number of sectors obtained from diploids Hbnc//MSE and H//MSE at different temperatures*[a]

	Temperature					
	28 °C		37 °C		44 °C	
Sectors	Hbnc//MSE	H//MSE	Hbnc//MSE	H//MSE	Hbnc//MSE	H//MSE
Yellow	7	25	19	58	10	53
White	17	53	39	77	22	34
Green	0	3	1	13	17	5
Total	20	81	59	148	49	72
Mean sectors/colony	0·5	2·02	1·48	3·70	1·22	1·80

[a] 40 colonies per strain.

Table 6. *Percentage of haploid and diploid sectors derived from diploid strains with and without* bncA *gene at different temperatures*

	Temperature								
	28 °C			37 °C			44 °C		
Diploid strains	% haploid	% diploid	Number of sectors analysed	% haploid	% diploid	Number of sectors analysed	% haploid	% diploid	Number of sectors analysed
A//MSE	80	20	25	89	11	37	92	8	24
Abnc//MSE	80	20	25	82	18	22	83	17	23
H//MSE	71	29	31	77	23	64	85	15	101
Hbnc//MSE	67	33	21	68	32	41	79	21	33

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loid sectors in the four diploids analysed seems not to be seriously disturbed by the presence of *bncA* gene. However the percentage of haploid sectors increased in all cases with the increase in temperature (Table 6).

4. Discussion

The frequency of aneuploids obtained for the bnc strain (0·138%) was similar to that obtained for MSE (0·112%) indicating that the presence of *bncA* does not interfere with the frequency of mitotic nondisjunction that gives rise to aneuploids. However, disomics carrying *bncA* produced approximately five times more haploid colonies than *bncA*$^+$ disomics (Table 1). Although both processes probably involve nondisjunction, the *bncA* gene acts differently in each one, not modifying the instability of haploid strains but increasing it in disomic strains.

The expression of gene *bncA* in *A. nidulans* strains other than that in which the mutation originated disclosed that the haploid strain Hbnc did not differ from the MSE strain carrying the *bncA* gene in terms of the percentage of conidia having more than one nucleus. However, the strain with chromosome duplication Abnc exhibited higher percentages of multinucleated conidia in relation to bnc and Hbnc strains (Table 2). It was previously shown that duplication strain A produces a small percentage (0·27%) of binucleate conidia (Paes de Barros, 1977). So, it is not surprising that the combined effects of gene *bncA* and the chromosome duplication may have contributed to increase the formation of multinucleate conidia in this strain.

The instability detected as sectoring in chromosome duplication strains and in diploids showed that *bncA* had a marked stabilizing effect on both (Tables 3, 4 and 5). This stabilization occurred at all temperatures tested, practically for all types of sectors produced and in diploids with and without chromosome duplications. Strains carrying *bncA* are practically identical in morphology and have the same growth rate when compared to the respective *bncA*$^+$ strains. So, the presence of the *bncA* gene seems to have no effects on the detectability of sectors from disomic, duplication and diploid strains. However in diploids, *bncA* reduces the frequency of both mitotic recombination and haploidization (Table 6). Since these two processes are supposed to be independent mitotic events, it is possible that the multinucleate condition affects the recovery of both types of segregants in diploid strains. Also it could be postulated that a product of the mutant gene interferes with mitotic pairing. On the other hand, the *bncA* gene may produce effects on the stability as a result of changes in the nuclear volume simultaneously with cell volume. This gene causes reduction of instability in strains with duplication and in diploids, thus suggesting that the same causes may be responsible for the stabilization process in both.

The stabilization that can be obtained by using stabilizing genes is of interest because it can permit a better understanding of the real causes of genetic instability and can also permit the crossing of highly unstable strains. It could also be a means of reducing the instability of industrially utilized strains by genetical methods (Azevedo, 1975; Ball & Azevedo, 1976).

Regardless of gene *bncA*, the instability of strains with chromosome duplications is affected by different temperatures (Table 3) with the greatest instability being observed at 44 °C, thus confirming data obtained by Niffineger-Souza (1979) and Favraud (1984). For diploids, however, the greatest instability occurred at 37 °C and not at higher or lower temperatures (Tables 4 and 5). Changes in incubation temperature could then be used for partial stabilization of *A. nidulans* diploids in the laboratory. If this phenomenon could be extended to other species of industrial interest, this could also be a process of diploid strain stabilization similar to another that utilizes a balanced lethal system (Azevedo & Roper, 1967). This reduced instability, however, may be related to growth at temperatures that are not ideal for the species.

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