Theor. Appl. Genet. 61, 171–175 (1982)



# Behaviour of DNA-induced Inositol-independent Transformants of *Neurospora crassa* in Sexual Crosses

G. Szabó and M. Schablik
Institute of Biology, University Medical School, Debrecen (Hungary)

**Summary.** Treatment of inositolless (*inl*) strains of *Neurospora crassa* with DNA from the wild type (allo-DNA) gives rise to inositol-independent ($inl^+$) colonies. Some of these DNA-induced $inl^+$ strains (transformants) are sterile in sexual crosses on minimal medium that selects for the maintaining of the $inl^+$ character. The same $inl^+$ transformants, when crossed with an *inl* standard strain, are fertile on complete (inositol-containing) medium. There are, however, an increased number of unusual non-Mendelian tetrads (24%) among the progeny. The $inl^+$ and *inl* progeny from these complete non-Mendelian tetrads were further examined for the inheritance of the $inl^+$ trait. Several $inl^+$ progeny of these tetrads segregate *inl* conidia if growing on inositol-containing medium. The number of $inl^+$ conidia in certain $inl^+$ cultures decreases quickly under non-selective conditions. In transformants carrying mutant markers in linkage groups III, IV and VI non-Mendelian segregation of these traits can also be detected.

The mechanism of the development of sterility and of the aberrant segregation is discussed.

**Key words:** Transformation – *Neurospora crassa* – Aberrant segregation

## Introduction

Inositol-independent ($inl^+$) strains have earlier been obtained from inositolless (*inl*) *Neurospora crassa* by treatment with DNA from the wild-type *N. crassa* (Case et al. 1979; Mishra et al. 1973; Mishra and Tatum 1973; Schablik et al. 1977). These strains were heterokaryotic, having both $inl^+$ and *inl* nuclei, had lower growth rates, even in the presence of inositol, and the DNA-induced new character often did not follow Mendelian inheritance. Crosses of $inl^+$ and *inl* strains revealed an unusual increase in the number of non-Mendelian (6:2, 2:6, 5:3) tetrads ("gene conversions").

In this paper we report on the genetic analysis of the DNA-induced $inl^+$ strains.

## Materials and Methods

*Strains:* The following *Neurospora crassa* strains were obtained from the collection of the Rockefeller University or from the Fungal Genetics Stock Center: R 2,506 ($mt^a$, *rg, inl*), 89,601 ($mt^A$, *inl*), 89,601-36,104 ($mt^A$, *inl, met*-3), 1,987 ($mt^a$, *trp*-l, *cot*-l, *inl, ylo*-l), 987 ($mt^a$) and 988 ($mt^A$). Inositol requirement (*inl*) is a specific mutation (Giles 1951; Mishra et al. 1973) having a very low frequency of reversion and producing a defective inositol synthase. The ragged (*rg*) mutant (Mishra 1973) has a distinctive colonial morphology characterized by a relatively thin cell wall that makes this strains specifically suitable for DNA uptake (unpublished data).

Transformants and spontaneous revertants were obtained in the authors' laboratory from R 2,506 ($mt^a$, *rg, inl*) by the mycelial fragment method (Mishra et al. 1973), and from 1,987 ($mt^a$, *trp*-l, *cot*-l, *inl, ylo*-l) by treating protoplasts with DNA as described by Case et al. (1979). Colonies appearing on minimal medium after treatment with DNA from the wild type strain were marked with a capital letter T and a figure, e.g., T5, T6. Spontaneous revertants that appeared without DNA treatment were marked with letter K and a figure e.g., K2. The serial number of the ascus studied and those of the progeny from the same tetrad were also added; e.g., T5-1-1, 2, 3 . . .8 designated clones No. 1–8 from ascus No. 1 originating from a cross between T5 and 89,601.

*Media:* The media used have been described in a previous article (Szabó et al. 1978).

*Genetic Analysis:* Random spore analysis was performed as follows. An ascospore suspension was heat-shocked at 60 °C for 30 min, then spread on complete medium containing inositol and/or methionine, tryptophan 100 µg/ml and 4% v/w L-sorbose. The number of colonies growing on agar plates was counted after incubation at 27 °C for 72 h and then replicated upon minimal and supplemented medium. For tetrad analysis, 21-day-old perithecia were dissected and ascospores were isolated from complete asci. The morphology (ragged or wild type, orange or yellow), colonial character at 37 °C (*cot*-l), mat-

0040-5752/82/0061/0171/$ 1.00

ing type and nutritional requirements (*inl, met, trp*) of the colonies grown from the ascospores of the tetrads were studied.

The viability of the ascospores or conidia was determined by relating the number counted in Buerker chamber to colonies grown on complete media.

*Detection of Segregation of Alleles by Conidial Analysis:* Suspensions of conidia (50–4,000) were plated on complete medium in an appropriate number of Petri dishes and incubated at 37 and 27 °C. Auxotrophic markers were detected by replicating all single colonies on selective media.

## Results

### *Fertility of DNA-induced Transformants in Crosses*

*Inl*$^+$ DNA-induced transformants (20 strains) and spontaneous revertants (8 strains) were crossed on crossing medium lacking inositol. The spontaneous revertant *inl*$^+$ strains (obtained without DNA treatment) gave fertile crosses on this medium, whereas out of 20 DNA-induced *inl*$^+$ strains 13 were barren. In a few cases empty perithecia were detected. Both the DNA-induced and the spontaneous revertant strains were fertile on inositol-containing medium.

### *Inheritance of the inl*$^+$ *Trait of DNA-induced Transformants*

The *inl*$^+$ transformants generated a large number of non-Mendelian tetrads, thus confirming our previous observations (Table 7 in: Szabó et al. 1978, and Table 1).

When strain T5 was crossed with a standard *inl* strain, 24% non-Mendelian tetrads resulted, the rest of the tetrads were regular Mendelian ones showing a 0:8 or a 4:4 (*inl*$^+$:*inl*) ratio. The 0:8 tetrad ratio indicated the mating of the *inl* nuclei and the 4:4 ratio indicated the union of the *inl*$^+$ nuclei of the heterokaryotic hyphae with the *inl* nuclei of the standard strain, respectively (Table 1). (*Inl*$^+$ transformants and revertants were found to be heterokaryotic (Szabó et al. 1978).) Clones from the ascospores of some non-Mendelian and Mendelian tetrads (Table 1) were analyzed further in crosses with *inl*$^+$ standard strains.

All *inl*$^+$ progeny from two Mendelian tetrads, when crossed to an *inl*$^+$ standard strain, proved to be fertile both on minimal and on inositol-containing media, and all of the ascospores were *inl*$^+$. The *inl*$^+$ progeny of the non-Mendelian tetrads were also fertile on both media, but four progeny from the complete tetrads behaved as if they were *inl* (Table 2). Only about 50% of the ascospores (from crosses between *inl*$^+ \times$ *inl*$^+$ standard strains) were found by random spore analysis to be *inl*$^+$. These F 1 ascospores were *inl*$^+$ after germination but behaved as *inl* in crosses with an *inl*$^+$ standard strain. They were also found to often lose *inl*$^+$ character during storage and on vegetative transfers (Table 3). Two of the cultures (T5-1-7, T5-6-4), however, did not lose their *inl*$^+$ character so quickly. One of these cultures, which generated only *inl*$^+$ ascospores when crossed with an *inl*$^+$ standard strain (T5-6-4), even segregated *inl* conidia (Table 3). The conidia from these

**Table 1.** Tetrad analysis of *Neurospora crassa* strains

| Cross | Ratio of *inl*$^+$: *inl* ascospores | | | | | | No. of tetrads examined |
|---|---|---|---|---|---|---|---|
| | 4:4 | 6:2 | 2:6 | 5:3 | 3:5 | 0:8 | |
| T21 transformant (*inl*$^+$)[a] | – | – | 2 | – | – | 3 | 5 |
| T5 transformant (*inl*$^+$)[b] | 38 | 1 | 3 | 8 | – | 54 | 104 |
| K2 spontaneous revertant[b] | 27 | – | – | – | – | – | 27 |

[a] Crossed with strain 89,601-36,104 (*mt*$^A$; *inl, met*-3)
[b] Crossed with strain 89,601 (*mt*$^A$; *inl*)

**Table 2.** Crosses[a] of cultures from non-Mendelian tetrads

| Ascus | $F_1$ | Aberrant spore pairs | Ascospores ($F_2$) | |
|---|---|---|---|---|
| | | | germination % | *inl*$^+$ % |
| T5-1 | Spore 1: *rg*; *inl*$^+$ | Spores 1 and 2 | 53 | 100 |
| | Spore 2: *rg*; *inl* | | n.d. | 69 |
| | Spore 3: *rg*; *inl* | Spores 3 and 4 | 65 | 67 |
| | Spore 4: *rg*; *inl*$^+$ | | 38 | 100 |
| | Spore 5: *rg*$^+$; *inl* | Spores 5 and 6 | 53 | 42 |
| | Spore 6: *rg*$^+$; inl$^+$ | | 54 | 100 |
| | Spore 7: *rg*$^+$; *inl*$^+$ | Spores 7 and 8[b] | n.d. | 100 |
| | Spore 8: *rg*$^+$; *inl*$^+$ | | 45 | 56 |
| T5-6 | Spore 1: *rg*$^+$; *inl*$^+$ | Spores 1 and 2[b] | n.d. | 57 |
| | Spore 2: *rg*$^+$; *inl*$^+$ | | 100 | 100 |
| | Spore 3: *rg*$^+$; *inl*$^+$ | Spores 3 and 4[b] | 51 | 50 |
| | Spore 4: *rg*$^+$; *inl*$^+$ | | 32 | 100 |
| | Spore 5: *rg*; *inl* | | 64 | 48 |
| | Spore 6: *rg*; *inl* | | 45 | 58 |
| | Spore 7: *rg*; *inl*$^+$ | | 58 | 100 |
| | Spore 8: *rg*; *inl*$^+$ | | 87 | 100 |
| T5-47 | Spore 1: *rg*$^+$; *inl*$^+$ | Spores 1 and 2 | 65 | 57 |
| | Spore 2: *rg*$^+$; *inl* | | 58 | 57 |
| | Spore 3: *rg*$^+$; *inl* | | 45 | 52 |
| | Spore 4: *rg*$^+$; *inl* | | 44 | 45 |
| | Spore 5: *rg*; *inl*$^+$ | | 37 | 100 |
| | Spore 6: *rg*; *inl*$^+$ | | 12 | 100 |
| | Spore 7: *rg*; *inl*$^+$ | | 48 | 100 |
| | Spore 8: *rg*; *inl*$^+$ | | – | – |

[a] Crossed with strains 987 and 988
[b] Spore pairs being pseudo-wild types are revealed in $F_2$

**Table 3.** Viability of conidia from mycelia of pseudo-wild ascospores

| Ascospore | $F_1$ (*inl*) | Viable count on medium | | % germination on complete medium |
|---|---|---|---|---|
| | | minimal | complete | |
| | | after 12 – 14 weeks of storage | | |
| T5-1-7 | + | $1.7 \times 10^6$ | $1.6 \times 10^6$ | 19 |
| T5-1-8 | + | 0 | $7.0 \times 10^4$ | 3 |
| T5-6-3 | + | 0 | $1.5 \times 10^2$ | n.d. |
| T5-6-4 | + | $1.4 \times 10^4$ | $2.9 \times 10^5$ | 20 |
| T5-47-1 | + | 0 | $2.9 \times 10^6$ | 36 |

quickly segregating cultures also had a low viability when they were stored for 12–14 weeks (Table 3). Ascospores segregating both the wild-type and mutant alleles may be considered as pseudo-wild types (Case and Giles 1964).

In order to examine the behaviour of the *inl*$^+$character in the next (F2) generation of non-Mendelian tetrads, we also crossed two *inl*$^+$ members of a 2:6 tetrad with an *inl* standard strain. One of these *inl*$^+$ strains (T5-133-7), when crossed with a standard *inl* strain, was sterile on minimal but fertile on inositol-containing medium. Out of 30 asci only one had *inl*$^+$ ascospores and that one gave 2:6 (*inl*$^+$:*inl*) ratio. When conidia from one of the *inl*$^+$progeny (T5-230-5) were plated, the same number of colonies grew both on minimal and on inositol-containing medium. However, after three transfers on inositol-containing medium, only one *inl*$^+$ conidia was found among $10^5$. The other member of this pair (T5-133-8) produced no *inl*$^+$ascospores in a cross with an *inl* standard strain (13 complete tetrads were analyzed).

*Inheritance of some Chromosomal Traits of inl$^+$ Transformants*

The segregation of the mutant and wild type alleles in the non-Mendelian progeny of the transformants may be explained by the integration and elimination of the transforming DNA, by the presence of nonintegrated DNA, and by the disomy of the relevant chromosome. In order to distinguish between these alternatives, strains with multiple marked chromosomes were treated with DNA and selected for the *inl*$^+$ character. Strain 1,988 (*mt$^a$*, *trp*-1, *cot*-1, *inl*, *ylo*-1) was treated with wild type DNA, and one of the *inl*$^+$ transformants (T21) was crossed with an *inl*, *met*-3 (89,601-36,104) standard strain. Two out of the five ordered tetrads (Table 1) segregated in a non-Mendelian fashion if only the *inl* locus was considered, but the number of non-Mendelian tetrads was greater if the segregation of other loci were also taken into account (Table 4).

Further transformants (T1 and T9) of the same genotype were also examined. Two and four complete tetrads were analyzed, respectively. Two tetrads from both crosses (T1-22, T1-29 and T9-33, T9-38) segregated in a non-Mendelian way if markers of all four linkage groups were examined (Table 5). Non-Mendelian segregation was found with every linkage group investigated. Conidial analysis of the progeny from sister spores indicated the presence of traits from both parents. (From T1-22 *met*$^+$ and *met*, from T1-29 *met*$^+$ and *met*, from T9-38 *trp*$^+$and *trp*, *cot*$^+$ and *cot*.) The following cultures T1-22-7, T1-29-1, T1-29-4, T1-29-7, T1-29-8, T9-33-5, T9-33-6, T9-33-7, T9-33-8 (Table 5) were also analyzed, but no segregation could be detected.

**Table 4.** Non-Mendelian tetrads from a multiple marked transformant

| Ascus | | Genotypes | Non-Mendelian segregants |
|---|---|---|---|
| T21-46 | Spores 1 and 2: | *trp*-1; *cot*-1$^+$; *inl*, *met*-3; *y* | *inl*$^+$: *inl* |
| | Spores 3 and 4: | *trp*-1; *cot*-1; *inl*, *met*-3; *y* | *met*-3$^+$: *met*-3 |
| | Spores 5 and 6: | *trp*-1$^+$; *cot*-1; *inl*$^+$, *met*-3; *o* | |
| | Spores 7 and 8: | *trp*-1$^+$; *cot*-1$^+$; *inl*, *met*-3$^+$; *o* | |
| T21-47 | Spores 1 and 2: | *trp*-1; *cot*-1; *inl*, *met*-3; *o* | *trp*-1$^+$: *trp*-1 |
| | Spores 3 and 4: | *trp*-1$^+$; *cot*-1; *inl*, *met*-3$^+$; *o* | *met*-3$^+$: *met*-3 |
| | Spore 5: | *trp*-1$^+$; *cot*-1$^+$; *inl*, *met*-3$^+$; *y* | |
| | Spore 6: | *trp*-1; *cot*-1$^+$; *inl*, *met*-3; *y* | |
| | Spore 7: | *trp*-1$^+$; *cot*-1$^+$; *inl*, *met*-3$^+$; *y* | |
| | Spore 8: | *trp*-1; *cot*-1$^+$; *inl*, *met*-3; *y* | |
| T21-50 | Spores 1 and 2: | *trp*-1$^+$; *cot*-1$^+$; *inl*, *met*-3$^+$; *o* | *inl*$^+$: *inl* |
| | Spores 3 and 4: | *trp*-1; *cot*-1; *inl*, *met*-3; *o* | *cot*-1$^+$: *cot*-1 |
| | Spore 5: | *trp*-1; *cot*-1; *inl*, *met*-3; *y* | *met*-3$^+$: *met*-3 |
| | Spore 6: | *trp*-1; *cot*-1; *inl*, *met*-3$^+$; *y* | |
| | Spores 7 and 8: | *trp*-1$^+$; *cot*-1, *inl*$^+$, *met*-3$^+$; *y* | |

**Table 5.** Conidial analysis of clones from non-Mendelian tetrads

| Ascus | | Genotypes | Aberrant spore pairs | Genotypes of pseudo-wild types conidia recovered |
|---|---|---|---|---|
| T1-22 | Spore 1: | *trp*-1$^+$; *cot*-1; *inl*, *met*-3$^+$; *o* | Spores 1 and 2 | *trp*-1$^+$; *cot*-1; *inl*, *met*-3$^+$; *o*<br>*trp*-1$^+$; *cot*-1; *inl*, *met*-3; *o* |
| | Spore 2: | *trp*-1$^+$; *cot*-1$^+$; *inl*, *met*-3; *o* | | |
| | Spores 3 and 4: | *trp*-1$^+$; *cot*-1$^+$; *inl*, *met*-3; *o* | | |
| | Spores 5 and 6: | *trp*-1; *cot*-1; *inl*, *met*-3; *y* | | |
| | Spores 7 and 8: | *trp*-1; *cot*-1$^+$; *inl*, *met*-3$^+$; *y* | | |
| T1-29 | Spores 1 and 2: | *trp*-1$^+$; *cot*-1$^+$; *inl*$^+$, *met*-3$^+$; *y* | | |
| | Spores 3 and 4: | *trp*-1$^+$; *cot*-1; *inl*$^+$, *met*-3$^+$; *y* | | |
| | Spore 5: | *trp*-1; *cot*-1; *inl*, *met*-3$^+$; *o* | Spores 5 and 6 | *trp*-1; *cot*-1; *inl*, *met*-3; *o*<br>*trp*-1; *cot*-1; *inl*, *met*-3$^+$; *o* |
| | Spore 6: | *trp*-1; *cot*-1; *inl*, *met*-3; *o* | | |
| | Spores 7 and 8: | *trp*-1; *cot*-1$^+$; *inl*, *met*-3; *o* | | |
| T9-33 | Spore 1: | *trp*-1; *cot*-1; *inl*, *met*-3; *o* | Spores 1 and 2 | |
| | Spore 2: | *trp*-1$^+$; *cot*-1; *inl*, *met*-3; *o* | | |
| | Spores 3 and 4: | *trp*-1$^+$; *cot*-1; *inl*, *met*-3$^+$; *o* | | |
| | Spore 5: | *trp*-1; *cot*-1$^+$; *inl*, *met*-3; *y* | Spores 5 and 6 | |
| | Spore 6: | *trp*-1; *cot*-1$^+$; *inl*, *met*-3$^+$; *y* | | |
| | Spore 7: | *trp*-1; *cot*-1$^+$; *inl*, *met*-3$^+$; *y* | Spores 7 and 8 | |
| | Spore 8: | *trp*-1; *cot*-1$^+$; *inl*, *met*-3; *y* | | |
| T9-38 | Spores 1 and 2: | Failed to germinate | | |
| | Spores 3 and 4: | *trp*-1; *cot*-1; *inl*, *met*-3$^+$; *y* | | |
| | Spores 5 and 6: | *trp*-1$^+$; *cot*-1$^+$; *inl*, *met*-3; *o* | | |
| | Spore 7: | *trp*-1; *cot*-1$^+$; *inl*, *met*-3$^+$; *o* | Spores 7 and 8 | *trp*-1$^+$; *cot*-1$^+$; *inl*, *met*-3$^+$; *o* |
| | Spore 8: | *trp*-1$^+$; *cot*-1$^+$; *inl*, *met*-3$^+$; *o* | | *trp*-1; *cot*-1; *inl*, *met*-3$^+$; *o* |

## Discussion

The DNA-induced transformants are distinguished from the spontaneous revertants by two features. They do not give fertile crosses on selective medium, and they produce such a large number of non-Mendelian tetrads that has not been hitherto reported with *N. crassa.*

Upon treatment of *N.crassa* with DNA special conditions have been established that are favourable for DNA uptake (Aradi et al. 1978; Schablik et al. 1977), and need to be observed in order to obtain transformants. Under these circumstances the amount of DNA taken up could be nearly the same as that of the DNA content of the hyphae. In addition to transforming the *inl* locus, the incorporated DNA may also be integrated into several other loci. The *inl* and *met*-3 loci are closely linked on chromosome No. 2, but the results in Table 4 (ascus T21-46, spores Nr. 5, 6 and 7, 8) point to the presence of the *inl*$^+$gene as an independently segregating marker. This possibility has been proved in eukaryotic organisms (Wigler et al. 1979).

In experiments carried out with DNA-DNA hybrids at least 1–2% of the DNA of the transformed strain (T5) were mismatched after hybridization to the DNA of the recipient or to the DNA of the wild-type strain (Fehér et al. 1979).

The integration of a substantial amount of high molecular weight DNA may cause non-specific insertions, gene duplications, rearrangements, etc., that may be responsible for meiotic disorders i. e., for the appearance of barren perithecia. (Raju and Perkins 1978). If transformations were carried out with specific DNA segments, sterility perhaps could be avoided. Alteration of the normal DNA sequences may explain the low germination ratio of some of the ascospores from crosses of transformants (Table 2).

When the *inl*$^+$ transformed strains were crossed with an *inl*$^+$ standard strain, some of the *inl*$^+$ ascospores of the progeny were stable *inl*$^+$ but several segregated *inl* progeny.

The segregation of the (*inl*) mutant allele from the wild type transformant may be explained by integration and quick elimination of the transforming DNA, by the genetic activity of a non-integrated DNA fragment or by the disomy of the chromosome in question. Similar segregation of alleles (Table 2, 4) from ascospores of complete tetrads obtained without DNA treatment has been described by others (Case and Giles 1964; Martin 1959; Threlkeld 1962). These segregating ascospores were called pseudo-wild types. The source of pseudo-wild types in the above cases cannot be non-disjunction during meiosis because the pseudo-wild type ascospores

were found in asci containing eight viable spores. The gratuitous allele may be located on an extra chromosome or on a chromosomal fragment. The extra chromosome or fragment may have originated by an extra replication during meiosis. The result of an extra replication may be disomy or diploidy.

Stable disomics or diploids are unknown in *N. crassa.* Diploids break down to haploids very quickly (Smith 1974). We assume that haploidization is responsible for the different $inl^+$:*inl* ratios of non-Mendelian tetrads, for the differences between sister-spores and for the segregation of the alleles.

To see whether disomy or diploidy was responsible for the segregation of the $inl^+$ and *inl* alleles we studied multiple marked strains. If strains obtained after DNA treatment and selected for the $inl^+$ trait were disomic only for linkage group V no other pseudo-wild ascospores would be expected for the other markers.

The experiments shown in Table 5 indicate that multiple marked strains, after transformation with DNA, also produced a large number of non-Mendelian tetrads and pseudo-wild type ascospores concerning every trait (chromosome) examined (except for *ylo*-1). These results support the assumption that non-Mendelian ratios and irregular segregation obtained with transformed strains are the consequence of diploidization.

It can not be excluded, however, that the segregating markers were carried only by chromosomal fragments.

This latter mechanism and the above assumption are not mutually exclusive, in different transformants both may work.

## Acknowledgement

This work was supported by a grant from the Hungarian Academy of Sciences.

We are grateful to Ms. Zsuzsanna Fekete and to Ms. Margit Kántor for their skilful technical assistance.

## Literature

Aradi, J.; Schablik, M; Zsindely, A.; Csongor, J.; Szabó, G. (1978): Factors influencing the DNA uptake by *Neurospora crassa.* Acta Biochem. Biophys. Acad. Sci. Hung. **13,** 259–267

Case, M.E.; Schweizer, M.; Kushner, S.R.; Giles, N.H. (1979): Efficient transformation of *Neurospora crassa* utilizing hybrid plasmic DNA. Proc. Natl. Acad. Sci., U.S. **76,** 5259–5264

Case, M.E.; Giles, N.H. (1964): Allelic recombination in *Neurospora:* tetrad analysis of a three-point cross within the *pan-2* locus. Genetics **49,** 529–540

Fehér, Zs.; Schablik, M.; Szabó, G. (1979): Hybridization of DNA's from *Neurospora crassa* strains indicate base sequence alterations as a consequence of genetic transformation. Acta Biol. Acad. Sci. Hung. **30,** 387–392

Giles, N.H.jr. (1951): Studies on the mechanism of reversion in biochemical mutants of *Neurospora crassa.* Cold Spring Harbor Symp. Quant. Biol. **10,** 283–313.

Martin, P.G. (1959): Apparent self-fertility in *Neurospora crassa.* J. Gen. Microbiol. **20,** 213–222

Mishra, N.C.; Szabó, G.; Tatum, E.L. (1973): Nucleic acid induced genetic changes in *Neurospora.* In: The role of RNA in reproduction and development. (eds. Niu, M.C.; Segal, S.J.) pp. 259–268. Amsterdam: North-Holland

Mishra, N.C.; Tatum, E.L. (1973): Non-Mendelian inheritance of DNA-induced inositol independence in *Neurospora.* Proc. Natl. Acad. Sci. U.S. **70,** 3875–3879

Raju, N.B.; Perkins, D.D. (1978): Barren perithecia in *Neurospora crassa.* Can. J. Genet. Cytol. **20,** 41–59

Schablik, M.; Szabolcs, M.; Kiss, A.; Aradi, J.; Zsindely, A.; Szabó, G. (1977): Conditions of transformation by DNA of *Neurospora crassa.* Acta Biol. Acad. Sci. Hung. **28,** 273–279

Smith, D.A. (1974): Unstable diploids of *Neurospora* and a model for somatic behaviour. Genetics **76,** 1–17

Szabó, G.; Schablik, M.; Fekete, Zs.; Zsindely, A. (1978): A comperative study of DNA induced transformants and spontaneous revertants of inositolless *Neurospora crassa.* Acta Biol. Acad. Sci. Hung. **29,** 375–384

Threlkeld, S.F.H. (1962): Some asci with non-identical sister spores from a cross in *Neurospora crassa* Genetics **47,** 1187–1198

Wigler, M.; Sweet, R.; Gek Kel Sim, Wold, B.; Pellicer, A.; Lacy, E.; Maniatis, T.; Silverstein, S.; Axel, R. (1979): Transformation of mammalian cells with genes from prokaryotes and eukaryotes. Cell **16,** 777–785

Received June 16, 1981
Communicated by L. Alföldi

Prof. Dr. Gábor Szabó
Dr. M. Schablik
Department of Biology
University Medical School Debrecen
P.O.B.1 H-4012 Debrecen (Hungary)