DNA-mediated Genetic Changes in *Neurospora crassa*

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Evidence for genetic transformation in *Neurospora crassa* is based on the observations that allo-DNA has a specific effect in producing transformants which is abolished by DNAase treatment and that iso-DNA is not effective in transformation. Here, unambiguous evidence for genetic transformation is provided by transfer of a temperature-sensitive inositol requirement from a donor to a recipient strain. Data provided also suggest the role of growth conditions and the involvement of a nuclease gene in the DNA uptake and transformation of *N. crassa*.

INTRODUCTION

Transformation by nucleic acid molecules or isolated chromosomes provides unusual means for the transfer of genetic information in both prokaryotes and eukaryotes (Avery *et al.*, 1944; Cosloy & Oishi, 1973; Hotchkiss, 1976; Ottolenghi-Nightingale, 1974; Fox *et al.*, 1970; McBride & Ozer, 1973; Mishra *et al.*, 1973; Mishra & Tatum, 1973; Mishra, 1976*a, b*; Mishra, 1977; Ruddle & Fournier, 1977). We have recently described the characteristics of such a genetic system in *Neurospora crassa* (Mishra *et al.*, 1973; Mishra & Tatum, 1973; Mishra, 1976*a, b*; Mishra, 1977). Our original result [the transformation of an inositol-requiring (*inl*) strain to inositol independence (*inl*⁺)] has recently been confirmed (Mishra, 1977; Schablik *et al.*, 1977). In these studies, the evidence for genetic transformation was: (i) that allo-DNA had a specific transforming effect which was abolished by treatment with DNAase and (ii) that iso-DNA (recipient DNA preparation) did not transform. Unequivocal proof of transformation, provided by transfer of a temperature-sensitive (*ts*) character to a recipient strain, is presented in this paper. Previously, such experiments could not be performed due to the lack of temperature-sensitive mutants, but we have recently isolated *inl ts* mutants of *N. crassa*, which require inositol for growth only at 37 °C and not at 25 °C (unpublished results); DNA from this *inl ts* strain has been used in transformation. Our results show that an *inl* mutant of *N. crassa*, which requires inositol for growth at any temperature, can acquire temperature sensitivity for this requirement following treatment with DNA from a temperature-sensitive strain. There is evidence also that a young *N. crassa* culture is 'physiologically competent' for DNA uptake and transformation. A nuclease gene may be involved in transformation in *N. crassa*, since a nucleaseless (*nuc-1*) mutant when used as recipient showed both reduced DNA uptake and reduced frequency of transformation. Study of this transformation system could lead to an understanding of the molecular basis of transformation in eukaryotes.

METHODS

The multiply marked strain 2506 (*A, rg, arg, pan, nuc-1*⁺) of *Neurospora crassa* carrying an *inl* allele (89601) was used as the recipient. A newly isolated temperature-sensitive strain (RL3-8A-2), which required inositol for growth at 37 °C but not at 25 °C, was used as the donor in transformation. The recipient strain

(2506) required inositol, arginine and pantothenic acid for growth at 25 and 37 °C. The inositol loci in strains 2506 (*inl*) and RL3-8A-2 (*inl ts*) are allelic (unpublished results). These strains were grown on appropriately supplemented medium (Vogel, 1964). DNA prepared from the temperature-sensitive strain (*inl ts*) (Mishra *et al.*, 1973) was designated allo-DNA; DNA prepared from the recipient strain (2506) was designated iso-DNA. Young cultures (30 h) of the recipient strain were treated with allo-DNA (50 µg ml⁻¹) and CaCl₂ (80 mM). Recipient cultures without any DNA or treated with iso-DNA (50 µg ml⁻¹) served as controls. Presumptive transformants from the treated culture were selected by plating on medium without inositol and incubating at 25 °C. Viable counts were performed by plating on medium containing inositol (Mishra *et al.*, 1973). Transformant colonies growing on medium without inositol were isolated and their temperature sensitivity was determined. Those unable to grow on medium without inositol but able to grow with it at 37 °C were designated temperature-sensitive (*ts*) for inositol requirement. Only transformation of the inositol locus of the recipient strain was studied.

In other experiments, strains 2506 and 2506M were used as recipients; these were isogenic except that 2506M carried the mutant (*nuc-I*) allele of the nuclease gene (*nuc-I*⁺) (Ishikawa *et al.*, 1969). The two strains were separately treated with donor DNA from the wild-type (*inl*⁺) strain RL3-8A in the presence of CaCl₂ (80 mM). *Inl*⁺ transformants were then selected on medium without inositol (Mishra *et al.*, 1973). In the DNA uptake experiment, young (30 h) cultures of the recipients were incubated with ³²P-labelled DNA (50 µg ml⁻¹; sp.act. 6.7 × 10⁸ c.p.m. µg⁻¹) for 1 h and then the DNAase-resistant radioactivity was measured in a Beckman LS230 scintillation counting system.

RESULTS AND DISCUSSION

Transfer of a temperature-sensitive character

DNA from a temperature-sensitive donor was used in transformation to examine whether the donor-specific genetic information could be transferred to a recipient. The results from three separate experiments are presented in Table 1. The frequency of transformation was 2.3×10^{-6} . A significant number of the transformants (74 out of 95 examined) were unable to grow at 37 °C in the absence of inositol. Since these transformants could grow at 37 °C when inositol was added to the medium, their temperature-sensitive character was specific with respect to the inositol requirement. Thus, they had acquired the *ts* character of the donor DNA. The results also show that the *ts* character of the transformants was a specific effect of the donor DNA since colonies obtained without any DNA or following treatment of the recipient with its own DNA (iso-DNA) were not temperature-sensitive. Some of the transformants which were not temperature-sensitive with respect to inositol requirement for growth could have originated from reversion of the *inl* locus (*89601*) of the recipient strain (2506). Others could have resulted from recombination between donor and recipient DNA molecules (Whitehouse, 1965; Yoshikawa, 1966; Mishra, 1977). The above results provide conclusive evidence for transformation at the inositol locus of *N. crassa*. To our knowledge, no such transfer of temperature-sensitive character mediated by allo-DNA in eukaryotes has been reported previously.

Table 1. *Transformation of the inl recipient*

Recipient (*inl*) was treated with allo-DNA (*inl ts*) or iso-DNA (*inl*) as described in Methods; colonies growing on medium without inositol at 25 °C were designated as transformants. Data from three or more experiments are presented.

Treatment	No. of transformants examined	No. of transformants of each genotype *	
		<i>inl ts</i>	<i>inl</i> ⁺
Allo-DNA	95	74	21
Iso-DNA	7	0	7
No DNA	9	0	9

* *inl ts* requires inositol for growth at 37 °C but not at 25 °C; *inl*⁺ does not require inositol for growth at any temperature.

Table 2. [^{32}P]DNA uptake by mycelium

A young culture (30 h) of strain 2506 (*nuc-1*⁺) was incubated for 1 h with [^{32}P]DNA (sp.act. 6.7×10^3 c.p.m. μg^{-1}) in the growth medium.

DNA ($\mu\text{g ml}^{-1}$)	[^{32}P]DNA uptake [c.p.m. (mg dry wt mycelium) ⁻¹]
16	13 100
25	26 300
50	39 600
100	43 200

Effect of growth conditions on DNA uptake and transformation

Since genetic transformation is related to the amount of DNA taken up, we investigated the growth conditions that influence DNA uptake by *N. crassa* mycelium. DNA uptake by a young culture (30 h) was linearly dependent on the amount of DNA added up to $50 \mu\text{g ml}^{-1}$ in the growth medium (Table 2), and on time up to 1 h incubation. The young culture, under optimal conditions, took up a maximum of $6 \mu\text{g DNA (mg dry wt mycelium)}^{-1}$ which is equivalent to the DNA content of 1 mg mycelium (Minagawa *et al.*, 1959). DNA uptake by an old culture (40 to 60 h) was much lower and was not proportional to the amount of DNA added (data not shown). In the presence of CaCl_2 (80 mM), DNA uptake by the young mycelium was doubled. Addition of cyclic AMP or polyethylene glycol had no effect. However, in the presence of ATP (2 mM), the amount of DNA uptake by young mycelium increased by 64 %, which may reflect the energy dependence of the process (Lacks, 1976).

The competence of young cultures suggested that they could be more efficiently transformed by allo-DNA. In three parallel experiments, young cultures (10×10^6 cells) treated with allo-DNA yielded *inl*⁺ transformants at a frequency of 1.8×10^{-6} , which was raised to 2.3×10^{-6} to 2.7×10^{-6} by the addition of CaCl_2 . Such a stimulatory effect of CaCl_2 in bacterial transformation is well known but its role in transformation is unknown (Oishi & Irbe, 1976). The frequency of transformation by allo-DNA was consistent in parallel experiments using young cultures as recipients. Neither young cultures without any DNA (24×10^6 cells analysed) nor those with allo-DNA previously treated with DNAase (5×10^6 cells analysed) yielded *inl*⁺ colonies. The frequency of transformation by allo-DNA varied in experiments involving old cultures (40 to 60 h) of the recipient. These observations suggest that young *N. crassa* cultures possess a 'physiological preparedness or competence' for DNA uptake and transformation. Thus, the negative effect of allo-DNA reported previously (Mishra *et al.*, 1973) can perhaps be ascribed to differences in the physiological conditions of the recipient cultures used which were of various ages.

Role of nucleases in genetic transformation

At least three groups of enzymes (nucleases, DNA-polymerases and ligases) involved in recombination are also known to be involved in the uptake and integration of donor DNA molecules during transformation in bacteria (Lacks *et al.*, 1974; Lacks, 1976). We therefore compared the efficiency of a nuclease-less (*nuc-1*) mutant (2560M) with that of the wild-type (*nuc-1*⁺) strain (2506) in DNA uptake and transformation. These multiply marked strains are isogenic except for the *nuc-1* locus; *nuc-1* mutants are deficient in certain nucleases (Ishikawa *et al.*, 1969). The two strains were compared for their ability to take up [^{32}P]DNA and for the transformation of the *inl* locus.

The amount of DNA taken up by the nuclease-less (*nuc-1*) strain (Table 3) was only about 40 % of that taken up by the *nuc-1*⁺ strain. The *nuc-1* recipients showed a 90-fold reduction in the frequency of transformation compared with the wild-type recipient (*nuc-1*⁺). In genetic crosses between *nuc-1*⁺ (*inl*) and *nuc-1* (*inl*) strains, the *nuc-1* (*inl*) progeny always

Table 3. *Effect of a nuclease-less mutation (nuc-1) on DNA uptake and the frequency of transformation*

Uptake of [32 P]DNA ($50 \mu\text{g ml}^{-1}$; sp.act. 6.7×10^3 c.p.m. μg^{-1}) by young cultures (30 h) was measured after 1 h incubation. Data are based on three or more experiments.

Strain	[32 P]DNA uptake [c.p.m. (mg dry wt mycelium) $^{-1}$]	Frequency of <i>inl</i> $^{+}$ transformants
2506 (<i>nuc-1</i> $^{+}$)	39600	2.7×10^{-6}
2506M (<i>nuc-1</i>)	15840	0.03×10^{-6}

showed a reduced frequency of transformation at the *inl* locus and a reduced DNA uptake in addition to nuclease deficiencies. These findings are similar to the effects of nuclease-less mutation on the frequency of bacterial transformation and suggest that nucleases may play a role in the uptake and integration of the donor DNA into recipient genomes during transformation in *N. crassa* as in bacteria (Lacks *et al.*, 1969; Lacks, 1976). Schablik *et al.* (1977) reached the same conclusion by showing that DNA uptake by a young *N. crassa* culture was significantly reduced when the culture was repeatedly washed to remove extracellular nucleases. We have recently developed a large number of nuclease-less mutants of *N. crassa* (Forsthoeft & Mishra, 1977) and with the use of these as recipients it may be possible to determine the role of nucleases in transformation. In addition, conditions which may lead to the evaluation of the DNA polymerases during transformation in *N. crassa* have been described (El-Assouli & Mishra, 1978). Continuation of these studies should provide some understanding of the molecular basis of transformation (including recombination of DNA molecules) in higher organisms.

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