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The effect of DNA structure and restriction enzymes on transformation efficiencies in Neurospora crassa

Kristina Garnand
University of New Mexico

Mary Anne Nelson
University of New Mexico

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Abstract

Addition of the appropriate restriction enzyme to linearized transforming DNA has been shown to increase transformation efficiencies in organisms as diverse as *Saccharomyces cerevisiae* (Schiestl and Petes 1991 Proc. Nat. Acad. Sci. USA **88**:7585-7589) and *Dictyostelium discoideum* (Kuspa and Loomis 1992 Proc. Nat. Acad. Sci. USA **89**:8803- 8807). This process has been described as REMI, for restriction enzyme-mediated integration. We have examined the effect of restriction enzyme addition on transformation efficiencies in *Neurospora crassa*. The frequency of cotransformation of a *qa-2 inl* double mutant with two plasmids [one containing the selectable marker *qa-2+* (quinate utilization) and the other containing *inl+* (inositol)] was also examined, as was the generation of stable versus abortive transformants.

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Kristina Garnand and Mary Anne Nelson - Department of Biology, University of New Mexico, Albuquerque, NM 87131

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Circular (plasmid) DNAS, linear DNAs and linear DNAs plus the restriction enzyme that had been used to linearize the plasmids were used in these transformation experiments. The PMSNI plasmid contained the selectable *qa-2*+ marker (Nelson and Metzenberg 1992 Fungal Genet. Newsl. 39:59-60). The pOKE01 plasmid contained the selectable *inl*+ marker in an 8.5 kbp *Pst*I insert in pUC18; this plasmid was a gift from Dr. R.L. Metzenberg. Plasmid DNAs were prepared using the Qiagen, Inc. Plasmid Midi kit. The restriction enzymes in the digested DNA samples were inactivated by heating at 60 C for 10 minutes.

Preparation of spheroplasts [from a *qa-2 aro-9 inl al-2 a* (RLM 63-01, a gift of Dr. R.L. Metzenberg) strain of *N. crassa*] and their transformation were as previously described (Schweizer et al. 1981 Proc. Nat. Acad. Sci. USA 78:5086-5090). For the single transformation experiments, 1 ug of DNA (circular or linear) was mixed with 100 ul spheroplasts; where noted, 1 ul of the appropriate restriction enzyme, at 15 U/ul, was added to the linear DNAs immediately prior to transformation. In cotransformation experiments, a total of 2 ug DNA (1 ug each plasmid), plus 15 U each enzyme (where specified) was added to 100 ul spheroplasts. Transformants were regenerated in liquid medium and spread onto selection plates (Akins and Lambowitz 1987 Cell 50:331-345). In the cotransformation experiments, the regenerated spheroplasts were divided and spread onto plates selective for *qa-2*+, *inl*+ or both *qa-2*+ and *inl*+.

Since different preparations of spheroplasts often give quite dissimilar transformation efficiencies, we controlled for chance variations in transformation efficiencies by doing all transformations for a given experiment at the same time with a single preparation of spheroplasts. The results of a typical experiment are shown in Figures 1 and 2. The use of linear instead of circular transforming DNA did not significantly increase the generation of stable transformants (Fig. 1 and 2). The addition of restriction enzymes to the transforming DNA did not have a dramatic or consistent effect in transformations with single DNAs. Also, transformation with linear and circular DNAs gave approximately the same efficiencies of cotransformation of the *qa-2*+ and *inl*+ markers. However, the addition of restriction enzymes in the cotransformation experiments resulted in the generation of fewer singly and doubly-

transformed strains (Fig. 1). The reason for the reduction in transformation efficiencies in cotransformation experiments when restriction enzymes were added is unclear; the enzymes do not destroy the selectable markers that were used.

The effect of DNA structure on the generation of abortive transformants is shown in Fig. 2. Abortive transformants are defined as those which begin to grow and form small colonies on the selection plates, but stop growth prior to making a large colony; these transformants fail to grow upon transfer, and are thought to reflect cases in which the transforming DNA enters the cell but fails to become stably integrated into the genome. The use of linear instead of circular DNA caused a great reduction in the numbers of abortive transformants that were obtained; the addition of restriction enzyme did not have a significant effect on the stable/abortive ratio.

In *N. crassa* the addition of restriction enzymes to linearized transforming DNA was not shown to increase the efficiency of transformations consistently. This is in contrast to results obtained with *S. cerevisiae* and *D. discoideum*. Although viability was not directly measured, the similar numbers of transformants obtained when single transforming DNAs were used suggests that the addition of *Pst*I or *Hin*dIII is not resulting in decreased viability. DNA structure had no significant effect on the frequency of cotransformation with the selectable markers that were examined. However, the use of linear instead of circular transforming DNA was shown to significantly decrease the background of abortive (unstable) transformants.

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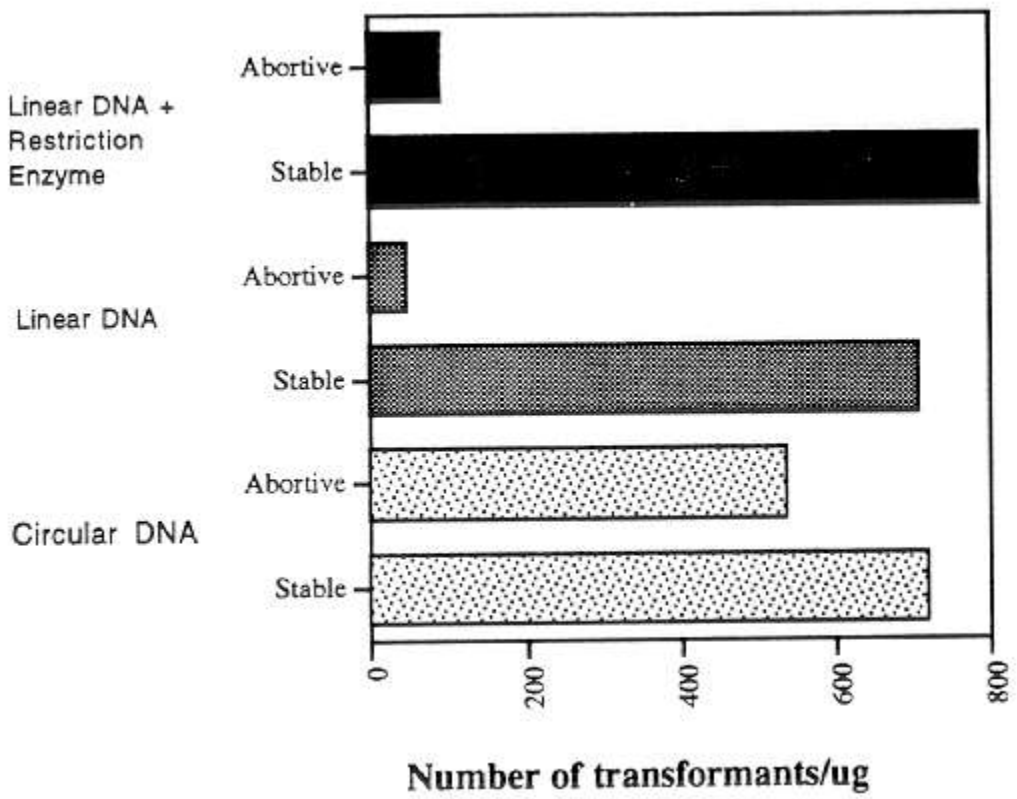

Figure 1. The effect of DNA structure and addition of restriction enzymes on transformation and cotransformation efficiencies. The numbers of stable transformants obtained per ug DNA are shown. In the cotransformation experiments, 1 ug each DNA (2 ug total) was used for the same volume (100 ul) spheroplasts, so the numbers of transformants are per ug each DNA. In the Transforming DNA column, the symbols are as follows: Co, cotransformation; L, linearized plasmid DNA; C, circular plasmid DNA; L+RE, linearized plasmid DNA plus restriction enzyme; inl+, transformation with the selectable *inl*+ marker (pOKE01); *qa-2*+, transformation with the selectable *qa-2*+ marker (pMSN1). The pMSN1 plasmid was linearized with *Hin*dIII, and the POKE01 plasmid with *Pst*I.

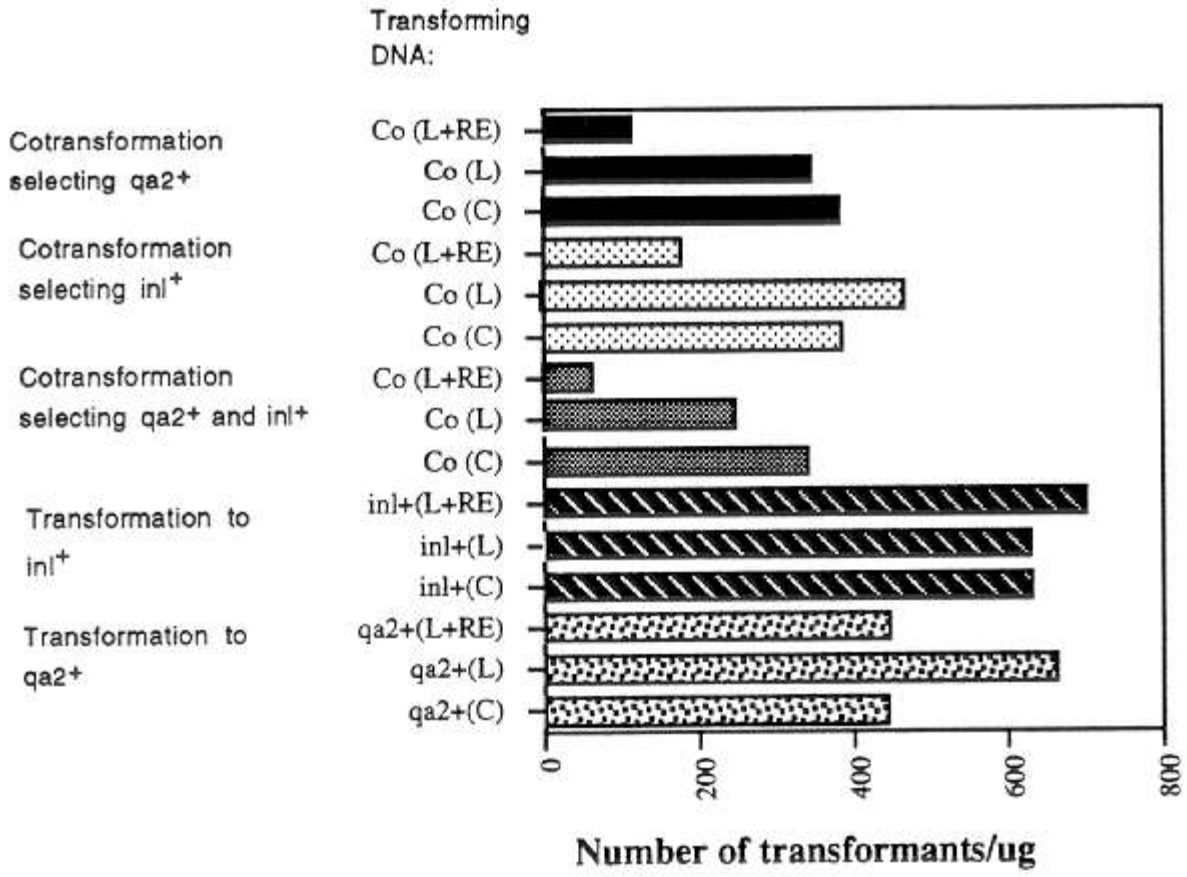

Figure 2. The effect of DNA structure on the generation of stable and unstable (abortive) transformants. The *qa-2 aro-9 inl al-2 a* spheroplasts were transformed to *inl*+ with circular or *Pst*I linearized forms of the POKE01 plasmid, and the restriction enzyme *Pst*I was added as noted.

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