

The ribosomal repeat of *Aspergillus niger* and its effects on transformation frequency

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The ribosomal RNA genes have been used in studies of a variety of phenomena. These include studies of molecular phylogeny, transcription, recombination and transformation. In *Saccharomyces cerevisiae*, where integrative transformation is achieved by homologous recombination, the presence of the ribosomal repeat unit in the transformation vector greatly increases the frequency of transformation (Szostak and Wu 1979. Plasmid 2:536-554; Smolik-Utlaut and Petes 1983. Mol. Cell. Biol. 3:1204-1211). In the filamentous fungus *Aspergillus nidulans*, where integrative transformation can be achieved either via homologous or heterologous recombination, the presence of ribosomal repeat sequences in the transformation vector has no effect on the frequency of transformation (Tilburn et al. 1983. Gene 26:205-221). [Ed.: This is also the case in *Neurospora crassa*: Russell et al. 1989. BBA 1008:243-246]. Here we report the molecular cloning of the ribosomal repeat unit from *Aspergillus niger*. We have found that the presence of cloned ribosomal DNA sequences from *A. niger* increases the frequency of transformation in *A. niger*.

1. Cloning of the ribosomal repeat unit of *A. niger*

A genomic library of wild type *A. niger* (Kelly and Hynes 1985. EMBO J 4:475-479) DNA, partially digested with *Mbo*I, was constructed in the lambda based replacement vector EMBL 3. The library was screened with a ³²P labelled plasmid probe, pMN1, containing the *A. nidulans* ribosomal repeat unit (Borsuk et al. 1982. Gene 17:147-152). Several plaques were identified that hybridized to pMN1. *Sal*I digested DNA prepared from these plaques showed a band of approximately 7.8 kb, and Southern blot analysis showed this band to have homology to pMN1. That this band represented an entire ribosomal repeat unit was inferred from the finding from Southern blot analysis that *A. niger* DNA cut with a series of enzymes, including *Sal*I, *Bam*HI and *Bgl*II, gave a hybridizing band of approximately 7.8 kb representing one ribosomal repeat unit. This 7.8 kb sequence was cloned into the *Sal*I site of pBR322 and the recombinant plasmid was designated pANiR1. A partial restriction map was determined and is shown in Figure 1. The coding regions are also shown as determined both by hybridizing restriction fragments of pANiR1 to Northern blots of total RNA extracted from a wild type strain of *A. niger* and by hybridizing ribosomal RNA probes to Southern blots of various digests of pANiR1. The position of the genes, and of restriction sites, was compared with those found by Borsuk et al. (1982. Gene 17:147-152) and Lockington et al. (1982. Gene 20:135-137) in the cloned *A. nidulans* ribosomal repeat DNA. The position of the genes was found to be conserved, as were the restriction sites within the coding regions. The restriction sites within the non-transcribed spacer region were not conserved.

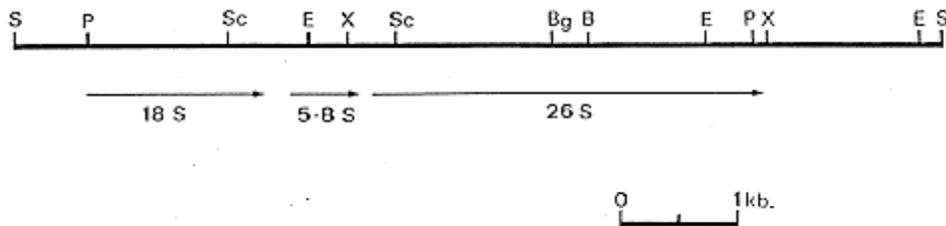


Figure 1. Restriction endonuclease map of the *A. niger* ribosomal repeat. Restriction endonuclease sites: S - *Sal*I; P - *Pst*I; Sc - *Sac*I; E - *Eco*RI; X - *Xho*I; Bg - *Bgl*II; B - *Bam*HI The lines below the map indicate the positions of the rRNA genes.

2. The effect of the ribosomal repeat unit of *A. niger* of the frequency of transformation

Transformations of *A. niger* were performed using the *amdS* gene of *A. nidulans* as a dominant selective marker (Kelly and Hynes 1985. EMBO J 4:475-479). The plasmid used, p3SR2, contained the *amdS* gene cloned into pBR322 (Hynes et al. 1983. Mol. Cell. Biol. 3:1430-1439). Transformation frequencies were compared in experiments using p3SR2 alone, p3SR2 plus equimolar amounts of pMN1, and p3SR2 plus equimolar amounts of pANiR1 (Table I). Higher frequencies of transformation were consistently found when pANiR1 was present, but not when pMN1 was present. This result was repeatable, and did not depend on the particular *amdS* containing plasmid used. Transformation frequencies of *A. niger* using *amdS* selection were low, possibly due to the observation that transformants contain multiple copies of the *amdS* gene, and a transformant containing a single copy of the *amdS* gene may not grow sufficiently to be detected.

Table I: Transformation of *A. niger* in the presence of the cloned ribosomal repeat from *A. niger* and *A. nidulans*

Transforming DNA	Number of <i>amdS</i> ⁺ transformants per microgram DNA	
	Experiment 1	Experiment 2
p3SR2	15	26
p3SR2 + pMN1	24	30
p3SR2 + pANiR1	167	138

Results shown are of two independent experiments, each with 2×10^4 viable protoplasts per treatment. In six experiments, the average fold increase was five.

DNA was extracted from eight strains cotransformed with pANiR1 and p3SR2, digested with *Hind*III, separated by agarose gel electrophoresis, transferred to nylon membrane, and hybridized with the insert of p3SR2. The membrane was washed and rehybridized with the insert of pANiR1. There are no *Hind*III sites in the ribosomal repeat unit, and as expected, the insert of pANiR1 hybridized to a band of very high molecular weight, too large to quantify on standard agarose gels. There are no *Hind*III sites in p3SR2, and no hybridization is observed between p3SR2 and wildtype *A. niger* DNA. In the transformant DNA, the *amdS* probe also hybridized to a band of very high molecular weight. Thus it is probable that the *amdS* sequences integrated at the ribosomal repeat region.

In order to determine if this result was entirely dependent upon the selectable marker in the recipient strain, transformations were performed using the cloned *A. niger pyrG* gene (pAB4.1) as a selectable marker in a *pyrG* auxotrophic strain of *A. niger* (van Hartingsveldt et al. 1987. Molec. Gen. Genet. 206:71-75). The plasmid pAB4.1 transformed the *A. niger* strain AB4.1 at a much higher frequency than observed for p3SR2 and the wild type strain. Numbers of transformants in experiments using pAB4.1 alone were compared with numbers of transformants using both pAB4.1 and pANiR1. In four independent experiments, the transformation frequency was 3.7-, 3.9-, 4.0- and 4.5-fold higher in the presence of pANiR1 than in its absence. These experiments showed that the effect of pANiR1 on transformation frequency was neither recipient strain nor selectable marker dependent. Various subclones of pANiR1 were tested in an attempt to localize the effect. Neither the *EcoRI-EcoRI* fragment nor the small *EcoRI-SalI* fragment had an effect on transformation frequency when present as inserts in pBR322 in cotransformation experiments. Further, the increase in transformation frequency was not apparent when the ribosomal repeat unit was inserted into the same plasmid as the *amdS* selectable marker, but this may be due to the reduction in transformation frequency due to the very large size of the plasmid masking the five fold effect.