

## How many 5S rRNA genes and pseudogenes are there in *Aspergillus nidulans*?\*

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We have estimated the number of 5S rRNA genes in *Aspergillus nidulans* using two-dimensional agarose gel electrophoresis and hybridization to appropriate probes, representing the 5'-halves, the 3'-halves of the 5S rRNA sequence and a sequence found at the 3'-end of all known *A. nidulans* pseudogenes (block C). We have found 23 5S rRNA genes, 15 pseudogenes consisting of the 5'-half of the 5S rRNA sequence (of which 3 are flanked by block C) and 12 copies of block C which do not seem to be in the vicinity of 5S rRNA sequences. This number of genes is much lower than our earlier estimates, and makes our previously analyzed sample of 9 sequenced genes and 3 pseudogenes much more representative.

For a number of years we have been analyzing the sequence and organization of 5S rRNA genes in *Aspergillus nidulans*. In contrast to most eukaryotes, whose 5S rRNA genes are organized in the form of tandem repeats, in *A. nidulans* these 120 bp long genes are dispersed and show sequence microheterogeneity. Moreover, of the cloned sequences which were isolated as hybridizing to 5S rRNA three turned out to be unusual pseudogenes, consisting of the 5'-half of the 5S rRNA sequence (block A) flanked by a longer fragment (block C) instead of the normal 3'-half of the 5S rRNA gene (block B). Block C was highly conserved in the three pseudogenes [1-4]. Very similar results were obtained for two other *Aspergillus* species, *A. wentii* and *A. awamori* [5]. In each species one truncated 5S rRNA pseudogene flanked by block C was found; in addition, we found a more typical pseudogene with changes in the whole 5S sequence and a pseudogene lacking the first 40 nucleotides of the 5S rRNA sequence

[5]. The analysis of these 23 genes and 7 pseudogenes led us to speculations on the evolution of these dispersed sequences. However, initial estimations made in the late 70-ies by reassociation curves (unpublished) indicated that there were about 100 copies of 5S rRNA genes in *A. nidulans*, thus the 9 genes and 3 pseudogenes we had cloned and sequenced in this organism were not really a representative sample. The experiments described in this paper were aimed at determining the number of copies of 5S rRNA genes and pseudogenes in *A. nidulans*.

### MATERIALS AND METHODS

All the methods used were described previously [5] with the exception of two dimensional electrophoresis which was performed according to Yi *et al.* [6] with some modifications. Total *A. nidulans* DNA digested with *EcoRI* (prepared at the Department of Genetics)

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was subjected to electrophoresis in a 0.5% agarose (Sigma or FMC Bioproducts Corporation) gel in siliconized glass capillaries 2.5 mm in diameter and 15 cm long, for 24 h at 40 V. The buffer was circulated using a peristaltic pump. After electrophoresis the agarose was pushed out from the tubes into a glass tube 3 mm in diameter and 12 cm long. *Hae*III (BRL) and an appropriate digestion buffer were added to the glass tube, its ends were sealed with parafilm and incubated in a water bath for 2.5 h at 37°C. After digestion the agarose fragment was placed in a 1.5% agarose gel and electrophoresed for 20 h at 40 V in the second direction. After electrophoresis the DNA was transferred to Hybond C Extra (Amersham) and hybridized to appropriate probes, using conditions recommended by the manufacturer. Each filter was used for hybridization 3 times, and after each hybridization the probe was removed using 0.2 M NaOH containing 0.1% SDS. Autoradiography was performed using Foton XR-1 film and X-omatic intensifying screens.

The probes for block A and B were derived from the pMN912 plasmid [2], in which a *Hind*III fragment of 0.3 kb containing a *A. nidulans* 5S rRNA gene is present. This fragment was isolated from a 1.5% agarose gel, and digested with *Hpa*II which cleaves the gene at position 65 yielding two fragments, a 0.1 kb fragment containing block A and a 0.2 kb fragment containing block B. The two fragments were isolated after electrophoresis in a 1.5% agarose gel. The two fragments were each subcloned in the Bluescript plasmid pKS+ cleaved with *Cla*I and *Hind*III. Plasmids pKS/A and pKS/B containing, respectively, the left and right half of the 5S rRNA gene were obtained and used for hybridization.

The C block had previously been cloned in plasmid pMG1 [4]. However, the cloned fragment contained an *Hae*III site which made it unsuitable for analysis of *Hae*III-cleaved DNA. The pMG1 plasmid was cleaved with *Sal*I and *Eco*RI and a 220 bp fragment containing block C was isolated and cleaved with *Hae*III. After electrophoresis in a 1.5% agarose gel a 160 bp fragment was isolated and cloned in pKS+ digested with *Eco*RI and *Eco*RV, yielding the pKS/C plasmid.

## RESULTS

Each filter was hybridized three times to probes representing the 5'-half of the 5S rRNA gene (block A), the 3'-half (block B) and the block C sequence which has been found to flank all three *A. nidulans* 5S rRNA pseudogenes identified so far. The hybridization results are shown in Fig. 1 a, b and c, respectively. The results are based on the assumption that neither *Eco*RI nor *Hae*III cut within the 5S rRNA genes; we also know that they do not cut within the block C sequence used as a probe.

Altogether 41 different fragments hybridizing to the probes have been found; they can be divided into the following categories:

| Class | Number |
|-------|--------|
| AB    | 21     |
| ABC   | 2      |
| AC    | 3      |
| A     | 12     |
| C     | 3      |

The first two classes represent *A. nidulans* 5S rRNA genes, of which two are flanked by block C sequences. The third class are the three known pseudogenes of the AC type which we have cloned and sequenced [2]. The next class represents A sequences which do not appear to be flanked by the C block (so far we have not cloned any such fragments), and the last group are C sequences alone.

Altogether 8 C sequences were detected under the stringent conditions used in these experiments, and it is of course possible that there are more of them in the *A. nidulans* genome, but their homology is insufficient to allow them to be detected under the stringent conditions used.

With the number of *A. nidulans* 5S rRNA genes being only 23, our results obtained so far — that is the sequences of 9 genes — represent quite a sizeable portion of these sequences in the *A. nidulans* genome. The unexpected finding is that there are probably more pseudogenes than we had expected on the basis of our sequencing studies — 15, almost as many as there are genes. We had expected that the pseudogenes would form altogether about 1/4 of the number of the gene sequences, as in the three *Aspergillus* species analyzed so far we had detected 7 pseudogenes and 25 genes. Thus if

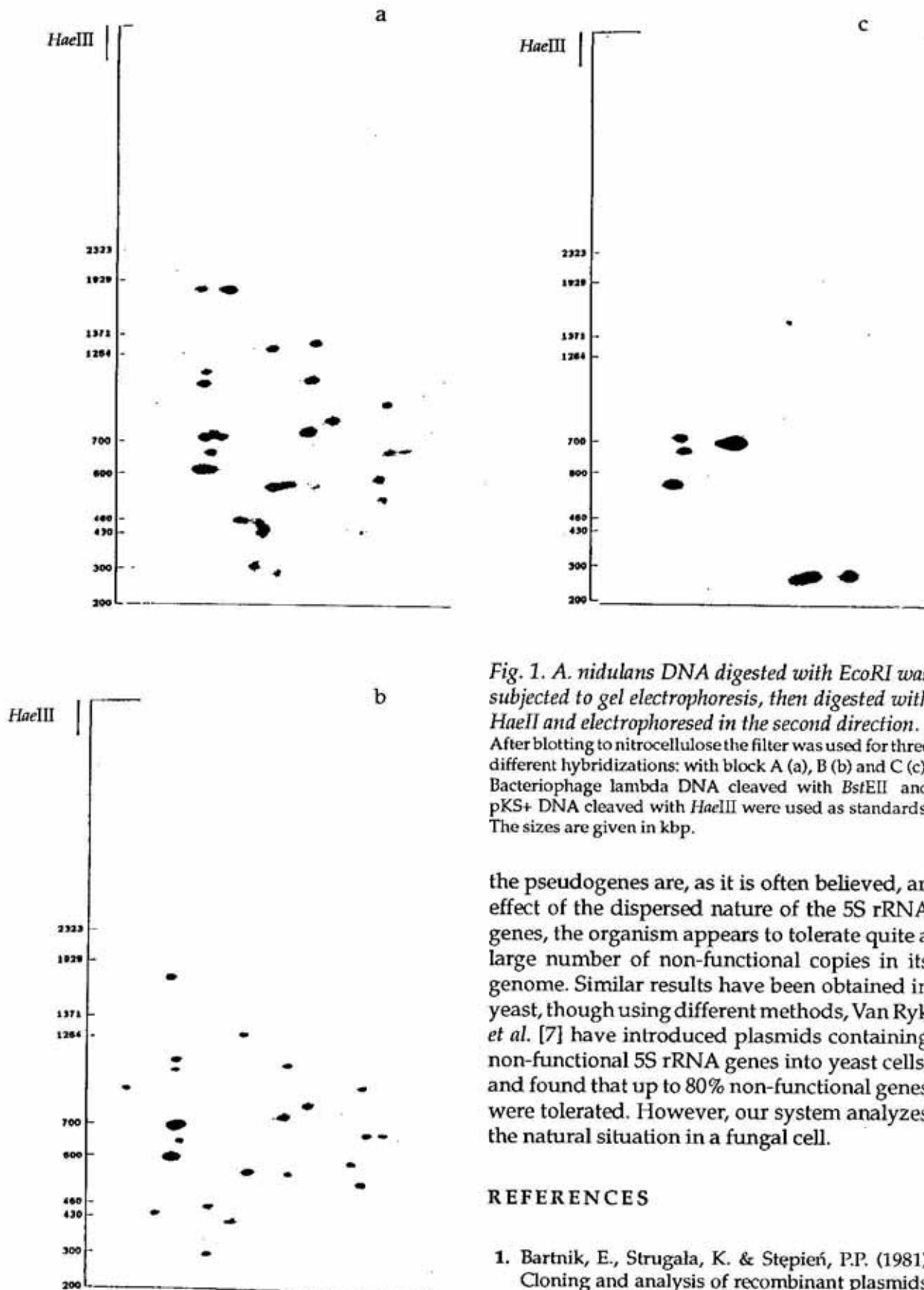


Fig. 1. *A. nidulans* DNA digested with *EcoRI* was subjected to gel electrophoresis, then digested with *HaeII* and electrophoresed in the second direction. After blotting to nitrocellulose the filter was used for three different hybridizations: with block A (a), B (b) and C (c). Bacteriophage lambda DNA cleaved with *BstEII* and pKS+ DNA cleaved with *HaeII* were used as standards. The sizes are given in kbp.

the pseudogenes are, as it is often believed, an effect of the dispersed nature of the 5S rRNA genes, the organism appears to tolerate quite a large number of non-functional copies in its genome. Similar results have been obtained in yeast, though using different methods, Van Ryk *et al.* [7] have introduced plasmids containing non-functional 5S rRNA genes into yeast cells, and found that up to 80% non-functional genes were tolerated. However, our system analyzes the natural situation in a fungal cell.

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