

Sequencing of the non-transcribed spacer region of the ribosomal RNA genes of *Neurospora crassa*

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The nontranscribed spacer (NTS) region of the ribosomal RNA (rRNA) genes is the most important region of the rDNA because it contains the nucleotide sequences that trigger and/or terminate transcription. To understand the structural organization of the NTS region of *Neurospora crassa* rDNA we have cloned and sequenced the complete NTS region and compared it with the corresponding sequences from other organisms.

One 3592 nucleotide long *N. crassa* nuclear DNA containing the nontranscribed spacer region and part of 26S rRNA (3' end) and 17S (5' end) was isolated and cloned in the plasmid pBR325 (Chambers, C., S.K. Dutta and R.J. Crouch 1986. Gene 44:159). The clone, namely pCC3400, is resistant to ampicillin and tetracycline and sensitive to chloramphenicol.

The complete NTS region of *N. crassa* rDNA (comprising 3592 nt), determined by the chain termination method of nucleotide sequencing, shows many significant features and a brief account of these features follows:

1. Like other rDNA sequences in the NTS region (e.g. *Xenopus*, human, rat, mouse etc.) the NTS region of *N. crassa* is GC rich (65% G+C).
2. In the NTS region the rRNA processing site 6 is present (indicating that the clone pCC3400 contains the 3' end of the coding region of the 26S rRNA and flanking sequences) and the sequence GGTGCGAGAACCCGGA (nucleotide residue 226 to 240) is similar to the consensus sequence reported for the rRNA processing site 6 for yeast, *Xenopus*, mouse and human (but not for *Drosophila* where the transcription of the tandem array of ribosomal DNA does not terminate at any fixed point).
3. The NTS region contains long stretches of pyrimidines all over, and this type of structural organization is present in maize, wheat, *Raphanus sativus*, *Vicia faba*, pea, cucumber and rice.
4. The transcription termination site, starting with the conventional "SalI box" is present in the NTS region of *N. crassa* as has been reported for mouse and human. However, compared to 8 SalI boxes in mouse rDNA in the NTS region, only one SalI box is present in *N. crassa* (from nucleotide 1469 to 1477). Sequence comparison of the SalI boxes and neighboring sequences from different sources suggest that motif TCGAC is common to all.
5. The sequence motif CTTCCCT (from nucleotide residue 512 to 517) shows similarity with the human transcription termination site T-2 of its pre-rRNA.
6. The overall sequence of the NTS region of *N. crassa* is closer to that of the *Saccharomyces cerevisiae* NTS region than to those of human, *Xenopus*, wheat, rice, cucumber, *Vicia faba*, mouse, rat and *Drosophila*.

7. In the NTS region of *N. crassa* certain sequences like TCTC, TTTT and TTGC reiterate several times. Similar repetition of sequences has been reported for human rDNA although the functional significance of these sequences is not yet established.

Briefly, the information gained in the present studies should be very useful in studying the regulation of transcription of rRNA genes of *N. crassa* and the conserved sequences in the NTS region might be exploited in the evolutionary studies. Determination of the *N. crassa* transcription initiation and termination sites *in vivo* and *in vitro* using clone pCC3400 characterized in the present studies await the results of the appropriate experimental design and as such are a continuous focus of our laboratory. The entire sequence is being published elsewhere and is available upon request.

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