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DNA SEQUENCE OF THE REGION ADJACENT TO THE oxil AND oxi2 GENES FROM ASPERGILLUS NIDULANS MITOCHONDRIAL DNA

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The nucleotide sequence of 5 different fragments from the HpaII-6 region of Aspergillus nidulans mitochondrial DNA were established to provide more information on the position of genes coding for subunit 1 (oxi1) and subunit 2 (oxi2) of cytochrome oxidase. A relatively high GC content and open reading frames have been found but none of the sequences is homologous to the exon parts of the oxi1 or oxi2 genes from Saccharomyces cerevisiae.

Mitochondrial DNA from Aspergillus nidulans consists of a circular molecule 31 kb in length and of GC content 31% (Lopez-Perez & Turner, 1975), whose restriction enzyme map (Stepien et al., 1978) and the positions of genes coding for ribosomal RNAs have been established (Lazarus et al., 1980). Cloning of this mitochondrial DNA in E. coli on pBR322 plasmid (Bartnik et al., 1979, 1981) made possible fine mapping and sequencing of all genes coding for tRNAs, rRNAs, and ATPase subunit (Kochel et al., 1981). By hybridization of the mitochondrial DNA fragments to probes representing various mitochondrial genes from yeast, Macino et al. (1980) established approximate positions of genes oxi1, oxi2 and oxi3 coding for cytochrome oxidase subunits 1, 2 and 3, respectively, and for apocytochrome b. In the above-mentioned studies, short yeast exon probes representing oxi1 and oxi2 genes were shown to hybridize to HaeIII-3 fragment of A. nidulans mitochondrial DNA. The arrangement of the two genes was not established, however, and it seems possible that one of them, most probably oxi1, could extend into the HaeIII-5 region.

The present study was designed to test whether DNA sequences within the HpaII-6 fragment of A. nidulans mitochondrial DNA are homologous to yeast

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oxil or oxi2 sequences (Coruzzi & Tzagoloff, 1979; Bonitz et al., 1980). Five fragments from a 1.7 kb long HpaII-6 region were sequenced using the technique of Maxam & Gilbert (1980), and the sequences obtained were compared with the yeast oxi1 and oxi2 sequences using the computer program of Staden (1977).

MATERIALS AND METHODS

Strains. Clone BamHI-2 was from the collection of the Department of Genetics, Warsaw University. It consists of an A. nidulans mitochondrial DNA fragment BamHI-2 maintained in pBR322 plasmid in E. coli strain HB101, and it was obtained as described by Bartnik et al. (1981).

Reagents. [γ-32P]ATP (spec. act. 3000 Ci/mmole) was purchased from Amersham; restriction enzymes and polynucleotide kinase were from Bethesda Research Laboratories (U.S.A.).

Preparation and labelling of DNA fragments. Several hundred micrograms of the plasmid BamHI-2 were routinely digested with HpaII restriction enzyme to produce 0.9 kb, 1.7 kb and 3.2 kb fragments. Following preparative agarose electrophoresis, the 1.7 kb fragment was isolated, dephosphorylated using alkaline phosphatase, and labelled at 5' ends using polynucleotide kinase and [32P]ATP. After digestion with TaqI restriction endonuclease, labelled fragments were separated on 5% acrylamide gel and sequenced. All the above procedures were performed as described by Maxam & Gilbert (1980). Alternatively, the 1.7 kb band was digested with TaqI, the mixture of five subfractions was dephosphorylated, labelled at 5' ends, resolved on a 5% polyacrylamide gel, and the reisolated fragments were denatured and subjected to strand separation on 8% polyacrylamide gel. Single-stranded labelled fragments were eluted from the gel and sequenced. Comparison of DNA sequences was performed using the program designed by Staden (1977).

DNA sequencing. DNA fragments labelled at one end with 32P were divided into four parts, 10 000 - 20 000 cpm each, and the following four reactions were carried out: C and T with hydrazine; C with hydrazine in 5 M-NaCl; A with NaOH; and G with dimethyl sulphate, according to Maxam & Gilbert (1980). The reaction products were separated on 17% or 8% urea-polyacrylamide gels. Temperature of separation was 45 - 55°C, and gel dimensions 1 × 300 × 1000 mm. Autoradiograms were exposed for 10 - 14 days at −20°C using Agfa Osray films. No pre-flashing or intensifying screens were used.

RESULTS AND DISCUSSION

Figure 1 presents a map of A. nidulans mitochondrial DNA (Steptien et al., 1978; Lazarus et al., 1980; H. Küntzel, personal communication), and a scheme of the sequencing strategy. Figure 2 presents an example of the sequencing gels. The sequences obtained are presented from 5' ends (Fig. 3) in the direction indicated by the arrow on Fig. 1. For sequences obtained from the separated strands of DNA
from the fragment 3 it is impossible to assign the orientation, and the arrows C and D have been located arbitrarily.

Table 1 presents the numbers of termination codons TAG and TAA found in each of the six possible reading frames for each sequence and the GC content. In *A. nidulans* all the known mitochondrial genes are transcribed clockwise (Köchel

![Diagram showing restriction enzyme map of *Aspergillus nidulans* mitochondrial DNA and the sequencing strategy. Outer circle — HaeIII fragments; inner circle — HindIII fragments; two arrows inside — BamHI sites; two bars — HpaII sites within the BamHI-2 fragment; on the enlarged HpaII-6 fragment (below) HpaII sites (bars) and TaqI sites (arrows) are indicated. Open arrow — site not mapped.]

**Table 1**

The termination codon occurrence and the GC content of 5 sequences from the HpaII-6 region of *Aspergillus nidulans* mitochondrial DNA

<table>
<thead>
<tr>
<th>Name of the sequence and its orientation</th>
<th>Length in base pairs</th>
<th>Number of termination codons in each reading frame</th>
<th>The longest amino acid sequence possible</th>
<th>GC content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A →</td>
<td>221</td>
<td>α  β  γ  0  6  7</td>
<td>73</td>
<td>27</td>
</tr>
<tr>
<td>B ←</td>
<td>211</td>
<td>3  7  0  1  3  7</td>
<td>69</td>
<td>40</td>
</tr>
<tr>
<td>C →</td>
<td>241</td>
<td>11  5  1  6  5  1</td>
<td>70</td>
<td>27</td>
</tr>
<tr>
<td>D ←</td>
<td>187</td>
<td>2  2  8  5  1  6</td>
<td>54</td>
<td>29</td>
</tr>
<tr>
<td>E ←</td>
<td>138</td>
<td>0  4  2  5  2  1</td>
<td>45</td>
<td>38</td>
</tr>
</tbody>
</table>
et al., 1981) and therefore it seems likely that for the sequences A, B and E only reading frames $\alpha$, $\beta$ or $\gamma$ may function in situ. Sequences C and D cannot be oriented on the map and thus both clockwise and counterclockwise reading frames are equally probable.

![DNA sequence](image)

Fig. 2. An example of 8% polyacrylamide gel showing a sequence ladder. Autoradiogram after 14 days of exposure.

A comparison of the obtained sequences with the exon sequence of the genes coding for subunit 1 and subunit 2 of cytochrome oxidase from *Saccharomyces cerevisiae* was performed using Staden's computer program to search for homologies. Both clockwise and counterclockwise orientations starting from 5' end
were compared, and in each case the whole length of *Aspergillus* sequence was used as a frame.

For each comparison the highest coincidence number was equal $\pm 2\sigma$ for both orientations of DNA fragments and in no case was it higher than 43%, thus indicating no significant homology between the sequences tested. For comparison, the DNA sequence of the cytochrome $b$ gene from the *A. nidulans* mitochondrial genome shows 66% homology with the *S. cerevisiae* gene (R. Wayne-Davies, personal communication).

A

AAAGTAAATAATGCTATCTUTGACCGG6GTGTAAGTAGTATAATATATCATAAAATTTGAAACAGAAATAAT
TTAATCAAAAATATCAATTATTAT1ATACGTGAAGATT1ATGCTGATTTATAAT1AACCAAT1AGTTTAAA
ATTAAATTAAATATTATTTAATCATAGTGGTAGTGG11T1CTGATGCTGATCGTACGCGAGCY6
CCCTTCACCC

B

AAGTACGGCTGTGGTGG1GTCCTATCTTATTCGCCAATCC1TCCG66CACCACCGAG6GTCAA
AAAATAGGCT1TTTCAAGTTCGATGTC11CG66ACCACACTCAATTATAGTT111AA1ACTCAATTCTTA
AA1GACAAGTATAAAAGTTTAACGATGATGTAAGCAGAGCATTATATG66ACCACG61ACGCAAG1T1T
G

C

TAAGTTGGAATATCTCAAAAGCACAAGATCAATAAGGTTTAGTATTCAAGGAG11T111AAAT1AGT
CGAGTTTCTTTAATACACTGAGGATANAAAGAACAAGATAAAATAGATCTACACCCAGAGTAGTATTAGTT1AA:
TACTAAATTCAAGGTATAATACAGCAGCAAG61ATTTTAT11AAATATCCATTTTGGGAAACAGAAG1TT
TAGATCAGATTTGAGAAGGTTG11AGAL11

D

GTTGTGCGTATATCTCTTAA1AAAGTATACTTACACCAAAATATTGCTACATAAA1AAAGGTTTTTTTGT
T1AAGATAAAAAGACGCTACTGGTAGTGTTCGATACCGCGCTGA
CAGCAT1GTATATTATAATAGTAAAGACT11CA1AA1CCGCTCA1

E

TTGGCTTGGCT1AA1ACTGGAAGACTG1TAATAT1TATAGATATATATCACA1CAGTAGGAAACACAT
CTAGGGTTAAAACCTAAATTGGTCTT1CAGACGACTACACCCAGAGTAGTT11TTCATTTAATTCT1ACAT

Fig. 3. Five sequences from the HpaII-6 fragment of the *Aspergillus nidulans* mitochondrial DNA. The letters represent fragment denomination shown in Fig. 1.

The results suggest that no major part, if any, of the ox1l and ox1l2 exons map within the HpaII-6 fragment of *A. nidulans* mitochondrial DNA. The sequences obtained do not cover the whole fragment. Thus a possibility that they represent an intron(s) of ox1l gene while the short exon parts within HpaII-6 region were not sequenced, cannot be completely ruled out. It is likely, however, that both ox1l and ox1l2 map to the left of the HpaII-6 fragment. Schematic presentation of the heteroduplex studies performed by Kintzel et al. (1980) can be interpreted in favour of this hypothesis. In Southern blots presented by Macino et al. (1980) a weak hybridization of the ox1l probe to the HaeIII-1 band can be observed. If this is due to a specific hybridization, the position of ox1l would be on the boundary
of the HaeIII-3 and Hae-III-1a fragments. This would be also consistent with the data presented in this paper and with the observation that on Northern blots of mitochondrial RNA from *A. nidulans*, RNA classes of the same size hybridize both to labelled DNA fragments BamHI-2 and to HindIII-BamHI-1 (N. J. Pieniak, P. P. Stepień, E. Bartnik, in preparation). In the light of this study such hybridization would suggest that a part of the oxI gene is located within the HindIII-BamHI-1 fragment.

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REFERENCES


SEKWENCJA DNA OBSZARU PRZYLEGŁEGO DO GENÓW ox1 i ox2 Z MITOCHONDRIALNEGO GENOMU ASPERGILLUS NIDULANS

Streszczenie

Ustalono sekwencje nukleotydowe pięciu fragmentów pochodzących z obszaru HpaII-6 mitochondrialnego DNA Aspergillus nidulans. Stwierdzono występowanie otwartych ramek odczytu oraz stosunkowo wysoką zawartość par G-C, ale żadna z badanych sekwencji nie była homologiczna do części kodujących genów ox1 i ox2 z Saccharomyces cerevisiae.

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