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Short Sequence-Paper

cDNA sequence of subunit VIII of ubiquinol–cytochrome-*c* oxidoreductase from *Schizosaccharomyces pombe* ☆

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Abstract

We have cloned a cDNA coding for subunit VIII of the ubiquinol–cytochrome-*c* oxidoreductase of *Schizosaccharomyces pombe* by functional complementation of the null mutant in the *QCR8* gene of *Saccharomyces cerevisiae*. DNA sequence analysis reveals an open-reading frame of 276 bp encoding a 10.5 kDa protein with 51% amino acid sequence identity to its counterpart in *S. cerevisiae*.

Keywords: cDNA sequence; Ubiquinol–cytochrome-*c* oxidoreductase; Subunit VIII; (*Schizosaccharomyces pombe*)

The ubiquinol–cytochrome-*c* oxidoreductase of the respiratory chain, also referred to as the bc_1 complex or complex III, transfers electrons from ubiquinol to cytochrome *c* coupled to proton translocation. It consists of three or four subunits in prokaryotic organisms [1] up to eleven subunits in bovine heart [2]. For the *S. cerevisiae* bc_1 complex ten subunits have now been described [3,4]. Subunit VIII is an 11 kDa protein [5] and is thought to be closely associated to cytochrome *b*, the central subunit of the bc_1 complex [6]. It is proposed that cytochrome *b* together with subunits VII and VIII forms a subcomplex, as a first step in the assembly of the complex [6]. Subunit VIII has recently been shown to contribute to the quinone oxidation centre of the enzyme [5] in agreement with the identification of its bovine heart homologue as (part of the) binding site of azido-quinone analogues [7,8]. The gene encoding this protein is designated *QCR8*.

In order to study the function of subunit VIII and its separate domains within the bc_1 complex we have carried out DNA sequence analysis on the *QCR8* genes of other lower and higher eukaryotes. Here, we report the characterisation of this gene in the fission yeast *S. pombe*. This yeast has diverged extensively in evolution from the budding yeast *S. cerevisiae*. The two yeasts exhibit many differences at the molecular level as shown by the extensive sequence divergence of RNAs and protein sequences [9].

Sequences present in a cDNA library from *S. pombe* (gift by John Fikes) were inserted adjacent to the promoter of the *S. cerevisiae* alcohol dehydrogenase-1 gene, thus ensuring high level, constitutive expression [10]. The *S. cerevisiae* subunit VIII null mutant (DLL80) [11] was transformed with this library to create approximately 10,000 transformants. These colonies were replicated on ethanol/glycerol (EG) medium to screen for clones capable of restoring the EG-negative phenotype of the DLL80 strain. Two positive clones were identified and were found to be identical on subsequent partial DNA sequence analysis [12]. The clone was transferred into a suitable sequencing vector (pBluescript SK) using the *Hin*dIII restriction endonuclease. The cDNA was sequenced on both strands. The sequence shown in Fig. 1 is incomplete due to the occurrence of a *Hin*dIII site at position −44 relative to the initiation ATG. Additional restriction analysis showed that the complete cDNA is approximately 20 bp longer. No effort was undertaken to sequence this additional part of the cDNA.

The cDNA contains an open reading frame of 276 bp encoding a 92-amino-acid protein with a predicted molecular mass of 10.5 kDa. The ATG codon at position 1 is preceded by an in-frame stop codon (position −36). The predicted amino acid sequence, as aligned using the Clustal V computer program [13], displays 51% identity with

☆ The sequence data reported in this paper have been submitted to the Genbank Data Library under accession number U18794.

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-44  AAGCTTTGTAACGGCAATCATCAATATATTATTGTTTTAACAAA
  1 ATGGGTGGTGCTGCTGGAGGAAAAACTTATTTAGGATGGTGGGC
      M  G  G  A  A  G  G  K  T  Y  L  G  W  W  G
 46 CATCTAGGTGGACCTAAACAAAAAGGAATCATAACATATTCCCTT
      H  L  G  G  P  K  Q  K  G  I  I  T  Y  S  L
 91 TCCCCATTTCAACAGAGACCAATGGCCGGCTTCTTCAAAACTTCT
      S  P  F  Q  Q  R  P  M  A  G  F  F  K  T  S
136 ACGCAAAATATGTTTCGTCGCGTCATGACTGAAGGTCTATACGTA
      T  Q  N  M  F  R  R  V  M  T  E  G  L  Y  V
181 GCAATTCCGTTTGGAATAGCTTACTATATTTACTGTTGGGGGAAG
      A  I  P  F  G  I  A  Y  Y  I  Y  C  W  G  K
226 GAGCGTAATGAATTCCTCAATTCCAAACATGGAAGACACTTGGTG
      E  R  N  E  F  L  N  S  K  H  G  R  H  L  V
271 GAGGAATAATGATGAGAATGAAGTATTCATTTTGGTAGAAGGATT
      E  E  *
316 TACAAAATTCTTCCCTACCCTTTCCTTATTGGGTTTACAATTACT
361 ATCTCTCTCCTCTCATTATGCTTAGTATCAGAAATCTATATATCC
406 AACAAAATTACTTTGTGATATG
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Fig. 1. cDNA sequence and deduced amino acid sequence of the 10.5 kDa subunit VIII of the bc_1 complex from *S. pombe*. The stop codon is indicated (*). A poly(A) tail was found directly downstream of the 3′-terminus.

subunit VIII from *S. cerevisiae*. Two additional sequences are included in the multiple sequence alignment in Fig. 2. The *Kluyveromyces lactis* protein [14] is highly similar to *S. cerevisiae* subunit VIII (70% identical) but shows only 37% sequence identity with the *S. pombe* homologue. The fourth sequence is the 9.5 kDa subunit from bovine heart [15], the subunit that can be labelled by azido-Q derivatives [7,8]. Despite low similarity with the *S. cerevisiae* and *K. lactis* proteins (18% and 19% identity, respectively), it is generally regarded as the functional homologue of the yeast 11 kDa proteins [5]. The *S. pombe*

Fig. 2. Protein sequences of the subunit VIII homologues from *S. cerevisiae* (*S. c.*) [11], *K. lactis* (*K. l.*) [14], *S. pombe* (*S. p.*) (this study) and bovine heart (b. h.) [15] aligned using Clustal V [13]. Amino acids conserved in all four sequences (black background) and amino acids appearing in three sequences (grey background) are indicated. Gaps (indicated by dots) were introduced to optimise the alignment.

sequence displays 33% identity to the bovine heart protein, thereby confirming functional equivalence.

Inspection of the sequence shows that it can be roughly divided into 3 domains displaying different extents of conservation, the central domain being the most highly conserved. The N-terminal domain (residues 1–25) displays low overall identity. However, amongst the three yeast species it is the most highly conserved region. Since this part of the protein has not been studied in detail, it is not known whether this region is important for function of the protein.

The C-terminal domain (residues 64–92) displays a very low level of sequence conservation, even if only the three yeast species are considered. This may imply that this region is not so important for the function of the protein. In confirmation of this idea, Schoppink and co-workers have shown that a C-terminal deletion mutant of subunit VIII consisting of the first 66 residues fused to the sequence SCSQAC [1] coming from a stop-oligonucleotide was still able to partially restore the wild type phenotype when transformed into DLL80 [16]. They showed that the turnover of the bc_1 complex in this mutant was not changed and they concluded that this part of the protein is not necessary for activity, although the assembly of the complex was strongly affected.

The C-terminal domain contains two regions that have been submitted to a more detailed study. Hemrika and co-workers [17] have constructed a mutant in which the aromatic region 66-YWYWW-70 in the *S. cerevisiae* subunit VIII was replaced by non-aromatic residues. They show that especially the aromatic nature of residue 66 is of great importance for the assembly of a functional enzyme. As shown in Fig. 2, the *S. pombe* protein is the only one to lack an aromatic residue at this position. Four aromatic residues are however present at other positions in the same region (residues 65–70). Since 3–4 aromatic residues are present in all 4 sequences, presence rather than exact position may be more important.

The second region that was studied in detail consists of residues 69–73. These 5 residues (WWKNG) were replaced by a cysteine in a mutant obtained by random mutagenesis [5]. The mutant showed a reduced enzyme activity and it was concluded that this region of subunit VIII contributes to the Q_{out} binding domain. This region, however, is not conserved. Hemrika and co-workers [5] already discussed the possibility that the reduced enzyme activity is due to the introduction of a cysteine residue. The other three proteins all contain a cysteine residue in this region. It may be speculated that indeed this cysteine is important for the activity of the enzyme.

Fig. 3 shows hydrophilicity plots of all four sequences

[1] Due to the introduction of an extra cytosine at position 185 [5], the amino acid sequence differs from the sequence given by Schoppink et al. after the isoleucine at position 61 [16].

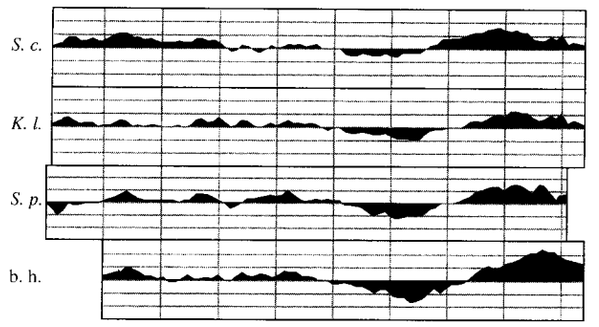

Fig. 3. Hydrophilicity plots of the four proteins according to the Kyte-Doolittle scale [18]. A window size of 19 was used. Hydrophilic regions are depicted by positive values, hydrophobic regions by negative values. The plots were grouped according to the multiple sequence alignment.

using the Kyte-Doolittle scale [18], grouped according to the multiple sequence alignment. In contrast to the relatively low sequence conservation, the secondary structure is highly conserved. This feature further strengthens the idea that these proteins are functional equivalents. We speculate that the regions in the protein containing a relatively high number of conserved amino acids are important for function of the protein, while other regions contribute to a specific structural feature.

In order to quantitate the extent of functional complementation of DLL80 by the *S. pombe QCR8* gene the protein-coding sequence was cloned under control of the *S. cerevisiae QCR8* promoter in the centromeric *E. coli–S. cerevisiae* shuttle vector pRS313 [19]. Fig. 4 shows a comparison with the same set-up using the *S. cervisiae*

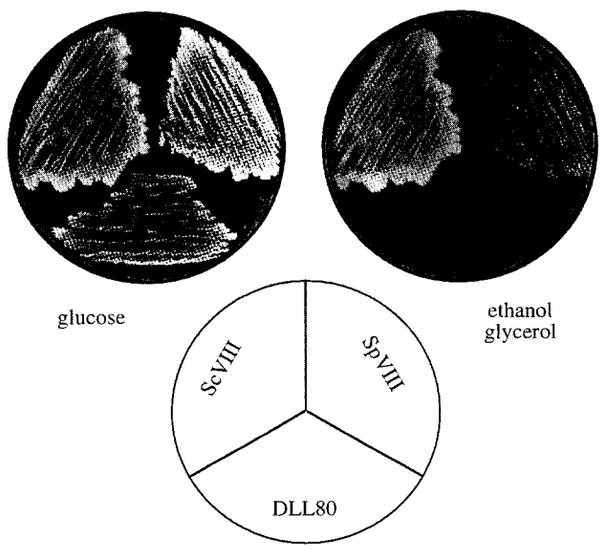

Fig. 4. Analysis of the growth characteristics of the *S. cerevisiae* subunit VIII null mutant (DLL80), DLL80 transformed with the *S. cerevisiae QCR8* gene on a single-copy plasmid (wild type) and when transformed with the *S. pombe QCR8* gene (SpVIII). Cells were first grown on medium containing glucose and subsequently replicated on ethanol/glycerol medium.

QCR8 gene (ScVIII). This indicates that the *S. pombe* subunit VIII can only partly suppress the disruption on the *S. cerevisiae QCR8* gene. Growth rate analysis in liquid medium containing lactate as the carbon source gave a generation time of 8.7 h for the SpVIII mutant. This corresponds to 65% of wild-type growth.

Transformation of DLL80 with the *K. lactis QCR8* gene does not alter the growth characteristics found with the wild-type strain [20]. Properties of this heterologous bc_1 complex are similar to those of the *S. cerevisiae* complex. The heterologous bc_1 complex consisting of *S. cerevisiae* subunits supplemented with subunit VIII from *S. pombe* is now under investigation to determine what causes the reduced growth rate in this mutant.

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