ELSEVIER

Biochimica et Biophysica Acta 1282 (1996) 179–181

BBA Biochimica et Biophysica Acta

Short sequence-paper

cDNAs from *Onchocerca* sp. encoding members of the MRS3/MRS4 class of mitochondrial solute carriers [1]

Julian Catmull [a,b], David J. Miller [a,*]

[a] *Department of Biochemistry and Molecular Biology, James Cook University of North Queensland, Townsville, Qld. 4811, Australia*
[b] *Department of Biochemistry, University of Iowa, Iowa City, IA 52242, USA*

Received 8 March 1996; revised 6 May 1996; accepted 8 May 1996

Abstract

cDNA clones from the parasitic nematodes *Onchocerca volvulus* and *Onchocerca gibsoni* encode homologs of the yeast proteins MRS3 and MRS4. Together with an uncharacterised ORF on chromosome III of *Caenorhabditis elegans*, these constitute a new class of proteins belonging to the mitochondrial solute carrier protein superfamily. So far, five other members of this protein family have been identified in *C. elegans*, but levels of identity between these and the *Onchocerca* proteins were considerably lower. Consideration of cysteine content and overall charge implies that the natural substrates of the nematode proteins are small ions.

Keywords: Nematode; Mitochondrial solute carrier; Transport protein; (*Onchocerca* sp.); (*C. elegans*)

The parasitic nematode *Onchocerca volvulus* is the causative agent of the human disease known as onchocerciasis or 'river blindness'. *Onchocerca gibsoni* is a common cattle parasite in northern Australia and, in terms of biochemical, antigenic and genetic criteria, is the best available animal model for the human pathogen. Whilst searching for genes expressed in specific larval stages of *Onchocerca* sp., we cloned a cDNA from uterine microfilariae of *O. gibsoni* [1] which was found to encode a protein homologous with the mitochondrial solute carrier superfamily. cDNA clone OG#7 had an insert of 1248 bp containing an open reading frame of 903 bp. The 301 residue hypothetical protein encoded by this open reading frame has a predicted molecular weight of 34200 and p*I* of 9.8. Comparison of the hypothetical protein encoded by OG#7 with the databases indicated significant matches with members of the mitochondrial solute carrier protein superfamily. This extensive family includes proteins which exchange ATP and ADP (the AAC proteins) or transport specific small ions across the inner mitochondrial membrane. The most significant matches with the OG7 protein were an uncharacterised *Caenorhabditis elegans* open reading frame (ORF) and the yeast proteins MRS3 and MRS4 (Fig. 1), which are thought to be mitochondrial cation transport proteins (MCTs) [2]. The *C. elegans* ORF is on cosmid W02B12, corresponding to a segment of chromosome III (Swinburne, unpublished data). The *O. gibsoni* clone was used as a hybridisation probe to identify and isolate the *O. volvulus* homolog of OG#7 (referred to here as OV#7) from cDNA libraries [3]. Table 1 summarises identity data between the two yeast and three nematode proteins.

This group of nematode proteins has key structural features which identify them as members of the mitochondrial solute translocator protein superfamily. Members of this family are all approximately 300 amino acid residue polypeptides, with a characteristic tripartite primary structure, first identified by Aquila et al. [4] in the ATP/ADP carrier. Each of the three domains contains two hydrophobic regions with the potential to form membrane-spanning α-helices, and around 16 to 18 positions are highly conserved in each of the domains, several of which flank the hydrophobic regions. Variability between members of this protein superfamily apparently increases towards the C-terminus, occurring particularly within the central hydrophilic region of the third repeat. Five other members of the mitochondrial solute transport protein superfamily have been identified in *C. elegans*, including a putative AAC

* Corresponding author. Fax: +61 77 251394; e-mail: david.miller@jcu.edu.au

[1] The *O. gibsoni* and *O. volvulus* sequence data have been submitted to the GenBank nucleotide sequence database under the respective accession numbers: U45997 and U45998.

PII S0005-2736(96)00088-0

Table 1
Identity matrix

	1	2	3	4	5
1. *O. volvulus*	–	82.33	60.92	34.91	33.44
2. *O. gibsoni*	90.74	–	57.00	32.20	31.67
3. *C. elegans*	66.30	62.22	–	34.91	33.00
4. MRS3 yeast	37.64	34.44	36.90	–	74.91
5. MRS4 yeast	36.23	34.44	34.77	76.38	–

Identities are given as percentages and were calculated from the alignment shown as Fig. 1. Figures above the diagonal represent overall identity; those below the diagonal were calculated after removal of the highly divergent domain (see Fig. 1).

protein, candidate phosphate and oxoglutarate/malate carriers, and two proteins of unknown function [5]. Matches between the *O. gibsoni* and *O. volvulus* proteins and these were much less significant.

Members of the MRS3/MRS4 class of proteins are relatively cysteine-rich, and there are major differences in the distribution of cysteine residues between the related nematode and yeast proteins. Of the 8–11 cysteine residues present in the nematode proteins, 6 or 7 occur in the central hydrophilic regions of the repeat units, suggesting that these segments of protein, which protrude into the mitochondrial matrix [6,7], are likely to be relatively rigid structures due to intramolecular disulfide bond formation. By contrast, only single cysteine residues in the yeast homologs are predicted to be in this compartment; 3 of 6 cys in MRS4 and 4 of 7 cys in MRS3 are likely to be located in membrane-spanning domains, compared with only 1 of 8 in the *C. elegans* ORF and 2 of 11 in both OG7 and OV7. These differences are sufficient to suggest different functions for the yeast and nematode proteins.

In general, AAC proteins have lower cysteine contents than do those proteins which transport smaller ions, and this may be related to differing flexibility requirements. Inhibitor studies indicate that, although the phosphate carrier (P_iC) and uncoupler protein (UCP) are able to bind ADP and ATP, the resulting protein-substrate analog complexes are unable to reorientate the binding centre to allow solute transfer across the membrane [8]. The higher rigidity in P_iC and UCP implied by the higher cysteine contents may be required to facilitate binding of the much smaller substrates (phosphate and hydrogen ions, respectively) of these transporters [7,8]. A number of other considerations imply that ADP and ATP are unlikely to be the natural substrates for the novel nematode proteins, including overall charge (the ATP/ADP carriers have higher overall charge than do OG7 and related proteins with smaller substrates) and the absence of specific sequence motifs (e.g., RRRMMM is conserved in all ATP/ADP carriers; [9]).

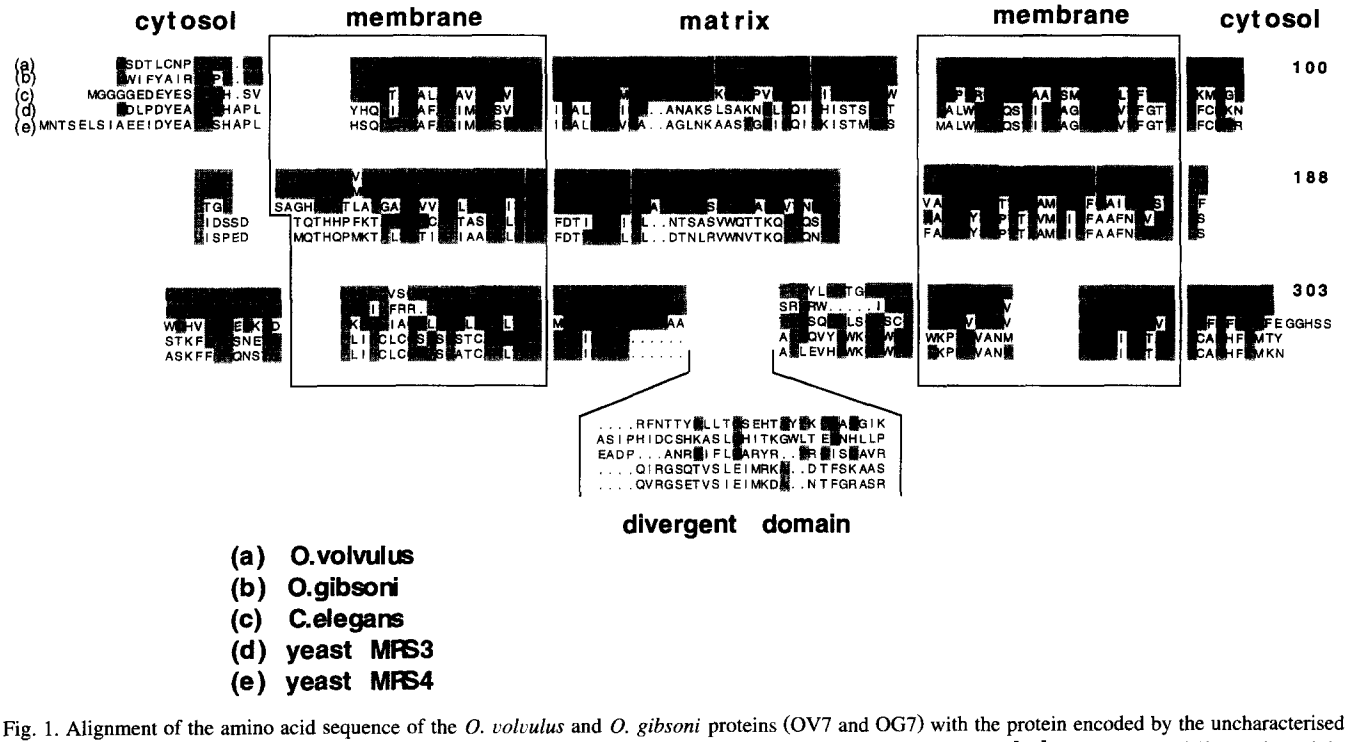

Fig. 1. Alignment of the amino acid sequence of the *O. volvulus* and *O. gibsoni* proteins (OV7 and OG7) with the protein encoded by the uncharacterised *C. elegans* ORF W02B12.9 and the yeast proteins MRS3 and MRS4. The alignment was generated using ClustalV [11] from the PAM250 protein weight matrix (gap weighting 10). Hydrophobicity profiling and comparison with related proteins [12] allowed the assignment of probable cellular locations of various protein segments. Numbers at the right of the figure refer to residues in the OV7 deduced amino acid sequence, and shading is used to emphasise identity with that sequence. Members of the mitochondrial solute carrier superfamily have a characteristic three domain structure, each domain (approximately 100 amino acid residues) containing two hydrophobic areas which are predicted to form membrane-spanning α-helices. The boxed regions in the figure indicate the six predicted hydrophobic segments.

Analysis of the literature suggests that the novel nematode proteins are likely to be located in the inner mitochondrial membrane, and that they may function in transport of a small ion. MRS3 and MRS4 are believed to be cation carriers, but there is only limited and indirect evidence to support this. These were identified as suppressors of some mitochondrial RNA splicing defects and, although deletion of either or both of these genes has no obvious phenotypic effect, a temperature-sensitive petite morphology results from their overexpression. Rationalising the phenotypic effects and probable location suggested that the yeast proteins function in transport of polyamines, Mg^{2+} or possibly another cation [2]. There is no significant sequence homology, and no obvious similarity in higher-order structure, between any known Mg^{2+} carrier and the newly-identified class of proteins. Two of the components of the *Escherichia coli* putrescine transport system are of similar size to mitochondrial solute carriers and, although having little sequence similarity, are predicted to each contain six membrane-spanning α-helices [10]. Clearly, if OG7 and its homologs do function in cation transport, there are likely to be novel mechanisms involved.

References

[1] Catmull, J., Zhang, D. and Miller, D.J. (1992) Int. J. Parasitol. 22, 685–687.
[2] Wiesenberger, G., Link, T.A., Von Ahsen, U., Waldherr, M. and Schweyen, R.J. (1991) J. Mol. Biol. 217, 23–37.
[3] Donelson, J.E., Duke, B.O.L., Moser, D., Zeng, W., Erondu, N.E., Lucius, R., Renz, A., Karam, M. and Flores, G.Z. (1988) Mol. Biochem. Parasitol. 31, 241–250.
[4] Aquila, H., Misra, D., Eulitz, M. and Klingenberg, M. (1982) Hoppe-Seyler's Z. Physiol. Chem. 363, 345–349.
[5] Runswick, M.J., Philippides, A., Luria, G. and Walker, J.E. (1994) DNA Seq. 4, 281–291.
[6] Klingenberg, M. (1989) Arch. Biochem. Biophys. 270, 1–14.
[7] Aquila, H., Link, H.A. and Klingenberg, M. (1987) FEBS Lett. 212, 1–9.
[8] Aquila, H., Link, H.A. and Klingenberg, M. (1985) EMBO J. 4, 2369–2376.
[9] Colleaux, L., Richard, G.-F., Thierry, A. and Dujon, B. (1992) Yeast 8, 325–336.
[10] Pistocchi, R., Kashiwagi, K., Miyamoto, S., Nukui, E., Sadakata, Y., Kobayashi, H. and Igarashi, K. (1993) J. Biol. Chem. 268, 146–152.
[11] Higgins, D.G., Bleasby, A.J. and Fuchs, R. (1992) Comp. Appl. Biosci. 8, 189–191.
[12] Walker, J.E. and Runswick, M.J. (1993) J. Bioenerg. Biomembr. 25, 435–446.