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The Mitochondrial Transport Protein Superfamily

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The ADP/ATP, phosphate, and oxoglutarate/malate carrier proteins found in the inner membranes of mitochondria, and the uncoupling protein from mitochondria in mammalian brown adipose tissue, belong to the same protein superfamily. Established members of this superfamily have polypeptide chains approximately 300 amino acids long that consist of three tandem related sequences of about 100 amino acids. The tandem repeats from the different proteins are interrelated, and probably have similar secondary structures. The common features of this superfamily are also present in nine proteins of unknown functions characterized by DNA sequencing in various species, most notably in *Caenorhabditis elegans* and *Saccharomyces cerevisiae*. The high level expression in *Escherichia coli* of the bovine oxoglutarate/malate carrier, and the reconstitution of active carrier from the expressed protein, offers encouragement that the identity of superfamily members of known sequence but unknown function may be uncovered by a similar route.

KEY WORDS: Mitochondria; transport proteins; sequences; bacterial expression.

INTRODUCTION

The sequences of the ADP/ATP, phosphate, and oxoglutarate/malate carriers from the inner membranes of mitochondria, and of the uncoupling protein from brown fat mitochondria, demonstrate that they are a superfamily of related proteins (Walker, 1992; Krämer and Palmieri, 1992). Their sequences are all about 300 amino acids long and they are characterized by the presence of a threefold sequence repeat of about 100 amino acids, first noted (Saraste and Walker, 1982) in the published sequence of the ADP/ATP carrier (Aquila *et al.*, 1982), and subsequently in the uncoupling protein (Aquila *et al.*, 1985), in the phosphate carrier (Runswick *et al.*, 1987), and in the oxoglutarate/malate carrier (Runswick *et al.*, 1990).

The relationships between the sequences of the superfamily members of known sequence is illustrated by pairwise comparisons of their sequences with the computer program DIAGON (Fig. 1). By looking along the strong central diagonal line (which represents the perfect relationship obtained by aligning a sequence with itself), other weaker parallel diagonal lines can be seen to persist through the diagram. These lines are about 100 amino acids apart, and they represent the weaker but significant relationships between tandem repeats in the same protein, and between tandem repeats in different superfamily members.

SECONDARY STRUCTURES OF SUPERFAMILY MEMBERS

Although the various sequence repeats in the superfamily are rather weakly related in some cases, it is a reasonable assumption that each of the related sequences will be folded into the same structural element or domain. The secondary structure of that element is not known, as no member of this protein superfamily has been induced to form either 2-dimensional or 3-dimensional crystals, but clues are present in their hydrophobic profiles, which are also conserved (Fig. 2). These profiles suggest that the second and third domains in the sequence could consist of two hydrophobic transmembrane α-helices joined by

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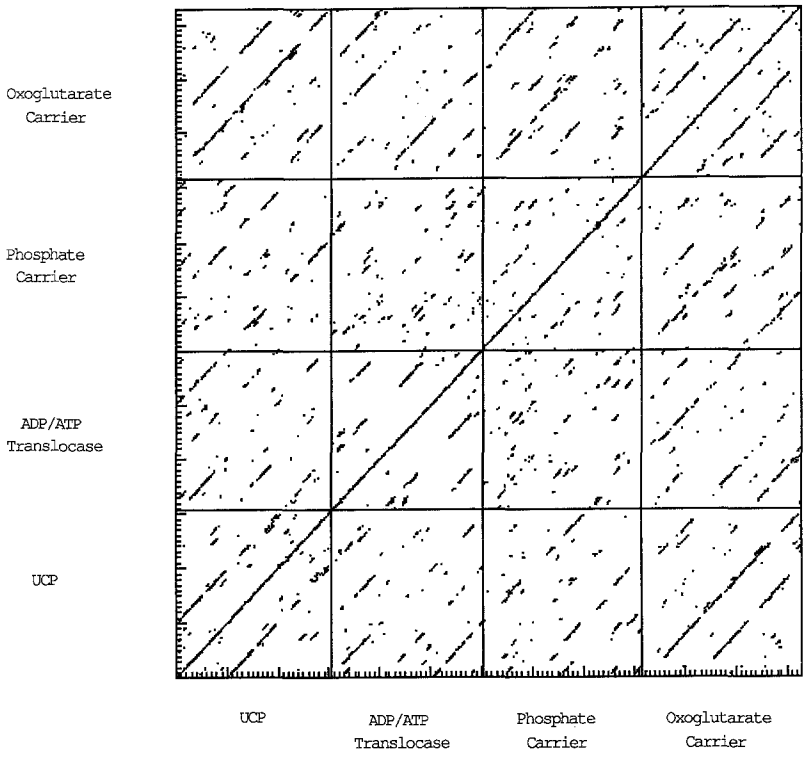


Fig. 1. Pairwise comparison of sequences of mitochondrial carrier proteins of known transport specificity. Bovine sequences were used, except for the uncoupling protein sequence (UCP), which is from hamster. The comparisons were made with the computer program DIAGON. Dots are recorded where the score exceeds a threshold of 270 in a window 25 amino acids long, using the MDM78 scoring matrix.

an extensive hydrophilic region, with each domain joined by a shorter hydrophilic region. This secondary structure is less evident in the first repeat, as the region that corresponds to the second helix of the first repeat tends to be less hydrophobic than in domains II and III. However, the requirement that the sequence repeats should have the same fold makes it likely that this region will also form a transmembrane α-helix, and that the same structural motif will be present in the first third of the polypeptide chain as in the second and third repeats. Other models not conforming to this principle have been proposed and are discussed elsewhere (Walker, 1992).

These considerations have led to a common structural model for the mitochondrial transport proteins in which the predominant features are six transmembrane α-helices and three extensive hydrophilic regions exposed to the aqueous environment (see Fig. 3). A prediction of this model is that the N- and C-termini lie on the same side of the inner mitochondrial membrane, and, in confirmation, it has been demonstrated that both extremities of the phosphate carrier are exposed on the cytoplasmic surface (Ferreira *et al.*, 1990; Capobianco *et al.*, 1991). The N-terminal region of the sixth proposed α-helix of the uncoupling protein is on the matrix side of the membrane (Miroux *et al.*, 1992), which is also consistent with the model. Other evidence suggests that the conformations of the large hydrophilic regions change during transmembrane transport, and so some topological experiments on the ADP/ATP carrier have given rise to data that seem at first sight to conflict with the model (Walker, 1992; Krämer and Palmieri, 1992).

Alignment of sequences from various species of each of the four carriers of known function (Fig. 4) show that conserved amino acids tend to cluster in the potential α-helices and immediately after them (at the beginning of loops A, B, and C). Alignment of the repeated sequences across eleven members of the superfamily (Fig. 5) shows that only a small number of amino acids are absolutely conserved. Most notable among these are conserved proline residues at the end of proposed helices I, III, and V, followed after an interval of one amino acid by an acidic residue

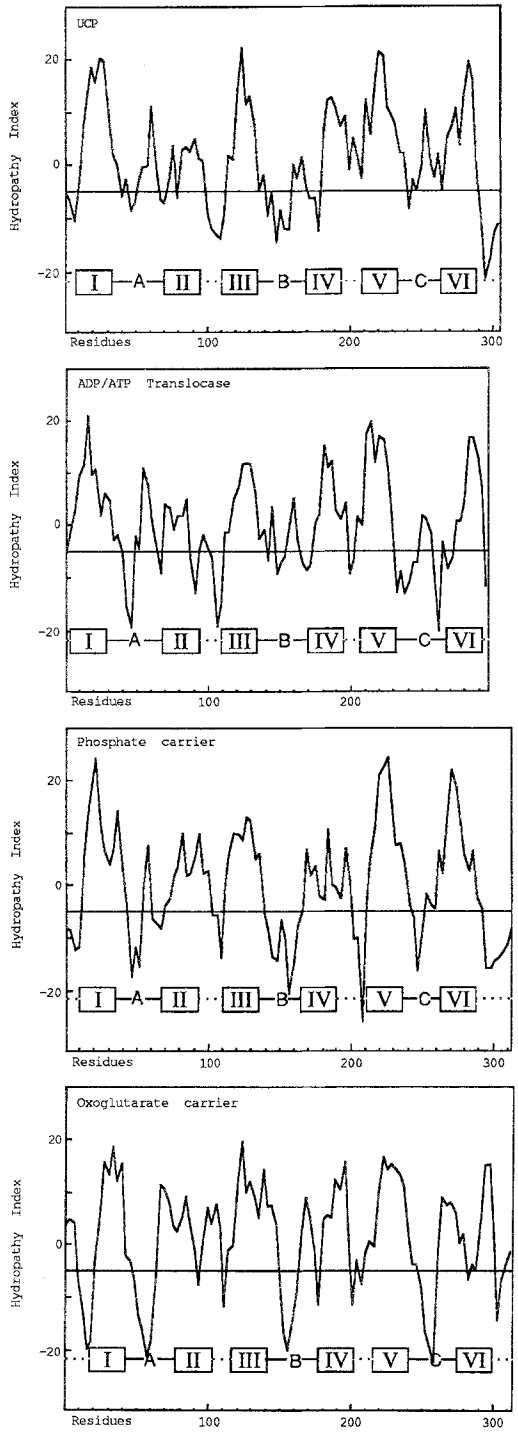


Fig. 2. Hydrophobic profiles of the four mitochondrial transport proteins of known transport specificity and sequence. The sequences used in the calculation are the same as those employed in Fig. 1. Beneath each profile, boxes I–IV indicate the positions of possible α-helical membrane spanning segments. Segments A, B, and C represent more hydrophilic regions. The three related domains present in each sequence consist of I–A–II, III–B–IV and V–C–VI.

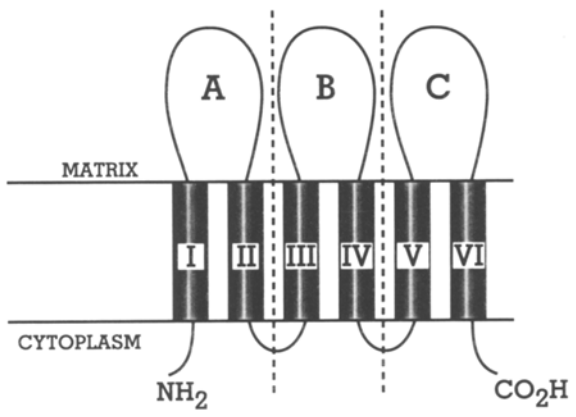


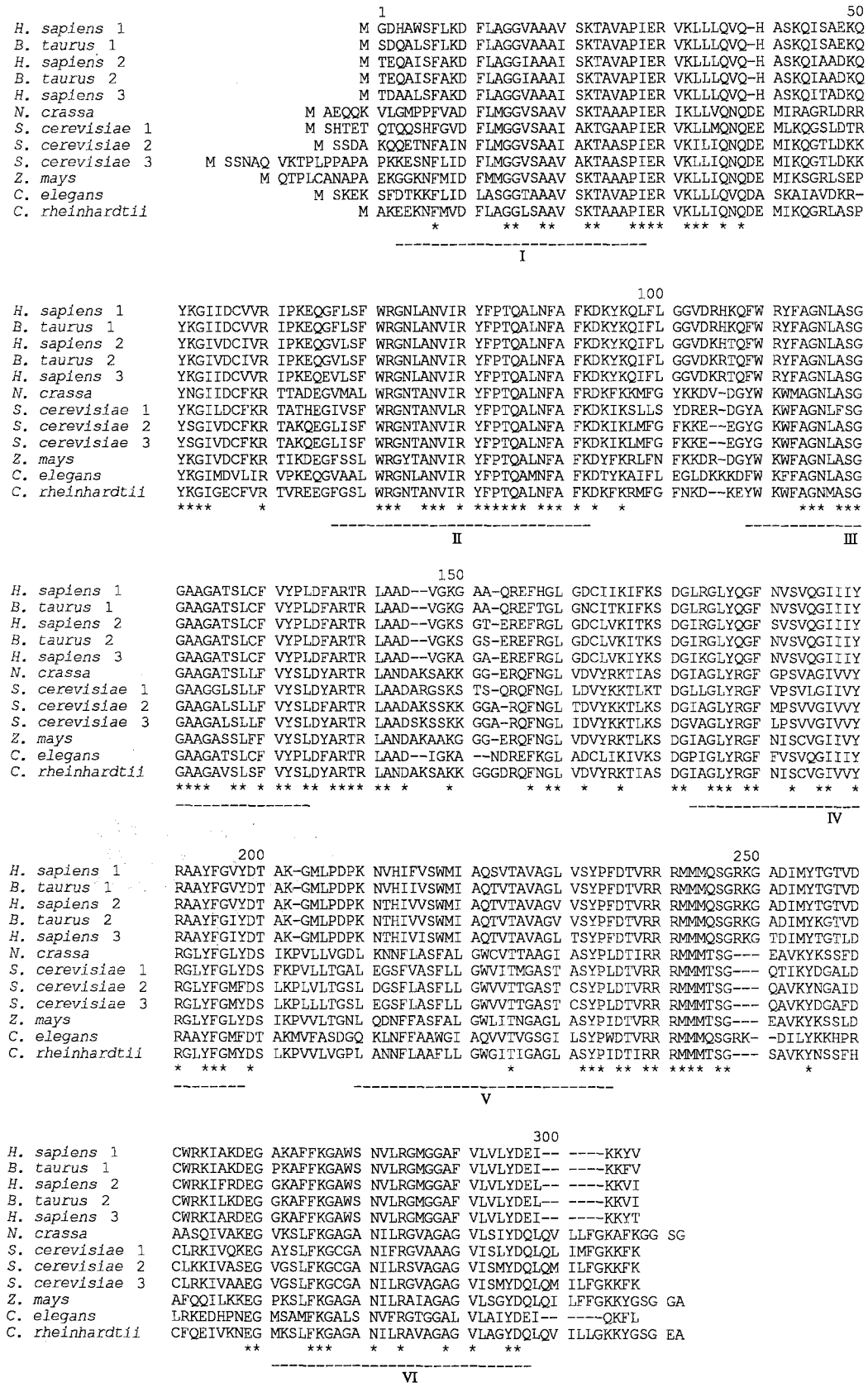
Fig. 3. A six-helix model of mitochondrial transport proteins. The dotted lines indicate that the proteins have three tandem structural repeats. Each repeat is proposed to consist of pairs of transmembrane α-helices (I and II, III and IV, V and VI) linked by extensive hydrophilic regions (A, B, and C). The cytoplasmic locations of the N- and C-terminal extremities in the phosphate carrier, of the N-terminal of the ADP/ATP carrier, and of the C-terminal of the uncoupling protein have been demonstrated (see text).

(Walker, 1992). It is likely that these amino acids are important in the overall structure of the members of the superfamily.

RECOGNITION OF NEW SUPERFAMILY MEMBERS

The sequence motif P.X.D/E-(20–30 acids)-D/E.G-(4 amino acids)-aromatic amino acid-K/R.G, which includes the conserved proline and the conserved acidic amino acid, is found in many of the repeated domains. This motif, the threefold 100 amino acid sequence repeats, the repetitive feature, and the hydrophobic profile together provides characteristic features for the recognition of new superfamily members of unknown biochemical function. They have been noted, for example, in two closely related proteins MRS3 and MRS4 from *Saccharomyces cerevisiae* (Weisenberger *et al.*, 1991), in a protein detected by screening a human cDNA expression library with a circulating autoantigen associated with Graves' disease (Zarrilli *et al.*, 1989), and in a protein encoded in a hypotrychous ciliated protozoan, *Oxytricha fallax* (Williams and Herrick, 1991; see Fig. 5 for sequences). The yeast MRS3 and MRS4 protein sequences are sufficiently similar to suggest that they may be isoforms of the same protein. They have both been demonstrated to be targetted to the inner mem-

a


H. sapiens 1
B. taurus 1
H. sapiens 2
B. taurus 2
H. sapiens 3
N. crassa
S. cerevisiae 1
S. cerevisiae 2
S. cerevisiae 3
Z. mays
C. elegans
C. rheinhardtii
I
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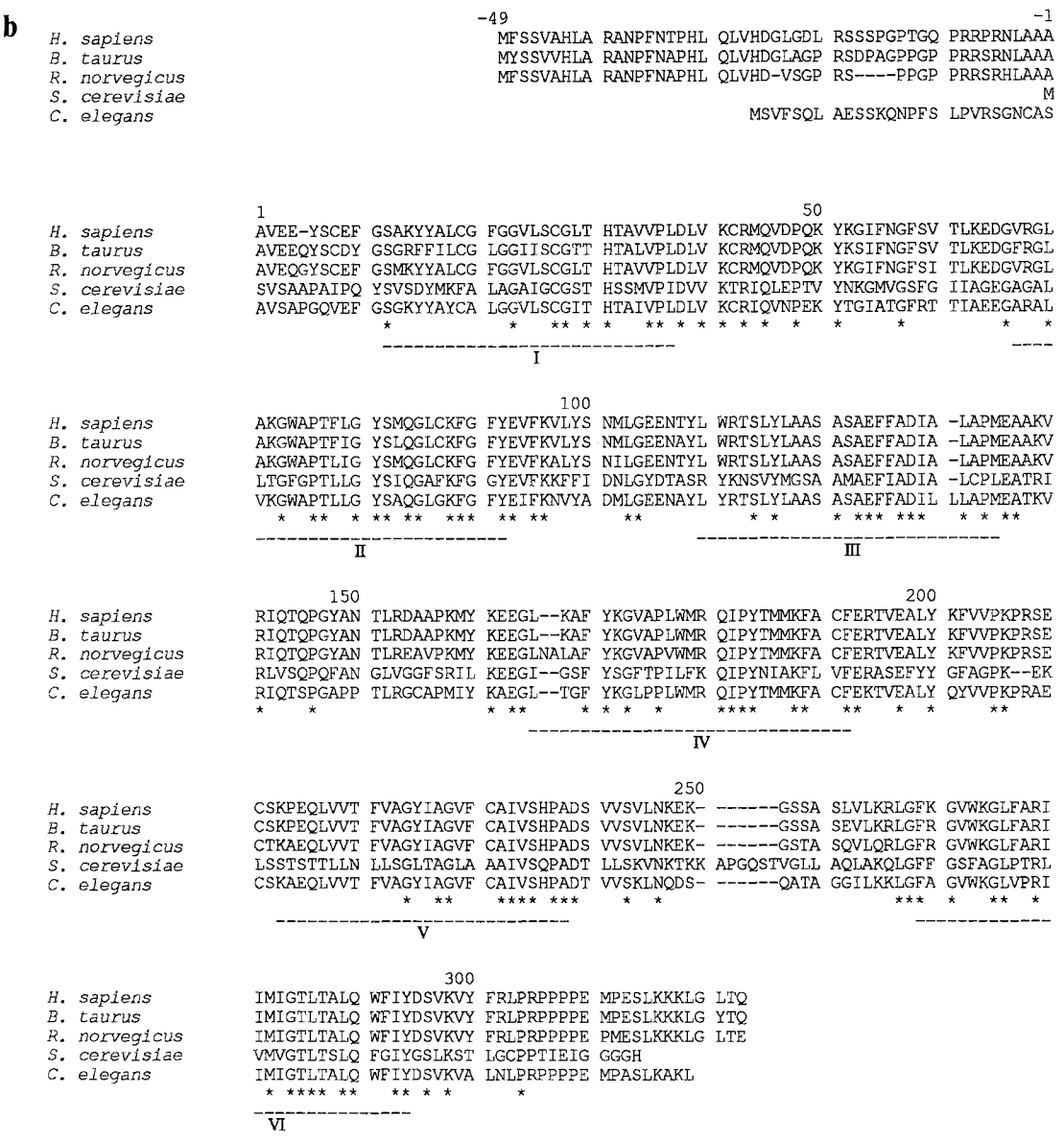

Fig. 4. Comparisons of sequences of each of the four mitochondrial carrier proteins of known function. Asterisks denote conserved residues, and I–VI are the positions of proposed α-helical segments. Parts (a)–(c) show alignments of the ADP/ATP, phosphate, and oxoglutarate carrier proteins, and part (d) of the uncoupling protein from brown fat. The sequences are taken from the EMBL database, and the following representative species are shown: man, *Homo sapiens*; cow, *Bos taurus*; the fungi, *Neurospora crassa* and *Saccharomyces cerevisiae*; maize, *Zea mays*; the nematode worms, *Caenorhabditis elegans* and *Chlamydomonas rheinhardtii*; rat, *Rattus norvegicus*; hamster, *Mesocricetus auratus*; rabbit, *Oryctolagus cuniculatus*; mouse, *Mus musculus*. Conserved amino acids are marked with asterisks, and I–VI show the positions of potential membrane-spanning α-helices.

brane of the organelle. Although their exact function is unknown, they influence RNA splicing in the organelle, perhaps by translocating cationic polyamines (Weisenberger *et al.*, 1991). The Graves' disease protein is expressed primarily in the thyroid gland, but transcripts of the bovine homologue are also present in other tissues, albeit at lower levels than are found in the thyroid (Fiermonte *et al.*, 1992). Its subcellular location and function are not known, nor are they for the protozoan protein. In the absence of further information, it is a reasonable assumption

c

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                1                                                   50
H. sapiens    M AATASAGAGG MDGKPRTSPK SVKFLFGGLA GMGATVFVQP LDLVKNRMQL SGEGAKTREY KTSFHALTSI
B. taurus     M AATASPGASG MDGKPRTSPK SVKFLFGGLA GMGATVFVQP LDLVKNRMQL SGEGAKTREY KTSFHALISI
C. elegans                   MAEDKTKR LGRWYFGGVA GAMAACCTHP LDLLKVQLQT QQQGKLTIGQ LS-----LKI
                                           *** * *  *     * *** *   *     *  *             *
                                ------------------------------
                                             I

                                             100
H. sapiens      LKAEGLRGIY TGLSAGLLRQ ATYTTTRLGI YTVLFERLTG ADGTPPGFLL KAVIGMTAGA TGAFVGTPAE
B. taurus       LRAEGLRGIY TGLSAGLLRQ ATYTTTRLGI YTVLFERLTG ADGTPPGFLL KAVIGMTAGA TGAFVGTPAE
C. elegans      YKNDGILAFY NGVSASVLRQ LTYSTTRFGI YETVKKQLPQ D--QPLPFYQ KALLAGFAGA CGGMVGTPGD
                            * *   ***  ** *** ** *      *       *  *   **     ***  *  ****
                     -----------------------------             ----------------------------
                                   II                                          III

                       150                                                    200
H. sapiens      VALIRMTADG RLPADQRRGY KNVFNALIRI TREEGVLTLW RGCIPTMARA VVVNAAQLAS YSQSKQFLLD
B. taurus       VALIRMTADG RLPVDQRRGY KNVFNALFRI VQEEGVPTLW RGCIPTMARA VVVNAAQLAS YSQSKQFLLD
C. elegans      LVNVRMQNDS KLPLEQRRNY KHALDGLVWI TREEGFMKMF NGATMATSRA ILMTIGQLSF YDQIKQTLIS
                      **  *   **  *** * *     *  *   ***              **       **   *   ** *
                                                      -----------------------------
                                                                     IV

                                                        250
H. sapiens      SGYFSDNILC HFCASMISGL VTTAASMPVD IAKTRIQNMR MIDGKPEYKN GLDVLFKVVR YEGFFSLWKG
B. taurus       SGYFSDNILC HFCASMISGL VTTAASMPVD IVKTRIQNMR MIDGKPEYKN GLDVLVKVVR YEGFFSLWKG
C. elegans      SGVAEDNLQT HFASSISAAS VATVMTQPLD VMKTR----- MMNAGPGEFK GILDCFMFTA KLGPMGFFKG
                **   **    **  *      * *    * *   ***      *    *     *            *     **
                   ----------------------------                                      -------
                                 V

                                    300
H. sapiens      FTPYYARLGP HTVLTFIFLE QMNKAYKRLF LSG
B. taurus       FTPYYARLGP HTVLTFIFLE QMNKAYKRLF LSG
C. elegans      FIPAWARLAP HTVLTFIFFE QLRLKFGYAP PVKAS
                * *  *** * ******** *
                --------------------
                      VI
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Fig. 4. Continued.

that these proteins will also prove to be transport proteins in the inner membranes of mitochondria.

NEW SUPERFAMILY MEMBERS DISCOVERED BY GENOMIC SEQUENCING

With the advance of genomic DNA sequencing projects, new members of the superfamily are beginning to emerge. For example, two new members have been discovered by genomic sequencing of chromosomes IX and XI of the yeast *Saccharomyces cerevisiae* (Colleaux *et al.*, 1992; B. G. Barrell, personal communication; see Fig. 5). Therefore, a total of three carrier proteins of unknown function have been revealed in *Saccharomyces cerevisiae* (MRS3 and MRS4 being considered as isoforms of the same protein). The sequences of the yeast ADP/ATP (Adrian *et al.*, 1986) and phosphate carriers (Phelps *et al.*, 1991) have also been described earlier, and there are at least three different isoforms of the ADP/ATP carrier (Lawson and Douglas, 1988; Gawaz *et al.*, 1990; Kolarov *et al.*, 1990; see below). Excluding the isoforms, therefore, a total of six different members of the superfamily have been sequenced in *S. cerevisiae*.

Extensive genomic sequencing is also being carried out in the nematode worm, *Caenorhabditis elegans*. Part of this project has been concerned with the random sequencing of the 5′ ends of cDNAs, and has generated 1,517 partial cDNA sequences with an average length of 388 base pairs (Waterston *et al.*, 1992). Among them are sequences encoding short stretches of protein sequences closely related to the mitochondrial ADP/ATP and phosphate carriers, and other clones encode unidentified members of the mitochondrial carrier superfamily (Runswick *et al.*, 1993). Subsequently, the partial cDNA sequences from *C. elegans* have been completed (Runswick *et al.*, 1993), and have been found to encode the nematode homologues of the mitochondrial ADP/ATP, phosphate, and oxoglutarate/malate carriers, and two new members of the superfamily of unknown transport specificity, referred to as carriers C1 and C2 (see Fig. 5). The partial cDNA clone for a third carrier of unknown function (C3) appears to have been lost during storage (see Runswick *et al.*, 1993). A fourth carrier of unknown function in *C. elegans* is encoded by another partial cDNA sequence (H. Wang, personal communication). Its sequence differs

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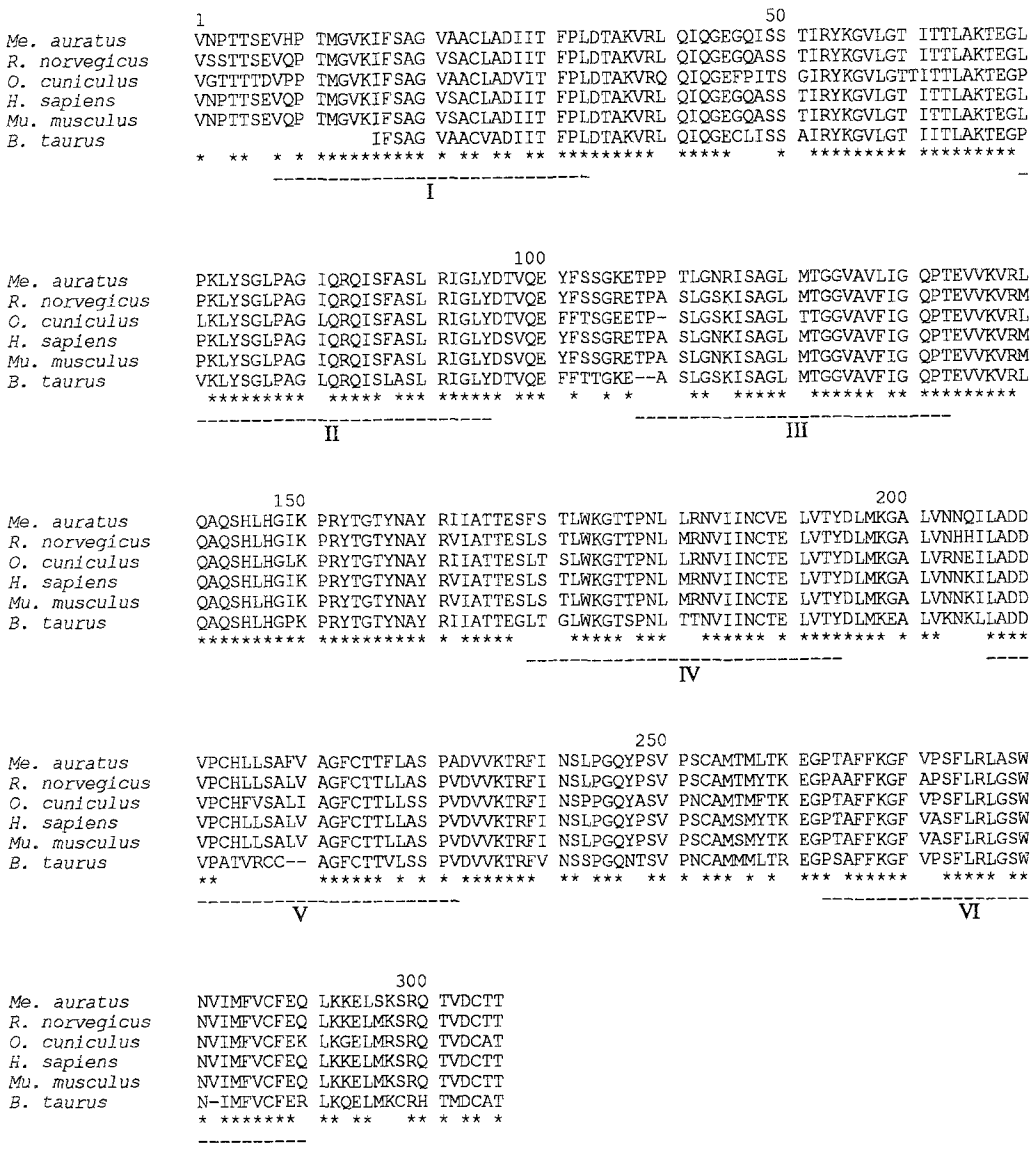

Fig. 4. Continued.

from those of C1, C2, and C3, and therefore, so far, seven members of the mitochondrial carrier superfamily have been found in *C. elegans*.

The four *C. elegans* carriers of unknown function differ extensively in sequence from those of the three carriers of unknown function from *S. cerevisiae*, from the Graves' disease carrier (Zarrilli *et al.*, 1989; Fiermonte *et al.*, 1992), and from the *O. fallax* carrier (Williams and Herrick, 1991). In consequence, each of these proteins is likely to have a different function. Therefore, there are currently 13 different members of the carrier superfamily (excluding isoforms). The transport properties of nine of the members remain to be established. As discussed in the following section, it is possible that some of them correspond to the unsequenced mitochondrial carriers of well-characterized transport specificity.

UNSEQUENCED MITOCHONDRIAL CARRIER PROTEINS OF KNOWN FUNCTION

In addition to the ADP/ATP, phosphate, and oxoglutarate/malate carriers, at least seven other mitochondrial carriers have been biochemically characterized in mammalian mitochondria (Walker, 1992;

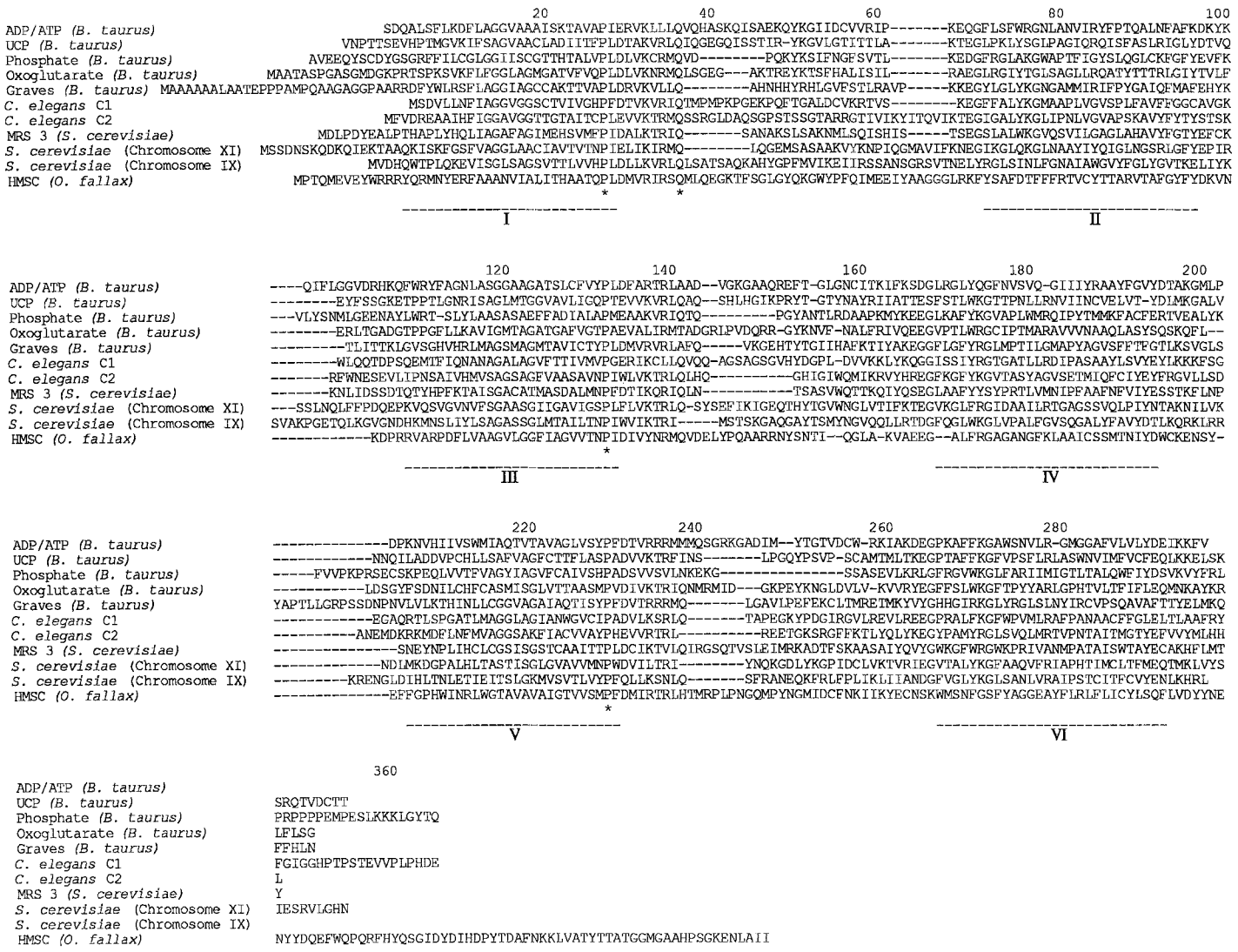

Fig. 5. Alignment of sequences of eleven members of carrier superfamily with different specificities. Beneath the bovine sequences of the four carriers of known function are placed the sequence of seven members of the carrier superfamily with unknown functions. They are the Graves' disease carrier, carriers C1 and C2 from *C. elegans*, MRS3 and carriers on chromosomes XI and IX from *S. cerevisiae*, and the homologue of the mitochondrial solute carrier (HMSC) from *Oxytricha fallax*. For other information, see legend to Fig. 4.

Krämer and Palmieri, 1992). For example, pyruvate and acyl-carnitine are exchanged for OH^- and carnitine, respectively, by their carriers. The aspartate/ glutamate carrier, and the oxoglutarate/ malate carrier, participate in the aspartate-malate shuttle, a device for reducing NAD^+ in the matrix, and there are carriers for citrate, glutamate, ornithine, and dicarboxylate. Some of them, such as those involved in oxidative phosphorylation, are found in mitochondria in all tissues, whereas others have a more limited tissue distribution; for example, the dicarboxylate and citrate carriers have little if any activity in heart mitochondria, but are particularly active in liver (LaNoue and Schoolwerth, 1984).

It is likely that these carriers belong to the carrier protein superfamily since they have features in common with the established members. For example, their molecular weights fall within the same range of values (28–32 kDa) as known superfamily members, and they also tend to copurify with them (Krämer and Palmieri, 1989, 1992). However, until their sequences are known, it will remain uncertain that they are members of the superfamily. A number of practical problems have made the determination of the sequences of some carriers a rather difficult undertaking. For example, many of them can be isolated in a homogeneous state only with difficulty (Krämer and Palmieri, 1989), and often in insufficient amounts for direct protein sequencing. In addition, their N-terminals may be modified, making it necessary to isolate peptides from digests.

Another possible strategy for cloning and sequencing these proteins is to use the DNA sequences of the nematode or yeast carriers of unknown function to amplify the homologues from bovine cDNA populations, and then to express the bovine cDNA sequences in *Escherichia coli*. The expressed proteins could then be isolated and used to prepare antibodies, and these antibodies could be employed in Western blot analyses to follow carrier activities during their isolation from mammalian mitochondria. It might also be possible to obtain the expressed carrier protein in an active form, and to study its transport properties directly. This latter approach could be applied directly to the nematode sequences and to other sequences encoding unidentified superfamily members, without the intermediate step of cloning the bovine homologues. Encouraging progress has been made toward expressing mitochondrial carrier proteins in bacteria, as described in the following section.

BACTERIAL EXPRESSION OF MITOCHONDRIAL CARRIER PROTEINS

In contrast to the success enjoyed in the bacterial expression of many eukaryotic globular proteins, the expression of eukaryotic membrane proteins in bacteria has proved to be a difficult undertaking. In all but two of the few examples where bacterial expression of a eukaryotic membrane protein has been observed, the levels of expression are far below those achieved for globular proteins (Schertler, 1992). The expression of 30–400 adrenergic receptor molecules per bacterial cell is typical (Strosberg, 1992). Various suggestions have been advanced to account for these difficulties, including the possibilities that the expressed proteins are toxic to the cell, or that they are unstable and are degraded rapidly in the bacterium, or that the codon usage in the eukaryotic gene is incompatible with a high level of expression in *E. coli*.

The exceptions are the membrane-bound bovine 17α-hydroxylase cytochrome P450 from microsomes (Barnes *et al.*, 1991), and the oxoglutarate/malate carrier protein from bovine mitochondria (Fiermonte *et al.*, 1993; see Fig. 6), both of which were expressed to a level of about 15 mg/liter of *E. coli* culture. Significant but lower levels of bacterial expression were observed with the bovine ADP/ATP carrier (Fiermonte *et al.*, 1993). Both the oxoglutarate/malate and the ADP/ATP carriers accumulated as insoluble inclusion bodies in the bacterial cytoplasm. Those containing the oxoglutarate/malate carrier have been solubilized in the presence of detergents, and have been reconstituted into phospholipid vesicles. The transport properties of this reconstituted carrier were shown to be very similar to those of the natural carrier in mitochondrial membranes (Fiermonte *et al.*, 1993).

It is not yet known whether other members of the superfamily can be expressed in bacteria in a similar way. The effect of the expression of the carrier on bacterial physiology may differ from one carrier to another; for example, the presence of a vector containing the oxoglutarate/malate carrier had no observable effect on bacterial growth, whereas the presence in the bacterium of a vector containing the ADP/ATP

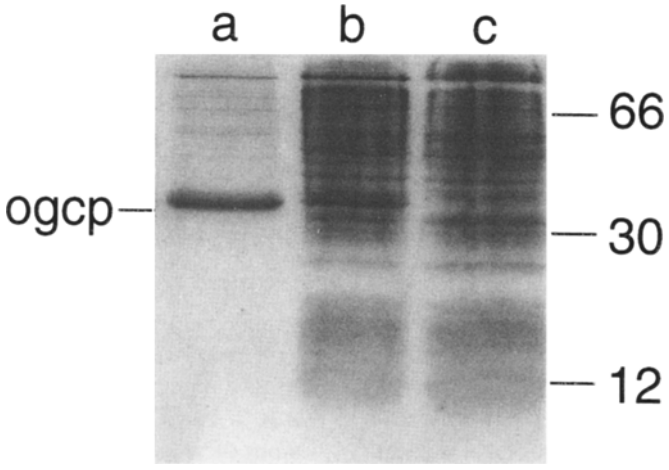

Fig. 6. Expression of the oxoglutarate carrier from bovine heart mitochondria in *E. coli*. (a) Purified inclusion bodies containing the oxoglutarate carrier (ogcp); (b) *E. coli* cells at the end of the induction period; (c) total proteins from a control bacterial culture containing the expression plasmid without an insert. Proteins were separated by denaturing polyacrylamide gel electrophoresis and stained with Coomassie blue dye. The oxoglutarate carrier protein was identified by N-terminal sequence analysis and by immunoblotting (Fiermonte *et al.*, 1993). The positions of molecular weight markers are shown at the right-hand side.

carrier slowed down bacterial growth considerably, even though expression of the carrier had not been induced (Fiermonte *et al.*, 1993).

A different problem was encountered in attempts to express the intact mitochondrial phosphate carrier in *E. coli*; it appeared that although the gene was transcribed, the mRNA was not translated at detectable levels. However, reasonable levels of expression were obtained by fusing fragments of the phosphate carrier lacking its C-terminal region to part of the α-subunit of ATP synthase (Ferreira and Pedersen, 1992). Fragments of the uncoupling protein from brown fat have been expressed in *E. coli* by fusing them to Mal E (Miroux *et al.*, 1992). If fragments of the uncharacterized carriers could be similarly expressed, they might prove to be useful in the production of antibodies that could aid in the characterization of the carriers' functions.

ISOFORMS OF MITOCHONDRIAL CARRIER PROTEINS

An additional complexity of the mitochondrial transporter superfamily is that there are isoforms of the ADP/ATP translocase. Two isoforms, named T1 and T2 (or alternatively ANT1 and ANT3), were first characterized in cows (Walker *et al.*, 1987; Powell *et al.*, 1989), and subsequently in humans (Neckelman *et al.*, 1987; Cozens *et al.*, 1989), in part with the aid of the bovine cDNAs (Houldsworth and Attardi, 1988). Subsequently, a third expressed human gene T3 (or ANT2) and at least seven pseudo-genes were also found (Battini *et al.*, 1987; Ku *et al.*, 1990). Three expressed genes (AAC1, AAC2, and AAC3) for the translocase are present in *Saccharomyces cerevisiae* (Lawson and Douglas, 1988; Kolarov *et al.*, 1990) and two genes have been characterized in *Zea mays* (Bathgate *et al.*, 1989). In contrast, it appears from Southern blots and from genomic DNA sequencing experiments that the uncoupling protein (Ricquier *et al.*, 1991) and the phosphate and oxoglutarate/malate carriers (Runswick *et al.*, 1990; Iacobazzi *et al.*, 1992) are each encoded by a single gene in mammals.

The functional significance of the mammalian isoforms remains a mystery. They are expressed differently in various tissues. The T1 isoform appears to be generally expressed in all bovine tissues that have been examined, but predominantly in heart tissue (Powell *et al.*, 1989). In human cell lines grown in tissue culture, T2 and T3 were expressed in equal amounts, but T1 expression was not observed (Lunardi and Attardi, 1991). During human muscle development, T2 was found at high levels in myo-

blasts and myotubes, but was decreased markedly in mature adult muscle; T3 was present in high amounts in myoblasts, was decreased in myotubes, and was barely detectable in adult muscle; T1 expression was found in adult muscle but not in myoblasts or myotubes. These differences in the expression of the three human genes may reflect the adaptation of muscle cells to changing energetic requirements during their development (Lunardi *et al.*, 1992). The human T2 and T3 genes are both found on the X chromosome (Chen *et al.*, 1990; Schiebel *et al.*, 1993) and T1 is on chromosome 4 (Li *et al.*, 1989), but T2 is in the pseudoautosomal region and escapes X-inactivation, whereas T3 is at Xq and undergoes X-inactivation (Schiebel *et al.*, 1993). The biological significance of this observation is not understood at present.

In the case of the yeast genes it was found by gene disruption that AAC1 was nonessential for growth on a respiratory carbon source. When AAC2 was disrupted, the mutant could not grow on glycerol as a carbon source, but the deletion was compensated by overexpression of AAC1. Expression of AAC3 occurs under anaerobic conditions (Kolarov *et al.*, 1990). The biochemical properties of the yeast AAC1 and AAC2 isoforms have been compared. The transport activity of AAC1 was 40% that of AAC2 (Gawaz *et al.*, 1990).

EXTENT OF THE MITOCHONDRIAL CARRIER SUPERFAMILY

All members of the mitochondrial carrier superfamily of characterized specificity are confined to mitochondria, and none of them has been detected in any other subcellular compartment. However, the possibility remains that members of the superfamily as yet uncharacterized may be in other cellular compartments. One possible candidate is an ATP transporter of unknown sequence found in the endoplasmic reticulum (Mayinger and Meyer, 1993). Chloroplasts and bacteria also have a very active transport of metabolites across their membranes. The outer membrane of chloroplasts is permeable to small molecules, but important metabolites are transported through the inner membrane by proteins. The major transport protein is the triose phosphate-3-phosphoglycerate/phosphate translocator, which provides the cell with fixed carbon in the form of triose phosphate. In its role of moving the main product of the organelle to the cytosol, the chloroplast phosphate transporter is analogous to the mitochondrial ADP/ATP translocase, and it also has a similar apparent molecular mass (29 kDa in spinach chloroplasts). However, its protein sequence does not contain the threefold repeat that is characteristic of the mitochondrial carriers, and appears to be unrelated to the mitochondrial superfamily (Flügge *et al.*, 1989). Nor are any of the three types of bacterial transport proteins (Lengeler *et al.*, 1990; Higgins *et al.*, 1990; Henderson, 1991) related in sequence to the mitochondrial carriers.

Maloney (1990) has pointed out that many membrane transport proteins have two common characteristics: they operate as a dimer, and the dimer has usually 12 transmembrane α-helices. The monomers may be separate chains as in the mitochondrial carriers, or within the same chain, as in the bacterial sugar transporters, for example. Therefore, it is proposed that common structural and functional principles may unify this wide range of transport proteins. The validity of this suggestion will be assessed when more structural evidence becomes available.

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REFERENCES

Adrian, G. S., McCammon, M. T., Montgomery, D. L., and Douglas, M. G. (1986). *Mol. Cell. Biol.* **6**, 626–634.

Aquila, H., Misra, D., Eulitz, M., and Klingenberg, M. (1982). *Hoppe-Seyler's Z. Physiol. Chem.* **363**, 345–349.

Aquila, H., Link, T. A., and Klingenberg, M. (1985). *EMBO J.* **4**, 2369–2376.

Barnes, H. J., Arlotto, M. P., and Waterman, M. R. (1991). *Proc. Natl. Acad. Sci. USA* **88**, 5597–5601.

Bathgate, B., Baker, A., and Leaver, C. J. (1989). *Eur. J. Biochem.* **183**, 303–310.

Battini, R., Ferrari, S., Kaczmarek, L., Calabretta, B., Chen, S. T., and Baserga, R. (1987). *J. Biol. Chem.* **262**, 4355–4359.

Capobianco, L., Brandolin, G., and Palmieri, F. (1991). *Biochemistry* **30**, 4963–4969.

Chen, S. T., Chang, C. D., Huebner, K., Ku, D. H., McFarland, M., DeRiel, J. K., Baserga, R., and Wurzel, J. (1990). *Som. Cell Mol. Genet.* **16**, 143–149.

Colleaux, L., Richard, G. F., Thierry, A., and Dujon, B. (1992). *Yeast* **8**, 325–326.

Cozens, A. L., Runswick, M. J., and Walker, J. E. (1989). *J. Mol. Biol.* **206**, 261–280.

Ferreira, G. C., Pratt, R. D., and Pedersen, P. L. (1990). *J. Biol. Chem.* **265**, 21202–21206.

Ferreira, G. C., and Pedersen, P. L. (1992). *J. Biol. Chem.* **267**, 5460–5466.

Fiermonte, G., Runswick, M. J., Walker, J. E., and Palmieri, F. (1992). *DNA Seq.* **3**, 71–78.
Fiermonte, G., Walker, J. E., and Palmieri, F. (1993). *Biochem. J.*, **294**, 293–299.
Flügge, U. I., Fisher, K., Gross, A., Sebald, W., Lottspeich, F., and Eckershorn, C. (1989). *EMBO J.* **8**, 39–46.
Gawaz, M., Douglas, M. G., and Klingenberg, M. (1990). *J. Biol. Chem.* **265**, 14202–14208.
Henderson, P. J. F. (1991). *Curr. Opinion Struct. Biol.* **1**, 590–601.
Higgins, C. F., Hyde, S. C., Mimmack, M. M., Gileadi, U., Gill, D. R., and Gallagher, M. P. (1990). *J. Bioenerg. Biomembr.* **22**, 571–592.
Houldsworth, J., and Attardi, G. (1988). *Proc. Natl. Acad. Sci. USA* **85**, 377–381.
Iacobazzi, V., Palmieri, F., Runswick, M. J., and Walker, J. E. (1992). *DNA Seq.* **3**, 79–88.
Kolarov, J., Kolarova, N., and Nelson, N. (1990). *J. Biol. Chem.* **265**, 12711–12716.
Krämer, R., and Palmieri, F. (1989). *Biochim. Biophys. Acta* **974**, 1–23.
Krämer, R., and Palmieri, F. (1992). In *Molecular Mechanisms in Bioenergetics* (Ernster, L., ed.), Elsevier Science Publishers, Amsterdam, pp. 359–384.
Ku, D., Kagan, J., Chen, S., Chang, C., Baserga, R., and Wurzell, J. (1990). *J. Biol. Chem.* **265**, 16060–16063.
LaNoue, K. F., and Schoolwerth, A. C. (1984). In *Bioenergetics* (Ernster, L., ed.), Elsevier Science Publishers, Amsterdam, pp. 221–268.
Lawson, J. E., and Douglas, M. G. (1988). *J. Biol. Chem.* **263**, 14812–14818.
Lengeler, J. W., Titgemayer, F., Vogler, A. P., and Wöhrl, B. M. (1990). *Philos. Trans. R. Soc. London B* **326**, 489–504.
Li, K., Warner, C., Hodge, J. A., Minoshima, S., Kudoh, J., Fukuyama, R., Maekawa, M., Shimizu, Y., Shimizu, N., and Wallace, D. C. (1989). *J. Biol. Chem.* **264**, 13998–14004.
Lunardi, J., and Attardi, G. (1991). *J. Biol. Chem.* **266**, 16534–16540.
Lunardi, J., Hurko, O., Engel, W. K., and Attardi, G. (1992). *J. Biol. Chem.* **267**, 15267–15270.
Maloney, P. C. (1990). *Res. Microbiol.* **141**, 374–383.
Mayinger, P., and Meyer, D. I. (1993). *EMBO J.* **12**, 659–666.
Miroux, B., Casteilla, L., Klaus, S., Raimbault, S., Grandin, S., Clément, J. M., Ricquier, D., and Bouillaud, F. (1992). *J. Biol. Chem.* **267**, 13603–13609.
Neckelmann, N., Li, K., Wade, R. P., Shuster, R., and Wallace, D. C. (1987). *Proc. Natl. Acad. Sci. USA* **84**, 7580–7584.
Phelps, A., Schobert, C. T., and Wohlrab, H. (1991). *Biochemistry* **30**, 248–252.
Powell, S. J., Medd, S. M., Runswick, M. J., and Walker, J. E. (1989). *Biochemistry* **28**, 866–873.
Ricquier, D., Casteilla, L., and Bouillard, F. (1991). *FASEB J.* **5**, 2237–2242.
Runswick, M. J., Powell, S. J., Nyren, P., and Walker, J. E. (1987). *EMBO J.* **6**, 1367–1373.
Runswick, M. J., Walker, J. E., Bisaccia, F., Iacobazzi, V., and Palmieri, F. (1990). *Biochemistry* **29**, 11033–11040.
Runswick, M. J., Philippedes, A., Lauria, G., and Walker, J. E. (1993). In preparation.
Saraste, M., and Walker, J. E. (1982). *FEBS Lett.* **144**, 250–254.
Schertler, G. F. X. (1992). *Curr. Opinion Struct. Biol.* **2**, 534–544.
Schiebel, K., Weiss, B., Wöhrle, D., and Rappold, G. (1993). *Nature Genet.* **3**, 82–87.
Strosberg, A. D. (1992). *Mol. Neurobiol.* **4**, 211–250.
Walker, J. E. (1992). *Curr. Opinion Struct. Biol.* **2**, 519–526.
Walker, J. E., Cozens, A. L., Dyer, M. R., Fearnley, I. M., Powell, S. J., and Runswick, M. J. (1987). *Chem. Scr. B* **27**, 97–105.
Waterston, R., Martin, C., Craxton, M., Huynh, C., Coulson, A., Hillier, L., Durbin, R., Green, P., Showkeen, R., Halloran, N., Metzstein, M., Hawkins, T., Wilson, R., Berks, M., Du, Z., Thomas, K., Thierry-Mieg, J., and Sulston, J. (1992). *Nature Genet.* **1**, 114–122.
Weisenberger, G., Link, T. A., van Ahsen, U., Waldherr, M., and Schweyen, R. J. (1991). *J. Mol. Biol.* **217**, 23–37.
Williams, K. R., and Herrick, G. (1991). *Nucleic Acids Res.* **19**, 4717–4724.
Zarrilli, R., Oates, E. L., McBride, O. W., Lerman, M. I., Chan, J. Y., Santisteban, P., Ursini, M. V., Notkins, A. L., and Kohn, L. D. (1989). *Mol. Endocrinol.* **3**, 1498–1508.