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A leucine motif in the amino acid sequence of subunit 9 of the mitochondrial ATPase, and other hydrophobic membrane proteins, that is highly conserved by editing

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Abstract Subunit 9 of the mitochondrial ATPase, but also other hydrophobic mitochondrially encoded proteins, contains a high frequency of the leucine motif, -Leu-X_9-Leu-, which is highly conserved through RNA editing. The leucine motif may provide specific recognition sites between membrane-spanning domains of the F_0-ATPase and between other hydrophobic subunits during the assembly of multienzyme complexes in the inner mitochondrial membrane.

Key words: Amino acid sequence analysis; F_0F_1-ATPase; Helix–helix interaction; Mitochondrion; Quaternary protein structure; RNA editing

1. Introduction

Four of the five mitochondrial respiratory complexes (ATP synthase, NADH dehydrogenase, bc_1 complex and cytochrome oxidase) are assembled both from subunits encoded and synthesized within the mitochondria and from nuclear-encoded subunits imported from the cytoplasm. Little is known about the molecular mechanism of assembly of these mitochondrial complexes.

A leucine zipper motif mediates dimerization of a number of different proteins, including a class of DNA-binding proteins [1–7]. The leucine zipper motif is a sequence of leucine residues spaced every seventh amino acid residue along an α-helix [1]. As a result, all the leucine residues of the motif are placed along one side of the α-helix. According to the zipper model, the leucine side chains extending from the leucine repeat are able to interdigitate with leucine side chains of a second polypeptide which contains the same motif [1,2]. Therefore, the hydrophobic surfaces of two leucine repeating sequences of the protein molecules might interact to form homo- or heterodimers [1–3]. The leucine zipper model for protein dimerization is strongly supported by experimental data which shows that replacement of leucine at any position of the motif by site-directed mutagenesis destabilises dimer formation as well as the DNA-binding potential of the protein [2].

In addition to DNA-binding proteins, leucine zipper motifs have recently been reported in photosystem I reaction centre polypeptides of higher plants [5], glucose-transporter glycoproteins [6] and voltage-gated Ca^{2+} channels [7]. It has been suggested [5] that the leucine zipper motif is a common mechanism for mediating protein dimer formation in a wide range of different systems when specific interactions are required.

In the present communication we report the presence of a new leucine motif formed by leucine repeats (-Leu-X_9-Leu-) in subunits 6 and 9 of ATP synthase, subunits 3 and 4 of NADH dehydrogenase, and subunit II of cytochrome oxidase in mitochondria of several plant species, and we suggest that this new leucine motif is also involved in the assembly of the hydrophobic part of membrane-bound multisubunit complexes.

2. Materials and methods

Amino acid sequences of mitochondrially encoded polypeptides (subunits 6 and 9 of ATP synthase, subunits 3 and 4 of NADH dehydrogenase, and subunit II of cytochrome oxidase) before and after editing of the appropriate mRNAs are taken from [8–15]. The sequences of the F_1-ATPase synthase α- and β-subunits are from [16,17]. The sequence of ATP synthase subunit 9 from yeast and bovine is taken from [18,19]. Other sequences are from the Swissprot database (release 17) and were analyzed by the program PSEARCH of the PCGENE package.

3. Results and discussion

An analysis of the distance between neighbouring leucine residues in subunits of the mitochondrial ATPase is shown in Fig. 1. The sequence -Leu-X_9-Leu- is found with a very high frequency compared with other -Leu-X_n-Leu- in the two hydrophobic subunits 6 and 9 of the F_0-ATPase (Fig. 1A,B) but with a low frequency in the hydrophilic subunits α and β of the F_1-ATPase (Fig. 1C,D).

In subunit 9 the leucine residues appear in a very regular pattern of five consecutive -Leu-X_9-Leu- which are found in most of the sequences available (Fig. 2) [20,21]. This pattern is maintained by RNA editing to a very significant extent (Fig. 2, Table 1).

Most of the leucine motifs in subunit 9 of the ATPase appear in the membrane-spanning regions of the subunit [8,18]. Since leucines 10 amino acids apart will appear on the same side of an α-helical wheel, this suggests that the leucine motif is perhaps involved in subunit–subunit interactions through intramembrane helix–helix association by analogy with the leucine zipper motif [1,2].

The -Leu-X_9-Leu- motif is found in other mitochondrially encoded subunits of the respiratory complexes and here it is also conserved through RNA editing (Table 1). A significant proportion of all editing events in subunits of the NADH dehydrogenase, cytochrome *c* oxidase and ATPase are involved in

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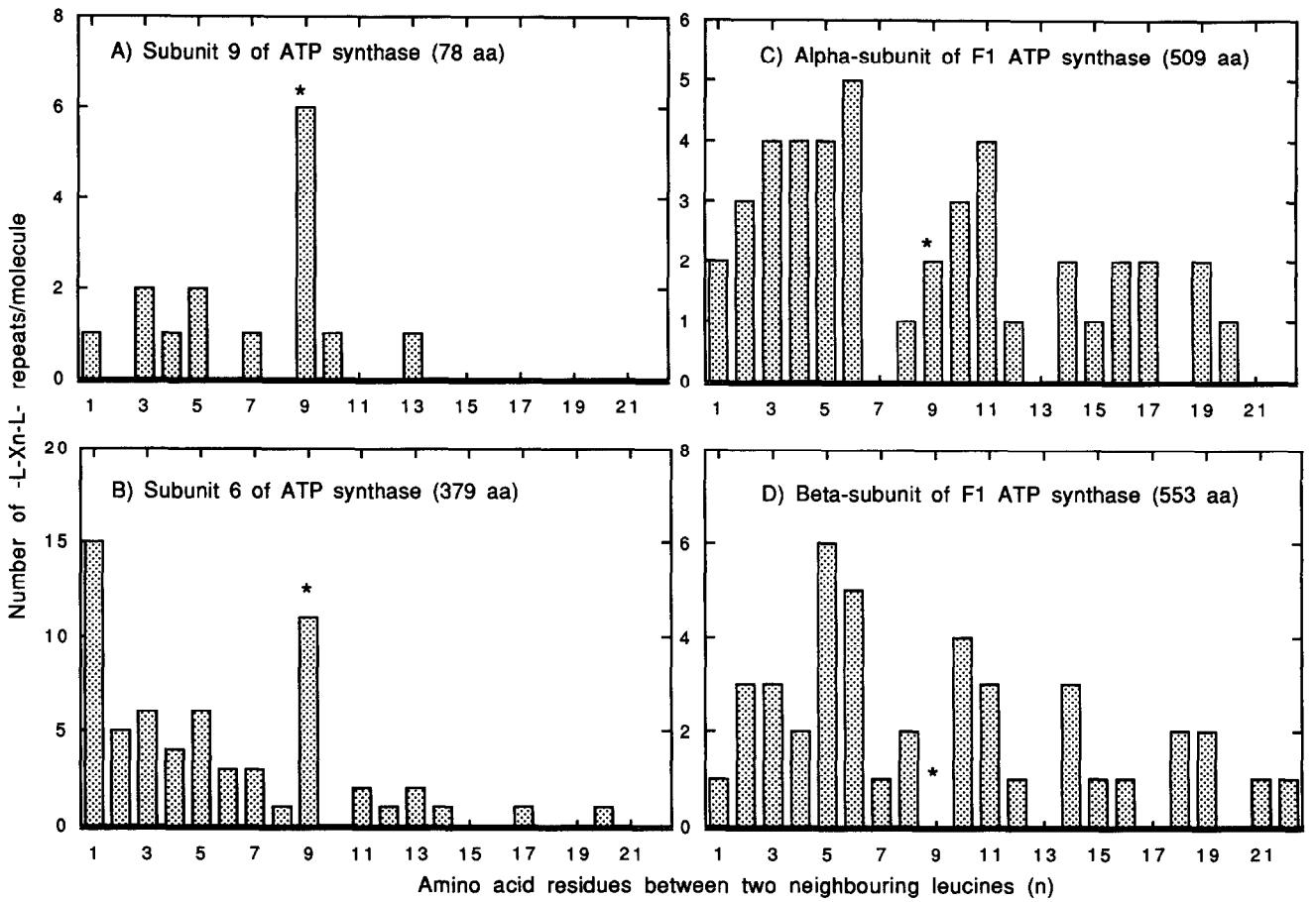

Fig. 1. The frequency of the -Leu-X_n-Leu- motif in different subunits of mitochondrial ATPase. (A) Subunit 9 of the F_0 (*Oenothera*, cDNA sequence) [9]; (B) subunit 6 of the F_0 (sorghum, cDNA sequence) [12]; (C) α-subunit of the F_1 (*Nicotiana plumbaginifolia*, DNA sequence) [16]; (D) β-subunit of the F_1 (maize, DNA sequence) [17]. *n*, number of amino acids between two neighbouring leucine residues.

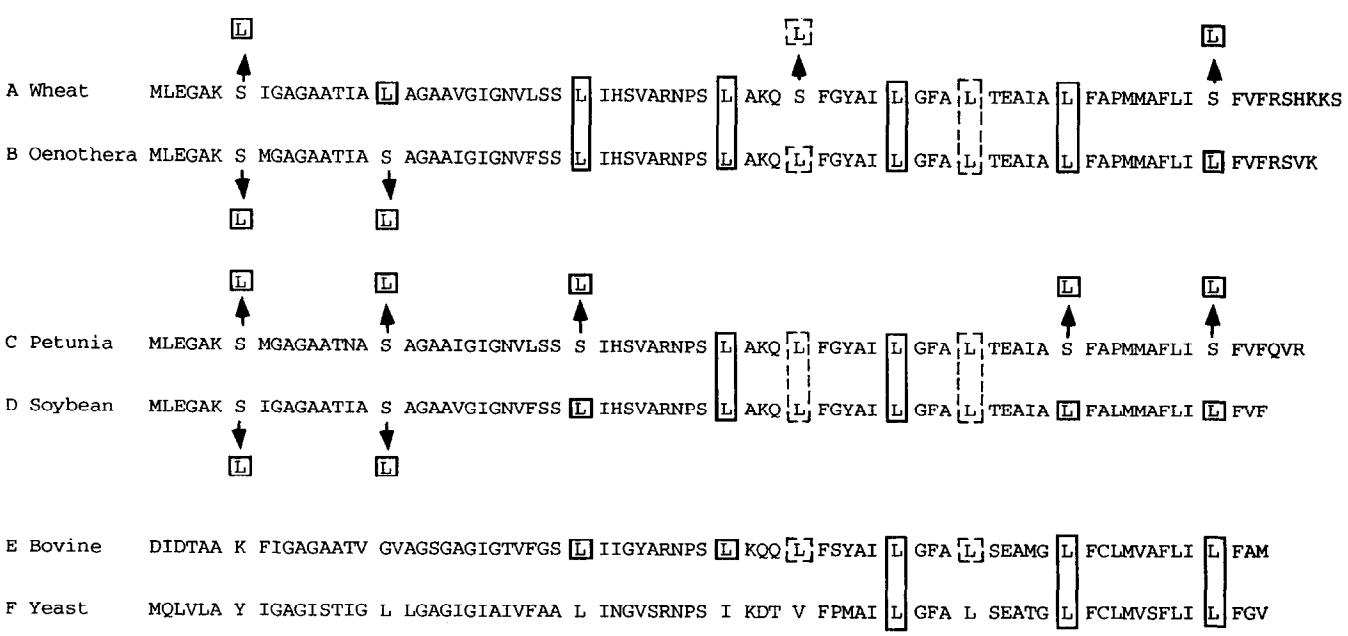

Fig. 2. Leucine motif in amino acid sequences of subunit 9 of ATPase from mitochondria of wheat (A), *Oenothera* (B), *Petunia* (C), soybean (D), before and after editing the corresponding mRNA. Sequences of subunit 9 of bovine ATPase (nuclear-encoded) (E) and yeast ATPase (mitochondrially encoded) (F) are given for comparison. Leucine residues which constitute copies of the repeating sequence appear within boxes (solid lines). Boxes with broken lines show an overlapping leucine repeat. Leucine residues appearing as a result of editing of mRNA (based on the cDNA sequence) are shown on a separate line and indicated by arrows. The sequences are from A [8], B [9], C [10], D [11], E [19] and F [18].

Table 1
The effects of mRNA editing on the presence of leucine repeat in proteins of plant mitochondria

Protein	Leucine repeat, no.		Total number of editing events	Editing events	Ref.
	Before editing	After editing			
ATP9					
Oenothera	5	6	3 (67%)		see Fig. 2
Petunia	2	6	7 (71%)		
Soybean	5	6	2 (100%)		
Wheat	3	6	5 (60%)		
ATP6 (sorghum)	7	11	15 (27%)	195 P→L 302 S→L 316 S→L 359 S→L	[12]
ND3 (*Oenothera*)	2	5	12 (25%)	72 P→L 89 P→L	[15]
ND4 (wheat)	8	15	22 (36%)	15 P→L 26 P→L 36 P→L 106 S→L 139 P→L 150 P→L 326 P→L 478 P→L	[14]
COX2 (*Oenothera*)	1	3	17 (12%)	93 P→L 186 P→L	[13]

Amino acid sequences of plant mitochondrial proteins before and after editing of appropriate mRNAs are taken from [8–15]. The relative activity of leucine repeat formation which was calculated as a percentage of amino acid changes per molecule is shown in parentheses.

creating (conserving) the leucine motif, mostly through serine-to-leucine or proline-to-leucine changes (Table 1).

The leucine motif is, in general, much more common in membrane proteins than in non-membrane proteins. However, it is more common in mitochondria than in chloroplasts (Table 2) which may reflect their different evolutionary origin. An analysis of the frequency of the leucine motif in the ATPase from different sources shows that it is very common in the mitochondrial F_0, much more than the average for mitochondrial proteins, and less common in F_0 of chloroplasts and prokaryotes (results not shown).

We conclude the following: (i) the -Leu-X_9-Leu- motif is characteristic for membrane proteins particularly in mitochondria; (ii) the leucine motif is conserved through RNA editing in mitochondrially encoded subunits; (iii) the new leucine motif may function in a similar way as the leucine zipper by providing specific recognition sites between membrane-spanning domains of hydrophobic subunits during the assembly of multisubunit complexes.

Table 2
Specific content of the -Leu-X_9-Leu- motif in amino acid sequences of membrane and non-membrane proteins of plant organelles

Type of proteins	Specific content of repeat (Number of repeats per 100 aa)	Number of sequences analyzed
Non-membrane proteins in mitochondria	0.74 ± 0.16	16
Non-membrane proteins in chloroplasts	0.78 ± 0.06	122
Membrane proteins in mitochondria	2.20 ± 0.33	41
Membrane proteins in chloroplasts	1.41 ± 0.11	178

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