Letter to the Editor

The N-Terminal, Putative, Mitochondrial Targeting Domain of the Mitochondrial Genome Maintenance Protein (MGM1) in Yeast Is Homologous to the Bacterial Ribonuclease Inhibitor, Barstar

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Little is known concerning the evolutionary origins or three-dimensional structures of mitochondrial targeting domains (Saier et al. 1989). The mitochondrial genome (mtDNA) of eukaryotes is maintained by nuclear-encoded proteins that replicate the mtDNA and partition it to daughter cells at division. Of the few such proteins that have been characterized, MGM1 apparently functions exclusively in mtDNA synthesis (Jones and Fangman 1992). This protein has a modular structure consisting of (1) a short, N-terminal, putative, targeting domain linked by an aspartyl-rich flexible linker to (2) a large GTP-binding protein domain homologous to the microtubule-binding protein, dynamin D100, which in turn is linked to (3) an essential but functionally uncharacterized C-terminal domain (Jones and Fangman 1992). The observations reported here provide evidence concerning the evolutionary origin and three-dimensional structure of the N-terminal targeting domain of MGM1.

The N-terminal domain of MGM1 resembles typical mitochondrial targeting sequences that are usually 20–80 amino acyl residues long, are rich in positively charged and hydroxy amino acids, and contain amphipathic α helices with characteristic properties (Saier et al. 1989; Jones and Fangman 1992). We here show that the N-terminal domain of MGM1 is homologous to barstar, the single domain RNase (barnase) inhibitor from Bacillus amyloliquefaciens that, like the N-terminal domain of MGM1, contains an amphipathic α-helix. Availability of the three-dimensional structure of barstar (Guillet et al. 1993; Hartley 1993; Schreiber and Fersht 1993) renders this finding particularly interesting as regards the structural basis for mitochondrial targeting.

Figure 1 shows a binary alignment of the sequence of barstar with that of the N-terminal, putative, mitochondrial targeting domain of MGM1. The alignment algorithm was SEQHP of the Los Alamos package. Statistical analyses to establish homology were performed with the SEQDP program of the Los Alamos package as well as the RDF2 program (Kanehisa 1982; Pearson and Lipman 1988). The comparison score obtained with these two programs was the same. The two sequences exhibit 25% identity and 61% similarity in 73 amino acids overlap, with a comparison score of 12 SDs (RDF2 and Los Alamos programs; 1,000 random shuffles each). These values are sufficient to establish that these two sequences arose from a common ancestral sequence (Doolittle 1986; Saier 1994).

N-terminal, amphipathic, α helices of (A) MGM1 (residues 19–36) and (B) barstar (residues 11–28), corresponding to the boxed sequences in figure 1, are depicted in figure 2. The helix in MGM1 exhibits a large hydrophobic moment (13.8), contains only positively charged residues, and conforms in composition to that expected for a mitochondrial targeting sequence (Saier et al. 1989). That in barstar possesses a hydrophobic moment of lesser magnitude (8.6), contains two negatively charged residues, and exhibits one strongly polar residue within its hydrophobic half. The similarity in residue position and content is striking (identical residues are shown: the alignment algorithm was SEQHP of the Los Alamos package. Statistical analyses to establish homology were performed with the SEQDP program of the Los Alamos package as well as the RDF2 program (Kanehisa 1982; Pearson and Lipman 1988). The comparison score obtained with these two programs was the same. The two sequences exhibit 25% identity and 61% similarity in 73 amino acids overlap, with a comparison score of 12 SDs (RDF2 and Los Alamos programs; 1,000 random shuffles each). These values are sufficient to establish that these two sequences arose from a common ancestral sequence (Doolittle 1986; Saier 1994).

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Key words: mitochondria, targeting, amphipathic helix, homology, evolutionary opportunism.

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0737-4038/94/1106-0015$02.00
FIG. 2.—Helical wheel depictions of the boxed regions of the sequences aligned in fig. 1, revealing the amphipathic nature of these sequences when arrayed in $\alpha$ helices. A, MGM1; B, barstar. Residues common to both proteins are presented in boldface print. The dashed lines delineate the borders between the hydrophilic and hydrophobic halves of the helices.

Because N-terminal amphipathic helices are believed to function generally in macromolecular recognition (Saier and McCaldon 1988), it can be postulated that the hydrophilic faces of the amphipathic helices in MGM1 and barstar are localized to the protein surface where they mediate interaction with the protein translocation machinery of the mitochondrion or with barnase, respectively. X-ray crystallographic analyses have shown that residues 12–25 in barstar, encompassing all but the first residue and the last three residues of the helix shown in figure 2B, are, in fact, in helical configuration as part of an open-faced sandwich, with the hydrophobic face of the helix buried in the interior of the protein and the hydrophilic face exposed to the surface (Guillet et al. 1993).

The structure of barstar undoubtedly corresponds closely to that of the homologous targeting domain in MGM1. MGM1 therefore may provide an example of the principle of evolutionary opportunism: an amphipathic $\alpha$ helix within a preexistent protein domain of bacterial origin was evidently modified to allow this domain to gain a mitochondrial targeting sequence. Interestingly, the principle of evolutionary opportunism is also exemplified by the target of barstar function, barnase, which exhibits sequence similarity to a domain in eukaryotic RNA polymerases II (Shirai and Go 1991).

LITERATURE CITED


MITIKO GO, reviewing editor

Received March 24, 1994

Accepted July 22, 1994