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DNA sequence of the *putA* gene from *Salmonella typhimurium*: a bifunctional membrane-associated dehydrogenase that binds DNA

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Catabolism of proline is widespread in prokaryotes and in mitochondria of eukaryotic cells. In enteric bacteria, the *putA* gene encodes a membrane-associated dehydrogenase (EC 1.5.99.8) that oxidizes proline to glutamate for use as the sole carbon, nitrogen or energy source (5). In addition, PutA protein also functions as the autogenous transcriptional repressor of the *put* operon (6, 10). The structure and function of PutA protein are interesting because it is a very large protein with multiple functions: (i) it can be isolated in both monomeric and dimeric forms (8), (ii) it has two separate enzymatic activities — proline dehydrogenase and pyrroline-5-carboxylic acid (P5C) dehydrogenase (8), (iii) it specifically binds *put* control region DNA and acts as an autogenous transcriptional repressor of the *put* operon (10), (iv) it binds to the cytoplasmic membrane and interacts with the electron transport chain (5), (v) it binds a number of substrates — proline, P5C, the cytoplasmic membrane, DNA, FAD, and NAD (5).

In this paper we describe the complete DNA sequence of the *putA* gene from *Salmonella typhimurium*. Based on the predicted amino acid sequence of the protein, the *putA* gene encodes a single polypeptide of 1202 amino acids (132 kD), in agreement with direct biochemical characterization of the PutA protein (8). PutA protein has sequence similarity to a number of proteins (Figure 1). The N-terminus of the *S.typhimurium* PutA protein has 100% identity to deduced amino acids from the partial DNA sequences of the *putA* gene from both *Klebsiella pneumoniae* (3) and *Escherichia coli* (9) indicating that *putA* may be conserved in these bacteria. Within the PutA protein, the region from 760–860 amino acids has high identity (43–54%) to a number of NAD dehydrogenases from a wide variety of organisms (Figure 1) suggesting that this domain of PutA protein may contain the dehydrogenase activity. Residues 877–896 of the PutA protein contain the consensus sequence and conform to the stereochemical constraints of the helix–turn–helix motif of prokaryotic DNA-binding proteins (2). However, searches of the EMBL database and GenBank failed to show sequence similarity to any known DNA-binding proteins. In contrast to the *S.typhimurium putA* gene, in *Saccharomyces cerevisiae* each dehydrogenase activity and the regulatory activity are encoded in separate proteins: the *PUT1* gene encodes only proline dehydrogenase while the *PUT2* gene encodes P5C dehydrogenase, and the *PUT3* gene encodes an activator protein (4, 7, 13). The *S.typhimurium* PutA protein has no significant homology to either PUT1 or PUT3 of *S.cerevisiae*. However, amino acid residues 1069–1102 of the *S.typhimurium* PutA protein show 58% identity with the *S.cerevisiae* PUT2 protein which has P5C dehydrogenase activity. We are analyzing the PutA protein genetically to identify each of the functional domains.

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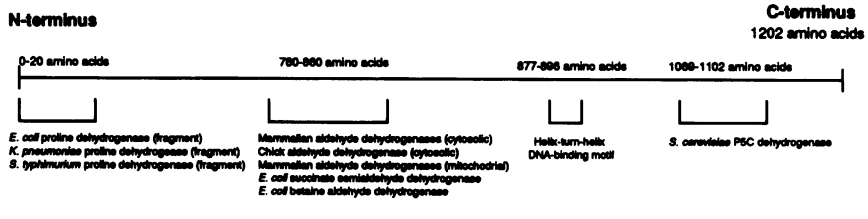

Figure 1. Amino acid alignment of *S.typhimirium* PutA protein with other proteins.