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Solution Structure of the Catalytic Domain of the Mitochondrial Protein ICT1 That Is Essential for Cell Vitality

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The ICT1 protein was recently reported to be a component of the human mitoribosome and to have codon-independent peptidyl-tRNA hydrolysis activity via its conserved GGQ motif, although little is known about the detailed mechanism. Here, using NMR spectroscopy, we determined the solution structure of the catalytic domain of the mouse ICT1 protein that lacks an N-terminal mitochondrial targeting signal and an unstructured C-terminal basic-residue-rich extension, and we examined the effect of ICT1 knockdown (mediated by small interfering RNA) on mitochondria in HeLa cells using flow cytometry. The catalytic domain comprising residues 69–162 of the 206-residue full-length protein forms a structure with a β1–β2–α1–β3–α2 topology and a structural framework that resembles the structure of GGQ-containing domain 3 of class 1 release factors (RFs). Half of the structure, including the GGQ-containing loop, has essentially the same sequence and structure as those in RFs, consistent with the peptidyl-tRNA hydrolysis activity of ICT1 on the mitoribosome, which is analogous to RFs. However, the other half of the structure differs in shape from the corresponding part of RF domain 3 in that in ICT1, an α-helix (α1), instead of a β-turn, is inserted between strand β2 and strand β3. A characteristic groove formed between α1 and the three-stranded antiparallel β-sheet was identified as a putative ICT1-specific functional site by a structure-based alignment. In addition, the structured domain that recognizes stop codons in

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Abbreviations used: RF, release factor; PTC, peptidyltransferase center; PTH, peptidyl-tRNA hydrolysis; FCM, flow cytometry; siRNA, small interfering RNA; NOE, nuclear Overhauser enhancement; PI, propidium iodide; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; PBS, phosphate-buffered saline; EDTA, ethylenediaminetetraacetic acid; FITC, fluorescein isothiocyanate; PDB, Protein Data Bank.

RFs is replaced in ICT1 by a C-terminal basic-residue-rich extension. It appears that these differences are linked to a specific function of ICT1 other than the translation termination mediated by RFs. Flow cytometry analysis showed that the knockdown of ICT1 results in apoptotic cell death with a decrease in mitochondrial membrane potential and mass. In addition, cytochrome *c* oxidase activity in ICT1 knockdown cells was decreased by 35% compared to that in control cells. These results indicate that ICT1 function is essential for cell vitality and mitochondrial function.

Introduction

The ICT1 gene was originally found by a comparison of gene expressions between undifferentiated and differentiated HT29-D4 human colon carcinoma cells.[1,2] Among many differentially expressed genes, its mRNA was strongly downregulated during *in vitro* differentiation of HT29-D4 cells; thus, the gene was named ICT1 (*i*mmature *c*olon carcinoma cell *t*ranscript *1*). Homologous genes are conserved not only in eukaryotes but also in bacteria, although few studies on ICT1 have so far been carried out.

According to the Pfam database generated by multiple-sequence alignments using hidden Markov models,[3] the ICT1 protein belongs to the release factor (RF)-1 family, which includes class I polypeptide chain RFs from bacteria and mitochondrial RFs (Fig. 1a). For translation termination, bacteria have two RFs, RF1 and RF2, which specifically recognize UAG/UAA and UGA/UAA stop codons, respectively, while mitochondria have mtRF1a (also called HMRF1L), which recognizes UAG/UAA stop codons. The bacterial-type RFs are composed of four domains, each of which has specific roles when bound to a ribosome for translation termination (Fig. 1b). For recognition of the stop codon, domains 2 and 4 interact with the decoding center in the small ribosomal subunit.[4,5] Domain 3 interacts with the peptidyltransferase center (PTC) of the large ribosomal subunit to trigger peptidyl-tRNA hydrolysis (PTH), which releases the nascent polypeptide chain from the P-site-bound peptidyl-tRNA. The catalysis involves a universally conserved Gly-Gly-Gln motif (the GGQ motif) in domain 3.[6,7] The interdomain space on the ribosome is bridged by domain 1. It is notable that although all ICT1 proteins have the GGQ motif, they are shorter than the RFs (Fig. 1a). The Pfam database shows that the sequence in the vicinity of the GGQ motif (~25 residues) that defines the RF-1 family can be aligned well between ICT1 proteins and RFs, whereas the other sequence regions are poorly aligned.

Comprehensive analyses of subcellular localizations using green fluorescent protein tagging have shown that the human ICT1 protein and its yeast homologue are localized in mitochondria.[8,9] The N-terminal extension that exists in all eukaryotic ICT1 proteins is a mitochondria-targeting presequence (Fig. 1a).[9,10] Recently, it has been reported that the human ICT1 protein is a component of mitochondrial ribosome (mitoribosome) and that it has PTH activity via the GGQ motif.[10] Intriguingly, unlike RFs, the PTH activity of ICT1 is codon-independent. Thus, ICT1 has been suggested to be involved in some translation process other than the translation termination process mediated by RFs.[10]

To obtain insights into the functional differences between ICT1 and RFs, we determined, using heteronuclear NMR methods, the solution structure of a truncated ICT1 protein from a mouse that lacks the N-terminal mitochondrial targeting signal and an unstructured C-terminal extension. This structured region contains the catalytic GGQ motif and thus is referred to as the catalytic domain. The determined

Fig. 1. Comparisons between ICT1 proteins and bacterial-type RFs from various organisms. (a) Schematic domain representations of RF1 family proteins: *E. coli* RF2, mouse ICT1 protein, and its *E. coli* homologue *yaeJ*. Numbering is according to the ICT1 structure determined in this study and the structure of free *E. coli* RF2 (PDB code 1GQE). (b) Structure of RF2 bound to the 70S ribosome from *T. thermophilus* (PDB code 2WH1). The ribosome structure is not drawn. Domains 1, 2, and 4 are shown in the same colors as in (a). In domain 3 containing the GGQ motif, the α-helices and the β-sheet are shown in pink and light green, respectively. (c) Structure-based sequence alignment of ICT1 proteins from various organisms (from bacteria to eukaryotes) and bacterial and mitochondrial RFs. The secondary structure elements of the ICT1 structure determined in this study and of the RF2 structure (PDB code 1GQE) are indicated. The truncation positions for the ICT1 protein used in this structural determination are marked by vertical arrows. Asterisks indicate highly conserved residues (>94%) among both ICT1 proteins and RFs. (#) Highly conserved residues among the ICT1 proteins from bacteria to eukaryotes. Alignments are as follows: purple: glycine (G); yellow: proline (P); green: small and hydrophobic amino acids (A, V, L, I, M, F, W); gray: hydroxyl and amine amino acids (S, T, N, Q); red: charged amino acids (D, E, R, K); cyan: histidine (H) and tyrosine (Y).