

## Molecular Characterization of the Human SECp43 Protein

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Selenium exerts its biological activity as Selenocysteine, the 21<sup>st</sup> amino acid to participate in ribosome-mediated translation. The pathway of Selenocysteine biosynthesis has been elucidated in considerable detail for prokaryotes. However, the Selenocysteine biosynthesis pathway in eukaryotes is still being resolved, many of the proteins involved have been identified based on the prokaryotic pathway. In eukaryotes, selenocysteine incorporation occurs cotranslationally at UGA stop codons via the interactions of RNA-protein complexes, one comprises of selenocysteyl (Sec)-tRNA<sup>[Ser]Sec</sup> and its specific elongation factor, EFsec, and another consisting of the SECIS element and SECIS binding protein, SBP2. Others factors implicated in this pathway include two selenophosphate synthetases, SPS1 e SPS2, ribosomal protein L30, and two factors identified as binding tRNA<sup>[Ser]Sec</sup>, SEPSECS and SECp43. The specific function of SECp43 remains elusive. Therefore, the molecular characterization of the Human SECp43 protein is very important for understanding the role this protein in the Selenocysteine biosynthesis pathway. In this work, human SECp43 gene was synthesized based on oligonucleotide fragments according to the codon preference of *Escherichia coli*. The whole synthetic gene was constructed by polymerase chain reaction. The human SECp43 open reading frame was cloned in the pET-28a(+) (Novagen) expression vector and the recombinant protein expressed in *Escherichia coli* at 20°C for 14 hours by IPTG induction. A purification protocol is been established to obtain pure and stable human recombinant SECp43 for future tRNA<sup>[Ser]Sec</sup> interaction studies by fluorescence spectroscopy and isothermal titration calorimetry (ITC). Structural studies by small-angle X-ray scattering (SAXS) and by protein crystals X-ray diffraction will be performed.

Keywords: SECp43, Selenocysteine biosynthesis pathway, tRNA<sup>[Ser]Sec</sup> binding protein

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