Selenoproteins Biosynthesis: Regulation by RNA/Protein Interactions

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Selenium is an essential micronutrient incorporated into proteins during mRNA translation as selenocysteine (Sec), which is synthesized by transfer of Se from selenylphosphate to serine on a specific t-RNA. Se-proteins require Se for activity, most of which involves maintenance of redox potentials in living cells[1]. Sec insertion depends on the conversion of an mRNA stop codon (UGA) to a readable codon and requires t-RNA^{sec}, a special stem/loop mRNA structure (SECIS: selenocysteine insertion sequence), a protein elongation factor specific for t-RNA^{Sec}, and SBP2, a SECIS binding protein. Since only the SECIS are mRNA-specific, they are likely related to the graded or hierarchal responses to [Se] observed in Se-protein biosynthesis. SECIS are located in the 3'-UTR regions of the mRNA and are classified in two groups according to their terminal loops: Form 1, large and variable size; Form 2, small (3-6 nt) with an internal loop.

Structural properties of different 5'-^{32}P-SECISs were analyzed as binding affinity and protein "footprinting" of rat SBP2, expressed in significant amounts as soluble, full length protein after subcloning cDNA kindly provided by P. Copeland. Form 1 SECISs were: Glutathione Peroxidase 1 (GPX1) and Form 2: Thioredoxin Reductase (TR) 1, TR3 and SelP. Short RNAs (42-47nt) were synthetic oligomers and longer RNAs (142-144nt) were transcribed from cDNA clones kindly provided by M. Berry, and R. Sunde.

The results show that the SPB2 binding site was complete within 42nt SECIS, since affinities were indistinguishable between GPX1 142 and 42 nt SECISs. At SBP2/SECIS molar ratios 5:1 to 10:1, 50% of RNA was bound to protein, corresponding to apparent K_d 40 to 80 nM (at [RNA] = 8 nM), for all the SECIS elements, showing that SBP2/SECIS stability is high and comparable for Form 1 and From 2 SECIS. However, when GPX1, SelP, TR1 and TR3 (42-47 nt) were probed with nucleases, Cu-$(Phen)_2$, Fe(EDTA), and RNAses T1, V1, S1 and A in the presence and absence of SBP2, an higher order structure was revealed by different patterns of nuclease access for Form 1 and Form 2 SECIS. SECIS-specific variations in SBP2 "footprints" coincided with differences in the terminal loops for GPX1 and TR1 and with Se responses *in vivo*. (Support: NIH-DK 20251).

[1] Sun Q-A et al., J.Biol.Chem (1999) 274:24522.