Biosynthesis of selenoproteins

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Selenocysteine is co-translationally incorporated into the nascent polypeptide chain, in both prokaryotes and eukaryotes, through a process directed by a UGA codon. Recently it was shown that special elongation factor, novel tRNA and insertion elements at 3′ non-coding region were involved in this biochemical process.

IN the recent years, the importance of selenium (Se) as an essential trace element has progressively emerged due to the analysis of selenium deficiency diseases (Keshan disease and Kaschin-Beck disease) and identification of a number of selenoproteins. This element is present within proteins in the form of selenocysteine ($HSeCH_2CH(NH_2)COOH$), an analogue of cysteine in which the sulphur atom has been replaced by an atom of selenium. In contrast to random incorporation of selenomethionine in the place of methionine in proteins, selenocysteine residues occupy unique positions and are not simply cysteine replacements[1,2].

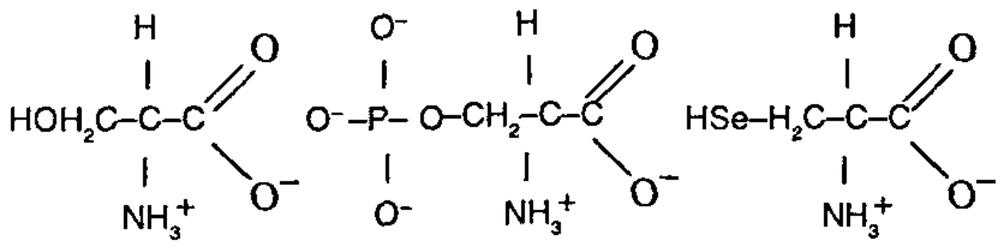
Ever since it was first suggested that Se is an essential component of these proteins, there has been much attention for the mode of incorporation of the Se moiety into the proteins, which occurs by a co-translational process[3]. With the exception of thiolase and selenoprotein P, glutathione peroxidase, bacterial formate dehydrogenase, Type I iodothyronine deiodinase, protein PA of glycine reductase complex are known to contain selenocysteine, which is also involved in the catalytic function of the above.

Biosynthesis of selenocysteine: the 21st amino acid

Sunde and Evenson[4] identified serine as the ultimate source of carbon for selenocysteine, and proposed that an additional metabolic step involving the replacement of the phosphate of phosphoserine with selenol (Se–H) group, either while phosphoserine is esterified to the tRNA or perhaps after phosphoserine incorporation into the polypeptide chain, would lead to the synthesis of selenocysteine.

Synthesis of selenocysteine

Serine ⟶ Phosphoserine ⟶ Selenocysteine



Expansion of genetic code

Chambers and associates[5] showed selenocysteine, found in the active site of glutathione peroxidase, is encoded by a TGA codon. The existence of a TGA codon in the middle of otherwise an open reading frame was also reported for Type I iodothyronine deiodinase[6]. Berry *et al.*[6] constructed deletion mutants and frame shift insertions and confirmed that the putative stop (TGA) codon is in-frame, corresponding to selenocysteine. Zinoni *et al.*[7] also demonstrated an in-frame UGA opal nonsense codon, which directs the incorporation of selenocysteine in the formate dehydrogenase gene. A multiple selenocysteine containing protein, selenoprotein P from rat, was characterized[8,9] and the corresponding gene[10] sequences were shown to contain ten TGA codons in the open reading frame. Three in-phase TGA codons were also identified in the gene encoding the mitochondrial capsule selenoprotein from mouse sperm[11]. These results suggest that incorporation of selenocysteine into these proteins can occur by suppression of the UGA codon.

Novel tRNA

The existence of two UGA suppressor tRNAs that can carry phosphoserine in mammalian, avian and xenopus species was demonstrated[12,13]. Tappel *et al.*[14,15] identified a tRNA in rat liver that is specific for selenocysteine insertion. Leinfelder *et al.*[16,17] discovered the *sel C* gene from *E. coli*, encoding an unusual seryl tRNA with UCA at the anticodon loop. This tRNA deviated in several positions from sequences previously considered

invariant in other tRNA species[18] and is the longest tRNA species with 95 nucleotides, with an acceptor stem of 8 base pairs[18]. The novel tRNA accepts L-serine and cotranslationally inserts selenocysteine by recognizing the 'specific' UGA codon. The serine residue, which is attached to unique $tRNA^{uca}$, is converted to selenocysteine in a reaction dependent on the *selA* and *selD* gene products[19,20]. This is considered another important pathway for providing additional amino acids like the formylation of methionine attached to the prokaryotic initiator tRNA[21] and the conversion of glutamate to glutamine during the synthesis of glutaminyl tRNA in several organisms[22].

Unique translational factor

Forchhammer *et al.*[23] discovered a unique translation factor (SELB), the *selB* gene product, exhibiting extensive homology with sequences of translation initiation factor-2 (IF2) and elongation factor Tu (EF–Tu). Furthermore, purified SELB protein binds guanine nucleotides in a 1:1 ratio and specifically complexes with selenocystinyl $tRNA^{uca}$. Thus, SELB could be an amino acid-specific elongation factor, replacing EF–Tu in a special translation step. A relevant question for understanding translation and the evolution of the genetic code is, how the translational machinery can cope with the situation that one and the same triplet codon can signal either chain termination or selenocysteine insertion?

Context effects

Bock *et al.*[24,25] made deletions in *E. coli* formate dehydrogenase gene and fused in-frame to the *lacZ* reporter gene. Their analysis revealed that a sequence of 40 bases in the mRNA at the 3′ side of the UGA codon can be folded into a putative hair pin structure, and is required for selenocysteine incorporation. Mutagenesis of the hair pin structure showed that sequences of the loop region are particularly important and serve as a recognition element for special elongation factor, directing the selenocysteine-inserting tRNA species to decode a particular UGA.

Similar structures are conserved in the 3′ untranslated regions in many, albeit not all, selenoprotein mRNAs[26]. Shen *et al.*[27] abolished selenocysteine insertion to glutathione peroxidase by deleting four nucleotides at the 3′ untranslated region of glutathione peroxidase gene and suggested hairpin-forming region was essential for translational insertion of selenocysteine at a UGA codon. Similar functional elements in the 3′ untranslated region of the rat selenoprotein P mRNA, with predicted stem-loops were observed[28,29]. Footprinting experiments showed that a special elongation factor, SELB binds specially at the loop region of the hairpin structure[30]. The targeted insertion of selenocysteine is accomplished by the recognition and binding of ternary complex (special elongation factor and charged novel tRNA) to the mRNA insertion elements in the immediate neighbourhood of in-frame UGA codon[30,31]. Removal of insertion elements from the 3′ end leads to termination of protein at the UGA codon.

Expression of gene during selenium deficiency

Western blot analysis for the detection of immunoreactive protein in liver cytosol obtained from rats fed on selenium deficient diets revealed that glutathione peroxidase protein was not expressed[32]. Northern blot analysis confirmed three-fold induction of mRNA coding for glutathione peroxidase in livers of selenium-deficient rats[33,34]. These results indicate that mRNA is synthesized and accumulated to elevated levels in tissues of rats fed on selenium-deficient diets and that selenium status appears to regulate the translation of selenoproteins.

Conclusion

As we trace the pathway of the development of our understanding of biosynthesis of selenoproteins, several interesting features emerge – 'extra' coding capacity by UGA codon[3], diversified tRNA[18], existence of specific elongation factor[23], insertion elements in non-coding region[29,30] and elucidation of selenocysteine as being the 21st amino acid[35] – making the protein-synthesizing system a more flexible one. What more surprises could the selenoprotein molecule hold?

1. Stadtman, T. C., *Annu. Rev. Biochem.*, 1980, **49**, 93–110.
2. Stadtman, T. C., *FASEB J.*, 1987, **1**, 375–379.
3. Bock, A., Forchhammer, K., Heider, J. and Baron, C., *Trends Biochem. Sci.*, 1991, **16**, 463–467.
4. Sunde, R. A. and Evenson, J. K., *J. Biol. Chem.*, 1987, **262**, 933–937.
5. Chambers, I., Frampton, J., Goldfarb, P., Affara, N., McBain, W. and Harrison, P. R., *EMBO J.*, 1986, **5**, 1221–1227.
6. Berry, M. J., Banu, L. and Larsen, P. R., *Nature*, 1991, **349**, 438–440.
7. Zinoni, F., Birkman, A., Stadtman, T. C. and Bock, A., *Proc. Natl. Acad. Sci. USA*, 1986, **83**, 4650–4654.
8. Matchnik, P. A. and Tappel, A. L., *J. Inorg. Biochem.*, 1990, **40**, 265–269.
9. Read, R., Bellow, T., Yang, J. G., Hill, K. E., Palmer, I. S. and Burk, R. F., *J. Biol. Chem.*, 1991, **265**, 17899–17905.
10. Hill, K. E., Llyod, R. S., Yang, J. G., Read, R. and Burk, R. F., *J. Biol. Chem.*, 1991, **266**, 10050–10053.
11. Karimpour, I., Cutler, M., Shih, D., Smith, J. and Kleene, K. C., *DNA Cell Biol.*, 1992, **11**, 693–699.
12. Hatfield, D., Diamond, A. and Dudock, B., *Proc. Natl. Acad. Sci. USA*, 1982, **79**, 6215–6219.

13. Hatfield, D., *Trends Biochem. Sci.*, 1985, **10**, 201–204.
14. Hawkes, W. C. and Tappel, A. L., *Biochem. Biophys. Acta*, 1983, **739**, 225–234.
15. Hawkes, W. C., Lyons, D. E. and Tappel, A. L., *Biochem. Biophys. Acta*, 1982, **699**, 1983–1991.
16. Leinfelder, W., Zehelein, E., Mandrand-Berthelot, M. A. and Bock, A., *Nature*, 1988, **331**, 723–725.
17. Heider, J., Leinfelder, W. and Bock, A., *Nucleic Acids Res.*, 1989, **17**, 2529–2540.
18. Schon, A., Bock, A., Ott, G., Sprinzl, M. and Soll, D., *Nucleic Acids Res.*, 1989, **17**, 7159–7165.
19. Leinfelder, W., Stadtman, T. C. and Bock, A., *J. Biol. Chem.*, 1989, **264**, 9720–9723.
20. Forchhammer, K., Rucknagel, K. P. and Bock, A., *J. Biol. Chem.*, 1990, **265**, 9346–9350.
21. Seong, B. I. and Raj Bhandary, U. L., *Proc. Natl. Acad. Sci. USA*, 1987, **84**, 334–338.
22. Schon, A., Kannangara, C. G., Gough, S. and Soll, D., *Nature*, 1988, **331**, 187–190.
23. Forchhammer, K., Leinfelder, W. and Bock, A., *Nature*, 1989, **342**, 453–456.
24. Zinoni, F., Heider, J. and Bock, A., *Proc. Natl. Acad. Sci. USA*, 1990, **87**, 4660–4664.
25. Heider, J., Baron, C. and Bock, A., *EMBO J.*, 1992, **11**, 3759–3766.
26. Berry, M. J., Banu, L., Chen, Y., Mandel, S. J., Kieffer, J. D., Harney, J. W. and Larson, P. R., *Nature*, 1991, **353**, 273–276.
27. Shen, Q., Chu, F. F. and Newburger, P. E., *J. Biol. Chem.*, 1993, **268**, 11463–11469.
28. Berry, M. J., Banu, L., Harney, J. W. and Larsen, P. R., *EMBO J.*, 1993, **12**, 3315–3322.
29. Hill, K. E., Llyod, R. S. and Burk, R. F., *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 537–541.
30. Baron, C., Heider, J. and Bock, A., *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 4181–4185.
31. Bock, A., Forchhammer, K., Heider, J., Leinfelder, W., Sawers, G., Veprek, B. and Zinoni, F., *Mol. Microbiol.*, 1991, **5**, 115–120.
32. Reddy, A. P., Hsu, B. L., Reddy, P. S., Li, N. Q., Kedam, T., Reddy, C. C., Tam, M. F. and Tu, C. P. D., *Nucleic Acids Res.*, 1988, **16**, 5557–5568.
33. Li, N. Q., Reddy, P. S., Kedam, T., Reddy, A. P., Tu, C. P. D. and Reddy, C. C., *J. Biol. Chem.*, 1990, **265**, 108–113.
34. Reddy, C. C., Li, N. Q., Reddy, P. S., Hildenbrandt, G. R., Reddy, A. P., Scholz, R. W. and Tu, C. P. D., in *Biological Oxidation Systems* (eds Reddy, C. C. and Madhyastha, K. M.), Academic Press, New York, 1990, vol. I, pp. 473–485.
35. Soll, D., *Nature*, 1988, **331**, 662–663.

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Hormones, cytoskeletal proteins and cell shape

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Recently attention has been focussed on the molecular mechanisms involved in cell shape changes brought about by chemical signals through cell surface proteins. The shape of any given cell type is maintained by various components of the cytoskeleton (microfilaments, intermediate filaments and microtubules), the extracellular matrix, the plasma membrane and associated proteins. Hormones and growth factors regulate the expression and post-translational modifications of these proteins which in turn depend on the phenotype of the cell. Action of hormones and growth factors on cells is also dependent on various factors such as phase and age of the cell, ion gradient and presence or absence of specific receptors at the right sites. The cause and effect in regulation of cell shape is not yet very clear.

THE change in cell shape can be considered as a primary adaptation of the cell to suit altered physiological demands. Cell shape changes are manifestations in many of the cellular events such as division, differentiation, transformation and death. A variety of observations suggest that cell shape changes exert specific effects on gene expression[1]. The exact mechanisms by which changes in cell shape affect the pattern of gene expression are not yet very clear but the cytoskeleton seems to play a key role in this process[2–4]. To understand functional and spatial relationship of the cell in a given tissue, it is necessary to know how the local microenvironment acts on the cell to regulate its phenotype. Cell phenotype is controlled by restructuring the cytoskeletal framework which in turn depends on interactions with neighbouring cells and extracellular matrix (ECM)[5–8]. A host of extracellular and intracellular proteins are involved in shaping the cellular architecture. The extracellular matrix proteins such as collagen, fibronectin, laminin, proteoglycans and fibrinogens[9], the transmembrane proteins such as integrins, cadherins[10], the cyto-