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Life with 22 amino acids

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Short Abstract: Selenocysteine (Sec) and pyrrolysine (Pyl) are two unusual amino acids co-traslationally incorporated. We analyze and compare Pyl and Sec decoding and particularly, we investigate especific features associated with Pyl synthesis and incorporation, making emphasis in their evolutionary aspects, and the implications for the genetic code evolution.

Long Abstract:
Selenocysteine (Sec), a selenium analog of Cys, and pyrrolysine (Pyl), are two unusual amino acids co-translationally incorporated into protein synthesis. Sec is a highly reactive nucleophile present at the redox active site of several selenoenzyme families. Pyl is a sui generis amino acid containing an electrophilic group, present at the active site of certain families of methyl-amino methyl transferases. Thus, Sec and Pyl expand the range of reactions of proteins. Although both amino acids are restricted to some taxa, Sec incorporation exhibits a wide phylogenetic distribution, being present in numerous phyla of the three domains of life. In contrast, Pyl incorporation is limited to four methylotrophic archaeal organisms Methanosarcina barkeri, M. acetivorans, M. mazei and Methanococcoides burtonii, in which pyrroproteins appear to have conferred an important selective advantage, and a single bacteria: Desulfitobacterium hafniense, which is the sole species incorporating both Sec and Pyl. Sec and Pyl are incorporated at UGA and UAG codons, respectively. Sec incorporation needs recoding of otherwise UGA termination codon; however, the mechanism for discrimination of UAGPyl and UAGstop is not fully understood.

We have analyzed and compared Pyl and Sec decoding, our results suggest that while Sec decoding strategy is similar in the three domains of life, Pyl-decoding strategy may be different in the bacterial and archaeal domains. We propose that in D. hafniense UAG codon is hardwired as a stop codon, and needs recoding to specify Pyl. In contrast, in Pyl-incorporating archaea the use of UAG as a coding word is very restricted, suggesting that a process of codon capture may be occuring to accommodate Pyl without need of recoding.

The mechanism of Pyl synthesis has not been elucidated, neither the genes involved have been identified. We reasoned that the enzymes responsible for the synthesis of the skeleton that derivitize lysine in Pyl (i.e. pyrroline-5-carboxylate) might be ‘common’ enzymes and not “Pyl specific enzymes”. We found that Pyl-incorporating archaea bears an almost exclusive pathway among the archaeal organisms for synthesizing pyrroline-5-carboxilate using glutamate as substrate whose genes (proA, proB and proC) are putatively arranged in an

operon. These genes are broadly distributed in the bacterial domain. So, we have performed phylogenetic analyses of these 3 genes to discern between two possible scenarios: i) Horizontal Gene Transfer (HGT) from bacterial species; or ii) several gene loss events occurred in the rest of the completely sequenced archaeal genomes. No evidence of recent HGT event can be observed, this may have certain implications for the genetic code evolution.

We also investigated the presence of additional genes linked to the Pyl trait, essentially by performing blast searches for genes that occur in organisms possessing the trait and are absent in organisms lacking it. Some genes were identified but the likelihood of being associated with this trait is rather low. This would reinforce the idea that the synthesis of pyrroline-5-carboxylate would be carried out by canonical enzymes.

Pyrrolysine introduces a further intricacy to the evolving genetic code, and gives additional hints as to how new amino acids and the genetic code might evolve from an already existing metabolic and informational machinery and how they may evolved entwined. Indeed, the current evidence that both LysS 1 and 2, and PylS can charge Lys and Pyl to tRNAPyl (respectively) supports the hypothesis that in situ synthesis of amino acids on a tRNA scaffold might be a simple means to expand the amino acid repertoire. This mechanism exists also for fMet, Asn, Gln, SeC and Cys and constitutes a strong support for the coevolution theory of the genetic code, which postulates that the formation of new amino acids through biosynthetic pathways guided and structured the genetic code. Indeed, these pathways may be considered as the quintessence of the CET since the informational apparatus constitutes an integral part of the metabolic pathway. In the case of Pyl, this situation may evolve to a scenario of competition between canonical and non-canonical synthesis of aminoacyl-tRNA, once the amino acid synthesis can occur free in solution and a canonical aminoacyl-tRNA synthetase has evolved, eventually one of the mechanisms became extinct. A second clue refers to which mechanisms might be involved while a codon capture process is taking place: the decreasing use of a particular codon, gene duplicaction and/or modification of translation key players (release factors, aminoacyl-tRNA synthetases, and perhaps, elongation factors).

The use of Pyl should have conferred an important selective advantage for methylotrophic archaea; pyrroproteins are highly expressed proteins involved in methanogenesis. Nevertheless, it is important to emphasize that the use of Pyl in these species is confined to four pyrroproteins families. It is possible that Pyl reactivity might be the cause of its restricted use. Maybe the old proverb “in the sin is the penitence” is valid for a highly reactive amino acid. In D. hafniense Pyl appears to be incorporated into a single polypeptide, and contrasting the situation of Pyl-incorporating archaea, there is no experimental evidence that this organism incorporates Pyl. In this context, it is important to highlight that D. hafniense is present in a high proportion in methanogenic film reactors and can share the same ecological niche that methylotrophic archaea; in turn, this would explain the taxa distribution of the trait and supports the idea that HGT between these archaea and bacteria have occurred, spreading the trait, similar to what has been described for Sec. Clearly, experimental evidence is needed about Pyl incorporation in D. hafniense.

Combining these lines of evidence, we could formulate a working hypothesis of an ongoing process of modification of the genetic code with the metabolic and informational adaptations

taking place. From this standpoint, Pyl addition to the genetic code appears to be a model case for carrying further studies concerning different aspects of the origin and evolution of the genetic code beyond the inherent interest in this case.