Synonymous codons are not the same with respect to the speed of translation elongation

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In the genetic code table, out of the 64 triplet codons, generally 61 codons are involved in coding for the 20 amino acids in organisms. Therefore, the genetic code table is an obvious degenerate: except Met and Trp, each of the 18 amino acids is coded by 2–6 synonymous codons. Synonymous codons (Box 1) not only encode the same amino acid, but are similar to each other with regard to their nucleotide sequences as well as distribution in the table. Therefore, study of these codons in different genomes has been of great interest to molecular evolutionary biologists[1–3].

Analysis of synonymous codons in different genomes in the early 1980s revealed that the general abundance values of different synonymous codons are significantly different from each other within a genome[4]. Interestingly, synonymous codons are also different from each other with respect to their distribution between the high and low expression genes in a genome[4]: certain synonymous codons, called preferred codons, are found more frequently in the high-expression genes than the low-expression genes, and the reverse is also true for the non-preferred codons. Based on this observation, evolutionary biologists had hypothesized mechanistic differences among synonymous codons during translation. One school of thought has been advocating in favour of the differential rates of decoding of synonymous codons by their cognate anticodons (involvement in the speed of translation elongation) leading to the above difference among the codons. This hypothesis argues for the requirement of fast translation (by the presence of preferred codons) of high-expression genes as more number of proteins are to be synthesized from its mRNA[5]. The other school of thought has been advocating in favour of the differential accuracy of decoding of synonymous codons by anticodons leading to the above difference among the codons. This hypothesis assumes that decoding inside the ribosome during translation is not a fool proof process. Considering the cellular economy involved in translation, the mis-incorporation of an amino acid in

a more abundant protein is more deleterious to the cell than the same in case of a less abundant protein (e.g. 1% error due to mis-incorporation of a protein with 100,000 copies will result in 1000 faulty molecules, while the same error of a protein with 100 copies will result in only one faulty molecule). As different synonymous codons are not decoded with equal accuracy, the codons with potential for most accurate translation are selected in the high-expression genes[6]. Though there has been the long-standing argument with regard to the nature of the difference among the synonymous codons, there is no doubt that synonymous codons are not same in all aspects[7].

Translation is an essential procedure in all cells. During this process, amino acids are brought to the site of protein synthesis by aminoacyl tRNAs, which recognize codons on the mRNA by their anticodons, and incorporate the correct amino acids in the elongating polypeptide. The recognition of the codon by the cognate anticodon, transfer of the amino acid to the nascent chain, and removal of the deacetylate tRNA are important attributes to the speed of translation elongation. Are synonymous codons different from each other with respect to the speed of translation elongation? The previous explanations in favour of this argument were mostly speculative. A recent experiment in yeast using ribosome profiling could not produce the anticipated result to appreciate this notion of translation speed difference among the synonymous codons[8]. So the difference among synonymous codons with regard to translational speed was not conclusive from any of the previously performed experiments. In this context, Yu *et al.*[9] have recently provided conclusive evidence in support of the notion that synonymous codons are different with respect to the speed of elongation of a polypeptide during translation.

The organism used in their study was *Neurospora crassa*, a common bread mould. The genome of this organism exhibits strong codon usage bias[10]. The fungus exhibits circadian rhythm and genes involved in this process have been reported to use selectively both optimal as well as non-optimal synonymous codons[10]. In *frq* gene, the non-optimal codons have been shown to be used for proper co-translational folding of the protein[10]. This is an interesting example of codon usage bias influencing both expression and function of genes. Moreover, protein structure has been shown to be related to codon usage bias at the genome level in this organism[11]. Therefore, studying the role of codon usage bias in this fungus is of significant interest to understand evolutionary significance of the bias.

Box 1.

Synonymous codons: Codons encoding the same amino acid, e.g. UUU and UUC are synonymous as both encode phenylalanine.

Codon usage bias: Unequal usage of synonymous codons in coding sequences of a genome.

Optimal codons (or preferred codons): The most frequently used codons among the synonymous codons for amino acids in high-expression genes than low-expression genes.

Non-optimal codons (or non-preferred codons): The synonymous codons which are less frequently used in high-expression genes than low-expression genes.

Ribosome profiling: A technique used to map an mRNA with respect to the regions that are bound with ribosomes.

Balanced translation model: Both preferred and non-preferred codons are translated with similar speed due to a balance between demand (preferred codon) and supply (cognate anticodon).

TFA: The time of first appearance of fluorescence which indicates completion of translation of the luciferase protein.

Yu *et al.*[9] used a cell-free translation of firefly luciferase gene (*luc* gene) in the cellular extract of *N. crassa* and the time of first appearance of fluorescence (TFA) as an indication of the completion of translation as well as protein folding. They made different constructs of the *luc* gene that are identical with respect to the coded protein sequence, but different with respect to the composition of synonymous codons among the constructs.

Though the *luc* gene was from a different organism (firefly), different constructs were made with regard to optimal and non-optimal codons of *N. crassa*. In the constructs composed of only optimal codons/preferred codons, TFA was faster in comparison to the construct that was composed of only non-preferred codons (Table 1). This result indicated that the speed of translation might be more in case of optimal codon than the non-

optimal codon (Figure 1), if mRNA structure and co-translational protein folding are ignored.

To find whether the different synonymous codon composition is resulting in faster translation due to translation kinetics but not due to different mRNA structure, Yu *et al.* performed *in vitro* translation with yeast cell extract. Codon usage bias is different between *N. crassa* and yeast: the preferred codon for an amino acid is different in the two organisms. Different cell extracts have naturally been optimized for the expression of genes according to the codon usage bias in its genome. Thus, the two constructs, OPT-*luc* (contains preferred synonymous codons according to *N. crassa* codon usage bias) and WT-*luc* (contains the wild-type *luc* gene sequence of firefly) (Table 1), produced TFA at different times when translation was carried out in yeast cell extract. The order of TFA of different constructs in the yeast cell extracts was different from that in the cell extract of *N. crassa*. This was an important proof that translation elongation is due to the decoding process and not due to other influences such as mRNA structure, protein folding, etc. To avoid further arguments about the impact of mRNA structure on TFA timing, Yu *et al.*[9] performed translation using constructs having non-preferred codons at different parts such as the N-terminal, middle and C-terminal regions. All the three constructs produced TFA with similar timings. The ^{35}S-methionine labelling experiment also proved that the complete protein is produced earlier in one of the constructs made up of preferred codons than that made up of non-preferred codons.

If a codon is decoded faster, then the ribosome retention time for that codon during translation will be less than in case the codon (Box 1) is decoded slower. Ribosome profiling is an elegant technique used to find the ribosome-bound mRNA part in a cell[12]. Earlier, this technique used in yeast was not able to find a negative correlation between preferred codon and ribosome retention time in that codon[8]. So the researchers had proposed the balanced translation model (Box 1)[8], which suggests that both preferred as well as non-preferred codons are decoded with similar speed inside the cell during translation. However Yu *et al.*[9] showed an anticipated reciprocal relation between ribosome retention in a

Table 1. Luciferase constructs with different composition of synonymous codons take different time for the completion of translation in cell-free system

| Type of *luc* construct	CAI$_{N.\ crassa}$	TFA$_{N.\ crassa}$ (min)	CAI$_{Yeast}$	TFA$_{Yeast}$ (min)
Wild type (WT-*luc*)	0.65	$x + 1.5$	0.709	y
OPT-*luc*	1.00	x	0.531	$y + 2$
de-OPT	0.36	$x + 7.5$	–	–

Three types of *luc* gene constructs were taken. Wild-type (WT-*luc*) contained the wild-type gene sequence of luciferase from firefly. The OPT-*luc* construct contained the entire gene sequence of luciferase optimized according to the codon usage bias (CUB) of *Neurospora crassa*. The de-OPT construct contained the sequence of *luc* gene composed of non-preferred codons according to the CUB of *N. crassa*.
CAI$_{N.\ crassa}$: Codon adaptation index of the three constructs calculated (according to the CUB of *N. crassa*).
CAI$_{Yeast}$: Codon adaptation index of the three constructs calculated (according to the CUB of yeast).
TFA$_{N.\ crassa}$: Time of first appearance of fluorescence of the three constructs noted in a cell-free translation system of *Neurospora*.
TFA$_{Yeast}$: Time of first appearance of fluorescence of the three constructs noted in a cell-free yeast translation system.
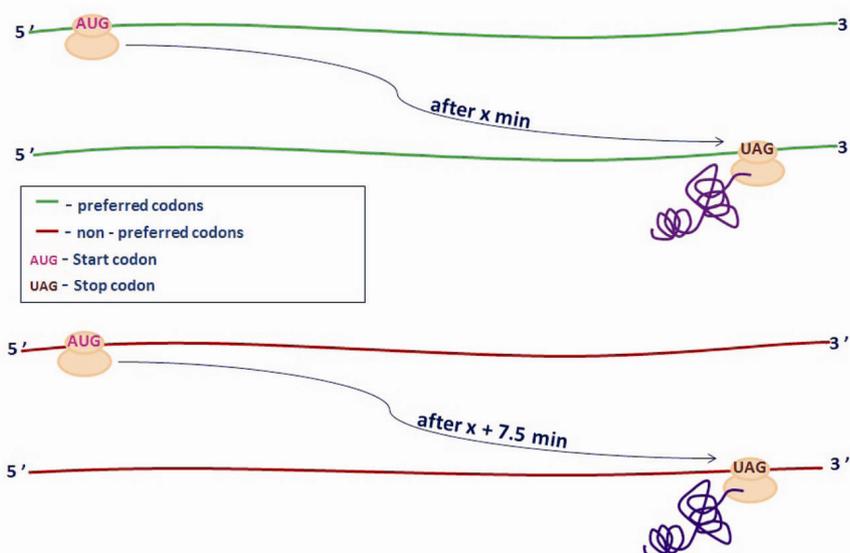
Figure 1. Schematic representation to explain the time difference seen in completion of translation of a gene composed of preferred and non-preferred codons. The green (upper one) and the red (lower one) lines represent the mRNA sequence of *luc* gene composed of preferred and non-preferred codons respectively. In case of the preferred codons, while it takes only *x* minutes to complete the translation, it takes 7.5 min more to complete the translation in case of the non-preferred codons. The sizes of the mRNA and protein sequence in both the cases are identical. The difference in elongation is proposed due to fast decoding of preferred codons in a cell.
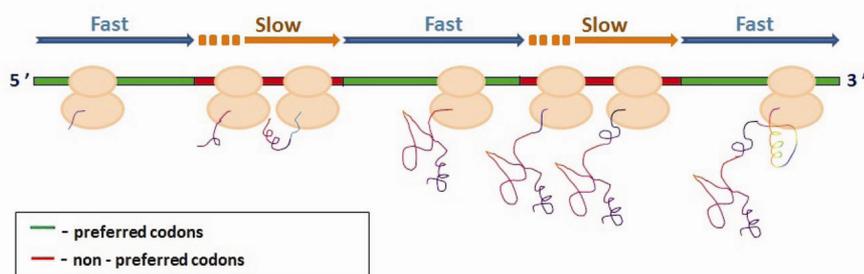
Figure 2. Schematic representation to show the relation between ribosome occupancy and different synonymous codons from ribosome profiling experiment. The green-red line represents an mRNA bound by a ribosome during translation. As the ribosome translates the regions of the mRNA, the difference in translation speed between the optimal and non-optimal codons can be observed. The green regions show sequences composed of preferred/optimal synonymous codons and the red regions show the non-preferred/non-optimal codons in the mRNA. More number of ribosome halting can be observed at the sequences containing non-preferred codons as it takes higher time to be decoded; consequently translation is slower at those sequences. However in the region composed of preferred codons, the translation speed is faster due to faster decoding of these codons leading to lesser number of ribosome to be associated with these codons.

codon and its preferred nature in the genome, in the ribosome profiling experiment carried out under *in vivo* and *in vitro* translation condition (Figure 2). The findings were same in both studies. Moreover, a change in concentration of mRNA had no impact on TFA, which was against the proposed balanced translation model. So the notion that ribosome retention in the preferred codon is less than that in the non-preferred codon was proved to be correct.

In conclusion, even though it was speculated earlier that synonymous codons are different with respect to the speed of translation elongation, there was no conclusive evidence. The findings by Yu *et al.*[9] support the fact that the speed of translation elongation due to preferred codons is higher than that of the non-preferred codons. It also explains well the reason behind the higher abundance of preferred codons in the high-expression genes. However, it does not eliminate the possible role of synonymous codons in accurate translation. In future, researchers will be looking forward to understanding the detailed mechanism of the fast and slow decoding processes of synonymous codons during translation.

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Ug99: saga, reality and status

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Wheat, the second most important cereal crop after rice, plays an important role in food and nutritional security worldwide. Wheat rusts, viz. black or stem rust (*Puccinia graminis* f. sp. *tritici*), brown or leaf rust (*P. triticina*) and yellow or stripe rust (*P. striiformis*), capable of spreading aerially over long distances, are highly variable and devastating pathogens. They evolve quickly to form new pathotypes/races, render resistant varieties susceptible and pose a serious threat to wheat production in different parts of the world[1]. Stem rust, also called 'polio of agriculture', has caused several severe epidemics in the past throughout the world. Developing rust-resistant varieties has been a continuous exercise over the years. One of the achievements of the green revolution of the 1960s was to reduce yield losses due to wheat rusts, as many resistance genes introduced in wheat during that period conferred resistance to most of the rust pathotypes of that time. A number of rust-resistant sources, including alien ones have been used to combat wheat stem rust. Introduction of rye (*Secale cereale* L.) gene (1B/1R translocation or substitution) into bread wheat[2,3], which carries *Lr26*/*Sr31*/*Yr9*, completely linked resistance gene has not only contributed 12–20% yield jump, but also imparted resistance to major biotic and abiotic stresses[4]. *Sr31* in combination with other stem rust resistance genes kept the stem rust fungus