Translational selection and molecular evolution

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An interplay among experimental studies of protein synthesis, evolutionary theory, and comparisons of DNA sequence data has shed light on the roles of natural selection and genetic drift in 'silent' DNA evolution.

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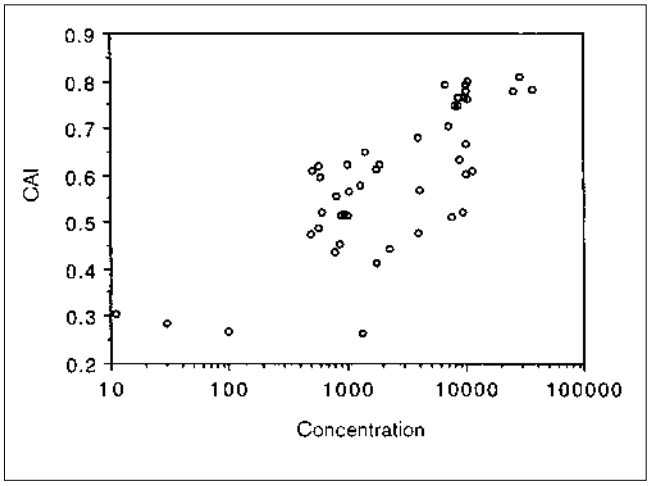
Introduction

Both functional and fitness consequences of synonymous codon usage have been identified in a number of taxa through a combination of biochemical studies of protein synthesis and analyses of DNA sequences. A balance among mutational processes, genetic drift, and selection to enhance the efficiency of protein synthesis, 'major codon preference', appears to explain patterns of codon usage at a large fraction of silent sites. In this review, we begin with a brief summary of patterns of codon usage that suggest translational selection for major codons. We then discuss the biochemical basis of adaptation at silent sites and present recent evidence both supporting and motivating modifications to the simple form of major codon preference. Finally, we discuss the role of translational selection in the evolution of both amino acid composition and protein size.

Patterns of codon usage: major codon preference

A number of patterns of base composition and DNA sequence variation suggest that natural selection discriminates among synonymous codons to enhance protein synthesis [1,2]. In *Escherichia coli* and *Saccharomyces cerevisiae*, genome-wide base composition at silent sites is biased toward a subset of 'major' codons for each amino acid. Among synonymous codons recognized by multiple tRNAs, major codons tend to be encoded by abundant tRNAs, and among synonymous codons recognized by a single tRNA, major codons generally show perfect Watson-Crick base pairing with the tRNA anticodon [3–8]. *In vivo* experiments in *E. coli* suggest that major codons can increase both the speed and the accuracy of translation [1,9]. Such codons could enhance fitness by increasing growth rates and/or by reducing the metabolic costs of protein synthesis. Correlations between tRNA pools and codon usage have also been noted in the genomes of *Salmonella typhimurium* [10], *Mycoplasma capricolum* [11], bacteriophage T7 [12], and, more recently, in the nuclear genome of *Drosophila melanogaster* [13•].

Figure 1



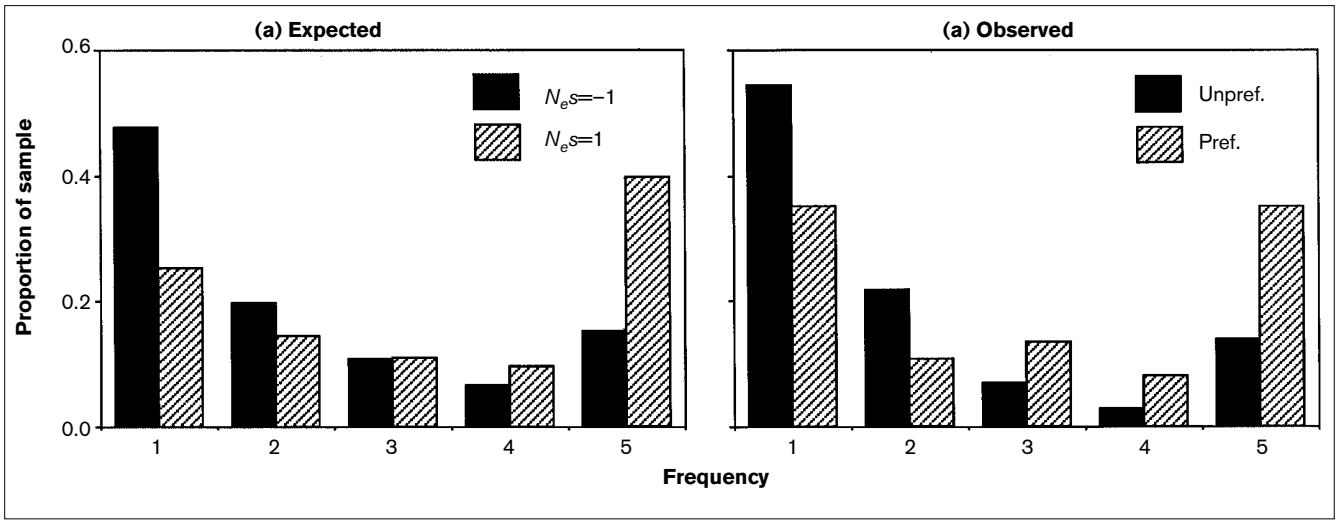
Codon bias and protein abundance in *E. coli*. Codon adaptation index (CAI) [72] values are plotted against protein concentration (molecules per cell) for 46 *E. coli* genes. CAI values are a positive function of codon bias. Figure from [14].

Although overall patterns of codon usage correlate with tRNA pools in many species, codon bias varies considerably among genes within their genomes. In *E. coli* and yeast, major codon usage shows a strong correlation with gene expression levels (Figure 1) [6,10,14–16]. Such patterns are consistent with major codon preference because the fitness benefit of a translationally superior codon should increase with the number of aminoacyl-tRNA selections it experiences. Similar relationships have been established in a number of other prokaryotes [17–20] and fungi [21–23], as well as in the nuclear genomes of *D. melanogaster* [24], *Arabidopsis thaliana* [25], *Dictyostelium discoideum* [26], *Caenorhabditis elegans* [27], and in the chloroplast genomes of a number of plant and algal lineages [28].

Population genetic tests of major codon preference

The simplest model of major codon preference is an evolutionary balance among natural selection favoring translationally superior major codons and mutation pressure and genetic drift allowing the presistence of slightly deleterious minor codons [29,30]. Population genetics theory predicts an inverse relationship between the efficacy of natural selection at a given nucleotide site and genetic linkage to other selected sites [29,31–35]. Under major codon preference, the effectiveness of selection, and thus levels of codon bias, should be a positive function of regional rates of

Figure 2



Population genetics of silent DNA mutations in *D. simulans*. **(a)** The expected proportions of preferred and unpreferred mutations found segregating within, or fixed between, populations were calculated according to the findings of Sawyer and Hartl [73] for a sample of five alleles and t_{div}, time of divergence scaled to N_e generations, of 0.6. The x-axis represents the number of alleles in which newly arisen mutations are present. The histogram shows the expected proportion of mutations in each frequency class under a two-state model of mutation-selection-drift with the scaled fitness difference, $N_e s$, between major and minor codons set to one. **(b)** Observed proportions of preferred and unpreferred silent mutations in a sample of five alleles of each of eight *D. simulans* genes. Data were pooled across loci. Adapted from [38•] (see reference for details of the method).

recombination. Kliman and Hey [36] showed that, in the *D. melanogaster* genome, genes in regions of reduced crossing-over show lower codon bias relative to genes located in regions of higher recombination.

Selection for codon bias also predicts differences in the evolutionary dynamics of putative fitness classes of silent mutations interspersed within a region of DNA [37]. Under major codon preference, silent mutations from non-major to major codons, 'preferred' mutations, should confer a small fitness benefit to the organism. Mutations in the reverse direction, 'unpreferred' mutations, should incur a fitness cost of the same magnitude. Comparisons of the within and between species evolutionary dynamics of preferred and unpreferred mutations have revealed patterns quite similar to those predicted by mutation-selection-drift [38•] (Figure 2).

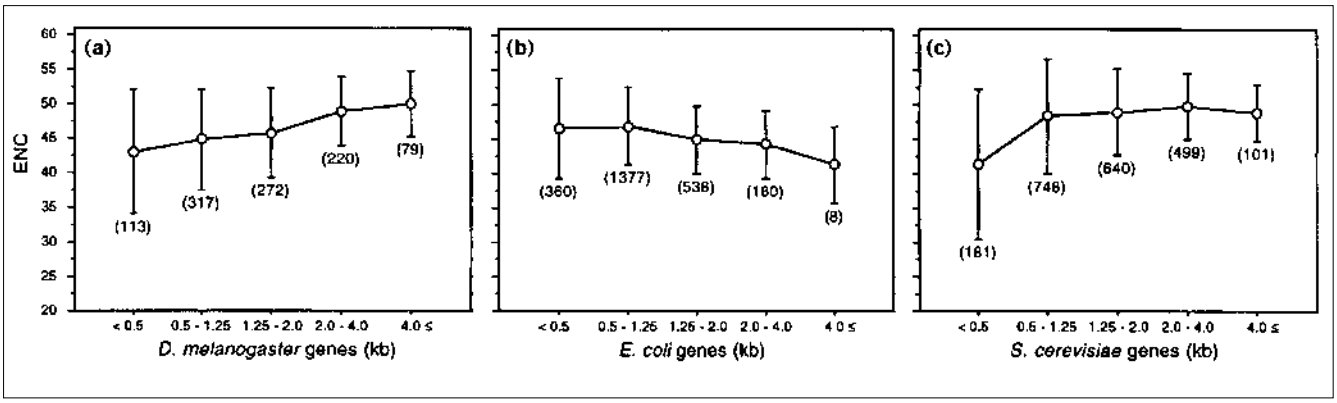
Finally, measures of silent DNA divergence are inversely related to synonymous codon usage bias between *E. coli* and *S. typhemurium* [39,40], *D. melanogaster* and *D. pseudoobscura* [41–43], *C. elegans* and *C. briggsae* [44,45], and among land plant chloroplast genes [46]. Although such patterns are qualitatively consistent with greater 'selective constraint' in highly expressed genes, the quantitative relationship between codon bias and silent divergence may not fit that predicted by major codon preference [47]. For some synonymous families in *E. coli*, the relationship between substitution rate and expression level does not appear to be related to codon bias.

Biochemical bases of selection at silent sites

Although the patterns discussed above strongly support the notion that major codons are generally favorable, the relationship between codon usage, the biochemistry of protein synthesis, and the fitness of organisms in populations is only beginning to emerge. At least three facets of translation could be affected by synonymous codon usage: the rate of elongation, the cost of proofreading, and the accuracy of translation (including rates of missense and processivity errors) [30,48]. During protein synthesis, a ribosome waits at a particular codon for the arrival of an aminoacyl-tRNA. This process is potentially costly: ribosomes are relatively expensive to synthesize, and the time for which they are idle should be minimized. Once the tRNA is bound to the RNA in the ribosome, elongation factor Tu is released with the hydrolysis of GTP. This reaction, known as 'substrate' or 'kinetic' proofreading, provides extra time to discern whether the correct tRNA is bound to the mRNA but the process is energetically costly: if the tRNA is rejected it must be recharged with elongation factor and GTP. In addition, despite proofreading, incorrect amino acids can be misincorporated into the growing peptide chain with the possibility that the protein produced will be either functionless or of reduced activity. Finally, the ribosome can undergo processivity errors while translocating, such as 'slipping' out of frame or 'dropping off' (premature termination) from the mRNA. Such errors are likely to result in dysfunctional proteins.

In vivo experiments in *E. coli* have shown that, during polypeptide chain elongation, the speed of aminoacyl-tRNA

Figure 3



Codon bias and gene length in **(a)** *D. melanogaster*, **(b)** *E. coli*, and **(c)** *S. cerevisae*. The means and standard deviations of a measure of codon bias, the effective number of codons (ENC) [74], are plotted for five length categories. The number of genes included in each category is shown in parentheses. ENC is inversely related to the degree of codon usage bias. Adapted from [58••].

selection at a given codon is proportional to the cognate tRNA's abundance [49]. Faster cognate tRNA recognition could confer a fitness advantage by enhancing any, or all, of the three facets of translation. But which, if any, of these processes determines the fitness effects of synonymous codon usage? The quantitative relationship between tRNA concentration and codon usage in *E. coli* grown under a variety of conditions [50] shows a remarkably good fit to the predictions of a model of growth optimization through selection for enhanced translational elongation rates [51••]. Berg and Silva [52••], however, have found that the correspondence between rates of elongation and patterns of synonymous codon bias in one synonymous family is not strong. The glutamic acid codon GAA is translated ~3.5 times faster than its synonym GAG, irrespective of context in *E. coli* [53]. Although selection to maximize the rate of elongation should favor GAA over GAG in all contexts, the frequency of GAA compared to GAG only increases with expression level among glutamic acid codons followed by G (i.e. GAA.G is increasingly favoured over GAG.G) [52••]. It is possible that mutational biases or other selection pressures, perhaps on local nucleotide composition, may act against GAG.nonG in *E. coli*.

Berg and Kurland's model of selection for translational elongation rates is consistent with the relationship between codon use and tRNA pools [51••] but similar models of selection on proofreading costs and translational accuracy have not been as thoroughly investigated. Two lines of evidence from DNA sequence comparisons suggest that the reduction of translational misincorporation rates also confers a fitness advantage to major codons. The fitness cost of errors in protein synthesis should be a function of the number and size of dysfunctional peptides produced. If natural selection biases codon usage to enhance translational accuracy, then, within a gene, selection should be stronger at codons encoding constrained amino acids — those at which a misincorporation would disrupt protein function — than at codons encoding less constrained amino acids. In *D. melanogaster*, higher major codon usage in DNA-binding motifs than elsewhere in transcription factor genes and in conserved than non-conserved amino acid positions in interspecific comparisons of proteins are consistent with codon selection for translational accuracy [54]. Such a pattern is not observed *in E. coli* [55]. Interestingly, although analyses of codon usage in mammals is complicated by large-scale patterns of base compositional bias [56], a similar correlation between silent and protein rates of evolution *within* loci suggests either that mutational biases vary within genes or that codon selection for translational accuracy also acts in mammals [57•].

Selection for translational accuracy also predicts a relationship between gene length and codon bias. Given the same level of expression, the energetic cost of producing dysfunctional peptides should be a function of their size. Eyre-Walker [14] showed that, for *E. coli* genes encoding proteins found in equimolar amounts, codon bias is indeed a function of gene length. Such a pattern has also been found among yeast ribosomal proteins [58••].

Moriyama and Powell [58••] have studied genome-wide relationships between codon bias and protein length in *E. coli*, *S. cerevisiae*, and *D. melanogaster*. Surprisingly, codon bias increases in larger proteins in *E. coli* but decreases in *S. cerevisiae and D. melanogaster* (Figure 3). The interpretation of such patterns is complicated by the multiplicity of factors that could influence the relationship between protein size and codon bias. Given the same level of expression and protein functional constraint, selection for translational accuracy predicts that longer genes should show greater codon bias. If expression levels differ among genes, however, then selection to decrease protein size — perhaps in the face of selection for protein stability or function — should be a positive function of expression levels. Finally, the efficacy of selection at a given nucleotide site is inversely related to the

mutation rate to deleterious mutations at linked sites [29,35]. Silent sites in longer genes may be linked to a greater number of deleterious mutations (at both silent and replacement sites) resulting in reduced codon bias relative to smaller genes ([29]; JM Comeron, M Kreitman, personal communication). As the three forces may act simultaneously, disentangling among their contributions may require analyses of more genomes to identify how recombination rates and gene expression levels influence the relationship between gene length and codon bias.

Conflicting selection pressures: evidence for antagonistic pleiotropy at silent sites

Although the benefits of major codon usage are well established, our understanding of the persistence of translationally suboptimal codons is less clear. Mutation pressures and genetic drift may account for many, if not most, minor codons, but both biochemical studies and sequence comparisons suggest that, at particular locations within genes, minor codons may be beneficial. For example, in *E. coli*, programmed frameshift events can require ribosomal pausing during elongation and slippage of the translational apparatus on the mRNA. Some such events depend on the use of minor codons [59,60•]. Furthermore, proper intracellular protein folding may require translational pauses, mediated by minor codon usage or mRNA structure, at specific locations within genes [61].

Several patterns of DNA sequence variation also support the notion that minor codons can be advantageous. In *E. coli*, reduced codon bias and silent divergence at the start of genes [62–65] may reflect constraints imposed by ribosome binding [64]. Similar patterns have been discovered recently in *D. melanogaster* [66•]. Major codon usage also decreases at the end of *E. coli* genes; apparently due, in part, to the fact that many genes overlap the Shine-Dalgarno, or coding sequence, of the next gene on the chromosome [65]. Finally, Maynard Smith and Smith [67] have shown that some sites in the middle of genes are occupied by a minor codon across a wide range of very diverged enteric bacteria. This suggests that natural selection may favor the persistence of apparently translationally inferior codons; however, the functional basis and relative frequency of minor codon preference remains to be established.

Conclusions: translation selection and protein evolution?

This review has focussed on translational selection at silent sites. The body of evidence supporting major codon preference has increased in the past several years through new methods and in a larger number of genomes. At the same time, some intriguing data have emerged that suggest that this model may require some refinement; minor codons may also be preferred at certain sites.

The contribution of translational selection in protein evolution has been the object of far less attention. As discussed above, protein length may be reduced to a greater extent in highly expressed genes in order to maximize the efficiency of translation. In addition, if tRNAs for some amino acids are translated more efficiently or accurately than others (or if some amino acids are less energetically costly to synthesize [68•]), then codons encoding such amino acids could also be favored at the level of translation. In *Mycoplasma capricolum* and *E. coli* [11], as well as in yeast [69•], tRNA pools match the amino acid usage of proteins, and in *E. coli*, the amino acid composition of proteins differs among highly and lowly expressed genes [70]. It is possible that tRNA pools are simply selected to match the amino acid composition of highly expressed proteins. Alternatively, tRNA pools and the amino acid composition of proteins may be co-adapted to enhance translational efficiency. Andersson and Kurland [71] have put forth convincing evidence that genomic evolution can drive the evolution of the translational system. It will be of great interest to determine the extent to which the converse holds; translational selection may play an important role in patterning both silent and replacement nucleotide composition as well as protein and genome size.

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