due to neutral drift. Here, we determine whether other, unknown factors, beyond neutral drift, affect the selection and/or distribution of rare codons. Such selective pressures could be used to control, for example, the rate of appearance of the nascent polypeptide, influencing co-translational folding pathways. We have developed a novel algorithm that evaluates the relative rareness of a nucleotide sequence used to produce a given protein sequence. We show that rare codons, rather than being randomly scattered across genes, often occur in large clusters. These clusters occur in numerous eukaryotic and prokaryotic genomes, and are not confined to unusual or rarely expressed genes: many highly expressed genes, including genes for ribosomal proteins, contain rare codon clusters. We show experimentally that such a rare codon cluster can impede ribosome translation of the rare codon sequence. These results indicate additional selective pressures govern the use of synonymous codons, and specifically that local pauses in translation can be beneficial for protein biogenesis.

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Analysis Of Ribosomal Dynamics As Revealed By Cryo-EM And Flexible Fitting

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Ribosomes are the molecular machines that translate the genetic message into nascent peptides, through a complex dynamics interplay with mRNAs, tRNAs, and various protein factors. A prominent example for ribosomal dynamics is the rotation of the small ribosomal subunit with respect to the large subunit, characterized as the ''ratchet motion'', which is triggered by the binding of several translation factors. Based on density maps of ribosomal complexes obtained by cryo-EM, we analyzed two kinds of ribosomal ratchet motions, induced by the binding of EF-G and RF3, respectively. By using the flexible fitting technique (real-space refinement) (1) and an RNA secondary structure display tool (coloRNA) (2), quasi-atomic models of the ribosome were obtained in these ratchet-motion-related functional states. The observed differences in rRNA were further mapped onto the highly conserved RNA secondary structure diagram. Comparisons between the two sets of ratchet motions revealed that, while the overall patterns of the RNA displacement are very similar, several local regions stand out in their differential behavior, including the highly conserved GAC (GTPase-associated-center) region. We postulate that these regions are important in modulating the general ratchet motion and bestowing it with the dynamic characteristics required for the specific function.

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Cryo-em Study Of Trna Hybrid States Stabilized By Viomycin

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Translocation is the step in translation where the peptidyl A-site tRNA on the ribosome moves to the P site and the deacylated P-site tRNA moves to the E site. Recently, several single-molecule FRET studies and cryo-EM studies have confirmed the existence of the tRNA hybrid states (A/P and P/E) and the spontaneous ratchet motion of the ribosome from Macrostate I to Macrostate II prior to translocation (Agirrezabala et al., 2008; Ermolenko et al., 2007a,b; J. Fu, J.B. Munro, S. Blanchard, J. Frank, unpublished). In one of the studies, antibiotic viomycin, which is known to block translation, was shown to promote MS II and the hybrid states the tRNA (Ermolenko et al., 2007B). To determine the mechanism by which viomycin blocks translation, and to further understand the intermediate states during translocation, we studied a pre-translocational complex prepared with viomycin. Single-particle reconstruction was used to determine the structure of the complex. Subsequent classification resulted in two distinct ribosome complexes in MS I and II. We believe that MS II represents the state in which translation is stalled by viomycin. Subsequent analysis revealed that the A-site tRNA is in the A/P hybrid state and the P site tRNA in a novel position in which it makes extensive contacts with the L1 stalk. The results confirmed the observation, by the single molecule FRET study, that viomycin locks the ribosome in the hybrid state. We are setting out for a more detailed analysis to understand the molecular details of the viomycin-induced hybrid states.

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2990-Pos Board B37

Polyelectrolyte Behavior And Kinetics Of The Aminoacyl-trna On The Ribosome

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A coarse-grained model is utilized to examine the changes in flexibility of aminoacyl-transfer RNA when it binds to elongation factor-Tu and guanosine-5′-triphosphate GTP. We predict that under appropriate conditions mode-coupling speeds-up the barrier-crossing rate for cognate (three base pairs that are matched) relative to near-cognate (one base pair mismatch) ternary complexes. We estimate the torque acting on the cognate ternary complex due to induced wrapping of the 30S subunit around the decoding site after correct codon-anticodon recognition. We predict by all atom grand Monte Carlo simulations the magnesium binding sites in tRNA-EF-Tu complex at low magnesium concentration. The prediction is in agreement with binding sites observed in x-ray structure (grown under high salt concentrations). We have used high level ab initio calculations to unravel the nature of interaction energy of magnesium with site-specific tRNAPhe bases. We find noticeable non-electrostatic contributions to the total interaction energy of the magnesium-base complex in gas phase and in polar solvent. Finally, we have developed stochastic techniques to elucidate fundamentally important rare events involving large thermal fluctuations along reaction pathways. These techniques will allow us to investigate the probability of forming contacts to stabilize GTPase activated state that involve configurational searches in the tail end of probability distribution.

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Four Amino Acids, Two Kinetic Steps, No Synthetase: The Original Genetic Code?

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Considering a theoretical genetic system with only four codon-anticodon pairs and four amino acids randomly assigned to the tRNAs, we show that an elementary form of translation allows the system to display coding rules for particular values of kinetic constants and reactants concentrations. We show that these values compare well with experimental data. The analysis suggests that only two types of amino acids could be efficiently differentiated at that level. While adding the contribution of a plausible form of tRNA aminoacylation inferred from studies on ribozymes, we show that the combination of both steps would allow this polymerization process to differentiate the four amino acids without aminoacyl-tRNA synthetase. Features of the genetic code support our analysis.

Protein Conformation

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Surface Modification Affects the Heme Planarity and Accessibility in Horseradish Peroxidase

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Some noncovalently linked hemes like those in the peroxidases have highly conserved characteristic distortions in the porphyrin plane. Conservation occurs even for some proteins with a large natural variation in the amino acid sequence. Thus it is reasonable to anticipate that nonplanar porphyrins and protein-induced changes in the planarity may provide a mechanism for protein modulation of biological properties.

We previously reported that covalent modification of three accessible charged lysines (Lys-174, Lys-232, Lys-241) to the hydrophobic anthraquinolysine residues successfully improves electron transfer properties, catalytic efficiency, and stability of HRP.