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.

2988-Pos Board B35

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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2989-Pos Board B36

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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2991-Pos Board B38

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

2992-Pos Board B39

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.

Here we report that this modification also alters the Heme planarity and accessibility. Using the known x-ray crystallographic structure of HRP, two 3D models of HRP representing the native and modified enzyme, were constructed. Molecular dynamics simulations showed that upon modification, the accessibility of the heme prosthetic group to the substrates increased from 39.6 to 99.7 $Å^2$. Moreover, the Heme Planarity also affected significantly. While in the native structure the heme plane is concave and curves toward the proximal His, in the modified enzyme the heme assumes a planar conformation and shifts the position of the proximal His. This change causes the heme iron to become more exposed on the distal side of the porphyrin plane and so more accessible to the peroxide substrate. This result agrees well with the experimentally observed enhanced reactivity of the modified enzyme to the peroxide substrates.

2993-Pos Board B40

Thermodynamic and Structural Analysis of Domain Interactions in PKR

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PKR (protein kinase R) is induced by interferon and is a key component of the innate immunity antiviral pathway. Upon binding dsRNA, PKR undergoes autophosphorylation reactions that activate the kinase, leading it to phosphorylate the translational initiation factor eIF2a, thus inhibiting protein synthesis in virally-infected cells. PKR contains a dsRNA binding domain (dsRBD) and a kinase domain. The dsRBD is composed of two tandem dsRNA binding motifs. An autoinhibition model for PKR has been proposed whereby dsRNA binding activates the enzyme by inducing a conformational change that relieves the latent enzyme of the inhibition that is mediated by interaction of the dsRBD with the kinase. We have probed the importance of interdomain contacts by comparing the relative stabilities of isolated domains with the same domain in the context of the intact enzyme using equilibrium chemical denaturation experiments. The two dsRNA binding motifs fold independently, with the C-terminal motif exhibiting greater stability. The kinase domain is stabilized by about 1.5 kcal/mole in the context of the holenzyme and we detect low-affinity binding of the kinase and dsRBD constructs in solution, indicating that these domains interact weakly. The p(r) distance distribution function obtained from small angle X-ray and neutron scattering experiments reveals that PKR populates extended conformations. Structural modeling using the published coordinates for the dsRBD and the kinase domain supports a model where PKR exhibits considerable flexibility and exists in a range of open and compact conformations. Our results do not support the autoinhibition model where latent PKR is locked in a stable, closed conformation and the dsRBD interacts with the kinase domain to block substrate binding.

2994-Pos Board B41

Substrate-induced Conformational Transition Of The CBS-PPase Of Moorella thermoacetica Is Regulated By pH And Adenine Nucleotide Binding

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CBS domains are putative energy-sensing modules, form a dimer in solution and have been shown to bind adenine nucleotides in a hydrophobic central cavity. Point mutations in either repeat can cause hereditary disorders, depending on the protein in which they are embedded. Inorganic pyrophosphatases (PPases), on the other hand, are ubiquitous phosphohydrolases which break down pyrophosphate (PPi) and release the resulting energy as heat into solution.

Previously we characterized a CBS-PPase from Moorella thermoacetica, containing a CBS repeat in its C-terminus. We showed that it has an absolute requirement for transition metal ions for maximal activity, is a dimer in solution and is regulated by adenine nucleotides where AMP and ADP inhibit activity, while ATP activates (2-fold). During the study, we noticed a lag in the time-course of mtCBS-PPase, where introduction of substrate to the reaction causes a putative conformational transition from inactive to active state. In the current study we characterized the lag in different conditions and with different methods, such as steady state kinetics, transient kinetics, fluorescence titration, filtration, equilibrium dialysis, and absorbance spectroscopy.

2995-Pos Board B42

Interaction Among The Stalk Modules Of Thrombospondin-1

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Thrombospondin-1 is a trimeric multi-module calcium-binding glycoprotein that is involved in important biological processes, such as angiogenesis, signal pathway, tumor formation and synaptogenesis. The subunit is composed of an N-terminal module, oligomerization module, the stalk modules, which including von Willebrand Factor type-C (vWF-C) module, three properdin or thrombospondin type 1 (TSR) modules, and three EGF-like modules, and finally the C-terminal calcium-binding wire module and the lectin-like module. Recent research indicates that conformational changes in the C-terminal modules influence ligand binding to the N-terminal modules. The second and the third EGF-like modules have been shown to interact closely with both the wire and the lectin-like modules. My work focuses on the interaction among the stalk modules and if they can propagate conformational changes between C-terminal and N-terminal elements of TSP-1 and interact with other extracellular molecules. Series of recombinant proteins have been generated based on the sequence of stalk modules. We have applied biophysical methods including far UV circular dichroism, intrinsic fluorescence, differential scanning calorimetry, isothermal titration calorimetry, and competitional ELISA. We have found there are strong interactions among the three EGF-like modules. There is one calcium binding site with in the second EGF-like module, the calcium concentration influences the stability of all the three modules from the ITC results. Based on DSC, far UV CD and fluorescence results, there are also strong interaction among the three TSRs and between TSRs and EGF-like modules. We did not find evidence for the interaction between vWF-C and TSR modules.

Overall, we have demonstrated that there are strong interactions among the stalk modules of TSP-1, which help relay conformational information between the N-terminal and C-terminal parts of the protein.

2996-Pos Board B43

pH Induced Conformational And Structural Alterations On Choline Oxidase

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Choline oxidase (ChOx) catalyzes the four-electron oxidation of choline to glycine betaine (GB). This reaction is of considerable importance for medical and biotechnological reasons; due to the accumulation of GB has been observed in a number of human pathogenic bacteria and the cytoplasm of many plants in response to hyperosmotic and temperature stresses, hence resulting in the prevention of dehydration and cell death. To complete the investigation, we employ different pH values subsequently measuring the function and activity of choline oxidase. Our results demonstrated that a reversible effect of pH on the ionization of amino acid residues at the active center of choline oxidase was observed near the optimum pH (8). The main inactivation of choline oxidase took place in the pH ranges 3-6 and 9-11, in which irreversible changes in the structure occurs leading to the enzyme inactivation. Furthermore, at higher pH a transition from α-helix to β-structure was appeared. It is interesting to point out that at lower pH the content of α-helix structure was increased. In addition, results of thermal denaturation of the enzyme at different pH by far-UV-CD evaluated that ChOx has the most stability structure at pH 8 and the most instability occur at higher pH values. Altogether low and high pH caused significant alteration on secondary and tertiary structures and activity of choline oxidase.

2997-Pos Board B44

Regulation of mGluR potentiation by Pin1 peptidyl-prolyl isomerase

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Group 1 metabotropic glutamate receptor (mGluR1/5) signaling has been implicated in mechanisms of cortical development, neurodegenerative diseases, associative learning, addiction and neural plasticity. These receptors are localized to the post-synaptic density and upon activation evoke a slow inward current. Strong stimuli, such as seizures, activation of dopamine receptors by amphetamines or exposure to new environment, act via the TrkB kinase cascade to phosphorylate the C-terminal domain of mGluR5 and result in subsequent prolonged potentiation of the mGluR-mediated slow inward current.

This phosphorylation dependent signaling pathway has been shown to rely on the rapid upregulation of an immediate early gene coding for Homer 1a protein and its interaction with a specific proline-directed phosphorylation site in the mGluR. Homer 1a was believed to act via simple competition with other Homer family proteins that contain a coiled-coil multimerization domain, absent in Homer1a, thus disrupting physical receptor-receptor couplings maintained by these proteins. More recently, electro-physiological experiments performed at Dr. Worley's lab have indicated that catalytic action of Pin1 peptidyl-prolyl isomerase is essential in the regulation of this pathway. However, the actual mechanism of this three-component switch remains unknown.

We are using NMR techniques to investigate the molecular basis for Pin1 and Homer 1a facilitated regulation of the mGluR5. Our results so far suggest a kinetic-trap based switching mechanism for mGluR5-mediated current potentiation.