processing step for proteins that contain a 'CAAX' motif, including Ras. Because inhibition of ICMT limits Ras-induced oncogenic transformation, ICMT is a potential target for cancer therapy. Human ICMT is predicted to have eight transmembrane helices and has no discernable homology with soluble methyltransferases. An outstanding question in understanding the mechanism of this enzyme is how both its water-soluble substrate (AdoMet) and lipophilic substrate (a prenylated cysteine) are recognized in the active site. A crystal structure of an integral membrane methyltransferase from a prokaryotic organism was recently determined. The prokaryotic methyltransferase has sequence similarity with ICMT in the region that binds AdoMet, but methylates different substrates, which are currently unknown. In order to identify regions of ICMT that are important for catalytic activity and delineate the CAAX binding site, we combined scanning mutagenesis with methyltransferase assays and interpreted the results with regard to the available structure. Of the 153 mutated positions, 61 reduced catalytic activity by $> 50\%$. Mutation of the conserved residues lining the AdoMet binding site reduced or eliminated catalytic activity, suggesting a similar mode of AdoMet recognition in the prokaryotic methyltransferase and ICMT. In addition, our studies have identified several residues, primarily aromatics, that may line the CAAX binding site. When substituted to alanine, the mutant enzyme is inhibited by higher concentrations of the lipophilic substrate. We propose that substrate inhibition results because these mutations alter the shape of the lipid-binding tunnel causing the substrate to bind nonproductively. Our results offer novel insights into the substrate binding sites of this unique enzyme.

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Regulation of Creatine Kinase by ASB9

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Creatine kinases (CKs) regulate ATP levels at sites of fluctuating energy demands. Reduced CK activity has been implicated in diseases such as heart failure and multiple sclerosis, however little is known about CK regulation. Ankyrin repeat and SOCS box-containing protein, ASB9, (part of an E3 ubiquitin ligase) has been shown to promote proteasomal degradation of CKs. We hypothesize that ASB9 and its splice variants control the levels and activity of CK at sites where energy is critically needed. Our results show that the ankyrin repeat domain of ASB9 (ASB9-ARD) binds CKB with very high affinity (nM) and 1:1 stoichiometry. Comparison of binding data from several N-terminal truncations of ASB9-ARD suggests that residues 19-35 of ASB9 contribute to the binding affinity. In addition, binding of ASB9-ARD to CK abolishes the enzymatic activity of CK. Hydrogen deuterium exchange mass spectrometry (HDXMS) has revealed that only one region in CKB (residues 182 - 203), was protected upon binding of ASB9 when the deuterium incorporation into CKB peptides alone was compared to those in complex with ASB9-ARDs. This region is right in front of the active site of the enzyme, and was shown previously to undergo a conformational change upon binding of creatine and ADP-NO3 to rabbit CKM. Results from the CK inhibition assay together with the HDXMS data strongly suggest that ASB9 binds directly to CKs and inhibits CK activity by altering regions near the active site of the enzyme. A model of this interaction generated using docking and computational modeling agrees with the binding and HDXMS data. We intend to completely characterize the ASB9-CK interaction and discover inhibitors that would disrupt the interaction, resulting in increased amounts of active CK to treat diseases in which there is a an increased energy demand and decreased CK function.

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Understanding Functional Evolution in the Alkaline Phosphatase Superfamily

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Over the past 40 years, it has been demonstrated that many enzymes are capable of promiscuous catalytic activities, facilitating the turnover of more than one chemically distinct substrate. This has been argued to play an important role in enzyme evolution, with highly promiscuous progenitor enzymes evolving under evolutionary pressure to modern day specialists, while still retaining some level of their former promiscuous activities[1]. This theory has been extensively tested by different experiments using *in vitro* evolution[2]. The alkaline phosphatase superfamily members provide a particularly attractive showcase for studying enzyme promiscuity, as they often show reciprocal promiscuity, in that the native reaction for one member is often a side-reaction for another[3]. While deceptively similar, their catalyzed reactions (cleavage of P-O and S-O bonds) proceed *via* distinct transition states and protonation requirements[4,5]. We present detailed computational studies of the promiscuous catalytic activity of three evolutionarily related members: the arylsulfatase from *Pseudomonas aeruginosa*[6], and the phosphonate monoester hydrolases from *Burkholderia caryophili*[7] and *Rhizobium leguminosarum*[8]. By tracking their structural and electrostatic features, and comparing to other known members of the superfamily, we provide an atomic-level map for functional evolution within this superfamily.

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Did Class 1 and Class 2 Aminoacyl Trna Synthetases Descend from Genetically Complimentary, Catalytically Active ATP-Binding Motifs?

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We examined the contribution of protein evolution to the beginning of life by performing a direct test of the Rodin and Ohno hypothesis that the two Aminoacyl tRNA synthethase (aaRS) classes descended from opposite strands of a single gene. Class I and Class II aaRS catalyze synthesis of aminoacyl-tRNA molecules for use in codon-directed protein synthesis. Validating that hypothesis would thus substantially simplify the origins of an elemental process necessary for life to occur. The test of the hypothesis was set up by constructing two genetically engineered genes for 46-residue ATP binding portions of Class I and II synthetases that are coded by fully complementary nucleic acid strands. I measured the catalysis of the amino acid activation reaction by these two simple active sites. Using experimental variations of amino acid concentration, peptide concentration and time of reaction, I confirmed that the observed rate of reaction was significantly higher than the background reaction and proportional to the concentration of catalytic peptide. We observed significantly higher catalytic activity from the Class I ATP binding peptide, as had been observed previously for the wild-type Class I sequence. Activity increased with time and with increase in amino acid and peptide concentrations. The enhanced rate of amino acid activation seen for the two classes of genetically altered peptides coded by opposite strands, thus supported Rodin and Ohno's hypothesis. Ancestral 46-residue peptides coded by opposite strands of a single RNA gene might therefore have helped launch protein synthesis by activating amino acids. This work suggests that the earliest peptide catalysts might have been quite short. Sense/antisense coding would have introduced unexpected diversity from a single gene. Supported by NIGMS 78227 to C. W. Carter, Jr, and the ABS.

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Analyses of the Interaction Between Lipocalin-Type Prostaglandin D Synthase and Substrate or Product

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Lipocalin-type prostaglandin D synthase (L-PGDS) catalyzes the isomerization of PGH_2 to PGD_2, which acts as a endogenous somnogen in the brain. This enzyme belongs to the lipocalin superfamily which consists of transporter proteins for lipophilic substances in the extracellular space. Our previous studies suggested that L-PGDS consists of a β-barrel structure with a hydrophobic pocket. The active thiol group of the Cys65 residue is located in this pocket and faces to the inside of the pocket.

A number of biochemical studies for L-PGDS, as a drug target for sleep disorders, have been reported in attempts to understand its catalytic mechanism, and several substrate recognition models of L-PGDS have been proposed. However, details of the mechanism by which L-PDGS recognizes its substrate are obscure, since essential information, such as its binding affinity and stoichiometry, of the interactions between L-PGDS and substrates remains unclear. Therefore, isothermal titration calorimetry (ITC) experiments were carried out to characterize the binding properties, including binding affinity and stoichiometry, of L-PGDS with respect to its substrates and products. In addition, to obtain thermodynamic information regarding the substrate and product binding, the enthalpy (ΔH) and entropy (ΔS) of the binding reactions were calculated.

The results of ITC experiments revealed that both the substrate and the product bind to L-PGDS with a stoichiometry of 2 to 1 and showed two binding sites