characterized aspartyl-(DRS) and asparaginyl-tRNA synthetase (NRS) from Plasmodium falciparum to determine the basis of their specificity towards L-asp and L-asn respectively. The negatively charged L-asp and its analogue L-asn differ only in their side-chain groups i.e., -OH and -NH_2. Further, the amino acid binding sites are highly conserved within these two enzymes. Analysis of the substrate (L-asp/L-asn) binding sites across species revealed two highly conserved residues in PfDRS (D408 and K372) and PfNRS (E395 and L360) that are involved in recognition of the $O^{\delta 2}/N^{\delta 2}$ of L-asp/L-asn respectively. These residues were mutated and swapped between the D408 → E in PfDRS and the corresponding E395 → D in PfNRS. A similar approach was employed for residue number K372 → L in PfDRS and L360 → K in PfNRS. The mutated $PfDRS^{D408E}$ retained its enzymatic activity during step 1 of aminoacylation reaction towards L-asp and L-asn and esterified $tRNA^{Asp}$ with L-asp like wild type enzyme, while the $PfDRS^{K372L}$ was rendered enzymatically inactive. The correspondingly mutated $PfNRS^{E395D}$ was enzymatically inactive. The mutated $PfNRS^{L360K}$ had an altered specificity and esterified $tRNA^{Asn}$ with non-cognate amino acid L-asp and not L-asn. These data suggest that the residue K372 is crucial for the enzymatic activity of PfDRS while the residue L360 in PfNRS imparts specificity towards L-asn.

2342-Pos

Activation of adenosine A2A receptors induces SUMOylation of HIF-1α

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Hypoxia is an established driver of numerous deleterious cell processes, including immunosuppression and tumor growth. Hypoxia-inducible factor 1α (HIF-1α) accumulates during periods of sustained hypoxia due to the action of multiple post-translational modifications, including the Small Ubiquitin-like modifier (SUMO) pathway. Although enhanced activity of the SUMO-pathway is upregulated during hypoxia, the mechanistic basis for this effect remains unclear. It has been proposed that the Gαs protein-coupled adenosine A2A receptor (A2AR), which is also activated during sustained hypoxia, mediates SUMOylation at a number of target proteins. We sought to determine if activation of A2AR by hypoxia induces SUMOylation of HIF-1α and which of three potential SUMO-sites on HIF-1α are modified by this mechanism. HEK293T cells expressing A2AR and treated with an A2AR agonist (CGS) showed significantly increased HIF-1α accumulation compared to untreated controls after exposure to hypoxia. Similarly, CGS and hypoxia enhanced SUMOylation of HIF-1α in human Jurkat T cells. Using site-directed mutagenesis and Förster resonance energy transfer (FRET), we determined that A2AR-mediated SUMOylation of HIF-1α occurs at lysine 532. Finally, we show that (1) this mechanism of regulation increases the mRNA levels of multiple HIF-1α transcriptional targets; and (2) this mechanism can be precluded by treating cells with a small molecule inhibitor of the SUMO pathway. Together, these data highlight a central role for A2AR-mediated SUMOylation of HIF-1α in mediating the effects of chronic hypoxia in Jurkat T cells.

2343-Pos

Biochemical characterization of LSD1 mutants with enhanced demethylase activity toward acetylated substrates

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Histone demethylase LSD1 regulates gene expression by catalyzing the removal of methyl marks at Lys4 on histone H3. LSD1 plays a critical role in promoting epithelial to mesenchymal transition, a process implicated in cancer progression. Acetyl marks present at Lys14 are known to slow LSD1 demethylase activity in cell and *in vitro*, decreasing gene repression. LSD1 mutants S390K and Y391K, located 30 Å from the flavin cofactor in the active site, were previously shown to display enhanced activity toward nucleosomes containing dually modified dimethylated Lys4 and acetylated Lys14 marks (H3K4me2K14ac). To gain mechanistic insights into how LSD1 mutants overcome Lys14 acetyl marks, wild-type LSD1 and mutants S390K and Y391K were biochemically characterized using a hydrogen peroxide and horseradish peroxidase coupled demethylase assay and H3K4me2 and H3K4me2K14ac peptide substrates. S390K and Y391K showed a ∼50% increase in catalytic activity toward the H3K4me2K14ac peptide while showing a ∼2-fold increase in catalytic efficiency toward the H3K4me2 peptide. The mutants' gain of catalytic activity toward the H3K4me2-K14ac peptide substrate was rather subtle, suggesting there are additional factors involved in LSD1 catalysis and substrate interactions that enable mutant LSD1 to overcome acetyl marks on nucleosome substrates. Interestingly, these mutants' enhanced catalytic efficiency toward the H3K4me2 peptide substrate is a rare example of gain of function LSD1 mutants that highlights the unique regulatory function of the helix encompassing S390 and Y391. This motivated us to further explore this region using small molecule screening experiments *in silico*.

2344-Pos

Identifying esterase and lipase activity in proteins of unknown function

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Over 3800 structures in the Protein Data Bank (PDB) have unknown function. The Biochemistry Authentic Scientific Inquiry Lab (BASIL) curriculum uses authentic inquiry to teach students to use structural bioinformatics tools to compare these structures to known enzymes and predict a function. We have predicted esterase or lipase function for several structures after performing both global and local structure alignments to identify similar structures in the PDB as well as specific amino acids that may be active site motifs. This function was supported by sequence alignment data from BLAST and PFam. SwissDock was then used for ligand docking to determine ester and lipid substrates to use for testing activity. Students in the biochemistry lab next used standard wet-lab biochemistry techniques to express and purify the target enzymes and performed kinetic assays using *p*-nitrophenyl acetate to test for esterase activity. After completing the BASIL curriculum, students continued with independent research projects to develop assays to test for lipase function. We have focused our efforts on the protein structures with PDB IDs 2O14 and 4Q7Q, as they showed the most promise through initial enzymatic testing. Both proteins show significant esterase activity in hydrolyzing both *p*-nitrophenyl acetate and *p*-nitrophenyl butyrate. Additionally, both proteins are able to hydrolyze *p*-nitrophenyl dodecanoate when sodium deoxycholate is present in the buffer solution for solubility of the substrate. We have also incorporated the *p*-nitrophenyl dodecanoate in DMPC vesicles, and testing of substrate hydrolysis with these vesicles is underway. We have also tested pancreatic lipase under all these conditions as a positive control for the experiments. Students will be creating a Proteopedia page for each protein. This project has been supported in part by NSF IUSE 1709355 and 2141908.

2345-Pos

Disease-associated mutations in Drp1 have fundamentally different effects on the mitochondrial fission machinery

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Patient mutations have been identified throughout dynamin-related protein 1 (Drp1), the key mediator of mitochondrial fission. These mutations generally impact children and often result in severe neurological defects and, in some cases, death. Previously, the underlying functional defect leading to patient phenotypes has been speculated based on comparisons with synthetic mutations at similar sites. Drp1 is comprised by four domains, including a GTPase domain (G-domain) that regulates GTP binding and hydrolysis and a middle domain (MD) that mediates Drp1 self-assembly. We analyzed six mutations, four in the MD and two in the G-domain. All the MD mutants studied were found to be dimer-limited in solution, and three of these MD mutants were found to be impaired in self-assembly. However, one of the MD mutants retains oligomerization capability despite being located within this self-assembly region. Further, this mutant retains its ability to interact with a pre-formed lipid template but is unable to reshape large, unilamellar vesicles (LUVs). This indicates that Drp1 dimers alone are sufficient to form a helical polymer on lipid template. Differences were also observed between the two G-domain mutations. One of the mutants is in a nucleotide-binding loop that is critical for functional hydrolysis. This mutant exhibited impaired GTP hydrolysis but can still oligomerize in solution and with a lipid template. In contrast, the other G-domain mutation is located relatively distant from the nucleotide binding site. This mutant poorly tubulates LUVs, highlighting the role of G-domain interactions in driving membrane curvature. Overall, the functional defects caused by mutations in Drp1 are highly variable even when the mutations occur within the same functional domain. This study provides a framework for characterizing additional Drp1 mutations to provide a comprehensive understanding of functional sites within this essential protein.

2346-Pos

Photo-control of small GTPase Ras GTPase cycle using photochromic molecule

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The small G protein Ras is a central regulator of cellular signal transduction processes, functioning as a molecular switch. Structure and regulatory mechanisms of Ras have been well studied at the molecular level. In this study, we have tried to photocontrol of Ras function using photochromic molecule as a regulatory molecular device. The two methods utilizing azobenzene derivatives as photochromic molecule were employed. The first one is that incorporating bifunctional azobenzene derivatives into the inhibitory peptides and photocontrol Ras function with